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Review Article

Autologous Bone Marrow Stromal Cell Transplantation for Central Nervous System Disorders – Recent Progress and Perspective for Clinical Application

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Abstract:

There is increasing evidence that the transplanted BMSC significantly promote functional recovery after CNS damage in the animal models of various kinds of CNS disorders, including cerebral infarct, traumatic brain injury and spinal cord injury. However, there are several shortages of information when considering clinical application of BMSC transplantation for patients with CNS disorders. In this review, therefore, we discuss what we should clarify to establish cell transplantation therapy as the scientifically proven entity in clinical situation and describe our recent works for this purpose. The BMSC have the ability to alter their gene expression profile and phenotype in response to the surrounding circumstances and to protect the neurons by producing some neurotrophic factors. They also promote neurite extension and rebuild the neural circuits in the injured CNS. The BMSC can be expanded in vitro using the animal serum-free medium. Pharmacological modulation may accelerate the in vitro proliferation of the BMSC. Using in vivo optical imaging technique, the transplanted BMSC can non-invasively be tracked in the living animals for at least 8 weeks after transplantation. It is urgent issues to develop clinical imaging technique to track the transplanted cells in the CNS and evaluate the therapeutic significance of BMSC transplantation in order to establish it as a definite therapeutic strategy in clinical situation in the future.

Keywords:

Bone marrow stromal cell, transplantation, cerebral stroke, spinal cord injury, translational study

Introduction:

Many of central nervous system (CNS) disorders can easily cause longstanding disability. There are few drugs that are effective for protect or repair the CNS tissue in clinical situation in spite of numerous numbers of basic researches [1]. However, recent studies have strongly suggested that cell transplantation therapy may potentially promote functional recovery after various kinds of CNS disorders, including cerebral infarct, spinal cord injury (SCI) and traumatic brain injury (TBI). A variety of cell types have been studied as cell source of transplantation into animal models of CNS disorders, including embryonic stem (ES) cells, neural stem cells, inducible pluripotent (iPS) cells, and bone marrow stromal cells (BMSC). Of these, the BMSC may have the most enormous therapeutic potential among them, because they can be harvested from the patients themselves without posing ethical or

immunological difficulties [2, 3]. There is increasing evidence that the transplanted BMSC enhance functional recovery by differentiating into the neural and endothelial cells and/or by producing various kinds of cytokines or growth factors that can rescue the host neurons [4, 5]. Although the results are encouraging, a variety of questions or problems still remains to be solved [4, 6-19]. In this review article, we present recent progress in basic and clinical research on the field of BMSC transplantation for CNS disorders and critically discuss what we should clarify to establish BMSC transplantation therapy as scientifically proven entity in clinical situation.

Recent Progress in BMSC Transplantation

Biological Aspects of The BMSC

Although the exact mechanisms underlying the beneficial effects of BMSC transplantation has not been fully clarified yet, several hypotheses have been proposed to explain it. First, BMSC *per se* are believed to differentiate into neural cells in the host's brain ('transdifferentiation theory'). This theory is based on the findings that BMSC simulate neuronal morphology and express the proteins specific for neurons *in vitro* [20, 21] or *in vivo* [22, 23]. Although the transdifferentiation theory is quite attractive, there still remain several questions: Thus, how is the mesenchymal cell fate of the BMSC oriented to the neuronal lineage? Are their morphological change and expression of neuronal phenotype identical to differentiation into functional neuronal cells? Actually, several studies posed a question about their *in vitro* differentiation into neurons [24, 25]. In order to seek the answers, we chemically treated the cultured BMSC with basic fibroblast growth factor (bFGF), retinoic acid, and DMSO, and found that they could potentially modify their gene expression profile in response to the surrounding environment [14]. Furthermore, a certain subpopulation of the BMSC morphologically simulates the neuron and expresses the neuron-specific proteins without evidence of cell fusion, when co-cultured with the neurons [4]. The findings strongly suggest that at least a certain subpopulation of the BMSC have the potential to alter their gene expression profile and to differentiate into the neural cells in response to the surrounding environment.

