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Review Article

Pharmacological approaches promoting stem cell-based therapy following ischemic stroke insults

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Abstract

Stroke can lead to long-term neurological deficits. Adult neurogenesis, the continuous generation of newborn neurons in distinct regions of the brain throughout life, has been considered as one of the appoaches to restore the neurological function following ischemic stroke. However, ischemia-induced spontaneous neurogenesis is not suffcient, thus cell-based therapy, including infusing exogenous stem cells or stimulating endogenous stem cells to help repair of injured brain, has been studied in numerous animal experiments and some pilot clinical trials. While the effects of cell-based therapy on neurological function during recovery remains unproven in randomized controlled trials, pharmacological agents have been administrated to assist the cell-based therapy. In this review, we summarized the limitations of ischemia-induced neurogenesis and stem-cell transplantation, as well as the potential proneuroregenerative effects of drugs that may enhance efficacy of cell-based therapies. Specifically, we discussed drugs that enhance proliferation, migration, differentiation, survival and function connectivity of newborn neurons, which may restore neurobehavioral function and improve outcomes in stroke patients.

Keywords: stroke; neurobehavioral function; neurogenesis; cell-based therapy; stem-cell transplantation; granulocyte colony-stimulating factor (GCSF); herbal medicine

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Introduction

Cerebral infarction (CI) is a major health problem worldwide. It is the second leading cause of death and the third most common cause of disability[1]. Numerous efforts have been made to reduce ischemia-induced neuron injury and restore neurological function by various mechanisms, such as inhibition of neuroinflammation[2], blocking *N*-methyl-*D*-aspartate (NMDA) receptor[3], opening of K_{ATP} channel[4] , suppression of melastatin-like transient receptor potential cation channel, subfamily M, member 7 (TRPM7) channel[5], and inhibition of postsynaptic density-95[6]. Despite of this, recombinant tissue plasminogen activator (rtPA) is still the only FDA-approved drug treatment for ischemic stroke and must be used within 4.5 h of onset[7]. Spontaneous neuroplasticity in perilesional tissue following ischemic insult may promote map reorganization abilities in human and animal models[8]. Neurogenesis was wildly accepted as a fundamental mechanism of neural plasticity[9, 10]. Recent studies suggest that after central nervous system (CNS) injuries, regeneration and reparation may occur in the brain through adult neural stem/progenitor cells. Stem-cell-based therapies, including cell transplantation and stimulation of endogenous neurogenesis, are potential strategies to repair and regenerate the injured brain and may provide the second therapeutic time window for ischemic stroke treatment[11]. However, whether stem cell transplantation would be beneficial for neuronal function following stroke insults is still indefinite. So in this review we will discuss 1) the evidence of neurogenesis in adult brain; 2) the contributions and limitations of ischemia-induced neurogenesis for cerebral repair; 3) the potentials and limitations of stem cell transplantation therapies; and 4) the potential role of drugs to enhance efficacy of the cell-based therapy by enhancing the proliferation, migration, differentiation, survival, and functional connectivity of newborn neuron.

The discovery of neurogenesis in normal adult brain

It was considered for a long time that neurogenesis ended in the period shortly after birth and adult neurogenesis was impossible. However, in 1992, adult neurogenesis in mouse brain was verified by Reynolds[12]. Only 6 years later, in 1998, adult neurogenesis in human brains was also found under

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physiologic conditions[13-17]. Then it was believed that proliferation of adult neural stem cells (NSCs) in the central nervous system (CNS) might have the ability to replace lost or damaged neural cells. Transplantation of exogenous or stimulation of endogenous stem cells could be potential treatments for human brain repair after an ischemic stroke or other neurodegeneration diseases[18-20].

Ischemic stroke promotes neurogenesis

The evidence of ischemic stroke-induced neurogenesis

Ischemic stroke is one of the most important causes of long-term disability and mortality worldwide. Vascular recanalization therapy is one of the few effective therapies; however, it can only be used within a narrow therapeutic time window (within 4.5–6 h post-stroke). The discovery of continuous adult neurogenesis in human brains provides a second therapeutic time window and gives hope to neural repair after ischemic stroke. Encouragingly, it was found that compared to quiescent state, the production of neuroblasts was significantly increased in the adult brain after ischemic stroke[21-26]. It was found that stroke gave rise to a 31-fold increase of the number of new-born neurons in the ipsilateral striatum[15]. The generated neuroblasts migrate toward the injured brain region, differentiate into mature striatal neurons, establish appropriate long-distance connections, integrate into the neuronal circuitry and may contribute to the recovery of ischemic stroke[27-29].

Cell responses associated with the ischemia-induced neurogenesis

While a series of evidence showing the presence of ischemic-enhanced neurogenesis in rodents and human, their mechanisms remain to be elucidated. For a better understanding of the promise and limitations of ischemia-enhanced neurogenesis on brain repair, the mechanisms underlying ischemia-enhanced neurogenesis, especially the cell response, are discussed in this section (Figure 1).

Ischemia-induced astrocyte-to-neuron conversion

After cerebral ischemia, astrocytes are activated, which indeed can give rise to neurons *in vivo* in the adult mouse striatum through Notch signaling pathway[30]. By local transduction of striatal astrocytes with adenoviruses expressing Cre under regulatory elements of the GFAP promoter in Connexin-30-CreER transgenic mice, researchers were able to visualize doublecortin (DCX)-positive neuroblasts striatal astrocyte origin[31]. Another study showed that striatal astrocytes could transdifferentiate into immature neurons at 1 week and mature neurons at 2 weeks after middle cerebral artery occlusion (MCAO). In addition, these astrocyte origin neurons could form synapses with other neurons at 13 weeks after MCAO. It has been shown that these astrocyte origin newborn neurons could produce connections with other neurons in the injured brain[32]. VEGF helps striatal astrocytes transdifferentiate into new mature neurons[33]. These results indicate that astrocytes were one of the sources of new-born neurons after ischemic stroke.

Astrocyte-derived neurotrophic factors involved in ischemia-included neurogenesis

Recently astrocytes are considered to be involved in adult neurogenesis through the releasing of neurotrophic factors[34, 35]. In stroke model, activated astrocytes enhanced the expression of BDNF[36], which enhanced the differentiation of CNS stem cell-derived neuronal precursors[37], resulted in higher initial NSCs engraftment and survival[38]. Glial cell line-derived neurotrophic factor (GDNF), another neurotrophic factor secreted by astrocytes, induces neural differentiation in neural progenitor cells[39], promotes striatal neurogenesis after stroke in adult rats[40]. Nerve growth factor (NGF) expressed in astrocytes and enhanced after ischemic stroke in peri-infarct area[41], has been shown to improve survival of newly generated cells in the ipsilateral striatum and subventricular zone (SVZ)[42].

