



## Neural Stem Cells and their Niches in Neuroregeneration

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### Abstract

Stem cell-based therapies in alleviating symptoms or reversing the progression of neurodegenerative diseases and nerve injuries have been investigated extensively. In this review, we first summarize our current understanding of the ontogeny of neural stem cells (NSCs) and their mobilization *in vivo*. We then focus on the cellular reprogramming in generation of iP-NSCs, iNeurons and iNSCs, and applications of NSCs and iNeurons in cell-based therapy, disease modeling and drug discovery. NSCs belong to the adult stem cells, which can self-renew and differentiate into neurons, astrocytes and oligodendrocytes. NSCs reside in specialized niches of the adult mammalian brain, which support their self-renew and differentiation throughout life. Two regions continuously generate new neurons, including subventricular zone (SVZ) of the lateral ventricles and subgranular zone (SGZ) of the hippocampus. For adult neurogenesis, sensitizing to the stress and physical stimulations or exposure to an enriched environment increases adult neurogenesis in animal models. Therefore, mobilizations of NSCs could improve the symptoms of neuronal degeneration disorders of the central nervous system. In peripheral nerve injury, transplantation of NSCs provided effective therapies for many neurological diseases and injuries. Mechanisms of peripheral neuroregeneration by NSCs administration include differentiation into Schwann cells, secretion of neurotrophic factors, and promoting myelination. Moreover, differentiated cells that have been genetically reprogrammed to an embryonic stem cell-like state by forced gene expression and/or factors treatment could maintain the defining properties of embryonic stem cells or functional neurons. Processes of cellular reprogramming were illustrated in the generation of induced pluripotent stem cells-derived NSCs (iP-NSCs), induced neuronal cells (iNeurons) and induced NSCs (iNSCs). The primary advantage in the use of cellular reprogramming is that adult cells are more easily obtained and with less ethical concerns. Thus, iP-NSCs, iNeurons and iNSCs are considered as a good cell source for cell-based therapies, disease modeling and drug discovery in neurodegenerative diseases.

### Keywords

Neural stem cells, Neuroregeneration, Mobilizations of NSCs, Cellular reprogramming, iP-NSCs, iNeurons, iNSCs

### Introduction

The prevalence of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy (SMA) is increasing rapidly. Stem cell-based therapies in alleviating symptoms or reversing the progression of neurodegenerative diseases and nerve injuries, including stroke, spinal cord injury, and peripheral nerve injury (PNI), have been investigated extensively [1-4]. In many tissues, stem cells serve as an internal repair system, dividing essentially without limit to replace damaged cells. Stem cells are classified into two categories, embryonic stem cells and adult stem cells (also called somatic stem cells). Embryonic stem cells are pluripotent, which can become all cell types of the body, and adult stem cells are thought to be limited to differentiate into different cell types of their tissue of origin [5-8]. Adult stem cells have been identified in many tissues and organs, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium and testis. It resides in specific areas of the tissue (called stem cell niche), which remain quiescent (non-dividing) for long periods of time until they are activated by a need for more cells to maintain tissues, or by disease or tissue injury. Therefore, scientists believe that if the activation and differentiation of stem cells can be controlled, these cells may become the basis of cell-based therapies.

Neural stem cells (NSCs) belong to the adult stem cells, which reside in the brain, and can self-renew and differentiate into neurons, astrocytes and oligodendrocytes [9]. NSCs were proposed as a promising cellular source for the treatment of diseases in nervous systems [10,11]. Two strategies were suggested in the cell therapy of nervous system disorders: 1) to transplant exogenous NSCs into the injury sites; 2) to stimulate the functional activity of endogenous NSCs *in vivo* [12,13]. Scientists reported that exposure to an enriched environment had been shown structural and functional changes in the brain and significant increase in neurogenesis *in vivo* [14,15]. Moreover, it has been demonstrated that pluripotency can be restored to adult somatic cells through ectopic co-expression of defined transcription factors, thus proving that the fate of somatic cells is not immutable and paving the way for modeling human diseases and personalized cell therapies. In recent years, scientists develop cell reprogramming methods in the generating of induced

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pluripotent stem cells-derived NSCs (iP-NSCs), induced NSCs (iNSCs) and induced neuronal cells (iNeurons or iNs) for cell-based therapy in neurodegenerative diseases and nerve injuries, disease modeling and drug discovery [16-19].

In this review, we first summarize our current understanding of the ontogeny of NSCs (including characterization, isolation and differentiation) and their mobilization *in vivo*. We then focus on the cellular reprogramming in generation of iP-NSCs, iNeurons and iNSCs, and applications of NSCs and iNeurons in cell therapy, disease modeling and drug discovery.

### NSCs and Generation of Neurons

Permanent loss of neurons with no possibility of cellular regeneration has been challenged recently by extensive evidence that certain brain areas retain the capability to generate new neurons into adulthood in rodents [20-23], nonhuman primates [24] and human [25]. New neurons are added to the olfactory bulb and the hippocampus of dentate gyrus in mammals, and to the neostriatum, paraolfactory and parahippocampal regions in birds [26]. Previous studies reported that implantation of NSCs promotes neuroregeneration in the animal models of nerve injury [27,28]. NSCs are highly desirable for clinical applications, which are based

