Chapter 3
Neural Stem Cells/Neuronal Progenitor Cells

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Abstract Neural stem/progenitor cells (NSCs) are defined as cells with the potential for self-renewal and differentiation into neurons, astrocytes, and oligodendrocytes. These cells can be derived from several sources, including embryonic stem cells and fetal tissue. NSCs have been found to exist not only in the developing brain but also in the mature mammalian brain. NSCs were initially cultured as floating neurospheres in the presence of epidermal growth factor from adult and embryonic murine forebrain. Cell transplantation using these cells has evolved as a promising experimental treatment approach for stroke. Additionally, the activation of endogenous neural stem/progenitor cells has recently been employed for stroke treatment. This review provides an introduction to neural stem/progenitor cells and briefly describes some advances in neural stem cell transplantation for stroke.

Keywords Neural stem/progenitor cells • Neurosphere • Subventricular zone • Subgranular zone • Transplantation

3.1 Cell Biology

As initially observed by the pioneering neuroscientist Santiago Ramon y Cajal, the mature central nervous system (CNS) was thought to be distinguished from the developing nervous system by the lack of growth and cellular regeneration; it was believed that nerve paths were something fixed, ended, and immutable and had no regeneration potential in the adult CNS [1]. However, recent advances in neuroscience have revealed the falsehoods in this myth. In 1992, Reynolds, Weiss, and colleagues for the first time isolated neural stem cells (NSCs) and propagated them in the presence of epidermal growth factor (EGF) to give rise to large cellular spheres that they termed "neurospheres" [2, 3]. Neurons and glial cells are derived from common immature NSCs, which are defined as self-renewing and multipotential cells (Fig. 3.1). NSCs have been found to exist not only in the developing

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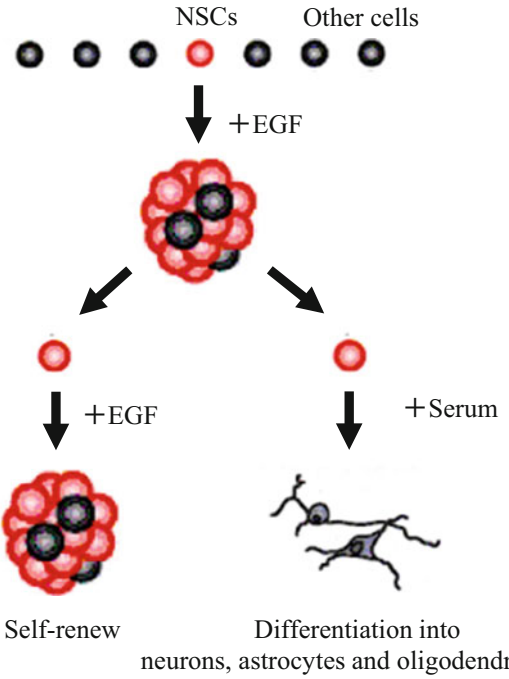

Fig. 3.1 NSC and neurosphere method

brain but also in the mature mammalian brain. Cultured NSCs derived from murine embryonic brains can be propagated by incubation in serum-free medium containing EGF and subsequently differentiated into neurons and astrocytes by incubating in low-serum medium (e.g., 1 % fetal bovine serum-containing medium without EGF) [4].

NSCs exist in at least two regions of the adult brain – the subventricular zone of the lateral ventricle and the subgranular zone of the hippocampus. Newborn neurons are incorporated into existing functional networks and are thought to have important innate and adaptive roles in cognition, behavior, and tissue repair [5]. Notch signaling, which is highly active in quiescent NSCs in these areas, plays a pivotal role in maintaining the undifferentiated and quiescent state of NSCs [6–8]. Interestingly, NSCs give rise to their own niche cells through asymmetric segregation of Notch ligand Delta-like 1 during mitosis, a process that may contribute to initialization of activated NSCs to return to a basal NSC state (undifferentiated and quiescent) [9]. Conversely, transcription factors including basic helix-loop-helix (bHLH) transcription factors regulate NSC proliferation and differentiation of each cell type [10]. Proneural bHLH genes, such as Ascl 1 (as Mash 1) and Neurogenin 2, promote neuronal fate determination and suppress astrocytic gene expression [11, 12]. The bHLH gene Olig 2 regulates oligodendrocyte specification, whereas the bHLH genes Hes 1 and Hes 5 maintain NSCs by repressing proneural gene expression [13, 14]. In addition, Ascl 1 and Olig 2 regulate oligodendrocyte and motor neuron development, respectively [13, 14]. A

recent report showed that oscillatory control of these factors determines NSC multipotency and fate [15].

