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# Stem Cell Therapy for Traumatic Brain Injury: A Progress Update

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## Abstract

Stem cell transplantation is a promisng therapy for traumatic brain injury. Several phase I or IIa clinical trials in recent years have indicated that this treatment is safe and feasible in patients. Functional outcome was partially improved by this treatement. This review collected 112 preclinical animal studies conducted from 1996 to 2017 and analyzed the key factors affecting the efficacy of stem cell transplantation in treating traumatic brain injury (i.e., animal models, types and doses of transplantated stem cells, intervetion routes, time window of treatment and period of follow up etc.), and the associated action mechanism of transplanted stem cells (i.e., cell replacement, secretion of neurotrophic factors and suppresion of inflammtion). The strategies for enhancing optimal stem cell investigations are discussed and the data collected here would be of great value for planing future clincal trials.

**Keywords: Stem cells; Traumatic brain injury; Preclinical; Transplantation**

## Introduction

Traumatic Brain Injury (TBI) is an unpleasent accident frequently encounted in daily life. Strong force suddenly destructs brain tissue and vascular struction that result in immediate tissue contusion, cellular edema and hemorrhage. The intracranial pressure is rapidly elevated, blood flow is simultaneously decreased. These events initiate various subsequent responses and amplify the severity of primary brain damage. Injured neurons, axons and tracts fail to conduct normal activity and eventually lead to a high rate of disability. Currently there is no effective pharmacological agents to completely rescue the damaged brain tissue. Stem cells have the ability of self-renew and produce differented tissues that are proposed to rebuild this impaired biological structure. In response to traumatic injury, endogenous neural stem cells in the subventricular zone and dentate gyrus are activated to produce neurogenesis and angiogenesis [1,2]. Animal studies have demonstrated that these cells are proliferated and migrate toward to the injury site. They are able to differentiate into neurons and glia and integrate with the local cells. Unfortunately, the number of new generated cells is not sufficient to reach the urgent need of the body. Transplantation is the efficient way to deliver stem cells into the injury site and solve this shortage. Preclincial animal studies were done in several disease models with different types of stem cells [3]. Clinical trials have been performed in stroke [4,5], spinal cord injury [6-8], neurodegenerative diseases [9] and other diseases [10-13]. Both preclinical and clinical trials indicate that stem cell transplantation has the potential to be used for treating patients.

## Clinical Trials

Up to date, seven clinical trials of stem cell transplantation for TBI have been reported from China [14-16], India [17] and US [18-20] (Table 1). There are 6 studies using autologous bone marrow mesenchymal stem cells or mononuclear cells were used and one study using umbilical cord blood mesenchymal stem cells. First clinical trial was done in 2008 by a Chinese group in which they directly injected autologous bone marrow derived mesenchymal stem cells into the site of brain damage when second surgery for skull repair was performe [14]. Second dose of stem cells was administrated intravenuously at 5-12 days after surgery. Laboratory and clinical assessments indicated no any immediate and delayed toxicity in these patients during a 6 months of follow up. One patient had an epilepsy occurred twice in the first 2 months and relieved by phenytoin sodium and topamax. It is not sure whether it was related with the treatment. The Barthel index, a parameter of neurological functions, in these patients was significantly improved at the 6$^{th}$ month

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**Table 1:** Clinical trials of stem cell therapy in traumatic brain injury.

