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Stem Cells as Promising Therapeutic Options for Neurological Disorders

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ABSTRACT

Due to the limitations of pharmacological and other current therapeutic strategies, stem cell therapies have emerged as promising options for treating many incurable neurologic diseases. A variety of stem cells including pluripotent stem cells (i.e., embryonic stem cells and induced pluripotent stem cells) and multipotent adult stem cells (i.e., fetal brain tissue, neural stem cells, and mesenchymal stem cells from various sources) have been explored as therapeutic options for treating many neurologic diseases, and it is becoming obvious that each type of stem cell has pros and cons as a source for cell therapy. Wise selection of stem cells with regard to the nature and status of neurologic dysfunctions is required to achieve optimal therapeutic efficacy. To this aim, the stem cell-mediated therapeutic efforts on four major neurological diseases, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and stroke, will be introduced, and current problems and future directions will be discussed. J. Cell. Biochem. 114: 743–753, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: STEM CELL; NEUROLOGICAL DISORDERS; PARKINSON'S DISEASE; HUNTINGTON'S DISEASE; AMYOTROPHIC LATERAL SCLEROSIS; STROKE

Neurological diseases encompass any disorder in the central nervous system (CNS) or the peripheral nervous system (PNS) that is caused by structural, biochemical, and electrophysiological dysfunctions of neurons or glial cells. Neurodegenerative diseases are common types of neurological disorders resulting from progressive degeneration or functional loss of neurons. Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) are included in the category of neurodegenerative diseases. In contrast, neurological disorders that are caused by dysfunctional blood circulation in the brain are categorized as neurovascular diseases. Both ischemic and hemorrhagic strokes are representative disorders for neurovascular disease.

Regardless of the type of neurological disease, all symptoms result from the significant loss of neurons or glial cells in the nervous system. For example, PD is caused by the death of dopaminergic (DA) neurons in the substantia nigra pars compacta of the midbrain, and HD results from the death of medium spiny neurons in the basal ganglia. ALS, which is referred to as Lou Gehrig's disease after a famous baseball player in the US who suffered the disease, is caused by the death of motoneurons. Neurovascular diseases such as stroke are another form of neurologic disease resulting from the death of neurons or other brain cells near dysfunctional blood vessels, mostly in the CNS.

Stem cell therapy draws attention as a promising therapeutic option for the treatment of various neurologic diseases. Stem cells by themselves are characterized as cells with the capability of self-renewal as well as multipotency or pluripotency. Based on the differentiation potential and origin of the stem cells, there are a variety of stem cells, such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adipose-derived stromal cells (ADSCs), bone marrow-derived mesenchymal stem cells (BM-MSCs), and neural stem cells (NSCs). In addition to direct cell replacement, stem cells secrete various cytokines and growth factors that elicit a variety of beneficial effects such as anti-inflammatory effects, neural cell protection, and induction of the endogenic recovery system.

The effective treatment of a specific neurological disorder using stem cells requires an in-depth understanding of the etiopathophysiology of the disease and the functional characteristics of stem cells in the disease condition because the type of stem cells needed for efficient treatment of a certain type of neurological disease depends on which neural cells are affected and how the stem cells function in the disease condition.

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In this review, we will introduce basic, pre-clinical, and clinical research performed on certain neurological diseases and discuss the barriers that must be overcome to develop successful therapeutics to treat patients with neurological diseases.

PARKINSON'S DISEASE (PD)

PD is a progressive neurodegenerative disease caused by the preferential death of DA neurons in the substantia nigra pars compacta of the ventral midbrain (Fig. 1A). PD patients often display not only behavioral symptoms such as resting tremor, rigidity, bradykinesia, and postural instability but also abnormalities in speech, mood, and cognition. Current pharmacological therapy using L-DOPA is effective for the first several years after disease onset, although the drug begins to develop unwanted complications such as dyskinesia, waning of therapeutic effects, and "on–off" fluctuations at later time points.

Cell replacement therapy using fetal ventral mesencephalic tissue began approximately 30 years ago [Lindvall et al., 1992]. The main idea of this therapeutic approach is to increase DA levels in the basal ganglia by transplantation of the fetal DA precursors into the striatum. When human fetal ventral mesencephalic tissue was transplanted, a significant improvement in motor functions was detected along with the survival and integration of grafts into the host brain as examined by histopathological and radiological methods. To date, more than 300 cases of the fetal tissue engraftment have been performed worldwide, and the outcomes have been variable. Two double-blind and placebo-controlled clinical trials failed to prove the efficacy of fetal tissue transplantation in PD patients [Freed et al., 2001; Olanow et al., 2003]. Because of these unfavorable efficacy results, the ethical issues of using fetal tissues, and unexpected side-effects such as graft-induced dyskinesia, scientists began to evaluate stem cells as an alternative source for transplantation.

