Review paper

Folia Neuropathologica

Recent advances in the treatment of traumatic brain injury with autologous and non-autologous multipotent stem and progenitor cells: preclinical models and clinical trials

Mujahid Alizada[1,2], Shu Lin[1,3], Hongzhi Gao[2]

[1]Centre of Neurological and Metabolic Research, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China, [2]Department of Neurosurgery, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China, [3]Diabetes and Metabolism Division, Garvan Institute of Medical Research, Sydney, Australia

Folia Neuropathol 2021; 59 (3): 298-316 *DOI: https://doi.org/10.5114/fn.2021.108536*

Abstract

Traumatic brain injury (TBI) is a global health issue which causes millions of deaths and disabilities every year. The survivors of TBI may suffer from sensorimotor dysfunction, memory and cognitive disturbances, hearing and vision deficits, and various psychological problems. The primary insult may damage neurons, cerebral vessels and the blood-brain barrier, causing reactive astrogliosis and immune response with further damaging consequences. TBI lacks effective therapy. The currently available clinical treatment options include hyperbaric oxygenation, brain stimulation and rehabilitation. In recent years, the research on stem cell treatment of TBI has received extensive attention. Various types of stem cells, such as four types of mesenchymal stem cells, neural stem cells and olfactory ensheathing cells have been tried to treat TBI in clinical trials and preclinical models. This article reviews the research of autologous and non-autologous multipotent stem and progenitor cells for the treatment of TBI in both clinical and preclinical settings.

Key words: *traumatic brain injury, stem cells, transplantation, autologous, non-autologous, clinical trial, preclinical model.*

Introduction

Traumatic brain injury (TBI) is one of the main causes of death and disability in the world [19,91]. The annual incidence rate of TBI in various countries are 55-64 cases per 100,000 people in China [96], 823 cases per 100,000 people in the United States, 811 cases per 100,000 people in New Zealand [62], 107 cases per 100,000 people in Australia [43], 262 cases per 100,000 people in Europe [74]. Moreover, the TBI incidence rates of paediatrics varies greatly from country to country. For example, TBI affects more than 486 children per 100,000 people in Australia and more than 280 children per 100,000 people in the United Kingdom ever year [36,67].

The World Health Organization (WHO) reports that TBI will be the main source of morbidity and mortality with being one of the top health issues burdening financial platforms in the coming years [41,62]. TBI is mainly caused by traffic accidents, falls, gunshots, blasts, warfare and sports-related events [85]. Patients suffering from TBI can manifest motor, sensory and perceptive disturbances

Communicating authors

Prof. Shu Lin, Centre of Neurological and Metabolic Research, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China, e-mail: shulin1956@126.com, and Prof. Hongzhi Gao, Department of Neurosurgery, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China, e-mail: 1564747628@qq.com

along with severe depression, anxiety, and personality changes. In the long term, TBI may also give rise to neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease [81,93].

Damages of neuronal tissues associated with TBI fall into two categories: (i) Primary injury, which is directly caused by mechanical forces during the initial insult. The immediate impact of different mechanical insults to the brain can cause two types of primary injuries: focal and diffuse brain injuries. Studies have demonstrated that the co-existence of both types of injuries is common in patients who suffered from moderate to severe TBI however, diffuse axonal injury (DAI) accounts for approximately 70% of TBI cases [86]. (ii) Secondary injury, which refers to further tissue and cellular damages following the primary insult. The biochemical, cellular and physiological events that occur during the primary injury often progress into delayed and prolonged secondary damages which can last from hours to years. Mechanistically, a number of factors contribute to secondary injuries, which include excitotoxicity, mitochondrial dysfunction, oxidative stress, lipid peroxidation, neuroinflammation, axon degeneration and apoptotic cell death [76].

Based on severity, TBI is classified as mild, moderate and severe with mild being the most common one [33]. The Glasgow Coma Scale (GCS) (Table I) which is a clinical method for evaluating the consciousness level of patients after brain injury was used. The GCS for mild brain injury is 13-15, moderate brain injury is 9-12, and severe brain injury is 3-8 [33,34,94]. TBI initially causes focal or diffuse brain damage due to cutting, tearing and stretching [83,85]. If the violence is severe enough, it can cause damage of the blood-brain barrier (BBB), destruction of cerebral vessels, neurodegeneration, axonal injury, brain oedema and neuroinflammation [41,50,103]. Secondary events caused by TBI may occur hours to days to months post-trauma which leads to cellular, metabolic and molecular changes in the injured brain that further damages neurons [50,93]. Primary focal brain injury can be detected by computed tomography (CT) and magnetic resonance imaging (MRI), while diffuse brain injury requires diffusion tensor imaging (DTI) [46,56,68].

There is no effective therapy for the treatment of TBI. Currently, the clinically available treatment options include hyperbaric oxygen, brain stimulation and rehabilitation [15,19,100]. Due to the lack of promising curative options for TBI-induced primary injury, current research focuses on preventing secondary injury [100]. Moreover, the recent use of various stem cell types such as mesenchymal stem cells (MSCs), neural stem cells (NSCs) and their promising therapeutic effects have brought great hopes for curing both primary and secondary injures of TBI [16,48]. The two key factors affecting the effect of stem cell therapy are the optimal transplantation time and the transplantation route. Various time intervals, such as 1, 2, 7 and 14 days post-injury, as well as routes of administration such as intracranial, intrathecal, intravenous, intra-arterial have been tried, but the ideal time and route is yet to be agreed upon [20].

This article reviews the recent research progress in clinical trials and preclinical models of autologous and non-autologous pluripotent stem and progenitor cells for the treatment of brain trauma.

Olfactory ensheathing cells

Olfactory ensheathing cells (OECs) isolated in 1991 are unique glial cells that protect and support the regeneration of olfactory receptor neurons (ORNs) all the way from peripheral olfactory epithelium to the glomeruli of the olfactory bulb in the central nervous system [4,77]. OECs originally come

Table I. Glasgow Coma Scale (GCS)

Behaviour	Response	Score
Eye opening response	Spontaneously	4
	To speech	3
	To pain	2
	No response	1
Best verbal response	Oriented to time, place and person	5
	Confused	4
	Inappropriate words	3
	Incomprehensible sounds	2
	No response	1
Best motor response	Obeys commands	6
	Moves to localized pain	5
	Flexion withdrawal from pain	4
	Abnormal flexion (decorticate)	3
	Abnormal extension (decerebrate)	2
	No response	1
Total score	Best response	15
	Comatose	≥ 8
	Totally unresponsive	3

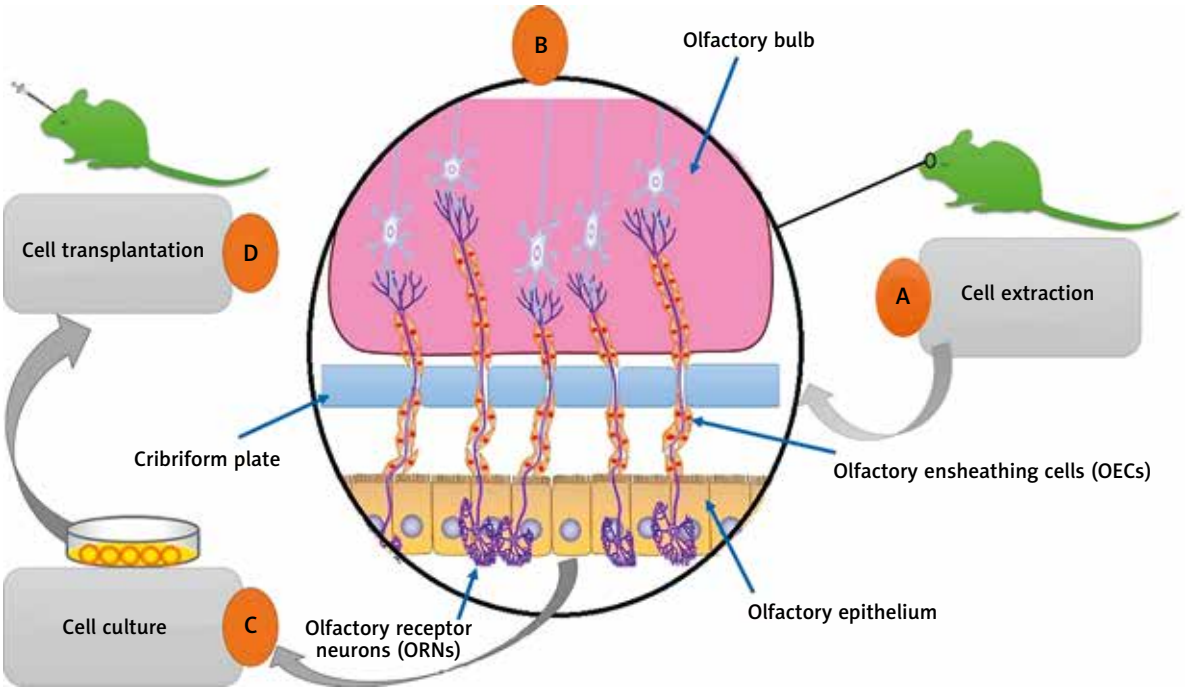

Fig. 1. Schematic view of olfactory ensheathing cells transplantation for the traumatic brain injury (TBI). **A**) Olfactory ensheathing cells (OECs) are usually isolated from the olfactory system of the rat in preclinical models. **B**) Components of the olfactory system showing the olfactory bulb, olfactory epithelium cells, olfactory ensheathing cells and olfactory receptor neurons. **C**) The cells are usually cultured to be identified and labelled before being engrafted. **D**) Specified and labelled cells are usually implanted around the lesion of the TBI model.

from neural crest and can be extracted either from the olfactory bulb (Fig. 1) called olfactory bulb-olfactory ensheathing cells (OB-OECs) or from olfactory mucosa named OECs [4,31,77]. The most specific markers for the identification of OECs are P75, S100b, and glial fibrillary acidic protein (GFAP) [3]. The expression of p75 which is a marker for OECs progenitor cells is inhibited by axonal contact. For example, the OECs will not express p75 if they are placed in contact with axons during culture [98,101].

To explore the neuroprotective effects of OECs in TBI, Wang *et al*. extracted cells from newborn Sprague-Dawley (SD) rats aged 2 to 3 days, identified by p75, labelled with Hoechst 33342, and immediately transplanted into the surroundings of the lesion of a modified controlled cortical impact (CCI) rat model. In order to prevent the occurrence of transplant rejection, the rats were injected with cyclosporine for three days before the injury until the tissue was harvested. On the 14th day post-implantation, neurological function was significantly improved. Histological analysis showed that OECs survived and migrated, growth-associated protein 43 (GAP43) and synaptophysin were enhanced, but the number of apoptotic cells was significantly reduced. In addition, at the molecular level, the Bcl2-associated agonist of cell death (BAD) gene is down-regulated, which is considered to be a potential mechanism for changes in OECs in transplanted brains [98].

