Neural stem cell therapy for brain disease

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Abstract

Brain diseases, including brain tumors, neurodegenerative disorders, cerebro-vascular diseases, and traumatic brain injuries, are among the major disorders influencing human health, currently with no effective therapy. Due to the low regeneration capacity of neurons, insufficient secretion of neurotrophic factors, and the aggravation of ischemia and hypoxia after nerve injury, irreversible loss of functional neurons and nerve tissue damage occurs. This damage is difficult to repair and regenerate the central nervous system after injury. Neural stem cells (NSCs) are pluripotent stem cells that only exist in the central nervous system. They have good self-renewal potential and ability to differentiate into neurons, astrocytes, and oligodendrocytes and improve the cellular microenvironment. NSC transplantation approaches have been made for various neurodegenerative disorders based on their regenerative potential. This review summarizes and discusses the characteristics of NSCs, and the advantages and effects of NSCs in the treatment of brain diseases and limitations of NSC transplantation that need to be addressed for the treatment of brain diseases in the future.

Key Words: Neural stem cell; Brain disease; Therapy; Animal experiment; Clinical trial; Cellular therapy

Core Tip: In this review, we elaborate on the characteristics of neural stem cells (NSCs) and their effects on the treatment of traumatic brain injury, hypoxic-ischemic brain injury, Alzheimer's disease and Parkinson's disease. At the same time, we discuss the

applications and limitations of NSCs to treat brain diseases.

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INTRODUCTION

Brain diseases are among the major disorders influencing human health. The main types of brain diseases include brain tumors, neurodegenerative disorders, cerebrovascular diseases, and traumatic brain injury (TBI). Previous studies have suggested that repair and regeneration is a complex process and is challenging due to the following reasons: (1) Nerve cells, including neurons, are highly differentiated terminal cells, with very low regenerative capability; (2) Insufficient secretion of neurotrophic factors is unable to sustain the homeostasis of local environment results in the failure to repair damaged nerve system; and (3) Following injury, the secretion of inflammatory factors and various cytokines is upregulated, which inhibits synaptic regeneration and aggravates hypoxia and ischemia. The major cause of nerve regeneration disorders is the scar formation at the injuries, which may act as a physical and chemical barrier, suppress nerve regeneration, and dysregulate the extension and growth of synapses. Therefore, various physiological processes, including the supply of neurotrophic factors, regeneration of axons, plasticity of synapses, and the microenvironment, are involved in the repair and regeneration of the central nervous system (CNS) after injuries, and the underlying mechanisms are very complex.

Cellular therapy uses neurogenic or non-neurogenic cells to replace, repair, or improve the functions of the injured nerve system, which are implemented mainly through transplantation of cells into the system. Stem cell transplantation therapy has been widely applied in treating CNS diseases because of its ability of regeneration in nerve repair and tissue damage. The mechanisms underlying the treatment of brain diseases with stem cell transplantation are similar: facilitating the local microenvironment, promoting blood vessel development, supporting neuron regeneration, and reducing inflammatory responses. The commonly used stem cells include neural stem cells (NSCs), mesenchymal stem cells (MSCs), adipose mesenchymal stem cells, and human-derived umbilical cord blood stem cells, among which NSCs have been widely used and has unique advantages in the treatment of brain disease.

In this review, we discuss the role and generation of NSCs for various neurodegenerative disorders. Recent studies using different types of NSCs and transplantation approaches have been discussed in detail, and the limitations of NSCs for neurodegenerative disorders are also discussed.

BASIC CHARACTERISTICS OF NSCS

During development, the brain and spinal cord are generated from a small number of NSCs lining the neural tube. These cells are undifferentiated cells and can differentiate into different cells[1]. The subgranular zone (SGZ) of the dentate gyrus (DG) and subventricular zone (SVZ) in adult brains are two neurogenic regions for neurogenesis [2]. The neurogenic regions, especially the hippocampus, participate in cell renewal by developing new neurons from the neural progenitor cells[3]. Several sources can be used for NSCs. They can be collected from brain tissue, reprogrammed from somatic cells[4,5], or differentiated from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)[6,7]. In addition, NSCs can differentiate into lineage-specific cells, such as neurons, oligodendrocytes and astrocytes[8]. They exist in highly-specific microenvironments, consisting of cell and extracellular components, such as ependymal cells, vasculature, extracellular matrix proteins, soluble factors, astrocytes, microglia, and pericytes[9,10]. Interaction of cells, transcription factors, neurotrophins, cytokines (such as growth factors, neurotransmitters, hormones and signaling molecules) have a crucial role in the proliferation and differentiation of NSCs. Cytokines (TNF-α) has been shown to induce proliferation of neural stem cells *via*

IKK/NF-κB signaling. While BMP4/LIF has been shown to induce neuronal stem cells in monkeys, it was shown to induce astrocyte-like differentiation of monkey NSCs[11-14]. Neural stem cells are involved in various biological functions and continue to play their role throughout the lifespan of an organism. Both intra and extracellular signals regulate the functional properties of NSCs. Sox2 is one of the major regulators among transcription factors that serve as molecular switches[15]. The association of NSCs and migration in blood vessels were recently studied and shown that blood vessels play a significant role in neuronal migration during brain development. Moreover, NSCs can migrate to designated regions, such as injured regions, following injury[16].