Vasculature is associated with neurogenesis

The vasculature is an important component of the adult neural stem cell niche. After cerebral ischemia, neurotrophic factors secreted by endothelial and pericyte affect the neurogenesis in a variety of aspects, such as promoting the proliferation, neuronal differentiation of NSCs[43]. Vascular endothelial growth factor (VEGF), which is secreted by endothelial cells and pericytes, is one of the most important neurotrophic factors stimulating cell proliferation in the SVZ[44, 45], facilitating the migration of immature neurons towards the ischemic tissue[46]. Besides VEGF, several other cytokines or growth factors have been implicated in poststroke neurogenesis. Betacellulin (BTC), placenta growth factor (PlGF-2) and Jagged1 were also found to induce NSCs proliferation during postnatal and adult neurogenesis[43, 47, 48]. Neurotrophin-3 (NT-3), a mediator of quiescence in the SVZ adult neural stem cell niche, promotes newly differentiated neurons in hippocampal dentate gyrus (DG)[49, 50] and cholinergic neuronal differentiation of bone marrow-derived neural stem cells[51]. Another endothelial-derived neurotrophic factor, pigment epithelium-derived factor (PEDF), was shown to promote the self-renewing cell division and multipotency maintenance of neural stem cells[52, 53].

Ischemia-induced pericytes-to-neuron conversion

Besides glial cells, pericytes were also found to be involved in neurogenesis. Studies found that 3 days after transient ischemia/reperfusion platelet-derived growth factor receptor beta-positive (PDGFR beta$^+$) pericytes within injured areas began to express the NSCs marker Nestin, and at day 7, some of them expressed the immature neuronal marker DCX. These findings suggest that brain pericytes may contribute to new neurons in response to ischemia condition[54, 55].

The polarization of microglia adjusts neurogenesis

Microglia, one of the resident immune cells in CNS, plays a crucial role in neurogenesis, which includes 1) Resting microglia in the neurogenic niche releasing neurotrophic factors such as insulin-like growth factor 1 (IGF-1) which are essential for new neurons proliferation and survival[56]; 2) activated microglia converting to neuron[57], and 3) bidirectionally adjusting

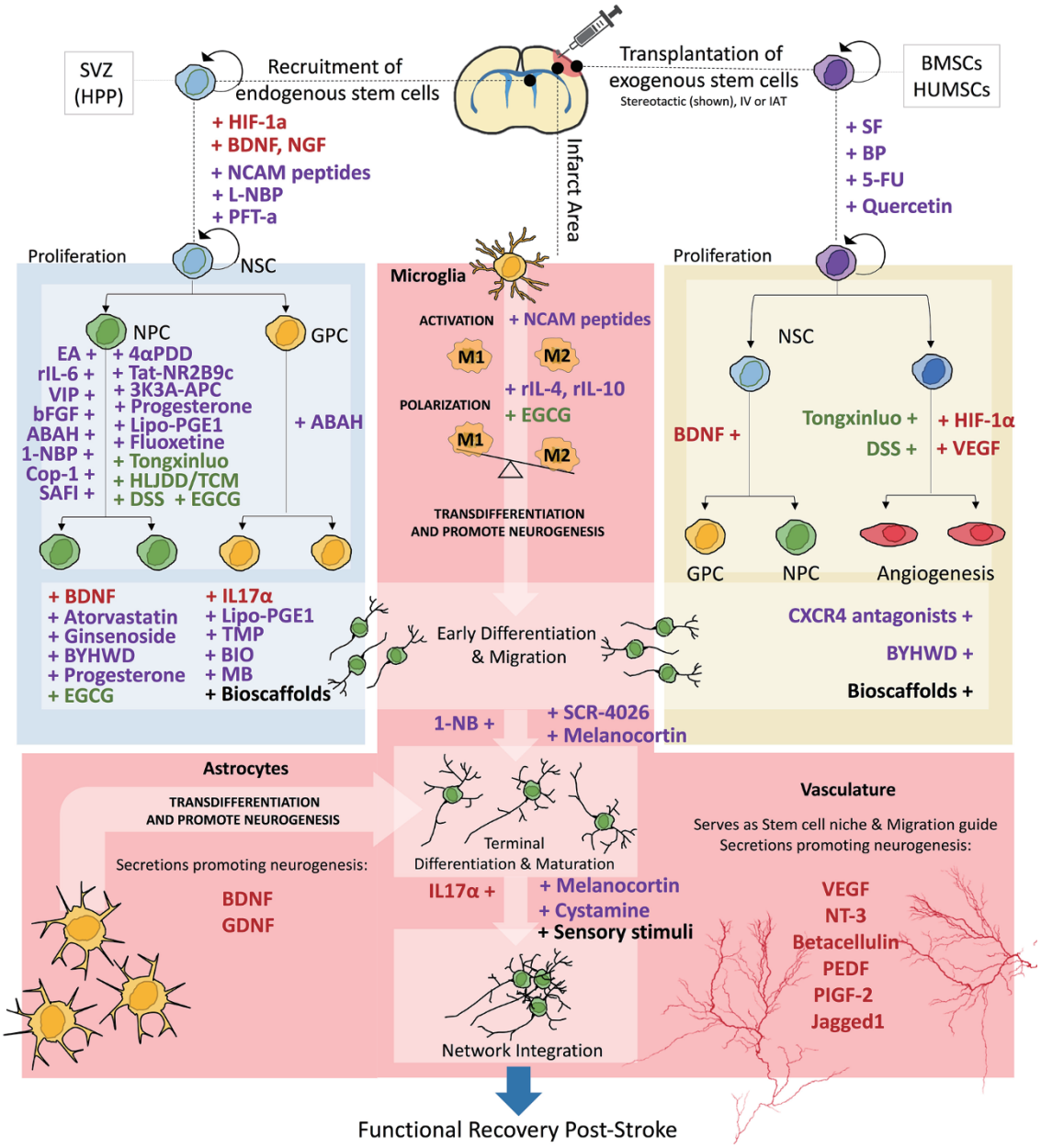

Figure 1. Schematic diagram of two major routes of stem cells and neurogenesis in stroke. Endogenous stem cells (Left) and transplanted exogenous stem cells (Right). Important processes towards improved neurological outcomes as shown are proliferation, differentiation, migration, and functional connection. Drug and peptides (Purple) and herbal medicines (Green) tested in animal and cell culture models are shown along side their suspected targeted processes. Endogenous compounds are denoted in Red text.

neurogenesis through polarization. In this section, we mainly discuss the third role of microglia, which is closely related to the regulation of neurogenesis and the recovery of neurological function.

