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Mesenchymal stem cell therapy for the treatment of traumatic brain injury: progress and prospects

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Abstract: Traumatic brain injury (TBI) is a major cause of injury-related mortality and morbidity in the USA and around the world. The survivors may suffer from cognitive and memory deficits, vision and hearing loss, movement disorders, and different psychological problems. The primary insult causes neuronal damage and activates astrocytes and microglia which evokes immune responses causing further damage to the brain. Clinical trials of drugs to recover the neuronal loss are not very successful. Regenerative approaches for TBI using mesenchymal stem cells (MSCs) seem promising. Results of preclinical research have shown that transplantation of MSCs reduced secondary neurodegeneration and neuroinflammation, promoted neurogenesis and angiogenesis, and improved functional outcome in the experimental animals. The functional improvement is not necessarily related to cell engraftment; rather, immunomodulation by molecular factors secreted by MSCs is responsible for the beneficial effects of this therapy. However, MSC therapy has a few drawbacks including tumor formation, which can be avoided by the use of MSC-derived exosomes. This review has focused on the research works published in the field of regenerative therapy using MSCs after TBI and its future direction.

Keywords: exosomes; mesenchymal stem cell (MSC); regenerative medicine; traumatic brain injury (TBI).

Introduction

Traumatic brain injury (TBI) is a major cause of injury-related deaths and disabilities in the USA and around the world. According to the Centers for Disease Control and Prevention, 30% of all injury-related deaths (153 people per day in the USA) are attributable to TBI (Taylor et al., 2017). Cognitive deficits, impaired memory, movement disorders, loss of hearing and vision, and psychological problems are common among the survivors. The primary injury damages the blood-brain barrier (BBB) and destroys or damages the neuronal and glial tissues which evokes local inflammation and causes secondary neurodegeneration. Restoration of the damaged tissue is required to prevent secondary neurodegeneration and to foster regeneration of the brain tissues. Although attempts were made to treat this condition as a single entity, TBI is, in fact, a syndrome which encompasses multiple damages requiring different targeted therapies and physiological goals. Clinical trials of drugs like erythropoietin (Robertson et al., 2014; Nichol et al., 2015) and progesterone (Robertson et al., 2014; Skolnick et al., 2014) have failed to improve brain damage in human patients in spite of their neuroprotective effects. Transplantation of mesenchymal stem cells (MSCs) is one of the promising approaches in regenerative therapy. These progenitor cells are found to migrate to the injury site, secrete trophic factors, and modify the regenerative process. This review is focused on different aspects of the MSC transplantation therapy for TBI and the future of this approach.

Types of TBI

Traumatic brain injury can occur due to any traumatic injury to the head, such as sports-related injuries, accidental fall, domestic violence, automobile accidents, or blast-related injuries. The direct impact or primary injury can cause focal injury occurring in a specific location or diffuse injury which is more widespread. The primary injury may spread over time and cause secondary injury with long-term consequences. Standard neuroimaging techniques such as computed tomography (CT) scan or magnetic resonance imaging (MRI) can detect the primary focal injury whereas the primary diffuse injury is normally detected by MRI (Lee and Newberg, 2005; Chalela et al., 2007; Moreau et al., 2013) including diffusion tensor imaging (DTI) (Ventura et al., 2014). The primary diffuse

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injury is an ongoing process that includes hypoxic brain damage, vascular injury, brain swelling, and axonal injury (Taber et al., 2006). The junction of gray matter-white matter, internal capsule, upper brainstem, and corpus callosum are among the areas mostly affected by the diffuse injury. Based on the severity of primary impact or damage as well as the extent of secondary damage, TBIs can be categorized into mild, moderate, and severe. Approximately 80% of TBI occurrences are mild, 10% are moderate, and 10% are severe cases as recorded (Blennow et al., 2016). TBI affects a significant part of the brain and the effect largely depends on the severity of the injury. In mild TBI (mTBI) the CT scan appears normal, Glasgow coma scale score (GCS) is 13–15, the patient remains unconscious for 30 min or less, and less than 24 h of mental alteration and amnesia were recorded (Ventura et al., 2014; Blennow et al., 2016). Even mTBI can cause death and damage of the neuronal and other cell types, and evoke immune responses in the body. Concussion, the most common form of TBI, can be found in a subset of patients diagnosed with mTBI and can induce transient disruption of brain function (Management of Concussion/m.TBI Working Group, 2009; Menon et al., 2010; Harmon et al., 2013; Ventura et al., 2014). The severity of secondary mechanisms of TBI includes cell death, axonal damage, inflammation, edema, and impaired neurogenesis (Chalela et al., 2007; Cernak and Noble-Haeusslein, 2010). Using DTI, investigators have found a high prevalence of diffuse injury in the white matter in the frontal lobes, corpus callosum, and corona radiata and in the deep white matter following mTBI (Maruta et al., 2010; Lipton et al., 2012; Ventura et al., 2014; Jang and Seo, 2015). Moderate TBIs are associated with a GCS score of 9–12 and severe TBIs are associated with GCS scores of 3–8 (Ventura et al., 2014; Blennow et al., 2016). In both these cases, patients suffer from problems with executive functions and attention deficits, cognition, memory, and concentration. Whether there is any difference between the damage caused by civilian and combat-related TBI is not yet fully elucidated, as blast-related TBI injuries are further complicated by blast-related burns, toxic inhalation, and radiation exposure (Ventura et al., 2014).

