



Narrative review of stem cell therapy for ischemic brain injury

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Abstract: Ischemic brain injury is a common cause of long-term neurological deficits in children as well as adults, and no efficient treatments could reverse the sequelae in clinic till now. Stem cells have the capacity of self-renewal and multilineage differentiation. The therapeutic efficacy of stem cell transplantation for ischemic brain injury have been tested for many years. The grafts could survive and mature in the ischemic brain environment. Stem cell transplantation could improve functional recovery of ischemic brain injury models in pre-clinical trials. The potential mechanisms included cell replacement, release of neurotrophic and anti-inflammatory factors, immunoregulation as well as activation of endogenous neurogenesis. Besides, many clinical trials were conducted and some of trials already had preliminary results. From the current published data, cell transplantation for clinical application is safe and feasible. No severe adverse events and tumorigenesis were reported. While the therapeutic efficacy of stem cell therapy in clinic still needs more evidences. In this review, we overviewed the studies about stem cell therapy for ischemic brain injury. Different types of stem cells used for transplantation as well as the therapeutic mechanisms were discussed in detail. The related pre-clinical and clinical trials were summarized into two separate tables. In addition, we also discussed the unsolved problems and concerns about stem cell therapy for ischemic brain injury that need to be overcome before clinic transformation.

Keywords: Ischemic brain injury; stem cell transplantation; therapeutic mechanism; clinical trials

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Introduction

Ischemic brain injury including stroke, hypoxic-ischemic encephalopathy (HIE) and traumatic brain injury is a high incidence health problem worldwide (1,2). It can cause massive brain tissue infarction, neuronal loss and gliosis, therefore resulting in the occurrence of cerebral palsy, motor defects and even death in immature and mature brain (3,4). Various clinical treatments have been used for ischemic brain injury, such as hyperbaric oxygen (5), medications (6), rehabilitation training (7) and therapeutic hypothermia (8). However, most of these therapeutic

methods are supportive care, and none can reverse the sequelae of ischemic encephalopathy.

Since the advent of stem cell biology, one approach to restore the function of ischemic brain injury is stem cell therapy. Many studies have already investigated the therapeutic potentials of stem cell transplantation in ischemic animal models (9-12). Stem cells have the capacity of self-renewal and multilineage differentiation, which could generate plenty of functional cells and migrate to the injured brain areas (13,14). The grafted cells could replace the lost cell type, reconstruct the neural circuitry with host cells, and therefore rebuild the functionality of ischemic

Table 1 Pre-clinical trials about stem cell transplantation for ischemic brain injury

Cell type	Animal model	Route & time	Graft maturation	Functional recovery	Therapeutic mechanism	Reference
Fetal brain tissue	HIE	Intracerebral & 3 days after HI	Neurochemical phenotype detected after transplantation for 10–12 weeks	Amelioration of motor deficits	No clarify	(19)
ESCs	Stroke	Intracerebral & immediately after stroke	No clarify	Improvement of the sensorimotor function	No clarify	(20)
iPSCs	Stroke	Intracerebral & 6 h after stroke	Differentiated into GFAP+ neural cells after transplantation for 28 days	Improvement of motor function and higher survival rate	Attenuate cerebral inflammatory and neural injury	(21)
NSCs	Stroke	Intracerebral & 7 days after stroke	Differentiated into neurons, astrocytes and oligodendrocytes after transplantation for 2 months	Improvement in the sensorimotor function	Graft-host interactions to stabilize differentiation and prevent gliotic host response	(22)
NSCs	Stroke	Intracortical & 48 h after stroke	20.6%±1.4% NeuN + neurons after transplantation for 3 months	Not tested	Grafts incorporated into injured cortical circuitry	(16)
BM-MSCs	Stroke	Intracerebral & 3 days after stroke	A small percentage neural-like cells after transplantation for 25 days	Significantly motor function improvement	Neurotrophic factor (BDNF, NT-3, VEGF) production	(23)
UCB-MSCs	HIE	Intravenous & 24 h after HIE	21.3%±3.5% differentiating into DCX+ neurons	Enhanced behavioral recovery	Cell replacement and suppression of gliosis	(24)
Adipose-derived MSCs	Stroke	Intracerebral & 24 h after stroke	Few cells expressed GFAP and NeuN after 3 days transplantation	Improvement of neurological function	Neurogenesis	(25)
Placenta-derived MSCs	HIE	Intracortical & 48 h after HIE	No clarify	Improvement of motor function	Modulation of immune responses and secretion the anti-inflammatory factors	(12)
Cerebral organoid	Stroke	Intracerebral & 6 h, 24 h, or 7 days after stroke	Multilineage differentiation to mimic <i>in vivo</i> cortical development	Improvement of neurological motor function	Neurogenesis, synaptic reconstruction and angiogenesis	(26)