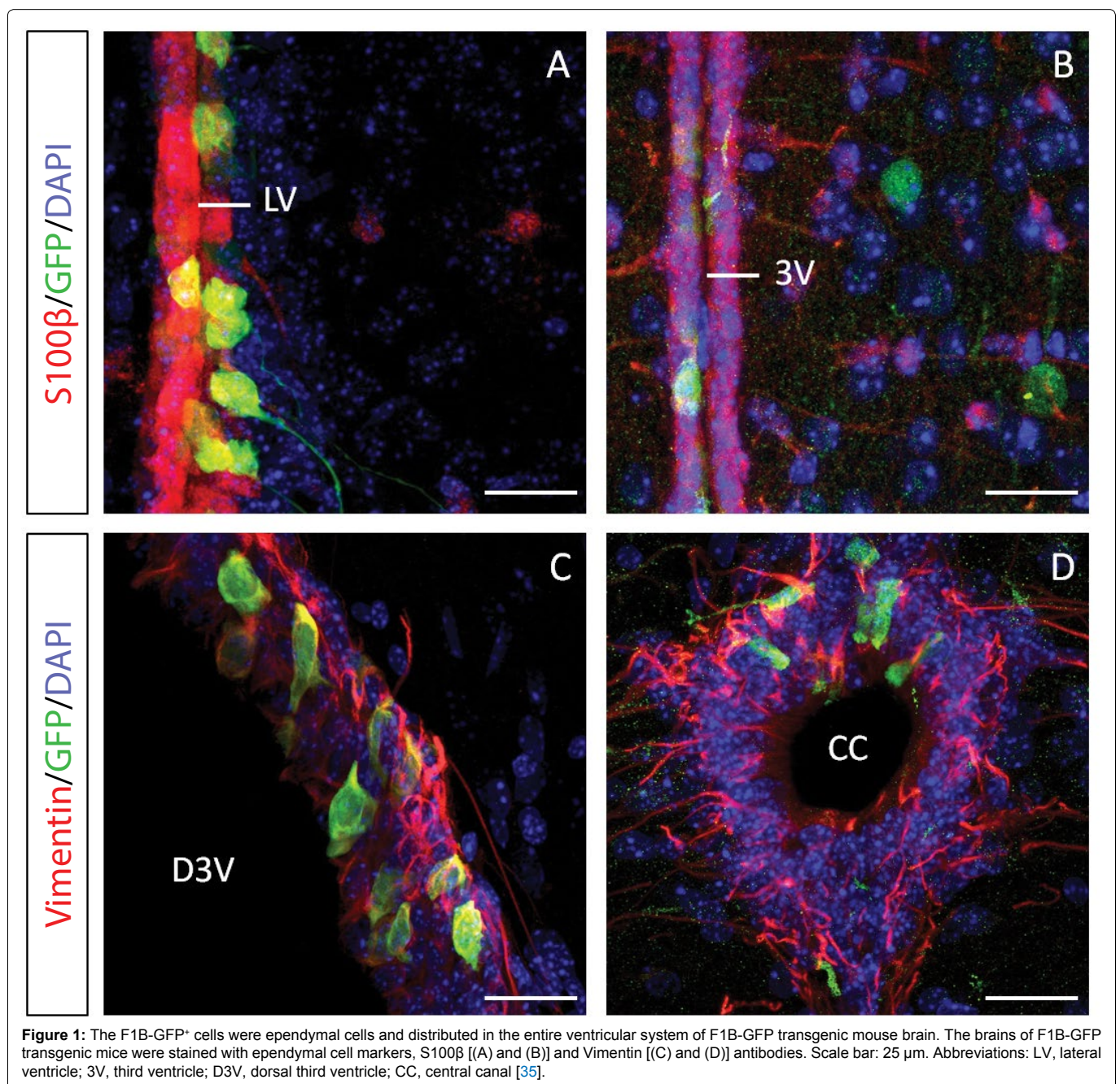
on two reasons: 1) NSCs are well-studied and well-characterized cells; 2) NSCs are capable of potentially unlimited self-renewal, and can be differentiated into neurons, astrocytes and oligodendrocytes both *in vitro* under defined conditions and *in vivo* after transplantation [29].

### Characterization of NSCs

NSCs are characterized by two properties, self-renewal and neuronal differentiation (multipotency). Self-renewal and differentiation of NSCs were regulated by the specialized microenvironment, or niche, in which these cells reside. Different signaling pathways have been implicated to play a role in the process [30,31]. Neurosphere formation is frequently used as a standard for evaluating self-renewal ability in NSCs. The mechanisms of neurosphere formation are related to the cell proliferation and cell-cell adhesion, which means that neurospheres can form from the clonal progeny of single NSCs, and/or from the adhered cells by cell-cell interactions initiated between NSCs [32]. The potential for neuronal differentiation of neurosphere-isolated cells upon withdrawal of growth factors was used to determine multipotency of NSCs [33,34].

### Isolation of NSCs

Fluorescence-activated cell sorting (FACS) has been applied





in isolation of different precursor and progenitor populations from tissues. NSCs can be isolated from brain tissues by cell-surface markers, such as CD133 or CD24, or by Sox1, Sox2 or Nestin promoter-driven GFP reporter. The cells cultured in the presence of growth factors and examined to determine whether they could expand to form neurospheres. Notably, fibroblast growth factor 1 (FGF1) is expressed in ventral cochlear neurons, olfactory bulbs, and hippocampal neurons. The brain-specific FGF-1B promoter is active only in the brain, and is active in ependymal cells of ventricles and dopaminergic neurons in the brains of FGF-1B promoter-driven GFP (F1B-GFP) transgenic mice (Figure 1) [35]. Brain-specific 1B promoter of FGF1 gene facilitates the isolation of NSCs with self-renewal and multipotent capacities [36]. Implantations of F1B-GFP-selected NSCs from mouse brains were able to repair the damaged sciatic nerve of rats [36,37]. Taken together, F1B-GFP could be used to directly isolate NSCs from mouse brain, and the F1B-GFP-selected NSCs could be used in neuroregeneration.