Preclinical studies on treating brain diseases with NSCs have reported promising results, while clinical trials in patients are still ongoing. Nevertheless, experiments on animal models or *in vitro* studies have shown that NSCs may be induced and activated to differentiate into neurons, consequently replacing the lost neurons, improving the local microenvironment, promoting blood vessel development, regulating inflammatory responses, and restored homeostasis of the brain.

NSCS AND ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive multifactorial brain disorder characterized by the amyloid-β (Aβ) deposition, as insoluble deposits or inclusions of proteins, accumulations of neurofibrillary tangles, and intracellular tau aggregation. It is the most common cause of dementia that slowly destroys memory and thinking skills. More than 26 million people are living with AD worldwide, and this number is expected to increase to 100 million over the next 35 years[17,18].

Targeting Aβ levels has been the central strategy to halt, retard, and reverse or cure AD pathology progression. Though great efforts have been made to cure AD symptoms and delay its progression, limited treatment options are available. Only four cholinesterase inhibitors (tacrine, donepezil, galantamine and rivastigmine are rarely prescribed due to its possible side effects) and NMDAR antagonists (memantine) have only been approved by United States Food and Drug Administration for AD. There is not a single drug approved in the last two decades. The available drugs (cholinesterase inhibitors), can only reduce the acetylcholinesterase activity to prevent the buildup of acetylcholine levels synaptic region. However, neither drug design to reverse the AD pathology nor immunotherapy that targets amyloid or Tau is the ultimate solution for Alzheimer's. Several lines of evidence have shown the successful approach of neural stem cells for the treatment of neurodegenerative disorders, including AD, amyotrophic lateral sclerosis and PD[19].

This approach of NSCs transplantation offers a tremendous therapeutic potential to cure neurodegenerative disorders based on its self-renewal ability and differentiate into neuronal, oligodendrocytes and astrocytes cells[20]. Tg2576 neural stem cells isolated from mice represent an Alzheimer disease model related to Aβ plaque. Tg2576 derived cells showed a disease model with reduced neuronal growth and MAP-2 expression. This model has been studied in various studies and offers to screen new molecules for the treatment of AD[21].

Ager *et al*[22] used NSCs derived from the fetal brain tissue and transplanted to the hippocampus of 3xTg-AD murine models and found that the transplanted NSCs improve the cognitive functions and enhanced synaptogenesis. The human neural stem cell population, HuCNS-SC, has been clinically tested before for different neurodegenerative disorders. Transplantation of HuCNS-SCs has been shown to improve cognition in two different models of neurodegeneration. Migration and differentiation of HuCNS-SC into immature neurons and glial cells were observed. Researchers have found the association of significant synaptic increase and other growth-associated markers were found in both 3xTg-AD and CaM/Tet-DTA mice models.

The hippocampus, which is critical for learning new memories, is normally affected at earlier stages of AD. Disruption of metabolic activities in hippocampal neurons has been demonstrated in earlier studies in AD[23]. The following diagram shows the different mechanisms of stem cells associated with AD (Figure 1).

A study conducted by Li *et al*[24], 2016 showed that metabolic activity was increased in the frontal cortex and hippocampal neurons. The human brain-derived NSCs (hNSCs) were transplanted into the hippocampus transgenic mouse model of AD to assess the role of hNSC on behavior and Alzheimer's pathology. Six weeks later, transplanted hNSCs migrated in different brain regions and slowly differentiated into neuronal cell types of CNS. These transplanted cells rescue AD symptoms, including
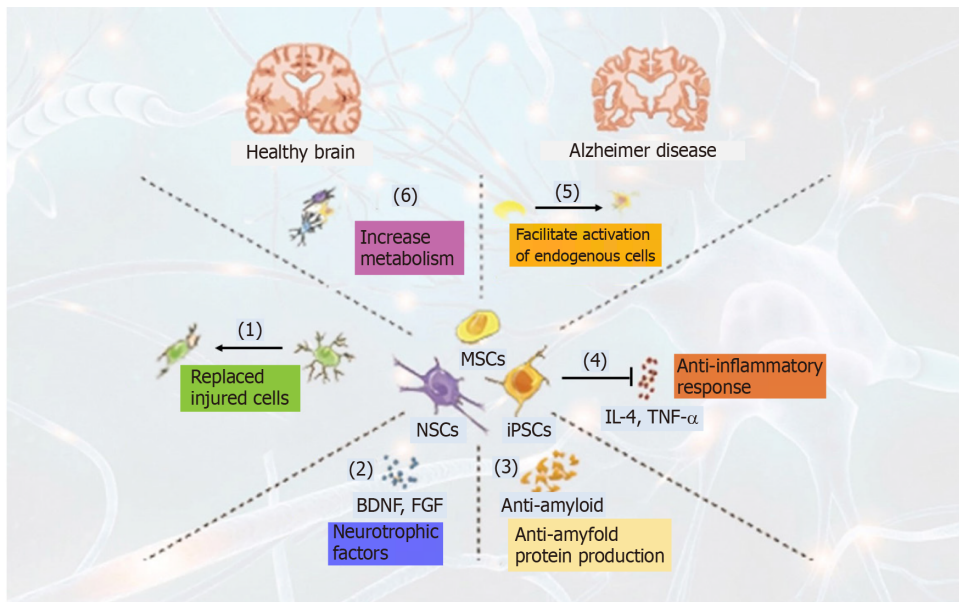
Figure 1 Mechanism of action of stem cells in Alzheimer disease. (1) Replaced injured or lost cells; (2) Enhanced secretion of neurotrophic factors (BDNF, GDNF, FGF, *etc.*); (3) Anti-amyloid protein production; (4) Inhibit inflammatory response; (5) Facilitate activation of endogenous cells; and (6) Enhanced metabolic activity of neuronal cells in the brain.