Second, the transplanted BMSC have also been reported to fuse with host cells and to simulate the differentiation into host cells ('cell fusion theory') [26]. Indeed, some of BMSC fuse with the neurons and acquire the phenotypes of both cells, when co-cultured with the neurons [4]. However, the roles and fates of the BMSC fused with the neurons are still completely unknown.

Third, there is increasing evidence that the BMSC produce some neuroprotective or neurotrophic factors and support the survival of the host neural cells [27]. This 'feeder theory' is quite natural because the BMSC *per se* support the homing and proliferation of the hematopoietic cells in the bone marrow by producing a variety of cytokines such as stromal cell-derived factor-1a (SDF-1a) [28]. Indeed, the conditioned medium of BMSC significantly promote neurite outgrowth from the dorsal root ganglion [29]. Very recently, we co-cultured the BMSC with the neurons exposed to an excitotoxic amino acid, glutamate, using three-dimensional co-culture paradigm. As the results, the co-cultured BMSC increased their release of soluble neuroprotective factors, including nerve growth factor (NGF), hepatocyte growth factor (HGF) and brain-derived neurotrophic factor (BDNF), and significantly ameliorated glutamate-induced neuronal injury [4]. Actually, previous reports have shown that the BMSC produced NGF, BDNF, neurotrophin (NT)-3 and glial cell line-derived neurotrophic factor (GDNF) [30, 31]. Furthermore, the BMSC-conditioned medium activates phosphorylation of mitogen-activated protein kinase/extracellular signal-regulated protein kinase and/or phosphoinositide 3-kinase/serine/threonine

kinase (PI3K/Akt) in primary culture of rat dorsal root ganglion (DRG) neurons [32]. The BMSC markedly promote the neurite extension from the neurons in the organotypic slice of the brain and spinal cord [11, 33]. Very recently, He et al. (2011) have reported that the BMSC significantly increase the expression of bFGF, BDNF, and vascular endothelial growth factor (VEGF) in the ischemic brain when transplanted with endothelial precursor cells [34]. These findings strongly suggest that the BMSC trigger endogenous signaling pathways of survival and repair in neurons by secreting soluble neurotrophic factors. Based on these biological aspects of the BMSC, we consider that the BMSC consist of heterogeneous cell populations and protect and/or repair the damaged CNS through multiple mechanisms, including transdifferentiation, cell fusion, and soluble factor production. This hypothesis may be quite natural because the cultured BMSC are isolated by their adhesion property to the culture dish and are not monoclonal [4].

Proliferation and Migration of the BMSC in CNS

Since several pioneering studies were reported [22, 23], numerous numbers of studies have confirmed that the BMSC can survive, migrate into the lesion, express the neural phenotypes and enhance functional recovery, when transplanted into animal models of CNS disorders. In the majority of these studies, the BMSC are transplanted *within 7 days after the insults*, and beneficial effects of BMSC transplantation can be observed approximately 4 weeks after transplantation [2, 3]. Recently, we have evaluated whether the transplanted BMSC can maintain their proliferation property in the CNS tissue or not. For this purpose, we labeled the GFP-expressing BMSC with a superparamagnetic iron oxide (SPIO) agent and transplanted into the ipsilateral striatum of the mice brain subjected to permanent focal ischemia at 7 days after the insult. Fluorescence immunohistochemistry revealed that many of the GFP-positive cells were widely distributed around infarct and partially expressed MAP2 and NeuN at 3 months after transplantation. However, only a smaller number of SPIO-positive cells could be detected on Turnbull blue staining. Surprisingly, the ratio of the SPIO- to GFP-positive cells was less than 3%. The value was comparable to the finding that the proportions of the SPIO-positive BMSC gradually decreased from 93.6% at P3 to 6.5% at P7 when the passages were repeated *in vitro*. The results have strongly suggested that the BMSC repeat their proliferation many times, migrate into the lesion, and partially express the neuronal phenotype in the host brain during 3 months after transplantation [16].