Under physiological circumstances, microglia retain a relative quiescent surveillance phenotype for constant monitoring of the brain parenchyma[58]. Shortly after ischemic stroke, due to the change of cellular environments, such as the deletion of ATP, microglia were activated to clear the cell debris[59]. The activated microglia present two polarization phenotypes, M1 and M2, which exhibit distinct roles in influencing neurogenesis. Acute M1 microglial activation along with secreted pro-inflammatory cytokines [interleukin 6(IL-6), tumor necrosis factor α (TNF-α), interferon gamma (IFN-γ), interleukin 23(IL-23), interleukin 12 (IL-12) and interleukin 1β (IL-1β), *etc.*][60-62] and reactive oxygen species (ROS)[63, 64]. It is widely considered that M1 microglia causes neuronal death, neurogenesis inhibition and exacerbates neuronal injury[65]. However, some studies do not fully support this notion. For instance, the neuro-inflammatory environment is not entirely harmful and

may have dual roles in regulating neurogenesis after stroke [66]. Advantages or disadvantages of neuroinflammation on neurogenesis depends on its severity and location. During mild, acute inflammation, activated, ramified or intermediate microglia in ipsilateral SVZ has been shown to accompany neuroblast migration after stroke, indicating a beneficial role in neurogenesis, while amoeboid microglia in the peri-infarct, accompanied with the uncontrolled inflammation, induced the death of newborn neuron, and inhibited neural progenitors from differentiating into neurons which were detrimental to neurogenesis [67-69].

In contrast, an increase in activated M2 microglia promotes neurogenesis[70]. In addition to cytokines and chemokines, microglial cells also synthesized and secreted neurotrophic factors like basic fibroblast growth factor (bFGF), brain derived neurotrophic factor (BDNF) and interleukin 4 (IL-4)[71] which are known to stimulate the proliferation, migration, differentiation, the survival of neuron[72] and regulate synaptic maturation[73]. According to the above studies, promoting M1 to M2 phenotype transition may be a promising strategy to minimize detrimental effects and/or maximizing protective effects.

In summary, ischemia induces astrocyte, pericytes and microglia to neuron conversion (Figure 1). Activated microglia bi-directionally adjusted the process of neurogenesis partly through the polarization. During the course of neurogenesis, molecules, such as ATP, glucose, signaling pathways, such as the notch, Ras/MAPK and PI3K/TOR/PTEN, and transcription-related factors, such as Hes1, miRNA 210, help regulate the process of neurogenesis[74]. Strategies to promote neuron conversion, microglia M2 polarization by targeting molecular pathways and transcription factors may promote neurogenesis.

The role of ischemia-induced neurogenesis in brain repair and recovery: favorable or harmful

Ischemia stroke enhances cell proliferation. Stroke-generated new neurons migrate into the severely damaged area, partly replenish the damaged or lost neuron, and promote the repair of injured-brain[15, 75]. Most studies suggest that ischemia-induced neurogenesis is a means of self-repairing which partly contributes to the neurological recovery and may be related to the spontaneous recovery after ischemic stroke insults. However, whether this injury-induced neurogenesis contributes to recovery after brain injury remains controversial.

Ischemia-induced neurogenesis promotes brain recovery

Most of the studies provided evidence that ischemia-induced neurogenesis is helpful to brain repair and recovery. After ischemia, spontaneous neurogenesis is enhanced and accompanied by the course of spontaneous recovery of neurological function, suggesting a possible relationship between neurogenic potential and recovery after injury[76, 77]. Once ischemia-induced neurogenesis and associated neuromigration was abolished in transgenic mice expressing herpes simplex virus thymidine kinase under control of DCX promoter by the antiviral drug ganciclovir (GCV), infarct size was enlarged, and post-ischemic sensorimotor behavioral deficits were measured by rotarod, limb placing, and elevated body swing tests were exacerbated[78]. In another study, when neuroprogenitor cells were conditionally ablated using a transgenic mouse model containing modified Herpes Simplex Virus Thymidine Kinase Gene (HSV-TK gene) driven by Nestin promoter, learning and memory outcomes were worsened and synaptic connectivity in the performant pathway reduced[79]. The above two studies present evidence that spontaneous ischemia-induced neurogenesis contributes to the recovery of neurological function and might therefore be a target for stroke therapy.

Spontaneous Ischemia-induced neurogenesis has its own limitation

Studies suggested the major limitation of spontaneous neurogenesis in the brain is the lack of surviving high-quality new-born neuron. Three major possibilities support this notion.

First, there are few surviving neurons. Despite the large number of new-born neurons that are generated following stroke, more than 80% of them die during the first 2 weeks, most of them do not differentiate to mature neurons after 4 weeks post-stroke[80], and no surviving differentiated mature neural cells were observed by 90 days[26]. These data indicate that although many neuroblasts are produced and migrate to the site of injury, the ability of new-born neurons to replace lost neurons is limited. The limited number of surviving NCSs is partly due to unfavorable microenvironment post stroke attack (high levels of detrimental inflammatory factors and lack of trophic factors). In addition, normal aging may lead to further decreases in the number and maturation of newly generated neurons in the ischemic penumbra. To this day, the mechanisms underlying the low survival rate of new-born neurons remain unclear.

Second, the morphological features of the new neurons remain abnormal. Despite the fact that stroke enhanced SVZ neurogenesis and attracted new-born neurons to the injury area in rodents[81, 82] and patients[23], approximately 5% to 10% of newborn granule cells display significant morphological abnormalities. The main features are additional basal dendrites, ectopic cell position, and an increased portion of mushroom spines in aberrant neurons, which suggests stable synaptic integration[83].

Finally, there is a lack of diversity in the new neurons. Ischemia-induced neurogenesis generates predominantly GABAergic interneurons in SVZ, which cannot replace the broad spectrum of neuronal subtypes damaged by stroke. Therefore, SVZ neurogenesis may not be sufficient to replenish the loss of neuron after ischemic stroke[16]

In summary, ischemia-induced neurogenesis promotes brain recovery to some extent; however, it has been proven weak. Endogenous neurogenesis by itself is insufficient for effective brain repair after stroke. More ideal strategies are needed to enhance the number of surviving neurons, alleviate morphological abnormalities, enrich the cell subtype and construct the new neural network.

Potential of cell-based therapy for clinical trans-formation

Numerous animal experiments provide evidence that promoting neurogenesis is a potential way to protect and repair damaged brain tissues post-stroke[82, 84-87]. However, the role of cell-based therapy in ischemic stroke still needs to be established, because of the demonstrated challenges of cell-based therapies for ischemic stroke. It has been proposed that transplantation of neurons could improve neurological function by a variety of mechanisms including neuron replacement, alleviation of the neuroinflammation[88], inhibition of MMP-9 activation[89], secretion of neurotrophic factors, thereby survival of newborn neurons in injured-brain. In recent years, many pre-clinical studies and clinical trials on stem cells transplantation have been performed (Figure 1). Stem cell-based therapies were found to reduce infarct size and improve neural functional recovery in pre-clinical studies; however, its efficacy in humans still needs to be determined.