Fu *et al*. tried either OECs or bone marrow derived-mesenchymal stem cells (BM-MSCs) alone or OECs and BM-MSCs co-transplantation to treat TBI. OECs were derived from SD rats, identified with p75 antibody, and labelled with Hoechst 33342. BM-MSCs were extracted from transgenic green fluorescent protein (GFP) mice and identified with CD44 antibody. Cells were transplanted singly or jointly around the lesion immediately after injury. To control the rejection of transplantation, the immunosuppressant cyclosporine A was given every day from 3 days before transplantation to the end of the experiment. Fourteen days after implantation, the neural function was improved in all three groups, being more obvious in the co-transplanted group.

Histological studies showed increased number of surviving neurons and reduced reactive astrocytosis in the host brain. Moreover, molecular studies have shown that the expression of ciliary neurotrophic factor (CNF) is low, and the Janus kinase/signal transducer and activator of the transcription (JAK/STAT) signalling pathway is activated [25].

Liu *et al*. were interested to know the therapeutic effects of combined NSCs and OECs in TBI. They isolated NSCs from mouse embryonic hippocampus and OECs from the olfactory bulb of neonatal rats. NSCs and OECs were labelled with Hoechst 33342 and chloromethyl-benzamidodialkyl carbocyanine (CM-Dil), respectively. Cells were engrafted into the penumbra of the rat TBI model alone or in combination. After 14 days of follow-up, the neurological function was greatly improved in the co-grafted group, with sparing more cortical neurons and minimum apoptotic cells. Furthermore, NSCs and OECs survived, migrated, and differentiated under inhibitory levels of interleukin 6 (IL-6) and BAD [57].

Transplantation of OECs for the treatment of TBI has attracted the attention of researchers in the field. Studies have shown that co-transplantation of MSCs and OECs or NSCs and OECs is more effective than transplantation of OECs alone in the treatment of rat TBI models. This might be due to the special biological features of OECs and their accompanying MSCs which have an apparent effect on the regeneration of axons. OECs, as a unique type of neuro-glial cells, are not only available in the epithelium of the nose and olfactory nerve but also in the olfactory bulb of the central nervous system. Although these studies have non-autologous preclinical properties, OECs are still a promising choice for autologous transplantation in clinical practice.

Neural stem cells

Neural stem cells are self-replicating, multipotent progenitors [49], and have the potential to differentiate into neurons, astrocytes and oligodendrocytes [70,108]. In the mouse model, it was found that the progenitor cells of NSCs were formed between 13.5 days and 15.5 days of the embryonic stage and remained inactive until birth [26]. There are two potential niches that host NSCs in the adult mammalian brain, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus [66]. The neurogenesis of SVZ and SGZ is essential for maintaining the olfactory bulb and spatial memory [9,42,110].

In order to study the long-term therapeutic effects of the human foetal neural progenitor cell (hfNPC) on TBI, Skardelly *et al*. injected human fetal neural progenitor cells (hfNPCs) directly into the lesion or intravenously to a CCI rat model 24 hours after the injury. Twelve weeks post-transplantation, the neurosensory and mental functions were improved in both groups and the volume of the lesion measured by MRI was also decreased with a prominent reduction in systemically transplanted rats. In histological investigations, reactive astrogliosis was decreased, angiogenesis and neuronal survival was increased, but engrafted cells differentiation was observed in very rare cases, indicating that the improvements are mainly due to inhibition of reactive gliosis and induced angiogenesis in the host brain [87]. Similarly Haus *et al*. investigated the long-term effects of human neural stem cells (hNSCs) on cognition improvement in the TBI model. They deployed hNSCs into the lesion of the CCI model of immunodeficient athymic nude (ATN) rats nine days after the injury. Five months after the operation, spatial memory improved, but non-spatial memory and emotional deficits did not boost significantly. Moreover, hNSCs differentiated into mature neurons and glial cells, but unlike Skardelly's findings, their observations showed that the size of the lesion did not decrease [35]. Furthermore, Ma *et al*. revealed the potential therapeutic effect of rat embryonic NSCs in TBI and its possible underlying mechanism. They extracted NSCs from the forebrain of a 14-day-old rat embryo and injected them directly into the injured brain of a CCI rat model 72 hours after the trauma. Eight weeks after transplantation, neuronal function was improved, NSCs survived, and their differentiation into neurons was observed. In addition, histological analysis demonstrated overexpression of synaptophysin (SYP) and regeneration-associated protein (GAP43) which is considered to be the underlying mechanism for NSCs mediated mental function improvement in the native brain [61].

Due to the variations of the clinical situation in TBI, different animal models have been developed. Even though, bigger animals are more similar in physiology to the human being, but the standardized methods of measuring outcomes and their reasonable price made rats the most used models in TBI [22,95]. The most commonly used models are

CCI, weight drop injury (WDI), fluid percussion injury (FPI), electronic device brain injury (EDBI) and blast injury (BI) [11,64]. In general, the rat is cleaned and anesthetized, fixed on injury platform, injured with the required parameters and then followed up for the targeted experimental time [1].

Sun *et al*. tried multipotent neural stem/progenitor cells (NS/NPCs) derived from the SVZ of adult Fisher 344 (F344) rats for the treatment of TBI. They injected NS/NPCs around the lesion of the FPI rat model two days post-injury. After two to four weeks, motor and cognitive function was improved, and NS/NPCs survived, migrated from the site of deposition and differentiated into location-specific astrocytes and oligodendrocytes [90]. As gap junctions provide direct communication between cells by means of tiny particles and ions transportation therefore Yu *et al*. were interested to investigate their connecting role between the transplanted NSCs and host brain cells of the TBI model. They implanted NSCs around the lesions of the CCI model 72 hours after the Wister rats were injured. Gap junction-associated connexin 43 (Cx43) was significantly overexpressed at the transplant site and the injury edge 1-4 weeks after transplantation, indicating that the transplanted cells may be involved in the circuit of native brain neurons [106]. In addition, Lin *et al*. demonstrated that human neural progenitor cells (hNPCs) can survive, differentiate and integrate into the neural circuit of the native brain in the rat TBI model [55]. In order to investigate the effects of hNSCs on the modulation of host immune response and their contribution to neuroprotection after TBI, Gao *et al*. primed hNSCs and injected them intracerebrally to the C57BL/6 mice CCI model 24 hours after injury. Six days after engraftment, there was no remarkable decrease in the volume of the lesion but there was a significant reduction in injury-dependent accumulation of the amyloid precursor protein (APP). Moreover, there was a decrease in activation of microglia and production of pro-inflammatory interferon γ (IFN-γ) while production of anti-inflammatory interleukin 4 (IL-4) was increased. Thus, primed hNSCs neuroprotection in the TBI model is at least partially due to modulation of the host immune response [27].

To reveal the effects of transplanted NSCs on the level of expression of B-cell lymphoma-extra-large (Bcl-xL) in the TBI model, Pang *et al*. introduced mouse embryonic NSCs into the traumatically injured brain of rats within 24 hours post-injury. Seven days after transplantation, motor function was improved, cellular apoptosis was reduced and Bcl-xL gene was overexpressed. Furthermore, herpes simplex virus (HSV) 1 carrying Bcl-xL recombinant alone was administered to the surroundings of the lesion, which resulted in the improvement of neuronal deficits as well as inhibition of apoptosis in the host brain. These findings suggest that Bcl-xL increased expression can be considered as an alternative for NCS engraftment in the treatment of TBI [73].

Xiong *et al*. attempted to justify the idea of brain-derived neurotropic factor (BDNF) mediated neuroplasticity being the underlying mechanism for transplanted NSCs therapy in TBI. They applied normal NSCs or BDNF knockdown NSCs to the traumatized brain regions of mice 24 hours after the injury. Two to four weeks post-operation, normal NSCs engrafted mice showed improvement of neurologic disabilities and upregulation of BDNF with synaptophysin, while rats that received BDNF knockdown NSCs not only demonstrated a lower level of synaptophysin but also exacerbated functions of the nervous system, indicating the critical therapeutic role of BDNF in the TBI model [102]. Likewise, Tao Chen *et al*. extracted NSCs from the rat embryo and introduced the BDNF gene to the cells. Encoded-NSCs or naïve-NSCs were injected directly into the damaged area of the rat's brain. One to four weeks after implantation, synaptic proteins such as TrkB and postsynaptic density protein 95 (PSD-95) were increased, the MAPK/Erk1/2 signalling pathway was activated, and the antioxidant protein thioredoxin (Trx) was upregulated [13]. Those results indicate that engrafted NSCs ameliorate TBI-induced neurologic deficits. The underlying mechanisms mostly seem to be inhibition of neuroinflammation and apoptosis, with increased expression of neurotrophic factors and synaptic proteins. The idea that transplanted NSCs substitute the damaged neurons and decrease the lesion size remains contradicting and unconvincing.

Even though the penetrating traumatic brain injury (PTBI) is less common than closed brain injury but its prognosis is far worse than for closed brain trauma. Therefore, Spurlock *et al*. tested the potential role of hNSCs in the improvement of PTBI. They implanted 4×10^5 hNSCs into the surroundings of the lesion of the Sprague Dawley (SD) rat TBI model one week after injury. The neurointellectual perfor-

mance improved after 8 weeks of transplantation, with significant differentiation and migration, but the differentiation was not obvious after 16 weeks of transplantation, and the cell morphology was very similar to that of native brain cells [88]. Similarly, Hu *et al*. wanted to know the ideal location for the deposition of hNSCs when treating PTBI. They transplanted hNSCs into the core or around the lesion one week after the injury. After 12 weeks, neuronal differentiation and motor function improvement were observed in both groups but sparing of a larger volume of intact brain including cortex makes the peri-lesion administration a preferable choice [40]. Beretta *et al*. made an attempt to see the effects of hNSCs on posttraumatic hyperexcitable brain. Due to lack of a reliable model for testing posttraumatic hyperexcitability of the brain, they traumatized one cerebral hemisphere of the immunodeficient ATN rat with CCI model and contralateral hemisphere was electrically stimulated to produce spontaneous seizures. Human NSCs were transplanted to either sham or injured brains. Fourteen weeks after implantation, they observed a twofold increase in survival and differentiation of hNSCs in injured brains compared to shams. Moreover, surviving and differentiation was the worst in stimulated hemispheres in either groups, indicating that kindling is harmful for differentiation and survival of hNSCs. It was also observed that mental function was ameliorated by hNSCs while these improvements were vanished by kindling meaning that hNSCs engraftment is not useful for animal or human with posttraumatic seizures [6]. Those results suggest that the implantation of the cells to the surrounding of the lesion is more effective than intralesional administration. Moreover, traumatized brains with a previous history of seizures may not be cured with NSCs therapy.