Due to their self-renewal ability and their pluripotent nature, ESCs are regarded as a promising candidate cell source for cell replacement therapy for neurological disorders, including PD. As a result, many differentiation protocols for ESCs into DA neurons have been established. The establishment of efficient DA neurons from ESCs was further facilitated by a deep understanding of the neural developmental process in vivo. In 2002, Kim et al. differentiated mouse ESCs (mESCs) into DA neural precursors and transplanted the cells into a rodent model of PD. The implanted neural precursors differentiated into neurons with midbrain DA neuronal characteristics in vivo and reversed behavioral dysfunc-

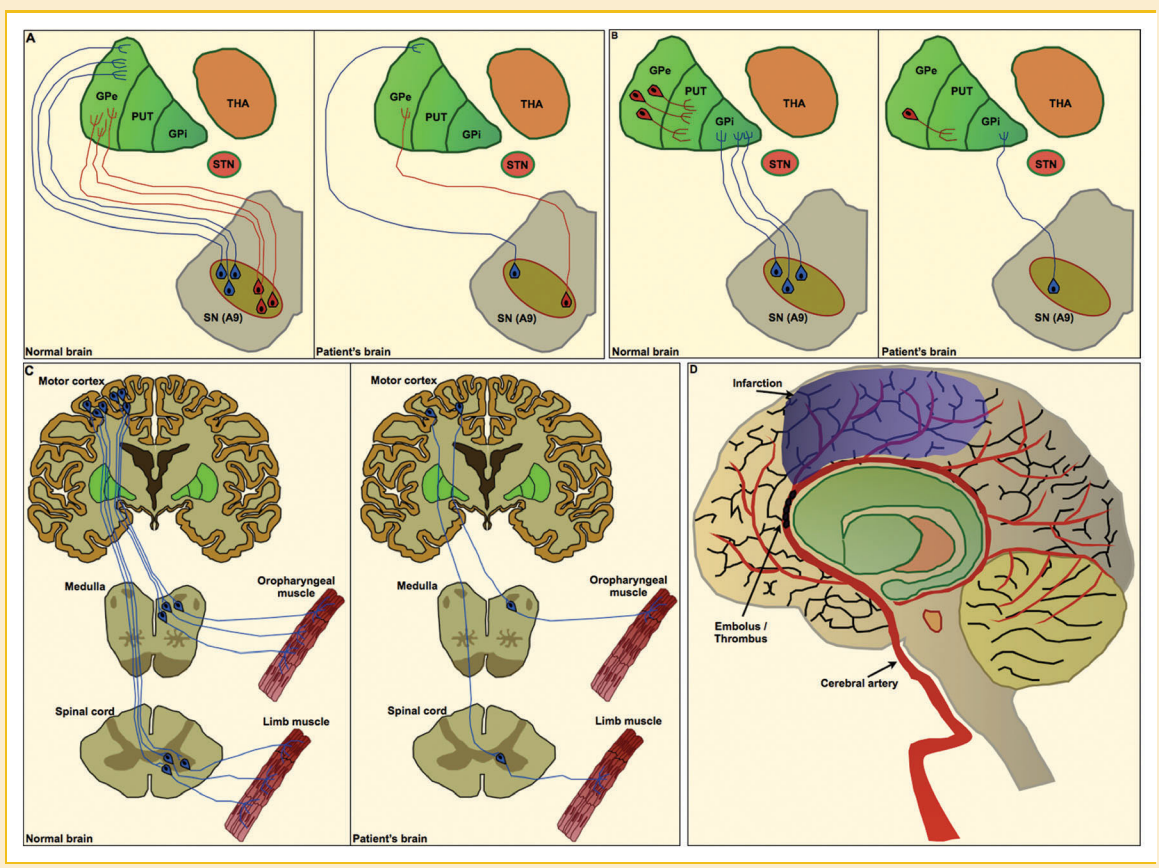

Fig. 1. Illustrations comparing normal and diseased brains. A: Parkinson's disease, (B) Huntington's disease, (C) Amyotrophic lateral sclerosis, and (D) Stroke. Blue line, excitatory neurons; Red line, inhibitory neurons; PUT, putamen; GPe, globus pallidus externa; GPi, globus pallidus interna; THA, thalamus; STN, subthalamic nucleus; SN, substantia nigra.

tion, showing the potential of ESC-mediated cell therapy for PD treatment [Kim et al., 2002]. Subsequently, Perrier et al. [2004] established a protocol for the efficient derivation of midbrain DA neurons from three human ESC (hESC) lines and two primate ESC lines. Roy et al. [2006] adopted a co-culture method in which hESCs were induced to acquire a DA neuronal fate by signaling molecules secreted from co-existing immortalized midbrain astrocytes. Further improvements in DA neuronal derivation from ESCs were achieved by the forced expression of exogenous Lmx1a and by the generation of midbrain floor-plate precursors using a floor-plate-based-strategy.

The possibility of teratoma formation derived from undifferentiated ESCs remaining after the differentiation process is a serious problem in applying ESCs to cell therapy. Several approaches have been attempted to resolve this issue. Schuldiner et al. demonstrated that hESCs containing the HSV-tk gene were selectively eliminated by the administration of the pro-drug ganciclovir in vitro. Furthermore, tumors formed in mice by injection of HSV-tk^+ hESCs regressed after ganciclovir treatment [Schuldiner et al., 2003]. The approach of sorting of midbrain DA neuronal precursors using specific markers such as Otx2 and Corin was also tested in rodent models of PD [Chung et al., 2011]. The undifferentiated ESCs in the embryoid body-derived cells expressed both octamer-type transcription factor (Oct4) and prostate apoptosis response 4 (PAR4) and demonstrated that these $Oct4^+/PAR4^+$ cells were removed by apoptosis using N-oleoyl serinol, a ceramide analogue, before transplantation [Bieberich et al., 2004]. Whether these selection methods are safe enough to be applied to clinical use requires further extensive and long-term follow-up investigation.