HIE, hypoxic ischemic encephalopathy; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; NSCs, neural stem cells; BM-MSCs, bone marrow mesenchymal stem cells; UCB-MSCs, umbilical cord blood mesenchymal stem cells.

injury brain (15,16). In addition, these transplanted stem cells could secrete neurotrophic factors to promote the survival of endogenous residual cells as well as endogenous neurogenesis (17). Besides, through releasing the anti-inflammatory cytokines, stem cells could modulate the inflammatory environment of ischemic brain (18). These properties make them as the most promising therapeutic treatment for ischemic encephalopathy in the future.

In this review, we discussed the related studies about stem cells transplantation for ischemic brain injury, summarized the pre-clinical (*Table 1*) and clinical trials (*Table 2*) in recent years. And the underlying mechanisms of

stem cell therapy, clinical trials as well as unsolved problems were also discussed.

We present the following article in accordance with the Narrative Review Checklist (available at <http://dx.doi.org/10.21037/tp-20-262>).

Stem cell types used for ischemic brain injury

Various types of stem cells including fetal cerebral tissues, embryonic stem cells (ESCs)/induced pluripotent stem cells (iPSCs), neural stem cells (NSCs), mesenchymal stem cells (MSCs) and brain organoid were tried for treating ischemic

Table 2 Main clinical trials about ischemic stroke that have finished or currently being carried out

Name of trials	Cells dosage	Patients recruited	Deliver route	Follow-up	Outcome measures	Therapeutic efficacy
Study of allogeneic BM-MSCs in subjects with ischemic stroke (NCT01297413)	0.5–1.5 million/kg	38	IV	12 months	Safety and tolerability	Function benefits
Transplantation of MSCs after stroke (NCT00875654)	100 or 300 million	31	IV	24 months	1. Clinical and functional effects; 2. The most effective dose	1. Improve motor recovery; 2. No effect of MSC dose on behavior scale
Infusion of BM-MNCs in subacute ischemic stroke patients (NCT03080571)	No description	20	IA	6 months	1. Change in NISS score; 2. Symptomatic intracranial hemorrhage; 3. New ischemic lesion; 4. Death	No relevant results published yet
Autologous BM-MSCs for patients with acute ischemic stroke (NCT01501773)	30–500 million	120	IV	12 months	1. Safety and efficacy variables; 2. Dose response gradient	1. Safe but ineffective in treatment of moderately severe subacute ischemic stroke; 2. No dose-response relationship
Autologous BM-MNCs in stroke patients (NCT00859014)	10 million/kg	25	IV	24 months	1. Safety and feasibility; 2. Functional outcome	1. No study-related severe adverse events; 2. A better outcome on NIHSS and mRS scale
Reparative therapy in acute ischemic stroke with allogeneic MSCs from adipose (NCT01678534)	1 million/kg	19	IV	24 months	1. Adverse events; 2. Neurological and systemic complications; 3. Tumorigenesis	No relevant results published yet
Pilot Investigation of NSCs in Stroke (NCT01151124)	2, 5, 10 or 20 million	13	Ipsilateral putamen injection	24 months	1. Incidence of adverse events; 2. Measure of functional outcome, cognitive impairment, overall disability and quality of life outcomes	1. Doses up to 20 million cells induced no adverse events; 2. Improved neurological function
Treatment of acute ischemic stroke with UC-MSCs (NCT04097652)	Low/medium /high dosage	9	IV	15 months	1. Changes in clinical function; 2. Incidence and frequency of adverse events	Ongoing
Effect of different transplantation Time for UC-MSCs in stroke patients (NCT04093336)	2 million/kg	120	IV	24 months	Assess the adverse events, neurological functional recovery and quality of life	Ongoing