Previous studies have shown that the transplanted BMSC migrate towards the lesion, although the underlying mechanisms are not clarified. Recent studies have shown that some chemokine such as monocyte chemoattractant protein-1 (MCP-1) and SDF-1a are expressed around the damaged CNS tissue and play an important role in the migration of the transplanted cells [35, 36]. Recently, we investigated the role of CXCR4, a specific receptor for SDF-1a, in the migration of the BMSC in the CNS. The BMSC were isolated from the wild type (WT) and the CXCR4-knockout (KO) mice, and were transplanted into ischemic brain of mice. As the results, functional recovery in the WT BMSC-transplanted mice was more pronounced than in the CXCR4-KO-transplanted mice. SDF-1a was extensively expressed in the reactive astrocytes around cerebral infarct. The transplanted cells were extensively distributed in the ipsilateral hemisphere in the WT BMSC-transplanted mice. However, most of them was found in the injection site in the CXCR4-KO BMSC-transplanted mice. The results suggest that the SDF-1a/CXCR4 system may play a critical role in the migration of the transplanted BMSC and contribute to recovery of neurological function [12]. Likewise, Son et al. (2006) also reported that SDF-1/CXCR4 and HGF/c-Met axes were involved in the recruitment of BMSC to the damaged tissue [37]. It may be quite valuable to elucidate the temporal profile of these chemokines around

damaged CNS tissue to determine the optimal timing of BMSC transplantation.

Mechanisms of Therapeutic Effects by BMSC Transplantation

Nowadays, as described above, there is increasing evidence that the BMSC significantly enhance the recovery of motor function when transplanted into the animal models of cerebral infarct, SCI, and TBI [2, 3]. In fact, we have confirmed that the BMSC significantly improve motor function on rotarod test, when stereotactically injected into the ipsilateral striatum at 7 days after the onset of permanent middle cerebral artery (MCA) occlusion [12, 38, 39]. The BMSC also promote functional recovery of the lower extremities, when directly transplanted into the injured spinal cord at 7 days after the onset of SCI [6, 7, 15, 40]. More interestingly, the BMSC have the potential to ameliorate cognitive dysfunction under certain conditions. Thus, Wu et al. (2007) directly transplanted the BMSC into the hippocampus and found significant improvement of cognitive function in Alzheimer' disease model of rats [41]. Maruichi et al. (2009) stereotactically transplanted the BMSC into the mice subjected to diffuse axonal injury, and concluded that BMSC transplantation significantly enhance the recovery of cognitive function on Morris Water Maze test [19]. Furthermore, Shichinohe et al. (2010) have also demonstrated that the BMSC significantly ameliorate white matter damage and improve cognitive function in chronic cerebral ischemia model of rats [42].

However, it is not fully understood through which mechanisms the engrafted BMSC enhance functional recovery after CNS damage. There are several explanations for this issue. First, the BMSC may also promote axon regeneration by secreting neuroprotective and/or neurotrophic factors. Thus, Hofstetter et al. (2002) transplanted the BMSC into the injured cord and found that the engrafted BMSC were tightly associated with longitudinally arranged immature astrocytes and formed bundles bridging the epicenter of the injury [43]. As aforementioned, the BMSC markedly promote the neurite extension from the neurons in the organotypic slice of the brain and spinal cord probably by secreting various kinds of neuroprotective or neurotrophic factors [11, 33]. Very recently, Chiba et al. (2009) have also demonstrated that the transplanted BMSC not only acquire neural cell phenotypes but also are integrated into the neural circuits of host around the injured spinal cord, promoting the recovery of neurological function [6].

Knowledge on the mechanisms underlying functional recovery after BMSC transplantation is largely based on histological findings. Alternatively, recent autoradiographic studies have demonstrated that the transplanted BMSC express the protein for gamma-aminobutyric acid (GABA) receptor and improve the binding potential for ^{125}I-iomazenil around the CNS lesion [13, 17]. Mori et al. (2005) also transplanted the BMSC into the rat cold injury model and found that the transplanted BMSC improved glucose metabolism in response to sensory stimuli, using autoradiography technique [44].