### Differentiation of NSCs

Several neurotrophic factors, such as leukemia inhibitory factor (LIF), FGF1, platelet-derived growth factor (PDGF), nerve growth factor (NGF)/brain-derived neurotrophic factor (BDNF)/neurotrophin-3 (NT-3), glial cell line-derived neurotrophic factor (GDNF), and retinoic acid (RA), promote differentiation of NSCs in mammalian neural development [1]. Previous studies also reported that levels of neurotrophic factors are changed in some neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis [38,39]. For examples, LIF is a member of the family of interleukin-6 cytokines, and mediated through a cell surface complex composed of LIF receptor  $\beta$  (LIFR $\beta$ ) and gp130. LIF induced differentiation of NSCs into astrocytes, and the LIF knock-out mice display reduced astrocyte and microglial activation in the hippocampus [40,41]. FGF1 is expressed predominantly in neural tissues, and presents its effect through high-affinity cell surface receptors. FGF1 has been shown to promote mitogenesis of neuroblasts, to enhance both neurite initiation and elongation of retinal ganglion cells, and to stimulate neuronal differentiation and neurite outgrowth of PC12 cells [42,43]. The presence of FGF1 in the adult brain suggests a role for FGF1 in neural maintenance in addition to neurogenesis [1]. Therefore, Identification of external signals involved in the regulation of NSCs differentiation may allow for improved transplantation methods and augment the treatment of neurodegenerative disorders.

### Mobilization of NSCs

NSCs resided in specialized niches of the adult mammalian brain that support their self-renewal and differentiation throughout life. Two regions continuously generate new neurons, which include subventricular zone (SVZ) of the lateral ventricles, and subgranular zone (SGZ) of the hippocampus. NSCs are largely quiescent *in vivo*, and occasionally become activated to proliferate. In SVZ, NSCs divide under both homeostasis and during regeneration to give rise to transit amplifying progenitors, which in turn generate neuroblasts that migrate to the olfactory bulb and give rise to olfactory bulb neurons [44-46]. NSCs that touch the ventricles are quiescent and are directly exposed to the cerebrospinal fluid (CSF). These cells contact ependymal cells via adherens junctions and thus anchor to the niche [47]. Vascular cell adhesion molecule 1 (VCAM1) receptors of the adherent junctions are located to the apical surface of NSCs in SVZ. Quiescent NSCs, upon disruption, could exit from the ependymal/CSF niche and result in neuron genesis in the SVZ [45,48]. In SGZ, new neurons are bred and functionally integrated into the granular cell layer of the dentate gyrus. Four types of neural progenitors (Type 1-4) are located in the SGZ during neurogenesis. Type 1 cells are likely the quiescent NSCs by the constant cell population size and proliferation rate. Types 2, 3 and 4 are daughter cells from Type 1 cells. Type 2 cells generally referred to as neural progenitor cells, while Type 3 cells are called neuroblasts. During the Type 3 stage, cells shift from the horizontal to vertical and begin radial migration into the granule cell layer. Type 4 cells cease mitosis, extend axons and lead to

the development of mature granule cells [49]. In birds, new neuronal precursors were born in the ventricular zone, migrate into high vocal center (HVC) where they differentiated into neurons, which projected to robust nucleus of the arcopallium or became interneurons [26]. Sensitization of NSCs had been demonstrated *in vivo* through singing, exercise, stress and physical stimulations.

**Learning (Singing):** As shown in adult canaries, a learning behavior model, they modify their songs every year and continue to learn new songs in adulthood. Song syllables are delivered in a very stereotyped manner during the breeding season, and become less frequent and stereotypy after the breeding season ends. In the observations of song change results, the most dramatic change that occurs in the song circuits of adult canaries is neurogenesis and neuronal replacement in the HVC of brain region, an important nucleus in the brain that controls this learning behavior of canaries [50-52]. Adult neurogenesis is thought to provide neural plasticity used in forming and storing new memories. New neurons continue to be added to the HVC of adult canaries, where they replace older neurons that have died [26,53-56]. Therefore, the neurogenesis of adult canaries is responsible for the seasonal plasticity of learning behavior. The activating mechanisms of neuronal replacement in songbirds may contribute to the understanding of the repair of human brain damages and neuronal degeneration diseases.

**Exercise:** Physical activity showed activation of neural precursors in the adult neurogenic niches [57-59], and enhanced the learning and memory function in both mice [60,61] and human [62]. Comparison of hidden-platform water-maze learning, forced-exercise (yoked-swim controls), voluntary-exercise (running wheel) or standard-living (control) conditions revealed that running increases NSCs proliferation and neurogenesis. Voluntarily physical activity was sufficient for enhanced neurogenesis in the dentate gyrus of hippocampus of adult mice [14]. Several signaling pathways have been implicated in the exercise-mediated activation of neurogenesis, including hippocampal PKC activity, vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), bone morphogenetic protein (BMP) and growth hormone pathway [57]. In therapeutic strategy, physical exercise can rescue the psychiatric illness, aging dementia and alcoholic-reduced adult neurogenesis [57,58,63].

**Response to stress (Stroke/ Muse):** Stroke results from the occlusion of a cerebral artery leading to brain infarction, which is the most common cause of death and long-term disability in adulthood. Stroke is followed by activating of NSCs in the SVZ, and newborn neuroblasts migrate towards the injury sites of brain [64-67]. The most obvious site for post-stroke neurogenesis is in the SVZ. Stroke interacts with the SVZ adult neurogenesis through several signaling pathways, including erythropoietin [68], stromal derived factor 1 (SDF1), angiopoietin 1 [69] and calpains [67]. However, scientists revealed that most neuroblasts die before differentiating into functional mature neurons, which fail to integrate into the neuronal circuitry [66,70,71]. Therefore, discovery of efficient strategies in promoting post-stroke neurogenesis for improvement of patient recovery after brain injury is needed.

The multilineage-differentiating stress-enduring (Muse) cells are stress-tolerant and nontumorigenic pluripotent stem cells, and show the ability to generate multiple cell types of the three germ layers. The Muse cells could be found in adult human mesenchymal population and adipose tissue without introducing exogenous genes [72-74]. Human Muse cells, intravenously injected, were able to integrate and differentiate into damaged sites including skin, muscle and liver tissues of mice [72,75]. Transplantation of human Muse cells into infarct brain of mice showed that Muse cells integrated into the injury sites and differentiated into neuronal cells in replacement of the lost neurons [76]. As shown in human adipose tissue-derived Muse cells (Muse-ATs), Muse-ATs reside within the adipocyte and stromal vascular fractions. Interactions of surrounding cells with Muse-ATs resulted in their lineage plasticity, adipose tissue differentiation and repair, and the production and recruitment of signaling molecules in times

of cellular stress [73,75]. Therefore, understanding of mechanisms in activating Muse and applications of Muse in replacement of the lost neurons are worthy for further investigations.