Different types of stem cells used in regenerative therapy of TBI

Stem cells are present in different tissues in the body and can be isolated and cultured. Based on their source or origin, stem cells can be categorized as hematopoietic stem cells, MSCs, neural stem cells (NSCs), epithelial or skin stem cells. Stem cells can also be categorized as exogenous or endogenous, embryonic stem cells, adult stem cells (ASCs), or induced pluripotent stem cells. Recently, ASCs have gained attention in regenerative therapies. Several researchers have shown the regenerative potential of MSCs for brain injuries. Although all stem cells have the potential to differentiate into adult cells, MSCs are promising because of their multipotent nature and potential to be extracted from any kind of tissues (da Silva Meirelles et al., 2006; Karp and Leng Teo, 2009). Moreover, when cultured *in vitro* they can be differentiated into cells other than cells of mesenchymal origin (Pittenger et al., 1999; Sanchez-Ramos et al., 2000; Hasan et al., 2017). MSCs were used synonymously for different types of cells, for example, MSCs, mesenchymal stromal cells, multipotent stromal cells, and also for mesenchymal progenitor cells regardless of their source of isolation. It is, therefore, important to specify the characteristics that define this particular cell type (Rojewski et al., 2008). According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT), the MSCs must be characterized based on their surface antigen characteristics, adherence to plastic, and differentiation potentials to osteoblasts, chondroblasts, or adipocytes. Other fibroblast like, plastic adherent cells, regardless of their tissue of origin, should be classified as mesenchymal stromal cells (Horwitz et al., 2005; Rojewski et al., 2008). ISCT delineates that MSCs must be plastic adherent and under normal culture conditions express surface antigens like CD105, CD73, and CD90 and lack expression of CD79α, CD11b, CD19, or CD45, CD34, CD14, and the HLA-DR to distinguish MSCs from other stem cells (Dominici et al., 2006; Hasan et al., 2017). Isolation of MSCs from tissues can be challenging. During isolation of MSCs from bone marrow cell suspensions, the major problem faced is the contamination with hematopoietic cells at early passages (Phinney et al., 1999; Peister et al., 2004) and phenotypic evolution of cells (Baustian et al., 2015). By phenotypic analysis of $CD45^-/Ter119^-$ bone marrow cells Baustian et al. (2015) showed that there was a change in the composition of cells expressing human MSC markers CD73, CD90, and CD105 and mouse MSC markers CD44 and Sca-1 (Baustian et al., 2015). Attempts were made to avoid these limitations and enrich MSCs in culture by using techniques including antibody-based cell sorting (Van Vlasselaer et al., 1994), positive and negative selection methods (Baddoo et al., 2003), frequent media change method (Soleimani and Nadri, 2009), and low and high density culture techniques (Eslaminejad et al., 2006; Eslaminejad and Nadri, 2009). Houlihan et al. (2012) isolated mouse

MSCs based on PDGFRα and Sca-1 as surface markers (Houlihan et al., 2012). By positive selection using a combination of antibodies to Sca-1, CD90 and PDGFRα, and culturing in hypoxia, Baustian et al. (2015) isolated a subpopulation of mouse $CD45^-/Ter119^-$ bone marrow cells and was able to enrich to a colony forming unit-fibroblast cloning efficiency of ¼ (Baustian et al., 2015).

The ability to home into the site of inflammation (Chamberlain et al., 2007; Alexanian et al., 2011; Chamberlain et al., 2011; Donega and Raineteau, 2017), reduced response to the immune system (Galindo et al., 2011), and secretion of growth factors to promote tissue regeneration (Parr et al., 2007; Galindo et al., 2011; Redondo-Castro et al., 2017) make MSCs an attractive candidate for regenerative therapies. Animal studies have shown enormous potential although MSC transplantation in humans is challenging (Menge et al., 2012; Nichol et al., 2015; Menon and Ercole, 2017), mainly because of lack of suitable transplantation methods as discussed in the section "Methods of delivery." However, this immense therapeutic potential of MSCs may not be fully exploited because of the effects of reactive oxygen species (ROS) during expansion. ROS may affect the *ex vivo* expansion of MSCs as well as their functioning after transplantation including immunomodulation and regeneration. ROS can affect the differentiation of MSCs *in vitro*. It is shown that a low ROS level is necessary to maintain survival, proliferation, and differentiation of cells *in vitro* while a higher level of ROS may be damaging to MSCs (Kobayashi and Suda, 2012; Atashi et al., 2015). Moreover, MSCs are lower in antioxidant activity and more sensitive to oxidative stress compared to many differentiated cell types (Orciani et al., 2010; Ko et al., 2012). Thus, excess ROS or exogenous H_2O_2 can impair MSC proliferation and differentiation (Alves et al., 2010; Ko et al., 2012; Choo et al., 2014). Although there is no direct evidence for ROS affecting MSCs' immunomodulation ability, there is sufficient evidence that during culture, oxidative stress increases and the levels of surface antigens of MSCs like CD13, CD 29, and CD 44 decrease along with the cells' ability to suppress T cell proliferation (Wagner et al., 2008; Zaim et al., 2012; Ren et al., 2013). Kizilay Mancini et al. (2015) have shown that MSCs isolated from human patients with atherosclerosis or diabetes type II, diseases known to be associated with elevated ROS levels, have reduced ability of T cell inhibition (Kizilay Mancini et al., 2015). As old age is associated with higher oxidative stress, the question comes whether donor's age affects the MSCs' ability of immunomodulation. Conflicting evidences exist regarding this question. Wu et al. (2014) observed that MSCs isolated from older patients had reduced ability of inhibiting T cell proliferation (Wu et al., 2014). On the other hand, studies conducted by Seigel et al. (2013) on human patients of different age groups did not find any significant correlation between age and T cell regulation by MSCs (Siegel et al., 2013; Denu and Hematti, 2016). In another study Landgraf et al. (2011) cultured peripheral blood mononuclear cells and MSCs obtained from healthy young and elderly donors to study the immunosuppressive effects of MSC. They did not observe any difference in the immunosuppressive properties of MSCs isolated from young or elderly donors and, therefore, concluded that MSCs from elderly donors were equally as suitable as those from younger donors (Landgraf et al., 2011).