cognitive defects, learning and memory impairment, by increasing neuronal connectivity and metabolic activity. This study suggests the role of hNSCs in modulating the metabolism of neuronal cells and validates the association between hippocampal neuronal metabolism and AD symptoms[24].

Chronic inflammation has a significant role and contributes to AD pathology in the brain. Transplantation of NSCs has been assessed to inhibit inflammatory processes. Researchers have shown that NSCs transplantation into the hippocampus attenuates inflammatory reactions and supports a neuroprotective role in beta-amyloid 42 (Aβ -42) peptide injected rat hippocampus, suggests an important role of NSCs in the inhibition of inflammatory reactions[25]. Neural stem cells are making a dominant appearance because of it neurogenic abilities, based on the recent findings that neurogenesis reduces significantly in AD patients compared to healthy subjects[26]. Progress is currently being made to differentiate the transplanted NSCs into cholinergic neurons, to compensate for the loss of injured neurons, the main research focuses on the treatment of AD.

A summary of preclinical studies of stem cells of different sources in rat and mice models of AD was showed in Table 1[27-37]. Ibotenic acid lesion or APP/PS1 transgenic mice were used in most of the AD model. Stem cells of different origin were used, which include rat, mouse and also from human. Genetically modified stem cells are also used in some studies, which have increased capacity to migrate from transplantation sites. Damage neuron replaced by transplanted stem cells. Stem cells migrate to the lesion site and differentiate to specific neurons *e.g.*, cholinergic neuron, clear beta-amyloid, and produced anti-inflammatory effects. These studies showed that transplantation of stem cells (ECS-derived, NSCs, and MSCs) improved or restored learning and memory in AD-model rats.

NSCS AND PARKINSON'S DISEASE

Parkinson's disease (PD) is a complex neurodegenerative disease that result from the loss of dopaminergic neurons in the substantia nigra, pars compacta (SNc) and mesencephalon, and the formation of α-synuclein-containing Lewy bodies, which consequently induce motor disorders[38]. The stem cell approach offers a significant therapeutic output to a wide range of neurodegenerative disorders including PD, because of the regenerative potential to renew the cells and replace the affected cells. Several studies have reported using neural stem cell approach to find a cure and explore the disease mechanism.

Table 1 Therapeutic potential of stem cell transplantation in Alzheimer's disease models

	Animal model	Transplanted cells	Density of transplanted cells	Transplantation site	Therapeutic effects	Unique features	Results	Ref.
1	Mice (Transgenic 3 x Tg- AD and Thy1-APP)	NSCs	100000 cells in 2 μL	Hippocampus	Aβ-clearance, increased synaptic density	Neprilysin gene transfer	Not assessed	Blurton-Jones *et al* [37]
2	Mouse (NBM lesion)	ESC-derived neurosphere	400 μL/injection, 1-5 × 10^4 cells/μL	Prefrontal and parietal cortices	ChAT and serotonin-positive neurons	ChAT + cells↑	Working memory ↑	Wang *et al* [27]
3	Rat (Forebrain), Okadaic acid	NSC (rat)	5 μL /injection site (2 injections) 2 × 10^4 cells/mL	Hippocampus and cerebral cortex	replace damaged or lost neuron	NGF(human), gene transfer	Memory ↑	Wu *et al*[28]
4	Mice (Transgenic Tg2576)	MSCs from human UCB	100000 cells/ Mouse (i.v.)	Systematic	Anti-inflammatory, anti-amyloidogenic	None	Not assessed	Nikolic *et al* [29]
5	Rat (NBM lesion) Ibotenic acid	ESC-derived NPC (mouse)	2 × 10^5 cells in 2 μL	Forebrain specially NBM	Forming cholinergic cell phenotype	Shh-primed	Water maze ↑; Spatial probe↑	Moghadam *et al*[30]
6	Mouse (3X TG-AD)	NSC (mouse)	100000 murine NSCs	Hippocampus	Neurotropic effects	BDNF-mediated effect	Working memory↑	Blurton-Jones *et al* [31]
7	Rat (Hippocampus) Kainic acid	Immortalized NSC (human, HB1.F3)	1 × 10^6 cells/rat	Hippocampal CA3 region	Migrate to injured site differentiate into neurons overexpressing ChAT	ChAT (human), gene transfer	Water maze ↑; Spatial probe↑	Park *et al*., 2012a[33]
8	Rat (NBM lesion) AF64A toxin	Immortalized NSC (human, HB1.F3)	1 × 10^6 cells/rat	ICV	migrate to various brain regions including cerebral cortex and hippocampus	ChAT (human) gene transfer	Water maze ↑; Spatial probe↑	Park *et al*[32]
9	Mice (Transgenic APP/PS1)	MSCs from human UCB	1 × 10^5 cells in 3 μL(3 injection once after 2 wk)	Hippocampus	Anti-inflammatory, anti-amyloidogenic, anti-phosphorylation of tau	None	Improved learning and memory	Lee *et al*[34]
10	Mouse (Hippocampus) Ibotenic acid	Immortalized NSC (human, HB1.F3)	2 × 10^5 cell suspension 2 μL	Hippocampus	migrated to lesion sites and differentiated into neurons and astrocytes	NGF (human); Gene transfer	Water maze ↑; Spatial probe↑	Lee *et al*[35]
11	Mice (Transgenic APP/PS1)	MSCs from human UCB	2 × 10^4 cells per head	Hippocampus, cortical region	Anti-inflammatory, Aβ-clearance	-	Not assessed	Kim *et al*[36]