**Physical stimulation (Magnetism/Ultrasound):** Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive approach of brain stimulation, which has been developed and demonstrated to be effective for Parkinson's disease and neuropsychiatric disorders [77-79]. Chronic rTMS promotes adult hippocampal neurogenesis and alleviates stress-related behaviors in mice with neuropsychiatric disorders. The studies provided further evidence that proliferation and neuronal differentiation of SVZ-derived NSCs of adult mice were increased after rTMS treatment [79-81].

BDNF is the neurotrophic factor involved in synaptic plasticity and neuron generation. Major depressive disorder patients showed lower BDNF levels in serum, and constitutively low rate of adult hippocampal cell proliferation [82]. Infusion of BDNF into the midbrain of rats showed antidepressant-like results [83]. Rats emit ultrasonic vocalizations in appetitive situations, like juvenile play or when tickled playfully. This tickling-induced ultrasound positively correlated with adult hippocampal cell proliferation as well as BDNF levels in mice [84-86]. Therefore, ultrasound stimulation might be an alternative strategy for treatment of major depressive disorder and Alzheimer's disease by increasing brain BDNF levels and neurogenesis in the hippocampus.

### Cellular Reprogramming in Generation of iP-NSCs, iNeurons and iNSCs

Cellular reprogramming was used in the generation of iP-NSCs, iNeurons and iNSCs. Cellular reprogramming rendered as adult cells to an embryonic stem cell-like state by forced gene expression and/or factors treatment to maintain the properties of stem cells (such as iP-NSCs and iNSCs) or functional neurons (such as iNeurons) [87-90]. The primary advantage in the uses of cellular reprogramming is that adult cells are easily obtained from living animals. Different cell sources for cellular reprogramming were developed in previous studies, including blood cells [91-94], skin-derived fibroblasts [95], urine [96,97] and hair follicles [98]. Two different reprogramming processes, including indirect and direct methods, had been explored in the induction of neural progenitor cells [29]. Indirect reprogramming uses the same iPSCs factors (such as Sox2, c-Myc, Klf4 and Oct-4) for cellular reprogramming, designated iP-NSCs. In this method, addition of specific growth factors to the reprogramming medium can overcome fibroblasts to go through an intermediate unstable state. Direct reprogramming uses the neural-specific reprogramming factors for cellular reprogramming, allowing direct obtaining of neural progenitor cells (iNSCs) from fibroblasts. Both iP-NSCs and iNSCs showed neuronal cell differentiation and functional recovery in the transplantation of rodents [87,99].

### iPSCs-derived neural stem cells (iP-NSCs)

The discovery of the technique that induces pluripotency in somatic cells (iPSCs) raised great hopes in regenerative medicine [100]. In the iPSCs studies, Kim et al. [101] first reported the cellular reprogramming of fibroblasts into induced neural precursor cells by using the same reprogramming factors of iPSCs. These transdifferentiated NSCs have the distinct advantage of being expandable *in vitro* and retaining the ability to give rise to multiple neuronal subtypes and glial cells. However, these NSCs-like colonies lose their self-renewal capacity after 3-5 passages *in vitro*. They did not observe oligodendrocytes differentiation [101]. Their et al. [87] developed neurosphere-like colonies that could be expanded for more than 50 passages and do not depend on sustained expression of the reprogramming factors, which were generated by constitutively inducing Sox2, Klf4 and c-Myc while strictly limiting Oct4 activity. The authors transplanted their iP-NSCs into the brain of neonatal myelin-deficient rats. The results revealed that grafted iP-NSCs survive and give rise to differentiated neural cells *in vivo* [87]. Neuronal lineage-specific reprogramming factors (Ascl1, Brn2 and Myt1l) also have been shown to induce iPSCs into neural cells [90].

In clinical applications, iP-NSCs were thought as a good cell source for cell therapies and/or disease modeling and drug discovery in ALS, SMA and Alzheimer's disease. For example, recent animal study demonstrated that human iP-NSCs transplantation prolong the lifespan in ALS mouse model. ALS is a progressive, fatal, neurodegenerative disease caused by the death of motor neurons in the cortex, brain stem and spinal cord. The progressive neurodegeneration of ALS resulted in muscle atrophy and weakness, and respiratory failure. Astrocytes become dysfunctional during the progression of ALS [102,103]. As shown in Kondo et al. [104], transplantation of human iP-NSCs into the spinal cord of ALS mice improves lifespan of the treated mice. The patient-derived iPSCs of SMA and Alzheimer's disease were also successfully used in disease modeling [105,106].