Lee *et al*. revealed the potential curative role of human parthenogenetic neural stem cells (hpNSCs) in the treatment of TBI. They implanted hpNSCs into the brains of a CCI rat model 72 hours post-trauma. Three months after engraftment, neurological, motor and intellectual functions were improved. In histological studies, biomarkers related to myelination increased, while reactive gliosis, neuroinflammation and splenic inflammation decreased [51]. To elucidate the difference between the effects of embryonic neural stem cells (ENSC) or differentiated cells (DC) in the treatment of TBI, ENSCs/DCs were isolated from E14 mouse embryo and injected into the lesion of CCI rat models 7 days post-injury. Following up for one to four weeks, it was noticed that microglia were activated, astrogliosis was inhibited and neurogenesis due to inducing of neuronal progenitor cells was enhanced. In addition, there was no significant difference between the ENSCs group and the dendritic cell group in terms of improvement, indicating that the implantation of ENSCs/dendritic cells can improve TBI by reducing neuroinflammation and inducing neurogenesis in the host brain [71]. Furthermore, Abad *et al*. derived human neural stem/progenitor cells (hNS/PCs) from the brain of a human undergoing epilepsy surgery. The cells were cultured in Pura Matrix hydrogel (PM) and deposited into the lesion of a TBI rat model 30 minutes post-injury. In the evaluation 1 to 28 days after transplantation, motor function and spatial memory improved, lesion volume reduced, neuroinflammation and reactive gliosis were inhibited, which strongly supports the use of PM-cultured hNS/PCs for the treatment of TBI [44].

After decades of conceptual assumption of the fixed regenerative capacity of the adult brain, the fascinating discovery of adult brain NSCs and their continued ability of neurogenesis throughout the life brings a new era for deeper understanding of the brain. NSCs not only maintain the neural tissue, but also provide expanded plasticity to the key brain structures that are vital for learning and memory. The rapid and vast progress in the field has elaborated on the biological as well as clinical potential of the cells. Although transplanted embryonic or adult NSCs can survive, migrate, differentiate and improve neurologic and cognitive functions in preclinical TBI models, the realistic and ethical sources of NSCs for autologous clinical translation have not yet been achieved. The clinical promise of NSCs still requires thorough understanding of their properties, mechanism of regulation and eventually application through collaborative efforts of both basic and translational research.

Mesenchymal stem cells

Mesenchymal stem cells are multipotent in nature, exist in adults, and have the ability to self-replicate and differentiate into various cell types [69]. As TBI is a very complex disease and there is no effective treatment available in the clinical practice, therefore, stem cell therapy has been the focus of research in the recent years. In spite of great prog-

Table II. Treatment of traumatic brain injury with bone marrow derived-mesenchymal stem cells

No. of participants	Age of participants (years)	Dose (cells)	Route and time of administration	Follow up (months)	Clinical outcome	Author	Ref.
7	6-55	10^8 and 10^9	Intra-lesional during surgery and IV (3-4) days post-intervention	6	Neurological function was significantly improved in all patients	Zhang 2008 Canada	[109]
10	5-14	6×10^6 per kg BW	IV, within 48 hours of injury	6	Neurological function was improved in all patients with a complete recovery in three patients	Cox 2009 United States	[17]
97	18-50	6×10^6	IT, about 3 months post-injury	14 days	27 of 73 patients showed motor function improvement and 11 of 24 patients demonstrated improvement in the conscious level	Tian 2013 China	[92]
14	12-65	1×10^6 per kg BW	IT, about 5 years post-injury	6	Neurologic function was improved in all patients	Sharma 2015 India	[79]
10	5-14	6×10^6 per kg BW	IV, within 48 hours of injury	6	Neurological function was improved in all patients	Liao 2015 United States	[54]
10	21-55	2×10^7 and 4×10^7	IT and IV, about 60 days post-injury	6	Neurological function was improved in all patients	Wang 2017 China	[99]
15	18-55	$(6\text{-}12) \times 10^6$ per kg BW	IV, within 48 hours of injury	6	Neurologic and cognitive functions were improved	Cox 2017 United States	[18]
50	7-64	1.28×10^8	IT, about 5 years post-injury	22	All patients got symptomatic improvement	Sharma 2020 India	[80]

IV – intravenous, IT – intrathecal, BW – body weight

ress in animal models, the clinical translation of stem cells for TBI is limited. However, there are some issues regarding the clinical trials, such as smaller sample size, majorly no controls, short follow-up time and being single-centred, but yet, it remains a great hope for the future of TBI patients. Especially, autologous stem cell therapy, which has no risk of immune reaction and rejection gives an enormous optimism to the future of the field.

Mesenchymal stem cells can differentiate into neural cells including neurons, but their differentiation without any stimulation is not very effective. In order to improve the survival and differentiation of MSCs into nervous cells, they are usually reprogrammed before being transplanted [59,63]. Four different strategies are used to reprogram MSCs towards neuronal cells lineage namely, small molecules, epigenetic modifications, psychotropic drugs and enriched media [7]. Moreover, retinoic acid which is a vitamin derivative is also known for its ability to differentiate MScs and progenitor cells into neurogenic cells [28]. In the following sections, we will review them according to their source of origin, such as autologous MSCs and non-autologous MSCs with their subtypes.

Autologous mesenchymal stem cells

Autologous MSCs studies are further sub-sectioned into clinical trials and preclinical models.

Autologous bone marrow-derived MSCs in clinical trials

In order to evaluate the safety and feasibility of autologous BM-MSCs in the treatment of TBI, a number of studies have been conducted (Table II). Zhang *et al*. conducted a prospective, non-randomized, open label clinical trial on seven patients aged six to fifty-five years old. The Barthel index (BI) (Table III) which is an ordinal scale used to assess the performance in activities of daily living (ADL) was used and all the patients have BI score less than 40. They administered 10^7-10^9 BM-MSCs directly into the lesion during the operation, and injected 10^8-10^{10} cells intravenously for the second time 4 to 12 days later. After 6 months of follow-up, the neurologic performance of all patients was significantly improved, except for one patient who had 2 seizures in the first 2 months after surgery and received conservative treatment. There was no death or serious

adverse reaction caused by transplantation [109]. Cox *et al*. have a total of four clinical trials, two of which have been completed, the other two are registered at ClinicalTrials.gov, and the final results are still on the way. The completed studies are prospective, non-random, open label, single-centre clinical trials on children and adults. In their first trial, ten children aged five to fourteen years with severe TBI were given intravenous 6×10^6 autologous bone marrow-derived mononuclear cells (BMMNCs) per kg body weight forty 8 hours after TBI. After 6 months of follow-up, the motor and sensory functions of all patients improved, and 3 patients recovered completely. No seizures, refractory intracranial pressure, altered cerebral perfusion or new ischemic events were noticed. MRI did not show any significant morphological changes at 1 and 6 months after surgery [17].

Moreover, in the second trial of Cox, 15 patients aged 18-55 with GCS scores of 5-8, age- and severity-matched controls, were treated with intravenous autologous BMMNCs. The patients were divided into three groups, and engrafted with 6, 9 and 12 million cells within 48 hours post injury. MRI and DTI were used to evaluate critical structures preservation in the brain. Six months post intervention, the patient's neurological and perceptive functions improved, the white matter anisotropy score (FA) was retained, and inflammatory biomarkers such as interleukin 1β (IL-1β) and IFN-γ, tumour necrosis factor α (TNF-α) and interleukin 10 (IL-10) were significantly reduced. There were no severe adverse events, except for mild pulmonary toxicity in high-dose recipients, which was not clinically significant [18]. Since Cox's first trial did not have a control group, Liao *et al*. tried a second retrospective, cohort, phase I clinical study. They included the patients treated in Cox research as the treatment group, and collected 19 children of matching age and severity as the control group. They were treated with standard treatment regimens without autologous bone marrow stromal cell transplantation. To evaluate and compare the clinical outcome between the two groups, paediatric intensity level of therapy (PILOT) and paediatric logistic organ dysfunction (PELOD) scores and length of neurointensive care unit (NICU) stay were considered. In patients treated with BMMNCs, PILOT and PELOD scores and NICU hospitalization were significantly reduced [54].

Table III. Barthel index (BI)

Activity	Response	Score
Feeding	Unable	0
	Needs help cutting, spreading butter, etc., or requires modified diet	5
	Independent	10
Bathing	Dependent	0
	Independent (or in shower)	5
Grooming	Needs help with personal care	0
	Independent face/hair/teeth/shaving (implements provided)	5
Dressing	Dependent	0
	Needs help but can do about half unaided	5
	Independent (including buttons, zips, laces, etc.)	10
Bowels	Incontinent (or needs to be given enemas)	0
	Occasional accident	5
	Continent	10
Bladder	Incontinent, or catheterized and unable to manage alone	0
	Occasional accident	5
	Continent	10
Toilet use	Dependent	0
	Needs some help, but can do something alone	5
	Independent (on and off, dressing, wiping)	10
Transfers (bed to chair and back)	Unable, no sitting balance	0
	Major help (one or two people, physical), can sit	5
	Minor help (verbal or physical)	10
	Independent	15
Mobility (on level surfaces)	Immobile or < 50 yards	0
	Wheelchair independent, including corners, > 50 yards	5
	Walks with help of one person (verbal or physical) > 50 yards	10
	Independent (but may use any aid; for example, a stick) > 50 yards	15
Stairs	Unable	0
	Needs help (verbal, physical, carrying aid)	5
	Independent	10

In order to investigate the efficacy and safety of bone marrow MSCs in the treatment of subacute TBI, Tian *et al*. conducted a prospective, non-randomized and open-label clinical trial. The subjects of the study were 97 patients aged 18 to 50 years with a persistent vegetative state or motor distur-

bance post-injury. The patients were injected intrathecally with 4 × 10^6 autologous BM-MSC within two months after trauma and followed up for fourteen days. 27 out of 73 patients showed improvement in motor function, and 11 out of 24 patients showed improvement in the conscious level. There were no serious transplantation-related complications or adverse effects. Younger and earlier treated patients benefited more from the treatment [92]. In order to evaluate the long-term efficacy and safety of autologous BMMNCs in the treatment of chronic TBI, Sharma *et al*. conducted two prospective, open-label, non-randomized clinical trials. In the first trial, the number of patients was fourteen, they were aged 12-65 years, and had a history of TBI for about five years. A dose of 1 × 10^6 BMMNCs per kg body weight were intrathecally injected *via* lumbar puncture. The follow-up was done for 6 months and the functional independence measure (FIM) scoring system was used to analyse the improvement of symptoms. The electroencephalograph (EEG) taken before transplantation was abnormal for ten patients of which seven patients had a history of seizures. Positron emission tomography (PET) brain scan showed improved metabolism in the brain. The neurological activities were enhanced with the rates of 7 of 13, 6 of 11, 7 of 13, 8 of 11, 9 of 13, 6 of 12 and 3 of 8 in upper limbs, lower limbs, trunk, balance, voluntary control, speech and cognition, respectively. Except for one case of epileptic seizures within 3 months after transplantation, there were no complications or side effects [79].

Furthermore, in Sharma's second trial, 50 patients aged between 7 and 64 were studied for 22 months after implantation. Fifteen patients received a second dose of cells after 6 months of intervention. The criteria for categorizing symptomatic improvement of less than 30%, 30-60%, and more than 60% into mild, moderate, and significant were adopted. Sixteen out of fifty patients had a history of seizures. 50% of the patients had significant improvement, 16% of the patients had moderate improvement, and 26% of the patients had mild improvement. Except for 2 cases of single seizures, which were cured by anti-epileptic drugs, there were no obvious adverse reactions. The patients who received a second dose of cells did not differ from single time recipients in their clinical outcome. The candidates who were younger and had acute mild injury showed better response [80]. In order to reveal the safety and feasibility of autologous BM-MSCs in non-acute severe TBI, Wang *et al*. attempted a prospective, non-randomised, open-label clinical trial on ten patients who had brain injury in about past sixty days and were aged 21-55 years. The patients were followed up for six months. Seven patients received 4 × 10^7 cells intravenously and 3 patients received 2 × 10^7 cells intrathecally. The motor and sensory deficits of 7 patients improved to varying degrees, and none of the patients deteriorated. There were no reports of deaths or serious adverse events. On the 3rd and 7th day after the intervention, serum nerve growth factor (NGF) and BDNF levels increased significantly [98].