With the advent of reprogramming technology, iPSCs have also been evaluated as a cell source for DA neurons. Wernig et al. [2008] derived DA neurons from iPSCs and detected behavioral improvement after engrafting the differentiated cells into a 6-OHDA rodent model of PD. Intriguingly, the methods of derivating iPSCs were reported to affect the differentiation process to midbrain DA neurons; human iPSCs generated by protein-mediated reprogramming, but not by lenti or retroviral vector-mediated reprogramming, were shown to differentiate into highly proliferative neural precursor cells (NPCs), indicating that the reprogramming factors remaining inside the iPSCs affected the neuronal differentiation process unfavorably. It would be of interest to determine whether other footprint-free iPSCs generated by adenovirus-, plasmid-, and mRNA-based reprogramming displayed similar phenotypes as the protein-based iPSCs.

Gene-editing technology using either zinc-finger nuclease (ZFN) or transcription activator-like effector nuclease (TALEN) was used to correct the point mutation A53T in the α-synuclein gene, suggesting the possibility that genetic defects causing familial PD could be repaired before cell-replacement therapy for PD [Soldner et al., 2011].

Reprogramming methods that convert fibroblasts or other types of somatic cells directly into neural precursors or DA neurons have recently been developed [Caiazzo et al., 2011; Kim et al., 2011]. Although this approach has limitations in securing a large number of pure DA neurons or DA neuronal precursors for cell therapy, it is regarded as a safer strategy for clinical application.

In addition to pluripotent stem cells, there have been attempts to use adult stem cells as a source for cell replacement therapy. DA neuron-like cells have been generated from a variety of adult stem cells such as BM-MSCs, placenta-derived MSCs, ADSCs, and Wharton's jelly MSCs, and in some cases, these cells have been shown to reverse behavioral dysfunctions in animal models of PD. However, more extensive characterization and evaluation of the DA neuron-like cells generated from adult stem cells would be needed before applying these cells in cell replacement therapy (Table I).

Furthermore, adult stem cells other than MSCs have been tested for the treatment of PD. For example, olfactory neuroepithelial cells located in the nasal passage drew attention because of their continuous regenerative capability, easy biopsy, capability for ex vivo expansion, and neurogenic differentiation potential [Roisen et al., 2001]. Furthermore, DA neurons were generated from the olfactory neuroepithelial cells in vitro and the DA neurons induced behavioral recovery after transplantation in a hemiparkinsonian rat model [Murrell et al., 2008].

Currently undergoing clinical trials for PD are using BM-MSCs as an autologous cell source for transplantation. A clinical trial is in progress in India to evaluate the therapeutic effect of BM-MSCs after transplantation into the striatum of PD patients (Study NCT00976430 on www.ClinicalTrials.gov). Another clinical trial in China is also underway to examine the effect of BM-MSCs after intravenous injection (Study NCT01446614 on www.ClinicalTrials.gov).

HUNTINGTON'S DISEASE (HD)

HD is a progressive autosomal dominant disease caused by the expansion of CAG triplet repeats (encoding polyglutamine) in exon 1 of the *huntingtin (htt)* gene on chromosome 4. One of the prominent pathological features of this devastating neurodegenerative disease is the selective degeneration of GABAergic medium spiny neurons in the striatum, resulting in abnormal involuntary movements, cognitive impairments, and psychiatric problems (Fig. 1B). The disease onset is usually between the ages of 40 and 50, with disease progression over 15–20 years before death. There is no known cure for this disease, and current treatment is focused on relieving symptoms and delaying disease progression. Recently, hope has risen for stem cell-mediated cell replacement therapy to cure this devastating disease.

As in the case of PD, fetal tissue transplantation has been tested extensively in animal models of HD. Fetal striatal tissues transplanted into the brain parenchyma of both rodent and primate models of HD were shown to survive and integrate well into the local environment of the host brain and reversed motor and cognitive dysfunctions [Hantraye et al., 1992; Dunnett et al., 1998; Nakao et al., 1999; Bachoud-Levi et al., 2000]. Inspired by the results from experimental animal models, several clinical trials have been performed. Bachoud-Levi et al. [2000, 2006] have shown in their 6-year follow-up study that intrastriatal transplantation of fetal striatal cells conferred a certain degree of motor and cognitive improvements for 2–3 years, but not permanently. Currently ongoing clinical trials using fetal tissue (e.g., Study NCT00190450

TABLE I. Summary of Representative Efforts to Evaluate Stem Cell-Mediated Therapeutic Strategies to Treat PD, HD, ALS, and Stroke