NSCs first expand by rapid cell division to generate a large number of different types of neurons during the early stage of brain development. After this neurogenic period, NSCs mostly lose their neurogenic potential and begin to preferentially generate glial cells during postnatal stages (astrogenic phase). Early stage NSCs have a greater capacity to proliferate and self-renew than late-stage NSCs [16]. This suggests that NSCs lose their neurogenic potential during development, which might be a disadvantage for neuronal repair in adult CNS. Kishi et al. found that neocortical NSC chromatin becomes globally condensed in a stage-dependent manner and that high-mobility group A (HMGA) proteins, which are chromatin architectural proteins, are necessary for the open chromatin state in early stage NSCs [17]. They also found that reduced HMGA protein levels and resultant global chromatin condensation are involved in restriction of the NSC differentiation potential during neocortical development [17]. Thus, HMGA proteins are capable of reprogramming late-stage NSCs into cells with early stage-specific capacities.

Developmental studies and experimental data have enabled us to determine that the terminal cell differentiation state is reversible and that altering the balance of specific transcription factors could be a powerful strategy for inducing pluripotency [18]. It has recently been demonstrated that induced neural stem cells (iNSCs) can be obtained from rodent and human somatic cells, such as fibroblasts, through forced expression of defined transcription factors [Sox2, Klf4, and Myc (also known as c-Myc) and Pou3f4 (also known as Brn4)] [19]. To date, two different approaches have been successfully used to obtain iNSCs: a direct method and an indirect method that involves an intermediate destabilized state. The possibility to induce characterized iNSCs from human cells, e.g., fibroblasts, has opened new horizons for research in human disease modeling and cellular therapeutic applications in the neurological field [20].

3.2 Ischemia-Induced NSC Activation

In vitro studies have shown that hypoxia enhances proliferation of cultured NSCs and modifies the ability of the cells to differentiate [21–24]. Conversely, reduced glucose has been shown to suppress proliferation and increase differentiation of murine neural stem cells [25]. It is now well known that endogenous neurogenesis occurs in certain brain areas after cerebral ischemia, such as the subgranular zone of the dentate gyrus in the hippocampus [26], subventricular zone of the lateral ventricle in the striatum [27], and cortical layer [28]. Some evidence indicates that these neurons reestablish connections and contribute to functional recovery [29, 30]. These new neurons migrate into the impaired lesion, where they express markers of projection neurons. However, the majority of new neurons die during the first weeks after stroke and are only capable of replacing a small fraction of necrotic mature neurons [31]. Recently, electrical stimulation has been reported to

elicit NSC activation and strengthen intrinsic neurogenesis as well as chemical stimulation, which could be suitable for the clinical application to stroke, because it is well established and its potential complications are manageable [32].