Despite the good clinical results and minor adverse events, the shortcomings of these trials, such as small sample size, short-term follow-up, being single-centre, mostly uncontrolled, and non-randomized, need to be addressed in future studies. Moreover, the ideal time of administration, the optimal dose and the potential therapeutic mechanism of the cells remain to be studied.

Autologous bone marrow-derived MSCs in preclinical models

To study the neuroprotective effects and the underlying mechanism of autologous BMMNCs in TBI, Bedi *et al*. intravenously injected 2 × 10^6 cells per kg body weight to the CCI rat model 72 hours post-injury. After 24 hours of implantation, the permeability of the blood-brain barrier was significantly decreased, and the apoptosis of activated microglia was noticeably increased. Four weeks later, neurocognitive function and spatial memory were remarkably improved, which is proposed to be due to the preservation of the blood-brain barrier and the inhibition of active gliosis [5]. Liu *et al*. were interested to know whether intrathecal injection of autologous BM-MSCs could reach the site of brain injury, and whether intrathecal or intravenous injection is more effective for the treatment of TBI. BM-MSCs were isolated from the rabbit, labelled with GFP and 2 × 10^6 cells per kg body weight were implanted either intravenously or intrathecally 24 hours post-injury. Four weeks after engraftment, the motor function was improved in all treated rabbits with greater enhancement in the intrathecal group, and the cells were observed in the lesion area of the brain with fluorescence microscopy [58]. Jiang *et al*. studied the therapeutic effects of autologous BM-MSCs transplantation on the CCI rat model along with their role

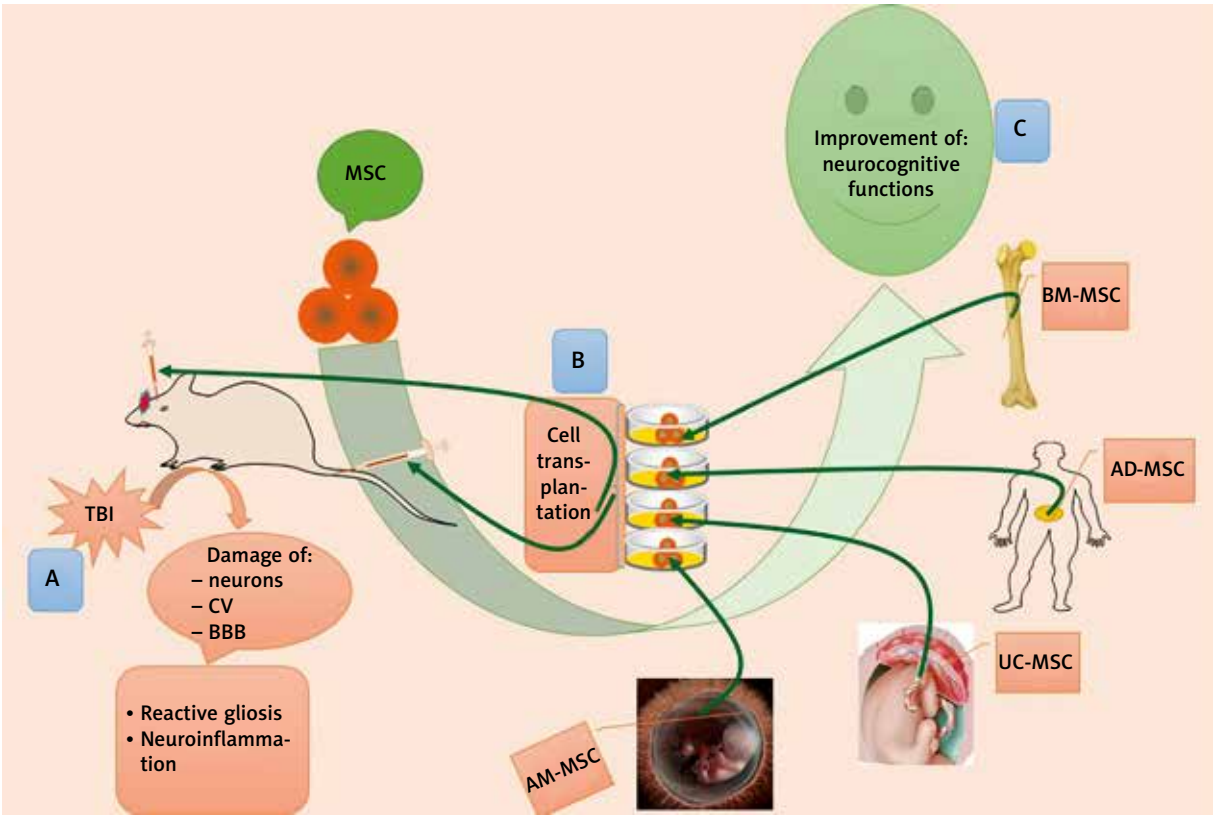

Fig. 2. Schematic of mesenchymal stem cells (MSCs) transplantation for traumatic brain injury (TBI). **A**) The rat model of TBI manifests a primary brain injury such as damage of neurons, cerebral vessels (CV) and the blood brain barrier (BBB) followed by a secondary brain injury, such as reactive gliosis and neuroinflammation. **B**) Four types of MSCs such as bone marrow-derived MSC (BM-MSCs), adipose-derived MSC (AD-MSCs), umbilical cord-derived MSCs (UC-MSCs) and amnion-derived MSCs (AM-MSCs) are isolated, cultured, identified, labelled and transplanted into the model *via* the intravenous route or intra/peri-lesional deposition. **C**) After intervention, cognitive, sensory and motor functions are improved

in ameliorating pathological changes. They injected a million cells into the site of injury during the surgery. The mice were scarified on day 1, 7, 14, 21, 28 and neurologic and pathologic studies were done respectively. Motor and intellectual functions improved fourteen days post engraftment. Histological studies showed that new blood vessels were formed, cerebral oedema was improved and reactive gliosis was inhibited. Moreover, the level of brain-derived neurotrophic factor was increased seven days post-implantation [45].

Despite multiple studies, the autologous nature of these studies makes them special among the preclinical models which can give a good insight into the underlying mechanism for autologous stem cell therapy. Furthermore, these data suggest that intrathecal transplantation is more effective than intravenous administration.

Non-autologous mesenchymal stem cells

Non-autologous MSCs research studies are categorized into the following four sub-types (Fig. 2).

Non-autologous bone marrow-derived MSCs

Bone marrow-derived MSCs have been proven to be effective in the treatment of TBI, and researchers have proposed several potential mechanisms (Table IV). Jiang *et al.* studied the therapeutic effects of human bone marrow stromal cells (hBMSCs) on TBI. They labelled the cells with superparamagnetic iron oxide (SPIO) and intravenously injected 3 million cells to

Table IV. Treatment of TBI with non-autologous bone marrow derived mesenchymal stem cells and their underlying mechanism

TBI model	Cell origin	Potential mechanism	Outcome	Ref.
CCI rat model	hBMSCs	Increased CBF and decreased brain atrophy	Neurocognitive function improved	[53]
CCI rat model	BMSCs	Increased expression of GDNF, GAP-43 and synaptophysin with downregulation of BAD and BAX proteins	Neurologic function and rat survival improved	[82]
WDI rat model	BMSCs	Increased expression of BDNF, VEGF and synaptophysin	Neuromotor function improved	[24]
EDBI rat model	BMMSCs	SDF-1 induced CXCR-4 in transplanted cells caused increased expression of NGF and BDNF in the host brain	Spatial memory and cognitive function improved	[21]
CCI rat model	hBMSCs	Early preservation of BBB and improvement of CBF	Neurocognitive function improved	[52]
CCI rat model	BMMSCs	SOD2 over-expressed cells preserved BBB and attenuated neuroinflammation in the host brain	Therapeutic effect was enhanced in recovering neurocognitive function	[84]
WDI rat model	BMMSCs	Increased neovascularization	Neuromotor function improved	[39]
WDI rat model	BMMSCs	Intralesional injection of MDL28170 prior to cell transplantation improved microenvironment for BMMSCs survival *via* inhibiting neuroinflammation and apoptosis	Neurologic function improved	[38]
CCI rat model	BMSCs	HP-BMSC transplantation unregulated HIF-1α, p-mTOR and VEGF in the recipient brain	Neurocognitive function improved	[107]
EDBI rat model	BMSCs	HIF-1α/SDF-1/CXCR4 axis decreases apoptosis of host neurons *via* improving the migration of engrafted cells	–	[32]

hBMSCs – human bone marrow stromal cells, CBF – cerebral blood flow, CCI – controlled cortical impact, GDNF – glial cell line-derived neurotrophic factor, BAD – BCL2-associated agonist of cell death protein, BAX – Bcl-2-associated X-protein, GAP-43 – growth-associated protein-43, VEGF – vascular endothelial growth factor, BDNF – brain-derived neurotrophic factor, WDI – weight-drop injury, BMMSCs – bone marrow-derived mesenchymal stem cells, SDF-1 – stromal cell-derived factor 1, CXCR-4 – C-X-C chemokine receptor type 4, NGF – nerve growth factor, BBB – blood-brain barrier, SOD2 – superoxide dismutase 2, HP-BMSC – hypoxic preconditioned-bone marrow stromal cell, HIF-1α – hypoxia-inducible factor-1α, p-mTOR – phosphorylated mechanistic target of rapamycin, EDBI – electronic device brain injury

the CCI rat model 5 days after the injury. Six weeks post-engraftment, mental function was improved. MRI demonstrated reduced systemic brain atrophy and increased cerebral blood flow (CBF) [53]. Osanai *et al*. tried intra-arterial implantation of bone marrow stromal cells (BMSCs) in the cortical freezing injury (CFI) rat model. Cells were extracted from rat's bone marrow, labelled with fluorescent dye and injected to the ipsilateral internal carotid artery 7 days post-trauma. After four weeks of follow-up, motor function was significantly improved. The implanted cells differentiated into neurons, astrocytes and endothelial cells. Optical imaging demonstrated that these cells were engrafted into the brain in their first pass before the systemic circulation [72]. Furthermore, in order to observe whether the severity of TBI determines the therapeutic efficiency of BMSCs, Bonilla *et al*. injected 5 million cells into moderate and severe chronic rat TBI models *via* intravenous route. Two months post-implantation, the neurologic performance of moderately injured animals improved significantly [8].