Delivery of MSCs to the brain

Routes of delivery

The success of stem cell therapy depends on optimal transplantation time point and route of administration. Although not many systematic studies on the delivery routes were done, researchers have delivered stem cells to host body through several routes including intravenous (i.v.), intra-arterial (i.a.), or intracranial (i.c.v.) delivery methods. For clinical applications i.v. route seems the most attractive. For brain delivery i.c.v. administration is the most targeted and frequently used approach although it is the most invasive one causing mechanical tissue disruption. Also, a limited number of cells can be delivered. As discussed by Xiong et al. (2017) i.v. injection of MSCs causes systemic distribution of cells while i.a. administration can cause cerebral ischemia (Xiong et al., 2017). On the other hand, i.c.v. injection limits the number of the injected cells to a sub-effective dose (Xiong et al., 2017).

The only noninvasive route of MSC administration is the intranasal (i.n.) route. Transplanted cells move through the nasal mucosa and either penetrate the cribriform plate and take the olfactory pathway to the brain (Galeano et al., 2018; Li et al., 2018) or migrate by the trigeminal nerve (Li et al., 2018). After crossing the cribriform plate, the cells either migrate to the olfactory bulb and other parts of the brain parenchyma or enter into the colony stimulating factor (CSF). Along with the CSF movement they move along the cortical surface and eventually enter the brain parenchyma (Danielyan et al., 2009). By trigeminal pathway the cells are taken up in the distal nasal cavity and migrate to the brain (Li et al., 2018). Using fluorescently labeled cells Danielyan et al. (2009) have shown the presence of rat MSCs in the brain 1 h after i.n. administration in mouse (Danielyan et al., 2009). The efficiency of i.n. delivery can

be enhanced by enzymatic permeabilization of the nasal mucosa with application of hyaluronidase. Because of the anatomical differences in mouse and human nasal cavity, and distance from the brain, the effect of i.n. administration of cells may vary. The supine position of anesthetized mice during cell administration allows a direct contact between administered cells and the nasal epithelium which possibly enhances the efficacy of the delivery method. On the other hand, in human subjects where repeated anesthesia is not possible finding optimal position of the head for cell delivery is essential (Li et al., 2018). Migration of cells from the nasal epithelium to the brain occurs within 2 h of cell delivery in rodents (Galeano et al., 2018). Danielyan et al. (2009) observed the presence of cells in subarachnoid space, olfactory bulbs, and other areas within 1 h of delivery in rodents (Danielyan et al., 2009; Galeano et al., 2018). In spite of all the measures of efficient delivery it should be noted that a large number of cells may be entrapped in the nasal cavity as observed by Galeano et al. (2018).

Time of delivery

Delivering MSCs at an optimum time is also important for efficient therapeutic effect. In a rat stroke model study, Omori et al. (2008) showed higher engraftment rate of infused MSCs when transplanted soon after the onset of injury (Omori et al., 2008). On the other hand, in a severe TBI model of rats, Han et al. (2013) observed histological and functional improvements when treated with human bone marrow-derived stem cells 7 days post-injury rather than 1 day post-injury (Han et al., 2013). In human subjects, Tian et al. (2013) have shown that the best result can be achieved when MSCs are transplanted in a window of efficacy after the onset of TBI. Ninety-seven TBI patients were transplanted with MSCs at different time points between TBI injury and the treatment start point. The patients with persistent vegetative state who received the therapy within 1.51 months of the injury showed better outcome than the patients with longer time lapse. Significant improvement was observed in patients with motor disorders who received cell treatment within 1.35 months after injury onset compared to those who received the treatment later (Tian et al., 2013). Therefore, the efficacy of MSC treatment was increased when it was implemented at an earlier time point.

Number of delivered cells

The number of transplanted MSCs plays an important role in the success of tissue repair. Although it may be expected that the higher the number, the greater will be the extent of tissue repair, that may not be the actual situation. Wu et al. (2008) have observed that following a brain injury in rats neurological functions were improved after infusion of a dose of 1×10^6 MSCs (Wu et al., 2008) which did not improve any further following infusion of 3×10^6 cells. In fact, a very small percentage of the administered MSCs survive in the system and reach the brain. In a systematic study in young mice, Danielyan et al. (2009) implanted 3×10^5 cells through i.n. route. They observed 584 ± 184 cells in the olfactory bulb, 227 ± 47 in the cortex, and 210 ± 60 cells arriving in the cortex within 1 h of application (Danielyan et al., 2009). Tian et al. (2013) transplanted 3.9×10^6 or 4.3×10^6 MSCs in subarachnoid space by lumber puncture technique after TBI in human patients and did not observe any correlation between number of cells administered and improvement of TBI outcome (Tian et al., 2013).