Stem cells

Bone marrow stem cells (BMSCs) are an array of different types of multipotent and pluripotent cells homed in the spongy tissue of almost all bones. Three basic lineages prevail: mesenchymal stem cells (MSCs), bone marrow mononuclear cells (BM-MNCs) and immortalized human neural stem-cell line. BMSCs were widely used to treat cerebral ischemic stroke in animal experiments and clinical trials for their advantages, such as easy collection, lack of ethical issues, pluripotency, and safely transplanted. MSCs transplantation was found to inhibit microglia activation, secrete growth factors, enhance angiogenic factor expression and vascular density, reduce scar size, limit apoptosis, and exert beneficial function on neurological recovery after ischemic brain injury in rats [11]. In the clinic, a long-term follow-up study for 5 years study of 16 patients showed that MSCs intravenous injection decreased modified Rankin score (mRS)[90]. Another study of 40 stroke patients by Bhasin group showed statistically significant improvement in modified Barthel Index (mBI) in stem cell group 6 months post-stroke[91]. MSCs also improved mBI at 39 and 52 months after transplantation[92] Despite the efficacy of MSCs proved in some clinical trials, the results from different clinical trials are partly contradictory (Table 1). For instance, a small clinical trial of 5 patients with acute middle cerebral artery (MCA) infarction by Band group reported a better Barthel index (BI) at 3 or 6 months but not at 12 months post-stroke when the patients received intravenous injection with autologous MSCs, In addition, MSCs transplantation did not improve mRS at 3, 6, 12 months post-stroke[93]. A randomized blinded phase II clinical trial showed at 6 months, there is no difference in BI, mRS, NIHSS and infarct volume between treatment group and control group[94]. In this study, 120 subacute stroke patients were enrolled, among them, 58 received 2.8×10^8 MSCs intravenously injected at a medium of 18.5 days post-stroke.

BM-MNCs are another type of stem cells widely used in clinical trials. In 2012, a prospective clinical trial was performed by Friedrich group. In this study, 20 patients with acute MCA infarct, spontaneous recanalization but persistent deficits were enrolled. BM-MNCs were intra-arterially injected between 3 and 7 days after stroke onset. At 3 months, clinical improvement occurred in 30% patients[95]. In the same year, a single-blinded (outcomes assessor) controlled Phase I/II trial was performed and a total of 20 MCA infarction patients were enrolled. Ten of them received MNCs injection. Results showed that BM-MNCs enhanced β-Nerve growth factor (β-NGF), however, did not improve neurological function at 6 months[96]. In 2015, a Phase I/IIa clinical Trial enrolled 12 severe embolic stroke patients[97]. At 6 months, intravenous MNCs enhanced cerebral blood flow (CBF), metabolic rate of oxygen consumption and neurologic outcomes. Another randomized, controlled, dose-finding, multicenter trial, IBIS, has sought to further test the efficacy of MNCs. This trial has just started the recruitment phase[98]. The findings are summarized in Table 1.

Besides mesenchymal stem cells, recently, immortalized human neural stem-cell lines were also used in clinical trials. Kalladka D enrolled 13 patients, among them, 11 received cell transplantation, and results showed that stem cell therapy with single intracerebral doses of up to 20 million cells significantly improved neurological function and was not associated with adverse events[99].

Neural progenitor cells (NPCs) transplant

Neural progenitor cells (NPCs) derived from adult brain and embryonic/fetal tissues can differentiate into neurons, astrocytes, or oligodendrocytes[100-102]. The transplantation of NPCs into brains with cerebral infarction increased dendritic length and the number of branch points and improved sensorimotor function[103]. These beneficial effects are thought to be associated with the secretion of trophic factors such as BDNF[104], vascular endothelial growth factor (VEGF)[105, 106], glial cell derived neurotrophic factor (GDNF)[107], basic fibroblast growth factor (FGF-2)[108, 109] and others by transplanted NPCs. Despite numerous animal experiments, the efficacy of progenitor cells on brain repair after stroke still need to be determined in more clinical trials. Recently a phase 2, randomized, double-blind, placebo-controlled, dose-escalation trial of intravenous NPCs was performed in 33 centers in the UK and the USA. Patients aged 18–83 years with moderately severe acute ischemic stroke were enrolled to treatment with intravenous NPCs (400 million or 1200 million cells) between 24 h and 48 h after symptom onset. Results showed that NPCs showed good efficacy in improvements in clinical functional scores (mRS and NIHSS score) and reductions in lesion volume[110].

Neuronal precursors transplant

Collective evidence showed that neuronal precursor cells improved animal survival following ischemic brain injury. Grafted neuronal precursor cells in ischemic stroke rats survived 3 months after transplantation and differentiated into neurons of diverse neurotransmitter-subtypes and the surviving neurons exhibit electrophysiological properties and ability to fire action potentials[111]. An independent group further

Table 1. Cell transplantation for ischemic stroke therapy (clinical trials in the recent five years).

Author	Name of Trial	Design	Patient	Cells	Time	Dosage	Deliver	Follow-up	Efficacy	Adverse Effects
Hess, 2017 [110] USA	Safety and efficacy of multipotent adult progenitor cells in acute ischemic stroke (**MASTERS**) (NCT 01436487)	RCT	Acute *n*=129 (*n*=67) Age: 18–83 Y	MAPC	24–48 h	4×10^8 12×10^8	IV	3 m	No	Safe
Bhasin [92] 2017, Indian	Safety and Feasibility of Autologous Mesenchymal Stem Cell Transplantation in Chronic Stroke in Indian patients. A four-year follow up	open-label	Chronic *n*=12 (*n*=6) Mean age: 42.8 Y	MSCs	3 m–2 y	N/A	IV	52 m	Clinical outcomes ↑	Safe
Steinberg [147] 2016 USA	Clinical Outcomes of Transplanted Modified Bone Marrow-Derived Mesenchymal Stem Cells in Stroke: A Phase 1/2a Study (NCT 01287936)	Open-label, single-arm	Stable, chronic stroke *n*=18 Mean age: 61 Y	BMSCs	6–60 m	2.5×10^6, 5.0×10^6, 10×10^6	Peri-infarct	24 m	ESS, NIHSS, F-M total score↑	Emergent adverse event↑
Kalladka, [99] 2016 UK	Human neural stem cells in patients with chronic ischemic stroke (PISCES): a phase 1, first-in-man study (NCT 01151124)	Open-label	Stroke *n*=13 (*n*=11) Age:≥60	CTX0E03	6–60 m	2×10^6, 5×10^6 10×10^6 20×10^6	Putamen	≥24 m	NIHSS↑	Hyperintensity in brain↑
Taguchi, [148] 2015 Japan	Intravenous Autologous Bone Marrow Mononuclear Cell Transplantation for Stroke: Phase 1/2a Clinical Trial in a Homogeneous Group of Stroke Patients	open-label study	Severe embolic stroke *n*=12, Age: 20–75	BM-MNCs	7–10 d	2.5×10^8 3.4×10^8	IV	6 m	Clinical outcomes↑	Safe
Moniche [98] 2015 Spain	IBIS trial (NCT 02178657)	RCT	MCA infarction *n*=76 (*n*=38) Age: 18–80	BM-MNCs	1–7 d	$2x10^6$ $5x10^6$	IAT	6–24 m	Going on	N/A
Qiao [149], 2014 China	A two-year follow-up study of cotransplantation with neural stem/progenitor cells, mesenchymal stromal cells in ischemic stroke patients	Case report	MCA or ACA infarction *n*=6 Age: 3–85	NSPCs or MSCs	1 w to 2 y	1) MSCs 0.5×10^6*4 2) NSPCs 6×10^6*3	CMCI	24 m	Clinical outcomes ↑	Low fever, dizziness
Prasad [150] 2014 India	Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial (NCT 0150177)	RCT	Subacute *n*=120 (*n*=58) Age: N/A	BMSCs	18.5 d (medium)	2.8×10^8 (Mean)	IV	6 m	No	Safe
Moniche [151] 2014 Spain	Intra-arterial bone marrow mononuclear cell transplantation correlates with GM-CSF, PDGF-BB, and MMP-2 serum levels in stroke patients: results from a clinical trial	RCT	Subacute MCA stroke *n*=17(*n*=8)	BM-MNCs	5–9 d	$1.59x10^8$	IAT	6 m	No	N/A