| Year | Patients | Cell type | Route | Time window | follow up | Result | References |
|---|---|---|---|---|---|---|---|
| 2008 | 7 (including 1 child) | ABMMSC | Direct injection + IV infusion | > 1 month | 6 months | All survived with good recovery and epilepsy was found in one patient | Zhang ZX et al. [14] |
| 2011 | 10 (children only) | ABMMC | IV infusion | < 48 hours | 6 months | Good recovery and no serious toxicity | Cox C et al. [19] |
| 2013 | 97 | ABMMSC | Lumbar puncture | > 1 month | 2 weeks | 39% patients improved, 2 had transient fever and 1 had headache | Tian C et al. [15] |
| 2013 | 20 treated and 20 controls | UCMSC | Lumbar puncture (4 times) | > 3 months | 6 months | Motor, sensory and balance functions Improved | Wang S et al. [16] |
| 2015 | 14 | ABMMC | Lumbar puncture | > one year | 6 months | Multiple functions improved in 33 to 73% patients | Sharma A et al. [17] |
| 2015 | 10 (children only) | ABMMC | IV infusion | < 48 hours | 3 weeks | Therapeutic intensity reduced | Liao GP et al. [18] |
| 2017 | 15 treated and 10 controls | ABMMC | IV infusion | < 48 hours | 6 months | No serious toxicity and cytokines downregulated. | Cox C et al. [20] |

ABMMC = Autologous Bone Marrow Mononuclear Cells; ABMMSC = Autologous Bone Marrow Mesenchymal Stem Cells; UCMSC = Umbilical Cord Mesenchymal Stem Cells