NBM: Nucleus basalis of Meynert; ESC: Embryonic stem cell; NGF: Nerve growth factor; 3XTG: Triple transgenic/APP-presenilin-tau; BDNF: Brain-derived growth factor; ChAT: Choline acetyltransferase; NPC: Neural precursor cell; NSC: Neural stem cell; SHH: Sonic hedgehog protein; UCB: Umbilical cord blood; Aβ: Beta-amyloid; MSCs: Mesenchymal stem cells; APP: Amyloid-β precursor protein; ICV: Intra-cerebro ventricular.

Induced neural stem cells (iNSCs) exhibited different stem cell biomarkers with self-renewal properties and has shown the potential to differentiate into dopaminergic (DA) neurons. Researchers have shown the role of grafted cells for the neuronal network by assessing synaptic markers. Analysis of 4 wk of post-transplantation showed an extensive network of presynaptic neurons. hESC-derived neural cells has been reported to reduce the tumorigenicity and function of DA neurons in a prolonged mature culture. The transfer of such grafts in monkeys improved behavior for 12 mo period, reflecting the significance of matured hESCs that can act as a source for DA neurons[39].

Studies have shown that transplantation of iNSCs transformed from somatic cells into PD mice brains improves motor manifestation behavior. Wernig *et al*[40] shown that iPS cells efficiently differentiate into neural precursor cells, further giving rise to neuronal and glial cells. Transplantation of iNSCs into the brain of fetal mice shows the potential of stem cell migration into different brain regions and its differentiation into glia and neurons, including glutamatergic, catecholamines and GABAergic subtypes. Moreover, induced iPS cells were differentiated into DA neurons after transplantation into the adult brain.

Researchers have shown that steroli cells can be directly converted into iPS cells, which exhibit different stem cell biomarkers with self-renewal properties and can differentiate into DA neurons. These grafted cells were validated for a matured neuronal network by assessing synaptic markers. Analysis of 4 wk of post-transplantation showed an extensive network of presynaptic neurons, suggest a crucial role of steroli based iNSCs may provide a source of replacement of affected cells with new fresh cells[41]. iNSCs derived from fibroblasts have been shown to improve PD symptoms. Transplantation of iNSC into the 6-hydroxydopamine (6-OHDA)-injected mice striatum shows substantial reduction in apomorphine mediated rotational symmetry. The engrafted iNSCs show the differentiation pattern to all neuronal lineages and differentiate to DA neurons[42].

Yang *et al*[43] shows that neural stem cells transplantation into a 6-hydroxydopamine-lesioned rat, migrate to the striatum and express dopaminergic traits. Studies demonstrated the role of single factors, (Platelet-derived growth factor (DGF-AA), -AB, and –BB) which plays a role in the differentiation of primary stem cells derived from fetal and adult CNS, differentiate C17.2 cells *in vitro*, suggesting its significance that C17.2 NSCs lead to the development of dopaminergic neurons and a source for transplantation[44].

Nurr1 is a transcription factor and is specifically required to induce DA neurons in the midbrain region[45,46]. However, later in another separate study, Wagner *et al*[47] used the same stem cell line C17.2 and demonstrated that Nurr1 alone was unable to induce the differentiation of C17.2 cells into dopaminergic neurons. While, in a combination of other factors derived from local type 1 astrocytes, overexpression of Nurr1 in NSCs (C17.2) generates dopaminergic neurons (Figure 2).

