Chapter 11
Stem Cells for Neurovascular Repair in CNS Trauma

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Abstract Stem cells exert therapeutic effects for central nervous system (CNS) trauma. Accumulating evidence reveals that stem cell-based therapies for CNS trauma can be achieved via transplantation of exogenous stem cells or stimulation of endogenous stem cells from the neurogenic niches of subventricular zone and subgranular zone, or recruited from the bone marrow through peripheral circulation. In this chapter, we review the different sources of stem cells that have been tested in animal models of CNS trauma, highlighting the research progress on stem cell-based therapeutics in stroke and their extension to traumatic brain injury (TBI). In addition, we discuss specific mechanisms of action, in particular neurovascular repair by endothelial progenitor cells, as key translational research for advancing the clinical applications of stem cells for CNS trauma.

11.1 Introduction

Traumatic brain injury (TBI) is the third leading cause of death and the leading cause of long-term disability in the United States [1]. In 2000, the direct and indirect costs of stroke in the United States were estimated to be $76.5 billion [2]. The mean lifetime cost of TBI to a single patient in the United States is estimated at $196,460; this includes inpatient care, rehabilitation, and follow-up care necessary for lasting deficits [3]. Approximately 1.7 million people sustain a TBI annually each year [1]. The numbers of affected individuals, the costs necessary to facilitate their care, and rehabilitation coupled with the lack of therapies indicate that TBI represents a current significant unmet medical need.

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The current therapy for TBI is limited, with decompressive craniectomy to relieve intracranial pressure as a treatment of choice for TBI patients [4–8] and thereafter patients relegated mostly to rehabilitation therapy [9–12] either via aerobic exercise and cognitive rehabilitation to improve learning and memory [13]. Recent clinical trials have targeted the acute phase of injury using neuroprotective drugs [14–18] and have also tested prophylactic treatments such as hypothermia to lessen TBI injury [19]. An opportunity exists for treatment regimens designed to abrogate the secondary cell death associated with TBI. Along this line of investigations, stem cell therapy may prove beneficial in treating TBI-secondary cell death beyond the acute phase of injury. Hematopoietic stem cells and mesenchymal stem cells have been used for many years to treat disorders with some observed degree of benefit for neurological disorders such as stroke and TBI [19].

To this end, we advance the approach that cell therapy can abrogate the blood–brain barrier (BBB) breakdown associated with TBI, and such BBB repair should directly benefit TBI in view of the BBB damage inherent in the disease itself. Our group has examined the BBB destruction accompanying the progressive pathology of TBI after the initial injury. The interaction between endothelial cells, pericytes, astrocytes, neurons, and smooth muscle cells create the neurovascular unit, which plays an important role as a barrier between the CNS and the blood stream [20]. The BBB not only serves as a barrier but it also transports nutrients back and forth crossing the endothelial layer; it also inactivates molecules that threaten to cross the barrier [20]. TBI is characterized mainly by the primary injury that results from blunt force to the brain matter, but we cannot neglect the fact that TBI is also associated with secondary events. These secondary cell death events, including BBB breakdown, occur with some delay and if left untreated can exacerbate the primary injury and can be detrimental with long-lasting adverse effects. Clinical and experimental animal models demonstrate that there are some behavioral changes that occur in the first days and changes in cerebral blood flow [21, 22]. As seen in stroke, TBI is also associated with pro-inflammatory components in the neurovasculature that accumulate within the brain. First, leukocytes accumulate within the first couple of hour after TBI followed by surplus in pro-inflammatory cytokines and oxidative stress [23]. After the initial injury in TBI, there is disruption of tight junctions, channels, pericytes, and astrocytic foot processes within the neurovasculature. For many years, it was believed that the opening of the BBB occur transiently after TBI but now we recognize that it is an event that occurs within the first day post-injury and that BBB permeability persists over time due to the progressive tight junctions alterations [24]. Targeting neurovascular repair has been explored recently but the results have been very limited in animal models. Further research needs to be performed focusing on the repair of neurovasculature in TBI. More recently, the inflammatory response has been implicated in the integrity of BBB after TBI, characterized by an increase in production of pro-inflammatory cytokines eventually leading to an increase in influx of inflammatory cells from blood to brain [25]. Altogether, these studies suggest that neurovascular alterations accompany TBI pathology, and that finding a strategy