BM-MSCs, bone marrow mesenchymal stem cells; BM-MNCs, bone marrow mononuclear cells; UC-MSCs, umbilical cord blood mesenchymal stem cells; NIHSS, national institute of health stroke scale; NSCs, neural stem cells.

brain injury (*Figure 1*).

Fetal cerebral tissues

The fetal cerebral tissues used for transplantation were obtained from different gestational ages and anatomical sites. Various cell types, such as NSCs, neurons, astrocytes

and oligodendrocytes can be isolated from fetal cerebral tissues (27,28). Studies had demonstrated the survival, migration and therapeutic efficacy of fetal cerebral tissues derived from rodents or human after being transplanted into the ischemic brain (19,29,30). Elsayed *et al.* (19) showed that transplants obtained from embryonic day 13 rat fetus survived in over 80% of the HIE animal models

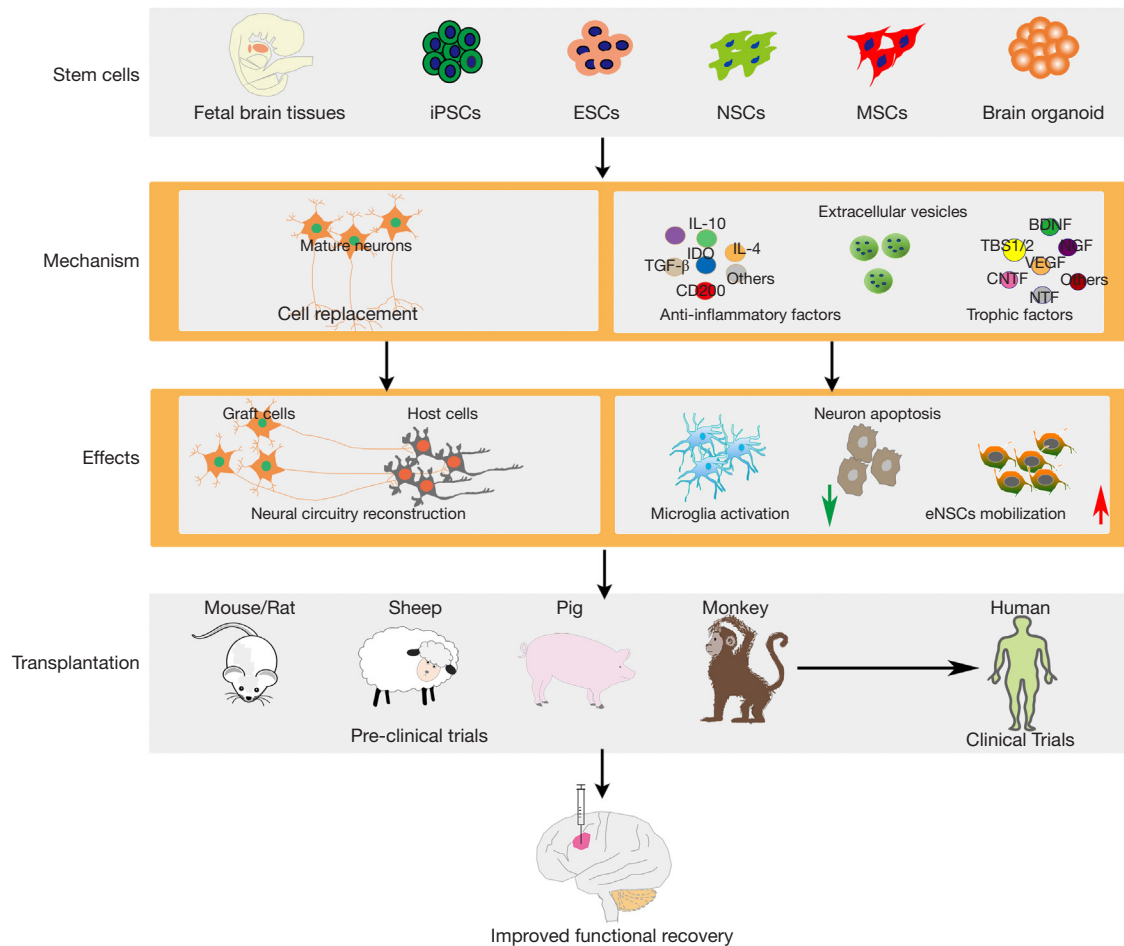


Figure 1 Stem cell types and therapeutic mechanism for treating ischemic brain injury. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; eNSCs, endogenous neural stem cells; MSCs, mesenchymal stem cells.

and acetylcholinesterase-positive fibers derived from transplants were extensively distributed at the graft-host interface after 6 weeks transplantation. Jansen *et al.* (27) transplanted cell suspension which were digested from fetal neocortical tissues into the motor cortex of HIE model. Survived cells could be detected in 72% of model animals, and the cell transplanted group performed better motor function than the control group 10–12 weeks after transplantation. Another study indicated that transplanting fetal frontal cortex into the injured brain could improve the motor defects, while surgical removal of transplants led to motor defects again (31). The fetal tissue was the first cell source tried for transplantation and demonstrated to be effective. While the usage of fetal cerebral tissues for treating ischemic brain injury in patients are limited due to the ethical issue.

ESC*s*/iPSC*s*