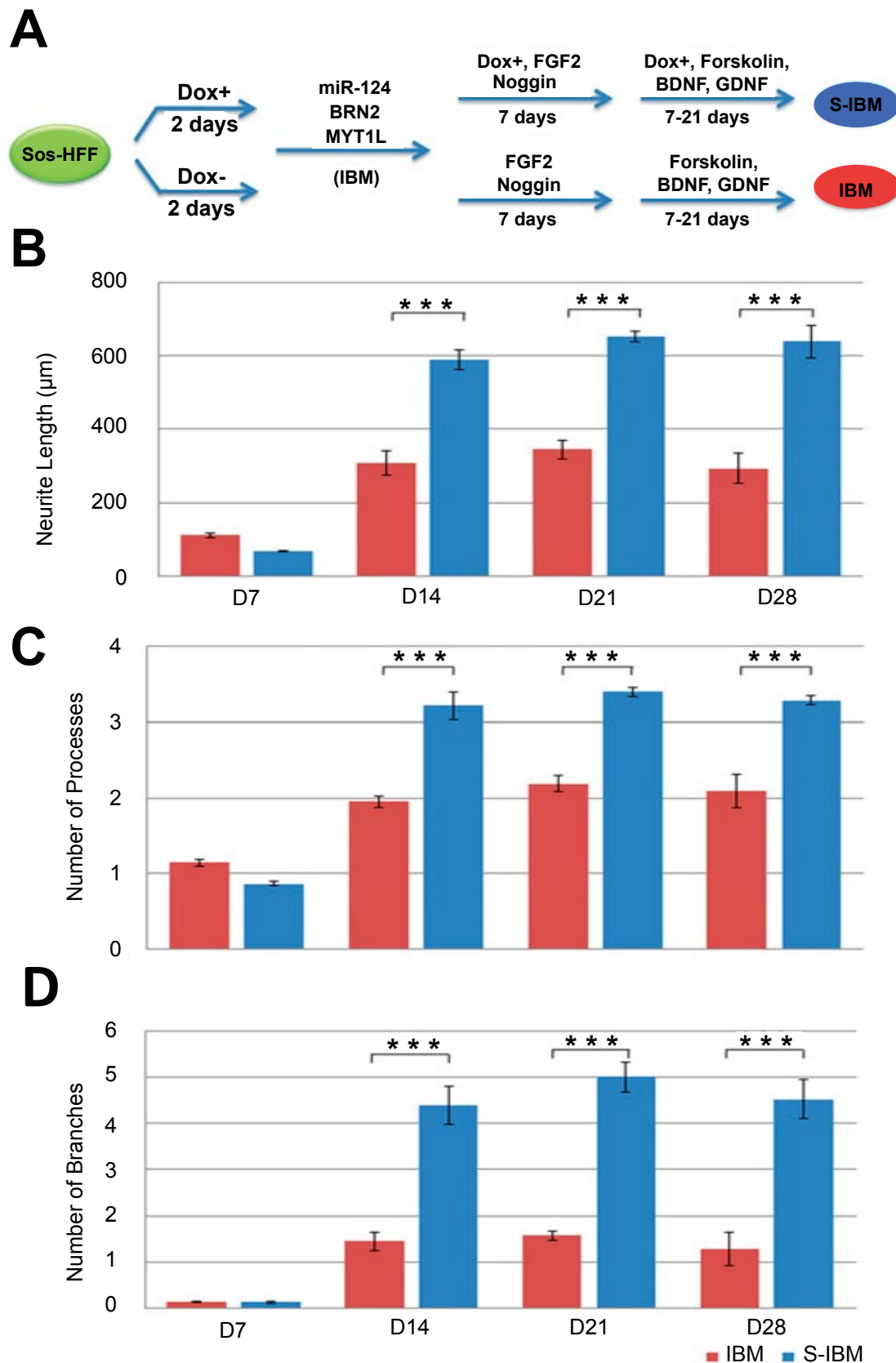
The concerns about iP-NSCs were due to viral infection with overexpression of c-Myc oncogene, which have shown increased tumorigenicity [17,107-110]. Therefore, to explore alternative stem cell sources that do not confer the disadvantage of iPSCs but are widely multipotent for clinical applications are needed in the future.

### iNeurons

To resolve the clinical hurdle of the iPSC derivations, the iNeurons were developed in overcoming the disadvantage of teratoma formation by the pluripotent cells. Recent studies showed that the generation of iNeurons is fast, efficient and devoid of tumorigenic pluripotent stem cells [89], which provide a novel and powerful tool that were chosen in studying neurological disease, drug discovery and regeneration medicine. Vierbuchen et al. [89] revealed the direct conversion of fibroblasts to functional neurons by defined factors. The results reported that most efficient conversions were achieved when Ascl1 and Brn2 were combined with Myt1l (termed BAM pool). In the BAM infection, iNeurons showed the expressions of neuronal markers, and were capable in forming functional synapses by co-cultured with astrocytes [89]. Moreover, hepatocytes-derived iNeurons by the BAM factors were developed and have acquired functional properties of mature neurons [111].

Encouraged by findings in mouse cells, human fibroblasts could also be directly induced to become functional neurons [90,112-114]. The direct conversion of human fibroblasts into functional neurons using BAM factors had been reported [115]. NeuroD1 co-infected with BAM (BAMN) factors generated the most mature neuronal cells in human fibroblasts by morphological and functional characterization [90]. The miRNAs (particularly the miR-124) showed significant contribution in reprogramming of iNeurons. Expression of miR-9/9\* and miR-124 in human fibroblasts induces their conversion into neurons. The miR-9/9\*-124-DAM-(miR-9/9\*-124 together with NeuroD2, Ascl1 and Myt1l)-converted iNeurons showed positive action potentials of functional evaluation [112,116]. Combination of miR-124, Brn2 and Myt1l (termed IBM pool) factors also reprogram human postnatal and adult primary dermal fibroblasts into functional neurons [113]. Small-molecule cocktails could convert mouse and human fibroblasts into functional neurons without exogenous genetic factors [117,118].

In particular, our finding demonstrated that SH2B adaptor protein 1 $\beta$  (SH2B1) can enhance neurite outgrowth of iNeurons which were reprogrammed from human foreskin fibroblasts, when combined with IBM factors under defined conditions. These SH2B1-enhanced iNeurons (termed SIBM-iNeurons) showed canonical morphology and expressing multiple neural markers and functional proteins for neurotransmitter release and exhibited action potentials. As shown in figure 2, SH2B1 enhances the length of neurite outgrowth and the numbers of processes and branches of human iNeurons induced by IBM factors. SIBM-iNeurons also showed calcium influx activity upon glutamate stimulation (Figure 3). This study demonstrated that the combination of SIBM factors in reprogramming of human fibroblasts is 10 times more efficiency than other combinations and can exhibit action potential as early as day 14 [114].