In addition, Anbari *et al*. injected 3 million bone marrow-derived mesenchymal stem cells (BMMSCs) intravenously into a rat model 20 hours after trauma. After fourteen days of transplantation, the neuronal function was significantly improved. The transplanted cells differentiated into neurons and astrocytes, and were observed in the traumatized region of the brain [2]. Shen *et al*. wanted to observe the effects and underlying mechanism of engrafted BMSCs on TBI. They isolated cells from GFP-transgenic mice and immediately injected them into the penumbra of the CCI rat model. At two weeks' follow-up, the brain activity and the survival rate of the rats were significantly improved. Histologic analysis manifested that apoptosis was significantly reduced and axonal regeneration increased. Molecular studies revealed increased levels of growth-associated protein 43 (GAP-43), synaptophysin and glial cell line-derived

neurotrophic factor (GDNF). In addition, the recipient brain showed a down-regulation of BAD, Bcl-2-associated X-protein (BAX) genes [82]. Feng *et al*. tried to use BMSCs to treat TBI and injected 3 million cells intravenously into mentally traumatized rats within 30 minutes after the trauma. After fourteen days, neuromotor function was significantly improved. Furthermore, the implanted cells migrated to the injured brain area and differentiated into neurons and astrocytes. The expression level of vascular endothelial growth factor (VEGF), BDNF and synaptophysin were up-regulated [24].

Deng *et al*. used stromal cell-derived factor 1 (SDF-1) to induce the expression of C-X-C chemokine receptor 4 (CXCR4) in BMMSCs. In the rat model of TBI, normal cells or modified cells were injected into the lesion. Four weeks post-implantation, the spatial memory and neurologic function of the mice treated with the stimulated cells were significantly improved. Furthermore, the levels of expression of NGF and BDNF were also remarkably increased in induced cells implanted rats [21]. Li *et al*. revealed the effects of hBMSCs on the cerebral hemodynamics of TBI. They injected 3 million cells intravenously into a CCI rat model 6 hours and 1 week after injury. Neurointellectual function improved three months post-transplantation. Magnetic resonance imaging demonstrated early preservation of the blood-brain barrier and enhanced cerebral blood flow [52]. Shi *et al*. over-expressed superoxide dismutase 2 (SOD2) in BMMSCs, and implanted normal or modified BMMSCs into a CCI rat model through the intravenous route. Twenty one days post-intervention, the neuronal and intellectual functions improvement was more remarkable in SOD2-adjusted cells. Furthermore, the preservation of the blood-brain barrier and the reduction of neuroinflammation were more significant in the rat brain treated with modified cells [84]. Hu *et al*. studied the effects of transplanted BMMSCs on neovascularization in TBI. They injected 10,000 BMMSCs into the lateral ventricle of the WDI rat model 12 hours post-surgery. Twenty one days post-intervention, neuromotor function was significantly improved. Moreover, endothelial progenitor cells, angiogenetic, and neuronal markers were increased in peripheral blood [39].

Since calpain activation plays a key role in TBI-induced neuroinflammation, its inhibitory effect has been proven to be neuroprotective. Therefore, Hu *et al*. searched the effects of combined therapy of BMMSCs and calpain inhibitor MDL28170 in a WDI rat model. MDL28170 was injected to the lesion thirty minutes post-injury followed by 1×10^5 GFP-labelled BMMSCs engraftment 24 hours after trauma. Histopathological investigations showed improved microenvironment for survival of transplanted BMMSCs *via* inhibition of neuroinflammation. After a 4-week follow-up, there was a greater improvement of neurologic function in cells and MDL28170 co-grafted group compared to mice treated with cells alone [38]. Yuan *et al*. investigated the effects of hypoxic preconditioning (HP) on BMSCs survival and their therapeutic role in TBI. They injected hypoxic preconditioned- bone marrow stromal cells (HP-BMSCs) or normal BMSCs into the CCI rat model 24 hours post-injury. Sensorimotor and cognitive function was significantly improved in HP-BMSCs treated mice 35 days after intervention. Moreover, hypoxia-inducible factor-1α (HIF-1α), phosphorylated mechanistic target of rapamycin (p-mTOR) and VEGF were overexpressed in the HP-BMSCs-treated brain [107]. Guo *et al*. investigated the effects of HIF-1α/SDF-1/CXCR4 axis on the neuroprotective role of transplanted BMSCs in TBI. BMSCs were treated with HIF-1α and SDF-1 prior to transplantation, and then injected into the lateral ventricle of the EDBI rat model. 14 days post-implantation, the mice were sacrificed and their brains were analysed. Histologic and molecular investigations revealed that the HIF-1α/SDF-1/CXCR4 axis promoted the migration of transplanted cells, thereby reducing cell apoptosis and enhancing the neuroprotective effect of the host brain [32].

Although bone marrow stem cells have been tried in clinical trials, but to know their underling therapeutic mechanisms such preclinical studies are still vital.

Non-autologous umbilical cord/umbilical cord blood-derived MSCs

Dong *et al*. studied the distribution of human umbilical cord mesenchymal stem cells (hUCMSCs) in a rat model of TBI. They isolated hUCMSCs from fresh human umbilical cords, labelled with GFP and injected into the lateral ventricles of WDI rats. The animals were followed up for 14 days, and the cells were tracked using immunohistochemistry (IHC) and small animal imaging system. On the 10^{th} day after transplantation, the cells were distributed in large blood vessels of the TBI model. Furthermore, VEGF was increased and neovascularization was

noticed in the brains of engrafted mice [23]. Gincberg *et al*. conducted two studies on human umbilical cord blood-derived CD45+ pan-hematopoietic cells (HUCB-CD45+) and the TBI injury. In their first study, they investigated the therapeutic effects of HUCB-CD45+ cells in a rat TBI model. HUCB-CD45+ cells were isolated from vaginal or caesarean delivery HUCB and injected intravenously to the WDI rat model 24 hours post-injury. After 6 weeks of transplantation, the motor and rational functions of the two groups of mice improved [29]. In their second study, they wanted to know the relationship between NGF and its receptors with engrafted HUCB-CD45+ cells in TBI. The interrelation of HUCB-CD45+ cells, NGF and its receptors and TBI was investigated both *in vivo* and *in vitro*. *In vitro* the cells were stimulated with TBI brain extracts while *in vivo* the cells were studied in the brains of the TBI rat model. In either case, the HUCB-CD45+ cells were found to be expressing NGF and its receptors TrkA, $p75^{NTR}$ and $\alpha 9\beta 1$ in response to TBI [30].

Qi *et al*. investigated therapeutic effects and the underlying mechanism of umbilical cord mesenchymal stem cells (UCMSCs) on TBI. They isolated cells from the umbilical cord of newborn rats, and transplanted three million cells into the lesion of the WDI rat model immediately post-injury. After 4 weeks of transplantation, motor and sensory functions were improved. In addition, neurotrophic factors such as GDNF and BDNF were up-regulated, while inflammatory factors such as IL-1β and TNF-α were down-regulated [75]. Xu *et al*. studied the importance of silencing histone deacetylase 1 (HDAC1) in hUCMSCs before their transplantation into the TBI model. The WDI rat model intravenously received three doses of 1 million normal hUCMSCs or HDAC1-silenced hUCMSCs (MSCs-siHDAC1) for three consecutive days 24 hours after injury. The mice were followed up for twenty eight days and the evaluation was done on day 1, 3, 7, 14, 21 and 28. Compared with the hUCMSCs treatment group, the intellectual and motor functions of the mice treated with MSCs-siHDAC1 were significantly improved. Furthermore, the survival rate, proliferation rate and migration rate of transplanted cells in the MSCs-siHDAC1 treatment group were significantly higher than those in the control group. The PI3K/AKT pathway was also activated in the MSCs-siHDAC1 treated group while inhibition of the pathway by LY294002 attenuated the neuroprotective effects of MSCs-siHDAC1 in the host brain [104].

Wang *et al*. explored the therapeutic effect of hUCMSCs in acute TBI. They isolated cells from fresh umbilical cord of human, labelled them with GFP and injected them into the lateral ventricle of the WDI rat model. Histological studies on day 10, 14, and 20 after transplantation showed increased expression levels of recipient brain microvessel density (MVD), BDNF and GFAP [97]. Srivastava *et al*. evaluated the role of human umbilical cord blood (HUCB) in TBI. They injected 25 million cells per kg body weight to the CCI rat model *via* the intravenous route either 24 or 72 hours post-injury. Far red fluorescent dye imaging showed that the integrity of the blood-brain barrier was improved 96 hours after injury. Furthermore, cytokine inhibition assay demonstrated reduced levels of TNF-α and IFN-γ [89]. Hu *et al*. investigated if TBI can cause cardiac dysfunction and if human umbilical cord blood cells (HUCBCs) therapy can improve TBI-related neurologic and cardiac dysfunctions. Echocardiography showed decreased left ventricular ejection fraction (LVEF) and fractional shortening (LVFS) in the CCI rat model. The mice were intravenously injected with one million HUCBCs three days post-injury. After 30 days' follow-up, neurocognitive function and cardiac dysfunction caused by brain trauma were significantly improved. Further studies revealed that myocardial cell apoptosis and myocardial fibrosis were reduced, and transforming growth factor β (TGF-β) and NADPH oxidase-2 (NOX2) were reduced [37].

Chen *et al*. studied the therapeutic effect of hUCMSCs in TBI and its underlying mechanism. One million cells were intravenously injected to the WDI rat model 3 hours post-injury. The neuronal deficit in the cell therapy group was significantly improved 28 days after transplantation. MRI showed that the volume of ischemic brain tissue in the treatment group was smaller than that in the untreated group. Furthermore, the expression levels of oxidative stress protein NOX2, apoptotic proteins BAX, Caspase-3 and brain oedema protein aquaporin-4 were down-regulated while the expression levels of angiogenesis biomarkers VEGF, SDF-1α, CXCR4 and antifibrotic proteins phospho-Smad1/5 and bone morphogenetic protein-2 were upregulated in the treatment group [12]. Caplan *et al*. explored the therapeutic effects of human umbilical cord blood-derived T-cells (hUCB-TCs) in TBI. Ten million cells per kg body weight were inject-

ed through the tail vein of the CCI rat model either 6 or 24 hours after the injury. The blood-brain barrier integrity was not preserved 96 hours post intervention. Alteration of chronic microglial activation and modulation of immune response were observed thirty days post-engraftment in the brains of treated rats [10]. These data suggest that umbilical cord-derived MSCs are effective in the treatment of brain injury in animal models but their clinical translation for autologous transplantation does not seem to be realistic.

Non-autologous amnion-derived MSCs

Chen *et al*. studied the effects of human amnion-derived multipotent progenitor cells (hAMPCs) on TBI-induced axonal degeneration in the penetrating brain injury (PBI) rat model. They extracted cells from human chorion, labelled with PKH26 and injected them into lateral ventricle at a dose of 2×10^6 immediately after the injury. Two weeks post-transplantation, the axonal degeneration of the corpus callosum and ipsilateral thalamus was significantly attenuated. In addition, cells were observed in the SVZ of both hemispheres, but cells were observed along the corpus callosum of the injured side, indicating that the cells first settled in the SVZ and then migrate towards the lesion [14]. Yan *et al*. explored the effects of human amnion-derived mesenchymal stem cells (AMSCs) and AMSC-derived neural stem-like cells (AMNSCs) in TBI. AMSCs were extracted from amniotic membrane of the human, co-cultured with differentiation medium *in vitro*, and segregated into AMNSCs. The CCI rat model was injected in penumbra either with AMSCs or AMNSCs four days post-injury. After 28 days' follow-up, the neuronal performance and spatial memory of the mice treated with AMNSCs was significantly improved. Furthermore, the neurotrophic factors BDNF, GDNF, ciliary neurotrophic factor (CNTF), NGF, and NT-3 were all up-regulated, but the survival and differentiation of the grafted cells were poor [105].