Stem cell	Model or patient	Phenotype	Refs.
Parkinson's disease			
Human fetal mesencephalic tissue	Patients with PD	Elevated dopamine level Engraftment in host tissue Improved motor function PET and fMRI	Freed et al. [2001], Lindvall et al. [1992]
Rat fetal mesencephalic tissue	6-OHDA-lesioned rat PD model	Engraftment in host tissue Improved motor function	Perlow et al. [1979]
Marmoset fetal mesencephalic tissue	6-OHDA-lesioned marmoset PD model	Engraftment in host tissue Improved motor function	Annett et al. [1990]
Marmoset fetal mesencephalic tissue	MPTP-lesioned marmoset PD model	Engraftment in host tissue Improved motor function	Fine et al. [1988]
Human ESC-derived dopaminergic neurons	6-OHDA-lesioned rat PD model	Engraftment in host tissue Dopaminergic neurons in the graft Electrophysiological properties Improved motor function	Kim et al. [2002]
Undifferentiated mouse ESCs	6-OHDA-lesioned rat PD model	Dopaminergic neurons in the graft Improved motor function PET and fMRI	Bjorklund et al. [2002]
Human iPSC-derived dopaminergic neurons	6-OHDA-lesioned rat PD model	Engraftment in host tissue Improved motor function	Wernig et al. [2008]
Human BM-MSC-derived neurons	6-OHDA-lesioned rat PD model	Engraftment in host tissue Improved motor function	Dezawa et al. [2004]
Autologous human BM-MSC	Patients with PD	Improved motor function	Venkataramana et al. [2010]
Huntington disease			
Cells from rat fetal striatum	Excitotoxic lesioned rat HD model	Electrophysiologic properties Engraftment in host tissue Elevated GABA level in the striatum	Nakao et al. [1999]
Cells from rat fetal striatum	Excitotoxic lesioned primate HD model	Survival and differentiation in host tissue Behavioral improvement Improved cognitive function	Hantraye et al. [1992], Nakao et al. [1999]
Cells from mouse fetal striatum	Transgenic HD model mice	Survival and differentiation in host tissue Improved motor function	Dunnett et al. [1998]
Cells from human fetal striatum	Patients with HD	Safety confirmed Engraftment in host tissue Improved motor function PET and fMRI	Furtado et al. [2005]
Human ESC-derived neural precursors	Excitotoxic lesioned rat HD model	Survival and differentiation in host tissue Improved motor function	Dihne et al. [2006]
Human umbilical cord blood mononuclear cells	Transgenic HD mouse model	Survival and differentiation in host tissue Improved motor function	Ende and Chen [2001]
Rat neural precursors	Excitotoxic lesioned rat HD model	Survival and differentiation in host tissue Improved metabolic function	Vazey et al. [2006]
Neurons derived from human NTera-2 cells	Excitotoxic lesioned rat and primate HD model	Survival and differentiation in host tissue Improved motor function	Hurlbert et al. [1999]
ALS			
Autologous BM-MSCs	Patients with ALS	Delayed decline of lung and motor function	Mazzini et al. [2008]
Autologous peripheral CD133+ cells	Patients with ALS	Prolonged survival rate	Martinez et al. [2012]
Allogenic CD34+ hematopoietic stem cells	Patients with ALS	No beneficial effects	Appel et al. [2008]
Human fetal neural stem cells	Patients with ALS	No disease progression Safety confirmed	Glass et al. [2012]
Human BM-MSCs	Transgenic ALS mouse model	Improved motor function Prevention of astrogliosis and microglial activation	Vercelli et al. [2008]
Human umbilical cord blood mononuclear cells	Transgenic ALS mouse model	Delayed disease progression Prevention of astrogliosis and microglial activation	Garbuzova-Davis et al. [2008]
Human neural stem cells	Transgenic ALS mouse model	Formation of functional synapses	Xu et al. [2009]
Mouse bone marrow cells	Transgenic ALS mouse model	Prolonged survival rate Delayed disease progression	Beers et al. [2008]
Mouse and rat GRP cells	Transgenic ALS mouse model	Delayed disease progression Prevention of microglial activation Engraftment into host tissue	Lepore et al. [2008]
Mouse ESC-derived motoneurons	Virus-induced paralyzed rat model	Engraftment into host tissue host Improved motor function Gain weight	Deshpande et al. [2006]
Stroke			
Human neural stem cells	Rat MCAO model	Migration and differentiation of the grafted cells	Kelly et al. [2004]
Primate ESC-derived neural precursors	Mouse MCAO model	Migration and differentiation of the grafted cells	Hayashi et al. [2006]
Mouse BM-MSCs	Rat MCAO model	Promoted angiogenesis Improved sensory and motor functions Decreased infraction volume	Chen et al. [2003]
Human BM-MSCs	Rat MCAO model	Engraftment into host tissue Improved motor function	Zhao et al. [2002]

(*Continued*)

TABLE I. (*Continued*)

Stem cell	Model or patient	Phenotype	Refs.
Neurons derived from human NTera-2 cells	Stroke patients	Safety confirmed Improved motor function	Borlongan et al. [1998]
Human ESC-derived neural stem cells	Rat MCAO model	Safety confirmed Improved motor function	Daadi et al. [2008]
Mouse ESC-derived neural stem cells	Rat MCAO model	Survival and differentiation in host tissue Enhanced neurogenesis	Buhnemann et al. [2006]
Human iPSC-derived neural stem cells	Rat MCAO model	Safety confirmed Survival and differentiation in host tissue Improved motor function Decreased infraction volume	Jensen et al. [2011]
Autologous BM-MSCs	Patients with stroke	Improved motor function	Bang et al. [2005]

on www.ClinicalTrials.gov) would provide useful information on the efficacy of the fetal tissue-mediated therapeutic approach.