Preliminary Clinical Trials of BMSC Transplantation for CNS Disorders

Based on these observations obtained from animal experiments, some preliminary clinical trials have already been started [45-49]. Thus, Bang et al. (2005) intravenously injected the autologous BMSC into 5 patients with severe neurological deficits due to ischemic stroke at 5 to 9 weeks after the onset, and concluded that autologous BMSC infusion is a feasible and safe therapy that may improve functional recovery [45]. Zhang et al. (2008) expanded the autologous BMSC in culture, and directly injected them into the brain during surgery in 7 patients with traumatic brain injury [49]. Saito et al. (2008) intrathecally infused the autologous BMSC into a 35-year-old patient with spinal cord injury 2 weeks after the onset [50]. Furthermore, Lee et al. (2008) transplanted the BMSC into 11 patients with multiple system atrophy through consecutively intra-arterial and three repeated intravenous routes, and found

significant improvement of their neurological scores [46]. Pal et al. (2009) injected the BMSC through lumbar puncture for 30 patients with SCI, and reported no serious adverse events [48]. Very recently, Mazzini et al. (2010) suspended the BMSC in the autologous cerebrospinal fluid (CSF) and directly transplanted into the spinal cord of 10 patients with amyotrophic lateral sclerosis (ALS). They concluded that BMSC transplantation is safe for patients with ALS [47]. These studies indicate that BMSC transplantation may at least cause no serious adverse effects.

However, further studies would be essential to scientifically confirm therapeutic effects of BMSC transplantation in CNS disorders. In fact, some investigators take a cautious attitude even if the preferable results are reported, because a blinded placebo-controlled study is very difficult to employ in these clinical trials on cell transplantation therapy [51, 52]. When considering better strategy to establish BMSC transplantation as a scientifically proven therapy in clinical situation, we should learn the lessons from the long (> 50 years) history of the development of neuroprotective drugs.

Lessons from Preclinical Studies for Neuroprotective Drugs

It is well known that despite much animal research concerning the pathophysiology of focal brain injury, little of this work has translated into effective treatment modalities for stroke in humans [53]. As recently pointed out by Savitz and Fisher [1], a large number of neuroprotective drugs have demonstrated varying degrees of effectiveness in preclinical models of ischemic stroke. Of these, totally 15 agents advanced to phase III clinical trials by now. However, none of them was proven to improve the outcome of patients with ischemic stroke. They raised some major problems that account for the failures of these phase III clinical trials [1]. First, inadequate preclinical testing may account for it. For example, in certain studies, some agents were administered just after the insult and their effects on infarct volume were examined only at 24 hr in the rat models of focal cerebral ischemia. Clinical trials were started on the basis of these results, although no data on long-term neurological outcome or testing in aged, diseased animals or a second species beyond rats have been published. Publication bias may partly be responsible for the failure of clinical trials, because any scientific journals hesitate to accept negative results in animal studies for neuroprotective drugs. Second, Savitz and Fisher [1] pointed out that inadequate clinical testing may explain why some phase III trials of neuroprotective drugs were not beneficial. Thus, many experimental studies have previously found their neuroprotective effects when the agents were administered before or just after the insult of cerebral ischemia. In the majority of phase III clinical trials, however, the agents were administered 6 to 8 hr or even longer after stroke onset. Therefore, a significant dissociation in therapeutic time window exists between animal experiments and phase III clinical trials. In addition to time window considerations, the stroke subtype of patients enrolled in phase III clinical trials widely varies. Thus, the majority of experimental studies have used transient or permanent middle cerebral artery (MCA) occlusion in rodents, which simulates embolic infarct in human. However, previous clinical trials have enrolled multiple stroke types such as lacunar infarct and subcortical white matter infarct. Some neuroprotective drugs may have no impact on white matter ischemic injury.