Microenvironment of the host tissue

The microenvironment of recipient tissue is also an important regulatory factor for the success of MSC transplantation therapy. The proinflammatory cytokines or ROS secreted by the immune cells in the inflammatory microenvironment of the injured tissue could reduce the success rate of stem cell therapy (Badiavas and Falanga, 2003; Garcia-Olmo et al., 2005). Post-trauma inflammation may also impair survival and homing efficiency of stem cells (Molcanyi et al., 2007). It was shown that proinflammatory cytokines and ROS secreted by the immune cells in the inflammatory microenvironment of the injured tissue reduced the success rate of MSC transplantation therapy (Garcia-Olmo et al., 2005). Regenerative efficacy of the stem cells transplanted with oral cox-2 inhibitor celecoxib have been shown to increase the engraftment efficacy and differentiation of stem cells leading to enhanced wound tissue repair (Geesala et al., 2017).

Mechanisms of MSC action in TBI

Figure 1 shows a schematic diagram of possible mechanisms of action of MSCs in the injured brain and is discussed below.

Transmigration of grafted MSCs

Following transplantation, a very small fraction of transplanted hMSCs actually reach the injury site. In a rat

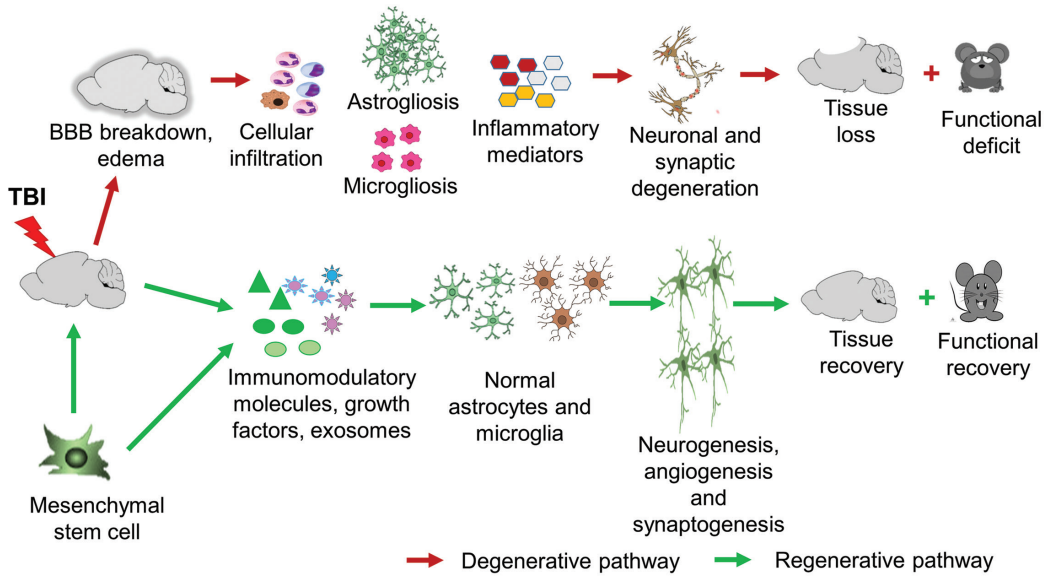

Figure 1: Possible mechanisms of action of mesenchymal stem cells in the brain after traumatic brain injury (TBI).
TBI disrupts the blood-brain barrier (BBB) causing edema and cellular infiltration, damages the neurons, and activates astrocytes and microglia. Activated astrocytes and microglia initiate inflammatory reactions causing further damages to the neurons and other cells resulting in tissue loss and functional deficits. Following implantation, MSCs cross the BBB, migrate to the damaged area of the brain and either secrete molecules themselves or help the neighboring cerebral tissues to secrete molecules to restrict tissue damage and promote neurogenesis and neuronal plasticity leading to structural and functional recovery.

model of TBI Mahmood et al. (2003) observed 495 cells in the perilesional area 28 days following the delivery of one million stem cells (Mahmood et al., 2003). After delivery of two million cells intravenously in Wister rats 24 h post TBI, they observed 25 cells/mm^2 after 1 month and 4 cells/mm^2 after 3-month post-administration. Following systemic delivery through i.v. injections, although MSCs are trapped by the lung, subsequently they migrate to the injured brain tissue (Chen et al., 2001; Mahmood et al., 2003; Sordi, 2009). Crossing the BBB is a challenge for the neuro-therapeutic drugs to be delivered in the brain and exert their effects. While different strategies were investigated to overcome this barrier (Abbott and Romero, 1996; Gaillard et al., 2012), MSCs seem to possess the ability to cross the BBB themselves (Schmidt et al., 2006; Steingen et al., 2008). Influenced by several secretory factors, e.g., chemokines and growth factors (Ponte et al., 2007), MSCs can transmigrate across the endothelium (Karp and Leng Teo, 2009) and move to the site of injury (Mahmood et al., 2006; Walker et al., 2009) where they can differentiate into adult cell types or promote tissue repair. MSCs express vascular adhesion molecule (VCAM-1) which helps them to adhere to the vascular endothelium (Ruster et al., 2006; Segers et al., 2006; da Silva Meirelles et al., 2009). Upon reaching the cerebro-vascular bed MSCs can adhere to the endothelial cells (ECs) of the BBB with the help of adhesion molecules like VCAM-1/VLA-4 and integrin-β1. Following adhesion, they use cytoplasmic podia (Steingen et al., 2008) to pass through the tight junctions of ECs (Schmidt et al., 2006) and invade the host tissue. MSCs express integrin-β1 (a subunit of VLA-4) but insufficient amount of integrin-α4 (ITGA4) causing significant cellular aggregation following transplantation leading to cerebral embolism. This results in limited homing ability (Maerz et al., 2016; Cui et al., 2017). Using ITGA4 overexpressing MSCs, Cui et al. have shown reduced cell aggregation and cerebral embolism following implantation in a rat model of stroke (Cui et al., 2017) thereby improving the transplantation outcome. A common outcome of TBI is the BBB disruption. In such cases, MSCs were shown to express tissue inhibitor metalloproteinase-3 which helps in restoring the integrity of the ECs and reduces the permeability of the BBB (Menge et al., 2012). In addition to adhesive interactions, chemokines released from tissues or ECs may cause adhesion ligand activation and transmigration of MSCs. MSCs expressing CCR2 [monocyte chemoattractant protein 1 (MCP-1) receptor] on the cell surface were found at higher frequency in the myocardium and other tissues like brain, kidney, and skeletal muscles of transgenic mice specifically expressing MCP-1 (Belema-Bedada et al., 2008). In a spinal cord lesion model, Hofstetter et al. (2002) observed that following i.v. administration,