3.3 NSC Transplantation for Stroke

3.3.1 Interaction Between Transplanted NSC and Host Brain

Transplantation of NSCs has been proposed as a promising therapeutic strategy in almost all neurological disorders, including Parkinson's disease [33], Huntington's disease [34], Alzheimer's disease [35], multiple sclerosis [36], amyotrophic lateral sclerosis [37], spinal cord injury [38], and ischemic stroke [39], which are characterized by the failure of CNS endogenous repair mechanisms to restore damaged tissue and rescue lost functions [40]. If the use of NSC transplantation is to be translated to clinical use, it is important to understand the mechanisms of action for improved recovery. The initial hypothesis assumed that NSCs would replace lost neurons and circuits. However, evidence for widespread afferent and efferent neuronal projections is lacking. NSCs prevent neuronal-programmed cell death and glial scar formation mainly via paracrine secretion of nerve growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, and glial cell-derived neurotrophic factor. Recent preclinical data confirmed that transplanted NSCs may exert a "bystander" neuroprotective effect. Results also identified a series of molecules – immunomodulatory substances, neurotrophic growth factors, stem cell regulators, and guidance molecules secreted from NSCs, which are temporally and spatially orchestrated by environmental cues [41]. The bystander effect is a multistep process that depends on the timing of cell injection and route of cell transplantation [42]. Once injected, NSCs migrate and home to injured sites [43, 44], likely due to constitutively expressed chemokine receptors, such as CXCR4, cell adhesion molecules, and integrins, which allows the NSCs to follow chemoattractant gradients and reach damaged lesion sites [45]. Following migration to the injured areas, transplanted NSCs survive in close proximity to blood vessels (Fig. 3.2), where they interact with inflammatory cells, endothelial cells, astrocytes, and microglia. If the NSCs are transplanted into a non-injured brain, NSC migration does not occur [43, 44]. Conversely, NSCs have the potential to integrate into the injured brain after differentiation into appropriate cells. However, this remains undetermined and it is unclear whether this contributes to functional recovery. The major concern in utilizing these cells is the capacity of NSCs to form tumors, although tumorigenicity is less for fetal-derived NSCs than for embryonic-derived NSCs [46].

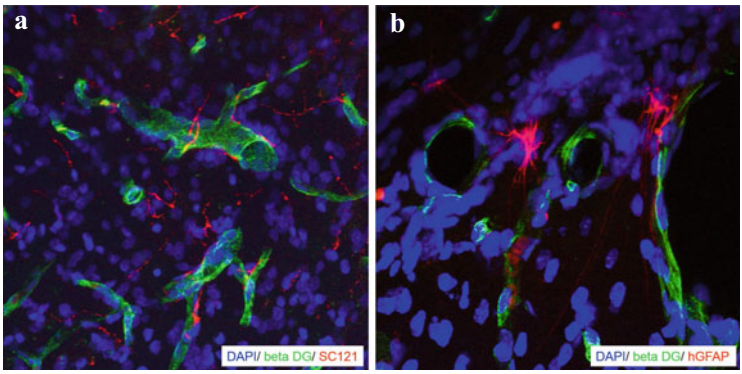

Fig. 3.2 NSCs survive in close proximity to blood vessels. Human NSCs (**a** *red*, SCS212) and NSC-derived astrocytes (**b** *red*, hGFAP) attached to vessels (*green*)

3.3.2 Endogenous Brain Repair After NSC Transplantation

3.3.2.1 Angiogenesis/Neovascularization

Transplanted NSCs migrate toward infarct lesions along existing vessels. Chemoattractants, such as stromal cell-derived factor-1 [45] and monocyte chemoattractant protein 1 [47], are reported to be critical factors associated with cell migration and homing to lesions, although the interaction between transplanted NSCs and existing vessels has not been fully elucidated. Nevertheless, the increased vascularization in the peri-infarct area after stroke is associated with functional recovery [48, 49]. Subacute NSC transplantation enhances neovascularization, and stem cell-induced vascular endothelial growth factor (VEGF) plays a critical role, as well as an anti-inflammatory effect [42]. Moreover, these vascular events correspond with two patterns of functional recovery: an early mode of recovery independent of neovascularization and delayed recovery that is NSC secreted and VEGF dependent and coincides with increased vascularization [42].

Transplanted NSCs upregulate expression of tight junction proteins, such as occludin, claudin 5, and Zo-1, and contribute to blood-barrier integrity by reducing leakage [42]. Although the functional role for neovessels has not been fully established, in addition to tissue perfusion, neovessels express trophic factors that remodel damaged tissues in the brain after ischemia, form new synapses, and attract endogenous neuroblasts originating in the subventricular zone [50].

3.3.2.2 Immunomodulation

Inflammation also plays an important role in ischemic stroke. Experimentally and clinically, the brain responds to ischemic injury with an acute and prolonged inflammatory process characterized by rapid activation of resident microglia,

production of proinflammatory mediators, and infiltration of various types of inflammatory cells into the ischemic brain tissue. However, these cellular events collaboratively contribute to secondary brain injury.