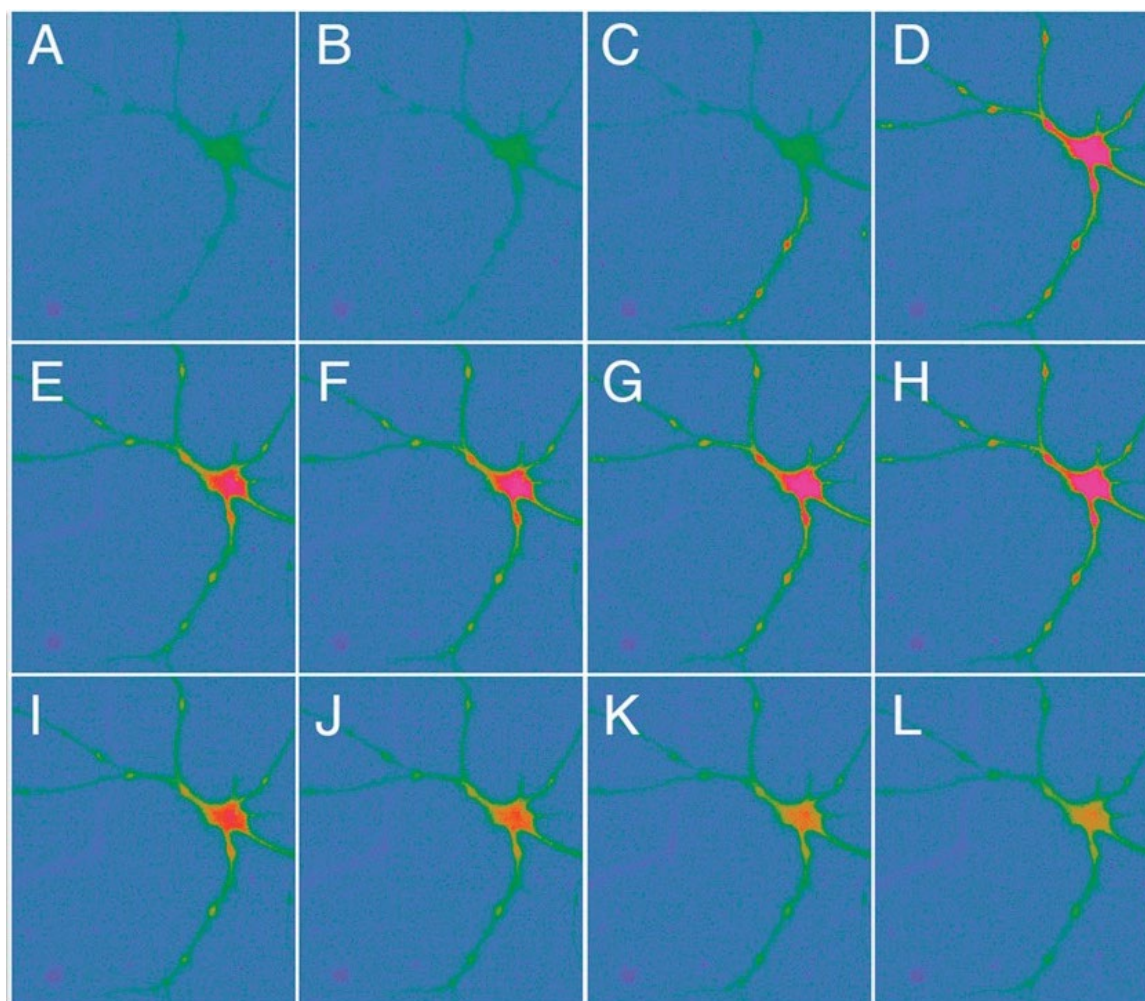
**Figure 2:** SH2B1 enhanced the length of neurite outgrowth and the numbers of processes and branches of human iNeurons induced by IBM factors. The schema showed the experimental protocol for reprogramming fibroblast cells into neurons (A). Within 28 days of infection, SIBM-iNeurons showed twofold more total neurite outgrowth than IBM-iNeurons at days 14, 21, and 28.  $n = 3$ ;  $***, p < 0.001$  (B). Within 28 days of infection, SIBM-iNeurons showed significantly more neuronal processes (C) and branches (D) than IBM-iNeurons at days 14, 21, and 28.  $n = 3$ ;  $***, p < 0.001$ . Abbreviations: SIBM comprises SH2B1, miR124, Brn2 and Myt1l [114].

## iNSCs

Unlike iNeurons, iNSCs are capable of self-renewing, as well as differentiating into neurons and astrocytes, which means that iNSCs (Table 1) would provide an unlimited source of neuronal cells for

clinical applications [99,119]. Lujan et al. [88] reported that Sox2 and FoxG1 are capable of generating clonal self-renewing, bipotent iNSCs that gave rise to astrocytes and functional neurons. When Brn2 was added to Sox2 and FoxG1, tripotent iNSCs that could be





**Figure 3:** SIBM-iNeurons showed  $\text{Ca}^{2+}$  influx activity upon glutamate stimulation at Day 28. SIBM-iNeurons were labeled with Fura2-AM, which is an intracellular  $\text{Ca}^{2+}$  indicator. Images were taken at consecutive intervals of 3.1 seconds after glutamate injection. The red color indicates the uptake intensity of Fura2-AM [114].

**Table 1:** Generation of iNSCs by various neuronal-specific transcription factors from somatic cells.

Ref.	Reprogramming factors	Self-renew	Differentiated cell types			Cell origin
			neurons	astrocytes	oligodendrocytes	
[101]	Sox2, Klf4, c-Myc, Oct4	+	+	+	n/a*	MEFs
[88]	Sox2, Brn2, FoxG1	+	+	+	+	MEFs
[87]	Sox2, Klf4, c-Myc, Oct4	+	+	+	+	MEFs
[99]	Sox2, Klf4, c-Myc, Brn4 with/without E47	+	+	+	+	MEFs
[120]	Sox2, Klf4, c-Myc, Brn4	+	+	+	+	MEFs
[123]	Sox2, Klf4, c-Myc, Brn2, Pax6, Id1, Hex1, Ngn2, Ascl1	+	+	+	+	Mouse Sertoli cells
[121]	Sox2	+	+	+	+	MEFs/HFFs
[122]	Sox2 or Oct4 or Nanog	+	+	+	+	Human astrocytes