In addition to the fetal tissue, scientists looked for alternative cell sources to treat HD. NSCs were used as a source for transplantation because of their proliferative ability and tendency to differentiate towards a neural lineage. An initial experiment showed differentiation of immortalized NSCs into pyramidal neurons with long axonal projections after transplantation into the striatum of the rat brain [Englund et al., 2002]. In another study, adult rat NSCs derived from the subventricular zone and transplanted unilaterally in the striatum of the QA-lesioned rat HD model were shown to differentiate in vivo into astrocytes and GABAergic neurons with accompanying behavioral improvements [Vazey et al., 2006]. Human NSCs (hNSCs) grown as neurospheres were also transplanted into the striatum of QA-lesioned HD rats. In this case, successful engraftment of the cells was also observed, which eventually led to reduced lesion volume and a significant improvement of behavioral performance over 8 weeks [McBride et al., 2004].

Stem cells derived from adult non-neural tissues also drew attention as a cell source for treating HD. Umbilical cord blood cells, ADSCs (hADSCs), BM-MSCs, and bone marrow cells were shown to be effective in a variety of HD animal models including transgenic mice and QA or 3-nitrosopropionic acid (3NP)-lesioned rats [Rossignol et al., 2011] (Table I).

To enhance the beneficial effects of cell transplantation, MSCs were genetically engineered to overexpress neurotrophic factors. For example, BM-MSCs overexpressing brain-derived neurotrophic factor (BDNF) or nerve growth factor were shown to be effective in restoring motor functions in a YAC128 transgenic mouse model of HD [Dey et al., 2010].

The current view of the function of adult stem cells is that the positive effects may not result from direct replacement of dead neurons by the stem cells themselves but may be due to indirect effects mediated by secreted beneficial factors that may protect against neuronal death, recruit endogenous stem cells, reduce inflammation, and stimulate neurogenesis in the brain.

Pluripotent stem cells such as ESCs have been tested for a potential therapeutic approach. In these studies, ESCs were differentiated into either NPCs or gamma-aminobutyric acid (GABA)-expressing neurons (GABAergic neurons) and transplanted into the striatum of rat models of HD. When engrafted into the striatum of QA-lesioned rats, ESC-derived GABAergic neurons were found to integrate into the local host brain for up to 6 weeks [Dinsmore et al., 1996]. Another group differentiated ESCs into NPCs and showed that these cells were able to survive in the QA-lesioned mouse striatum for up to 4 months without tumor formation [Dihne et al., 2006]. The therapeutic potential and challenges to overcome which are associated with pluripotent stem cells await further detailed investigation.

AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ALS is a fatal neurodegenerative disease caused by the death of upper and lower motoneurons in the cerebral cortex, brain stem, and spinal cord (Fig. 1C). The symptoms of this disease start with muscle weakness and atrophy, and the disease progresses rapidly, leading to death by respiratory problems. As a result, most ALS patients die within 3–5 years of diagnosis. Approximately 5–10% of ALS is the familial form, which is caused by mutations in superoxide dismutase 1 (SOD1, ALS1), alsin (ALS2), senataxin (SETX, ALS4), TAR DNA binding protein (TARDBP), or Fused in Sarcoma (FUS, ALS6). In spite of extensive efforts, there is no known effective treatment for ALS. The recent development of stem cell-mediated therapy offers the potential to treat this devastating disease. Stem cell-mediated therapeutic approaches target several effects such as direct cell replacement and modulation of a microenvironment that is favorable to motoneurons by secreting trophic factors.

The goals of stem cell therapies for ALS have been focused on either reconstructing the destroyed neural circuitry of motoneurons or delaying/preventing motoneuron death. The first goal appears more difficult than the second because stem cell-derived motoneuron precursor cells should project their axons to the right targets to form the circuitry for proper motor functions.

The methods of differentiation of ESCs into motoneurons have evolved with a better understanding of signaling pathways and transcription factors implicated in determining motoneuron fate. The initial effort of in vitro differentiation of ESCs into motoneurons was performed by the treatment with retinoic acid (RA). Systematic approaches were attempted based on sequential induction of motoneurons from mESCs by RA-mediated caudalization followed by a Sonic hedgehog (Shh)-induced ventralization process [Wichterle et al., 2002]. The motoneurons derived by this method