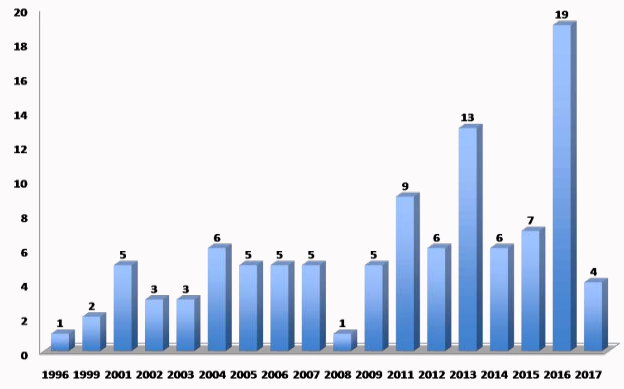


**Figure 1:** Number of published TBI preclinical studies of stem cells in each year.

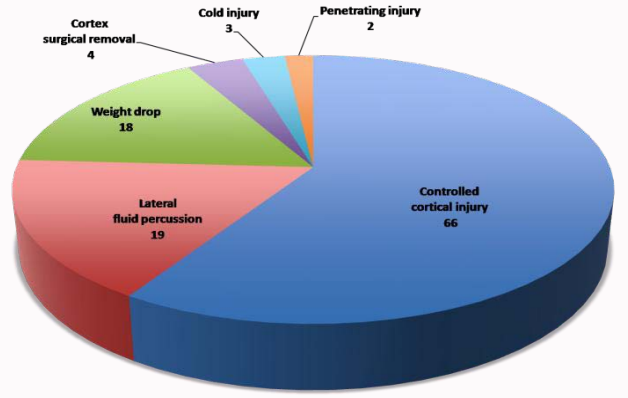


**Figure 2:** Number of published TBI preclinical studies of stem cells in each injury model.

following stem cell transplanatation. In other three trials of TBI patients, stem cells were given through lumbar puncture at subacute and chronic phases [15-17]. Most of patients did not have any symptoms assocaited with cell transplantation during the 6 months of follow up except two with transient fever and one with headache. The functions of balance, voluntary control, memory, limb activity, ambulation and gait patterns, speech, psychological status etc. were partially improved at the 6$^{th}$ month post treatment [17]. Metabolism measured by PET CT scan was improved as well. Eleven of 24 patients with persistent vegetative state showed a significant improvement of consciousness [15]. In the clinical trial of umbilical cord mesenchymal stem cell transplatation, the treated patients recovered better in motor, sensory, balance, self-care, sphincter control, communication, sococial cognition at the 6$^{th}$ month after treatment compared to the patients of control group ($p < 0.05$) [16]. The fate of transplantated cells was not tracked using MRI or CT image. It remains questions whether these transplanted cells survived and how well they participated in re-building local tissue structure. Three US trials were performed by the group in the University of Texas Medical School at Houston in which intravenous transplantation of autologous bone marrow mononuclear cells was given in the acute phase of brain trauma (<48 hours). First clinical trial was Phase I in ten children TBI patients aged 5 to 14 years old [19] and second clinical trial was Phase I/IIa in 25 adult TBI patients (15 treated and 10 sham) [20]. A 6 months of follow up was done in both clinical trials. No any serious toxicity was found in those patients except a mild pulmonary toxicity in the highest dose group of adult patients (12 X $10^6$ cells) that was considered not clinically significant. Plasma biomarker analysis had a dose-dependent trend for TNF-α suppression and a significant reduction in IL-1β, IL-10 and IFN-γ in the high dose group [20]. Third clinical trial was a retrospective cohort study [18] from the phase I pediatric patients and reported that autologous bone marrow mononuclear cells significantly reduced therapeutic intensity, the Pediatrix Intensity Level of Therapy (PILOT) scale. The result fits with the change of plasma biomakers reported in adult patients. The post-injury structure preservation of critical regions of interest in MRI image correlated with functional outcomes [20]. Dose dependent effect was not found in functional outcome assessment probably due to insufficient samples (n =5 per group). In this clinical trial, 217 of 232 patients with cell transplantation were excluded and only 15 patients enrolled in the study. Whether the stem cells transplanted at acute phase of TBI have the effect on functional recovery should be further evaluated in the following Phase II studies.

## Preclinical Trials

Using the Magnetic Particle Imaging (MPI), Zheng et al. [21] monitored the transplantation, biodistribution and clearance of mesenchymal stem cells after they were intravenously administrated in mice. Although this technology can be adapt to human use, the number of stem cells transplanted in patients still is difficult to be quantified. Originally, stem cell transplantation was designed to replace the damaged or lost cells so neurological deficit can be restored. However, the concept of cell replacement so far was not completely proved in animal studies. Neurotrophic factors increased and inflammatory responses suppressed following transplantation that are considered as two major action mechanisms at present. Whether the effect is persisent or can be replaced by other