A summary of preclinical studies of stem cells of different sources in rat, mice and monkey models of PD was showed in Table 2[42,48-54]. OHDA (rats and mice) and MPTP (monkey) drugs were used to create Parkinson's model in these studies. Transplantation cells of different origin were used, which include rat, monkeys and from human. Genetically modified stem cells are also used in some studies, which had unique features. Results of these studies showed that transplantation of stem cells in different cell densities (ECS-derived, NSCs and MSCs) in striatum decreased rotation and improved motor function in PD model.

NSCS AND TBI

Traumatic brain injury (TBI) refers to a disruption of normal function of the brain and/or pathological injuries of brain tissues caused by external forces instead of disorders of brain tissues. TBI has a complex pathological condition, which includes breakage of the blood-brain barrier, massive neuroinflammation, axonal injury and lesions[55]. It has been estimated that about 50-60 million patients globally are newly diagnosed with TBI every year. In developing countries, TBI is mainly caused by traffic accidents, while in developed countries, by the falling of the elderly[56]. Based on the population census in 2013, TBI mortality rates in China were 13/100000, while in the 27 United States, TBI accounted for 30% of all trauma-induced deaths. In the United States, about 5.3 million individuals are living with TBI-related disabilities[57, 58].

Despite having the higher frequency of TBI, a large proportion of molecular mechanisms and the basis of cognitive deficits and brain insults remain unknown.

Over the recent years, studies have demonstrated that neurogenesis in SVZ and SGZ was enhanced after TBI[59]. Endogenous NSCs get activated and migrate to regions of nerve injuries, which differentiate into neuroglial cells or oligodendrocytes and integrate into the injured local neurovascular network, promote the secretion of neurotrophic factors, and participate in nerve repair. Therefore, activating endogenous neurogenesis following TBI to contribute to post-injury functions may be a potential therapeutic approach[60,61]. On one hand, neurogenesis and nerve migration in human beings mainly exist in neonates younger than 18 mo but drastically decrease in adults, suggesting that neurogenesis following TBI in middle-aged and elderly people is substantially lower than in adolescents. While, glial scars have been reported to prevent the regeneration of axons and directly limit the repair of injuries in the late stage of TBI[62,63]. In addition, massive cell death and inflammatory responses in the late stage of TBI may disturb the local microenvironment, reduce the survival rate of new endogenous NSCs, and limit injury repair.

Transplantation of pre-differentiated human endogenous neural stem cells (ENSCs) has been reported to increase angiogenesis and neuronal survival in the lesion area

Table 2 Therapeutic potentials of stem cell transplantation in Parkinson's disease models

|	Disease model	Source of transplanted cells	Transplantation location	Density of transplanted cells	Unique feature or treatment	Results	Ref.
1	Rat, 6-OHDA	Immortalized NSC (mouse, C17-2)	Striatum	10^6 cells	TH/GTPCH1; Gene transfer	Rotation↓	Ryu *et al*[48]
2	Monkey, MPTP	ESC (monkey)	Bilateral putamen	3×10^5-6×10^5 cells per side	Stromal cell (mouse) feeder	PFS-parkinsonian factor score↓	Takagi *et al* [49]
3	Rat, 6-OHDA	Immortalized NSC (human, HB1.F3)	Striatum	3×10^5 cells/3 μL	TH/GTPCH1 gene transfer	Rotation↓	Kim *et al*[50]
4	Rat, 6-OHDA	Immortalized NSC (human, HB1.F3)	Striatum	2×10^5/3μL	NSC migration	Rotation↓	Yasuhara *et al* [51]
5	Rat, 6-OHDA	MSCs from human UCB	Striatum	1×10^5 cells/10 μL	FGF8/SHH	Rotation↓	Fu *et al*[52]
6	Rat, 6-OHDA	DA neurons from ESC (human)	Striatum	5×10^5 cells	None	Rotation↓, beam walking↓	Cho *et al*[53]
7	Mice, 6-OHDA	DA neurons from ESC (human)	Striatum	1.5×10^5 cells /1.5 μL	Wnt signal; SHH	Rotation↓	Kriks *et al*[54]
8	Mice, 6-OHDA	iNSCs (rat)	Striatum	1×10^5 cells	Tripotential differentiation capacity	Rotation↓	Choi *et al*[42]