geared towards neurovascular repair is likely to augment the progressive nature of the disease's secondary cell death. This chapter discusses the preclinical basis for testing stem cell therapy in CNS trauma. Because of significant research strides achieved in stem cell-based therapeutics in stroke, and with overlapping etiologies and pathologies between stroke and TBI, we provide relevant insights in stroke that may prove critical to extending the safety and efficacy of stem cell therapy for TBI. We outline below the potential of cell-based therapy in view of the current treatments for TBI. In particular, the wider therapeutic window for stem cell transplantation, which may allow neuroregeneration at the chronic stage of the disease as opposed to the acute phase targeted by neuroprotection, makes cell-based therapy an appealing strategy. Finally, we address the gap in knowledge concerning mechanisms underlying the therapeutic benefit of stem cells in CNS trauma. Here, we highlight the underexplored concept of neurovascular repair as a major mode of action of cell therapy, and emphasize the major role of endothelial progenitor cells (EPC) as an effective cell source for transplantation. Our strategy is to exploit this neurovascular repair mechanism via EPC transplantation as a standalone or as an adjunct therapy for augmenting existing treatments for TBI.

11.2 Stem Cell Sources

We attempt here to provide an overview of stem cell sources that have been investigated in stroke, then subsequently discuss stem cells that have been tested in TBI. Several sources of stem cells have been demonstrated as safe and effective in animal models of stroke. In a historical order, the major types of cells transplanted in stroke include fetal-derived cells, neuroteratocarcinoma cells (NT2N), xenogenic pig-derived cells, embryonic stem (ES) cells, adult stem cells (bone marrow, human umbilical cord, placenta, amnion fluid, menstrual blood), and induced pluripotent stem cells (iPS). Due to ethical and logistical concerns, the use of adult stem cells has flourished over the last decade, which was further aided by a moratorium for using federal funds on ES research. Interestingly, the ongoing FDA-approved stem cell clinical trials in stroke use adult stem cells. It is likely that adult stem cells may also be the frontrunner for cell therapy in TBI. For this chapter, we highlight the potential of adult bone marrow-derived EPC in neurovascular repair for stroke and TBI.

11.3 Stem Cell Therapy for CNS Trauma

Cell transplantation therapies and stem cell treatments have emerged as potential treatments for numerous diseases and medical conditions, including stroke. One approach using stem cells involved the direct transplantation of neural stem cells (NSCs) into the damaged region of the brain. NSCs transplanted following transient

global ischemia differentiated into neurons and improved spatial recognition in rats [26]. Post-mitotic neuron-like cells (NT2N) cells, derived from a human embryonal carcinoma cell line, migrated over long distances after implantation into brains of immunocompetent newborn mice and differentiated into neuron- and oligodendrocyte-like cells [27]. NT2N cells promoted functional recovery following focal cerebral ischemia after direct transplantation [28]. Similarly, MHP36 cells, a stem cell line derived from mouse neuroepithelium, improved functional outcome in rats after global ischemia [29] and also following focal cerebral ischemia or stroke [30]. NCSs grafted into brain developed morphological and electrophysiological characteristics of neurons [31].