transplanted MSCs formed a bridge between the injury epicenter and adjacent areas by forming bundles and thus guided regenerating fibers through the lesion area and promoted recovery (Hofstetter et al., 2002; Sordi, 2009).

Differentiation of transplanted MSCs

Differentiation of transplanted MSCs is an important aspect of successful therapy. MSCs have potential to differentiate into a variety of adult cells like chondrogenic, osteogenic, and neural progenitors, which can then be used in transplantation therapies. When growing *in vitro* without any differentiation cues, stem cells continue to divide in the culture. Although the transdifferentiation to neuronal cell types is questionable several studies have shown that under appropriate stimulation MSCs may differentiate into cells with neuronal fate. Cho et al. (2005) have shown that MSCs can be differentiated by retinoic acid (RA) into neuronal cells expressing neuron-specific markers and showing synaptic properties (Cho et al., 2005). Greco et al. (2007) have shown that following treatment with a cocktail of induction agents containing fibroblast growth factor (bFGF) and RA, 90% of hMSCs differentiated into cells expressing neuronal markers after 12 days of treatment. Furthermore, these cells were post-mitotic, were capable of neurotransmitter synthesis, and exhibited spontaneous post-synaptic currents (Greco et al., 2007). In another study, murine bone marrow-derived stromal feeder cells (MS5) were induced to produce cells with neuronal fate in which a graft-to-host axonal connection was created (Itoh et al., 1989; Wei et al., 2017). It is important that the transplanted MSCs cross the BBB, migrate, and home in the injured or inflamed brain tissues, and differentiate into neuronal or glial cells. Kopen et al. (1999) have observed that intra-cerebroventricular transplantation of MSCs in neonatal mice caused migration of the transplanted cells along definite routes and distribution throughout the brain. The cells differentiated into various cell types depending on the developmental stages of the part of the brain. Migration of the transplanted cells was also observed in the areas of the brain where neurogenesis was taking place. As the migrated cells were double positive for BrdU, a proliferation marker and neurofilament, these cells were likely involved in neurogenesis (Kopen et al., 1999). Differentiation of MSCs into glial cells was evidenced by different authors (Kopen et al., 1999; Sanchez-Ramos et al., 2000). In a rat TBI model after 30 days of i.v. administration of 1 million hMSCs, 6% ± 3.0% of cells were found to be Tuji1 positive and 12.7% ± 3.5% cells were GFAP positive (Mahmood et al., 2003). Nicholas et al. (2013) isolated and characterized MSC population from human peripheral blood. CD133 + ABCG2 + CXCR4 + MSCs primed with RA expressed markers of neural lineage. Transplantation of these cells in rats with TBI showed significant behavioral and histological improvements including production of neurotrophic factors as early as 2 days post-transplantation and neurogenesis at 1 and 3 months post-transplantation (Nichols et al., 2013).

Paracrine mechanism

Being immuno-privileged, MSCs can overcome the human leukocyte antigen barrier and facilitate transplantation (Qu and Zhang, 2017). Following injury, the brain attempts to regenerate by activating the endogenous neurogenesis process. It starts in the sub-ventricular zone (SVZ) and the sub-granular zone (SGZ) of the hippocampus. Neural progenitor cells originating in the SVZ migrate to the injury area but only a few of them survive and mature to aid in the repair process. The MSC therapy in rodents has been shown to increase the doublecortin positive new born neurons in the SVZ and SGZ areas (Pischiutta et al., 2014, 2016; Zanier et al., 2014; Mastro-Martinez et al., 2015) and increase GAP 43 expression indicating axonal growth (Zanier et al., 2014; Shen et al., 2016). Studies have shown that administration of growth factors like FGF-2, epidermal growth factor (Sun et al., 2009, 2010), or vascular endothelial growth factor (VEGF) (Thau-Zuchman et al., 2010) promotes post-TBI neurogenesis. The MSC treatment after TBI has increased the amount of these growth factors in the recipient tissues and aided in neurogenesis (Mahmood et al., 2004; Chen et al., 2005; Zanier et al., 2011; Wang et al., 2015; Shen et al., 2016; Feng et al., 2017), suggesting that the implanted MSCs worked as “bioreactors” to repair the injured tissues (Carbonara et al., 2018). Following transplantation, only a small proportion of MSCs was found to survive and whether these surviving cells differentiate into neural cell lineage is debated. Therefore, it is unlikely that their therapeutic effect is delivered by differentiating the grafted cells to neural cells. Rather, the therapeutic effect is likely due to diffused trophic factors secreted by transplanted MSCs that help the brain tissues surrounding the injury site to protect the uninjured cells (Caplan and Dennis, 2006) (Figure 1). These trophic factors include glial cell-line derived neurotrophic factor (Minnich et al., 2010), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), VEGF, CSF-1, and FGF-2 (Chau et al., 2014; Sun et al., 2015; Wei et al., 2017). Moreover, insulin-like growth factor has been shown to play a crucial role in supporting differentiation and proliferation