6-OHDA: 6-hydroxydopamine; MSC: Mesenchymal stem cell; ESC: Embryonic stem cell; FGF8: Fibroblast growth factor 8; GTPCH-1: GTP cyclohydrolyrase-1; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; iNSC: Induced neural stem cell; TH: Tyrosine hydrpxylase; NTN: Neurturin; UC: Umbilical cord blood; SHH: Sonic hedgehog protein; CN: Caudate nucleus; SN: Substantia nigra.
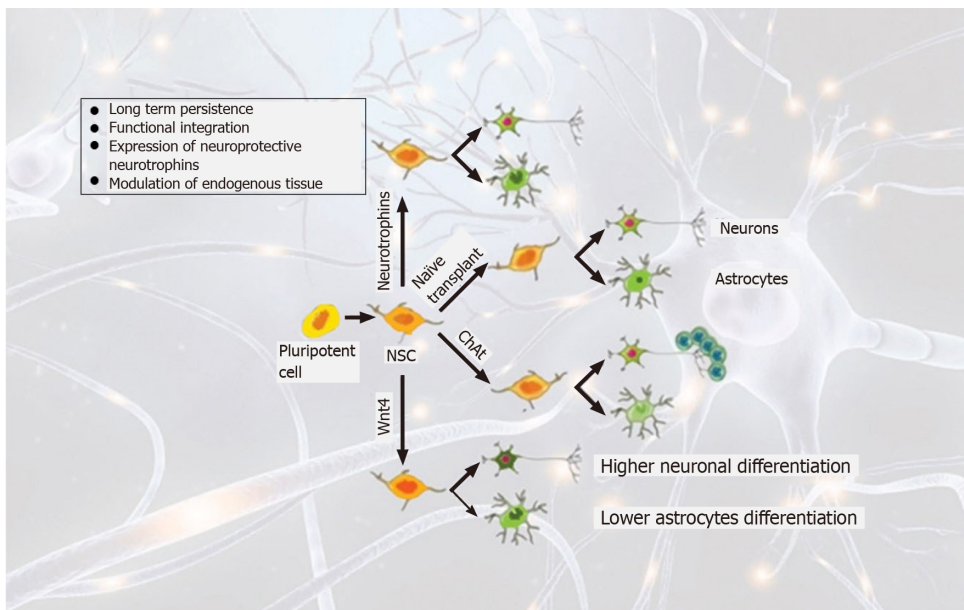
Figure 2 Overview of lineages of stem cells and transplantation strategies in Parkinson diseases. Pluripotent stem cells are directly converted to stem cells that can be further differentiated to long-term survival neurons by overexpressing neurotrophins. Wnt4 overexpression drives differentiation into neuronal cells while reducing glial scar formation.

and decrease astrogliosis, resulting in improved motor functions[64,65]. Moreover, researchers have shown that immediate transplantation of embryonic cortical neurons in the adult cortex after injury facilitates the restoration of injured motor pathways and supports the development of neuronal projections[66,67] (Figure 3).

Exogenous NSC transplantation can compensate for the disadvantage of insufficient endogenous NSCs to a certain degree and has a significant impact on the treatment of TBI[68,69]. Experiments in mice and rats have been demonstrated that, upon NSCs transplantation, the transplanted stem cells survive in affected regions and differentiate into mature astrocytes, oligodendrocytes, and neurons, which can then be
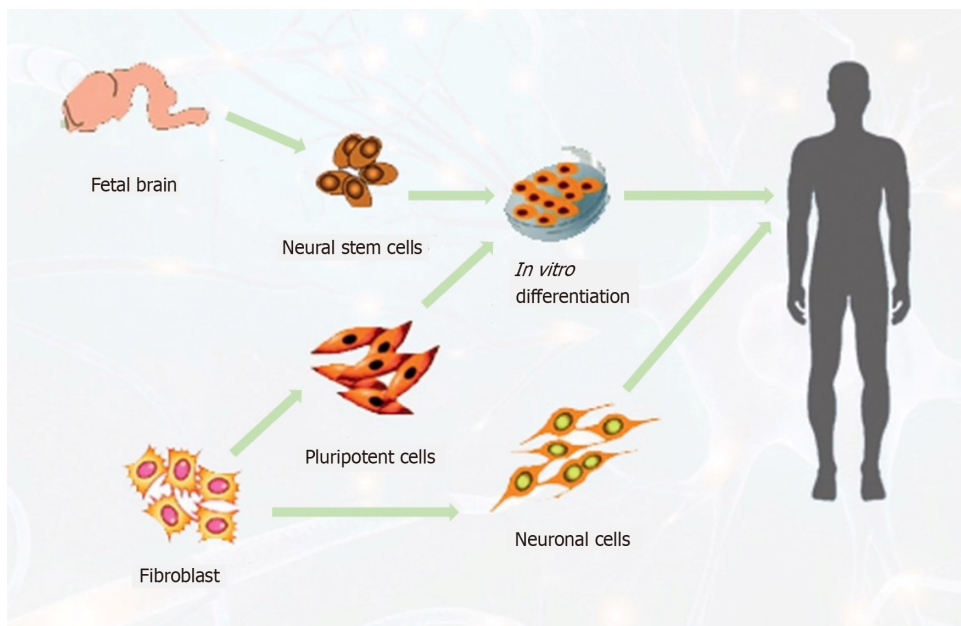
Figure 3 Schematic diagram of possible sources of neural stem cells to target stroke patients. (1) Neural stem cells from the fetal brain, differentiated to neuronal cells; (2) Neuronal cells directly generated from fibroblast cells, expanded to neuronal cells to replace the lost cells.

integrated into the neural circuit of the host to improve the injury-related cognitive and motor disorders[70,71]. When transferring human fetal NSCs to the hippocampus of TBI rats at 24 h post-injury, the transplanted cells survived. In addition, treating *in vitro* cultured NSCs with basic fibroblast growth factor, heparin, and laminin promote its differentiation into neurons at the injured area and the expression and secretion of glial-cell-line-derived neurotrophic factor *in vivo* from the transplanted cells, thus improving the internal environment of the brain, promoting the endogenous repair, and finally improving the cognitive functions of TBI rats[72]. The approach of cell therapy by transplanting ENSCs reduces neuroinflammation and supports neurogenesis in the adult injured cortex of the controlled cortical impact mouse model [69].