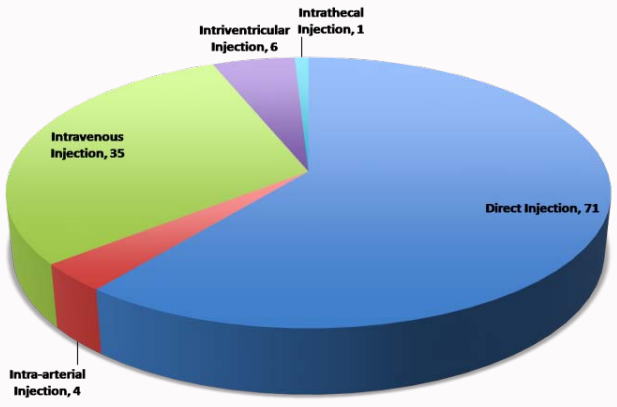


**Figure 3:** Number of published TBI preclinical studies of stem cells in each route of cell administration.

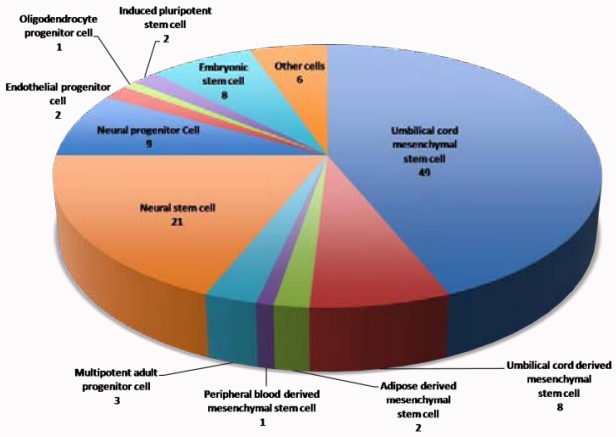


**Figure 4:** Number of published TBI preclinical studies of stem cells in each type of stem cells.

pharmaceutical agents needs to be investigated. Chang et al. [3] performed a meta-analysis of efficacy in pre-clinical human stem cell therapies for TBI and found that intra-lesional delivery resulted in larger effect than intravenous or intraventricular delivery. The details of those animal studies were not discussed. The purpose of this review is to summarize 112 peer reviewed stem cell papers for TBI published from 1996 to 2017, and analyze key factors affecting the outcome and discuss the strategies for enhancing translational stem cell research. There is a trend that more stem cell TBI studies performed in recent years, especially 2016 in which 19 of 112 papers were published (Figure 1), indicating the importance and clinical need. Rats and mice are the only species in these experiments. 91 rat studies and 21 mice stuides were included. Following transplantation, stem cells have to migrate toward the injury site in order to integrate with other local cells. As the size of brain in small animals is different from human, future preclinical trials should be also considered to do in large animal model so the therapy efficacy will be accurately assessed. Use of clinical revelant injury models is also important. Previous studies were done in various models, such as weight drop [22,23], controlled cortical injury [24,25], lateral fluid percussion [26,27], penetrating injury [28], surgical removal of cortex [29-33], and cold injury [34,35] (Figure 2). Closed TBI occurs more often in real world and is accompanied with a higher intracranial pressure. Open TBI has a high risk of infection because of the trauma wound. These factors should be considered when stem cells are transplanted at acute phase because the host microenvironment determines the survival rate of these cells after transplantation. Studies are recommended to conduct in both TBI models. Currently more than 80% studies were performed in open TBI models, including controlled cortical injury, lateral fluid percussion, penetrating injury, surgical removal of cortex and cold injury.

The administration route of stem cells is a critical factor that determines how effective they reach the injury site. Bonilla et al. [36,37] reported that direct injection of bone marrow stromal cells into the lesion site significantly improved neurologic deficit in animals with moderate TBI, not animals with severe TBI and this treatment effect was absent when same dose of bone marrow stromal cells was intravenuously given. Harting et al. [38] intravenosly administrated mesenchymal stem cells in rats at 24 hours following TBI and found that by 48 hours post-infusion, 1.5% to 3.7% cells traversed the lungs and reached the arterial circulation, 0.295% cells reached the carotid artery and a very small percentage reached the cerebral parechyma (0.0005%). One group reported that autologous bone marrow mononuclear cells they used are small and able to pass through the lungs [20]. They did not provide the numbers. Intravenous route and lumbar puncture are two preferred ways for stem cell delivery in previous TBI clinical trials. Although the direct injection is the best effective way to deliver stem cells into the lesion site for preclinical studies, other routes are recommended to be further tested when the effect of cell transplantation is present. Currently, 7 of 112 previous preclinical studies had stem cells delivered via CSF (Figure 3). Understanding the time courses these stem cells migrate and survive in the injured brain is helpful for designing the clinical trials.

There are more than 10 different types of stem cells employed in previous TBI studies (Figure 4) [25,39-47]. Bone marrow and umbilical cord mesenchymal stem cells are the most common transplanted cells accounting for 44% and 7% studies, respectively. Mahmood et al. [48] reported that bone marrow stromal cells was transplanted into the injury site at one week after controlled cortical injury in rats and these cells survived in the brain and expressed markers of neurons, astrocytes and oligodendrocytes at 3 months following treatment. Other commonly used cells are neural stem cells and progenitor cells accounting for about 30% studies. Haus et al. [49] reported that approximately 9% to 25% of Human Neural Stem Cells (hNSCs) transplanted directly into the injury site, survied for at least 5 months post-transplantation and differentiated into mature neurons (18% to 38%), astrocytes (13% to 16%) and oligodendrocytes (11% to 13%). Beretta et al. [50] directly injected hNSCs into the lesion and examined the brains at 14 weeks after injection. Of the initial 500,000 transplanted cells, 19,344 ± 3,816 cells survived in TBI brains. The predominant fate of these cells were neuronal (13.3% ± 2.2%), astroglial (11.5 % ± 1.7%), oligodendroglial (6.4% ± 2.1%) and undifferentiated state (8.3% ± 1.9%). Transplantation with undifferentiated embryonic stem cells has a risk of tumor formation in brain. Riess et al. [51] reported that two of 10 animals revealed tumor formation at 6 weeks following the procedure implanted stem cell directly at the injury site at 3 days post-injury. Such adverse event should be carefully avoided. Autologous stem cells are the best candidate for transplantation because the reject reaction is the biggest factor affecting the cell survival in additional to harmful substances in local microenviroment. Otherwise, immunosuppression is required for the rest of life. Cyclosporine and immunodeficient rats were used in preclinical studies [49,52,53]. It is reported that the survival