Based on these historical considerations, the first meeting of Stroke Therapy Academic Industry Roundtable (STAIR) was organized and the members published the recommendation statement for standards regarding preclinical neuroprotective and restorative drug development in 1999 [53]. The recommendations included that the effects of neuroprotective drugs should be assessed by analyzing both histological and functional outcome over an extended period, using appropriate animal stroke models. Precise evaluations of an adequate dose-response

effect over a reasonable time window are also recommended.

Subsequently, phase III clinical trial has been adopted to evaluate the beneficial effects of NXY-059 in acute ischemic stroke. NXY-059 is a newly developed spin trap agent that aggressively scavenges reactive oxygen species, and is proven to ameliorate tissue damage due to focal cerebral ischemia in both rodents and primates. The agent has clinically relevant therapeutic window, because it significantly reduces infarct volume and improves neurological function even when administered 4 to 6 hr after the insult [54, 55]. Many investigators believed that NXY-059 was the first neuroprotective agent that fulfilled many of the STAIR recommendations for preclinical testing. In the first phase III clinical trial named as Stroke-Acute-Ischaemic-NXY-Treatment (SAINT-I), 1,722 patients were enrolled up to 6 hr after stroke onset and the modified Rankin scale at 90 days was chosen to measure their functional outcome. As the results, NXY-059 significantly improved patients' outcome [56]. The second SAINT-II trials enrolled 3,195 patients, but could not reproduce the beneficial effects of NXY-059 for them [57]. Consequently, NXY-059 was then withdrawn from further development.

As pointed out by some investigators [1, 58], the failure of NXY-059 in the SAINT-II trial has cast a pall on the field of development of neuroprotective drugs for acute ischemic stroke. They have proposed that it is essential to bridge the still existing gap between preclinical studies and clinical investigations in order to achieve clinical application of neuroprotective drugs. Thus, most of animal studies measure infarct volume as a fundamental indicator to assess their neuroprotective effects. However, previous phase III clinical trials use disability and neurological deficit scales such as modified Rankin scale and Barthel Index. Even phase III clinical trials of NXY-059 did not determine the location and size of cerebral infarct. In their review article, therefore, they have suggested that the future clinical trials should include a biologically relevant end point. For example, it may be important to directly visualize the activity of neuroprotective agents on damaged tissue in clinical trials. Alternatively, stroke type was not considered when enrolling the patients even in the SAINT trials, although neuroprotective effects were examined in MCA occlusion model in the majority of animal studies. Therefore, they have proposed that future clinical trials should enroll only patients with MCA infarct, but not with small-vessel infarct.

Table 1

1. Evaluate the candidate drug in permanent and temporary occlusion models and in both rodent and gyrencephalic species.
2. Evaluate an adequate dose-response effect over a reasonable time window.
3. Appropriate physiological monitoring and blinding should be performed.
4. Histological and functional outcome measures should be assessed with prolonged survival to ensure that early treatment effects are not lost.
5. If feasible, treatment effects should be con-firmed in both sexes and aged animals.
6. Treatment effects should be replicated in several laboratories, including both industry and academic locations.
7. Data, both positive and negative, should be published.

STAIR Recommendations for Preclinical Stroke Drug Development

Recently, the neurologists and neurosurgeons in United States organized the first Stem cell Therapeutics as an Emerging Paradigm in Stroke (STEPS) meeting in 2007, and recommended the following translational criteria for designing laboratory studies on cell therapy for stroke (Table 2). They have concluded that the efficacy of cell transplantation therapy should be tested in multiple models of focal stroke, in two species, in both genders, and in multiple laboratories prior to clinical translation [59].

Table 2

1. The use of appropriate species and clinically relevant type of stroke models
2. The standardization of outcome measures and treatment protocols
3. The need for imaging of cell tracking and host response
4. The requirement for safety indices
5. A call for demonstrating mechanisms of action underlying restorative therapies in ischemic stroke

STEPS Recommendations for Preclinical Cell Therapy Studies

Questions in BMSC Transplantation To Be Answered

The authors strongly believe that there are several questions to be answered prior to clinical application of BMSC transplantation for CNS disorders in order to establish as the scientifically proven treatment entity. They include cell expansion technique, treatment protocol, and cell tracking technique.