(To be continued)

Author	Name of Trial	Design	Patient	Cells	Time	Dosage	Deliver	Follow-up	Efficacy	Adverse Effects
Diez-Tejedor [152] 2014 Spain	Reparative therapy for acute ischemic stroke with allogeneic mesenchymal stem cells from adipose tissue: a safety assessment: a phase II randomized, double-blind, placebo-controlled, single-center, pilot clinical trial	RCT	Acute stroke *n*=20 (*n*=10)	MSCs	≤2 w	N/A	IV	24 m	Going on	N/A
Chen[153], 2014, China Taiwan.	Intracerebral implantation of autologous peripheral blood stem cells in stroke patients: a randomized phase II study	Randomized Controlled Trial	MCA infarction *n*= 30 (*n*=15)	PBSCs	6 m-5 y	(3-8)×10^6	N/A	12 m	Clinical outcomes ↑	Safe
Banerjee [154] 2014 UK	Intra-Arterial Immunoselected $CD34^+$ Stem Cells for Acute Ischemic Stroke	Prospective, open-label,	Severe anterior circulation ischemic stroke *n*= 5 NIHSS ≥8	Autologous CD34+ selected stem/progenit-or cell	≤7 d	1×10^8	IAT	6 m	Clinical outcome ↑	Safe
Kim [155] 2013 South Korea	Intravenous transplantation of mesenchymal stem cells preconditioned with early phase stroke serum: current evidence and study protocol for a randomized trial STARTING-2 (NCT 01716481)	PROBE	Acute and chronic stroke *n*=60 (*n*=40) Age: 30-75	MSCs	≤90 d	N/A	IV	3 m	Going on	N/A
Jiang [156] 2013 China	Feasibility of delivering mesenchymal stem cells via catheter to the proximal end of the lesion artery in patients with stroke in the territory of the middle cerebral artery	Case report	Ischemic stroke *n*=3	UCMSCs	90-180 d	2×10^7	IAT	6 m	Clinical outcomes ↑	Safe
Chen [157] 2013 China	Multiple cell transplantation based on an intraparenchymal approach for patients with chronic phase stroke	Case report	Ischemic stroke *n*=6 Age: 42–87	OECs NPCs UC-MSCs SCs	6 m- 20 y	OECs (1.0-2.0)×10^6; NPCs: (2.0–5.0)×10^6; SCs: 2.0×10^6; UCMSCs (1-2.3)×10^7	intracranial	24 m	Clinical outcomes ↑	N/A
Bhasin [91] 2013 India	Stem cell therapy: a clinical trial of stroke	Prospective	Stroke, *n*=40	MSCs	3 m-2 y	(5-6)×10^7	intravenously	6 m	mBI ↑	Safe
Prasad [158] 2012 India	Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischemic stroke: a pilot study	Non-randomized phase-I clinical study	Subacute ischemic stroke *n*=11	MNCs	7-30 d	8×10^7	intravenously	13 m	Clinical outcome↑ Neurological function↑	Safe Seizure

(To be continued)

Author	Name of Trial	Design	Patient	Cells	Time	Dosage	Deliver	Follow-up	Efficacy	Adverse Effects
Moniche [96] 2012 Spain	Intra-arterial bone marrow mononuclear cells in ischemic stroke: a pilot clinical trial (NCT 00761982)	A single-blind Phase I/II trial	MCA stroke *n*=20 (*n*=10)	BM-MNCs	5–9 d	1.59×10^8	Intra-arterially	6 m	Clinical outcomes†	Safe
Friedrich [95] 2012 Brazil	Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke	Prospective	Acute MCA infarcts *n*=20	BM-MNCs	3–7d		IAT	6 m		

Abbreviation: BDNF, brain-derived neurotrophic factor; β-NGF, β-nerve growth factor; BMSCs, Modified Bone Marrow-Derived Mesenchymal Stem Cells; BI, Barthel index; CBF, cerebral blood flow; CMCI, cerebellomedullary cistern injection; DTI, diffusion tensor image; ESS, European Stroke Scale; FLAIR, Fluid-attenuated inversion recovery; F-M: Fugl-Meyer total score; FNA, fiber numbers asymmetry; GM-CSF, granulocyte-macrophage colony-stimulating factor; IAT, Intra-arterial therapy; IV, Intravenous; MAPC, multipotent adult progenitor cell, MCA, middle cerebral artery; MEP, motor-evoked potential; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; mRS, modified Rankin Scale; MRI, magnetic resonance imaging; MSC, Mesenchymal stem cells; N/A, not available; NIHSS, National Institutes of Health Stroke Scale; NPCs, Neural progenitor cells; OECs, Olfactory ensheathing cells; PDGF-BB, platelet-derived growth factor-BB; PROBE, Prospective, randomized, open-label, blinded-endpoint; SCs, Schwann cells; TMS, transcranial magnetic stimulation; UCMSCs, Umbilical cord mesenchymal stromal cells; VEGF, vascular endothelial growth factor.

showed that cell transplantation increased neurogenesis in the ipsilateral SVZ in both young adult (3 months old) and aged (24 months old) rats with focal cerebral ischemia[112], and improve sensory recovery after ischemic stroke[113]. Unfortunately, there is no clear clinical evidence to demonstrate the efficacy of neuronal precursors on cerebral ischemia recovery [92].

Despite significant improvements found in some case reports, prospective studies and single arm studies, no clear difference has been found in randomized, controlled, blinded clinical trials (RCT). These differences could be due, at least in part, to the diverse designs of experiments, such as the dose, route of administration, initial time of stem cells therapy, severity of disease, choice of neurological evaluation scales and design types of clinical trials. Yet it is possible that the limited capacity for neurogenesis in humans is the primary reason for the failure of stem cells on cerebral ischemia recovery in RCT clinical trials. Therefore, there is a pressing need to provide novel measures to improve the cell-based therapies for ischemic stroke. Because the consecutive process of cell-based therapy includes neuronal differentiation, migration, survival and functional connection, more effective measures need to be developed to successfully go through the full process and help us get out of the plight.