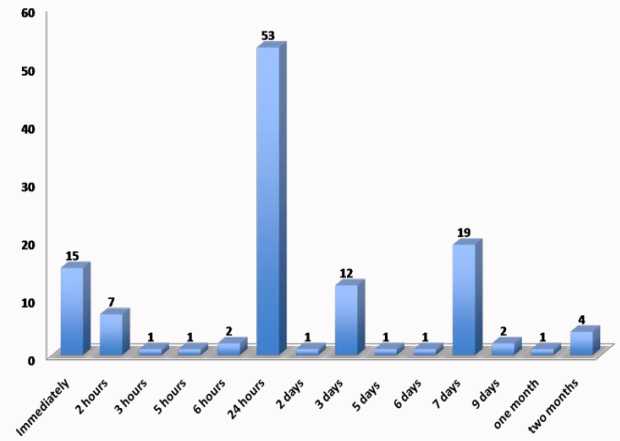


**Figure 5:** Number of published TBI preclinical studies of stem cells in each transplanation time window.

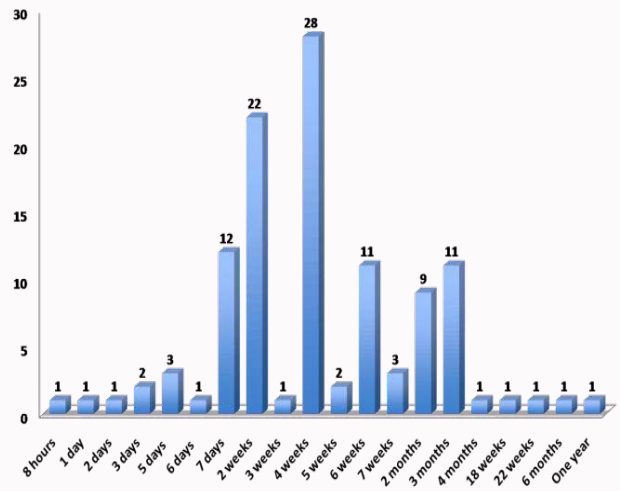


**Figure 6:** Number of published TBI preclinical studies of stem cells in each follow up duration.

of graft was extremely poor in the non-immunosuppressed group [41]. Transplantation time window varied among studies (Figure 5) [51,54-56]. In 53 of 122 studies, stem cells were transplanted at 24 hours post-TBI [57,58]. Other common time points are immediately [31,53], 3 days [59,60] and 7 days [28,61] post-TBI. Only in 7 of 112 studies, transplantion was delayed at 9 to 60 days post-TBI [36,37,49]. Injury progress in brain is complicated. Tissue dema, inflammatory response, oxidative stress and high intracranial pressure exacerbate the severity. The microenviroment at acute phase is poor for survival of transplanted stem cells. As Molcanyi et al. [52] reported, a significant post-traumatic inflammatory responses impaired survival and integration of implanted stem cells. However, neurological deficits were improved even when stem cells were transplanted at the acute phase of TBI, indicating the underlying mechanism of cell therapy not simply derived from cell replacement. Long-term effect was observed in most of previous TBI studies (Figure 6). Shear et al. [61] reported that approximately 100,000 Neural Progenitor Cells (NPC) were directly injected into the injury site at one week post-TBI in mouse controlled cortical injury model and follwed up 14 months. Transplanted NPCs survived in the host brain up to 14 months and enhanced motor and cognitive recovery. These cells were co-labeled for NG2, an oilgodendrocyte marker, but not for neuronal, astrocytic and microglial markers.