Although amniotic mesenchymal stem cell therapy can improve animal brain trauma models, due to the lack of clinical trials, poor survival and differentiating quality of grafted cells and their unclear therapeutic mechanism are issues which need to be resolved in future research.

Non-autologous adipose tissue-derived MSCs

Martinez *et al*. investigated the therapeutic effects of adipose tissue-derived mesenchymal stem cells (ADMSCs) in TBI. They isolated the cells from mouse peritoneal fat, labelled with GFP, and injected 200 cells around the wound in a CCI rat model 24 hours after injury. The rats were followed up for twenty one days with regular evaluation at day 2, 7, 14 and 21. TBI caused motor dysfunction was improved while sensory deficit was not ameliorated. Moreover, enhanced neurogenesis was observed in the dentate gyrus of hippocampus of the host brain [65]. Kappy *et al*. searched the effects of human ADMSCs in the TBI rat model. ADMSCs were extracted from human fats, and injected into the tail vein of rat with a dose of 1×10^6 cells three hours post-injury. Three days post-engraftment, TBI-induced neurologic impairment was not improved while the expression levels of TNF-α and β-amyloid precursor protein (β-APP) were decreased [47].

Ma *et al*. investigated whether HP can enhance therapeutic effects of ADMSCs in TBI. They isolated ADMSCs from transgenic-GFP mice, cultured under hypoxic conditions of 2.5% O_2 for 18 hours. The CCI rat model was treated with ADMSCs or HP-ADMSCs, respectively, by applying 2 million cells to the damaged cerebral cortex 1 hour after the injury. Fourteen days post-engraftment, the mice treated with HP-ADMSCs experienced the greatest mental function recovery. The survival and differentiation of HP-ADMSCs in the host brain were more obvious than those of other groups. Real-time polymerase chain reaction (RT-PCR) showed that anti-inflammatory IL-10 and anti-apoptotic genes were up-regulated in treated mice [60]. Ruppert *et al*. tried to use human ADMSCs to treat acute and subacute brain injury in a CCI rat model. Three million cells per kg body weight were injected intravenously to the animals either three or fourteen days post-injury. Thirty-two days after intervention, the neurological impairments caused by TBI in both groups were significantly improved [78].

MSCs in the treatment of TBI has achieved enormous attention over the last decade. It is because MSCs are convenient to harvest, mainly autologous so they do not cause immune response, and they can differentiate into neuronal cells, thereby help to repair brain tissue after trauma exposure. Their healing phenomena of TBI is mainly due to affecting the cells of the host tissue. They basically decrease the inflammation in the host tissue, stimulate the production of neural growth factors, enhance axonal growth and improve neuronal plasticity. MSCs have

the tendency to reside near the injury site and also have the ability to migrate across the injured tissue. However, these advantages are yet to be expanded to their full effect. More research is still required to better understand the mode of action of MSCS and their trophic effects on traumatized host tissue.

Conclusion and future directions

At present, there is no effective treatment for TBI in clinical practice. Currently available therapeutic options are hyperbaric oxygen, brain stimulation, and rehabilitation. Recent developments in the field of stem cell therapy have left a promising future for eliminating this fatal health problem. Although preclinical models have made great progress, clinical translation is still limited. There are few completed clinical trials only for BMMSCs with the drawbacks of smaller sample size, most of them without controls, short follow-up time and being single-centred. In addition, the issues concerning the exact mechanism of therapeutic effects, the limited available sources of autologous stem cells, and the determination of the optimal dose, time and route of administration for clinical use remain to be resolved.

Funding

This work was supported by the Science and Technology Bureau of Quanzhou (grant number 2020CT003).

Disclosure

The authors report no conflict of interest.

References

1. Albert-Weißenberger C, Várrallyay C, Raslan F, Kleinschnitz C, Sirén AL. An experimental protocol for mimicking pathomechanisms of traumatic brain injury in mice. Exp Transl Stroke Med 2012; 4: 1.
2. Anbari F, Khalili MA, Bahrami AR, Khoradmehr A, Sadeghian F, Fesahat F, Nabi A. Intravenous transplantation of bone marrow mesenchymal stem cells promotes neural regeneration after traumatic brain injury. Neural Regen Res 2014; 9: 919-923.
3. Au E, Roskams AJ. Olfactory ensheathing cells of the lamina propria in vivo and in vitro. Glia 2003; 41: 224-236.
4. Barraud P, Seferiadis AA, Tyson LD, Zwart MF, Szabo-Rogers HL, Ruhrberg C, Liu KJ, Baker CV. Neural crest origin of olfactory ensheathing glia. Proc Natl Acad Sci U S A 2010; 107: 21040-21045.
5. Bedi SS, Walker PA, Shah SK, Jimenez F, Thomas CP, Smith P, Hetz RA, Xue H, Pati S, Dash PK, Cox CS, Jr. Autologous bone marrow mononuclear cells therapy attenuates activated microglial/macrophage response and improves spatial learning after traumatic brain injury. J Trauma Acute Care Surg 2013; 75: 410-416.
6. Beretta S, Cunningham KM, Haus DL, Gold EM, Perez H, López-Velázquez L, Cummings BJ. Effects of human ES-derived neural stem cell transplantation and kindling in a rat model of traumatic brain injury. Cell Transplant 2017; 26: 1247-1261.
7. Bhang SH, Lee YE, Cho SW, Shim JW, Lee SH, Choi CY, Chang JW, Kim BS. Basic fibroblast growth factor promotes bone marrow stromal cell transplantation-mediated neural regeneration in traumatic brain injury. Biochem Biophys Res Commun 2007; 359: 40-45.
8. Bonilla C, Zurita M, Otero L, Aguayo C, Rico MA, Vaquero J. The severity of brain damage determines bone marrow stromal cell therapy efficacy in a traumatic brain injury model. J Trauma Acute Care Surg 2012; 72: 1203-1212; discussion 1211-1202.
9. Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 2001; 435: 406-417.
10. Caplan HW, Prabhakara KS, Kumar A, Toledano-Furman NE, Martin C, Carrillo L, Moreno NF, Bordt AS, Olson SD,Cox CS, Jr. Human cord blood-derived regulatory T-cell therapy modulates the central and peripheral immune response after traumatic brain injury. Stem Cells Transl Med 2020; 9: 903-916.
11. Cernak I, Savic J, Malicevic Z, Zunic G, Radosevic P, Ivanovic I, Davidovic L. Involvement of the central nervous system in the general response to pulmonary blast injury. J Trauma 1996; 40: S100-104.
12. Chen KH, Shao PL, Li YC, Chiang JY, Sung PH, Chien HW, Shih FY, Lee MS, Chen WF, Yip HK. Human umbilical cord-derived mesenchymal stem cell therapy effectively protected the brain architecture and neurological function in rat after acute traumatic brain injury. Cell Transplant 2020; 29: 963689720929313.
13. Chen T, Wu Y, Wang Y, Zhu J, Chu H, Kong L, Yin L, Ma H. Brain-derived neurotrophic factor increases synaptic protein levels via the MAPK/Erk signaling Pathway and Nrf2/Trx axis following the transplantation of neural stem cells in a rat model of traumatic brain injury. Neurochem Res 2017; 42: 3073-3083.
14. Chen Z, Tortella FC, Dave JR, Marshall VS, Clarke DL, Sing G, Du F, Lu XC. Human amnion-derived multipotent progenitor cell treatment alleviates traumatic brain injury-induced axonal degeneration. J Neurotrauma 2009; 26: 1987-1997.
15. Coronado VG, McGuire LC, Sarmiento K, Bell J, Lionbarger MR, Jones CD, Geller AI, Khoury N, Xu L. Trends in traumatic brain injury in the U.S. and the public health response: 1995-2009. J Safety Res 2012; 43: 299-307.
16. Cox CS, Jr. Cellular therapy for traumatic neurological injury. Pediatr Res 2018; 83: 325-332.
17. Cox CS, Jr., Baumgartner JE, Harting MT, Worth LL, Walker PA, Shah SK, Ewing-Cobbs L, Hasan KM, Day MC, Lee D, Jimenez F, Gee A. Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. Neurosurgery 2011; 68: 588-600.