NSCS AND HYPOXIC ISHEMIC BRAIN INJURY

Cerebrovascular disease is a global health issue, where the incidence and mortality rate of ischemic stroke are high levels. Thrombolytic therapy is considered the best treatment procedure for ischemic stroke[73,74]. Though it is not safe and tissue damage is usually inevitable. It is a complex process, which involves oxidative damage and apoptosis of neurons[73,75].

The sub-ventricular zone and dentate gyrus are the primary sites of endogenous NSCs. Exogenous NSCs are mainly extracted from three main sources for therapeutic purposes: extraction from brain tissue, differentiation from IPSc, and trans-differentiation from somatic cells[76]. Studies have been reported the methods of generating different types of NSCs and its applications in neurodegenerative diseases[76,77]. The SVZ NSCs have been shown its association with glioma progression and its occurrence. Effect of conditioned medium derived from NSCs has confirmed its association with SVZ NSCs, and found that conditioned medium from NSCs promote the glioma proliferation and invasion[78]. Earlier studies reported the characteristics of exogenous NSCs that it can migrate into ischemic brain regions, and differentiate into neurons and glial cells and facilitate endogenous NSCs differentiation and proliferation[79-81]. Transplantation of human NSCs in a stroke model of rats showed neuroprotective effects by enhancing dendrites branching, increasing corticospinal tract projections and inhibited inflammation[82,83]. It has been demonstrated that NSCs improved the condition of stroke rats when transplanted, suggesting a role of NSCs mediated regulation of angiogenesis and formation of brain microvasculature because of increased activity of proangiogenic factors[84].

Researchers conducted a small Phase 1 translational study and demonstrate the role of CTCoE3 human NSCs in stroke patients. Upon implantation of human NSCs into

the putamen, they found patients safe even for 2 years after transplantation and no side effects showed. However, a slight improvement showed in the NIH stroke scale [85]. The use of primary human tissue is limited because of the ethical and logistic complications to obtain large quantities of fetal neurons. Therefore, much effort is required to develop alternate sources of human cells for use in transplantation. One source is the NT2/D1 human embryonic carcinoma-derived cell line. These cells can proliferate and differentiate into human neuronal cells (LBS-Neurons) upon treatment with retinoic acid. These neuronal cells have been reported to survive, express neurotransmitters and regulate functional synapses.

Despite its significant role of NSCs in treating most neurodegenerative diseases, there are still some limitations. Modulation of cell dose is a critical factor, as low dose cannot provide therapeutic outcomes. While transplantation of high cell dose of tissue-derived NSCs can clot *in vivo* and may have a poor survival rate[2]. Furthermore, understanding molecular mechanisms of endogenous NSCs regulation largely remain unknown in patients with ischemic brain injury[86].

Due to the effectiveness of NSCs in animal models of cerebral stroke, clinical trials using NSCs have been conducted for the treatment of chronic ischemic cerebral stroke [87]. Although over 50 clinical trials have been registered for the treatment of cerebral stroke by stem cells, only human neural precursor cell line NT2/D1 and immortal human NSC line CTX have progressed to stage 1 and stage 2 phases. NT2/D1 cell, also known as NT2 cell, is a human teratoma-derived pluripotent embryonic carcinoma stem cell line, considered a neural precursor cell line. Treating NT2/D1 cells with tretinoin induces mitosis of anaphase neuron-like cell NT2N neuron (trade name: LBS-Neurons). A phase 1 clinical trial investigated the effects of NT2N neurons in basal ganglia stroke patients with severe motor disturbance. The 18-mo serum or imaging evaluations confirmed the safety and applicability of brain neuron transplantation in cerebral infarction patients with motor disturbance[88-90].

CTX0E03 is an immortalized human NSC line derived from human embryo brain tissues. CTX0E03 has been used as a clinical-grade NSC, based on which the commercial product CTX-DP was developed to treat chronic cerebral stroke (the ReNeuron PISCES trial)[90]. The 5-year follow up findings of phase 1 clinical trial of CTX0E03 in chronic cerebral stroke patients (PISCES I, NCT01151124) showed the following results: no immune or cell-related adverse events occurred, and only adverse influences from surgical procedures or complications were found; the overall NIHSS score improved by 2 points at 2 years after transplantation, which was associated with the improvement of neurological functions[85]. In another phase 2 clinical trial of CTX0E03 (PISCES II, NCT02117635), the 12-mo follow-up showed no cell-related safety events, while clinical related function improvement was found in 15 patients. CTX0E03 PISCESIII (NCT03629275), has already been approved, is a randomized, controlled, phase 2b clinical trial that aims to evaluate the safety and effectiveness of CTX cells in patients with chronic cerebral stroke (Figure 4).