\* n/a, not available; Abbreviations: MEFs, mouse embryo fibroblasts; HFFs, human foreskin fibroblasts

differentiated not only into neurons and astrocytes but also into oligodendrocytes were obtained [88]. *In vivo* assessment, iNSCs labeled with GFP were transplanted into the SVZ of adult mice. The results demonstrated that iNSCs exhibit cell morphology, gene expression, epigenetic features, differentiation potential and self-renewing capacity, as well as *in vitro* and *in vivo* functionality similar to those of wild-type NSCs. These iNSCs did not generate teratomas after injection into immunosuppressed mice, and the expression levels of the endogenous NSC factors were similar to those of control NSCs [99]. Furthermore, iNSCs achieve long-term survival and functional integration in the adult mouse brain. The results demonstrated that iNSCs long-term transplantation is a safe procedure and might be a valuable tool for cell therapies of neuroregeneration [120]. Moreover, the use of one transcription factor (such as Sox2, Oct4 or Nanog) can also reprogram the mature cells into iNSCs [121,122]. In Corti et al. [122], ectopic expression of the single reprogramming factor (Sox2, Oct4 or Nanog) into human cortical astrocytes gave rise to

colonies of small cells resembling NSCs as well as neurospheres. The number of colonies obtained was significantly greater with Nanog than with Sox2 or Oct4. In addition, Sheng et al. [123] showed that direct reprogramming of Sertoli cells into iNSCs by nine transcription factors (Sox2, Klf4, c-Myc, Brn2, Pax6, Id1, Hex1, Ngn2 and Ascl1) [123]. These iNSCs could differentiate into neurons, astrocytes and oligodendrocytes *in vivo*.

The iP-NSCs-like colonies were generated with low efficiency and could not self-renew, and the cells apparently lacked the ability to differentiate into oligodendrocytes [101]. Moreover, both undifferentiated iP-NSCs harbor potential tumorigenic risks [29,107,108,124]. iNSCs that were induced directly from fibroblasts using neuronal-specific transcription factors bypassed a partial or complete pluripotent state. Therefore, iNSCs reduced the risk of teratoma formation due to the persistent undifferentiated cells. Thus, it would be desirable to convert fibroblasts into proliferative neural precursor cells instead of neurons and iPSCs-derived cells.

## Applications of NSCs and iNeurons in Cell Therapy, Disease Modeling and Drug Discovery

Cell-based therapy could aid in alleviating symptoms or even reversing the progression of neurodegenerative diseases and nerve injuries. Neurodegeneration can disrupt molecular pathways, synapses, neuronal subpopulations, and local circuits in specific brain regions, as well as higher-order neural networks. Abnormal network activities may result in a vicious cycle in impairing the integrity and functions of neurons and synapses.

### NSCs treatment in PNI repair

Neuroregeneration of PNI is a complex process with Wallerian degeneration. After injury, the axonal skeleton disintegrates, and the axonal membrane breaks apart. The axonal degeneration is followed by degradation of the myelin sheath and infiltration by macrophages which serve to clear the debris from the degeneration [125,126]. Transplantation of NSCs provide effective therapies for many neurological diseases and injuries, which clearly explain the benefits observed after cell therapy [127]. The paracrine action of growth factors, cytokines, and hormones that are secreted or released by transplanted cells has been shown to provide most of the benefits after stem/progenitor cell administration. Mechanisms of peripheral neuroregeneration in NSCs administration include differentiation into Schwann cells, secretion of neurotrophic factors and promoting myelination [128]. Our studies showed that FIB-GFP-selected NSCs from mouse brain were able to repair the damaged sciatic nerve [9,37]. These NSCs were differentiated into Schwann cells, which contribute to the development of microenvironment that stimulates axonal regeneration in repairing of PNI (unpublished results).

### iNeurons in disease modeling and drug discovery

iNeurons generated via direct conversion of neuron do not pass through a pluripotent state, are not tumorigenic, and may serve as an interesting alternative to iPSC-derived cells for generating patient- and/or disease-specific neurons. In recent years, iNeurons have been utilized in modeling of neurodegenerative diseases such as Parkinson's disease [115,129,130], ALS and SMA [131], Dravet syndrome and mild febrile seizures [132], Glaucoma [133], Frontotemporal lobar degeneration (FTLD) [134], autism spectrum disorder [135] and Alzheimer's disease [118]. The results are been summarized in table 2.

A key feature of Parkinson's disease is progressive degeneration and death of dopaminergic neuron (DAs) in the substantia nigra region of the midbrain. Mouse and human fibroblast-derived DA showed positive DA markers, dopamine release and DA electrophysiology [115,129,130]. Injection of induced DA in rats presented attenuated rotational behavior at 8 weeks post injection. Long-term engraftment revealed that induced DA retained their DA neuron-like properties *in vivo* for up to 16 weeks post injection [130]. Therefore, directly reprogrammed DA are a promising source of cell-replacement therapy for PD.

In ALS and SMA, the motor neurons control the contraction of muscle fibers actuating movement, which are selective targets of degeneration in ALS and SMA. The induced motor neurons showed gene expression signature, electrophysiological characteristics of motor neurons and forming functional synapses with muscle and sensitivity to disease stimuli of ALS. *In vivo* functional tests demonstrated that induced motor neurons were able to engraft, migrate to appropriate sites of integration, and correctly respond