were shown to display several motoneuron phenotypes in both in vitro cell culture and animal models of motoneuron injury such as synapse formation with muscle fibers, expression of acetylcholine neurotransmitter and receptors in the synaptic junction, and motoneuron-like action potential [Harper et al., 2004]. However, axonal growth from the transplanted mESC-derived motoneurons in the spinal cord of a neuroadapted sinbis virus (NSV)-mediated rat model of motoneuron degeneration was inhibited by several myelin-associated proteins such as Nogo, MAG, and OMgp and repulsive axon guidance cues, preventing motoneuronal axons from reaching the skeletal muscle fibers [Harper et al., 2004]. Several approaches have been attempted to overcome the inhibitory effects of repulsive cues such as administration of dibutyryl cyclic AMP and Rho kinase inhibitors [Deshpande et al., 2006] and co-transplantation of cells secreting glial cell-derived neurotrophic factor (GDNF) [Deshpande et al., 2006]. Another group transplanted mESC-derived motoneurons into the PNS, which provided a favorable environment for axonal growth, and observed functional innervation of the motoneurons to the host muscle fibers with attenuated muscle atropy. Kerr et al. tested hESC-derived motoneurons in NSV-paralyzed rats and observed some functional recovery, probably resulting from neuroprotection by released trophic factors but not by direct cell replacement. Differentiation of hESCs into motoneurons was performed either by sequential exposure of hESCs to defined morphogens such as RA and Shh or by co-culturing with MS5 stromal feeders. Purmorphamamine, a Shh activator, instead of Shh itself improved the efficiency of motoneuronal generation from hESCs [Li et al., 2008].

hiPSCs derived from an ALS patient were also able to differentiate into motoneurons, providing a unique opportunity for autologous cell therapy, disease modeling, and drug screening [Dimos et al., 2008]. In combination with genome-editing technologies, hiPSC technology would provide a unique opportunity to correct genetic mutations in patients with familial ALS.

Due to the low risk of tumor formation, fetal neural precursors have been considered a therapeutic option for ALS treatment. As in other types of brain injury such as stroke, endogenous neurogenesis and migration of the newborn cells towards the injury sites were observed in the spinal cord of ALS patients. Neural precursors obtained from the embryonic rat spinal cord were shown to survive up to 4 months post-transplantation and retained multipotency to differentiate into neurons and glia [Chow et al., 2000].

Human NPCs originating from the fetal spinal cord were grafted into the spinal cord of $SOD1^{G93A}$ rats, a genetic model of ALS. These NPCs survived and integrated well into the local environment, extensively differentiated into neurons, and secreted growth factors such as GDNF and BDNF, resulting in delayed motoneuron degeneration and disease onset. Furthermore, the grafted NPCs were shown to innervate the host spinal cord motoneurons and form synaptic contacts and neuronal circuitry [Xu et al., 2009]. Interestingly, double grafting of NPCs into the lumbar and cervical segments of the $SOD1^{G93A}$ rat spinal cord increased the rat life span and prolonged disease onset, suggesting the potential benefit of multi-site transplantation approaches for ALS [Xu et al., 2011]. In a clinical phase I trial performed by Glass et al. [2012], 12 ALS patients who had a lumbar intraspinal injection of NPCs did not experience any side effects, demonstrating the safety and tolerability of the NPC transplantation. This group next plans to evaluate the efficacy of NPC transplantation.

In addition to direct cell replacement, trophic factors such as GDNF and BDNF secreted from the grafted neural precursors may play an important role in the amelioration of ALS symptoms. In an effort to boost grafting efficacy, NPCs were engineered to express exogenous GDNF and were transplanted into the spinal cord of $SOD1^{G93A}$ rats. The genetically modified human NPCs grafted well in the spinal cord and were shown to protect degenerating host motoneurons. Another study showed that an NSC subtype that expressed both Lewis X and CXCR4 protected host motoneurons, delayed disease progression, and increased the life span of the $SOD1^{G93A}$ mouse. In addition, hNSCs overexpressing vascular endothelial growth factor (VEGF) delayed disease onset and progression and prolonged the life span of the $SOD1^{G93A}$ mouse after transplantation. Furthermore, a study has suggested an interesting possibility that basic fibroblast growth factor (bFGF) could be used to drive differentiation of the grafted NSCs into motoneurons [Jordan et al., 2009].

Several studies have suggested the possibility of interactions between astrocytes and motoneurons in the etiopathology and progression of ALS [Clement et al., 2003]. Therefore, several efforts have been made to provide healthy astrocytes into the diseased area by transplanting glial-restricted precursors (GRPs) originating from the spinal cord [Rao and Mayer-Proschel, 1997]. In the cervical spinal cord of $SOD1^{G93A}$ rats, the grafted GRPs differentiated into astrocytes, resulting in attenuation of motoneuron death and other ALS-like symptoms [Lepore et al., 2008]. Therefore, co-transplantation of astrocytes appeared to generate beneficial effects when stem cell therapy was attempted.

Likewise, microglia appeared to be closely connected to the progression of ALS. Several studies have demonstrated that activated microglia and the accompanying inflammatory response might be contributing factors to the degeneration of motoneurons in the spinal cord. In addition, microglia with the $SOD1^{G93A}$ mutation were proven to be toxic and facilitated the loss of motoneurons, further supporting the important role of microglia in ALS pathophysiology [Beers et al., 2006]. In this regard, modulation of microglial activation state and inflammation should be considered carefully for successful stem cell-mediated therapy for ALS.

In addition to NSCs, other types of adult stem cells have been explored as a source for stem cell therapeutics for ALS. For example, whole bone marrow cells transplanted into irradiated $SOD1^{G93A}$ mice reduced ALS phenotypes and increased the life span of ALS mice. hMSCs were also used for transplantation. When transplanted into the spinal cord of $SOD1^{G93A}$ mice, hMSCs reduced the level of astrogliosis and microglial activation, enhanced the number of host motoneurons in the spinal cord, and improved behavioral functions [Vercelli et al., 2008] (Table I).