18. Cox CS, Jr., Hetz RA, Liao GP, Aertker BM, Ewing-Cobbs L, Juranek J, Savitz SI, Jackson ML, Romanowska-Pawliczek AM, Triolo F, Dash PK, Pedroza C, Lee DA, Worth L, Aisiku IP, Choi HA, Holcomb JB, Kitagawa RS. Treatment of severe adult traumatic brain injury using bone marrow mononuclear cells. Stem Cells 2017; 35: 1065-1079.
19. Dang B, Chen W, He W, Chen G. Rehabilitation treatment and progress of traumatic brain injury dysfunction. Neural Plast 2017; 2017: 1582182.
20. Das M, Mayilsamy K, Mohapatra SS, Mohapatra S. Mesenchymal stem cell therapy for the treatment of traumatic brain injury: progress and prospects. Rev Neurosci 2019; 30: 839-855.
21. Deng QJ, Xu XF, Ren J. Effects of SDF-1/CXCR4 on the repair of traumatic brain injury in rats by mediating bone marrow derived mesenchymal stem cells. Cell Mol Neurobiol 2018; 38: 467-477.
22. Denny-Brown DE, Russell WR. Experimental Concussion: (Section of Neurology). Proc R Soc Med 1941; 34: 691-692.
23. Dong HJ, Shang CZ, Li G, Niu Q, Luo YC, Yang Y, Meng HP, Yin HJ, Zhang HX, Zhao ML, Lin L. The distribution of transplanted umbilical cord mesenchymal stem cells in large blood vessel of experimental design with traumatic brain injury. J Craniofac Surg 2017; 28: 1615-1619.
24. Feng Y, Ju Y, Cui J, Wang L. Bone marrow stromal cells promote neuromotor functional recovery, via upregulation of neurotrophic factors and synapse proteins following traumatic brain injury in rats. Mol Med Rep 2017; 16: 654-660.
25. Fu XM, Liu SJ, Dan QQ, Wang YP, Lin N, Lv LY, Zou Y, Liu S, Zhou X, Wang TH. Combined bone mesenchymal stem cell and olfactory ensheathing cell transplantation promotes neural repair associated with CNTF expression in traumatic brain-injured rats. Cell Transplant 2015; 24: 1533-1544.
26. Fuentealba LC, Rompani SB, Parraguez JI, Obernier K, Romero R, Cepko CL, Alvarez-Buylla A. Embryonic origin of postnatal neural stem cells. Cell 2015; 161: 1644-1655.
27. Gao J, Grill RJ, Dunn TJ, Bedi S, Labastida JA, Hetz RA, Xue H, Thonhoff JR, DeWitt DS, Prough DS, Cox CS, Jr., Wu P. Human neural stem cell transplantation-mediated alteration of microglial/macrophage phenotypes after traumatic brain injury. Cell Transplant 2016; 25: 1863-1877.
28. Gao Y, Bai C, Wang K, Sun B, Guan W, Zheng D. All-trans retinoic acid promotes nerve cell differentiation of yolk sac-derived mesenchymal stem cells. Appl Biochem Biotechnol 2014; 174: 682-692.
29. Gincberg G, Shohami E, Lazarovici P, Elchalal U. Human umbilical cord blood CD45(+) pan-hematopoietic cells induced a neurotherapeutic effect in mice with traumatic brain injury: immunophenotyping, comparison of maternal and neonatal parameters, and immunomodulation. J Mol Neurosci 2018; 64: 185-199.
30. Gincberg G, Shohami E, Trembovler V, Alexandrovich AG, Lazarovici P, Elchalal U. Nerve growth factor plays a role in the neurotherapeutic effect of a CD45(+) pan-hematopoietic subpopulation derived from human umbilical cord blood in a traumatic brain injury model. Cytotherapy 2018; 20: 245-261.
31. Gladwin K, Choi D. Olfactory ensheathing cells: part I – current concepts and experimental laboratory models. World Neurosurg 2015; 83: 114-119.
32. Guo K, Yao X, Wu W, Yu Z, Li Z, Ma Z, Liu D. HIF-1α/SDF-1/CXCR4 axis reduces neuronal apoptosis via enhancing the bone marrow-derived mesenchymal stromal cell migration in rats with traumatic brain injury. Exp Mol Pathol 2020; 114: 104416.
33. Hackenberg K, Unterberg A. Traumatic brain injury. Nervenarzt 2016; 87: 203-214; quiz 215-206.
34. Harmon KG, Drezner J, Gammons M, Guskiewicz K, Halstead M, Herring S, Kutcher J, Pana A, Putukian M, Roberts W. American Medical Society for Sports Medicine position statement: concussion in sport. Clin J Sport Med 2013; 23: 1-18.
35. Haus DL, López-Velázquez L, Gold EM, Cunningham KM, Perez H, Anderson AJ, Cummings BJ. Transplantation of human neural stem cells restores cognition in an immunodeficient rodent model of traumatic brain injury. Exp Neurol 2016; 281: 1-16.
36. Hawley CA, Ward AB, Long J, Owen DW, Magnay AR. Prevalence of traumatic brain injury amongst children admitted to hospital in one health district: a population-based study. Injury 2003; 34: 256-260.
37. Hu H, Zhao Q, Liu X, Yan T. Human umbilical cord blood cells rescued traumatic brain injury-induced cardiac and neurological deficits. Ann Transl Med 2020; 8: 278.
38. Hu J, Chen L, Huang X, Wu K, Ding S, Wang W, Wang B, Smith C, Ren C, Ni H, ZhuGe Q, Yang J. Calpain inhibitor MDL28170 improves the transplantation-mediated therapeutic effect of bone marrow-derived mesenchymal stem cells following traumatic brain injury. Stem Cell Res Ther 2019; 10: 96.
39. Hu W, Jiang J, Yang F, Liu J. The impact of bone marrow-derived mesenchymal stem cells on neovascularisation in rats with brain injury. Folia Neuropathol 2018; 56: 112-123.
40. Hu Z, Gajavelli S, Spurlock MS, Mahavadi A, Quesada LS, Gajavelli GR, Andreoni CB, Di L, Janecki J, Lee SW, Rivera KN, Shear DA, Bullock RM. Human neural stem cell transplant location-dependent neuroprotection and motor deficit amelioration in rats with penetrating traumatic brain injury. J Trauma Acute Care Surg 2020; 88: 477-485.
41. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G,Kobusingye OC. The impact of traumatic brain injuries: a global perspective. NeuroRehabilitation 2007; 22: 341-353.
42. Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itohara S, Kageyama R. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat Neurosci 2008; 11: 1153-1161.
43. Jagnoor J,Cameron ID. Traumatic brain injury – support for injured people and their carers. Aust Fam Physician 2014; 43: 758-763.
44. Jahanbazi Jahan-Abad A, Sahab Negah S, Hosseini Ravandi H, Ghasemi S, Borhani-Haghighi M, Stummer W, Gorji A, Khaleghi Ghadiri M. Human neural stem/progenitor cells derived from epileptic human brain in a self-assembling peptide nanoscaffold improve traumatic brain injury in rats. Mol Neurobiol 2018; 55: 9122-9138.

45. Jiang J, Bu X, Liu M, Cheng P. Transplantation of autologous bone marrow-derived mesenchymal stem cells for traumatic brain injury. Neural Regen Res 2012; 7: 46-53.
46. Jung A, Johnson M, Veile R, Friend LA, Stevens-Topie S, Elterman J, Pritts T, Makley A, Goodman M. Variable saline resuscitation in a murine model of combined traumatic brain injury and haemorrhage. Brain Inj 2018; 32: 1834-1842.
47. Kappy NS, Chang S, Harris WM, Plastini M, Ortiz T, Zhang P, Hazelton JP, Carpenter JP, Brown SA. Human adipose-derived stem cell treatment modulates cellular protection in both in vitro and in vivo traumatic brain injury models. J Trauma Acute Care Surg 2018; 84: 745-751.
48. Kochanek PM, Tasker RC, Carney N, Totten AM, Adelson PD, Selden NR, Davis-O'Reilly C, Hart EL, Bell MJ, Bratton SL, Grant GA, Kissoon N, Reuter-Rice KE, Vavilala MS, Wainwright MS. Guidelines for the management of pediatric severe traumatic brain injury, third edition: update of the brain trauma foundation guidelines, executive summary. Neurosurgery 2019; 84: 1169-1178.
49. Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 2009; 32: 149-184.
50. Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav Immun 2012; 26: 1191-1201.
51. Lee JY, Acosta S, Tuazon JP, Xu K, Nguyen H, Lippert T, Liska MG, Semechkin A, Garitaonandia I, Gonzalez R, Kern R, Borlongan CV. Human parthenogenetic neural stem cell grafts promote multiple regenerative processes in a traumatic brain injury model. Theranostics 2019; 9: 1029-1046.
52. Li L, Chopp M, Ding G, Li Q, Mahmood A, Jiang Q. Chronic global analysis of vascular permeability and cerebral blood flow after bone marrow stromal cell treatment of traumatic brain injury in the rat: A long-term MRI study. Brain Res 2017; 1675: 61-70.
53. Li L, Jiang Q, Qu CS, Ding GL, Li QJ, Wang SY, Lee JH, Lu M, Mahmood A, Chopp M. Transplantation of marrow stromal cells restores cerebral blood flow and reduces cerebral atrophy in rats with traumatic brain injury: in vivo MRI study. J Neurotrauma 2011; 28: 535-545.
54. Liao GP, Harting MT, Hetz RA, Walker PA, Shah SK, Corkins CJ, Hughes TG, Jimenez F, Kosmach SC, Day MC, Tsao K, Lee DA, Worth LL, Baumgartner JE, Cox CS, Jr. Autologous bone marrow mononuclear cells reduce therapeutic intensity for severe traumatic brain injury in children. Pediatr Crit Care Med 2015; 16: 245-255.
55. Lin GQ, He XF, Liang FY, Guo Y, Sunnassee G, Chen J, Cao XM, Chen YY, Pan GJ, Pei Z, Tan S. Transplanted human neural precursor cells integrate into the host neural circuit and ameliorate neurological deficits in a mouse model of traumatic brain injury. Neurosci Lett 2018; 674: 11-17.
56. Lipton ML, Kim N, Park YK, Hulkower MB, Gardin TM, Shifteh K, Kim M, Zimmerman ME, Lipton RB, Branch CA. Robust detection of traumatic axonal injury in individual mild traumatic brain injury patients: intersubject variation, change over time and bidirectional changes in anisotropy. Brain Imaging Behav 2012; 6: 329-342.
57. Liu SJ, Zou Y, Belegu V, Lv LY, Lin N, Wang TY, McDonald JW, Zhou X, Xia QJ, Wang TH. Co-grafting of neural stem cells with olfactory en sheathing cells promotes neuronal restoration in traumatic brain injury with an anti-inflammatory mechanism. J Neuroinflammation 2014; 11: 66.
58. Liu W, Jiang X, Fu X, Cui S, Du M, Cai Y, Xu R. Bone marrow stromal cells can be delivered to the site of traumatic brain injury via intrathecal transplantation in rabbits. Neurosci Lett 2008; 434: 160-164.
59. Lu D, Li Y, Wang L, Chen J, Mahmood A, Chopp M. Intraarterial administration of marrow stromal cells in a rat model of traumatic brain injury. J Neurotrauma 2001; 18: 813-819.
60. Ma H, Lam PK, Tong CSW, Lo KKY, Wong GKC, Poon WS. The neuroprotection of hypoxic adipose tissue-derived mesenchymal stem cells in experimental traumatic brain injury. Cell Transplant 2019; 28: 874-884.
61. Ma H, Yu B, Kong L, Zhang Y, Shi Y. Transplantation of neural stem cells enhances expression of synaptic protein and promotes functional recovery in a rat model of traumatic brain injury. Mol Med Rep 2011; 4: 849-856.
62. Maas AIR, Menon DK, Adelson PD, Andelic N, Bell MJ, Belli A, Bragge P, Brazinova A, Büki A, Chesnut RM, Citerio G, Coburn M, Cooper DJ, Crowder AT, Czeiter E, Czosnyka M, Diaz-Arrastia R, Dreier JP, Duhaime AC, Ercole A, van Essen TA, Feigin VL, Gao G, Giacino J, Gonzalez-Lara LE, Gruen RL, Gupta D, Hartings JA, Hill S, Jiang JY, Ketharanathan N, Kompanje EJO, Lanyon L, Laureys S, Lecky F, Levin H, Lingsma HF, Maegele M, Majdan M, Manley G, Marsteller J, Mascia L, McFadyen C, Mondello S, Newcombe V, Palotie A, Parizel PM, Peul W, Piercy J, Polinder S, Puybasset L, Rasmussen TE, Rossaint R, Smielewski P, Söderberg J, Stanworth SJ, Stein MB, von Steinbüchel N, Stewart W, Steyerberg EW, Stocchetti N, Synnot A, Te Ao B, Tenovuo O, Theadom A, Tibboel D, Videtta W, Wang KKW, Williams WH, Wilson L, Yaffe K. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. Lancet Neurol 2017; 16: 987-1048.
63. Mahmood A, Lu D, Wang L, Chopp M. Intracerebral transplantation of marrow stromal cells cultured with neurotrophic factors promotes functional recovery in adult rats subjected to traumatic brain injury. J Neurotrauma 2002; 19: 1609-1617.
64. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J Neurosurg 1994; 80: 291-300.
65. Mastro-Martínez I, Pérez-Suárez E, Melen G, González-Murillo Á, Casco F, Lozano-Carbonero N, Gutiérrez-Fernández M, Díez-Tejedor E, Casado-Flores J, Ramírez-Orellana M, Serrano-González A. Effects of local administration of allogenic adipose tissue-derived mesenchymal stem cells on functional recovery in experimental traumatic brain injury. Brain Inj 2015; 29: 1497-1510.
66. Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell 2008; 3: 265-278.
67. Mitra B, Cameron PA, Butt W, Rosenfeld JV. Children or young adults? A population-based study on adolescent head injury. ANZ J Surg 2006; 76: 343-350.