**Table 2:** Applications of iNeurons in disease modeling and drug discovery

Type of iNeurons	Disease features	Cell origin	Reprogramming factors	Diseases model	Ref.
DAs	Progressive degeneration and death of dopaminergic neuron (DA) in the substantia nigra region of the midbrain.	MEFs/HFFs	Ascl1, Nurr1, Lmx1a	Parkinson's disease	[129]
		HFFs	Ascl1, Brn2, Myt1l, Lmx1a, FoxA2	Parkinson's disease	[115]
		HFFs	Ascl1, Ngn2, Sox2, Nurr1, Pitx3	Parkinson's disease	[130]
MNs	Progressive degeneration and death of motor neuron (MN) in the cortex, brain stem and spinal cord.	HEFs	Ascl1, Brn2, Myt1l, Hb9, Isl1, Lhx3, Ngn2, NeuroD1	ALS/SMA	[131]
Patient-derived GAs	Genetic mutation of <i>SCN1A</i> and impaired psychomotor development and ataxia.	Fibroblasts	Ascl1, Brn2, Myt1l, NeuroD1, Olig2, Zic1, rTA	Dravet syndrome	[132]
RGC	Accelerated death of retinal ganglion cells (RGC) leads to progressive visual field loss and eventual blindness.	MEFs	Ascl1, Brn3b, Ngn2	Glaucoma	[133]
iNeurons with <i>MAPT</i> mutation	Cause behavioural and executive impairment.	Human skin fibroblasts	Ascl1, Brn2, Myt1l, Lmx1a, FoxA2	Frontotemporal lobar degeneration	[134]
iNeurons with <i>CACNA1C</i> mutation	Cause social interaction, communication and behavioural impairment.	Human dermal fibroblasts	Ascl1, Pou3f2, Myt1l	Autism spectrum disorder	[135]
iNeurons with abnormal amyloid- $\beta$ peptide production	Aggregation and accumulation of amyloid- $\beta$ peptide, which is forming amyloid plaques in the brain.	Human foreskin/skin fibroblasts	VPA, CHIR, Repsox, Forskolin, SP600125, GO6983, Y-27632, Dorsomorphin	Alzheimer's disease	[118]

Abbreviations: MEFs, mouse embryo fibroblasts; HFFs, human foreskin fibroblasts

**Table 3:** Mobilization of NSCs in neurogenesis of the adult brain

Strategies for NSCs activation	Niches of NSCs activation	Expected improvement of CNS injuries
Learning	HVC (SVZ)	Developmental verbal dyspraxia; Mental retardation syndrome Fragile X
Exercise	Hippocampus	Major psychiatric disorders (depression, schizophrenia, drug addiction); Cerebral infarction; Aging-related cognitive decline
Stroke	SVZ	Stroke
Muse	Mesenchymal tissue	Cell-based therapy
Magnetism	SVZ/ hippocampus	Neuropsychiatric disorders (major psychiatric disorders, schizophrenia, epilepsy, neuropathic pain, amyotrophic lateral sclerosis, progressive muscle atrophy, multiple sclerosis); Neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease); Stroke
Ultrasound	Hippocampus	Major depression disorder; Alzheimer's disease

Abbreviations: Muse, multilineage-differentiating stress-enduring cells; HVC, high vocal center; SVZ, subventricular zone

to guidance cues, projecting their axons out of the central nervous system in the developing chick spinal cord [131]. Therefore, this induced motor neuron is a suitable substrate for *in vitro* studies of motor neuron function, disease, and injury.

The Dravet syndrome and mild febrile seizures arise due to genetic mutation of *SCN1A*, the gene encoding  $\alpha 1$  pore-forming subunit of the Nav 1.1 voltage-gated sodium channel. The patient-specific induced-glutamatergic neurons (GAs) showed delayed inactivation of sodium channels, indicating that these neurons can be potentially used for disease modeling and drug screening [132].

Glaucoma is one of the neurodegenerative disease and was characterized by accelerated death of retinal ganglion cells (RGC), which ultimately leads to progressive visual field loss and eventual blindness. Adenovirus-mediated transduction of three reprogramming factors (Table 2) can directly convert mouse fibroblasts to RGC-like neurons which expressed RGC markers and exhibited membrane action potential of functional RGC neurons. The cells showed great potential in retinal degenerative disease modeling [133].

### Mobilization of NSCs as a means for neuroregeneration

A milestone is marked in our understanding of the brain with the recent awareness that the adult nervous system can generate new neurons. Table 3 summarizes that neurogenesis in the adult brain can be mobilized via different ways, and the NSC mobilizations may improve the symptoms of neurodegenerative disorders. For adult brain neurogenesis, sensitization through stress and physical stimulations, and exposure to enriched environment can increase neurogenesis in animal models. Enriched environment consists of expanded learning opportunities, increased social interactions, more physical activity and larger housing [14,136,137]. As shown in table 3, learning, stroke and magnetism stimulation induced SVZ neurogenesis, and exercise and ultrasound stimulation induced hippocampal neurogenesis. For clinical investigations, exercise enhanced learning and memory function in both mice and human with neurodegenerative diseases [60-62]. Treatment of rTMS improves the symptoms of Parkinson's disease, and induced endogenous dopamine release in the ipsilateral dorsal striatum [78]. Ultrasound increased BDNF levels and cell proliferation in adult hippocampus of mice. Ultrasound might be used in treatment of major depressive disorder [138]. Therefore, mobilizations of NSCs could be a worthwhile strategy for regeneration of neurons in neurodegenerative diseases *in vivo*.

In spite of the rapid progress in stem cell research, several critical challenges of stem cell-based therapy in repairing neurodegenerative diseases remain to be resolved. These challenges include (1) the source of NSCs should be personalized; (2) the protocols to activate human NSCs should be properly established; (3) the isolation methods of human NSCs should be standardized; (4) the clinical efficacy of activations or transplants of NSCs should be evaluated in more adequate animal models; and (5) the mechanism of intrinsic repairing of neurodegeneration diseases needs to be better characterized. Mobilizations of NSCs by different means can provide unlimited cell sources in the adult brain. The mobilized NSCs could in turn regenerate neurons in repairing of neurodegenerative diseases *in vivo*. Moreover, patient-derived cells are ideal NSC sources for autologous cell transplantation because they can avoid immune rejection. In particular, generation of iNeurons from patient is fast, efficient and devoid of tumorigenicity that might be conferred by iP-NSCs. Therefore, mobilizations of NSCs *in vivo* and cellular reprogramming for cell transplantation are expected to alleviate symptoms or even reverse the progression of various neurodegenerative diseases.

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