The first application of stem cells to ALS patients was reported in 2003. In this study, Mazzini et al. evaluated the safety of the transplantation of ex vivo-expanded autologous BM-MSCs into the spinal cord of seven ALS patients and found no adverse side effects from the transplantation [Mazzini et al., 2003]. A recent clinical study further confirmed the safety and efficacy of the ex

vivo-expanded autologous BM-MSCs (Study NCT00855400 on www.ClinicalTrials.gov) and in-depth clinical study is underway to establish more efficient protocol (Study NCT01254539 on www.ClinicalTrials.gov). Another group infused bone marrow mononuclear cells (BM-MNCs) into the spinal cord of 11 patients with ALS. The number of motoneurons was increased in the transplanted patients, indicating a neurotrophic or neuroprotective effect of BM-MNCs [Blanquer et al., 2012]. BM-hematopoietic progenitor cells were also used for transplantation into the spinal cord of 13 ALS patients. Encouragingly, nine ALS patients were shown to improve during a 1-year follow-up after transplantation [Deda et al., 2009].

STROKE

Stroke is caused by an abrupt disturbance in blood circulation by either ischemia or hemorrhage, destroying brain cells near the damaged vasculature (Fig. 1D). This devastating disease is one of the top three causes of death and a leading cause of disability in Western countries. Currently, thrombolytic therapy is the only therapeutic option proven effective, but only approximately 5% of stroke patients benefit from this therapy due to its very narrow therapeutic window (i.e., 3–4.5 h after the onset of stroke).

Due to the degeneration of a heterogeneous population of brain cell types and vascular cells over large brain areas, stem cell therapy for stroke patients is a challenge and requires a coordination of complex restoration processes, including the replacement of a variety of lost brain cell types, the rebuilding of neural connections, and the reconstruction of the vascular system in the damaged area. Stem cell therapy has emerged as a promising therapeutic option to treat patients with stroke due to the multifunctional nature of stem cells. As in other brain diseases, a variety of stem cells have been tested as sources for transplantation.

NSCs with self-renewal ability and the potential to differentiate into neurons, astrocytes, and oligodendrocytes, serve as an ideal source for repairing ischemic injury where all three cell types in the brain are damaged. Toda et al. reported that only 1–3% of the grafted NSCs into the ischemic rat brain survived, and 3–9% of the surviving cells became neurons (NeuN+), suggesting that NSCs grafted in the ischemic brain were able to differentiate into neurons and promote functional improvements. Interestingly, regardless of the injection route (i.e., intrastriatal, intra-arterial, or intravenous) for transplantation, NSCs appeared to migrate to the ischemic region of MCAO rats and replace the dead neural cells. Human fetal NPCs were also shown to migrate toward the ischemic injury area, differentiate into neurons, and recover function and morphology in an ischemic rat model. This observation suggested the feasibility of using human fetal NPCs as a cell source for treating patients with ischemic stroke [Borlongan et al., 1998; Kelly et al., 2004].

When intravenously injected into an animal model of another type of stroke, intracerebral hemorrhage (ICH), hNSCs increased the number of neurons in the brain, enhanced functional recovery, decreased brain inflammation, and rescued degenerating neurons by adopting a chaperone-like role. Other types of stem cells such as BM-MSCs, hADSCs, human placenta-derived stem cells, and umbilical cord blood-derived progenitors have also been shown to be effective in reducing brain damage, decreasing inflammation, rescuing neuronal degeneration, and promoting long-term functional recovery [Chen et al., 2006; Brenneman et al., 2010] (Table I).

In an effort to enhance the beneficial effects, attempts have been made to genetically modify stem cells to overexpress trophic factors before transplantation. For example, overexpression of either VEGF, a potent angiogenic factor, or Akt1, an antiapoptotic factor, in hNSCs further enhanced the structural and functional recovery in stroke mice by promoting angiogenesis and increasing neuronal survival, respectively [Lee et al., 2009]. hMSCs that overexpress exogenous GDNF, placental growth factor (PlGF), and angiopoietin were also shown to be effective in behavior after transplantation in stroke animal models [Liu et al., 2006].

An interesting approach has been made by establishing a stable BM-MSC cell line, namely SB623 cell, which expressed Notch intracellular domain (NICD). The cell line was confirmed to express more cytokines such as BMP-4, HGF, MCP-1, VEGF than naïve BM-MSCs [Yan et al., 2007]. A clinical trial is in progress to examine the effect of SB623 cells in chronic stroke patients after transplantation into the basal ganglia of chronic stroke patients (Study NCT01287936 on www.ClinicalTrials.gov).