of MSCs into neural cells (Huat et al., 2014). In the injured brain MSCs release these bioactive molecules which either promote the survival and proliferation of existing cells or induce the neighboring cells to secrete molecules that help the survival and proliferation of the injured cells. These bioactive molecules fall into several categories such as chemoattractant, anti-fibrotic, immunomodulatory, or angiogenic factors (Table 1) and their mechanisms of action are well documented (Chen et al., 2002; Mahmood et al., 2004; da Silva Meirelles et al., 2016).

In addition to the activating endogenous neurogenesis in the SVZ, it is observed that MSCs are able to promote neural plasticity after TBI in rodents. Xiong et al. (2009) transplanted MSCs 7 days after TBI and injected fluorescent dye in the contralateral hemisphere 36 days after TBI. They found that infused fluorescent dye was transported from the contralateral hemisphere to the injured hemisphere through the corpus callosum, indicating recovered neuronal plasticity after MSC treatment in rats (Xiong et al., 2009). The MSCs possibly modulate glial activation following TBI. Thus, glial scar formation surrounding the injured area is reduced which in turn helps in the recovery of neuronal tissues leading to functional recovery (Zanier et al., 2011; Wang et al., 2013b; Pischiutta et al., 2014).

Immunomodulatory effects of MSCs in TBI

Following TBI, immune cells get activated and pro-inflammatory cytokines like interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNFα) are secreted which have both deleterious and beneficial effects. The immune system activation plays a role in the spread of secondary neurodegeneration and in later phases may help in the repair process. For example, microglial activation and migration help in clearing debris and tissue remodeling (Aloisi, 2001; Ziebell and Morganti-Kossmann, 2010; Zhang et al., 2013). T-cells have a role in tissue repair process in the later phases of injury (Brait et al., 2012). It is reported that MSCs can suppress the proliferation of T cells and differentiation of monocytes, affect the functions of dendritic cells, and also inhibit the production of TNF, and increase the production IL-10 (Nasef et al., 2008). By their ability to migrate to the injured tissues and secrete bioactive and trophic factors, MSCs help in cellular regeneration and modify the microenvironment to reduce inflammation and promote growth of the new tissue (Parr et al., 2007; Scuteri et al., 2011). Studies have shown that MSCs can modulate T-cell and microglial responses and the cytokine secretion profile of macrophages (Di Nicola et al., 2002; Meirelles Lda et al., 2009; Nemeth et al., 2009; Zhao et al., 2016a,b). Vasandan et al. (2016) observed that MSCs can reprogram microglia from pro-inflammatory (M1) to anti-inflammatory (M2) phenotype (Vasandan et al., 2016). Upon migration to injured tissues MSCs reduce the secretion of inflammatory cytokines to suppress the immune response and thereby promote the growth of healthy tissues (Uccelli et al., 2008; Lee et al., 2010; Sheikh et al., 2011). Zhang et al. (2013) studied the anti-inflammatory and immunomodulatory properties of MSCs by systemic transplantation into TBI model of rats. They observed a decrease in microglia and other inflammatory cells, reduction in pro-inflammatory cytokine production, and increase in anti-inflammatory cytokines possibly by activation of TNF-inducible gene 6 protein or TNF-stimulated gene 6, and suppression of nuclear factor

Table 1: Bioactive molecules secreted by mesenchymal stem cells (MSC) and their possible functions.

Molecules	Functions	References
CCL2 (MCP-1), CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (MCP-3), CCL20 (MIP-3a), CCL26 (eotaxin-3), CX3CL1 (fractalkine), CXCL5 (ENA-78), CXCL11 (i-TAC), CXCL1 (GROa), CXCL2 (GROb), CXCL8 (IL-8), CCL10 (IP-10), CXCL12 (SDF-1)	Chemokines/ chemoattraction	(da Silva Meirelles et al., 2008, 2009)
PGE-2, TGF-b, HGF, IDO, iNOS, HLA-G5, LIF	Immunomodulation	(Di Nicola et al., 2002; Meisel et al., 2004; Aggarwal and Pittenger, 2005; Sotiropoulou et al., 2006; Nasef et al., 2007; Sato et al., 2007; Di Ianni et al., 2008; Rizzo et al., 2008; Nemeth et al., 2009)
bFGF, PIGF, VEGF, MCP-1, IL-6	Angiogenesis	(Kinnaird et al., 2004; Hung et al., 2007; da Silva Meirelles et al., 2009)
VEGF, HGF, IGF-I, TGF-b, bFGF, GM-CSF	Anti-apoptosis	(Rehman et al., 2004; Togel et al., 2007)
SCF, LIF, IL-6, M-CSF, SDF-1	Growth and differentiation	(Haynesworth et al., 1996; Majumdar et al., 1998; Ohab et al., 2006; Sugiyama et al., 2006)

κB signaling pathway. Following administration of bone marrow derived MSCs 7 days after TBI, Kota et al. (2016) observed 50% reduction of IFN-γ and TNF-α (Kota et al., 2017) along with significantly reduced BBB permeability, edema, microglial activation, norepinephrine level, and increased neurogenesis (Kota et al., 2016). A recent study has found that MSC single injection in rats after TBI modulated gene expression profile of cytokines and chemokines in the brain and attenuated neurological injury (Lin et al., 2019). In another study MSCs were engineered to overexpress IL-10 and transplanted in the medial frontal cortex after TBI. This resulted in a significant improvement in motor function and a shift from classical inflammation (CD86) to alternative inflammation (CD163) state in the rats treated with engineered MSCs (Peruzzaro et al., 2019).