Drugs assisting stem cell-based therapy

Neurogenesis following ischemic stroke has been considered as a potential mechanism for neuronal restoration, however, endogenous neurogenesis by itself is insufficient for effective brain repair as most newborn neurons do not survive. Replenishment of stem cells does not perfectly solve the problem of neurogenesis and neurofunction restoration following ischemic stroke insults. Mobilization, promotion of migration, improvement of differentiation of neural stem/progenitor cells, and promotion of connection of newly-developed mature neurons may be a potential way for brain repair. In view of the clinical transformation of exogenous cells is not easy, using pharmacological drugs to improve stem cell-based therapies has recently become a new focus. In this section, we will discuss the drugs that promote neurogenesis via different ways in recent studies (Figure 1). Some drugs enhancing neurogenesis and improving neurological outcomes in animal experiments are shown in Table 2.

Chemical drugs

Proliferation of new stem cells

The proliferation of stem cells was the first step of neurogenesis. Neuroinflammation, neurotrophic factors and apoptosis-related signal pathway are involved in the process of proliferation (Figure 1).

Modulation of neuroinflammation

C-X-C chemokine receptor type 4 (CXCR4) is a receptor for a pleiotropic chemokine CXCL12. CXCR4 antagonist AMD3100 and CX549 mobilized bone marrow hematopoietic stem cells (HSCs) for transplantation by reducing neuroinflammation in stroke brain[114]. The neural cell adhesion molecule-derived

Table 2. Promotion neurogenesis with drugs after ischemic stroke.

Intervention	Related mechanism	Therapeutic effects	Neurological outcome
EGCG (predominant constituent of green tea) Zhang, Xu *et al* 2017 [84]	M2 phenotype of microglia	Proliferation of SVZ NPCs↑ Migration of SVZ neuroblasts↑	Functional recovery↑
MC-21(the anti-CCR2 antibody),Laterza, *et al*, 2017 [159]	Depletion of circulating monocytes; Reduced astrocyte activation in SVZ and adjacent striatum	Enhances striatal neurogenesis at one week post-insult, most likely by increasing short-term survival of the newly formed neuroblasts in the SVZ and adjacent striatum.	
Bumetanide (a selective Na^+-K^+-Cl--co-transporter inhibitor) ,Xu, Mu *et al*, 2017 [160]	Effects on inflammation−−−	Migration of neuroblasts in the SVZ towards the infarct area↑ Long-term survival of newborn neurons↑	Sensorimotor recovery↑
6-Bromoindirubin-3'-oxime (BIO)(GSK3β specific inhibitor) ,Wang, Li *et al*, 2017 [161]	N/A	Generation of neuroblasts in the SVZ↑ Neuroblasts migrated to the peri-infarct region↑ Newly formed neurons ↑	Sensorimotor recovery↑
Guanosine (GUO), Deng, Qiu *et al*, 2017 [162]	BDNF, VEGF↑	Neurogenesis and angiogenesis ↑	Functional recovery↑
4alpha-PDD (TRPV4 agonist), Chen, Hsu *et al*, 2017 [163]	eNOS expression and phosphorylation (serine 1177) ↑	NPC proliferation ↑ NPC migration in the ischemic hemisphere ↑	Functional outcomes on day 5↑
Fluoxetine ①,Sun, Zhou *et al*, 2016 [164]	N/A	Proliferation of newborn neurons in the SVZ↑ SGZ −−− Perilesional apoptosis ↓ Survival or differentiation of newly generated cells in the SVZ−−−	Behavioral outcome −−−
Fluoxetine ②,Sun, Sun *et al*, 2015 [165]	N/A	Neuroblasts in both the SVZ and DG↑ Dendritic complexity of newborn dentate granule cells↑ Survival or differentiation of newly generated cells−−−	Sensorimotor recovery −−−
Lipo-PGE1, Ling, Zhang *et al*, 2016 [166]	N/A	Proliferation↑ Migration of endogenous neural stem cells in the ipsilateral SVZ ↑	Neurological recovery ↑
100K/bFGF①,Li, Tsai *et al,* 2016 [167]	N/A	NSPCs proliferation↑ MAP-2 cells ↑ GFAP cells at the SVZ area and in the infarcted regions −−−	Motor coordination ↑
bFGF②,Wang, *et al* 2008[168]	N/A	Infarct size −−− Proliferation of progenitor cells in the subventricular zone and the subgranular zone of the dentate gyrus (DG) ↑	Neurobehavioral recovery ↑
ABAH (MPO Inhibitor), Kim, Wei *et al,* 2016 [169]	BDNF,Phosphorylation of cAMP response element-binding protein (Ser 133)↑, Acetylated H3 ↑, Chemokine CXC receptor 4↑	Neural stems cells ↑ Astrocytes↑ Neuroprogenitor cells↑ Neuroblasts ↑ in the ischemic SVZ, anterior SVZ striatum, and cortex	N/A
Progesterone .Jiang, Zuo *et al*, 2016 [170] Intervention	VEGF↓, BDNF↑	Newly generated neurons in the SVZ↑ Neuroblast cells in the peri-infarct region ↑	Neurologic function on days 7 and 14 post-occlusion ↑
MB ,Ahmed, Tucker *et al*, 2016 [171]	Reactive gliosis↓ , pro-inflammatory ↓, cytokines cytochrome c oxidase activity↑, ATP production in peri-infarct regions↑	Cell proliferation and neurogenesis in the peri-infarct zone ↓	Neurological deficits ↓

(To be continued)

Intervention	Related mechanism	Therapeutic Effects	Neurological outcome
l-NBP ,Yang, Li *et al* 2015 [119]	PKA↑, Akt↑, CREB↑,STAT3↓, cleaved Caspase-3↓, Bax↓	Neurogenesis (DG) ↑ Newborn cells and newly Mature neurons ↑	Behavioral recovery ↑
VIP ,Yang, Shi *et al* 2015 [172]	VEGF in the SVZ ↑	Stem cells and neuroblast in the SVZ at 7, 14 and 28 days after ischemia ↑	Neurological severity score ↓ infarct volume ↓
Melanocortin ,Giuliani, Zaffe *et al* 2011 [173]	Wnt-3A signaling pathways ↑	Stem cells co-localized with NeuN (used as indicator of mature neurons) and Zif268 (used as indicator of functionally integrated neurons) Day 50 post-stroke in the DG ↑	Learning, memory ↑
Ginsenoside Rd 5 ,Liu, Zhou *et al* 2015 [174]	p-Akt ↑,VEGF↑,BDNF↑,P-ERK↑,PC12 cell apoptosis ↓	BrdU/DCX and Nestin/GFAP double-positive cells in ischemic area ↑	N/A
Copolymer-1 (Cop-1) (Glatiramer acetate), Cruz, Lorea *et al* 2015 [175]	NT-3 ↑	Neurogenesis (at 7 and 60 days) in the SVZ, SGZ, and cerebral cortex ↑	Neurological outcome ↑
Huang-Lian-Jie-Du-Decoction (HLJDD) (TCM) ,Zou, Long et al. 2016 [139]	VEGF, Ang-1, Ang-2 ↑phosphorylation of AKT, and GSK-3beta↑	Alkaloids and Iridoids: neuronal differentiation in the cortex ↑ Alkaloids: neurogenesis↑	N/A
Huatuo Zaizao pill (TCM) ,Duan, Wang *et al* 2017 [140]	BDNF, phosphorylated PKA, CREB↑	Neurogenesis↑	Functional recovery ↑
Tongxinluo (TCM) ,Chen, Wang *et al* 2016 [176]	N/A	Neurogenesis ↑ angiogenesis in the peri-infarct area and SVZ↑	Neurological function deficit↑
Danggui-Shaoyao-San (TCM), Ren, Wang *et al* 2015 [142]	vascular endothelial growth factor ↑ eNOS phosphorylation ↑	Microvessel density in the peri focal region ↑ Stem cells and neuroblast in the SVZ↑	Neurobehavioral outcomes ↑