The action mechanisms of cell therapy were investigated in previous studies that focused on cell replacement, neurotrophic factors and suppression of inflammation. In fact, there was no solid evidence proving this concept of cell replacement in TBI because no histological observation or MRI image presented the injured brain being repaired with transplanted stem cells. Beretta et al. [50] reported the transplanted Human Neural Stem Cells (hNSC) survived and differentiated into neurons, astrocytes, oligodendrites and some undifferentiated cells at 14 weeks after injection. The survival rate was 3.8% and cognitive functions were also improved although the number of surviving hNSC was not correlated with the performance on either elevated plus maze or novel place recognition task. The lesion size was not decreased. Chen et al. [62] reported that implantated cells migrated toward the area of injury at 45 days and disappeared at 90 days. Riess et al. [51] reported that the embryonic stem cells transplanted at 3 days post-TBI survived 100% at 1 week and were only detected in one animal at 7 weeks. It was suggested that use of an immunosuppressant such as Asilo-GM1, optimal administration route and time window would improve the survival of transplanted stem cells. In immunosuppressed C57/Bl6 mice, NSC survived in the hippocampus and cortical area adjacent to the injury cavity for as long as 13 weeks after treanplantation and expressed neuronal or astroglial markers but no marker of oligodencrocytes [56]. Lu et al. [63] used hMSC in rats subjected to TBI and lesion volume was not significantly changed. Then collagen scaffold is selected as a cell delivery system for hMSC transplantation in their study. Collagen scaffold populated with 2 X $10^6$ cells were transplanted into the lesion cavity of the injured cortex 4 days after TBI and reduced the lesion volume at 35 days. Guan J et al. [64] also found that collagen scaffold efficiently improved cell survival and neurite growth *in vivo*, resulting in better neural functioal recovery and improved brain metabolism at 4 weeks. Xiong et al. [65] implanted collagen scaffold at 7 days after TBI, the hMSC improved spatial learning and sensorimotor function, enhanced angiogenesis at 6 weeks. However, Spurlock et al. [28] transplanted 400,000 hNSC into immunosupressed rats, neither cell proliferation nor glial lineage marker were detected at 16 weeks post-transplantation although rats had shorter latency to platform in a Morris water maze test at 8 weeks post-transplantion. It is not easy to enable stem cells replace the damaged or lost neurons.

The neurotrophic factors secreted from stem cells truly exist [54,66,67]. Gao et al. [24] reported the first direct demonstration of the release of a neurotrophic factor in conjunction with stem cell grafting. Microdialosis was performed in TBI rats at 7 days and Glial Cell Derived Neurotrophic Factor (GDNF) levels were detected in the samples from both intact and injured hippocampus. After grafting hNSCs into the injured hippocampus, there were 30% and 103% increases in GDNF secretion from the injured side when compared to TBI plus vehicle and TBI alone, respectively. Furthermore, an average 47% increase in GDNF levels was detected when comparing intact hippocampi of hNSC-grafted rats to intact hippocampi injected with the vehicle. In the cultured medium, human MSCs releases growth factors and hypoxia preconditioning significantly increased the releases of Hepatocyte Growth Factor (HGF) and Vascular Endothelial Growth Factor (VEGF) [68]. Because tissue replacement by MSCs cannot account for brain functional recovery after TBI, Chen et al. [62] examined whether MSCs produced Nerve Growth Factor (NGF) and other neurotrophic factor. After ICV injection, ELISA result of CSF