First, it would be critical to establish the feasible protocol to expand the human BMSC safely and rapidly. Thus, the BMSC harvested from the animals and humans are cultured in the medium including fetal calf serum (FCS) in the majority of animal experiments. Similar protocol has been applied even in clinical trials [45, 47-49]. However, the FCS carries the potential risk of prion, viral, or zoonoses contamination. The FCS may also provoke immunological reactions against xenogenic serum antigens. Based on these considerations, several attempts have been made to culture the BMSC in the medium without FCS. For example, autologous human serum is known to efficiently expand the human BMSC, but require huge amount of serum. Allogenic human serum, however, leads to growth arrest and death of hBMSC. Very recently, we have evaluated whether human platelet lysate (PL) would be useful to expand the BMSC as the alternative substitute. As the results, we have found that the BMSC expanded with the FCS-free, PL-containing medium retain their capacity of migration, survival, and differentiation, and significantly promote functional recovery when stereotactically transplanted into the infarct brain. The PL may be a clinically valuable and safe substitute for FCS in expanding the hBMSC to regenerate the infarct brain [60].

Second, it is still undetermined when the BMSC should be transplanted into the damaged CNS to achieve maximal therapeutic effects. As described above, the BMSC are transplanted *within 24 hours or 7 days* after the insults in the majority of animal studies. Baksi et al. (2006) reported that transplantation within 14 days of spinal cord injury provided significantly greater grafting efficiency than more delayed delivery, when the BMSC were intrathecally injected [61]. However, there are few studies that denote the effects of BMSC transplantation in chronic stage of CNS disorders except for spinal cord injury [62-64]. Despite these observations in animal experiments, the bone marrow-derived cells are usually transplanted several weeks (or even several months) after the onset in previous clinical trials [45, 46, 49, 50, 65, 66]. Therefore, a considerable gap of treatment protocol exists between animal experiments and clinical trials, which may

correspond to "*inadequate preclinical testing*" in the development of neuroprotective drugs (*see above*). The difficulty to rapidly expand the autologous BMSC may explain the "*delayed*" therapy protocols in clinical trials. Unfortunately, there is still no optimal culture protocol that enables to expand the autologous BMSC enough for transplantation therapy within 7 days after the onset of CNS disorders such as cerebral infarct. In recent clinical study, indeed, about 30 days were required to obtain 1×10^8 cells of autologous BMSC with conventional culture technique [45]. In order to solve the dissociation between animal experiments and clinical testing, we recently cultured the mice BMSC with granulocyte colony-stimulating factor (G-CSF). As the results, almost all of BMSC were immunologically positive for the G-CSF receptor. G-CSF significantly enhanced their proliferation by modulating their cell cycle and also upregulated their production of NGF, HGF, and SDF-1a [38]. Such pharmacological "*activation*" of the cultured BMSC may contribute to successful clinical application of BMSC transplantation therapy for CNS disorder in near future.