ESC*s* are derived from the inner cell mass of the blastocyst and have the broadest potentials to generate all cell types *in vivo* and *in vitro* (32). The totipotent ESC*s* could be differentiated into functional neurons and astrocytes with specific induction protocols *in vitro* (33,34). Lee *et al.* (20) explored the therapeutic efficacy of ESC*s* for ischemic encephalopathy. They found that there was a progressive reduction in infarction size in ESC*s* treatment group. Neurological severity score indicated an early beneficial effect and performed significantly better than control group. iPSC*s* are a population of cells which are reprogrammed from terminally differentiated somatic cells and possess the same self-renewal and differentiation capacity as ESC*s* (35,36). Reprogramming of somatic cells

to iPSCs produce patient-originated cells without concern of immunosuppression after autologous transplantation. Qin *et al.* (21) transplanted rat-derived iPSCs into stroke animal model. The transplanted iPSCs differentiated into GFAP⁺ neural cells after transplantation for 28 days. iPSCs treatment significantly reduced the number of neutrophils, microglia and the inflammatory cytokines including TNF- α , IL-1 β and IL-6. They also observed the motor function by MLPT scores, and the scores showed that iPSCs transplantation significantly improved ischemic rat's motor function from day 14 to day 42 post transplantation. Furthermore, the survival rate of rats in iPSCs group (83.33%) was higher than that of control group (66.67%) (37). Although the ESCs/iPSCs have the optimal differentiated potential toward neural cells, the side-effects including tumorigenesis and instability are severe safety concerns. These side-effects could significantly affect the functional recovery of ischemic animal models (38,39). Studies indicated that direct differentiation ESCs/iPSCs into NSCs/MSCs for transplantation could greatly reduce the risk of tumorigenesis and the therapeutic efficacy of NSCs/MSCs was obvious (40,41).