Another clinical trial using BM-MSCs has been performed by Bang et al. They transplanted autologous BM-MSCs into five patients suffering ischemic stroke via intravenous injection. After injection at 5–9 weeks post-ischemia, the BM-MSC transplantation was proven to be a safe therapy. BM-MSC transplantation improved early functional benefits, but the level of functional outcome declined with time during a 1-year follow-up [Bang et al., 2005]. In a subsequent report, the same group reported a long-term follow-up study with special emphasis on the aspect of safety and efficacy after transplantation of BM-MSCs in 85 patients. In this 5-year open-labeled, observer-blinded clinical study, no significant adverse effects were observed, and a long-term beneficial efficacy in behavioral performance and survival was evident [Lee et al., 2010]. However, clinical significance would require a more detailed analysis because the effects of BM-MSC transplantation appeared to be affected by many variables, such as patient age and stroke status.

In vitro-expanded autologous BM-MSCs were also transplanted into 12 stroke patients at 36–133 days post-ischemia. Neurological and neuroradiological analyses for 1-year demonstrated no significant side effects, including tumors, thromboembolism, and abnormal cell proliferation [Honmou et al., 2011]. A long-term follow-up study of functional efficacy would be of great interest.

Olfactory neuroepithelial cells have been examine for their capability to induce recovery from stroke injury. The cells were shown to secrete SDF1α and recruit endogenous bone marrow stem cells and NSCs, leading to improved behavioral functions [Shyu et al., 2008]. Clinical trial is currently underway by implanting ex vivo-expanded autologous olfactory neuroepithelial cells in ischemic stroke patients (Study NCT01327768 on www.ClinicalTrials.gov).

CONCLUSIONS AND PROSPECTS

Stem cell therapies have emerged as promising approaches to treat many incurable neurological diseases. A variety of stem cells have

been shown to be effective in preclinical studies using animal models; clinical trials have followed based on the animal studies. Through many studies using animals, we started to understand the characteristics and functional role of each type of stem cell. Overall, adult stem cells were considered safe in terms of tumor formation and immune rejection (i.e., autologous transplantation) and function in brain recovery primarily through paracrine effects. However, the limited amount of cells obtainable from an object even after ex vivo expansion and a lack of dramatic therapeutic effects within a short time period are weaknesses associated with adult stem cell-based cell therapy.

In contrast, pluripotent stem cells such as ESCs and iPSCs are regarded as a powerful source for cell therapy because these cells function both by direct cell replacement and also by paracrine effects. Unlimited availability of the cells is another advantage for ESCs and iPSCs. However, efficient differentiation technologies should be developed in parallel for applying these cells in the clinic because of the potential risk of unwanted side effects such as tumor formation. Luckily, this issue has been resolved in some cases such as differentiation into oligodendrocytes and retinal pigment epithelial cells; therefore, clinical trials using these cells for spinal cord injury and macular degeneration, respectively, were approved recently from FDA.

To develop effective stem cell therapeutic strategies for certain neurologic diseases, we need to fully understand the nature of the neurological disease and the behavior of stem cells in the animal model of the disease. Based on the functional and histological data from the animal study, the most appropriate stem cell type and detailed strategies for generating the stem cell therapeutics for the neurologic disease can be designed.

It should be considered that the transplantation of stem cells into the injured brain would not be efficient for brain recovery unless the hostile pathological environment is improved. For example, inflammation must be reduced to enhance engraftment and survival after stem cell transplantation. Therefore, efficient methods to modulate the microenvironment of the host brain, such as inflammatory control, neuroprotection, angiogenesis, and secretion of neurotrophic factors, need to be developed. This microenvironmental change can be achieved by the same stem cells grafted or by co-grafting with different types of cells such as astrocytes. The expected outcomes of microenvironmental control would be reduced inflammation, increased survival of the graft, enhanced differentiation into proper cell types, migration into the injury site, efficient integration into local environment, and formation of neural circuitry.

There are some caveats for translating data from animal models into human clinical trials because animal models sometimes do not directly reflect human conditions. For example, the assessment of the risk of tumor formation obtained from xenograft animal studies may not be directly applicable to human cases. Furthermore, the functional data from an animal model sometimes do not correlate with those from human cases.

Stem cell therapy for neurological diseases should meet several criteria to function effectively. First, ideally, most of the grafted cells should differentiate into a certain cell type of interest in vitro and, most importantly, in vivo. If not 100%, the composition of the cells produced after differentiation needs to at least be carefully defined. In the case of PD, contamination of seratonergic neurons in the cell population differentiated from the fetal graft in vivo contributed to the graft-induced dyskinesia, a serious side effect of fetal tissue-mediated transplantation therapy. Second, integration of the grafted cells into the local neural network and the formation of the functional circuitry might be important. The formation of neuronal circuitry could be confirmed by anterograde and retrograde tracing agents. Third, the grafted cells should survive for a long period of time. This issue can be improved by trophic factors secreted from the grafted cells with or without genetic modification or co-transplantation with other types of cells, such as astrocytes. Fourth, the risk of tumor formation needs to be considered seriously. Although hESCs/iPSCs are the cell types of great concern, adult stem cells are not free from this issue. In this sense, it should be carefully confirmed that cell division of the grafted cells does not occur vigorously post-engraftment. Fifth, efficacy and safety in vivo might be affected by the pathological microenvironment of animal models of each neurologic disease. Therefore, if possible, it would be desirable to evaluate stem cell transplantation in at least two different animal models of each disease, preferentially one for a genetic model and one for a toxin-lesioned model.

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