Third, the BMSC have been transplanted directly, intravenously or intrathecally in the majority of previous animal experiments. Although direct injection permits efficient delivery of the donor cells to the damaged tissue, it has potential risk for additional CNS injury. The injection of cells afloat in the medium may also result in limited cell retention and transplant survival. Intravenous or intrathecal transplantation is attractive because of its non-invasive, safe technique for the host CNS, but has been reported to result in less pronounced cell migration and functional recovery than direct cell transplantation [62]. Blood-brain barrier should also be permeable for the intravenously administered cells to migrate into the brain [67]. Therefore, optimal transplantation technique should be developed to serve maximally safe and efficient results, when applying BMSC transplantation into clinical condition. Alternatively, the intra-arterial injection of BMSC may be valuable to non-invasively deliver them to the damaged CNS [68]. Very recently, we have also confirmed the therapeutic effects of intra-arterial BMSC transplantation in TBI, using *in vivo* optical imaging (*submitted data*). More interestingly, the emerging field of tissue engineering may also provide promising alternatives. Thus, tissue-engineering approaches are designed to repair lost or damaged tissue through the use of cellular transplantation and biomaterial scaffold. Nowadays, the degradable biomaterials have been accepted as a valuable "scaffold" to fix and stabilize the transplanted cells in other organs such as bone, cartilage, heart and skin. Until recently, however, there have been only a small number of studies that denote effective scaffolds for cell transplantation for CNS disorders [69]. Therefore, we have recently assessed whether fibrin matrix can act as an injectable, valuable scaffold in BMSC transplantation for the injured CNS tissue. As the results, fibrin matrix markedly improved the survival and migration of the BMSC transplanted into the hemisected spinal cord or injured neocortex of rats [7, 70]. In addition, thermoreversible gelation polymer (TGP) hydrogel may also be one of candidates for the scaffolds to provide the suitable environment for the donor cells [71]. Such strategy of tissue engineering would be one of therapeutic options for CNS regeneration in patients with injured CNS.

Finally, it would be essential to develop the techniques to track the fate of the transplanted cells in the host CNS serially and non-invasively in order to guide further advancements in transplantation research and its future clinical application. Cell tracking technique would also be important as a "*biologically relevant end point*" in clinical situation (see above). Recent studies have suggested that magnetic resonance (MR) imaging, nuclear imaging, and optical imaging can be the candidates for it. In previous animal experiments, the donor cells can be identified on MR imaging by labeling with a SPIO agent [72-74]. MR imaging can image intact, opaque organisms in three dimensions with good spatial resolution, but requires

long imaging times and consequently slows date acquisition because of the low sensitivity. More importantly, magnetic nano-particles that label the donor cells cannot be succeeded to all the cells during their proliferation [16]. Nuclear imaging can also detect the transplanted cells by labeling them with radioactive tracers. Correa et al. (2007) recently labeled the autologous bone marrow mononuclear cells (BMMNC) with ^{99m}Tc-hexamethylpropylene (HMPAO), and injected them into a patient with ischemic stroke through a balloon catheter. The transplanted cells were visualized on single photon emission tomography (SPECT) [75]. Nuclear imaging can detect the target with high sensitivity, but has the difficulty to monitor the donor cells for several weeks because of relatively short half-life of clinically available tracers. On the other hands, optical imaging technique may also serve future technology to visualize the BMSC engrafted in the damaged CNS. Previously, we isolated the BMSC from the green fluorescence protein (GFP) transgenic mice, and transplanted the GFP-expressing BMSC into the mice brain subjected to cerebral infarct. As the results, *in vivo* fluorescence imaging technique could serially visualize the BMSC engrafted in the ipsilateral neocortex through the skull during 4 weeks [10]. Similarly, the GFP-expressing BMSC could be identified through the dura mater, when transplanted into the injured spinal cord of mice [15]. However, it is difficult to detect the fluorescence emitted from GFP through the skin because of its relatively short wavelength. Very recently, therefore, we have employed quantum dot as a novel cell tracer. The quantum dot emits near-infrared (NIR) fluorescence with much longer wavelength (800nm) that can easily penetrate the living tissue. We labeled the BMSC with quantum dots and directly transplanted them into the ipsilateral striatum of the rats subjected to permanent MCA occlusion. As the results, *in vivo* fluorescence imaging can clearly visualize the BMSC through the skull and scalp for at least 8 weeks after transplantation [39].

Concluding Remarks

In this review, we have discussed recent progress in basic and clinical research on the field of BMSC transplantation for CNS disorders. Furthermore, based on the history of the development of neuroprotective drugs, we have critically conferred the questions to be clarified in order to introduce BMSC transplantation therapy into clinical situation as a scientifically proven therapy. Further studies would warrant to provide essential information on full-scale clinical application of BMSC transplantation for patients with various CNS disorders.

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