Neural stem/progenitor cells

NSCs/NPCs are a population of cells in central nervous system which have the capacity of self-renewal and differentiation into neurons, astrocytes, and oligodendrocytes (42,43). In embryonic stage of mammals, NSCs are widely distributed in various regions of the brain, such as cerebral cortex, hippocampus, subventricular layer (44). While in adult brain, the NSCs mainly exist in subventricular and subgranular zone and maintain in a resting state (45). Once the brain injury occurs, these resting NSCs begin to proliferate and participate in the repair of brain injury (46). However, it is estimated that in patients with severe cerebral ischemia, about 120 million neurons are lost per hour during the acute ischemic period (47). Massive lost neurons cannot be replaced only relying on the endogenous NSCs proliferation. Therefore, transplantation of exogenous NSCs is a potential treatment for the repair of ischemic brain injury.

Most NSCs/NPCs used for cell transplantation are usually obtained by two ways, one is differentiated from ESCs or iPSCs (48,49), and the other is isolated from fetal or adult brain tissues (50,51). Many studies have demonstrated the therapeutic efficacy of NSCs transplantation for ischemic brain injury. In ischemic brain,

the survived NSCs differentiated into neurons expressing Tuj1, DCX, and GAD, and astrocytes expressing GFAP after transplantation for 4 weeks (22). In addition to differentiation into neurons, transplanted NPCs could also recruit the endogenous oligodendrocytes to myelinate the corpus callosum and reduce the glial scar formation (52), which were beneficial to the functional recovery of ischemic animal models. One ideal goal of stem cell therapy is to generate specific neurons to reconstruct the endogenous neural circuitry. Therefore, fate-committed neuronal progenitors seem to be much suitable for cell transplantation. Tornero *et al.* (53) generated neuronal progenitors with a cortical phenotype *in vitro*, and these fated cells could differentiate into TBR1⁺ cortical neurons after being transplantation in stroke-damaged cortex. And these cortical-fated cells induced motor function improvement. Importantly, no chromosome abnormalities and tumor formation were found after NSCs/NPCs transplantation for a long time.

MSCs

MSCs are adult stem cells which can be isolated from various tissues, such as bone marrow, umbilical cord blood, placental tissue and adipose tissue (24,25,54). MSCs derived from different tissues have similar biological characteristics, including low immunogenicity, capacity of proliferation, differentiation and secretion. Therefore, MSCs were largely used to treat ischemic brain injury in pre-clinical and clinical trials. The improvement of neurobehavioral functions were observed after transplantation from a short-term (48 h) (11) to a long-term (14 months) in ischemic animal models (55,56). However, the long-term survival of MSCs and its capacity of generating new "neuronal" cells after transplantation *in vivo* were controversial. van Velthoven *et al.* (57) found that less than 1% of transplanted MSCs could be detected after transplantation in HIE model for 18 days. One study reported that although bone marrow derived MSCs expressed markers of astrocytes (GFAP⁺), oligodendroglia (GalC⁺), and neurons (NF160⁺, NF200⁺, hNSE⁺, hNF70⁺) after 6 weeks transplantation. While these new "neuronal" cells were in nature with few processes and the functional recovery of ischemic rat was unlikely due to these new "neuronal" cells (58). Researchers considered the therapeutic efficacy of MSCs relying on cytokines secretion, immunoregulation and promotion of endogenous NSCs proliferation (23,59-61). No matter which mechanisms, the therapeutic efficacy of MSCs in pre-

clinical ischemic encephalopathy models were obvious.