Abbreviation: ABAH, 4-aminobenzoic acid hydrazide; Akt, protein kinase B; Ang-1, Angiopoietin-1; BDNF, brain-derived neurotropic factor; bFGF, basic fibroblast growth factor; BrdU, 5-bromo-2'-deoxyuridine; CREB, cAMP response element-binding protein; DCX, doublecortin; DG, dentate gyrus; EGCG, Epigallocatechin-3-gallate; GDNF, Glial cell-derived neurotropic factor; HBM-MSC ,Human bone marrow stem cell; H2S, hydrogen sulfide; L-NBP, L-3-n-butylphthalide; MB, methylene Blue; N/A , non-available; NT-3, neurotrophin 3; NPC: neural progenitor cell; PKA, protein kinase A; PGE1, Prostaglandin E1; SGZ, subgranular zone; STAT3, signal transducer and activation of transcription 3; SVZ, subventricular zone; TCM, Traditional Chinese Medicine; VEGF ,vascular endothelial growth factor; VIP, vasoactive intestinal peptide. ↑: enhanced or improved; −−−: no change; ↓decreased.

peptide FG loop significantly increased endogenous NSC mobilization in the neurogenic niches, which is associated with the modulation of the activation of microglia and modulation of neuroinflammation[115]. rIL-6 significantly increased the proliferation of NPCs in the ipsilateral SVZ[116].

Modulation of neurotrophic factors

Salvianolic acids for injection (SAFI) promoted the proliferation of NPCs, enhanced the number of surviving newborn neurons in the SVZ and led to the improvement of neurological outcome. In addition, SAFI activated sonic hedgehog–Patched–Gli (Shh-Ptch-Gli) signal pathway and induced the production of BDNF and NGF. The beneficial effect of SAFI was abolished by Cyclopamine (CYC) significantly through decreasing BDNF and NGF level. These data indicated that SAFI significantly improved long-term functional recovery by enhancing BDNF and NGF production and promoting neurogenesis[117]. Besides SAFI, in another study, cystamine was also found to significantly enhance neuronal progenitor cell proliferation and plasticity through BDNF/TrkB pathway after stroke[118] .

Modulation of apoptosis-related signal pathway

L-3-*n*-butylphthalide (*L*-NBP) was found to markedly increase 5-bromo-2′-deoxyuridine (BrdU)-positive cells in the hippocampal dentate gyrus (DG) on day 28 after ischemia by activating CREB and Akt and inhibiting STAT3 signaling [119]. Sodium ferulate (SF) and n-butylidenephthalide (BP) combined with BMSC can significantly improve neurogenesis following stroke through the enhancement of VEGF and BDNF expressions and activation of AKT/mTOR signal pathway[120].

3K3A-APC (3K3A-activated protein C) has been demonstrated to stimulate transplanted NSCs to neurons and promote neurological recovery via a protease-activated receptor-1 (PAR1)-protease-activated receptors(PAR3)-sphingosine-1-phosphate-receptor 1 (S1PRs)-Protein Kinase B (Akt) pathway *in vitro*[121].

Polyphenol ellagic acid (EA) was found to enhance the proliferation of NSCs and the content of nestin protein in the brain semidarkness zone through the Wnt/beta-catenin signaling pathway[122].

Tat-NR2B9c, a peptide disrupting the *N*-methyl-*D*-aspartate receptor-postsynaptic density protein-95 interaction, substantially increased neurogenesis in the dentate gyrus by reversing the ischemia-induced formation of *S*-nitrosylation-cyclin-dependent kinase 5 and increasing cyclin-dependent kinase 5 (CDK5) activity in the ipsilateral hippocampus[123].

Enhancement of migration of NPCs

Neurotrophic factors

BDNF enhanced the recruitment of NPCs into the lesioned site after ischemic stroke. Atorvastatin, a chemical that activates the expression of BDNF, enhanced migration of SVZ cells[124]. Similarly, overexpression of BDNF through gene therapy via Adeno-associated virus (AAV) infection facilitated endogenous NPC migration from the SVZ [125].

PI3K/AKT signal pathway

Tetramethylpyrazine (TMP) was found to promote NPC migration, this effect was reversed by inhibiting the molecular, such as phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK). These data show that the PI3K/AKT signaling pathway is involved in the process of migration of NPCs[126].

Promoting cell differentiation

nNOS-PSD-95 was found to be involved in the course of differentiation during ischemic stroke. A small-molecular inhibitor of nNOS-PSD-95 interaction, SCR-4026, was found to promote neural stem cells to differentiate into neuron-like cells[127]. Complement-derived peptide C3a regulates neural progenitor cell migration and differentiation *in vitro*[128] .

Enhancing the survival of stem cells

Transplantation of cells is a promising strategy for neuroregeneration, however cell survival is one of the key barriers to the success of cell implantation treatment. Studies have explored the protective effects of pharmacological agent preconditioning to enhance the viability of stem cells.

Modulation of neuroinflammation

Cerebral ischemia stimulated inflammatory processes and affected NSCs in multiple ways. Drugs modulating neuroinflammation are likely to provide neuroprotection during neurogenesis.

5-Fluorouracil (5-FU) pre-treatment enhanced the viability of transplanted bone marrow mononuclear cells (BMMNCs) in the hippocampus, which was found to be associated with the increased microvessel density (MVD), reduced levels of pro-inflammatory cytokines and increased levels of growth factors in the penumbra. These results indicate that 5-FU improve the local microenvironment and increase number of viable cells [129].

Quercetin is another drug found to be effective in improving the survival rate of human umbilical cord mesenchymal stromal cells (HUMSCs) in the injury site after local cerebral ischemia. The protective mechanisms include reducing proinflammatory cytokines (IL-1β and IL-6), increasing anti-inflammatory cytokines (IL-4, IL-10, and transforming growth factor-β1) and inhibiting cell apoptosis (caspase-3 expression)[130].

Apoptosis-related signal pathway

Although stroke stimulates the proliferation of NPCs, most of these cells die after injury. Alleviating apoptosis is a major way to improve the survival of stem cells. A number of anti-apoptotic drugs were found to improve the survival of neurons via a variety of singling pathways.

The tolerance of HWJ-MSC-derived neural-like cells was improved when they are preconditioned with deferoxamine (DFO). The tolerance may be due to the increase of HIF-1, BDNF, pAkt-1 and decrease of Bax/Bcl-2 ratio[131].