Although it is not very clear how immune cells of the host body react to the transplanted MSCs, a few authors have found that the MSC-immune cell interaction is bidirectional. Natural killer (NK) cells cultured in IL-15 supplemented medium or IL-2, IL-12/IL-15 or IL-12/IL-18 combinations could lyse the MSCs (Sotiropoulou et al., 2006; Zhao et al., 2016a,b). IL-2 activated NK cells were able to lyse MSCs (Spaggiari et al., 2006). Effects of Treg cells were shown by Engela et al. (2013). More research is needed in this field to elucidate the interactions between immune cells and MSCs under special circumstances of TBI.

Angiogenic and anti-apoptotic effects of MSCs

Angiogenesis is one of the important inherent potentials of stem cells which has made stem cell therapy a more promising and efficient strategy for treating various injuries including brain injuries. After homing in the injury site and adjacent areas, stem cells create a microenvironment that helps in modulating the angiogenic deficit due to the injury by releasing various proangiogenic growth factors (King et al., 2014). Administration of stem cells following brain injuries helps in the functional recovery of the brain. Engrafted stem cells cross the BBB, migrate to the site of injury, and release various paracrine angiogenic factors such as growth factors and cytokines including VEGF, transforming growth factor-β1, MCP-1, IL-6 (Kwon et al., 2014). Neovascularization is stimulated by these proangiogenic factors which play a significant role in the wound healing process. Pro-angiogenesis protects the brain tissue by increasing blood supply and together with neurogenesis and synaptic plasticity helps the injured brain to recover (Navaratna et al., 2009). The newly formed vessels, which are derived by means of sprouting and invagination, enter the injury zone and enhance the neurological recovery from injury (Chen et al., 2003; King et al., 2014). It is also speculated that hMSC treatment for conditions like ischemia can indirectly upregulate the release of endogenous VEGF and VEGF2 by means of releasing various factors which can promote the process of angiogenesis in the brain (Chen et al., 2003). Thus, MSCs respond to various models of injury such as brain, lung, heart, kidney, and skin enabling the process of tissue repair and self-renewal by means of releasing various proangiogenic factors (Niwa et al., 2009).

Another important mechanism by which implanted stem cells might efficiently control neuronal damage and survival is by interfering with apoptotic pathways. In a closed head injury model of TBI, Mettang et al. (2018) have shown the upegulation of pro-apoptotic mediators Bax and Bad (Mettang et al., 2018). In an *in vitro* excitotoxically injured slice culture model of spinal cord injury, Schizas et al. (2018) found stem cell treatment reduced the proportion of apoptotic cells and increased the survival of viable neurons (Schizas et al., 2018). The anti-apoptotic effect of MSCs is probably associated with the downregulation of caspase 3 (Dasari et al., 2007; Lin et al., 2013), production of antioxidants, inhibition of 12 lipoxygenases, and secretion of neurotrophic factors (Okouchi et al., 2007) that interfere with the apoptotic pathways after brain injury.

Functional recovery after MSC transplantation

Motor deficiency, cognitive deficiency, depression, and anxiety-related disorders are the common after-effects of TBI which have neurobiological underpinnings relating to TBI. Several authors have shown the recovery of motor and cognitive deficiencies following the MSC therapy after TBI in experimental animals (Mastro-Martinez et al., 2015; Pati et al., 2016). Jones et al. (2008) have shown that 1–3 months after TBI, rats showed anxiogenic behavior by exhibiting reduced entry and reduced time spent in the center area of an open field arena (Jones et al., 2008). Mahmood et al. (2005) injected hMSCs in rats after TBI and observed functional improvements after 3 months. They concluded that the functional improvement was attributed to stimulation of endogenous neurogenesis and synaptogenesis (Mahmood et al., 2005). Wang et al. (2013a) conducted a late onset (1 year after TBI) therapy with umbilical cord MSCs in a group of TBI patients and

observed improved motor activities 6 months after treatment (Wang et al., 2013a). As correctly suggested by Boltze et al. (2014) in analyzing the functional improvements in a rat stroke model, it is important to carefully choose the appropriate behavioral tests in animal models to avoid the false positive results of functional recovery following MSC transplantation. To this end, they have suggested that tests which are minimally affected by repeated testing should be selected in evaluation of functional recoveries following cell transplantation (Boltze et al., 2014). This is equally applicable to studying the functional recoveries after TBI.