Brain organoid

Brain organoid is an artificial mini brain differentiated from ESCs or iPSCs *in vitro*. It contains various cell types including NSCs, neurons, astrocytes and oligodendrocytes (26,62). Cerebral organoids showed potentials of multilineage differentiation in ischemic brain injury models after transplantation. They could mimic the cortical development, promote motor cortex region-specific reconstruction and form synaptic connection with host brain (63). Thus, these three-dimensional neural tissues which contain progenitor zone and rudimentary cortical layers are a potential resource for ischemic encephalopathy in the future.

Mechanisms of stem cell therapy for ischemic brain injury

Transplanted cells have been demonstrated to improve functional recovery in animal model of ischemic brain injury through various mechanisms such as cell replacement, neurotrophs, immunomodulation and neurogenesis (Figure 1).

Cell replacement and functional integration with host neural circuitry

Massive neuronal cells loss induced by the ischemic brain injury is the main cause of the secondary cerebral palsy and mental retardation. Replacement of the lost neurons is the most potential way to rebuild the functionality of injured brain after ischemia. Many studies have demonstrated that stem cells derived from various sources could survive and mature into different neuronal cell types in ischemic brain, such as GABA neurons (64), glutamate neurons, dopamine and serotonin neurons (65). Besides, astrocytes and oligodendrocytes could also be obtained in the grafts (66). Although transplanted cells could differentiate into mature neurons *in vivo*, the long-term survival and functional reconstruction of the neural circuitry in injured brain is the key point for stem cell therapy. Tornero *et al.* (53) showed that the grafted cells could mature into cortical neurons 2 months after transplantation and the grafted cells exhibited properties of mature neurons morphologically and electrophysiologically after being transplanted for 5 months. Importantly, the grafted cells

could receive direct inputs from host cells which indicated the functional neural circuitry formation (16). Furthermore, they demonstrated that grafted cells could integrate into the neural circuitry of the host and therefore affect animals' motor behavior (15). However, not all cell types could differentiate into mature neural cells and play the role of cell replacement after transplantation *in vivo* due to its original characteristics. Many studies indicated that NSCs/NPCs had the capacity of differentiation into mature neurons and integration into host neural circuitry (15,16), while it seemed that MSCs couldn't generate new functional "neuronal" cells after being transplanted in ischemic animal models (58).

Modulation of inflammatory microenvironment

Microglia and astrocytes are activated within minutes after ischemic brain injury and some cytokines (IL-6, TNF- α , IL-1 α , etc.) and chemokines (CXCL1, CCR2, CCL2) were released, which further attracted the immune cells migrating to the ischemic sites and worsened the injured brain (67-69). Many studies explored anti-inflammatory and immunomodulatory function of stem cells *in vitro* and *in vivo*. The stem cells inhibited pro-inflammatory cytokines (IFN- γ , TNF- α , IL-17, etc.) production in ischemic injured brain (12) by up-regulating the anti-inflammatory factors (TGF- β , IL-10, IL-4, CD200, etc.) (70,71). Chemokines MCP-1 and MIP-1 α are indicators of microglial/ macrophage activation. Huang *et al.* (18) demonstrated in their study that NSCs transplantation could significantly downregulate the expression of both chemokines, and therefore reduce transit of neutrophils and monocytes into the brain. Extracellular vesicles derived from MSCs are nanoscale vesicles which could modulate central and peripheral inflammation (72). Many studies showed that grafted MSCs could improve functional recovery by secreting extracellular vesicles to alleviate the neuroinflammation in ischemic brain injury models of monkeys (73), ovine (72), and rats (74). These results demonstrate the anti-inflammatory and immunomodulation effect of stem cells after transplantation *in vivo*. In addition, through modulating the inflammatory microenvironment, stem cells could inhibit apoptosis and therefore more residual neurons in ischemic area survived and benefited to the functional recovery (17). It is demonstrated that almost all stem cell types used for transplantation have the capacity of anti-inflammation and immunomodulation *in vivo* (18,37,75).