Pifithrin-a (PFT-a), a p53 inhibitor, starting from day 6 after MCAO, was found to enhance the survival of endogenous

NPCs in the SVZ. These data suggest that inhibition of p53 may extend the survival of endogenous NPCs after stroke.

Ginsenoside Rg1 prevents NSCs from oxygen-glucose deprivation (OGD) insult through inhibiting oxidative stress and the activity of p38/JNK2 signaling way in NSCs[132].

Relieve neuronal morphological damage

Noggin is a signaling molecule involved in embryonic development. Grafting NSCs modified by noggin gene reduced the percentage of apoptotic neurons and relieved neuronal morphological damage, which was accompanied by the decrease of the MDA levels, the SOD activity, and downregulation of the bone morphogenesis protein 4 (BMP4), VEGF, and bFGF proteins[133].

Promote the functional connection

Establishment of cell-cell interaction is considered to be crucial after stem cell transplantation. However, in recent studies, the functional connection, which is the key step for successful neurogenesis and was regarded as important for the repair of host brain architecture, is seldom considered. In order to promote the functional communication of neuron, the following methods may be useful in future studies. First, use of cell sheet as opposed to cell suspension. Previous transplant approaches have utilized injection of the cells in a cell suspension; however, these cells cannot establish a connection to the damaged tissues. However, the cell sheet was supposed to maintain cell-cell interactions and improve neurological functions [134]. Second, sensory stimuli promoted pluripotent stem cell-derived cortical neurons to incorporate into injured cortical circuitry and contribute to functional recovery in stroke[135].

Multiple functions on neurogenesis

Some pharmacological agents serve multiple functions in neurogenesis. For instance, after cerebral ischemia, IL-1Ra was found to increased stem cell proliferation, enhance neuroblast migration and promote the survival of newly born neurons[136]. IL-17A, secreted by astrocytes, augments survival of SVZ neural precursor cells (NPCs), neuronal differentiation and synaptogenesis via p38 MAPK/calpain 1 signaling pathway after ischemic stroke[137]. Indomethacin, a modulator of microglia activation, contributed to increased neuroblast proliferation in the SVZ and migration to the ischemic striatum following stroke[138].

Herbal medicine

Epigallocatechin-3-gallate (EGCG), the predominant constituent of green tea, was found to increase proliferation of SVZ NPCs and migration of SVZ neuroblasts, improve functional recovery, and attribute to the M2 phenotype induction in microglia[84].

Huang-Lian-Jie-Du-Decoction (HLJDD) is broadly used in Traditional Chinese Medicine (TCM) and shown to enhance neurogenesis. The main ingredients of HLJDD are alkaloids and iridoids. Alkaloids and iridoids enhance the level of VEGF, Ang-1, Ang-2, phosphorylation of AKT, and GSK-3beta, increasing the number of BrdU-positive cells[139].

Huatuo Zaizao pill (HT), a widely used TCM in clinic for the treatment of cerebrovascular disease, was also found to effectively enhance neurogenesis. HT treatment for 3 days increased neurogenesis in cerebral ischemia reperfusion animal models, and its effects may be associated with the increase of BDNF mRNA, PKA, and phosphorylated CREB[140].

Tongxinluo was shown to enhance neurogenesis and angiogenesis in the peri-infarct area and SVZ, which partly contributes to the amelioration of the neurological function deficit [141].

Danggui-Shaoyao-San (DSS) treatment significantly activated vascular endothelial growth factor, enhance microvessel density in the perifocal region, increased the numbers of $BrdU^+/DCX^+$ cells in the SVZ and improved neurobehavioral outcomes[142].

Buyang Huanwu Decoction (BYHWD) could markedly facilitated stem cell migration by increasing the expression of neurotrophic factors, such as stromal cell-derived factor-1, vascular endothelial growth factor, reelin, and BDNF in the ipsilateral infarct area after MCAO[143].

Enhance neurogenesis with GCSF

Stimulating the proliferation of neural stem/progenitor cells is another method to be used to improve neurobehavioral functions. Granulocyte colony-stimulating factor (GCSF), a glycoprotein that stimulates the bone marrow to produce and release granulocytes and stem cells into the bloodstream, has been considered as a promising cytokine to promote neurogenesis in ischemic stroke mice. A pre-clinical trial performed by Kawada in 2006 determined the role of GCSF in stimulating the proliferation of intrinsic neural stem/progenitor cells [144]. A randomized, blinded controlled trial enrolled 10 patients (7 for GCSF therapy) found that GCSF improved neurologic functioning (NIHSS, ESS, EMS, and BI) and fluorodeoxyglucose in the area surrounding the core[145]. To further determine the efficacy of GCSF on ischemic stroke, in 2016, a randomized controlled multicenter phase II trial enrolled more patients (49 patients, among them, 40 patients received GCSF therapy). However, this study found that GCSF neither improved functional recovery (NIHSS, ESS, EMS, and BI) nor reduced infarct volume[146] (Table 3). These results indicate that successful neurogenesis includes multiple steps, besides proliferation, other steps such as migration, differentiation, survival of mature neurons and functional connections are also critical. Pharmacological drugs targeting multiple steps of neurogenesis are potential ways to improve neurogenesis and neurological outcomes of ischemic stroke patients.

Conclusion ans future directions

Neurogenesis after stroke has been considered as an important mechanism for functional recovery. Numerous studies of animal experiments, prospective or pilot clinical trials and single arm trails showed that stem cell transplantation therapy improved neurological function. However, no clear evidence has validated the role of cell-transplantation therapy in improving stroke outcome from multicenter, large sample,

Table 3. Pre-clinical or clinical trials for endogenous neurogenesis.

Author Year Country	Trial	Design	Patients No. Total (Therapy)	Time (post-stroke)	Dosage Route	Follow-Up (month)	Efficacy	Adverse Effects
Shyu [145] 2006 Canada	Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial	Random-ized, blinded controlled trial	*n*=10 (*n*=7)	≤7 d	15 mg/kg per day*5 d, SC	12 m	Improve neurologic functioning.	Safe
Mizuma [146] 2016 Japan	Intravenous Low-Dose Granulocyte Colony-Stimulating Factor in Acute Ischemic Stroke	Random-ized Controlled Trial	*n*=49 (*n*=40)	≤24 h	150,300 mg/d*5 d, SC.	3 m	Did not improve functional recovery	Safe

MCA: middle cerebral artery; G-CSF, Granulocyte colony-stimulating factor; SC, Subcutaneous.

randomizes, controlled, and blinded clinical trials. Pharmacological drugs may enhance the efficacy of cell-transplantation therapy by promoting the proliferation, migration, differentiation, survival of newborn neuron and the function connection. Thus, using pharmacological drugs in combination with cell-based therapy can be a potential strategy to improve the post-stroke outcomes in clinical trials. Many drugs and herbal compounds that have been tested in animal models. Future studies could consider to selectively test these drugs and herbal compounds in well-designed randomized clinical trials.

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