Safety issues of MSC therapy

The MSC therapy has opened up a new horizon in the treatment of several diseases and conditions and has become more promising over the past few decades, but there are a number of questions tied to the potential risk behind these therapies which are still unresolved. The risk factors in the clinical application of stem cells start from the culturing and handling of cell lines because the purity and identity of the cells play a crucial role in safety and efficacy of the treatment. There is a chance of contamination of used hMSCs with bacteria, virus, fungi, or prion pathogens which lead to the transmission of diseases to the recipient (Kainer et al., 2004; Tugwell et al., 2005). In general, the risk factors are classified as intrinsic factors, that is, cell characteristics such as lifespan, viability, proliferative capacity, differentiation status, and tumorigenic potential, and extrinsic factors like handling protocols, contaminants, transport, and storage conditions (Herberts et al., 2011). It is reported that *in vitro* culturing of stem cells may change the characteristics by various means and there is a chance of deleterious mutations (Narva et al., 2010). Also, the type of stem cells, their route of administration, and their ability to differentiate and proliferate in the host system should be considered for safe transplantation. One of the very important potential risk factors involved in the administration of stem cells includes the risk of formation of tumors. Other risk factors include unwanted immune response and transmission of adventitious agents (Herberts et al., 2011). In general, the tumorigenic potential of the stem cells is basically induced by the local environment where the stem cell resides (Knoepfler, 2009). In order to obtain a potentially higher beneficial effect from stem cell therapy, sometimes a large number of cells are injected which itself may lead to unwanted effects. The site and mode of administration of stem cells also play a vital role in the efficacy of the treatment because when a higher number of cells are injected through small needle they may aggregate and may cause infarction (Herberts et al., 2011). Following administration, stem cells release certain soluble mediators which can modulate the effector function of the immune system leading to immunosuppressive and anti-inflammatory effects. The immune-suppression may, in turn, cause unwanted reactions (Djouad et al., 2003). On the other hand, in a study, Wang et al. (2017) transplanted hMSC-derived NSCs in a group of TBI patients. They observed improved neurological functions and increased serum levels of NGF and BDNF but did not observe any significant adverse effect in the 180 days follow-up period (Wang et al., 2017). In spite of involvement of various risk factors, stem cell therapy is found to be promising over the limited time, but further intensive studies still needs to be done to understand the long-term effect of stem cell therapy (Giordano et al., 2007).

MSC-derived exosomes as therapeutic strategy

In addition to different factors released from MSCs, recent research indicates that exosomes released from MSCs may play a vital role in exerting their therapeutic efficacy (Lai et al., 2010; Xin et al., 2013; Belting and Christianson, 2015; Zhang et al., 2015). Exosomes are nano-sized vesicles (30–120 nm in diameter) (Vlassov et al., 2012), derived from endosomes, and are important in intercellular communication (Pant et al., 2012). These vesicles pack and transfer proteins, RNAs, miRNAs, or long noncoding RNAs (lnc RNA) to other cells where they exert their effects and alter the functions of the recipient cells. There is compelling evidence that MSC-derived exosomes may exert therapeutic effects equivalent to MSC after brain injury in experimental animals (Xin et al., 2013; Doeppner et al., 2015; Zhang et al., 2015; Zhao et al., 2016a,b; Xiong et al., 2017). Zhang et al. (2015) have shown that i.v. injection of exosomes after TBI improved the functional recovery in rats (Zhang et al., 2015). Doeppner et al. (2015) reported the therapeutic efficacy was obtained by treatment with MSC-derived exosomes equivalent to MSC therapy in a rat stroke model (Doeppner et al., 2015). More recently, Kim et al. (2016) have shown improved cognitive functions following administration of MSC derived $CD63^+$ $CD81^+$ exosomes in a mouse model of TBI. In this study hMSC-derived exosomes rescued the pattern separation and spatial learning

skills in mice 1 month after experimental TBI (Kim et al., 2016). Using HT22 neuronal cell line El Bassit et al. (2017) showed that treatment of these cells with exosomes derived from human adipose derived MSCs after injury increased protein kinase C δII (PKCδII) expression and increased neuronal survival and proliferation (El Bassit et al., 2017). Human adipose derived MSCs containing metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) have shown beneficial effects in histological and motor function recovery in a mouse model of mild TBI (Patel et al., 2018). In this study investigators have also shown that lncRNA MALAT1 affects mRNA expression as well as expression of noncoding RNA, thereby pointing to multi-functionality of these exosomes (Patel et al., 2018). In a recent study C57BL/6 mice were treated with MSC-derived exosomes following CCI. The results showed that the exosome administration reduced the lesion size and improved the behavioral performance as assessed by modified Neurological Severity Score (mNSS) and Rotarod test. The treatment inhibited the expression of pro-apoptotic Bcl-2-associated X protein and proinflammatory cytokine like TNF-α and IL-1β, increased the expression of the anti-apoptotic B-cell lymphoma 2, and modulated microglia/macrophage polarization by downregulating the expression of inducible nitric oxide synthase, and upregulating the expression of CD206 and arginase-1 (Ni et al., 2019). In a combined TBI and hemorrhagic shock model of Yorkshire swine Williams et al. (2019) have shown that treatment with MSC-derived exosomes helped the animals to recover faster from neurological deficit (Williams et al., 2019).

The therapeutic efficacy of exosomes depends on the culture conditions of the parent cells (2D vs. 3D) (Zhang et al., 2016), their cargo such as mRNA, miRNA, lncRNA, protein, and mitochondrial DNA (Xiong et al., 2017). In addition to the cargo carried by the exosomes, they do not proliferate in the host tissue, are less immunogenic compared to live cells, and are easier to store and deliver to the recipient (Lai et al., 2011). These properties make exosomes a more attractive therapeutic option than their parent MSCs.

Conclusion and future direction

Mesenchymal stem cell therapy is one of the most potent and exciting options for regenerative therapies of TBI. It has shown its potential in clinical setting also. Predominantly, MSCs exert their therapeutic effects through secretion-based paracrine mechanism rather than direct cellular replacement. Following transplantation, live MSCs may cause adverse effects including immunological responses, tumor formation, microvascular embolism, seizures, and cell clotting (Boltze et al., 2015). Use of MSC-derived exosomes in regenerative therapy might be able to avoid these adverse effects and improve the therapeutic outcome.

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