Interestingly, experimental stroke leads to splenic atrophy and spleen-derived, proinflammatory, monocyte, and macrophage mobilization into the circulation, as well as subsequent accumulation in the ischemic brain. The decreased splenic size inversely correlates with the extent of infarct volume [51, 52]. Therefore, removal of the spleen might be effective for reducing infarct volume after stroke.

Transplanted NSCs have an anti-inflammatory effect even after 2–3 weeks poststroke, and interestingly, this effect is associated with the development of neovessels [42]. Similarly to other stem cell types, NSCs exert immunomodulatory effects outside the brain upon systemic transplantation, occurring within secondary lymphoid organs [53]. NSC-secreted leukemia inhibitory factor inhibits differentiation of pathogenic Th17 cells through the extracellular signal-regulated MAP kinase suppression of the cytokine signaling 3 inhibitory signaling cascade that, in turn, antagonizes interleukin 6-mediated phosphorylation of signal transducer and activator of transcription 3, both of which are required for Th17 cell differentiation in peripheral lymphoid organs [54].

3.3.2.3 Axonal Sprouting, Dendritic Branching, and Synaptogenesis

Following ischemia, enhanced axonal sprouting takes place in the vicinity of the lesion, which extends from the intact cortex toward the deafferented cortical area [55, 56]. In rats, NSC grafts demonstrated increased corticocortical, corticostriatal, corticothalamic, and corticospinal axonal rewiring from the contralesional hemisphere, with transcallosal and corticospinal axonal sprouting correlating with functional recovery [57, 58]. Functional imaging has also shown similar remapping of the brain after stroke, indicating recruitment of both ipsi- and contralesional brain areas at least during the first few weeks following injury [59, 60].

Chronic changes in dendritic structural plasticity after stroke have also been reported with increased contralesional layer V dendritic branching peaking at 18 days poststroke, while ipsilesional layer III branching decreases at 9 weeks poststroke [61, 62]. NSCs enhance dendritic branching, length, and arborization at 3 weeks poststroke in layer V cortical neurons in both the ipsi- and contralesional cortex [57]. In vitro and in vivo studies have demonstrated that VEGF, thrombospondins 1 and 2, and slit act as mediators and are partially responsible for the NSC-induced effects on dendritic sprouting, axonal plasticity, and axonal transport [57, 63].

Some studies have shown that NSC transplantation enhances synaptophysin immunoreactivity in the ischemic boundary area after transplantation, suggesting that NSC transplantation enhances synaptogenesis [64–66]. Satisfactory functional recovery as a result of transplantation has been associated with increased expression of synaptogenesis markers [65]. Daadi et al. showed that NSCs increase expression of synaptic markers and enhance axonal reorganization in injured

areas at 4 weeks after transplantation [67]. This was also confirmed with initial patch-clamp recording [67] and electron microscopy [66].

3.3.3 Modification of NSC Grafts for Transplantation

One of the main problems with NSC transplantation is the massive graft cell death, which is possibly due to a hostile host brain environment and reduced the effectiveness of this approach. It has been reported that only 1–3 % of grafted cells survive in the ischemic brain after grafting [68, 69], mainly due to inflammatory responses in the host brain after ischemia. To address these issues, approaches to modify NSCs for longer survival have been proposed. Minocycline-preconditioned NSCs have been reported to tolerate oxidative stress after ischemic reperfusion injury and express higher levels of paracrine factors [70]. Genetic manipulation of NSCs to overexpress copper/zinc-superoxide dismutase (SOD1) was also reported to enhance graft survival in an animal model with intracerebral hemorrhage [71]. This strategy could be a highly effective approach, although its safety should be validated.

3.4 Activation of Endogenous Neural Stem/Progenitor Cells

Animal studies have demonstrated that stem cell transplantation reduces ischemic brain injury by increasing endogenous neurogenesis and angiogenesis [50, 72, 73], even in the aging brain. Functional recovery has also been achieved using cell transplantation therapy, and results show that transplanted NSCs influence the host brain by increasing endogenous striatal neurogenesis [50]. It is important to note that graft-evoked neurogenesis varies depending on graft location and stroke type [74]. Nevertheless, it remains unclear how much stroke-induced or transplanted NSC-induced neurogenesis contributes to recovery or endogenous angiogenesis, axonal sprouting, dendritic branching, and synaptogenesis.

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