samples showed NGF significantly increased in both sham and injury mice at 14 and 45 days. Yan et al. [69] transplanted human amnion derived mesenchymal stem cells in the injury site at 4 days post-TBI and quantitative real-time PCR showed that the neurotrophic factors such as Brain Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), Neutrophin 3 (NT-3), Glial Cell Derived Neurotrophic Factor (GDNF) and Ciliary Neurotrophic Factor (CNTF) were markedly upregulated at 7 days post-transplantation [69]. These neurotrophic factors were confirmed by western blot analysis. Post-TBI inflammatory response was suppressed by transplanted stem cells in several studies [70-72]. Bedi et al. [73] intravenously administrated autologous bone marrow derived mononuclear cells at 72 hours post-TBI and found the blood brain barrier permeasbility reduced and the apoptosis of activated microglia increased by cell treatment at 24 hours post-injection. Kota et al. [74] transplanted MSCs into the brain at 72 hours after controlled cortical injury and measured the number of activated microglia at 7 days and 150 days post-transplantation. Activated microglia was significantly decreased by MSCs at both acute and chronic phase of TBI [74]. In another study, bone marrow MSCs were intraveously transplanted into rats 2 hours after TBI and also significantly reduced the number of microglia/macrophages, the density of infiltrated MPO neurtrophils, and lymphocytes at 72 hours post-TBI [72]. Levels of the proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 were significantly decreased at 12, 24 and 72 hours in the MSC treated group. The chemokines MCP-1, MIP-2 and RANTES were reduced at 12, 24 and 72 hours as well. The products of anti-inflammatory cytokines IL-10, TGF-β1 were increased at 12 and 72 hours in the MSC treated group. No changes in cytokines IL-1a and IL-4. MSC treatment upregulated TSG-6 expression, an inhibitory factor [72]. Galindo et al. [71] used a chilled needle and injured the motor cortex 4 times and then immediately implanted 100,000 MSCs and observed the inflammatory responses at 24 and 30 days. MSCs significantly decreased relative expression of TNF-α, IL-6, and IL-10 at 24 hours. There was no change of IL-4. MSCs also decreased TNF-α expression at 30 days, but IL-6 was significantly increased. IL-4 and IL-10 were not detectable [71]. The effect of MSCs on TBI induced key inflammatory cytokines was also reported in patients [20]. It is no doubt that transplanation of stem cells into the injured brain promotes neurological and cognitive functional recovery. The questions we have to answer at present include: 1) how to enhance the survival of transplanted stem cells? Pre-conditioning of stem cells or combination therapy with protective agents should be considered to increase the survival ability of cells and improve the microenvironment. 2) why the transplanted stem cells disappear from the brain even autologous stem cells? TBI induced immuno responses should be further studied because the stem cells survived better in immunosuppresion animals or when cyclosprone was used in previous studies. There is lack of direct comparison among different types of stem cells. Studies should be done to undestand the efficacy difference among them and guide the use of the optimal stem cells. 3) what is the best imaging technque for tracking the transplanted stem cells? New technology should be developed to allow the researchers monitor the stem cells participating in the repair process of brain. 4) Do rats and mice truly represent human? Studies should be also done in large animals. The stem cells may take a longer time to migrate into the targeted region in human. 5) what time courses of neurotrophic factors in injured brain? The levels of neurotrophic factors depend on the survival status of stem cells and will be decreased when the transplanted cells begin to degenerate at the site of implantation, affecting the remodeling. Studies should be used to determine the time courses of these neurotriphic factors and define whether exogenous growth factors may effectively serve as supplement. 6) Does closed TBI induce inflammatory responses different from open TBI? Most of previous studes were done in the model of controlled cortical injury, a transcranial operation. Studies should be done to determine the inflammatory responses in closed TBI and also to compare the efficacy between stem cells and anti-inflammation drugs on TBI induced inflammatory responses. Precise results we collected from preclincal studies would provide the best chance to advance the stem cell therapy in treating patients with TBI.

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