Neurotrophs and neurogenesis

Except the exogenous cell replacement, the survival and neurogenesis of endogenous cells is also very important for the functional recovery of ischemic brain injury (76). Stroke alone could stimulate neurogenesis at the subventricular zone. While stem cells treatment could significantly augment this neurogenic activity by secreting the neurotrophic factors (77). Neurotrophic factors are a group of short-lived proteins, showing efficacy of improving endogenous and exogenous stem cells survival and maturation previously (78,79). Many studies detected the secretion of neurotrophic factors by stem cells *in vitro* and *in vivo* (22,80). Oshita *et al.* (75) indicated in their research that bone marrow-derived MSCs promoted functional recovery through strong expression of BDNF and VEGF. Another study detected the increased secretion of NGF, BDNF, NTF3, FGF9, CNTF and TBS1/2 in NPCs culture medium under oxygen and glucose deprivation model. They postulated that the transplanted NPCs promoted functional recovery via secreting multiple factors which enhanced the neuronal survival and neuroplasticity (81). In addition, the neurotrophic factors secreted by grafted cells could enhance endogenous neurogenesis, more new cells (BrdU⁺/NeuN⁺) were observed 4 weeks after ischemic brain injury (55).

Clinical trials about stem cells therapy for ischemic brain injury

The therapeutic efficacy of stem cells has been demonstrated in lots of pre-clinical experiments. In recent years, various small or large-scale clinical trials have been underway, and many of them already showed preliminary results (see *Table 2*). Most stem cell types used for clinical trials were MSCs and NSCs. A clinical trial recruited 30 patients with severe stroke, and 5 patients received intravenous infusion of 1×10^8 autologous MSCs transplantation while another 25 patients were as the control group. After one-year follow-up, they found no adverse cell-related, serological, or imaging-defined effects in stroke patient. The Barthel index which indicates the functional recovery showed greater improvement than that of control patients from beginning to the end of the follow-up (82). A two-year open-label, single-site, and dose-escalation clinical trial (NCT01151124) about NSCs treating for stroke patients was completed. Analysis of clinical data showed that single intracerebral doses of NSCs up to 20 million cells induced no adverse events and were associated with improved neurological function (83).

Concerns about the zoonoses caused by the usage of xenogeneic culture medium and diminishment of functional recovery with time need to be verified in long-term follow-up. Lee *et al.* (84) conducted a five-year follow-up of autologous MSCs transplantation in patient with ischemic stroke. They demonstrated the beneficial effect of MSCs transplantation in terms of functional outcome and survival in stroke patients during long-term follow-up. Besides, many other clinical trials about stem cell therapy for ischemic patients are ongoing (<https://clinicaltrials>). From the current published data, cell transplantation for clinical application is safe and feasible. No severe adverse events and tumorigenesis were reported. While the functional outcomes still need to be assessed in more and larger cohort studies.

Perspectives

Currently, stem cell therapy is the most promising treatment for ischemic brain injury. And cell transplantation from bench to bed provides great hope for patients with ischemic brain injury. While many issues still need to be clarified before clinical application. For stem cell therapy to succeed in neurological deficits, the grafted cells should replace the lost neuronal type and reconstruct the corresponding neural circuitry with host cells (85,86). In ischemic brain injury, more evidences are needed to further support the conclusion that the long-term functional recovery is related to the neural circuitry reconstruction. In addition, which cell types should be chosen for treating ischemic injured patients at different stages? Is there any difference for surviving, proliferation, differentiation and functional integration of stem cells in mature and immature brain? Besides, the way and cell dosage for stem cell therapy also need to be clarified. Many studies tried the route including intracranial (15), intravenous (59), intranasal (76) or intraperitoneal injection (87). Each route has pros and cons. In clinical application, patient's physical status should be taken into consideration before choosing the injection route. In addition, ischemic encephalopathy always has many comorbidities such as hypertension, seizure and headache (88). Therefore, the stem cell therapy for ischemic brain injury with severe complications should be studied thoroughly before clinical application.

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Footnote

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