68. Moreau F, Asdaghi N, Modi J, Goyal M, Coutts SB. Magnetic resonance imaging versus computed tomography in transient ischemic attack and minor stroke: the more υou see the more you know. Cerebrovasc Dis Extra 2013; 3: 130-136.
69. Mundra V, Gerling IC,Mahato RI. Mesenchymal stem cell-based therapy. Mol Pharm 2013; 10: 77-89.
70. Nam HS, Benezra R. High levels of Id1 expression define B1 type adult neural stem cells. Cell Stem Cell 2009; 5: 515-526.
71. Nasser M, Ballout N, Mantash S, Bejjani F, Najdi F, Ramadan N, Soueid J, Zibara K, Kobeissy F. Transplantation of embryonic neural stem cells and differentiated cells in a controlled cortical impact (CCI) model of adult mouse somatosensory cortex. Front Neurol 2018; 9: 895.
72. Osanai T, Kuroda S, Sugiyama T, Kawabori M, Ito M, Shichinohe H, Kuge Y, Houkin K, Tamaki N, Iwasaki Y. Therapeutic effects of intra-arterial delivery of bone marrow stromal cells in traumatic brain injury of rats – in vivo cell tracking study by near-infrared fluorescence imaging. Neurosurgery 2012; 70: 435-444; discussion 444.
73. Pang AL, Xiong LL, Xia QJ, Liu F, Wang YC, Liu F, Zhang P, Meng BL, Tan S, Wang TH. Neural stem cell transplantation is associated with inhibition of apoptosis, Bcl-xL upregulation, and recovery of neurological function in a rat model of traumatic brain injury. Cell Transplant 2017; 26: 1262-1275.
74. Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma HF, Maas AI. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien) 2015; 157: 1683-1696.
75. Qi L, Xue X, Sun J, Wu Q, Wang H, Guo Y, Sun B. The promising effects of transplanted umbilical cord mesenchymal stem cells on the treatment in traumatic brain injury. J Craniofac Surg 2018; 29: 1689-1692.
76. Ray SK, Dixon CE, Banik NL. Molecular mechanisms in the pathogenesis of traumatic brain injury. Histol Histopathol 2002; 17: 1137-1152.
77. Roet KC, Verhaagen J. Understanding the neural repair-promoting properties of olfactory ensheathing cells. Exp Neurol 2014; 261: 594-609.
78. Ruppert KA, Prabhakara KS, Toledano-Furman NE, Udtha S, Arceneaux AQ, Park H, Dao A, Cox CS, Olson SD. Human adipose-derived mesenchymal stem cells for acute and subacute TBI. PLoS One 2020; 15: e0233263.
79. Sharma A, Sane H, Kulkarni P, Yadav J, Gokulchandran N, Biju H, Badhe P. Cell therapy attempted as a novel approach for chronic traumatic brain injury – a pilot study. Springerplus 2015; 4: 26.
80. Sharma AK, Sane HM, Kulkarni PP, Gokulchandran N, Biju H, Badhe PB. Autologous bone marrow mononuclear cell transplantation in patients with chronic traumatic brain injury – a clinical study. Cell Regen 2020; 9: 3.
81. Sharp DJ. The association of traumatic brain injury with rate of progression of cognitive and functional impairment in a population-based cohort of Alzheimer's disease: the Cache County dementia progression study by Gilbert et al. Late effects of traumatic brain injury on dementia progression. Int Psychogeriatr 2014; 26: 1591-1592.
82. Shen Q, Yin Y, Xia QJ, Lin N, Wang YC, Liu J, Wang HP, Lim A, Wang TH. Bone marrow stromal cells promote neuronal restoration in rats with traumatic brain injury: involvement of GDNF regulating BAD and BAX signaling. Cell Physiol Biochem 2016; 38: 748-762.
83. Shetty AK, Mishra V, Kodali M, Hattiangady B. Blood brain barrier dysfunction and delayed neurological deficits in mild traumatic brain injury induced by blast shock waves. Front Cell Neurosci 2014; 8: 232.
84. Shi X, Bai Y, Zhang G, Liu Y, Xiao H, Liu X, Zhang W. Effects of over-expression of SOD2 in bone marrow-derived mesenchymal stem cells on traumatic brain injury. Cell Tissue Res 2018; 372: 67-75.
85. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol 2010; 6: 393-403.
86. Skandsen T, Kvistad KA, Solheim O, Strand IH, Folvik M, Vik A. Prevalence and impact of diffuse axonal injury in patients with moderate and severe head injury: a cohort study of early magnetic resonance imaging findings and 1-year outcome. J Neurosurg 2010; 113: 556-563.
87. Skardelly M, Gaber K, Burdack S, Scheidt F, Hilbig H, Boltze J, Forschler A, Schwarz S, Schwarz J, Meixensberger J, Schuhmann MU. Long-term benefit of human fetal neuronal progenitor cell transplantation in a clinically adapted model after traumatic brain injury. J Neurotrauma 2011; 28: 401-414.
88. Spurlock MS, Ahmed AI, Rivera KN, Yokobori S, Lee SW, Sam PN, Shear DA, Hefferan MP, Hazel TG, Johe KK, Gajavelli S, Tortella FC, Bullock RM. Amelioration of penetrating ballistic-like brain injury induced cognitive deficits after neuronal differentiation of transplanted human neural stem cells. J Neurotrauma 2017; 34: 1981-1995.
89. Srivastava AK, Prabhakara KS, Kota DJ, Bedi SS, Triolo F, Brown KS, Skiles ML, Brown HL, Cox CS, Jr., Olson SD. Human umbilical cord blood cells restore vascular integrity in injured rat brain and modulate inflammation in vitro. Regen Med 2019; 14: 295-307.
90. Sun D, Gugliotta M, Rolfe A, Reid W, McQuiston AR, Hu W, Young H. Sustained survival and maturation of adult neural stem/progenitor cells after transplantation into the injured brain. J Neurotrauma 2011; 28: 961-972.
91. Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic brain injury-related emergency department visits, hospitalizations, and deaths – United States, 2007 and 2013. MMWR Surveill Summ 2017; 66: 1-16.
92. Tian C, Wang X, Wang X, Wang L, Wang X, Wu S, Wan Z. Autologous bone marrow mesenchymal stem cell therapy in the subacute stage of traumatic brain injury by lumbar puncture. Exp Clin Transplant 2013; 11: 176-181.
93. Titus DJ, Sakurai A, Kang Y, Furones C, Jergova S, Santos R, Sick TJ, Atkins CM. Phosphodiesterase inhibition rescues chronic cognitive deficits induced by traumatic brain injury. J Neurosci 2013; 33: 5216-5226.
94. Ventura RE, Balcer LJ, Galetta SL. The neuro-ophthalmology of head trauma. Lancet Neurol 2014; 13: 1006-1016.
95. Walker AE. The physiological basis of concussion: 50 years later. J Neurosurg 1994; 81: 493-494.

96. Wang CC, Schoenberg BS, Li SC, Yang YC, Cheng XM, Bolis CL. Brain injury due to head trauma. Epidemiology in urban areas of the People's Republic of China. Arch Neurol 1986; 43: 570-572.
97. Wang Y, Peng D, Yang X, Huang P, Ye H, Hui Y, Wang X, Sun W, Wu H, Zhang S, Wang L, Sha H, Shang C, Dong H, Hu Q. Study on umbilical cord-matrix stem cells transplantation for treatment of acute traumatic brain injury in rats. Turk Neurosurg 2019; 29: 750-758.
98. Wang YC, Xia QJ, Ba YC, Wang TY, Li NN, Zou Y, Shang FF, Zhou XF, Wang TH, Fu XM, Qi JG. Transplantation of olfactory ensheathing cells promotes the recovery of neurological functions in rats with traumatic brain injury associated with downregulation of Bad. Cytotherapy 2014; 16: 1000-1010.
99. Wang Z, Luo Y, Chen L, Liang W. Safety of neural stem cell transplantation in patients with severe traumatic brain injury. Exp Ther Med 2017; 13: 3613-3618.
100. Weston NM, Sun D. The potential of stem cells in treatment of traumatic brain injury. Curr Neurol Neurosci Rep 2018; 18: 1.
101. Wewetzer K, Kern N, Ebel C, Radtke C, Brandes G. Phagocytosis of O4+ axonal fragments in vitro by p75- neonatal rat olfactory ensheathing cells. Glia 2005; 49: 577-587.
102. Xiong LL, Hu Y, Zhang P, Zhang Z, Li LH, Gao GD, Zhou XF, Wang TH. Neural stem cell transplantation promotes functional recovery from traumatic brain injury via brain derived neurotrophic factor-mediated neuroplasticity. Mol Neurobiol 2018; 55: 2696-2711.
103. Xiong Y, Mahmood A, Lu D, Qu C, Kazmi H, Goussev A, Zhang ZG, Noguchi CT, Schallert T, Chopp M. Histological and functional outcomes after traumatic brain injury in mice null for the erythropoietin receptor in the central nervous system. Brain Res 2008; 1230: 247-257.
104. Xu L, Xing Q, Huang T, Zhou J, Liu T, Cui Y, Cheng T, Wang Y, Zhou X, Yang B, Yang GL, Zhang J, Zang X, Ma S, Guan F. HDAC1 silence promotes neuroprotective effects of human umbilical cord-derived mesenchymal stem cells in a mouse model of traumatic brain injury via PI3K/AKT pathway. Front Cell Neurosci 2018; 12: 498.
105. Yan ZJ, Zhang P, Hu YQ, Zhang HT, Hong SQ, Zhou HL, Zhang MY, Xu RX. Neural stem-like cells derived from human amnion tissue are effective in treating traumatic brain injury in rat. Neurochem Res 2013; 38: 1022-1033.
106. Yu B, Ma H, Kong L, Shi Y, Liu Y. Enhanced connexin 43 expression following neural stem cell transplantation in a rat model of traumatic brain injury. Arch Med Sci 2013; 9: 132-138.
107. Yuan X, Luo Q, Shen L, Chen J, Gan D, Sun Y, Ding L, Wang G. Hypoxic preconditioning enhances the differentiation of bone marrow stromal cells into mature oligodendrocytes via the mTOR/HIF-1α/VEGF pathway in traumatic brain injury. Brain Behav 2020; 10: e01675.
108. Zhang J, Jiao J. Molecular biomarkers for embryonic and adult neural stem cell and neurogenesis. Biomed Res Int 2015; 2015: 727542.
109. Zhang ZX, Guan LX, Zhang K, Zhang Q, Dai LJ. A combined procedure to deliver autologous mesenchymal stromal cells to patients with traumatic brain injury. Cytotherapy 2008; 10: 134-139.
110. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008; 132: 645-660.