CONCLUSION

The stem cells approach offers a significant output to a wide range of disorders, including neurodegenerative disorders, because of the regenerative potential to renew the cells and replace the affected cells. Neural stem cells are making a dominant appearance because of it neurogenic abilities, that neurogenesis reduces significantly in neurodegenerative patients compared to healthy subjects. Although studies on brain diseases with NSCs-based therapy are continuously increasing, and the NSC treatment strategy has provided an exciting and promising treatment method for brain diseases, there are still various uncertainties and potential risks involved in NSC transplantation, similar to the treatments with other stem cells: (1) Modulation of cell dose is a critical factor, as low dose is unable to provide the therapeutic outcomes. While transplantation of high cell dose of tissue derived NSCs can clot *in vivo*, and may have a poor survival rate; (2) Furthermore, understanding molecular mechanisms of endogenous NSCs regulation largely remain unknown in patients with neurodegenerative disorders; (3) Transplantation approaches can be improved by region specific regulation of local microenvironment in the brain: precise regulation of the microenvironment through genetic engineering techniques and combination transplantation may promote the proliferation and differentiation of transplanted NSCs, and greatly increase the treatment efficacy; and (4) Methods, timing, and doses of transplantation: strategies should be made to improve the transplantation methods to favor the aggregation of NSCs to the injured regions.
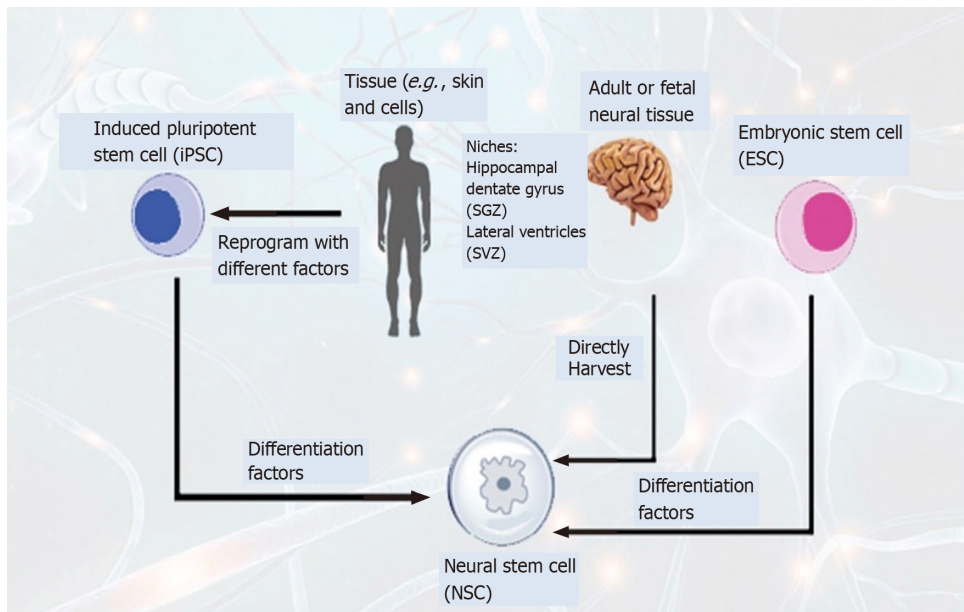
Figure 4 Schematic diagram of generation of neural stem cells *via* different methods to treat neurodegenerative disorders. Neural stem cells (NSCs) can be generated by extracting directly from the subgranular zone of the hippocampal dentate gyrus and subventricular zone of the lateral ventricles from fetal or adult brain. NSCs isolation from patients can be reprogrammed by using different factors such as transcription factors, small molecules, microRNAs, and other morphogens. NSCs can also be generated from blastocyst-derived embryonic stem cells by using differentiation factors. SGZ: Subgranular zone; SVZ: Subventricular zone.

However, based on the shortcomings of various *in vitro* and *in vivo* neurodegenerative disease models, the translational effects of NSCs into human patients remains unknown. Thus, a more definite role of NSCs in various transplantation settings further needs to be explored. Many studies provided the evidence of the association of cognitive improvement with increase in synaptic activity, which is closely correlated with increase in neuronal and glial cells. NSCs transplantations supports behavioral and cognitive functions. Although specific cell types that associate with improvements, that NSCs need to differentiate into, remains unknown. The selection of the best time window for stem cell treatment is closely associated with the clinical prognosis of patients; however, thus far, no studies have reported the best treatment time window. The differentiation potential of NSCs derived from different sources may also vary, and how to determine the doses of transplanted cells is, therefore, an important issue for future research studies. There are still great challenges in preventing immunological rejection responses, improving the survival rate of transplanted NSCs, and consequently obtaining activated young stem cells with a clinically effective grade.

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