Other direct transplantation experiments in the brain have utilized cells derived from bone marrow. Bone marrow stromal cells (MSCs), when injected into the lateral ventricle of the brain, migrated, and differentiated into astrocytes [32]. Fresh bone marrow transplanted directly into the ischemic boundary zone of rat brain improved functional recovery from middle cerebral artery occlusion [33]. Similarly, MSCs implanted into the striatum of mice after stroke, improved functional recovery [34]. MSCs differentiated into presumptive neurons in culture [35] and assumed functional neuronal characteristics in embryonic rats [36]. Intracerebral grafts of mouse bone marrow also facilitated restoration of cerebral blood flow and BBB after stroke in rats [37]. Indirect transplant methods, via intravenous or intra-arterial injection, also have been shown to afford positive effects. Following bone marrow transplantation with tagged donor cells, tagged bone marrow stem cells were shown to differentiate into microglia and astrocyte-like cells [38]. Intra-carotid administration of MSCs following middle cerebral artery occlusion in a rat model improved functional outcome [39]. Similarly, intravenous administration of umbilical cord blood cells ameliorated functional deficits after stroke in rats [40]. Rats, which had received tagged bone marrow cell transplantation, showed the tagged cells as putative neurons and endothelial cells following middle cerebral artery occlusion and reperfusion [41]. It has also been reported that intravenous administration of cord blood cells was more effective than intra-striatal administration in producing functional benefit following stroke in rats [42]. Intravenous administration of MSCs has also been found to induce angiogenesis in the ischemic boundary zone following stroke in rats [43].

Along this vein, stem cell therapy appears to be a promising treatment for TBI. We summarize below recent studies on cell-based therapeutics for TBI (Table 11.1). Here, we acknowledge that the field of stem cell therapy for TBI remains in its infancy. Stem cell sources range from cells derived from embryonic, fetal, and adult tissue sources (e.g., umbilical cord, placenta, amnion, bone marrow) [44–64]. Functional readouts have been mostly generated during short-term post-transplantation, thereby necessitating long-term investigations for monitoring of stable and robust benefits, as well as assessing any adverse effects over time in order to reveal both efficacy and safety profiles of stem cell therapy for TBI.

Table 11.1 Recent stem cell-based therapies for TBI

Author	Stem cell type	TBI animal model	Outcomes/results	References
Ma et al. 2011	Neural stem cells (NSCs) genetically modified to encode BDNF gene (BDNF/NSCs)	Controlled cortical impact (CCI) Rat	Enhanced neurite growth and upregulated synaptic proteins in BDNF/NSCs-transplanted TBI rats. Over expression of BDNF-mediated motor behavior improvement in transplanted TBI rats	[44]
Mahmood et al. 2006	Bone marrow stromal cells	Controlled cortical impact (CCI) Rat	Transplanted BMSCs were present in the injured brain 3 months after TBI and functional outcome was significantly improved	[45]
Qu et al. 2008	Marrow stromal cells (MSCs)	Controlled cortical impact (CCI) Mouse	Significant neurological improvements as revealed by morris water maze and foot fault test in MSC-treated TBI mice	[46]
Lu et al. 2007	Human marrow stromal cells (hMSCs)	Controlled cortical impact (CCI) Rat	hMSCs improved spatial learning and sensorimotor function, accompanied by reduced lesion volume in TBI animals	[47]
Harting et al. 2010	Mesenchymal stem cell (MSC)	Unilateral controlled cortical impact (CCI) Rat	Intravenous-delivered MSCs were identified in the lungs 48 h post-infusion; therefore, there was no functional improvement seen	[48]
Riess et al. 2002	Neural stem cells (NSC)	Controlled cortical impact (CCI) Mouse	TBI animals that received NSC transplant improved motor function with graft survival after 13 weeks post-transplantation	[49]
Hattiangady et al. 2012	Neural stem cell (NSC)	Unilateral partial hippocampal injury Rat	TBI animals that received SVZ-NSC grafts after injury exhibited improved mood and memory function as compared to control. The cells derived from grafts exhibited migration, survival, and neuronal differentiation	[50]

(continued)

Table 11.1 (continued)

Author	Stem cell type	TBI animal model	Outcomes/results	References
Nichols et al. 2013	Human peripheral blood derived (HPBD) MSC HPBD CD133+, ATP-binding cassette sub-family G member 2 (ABCG2)+, C-X-C chemokine receptor type 4 (CXCR4)+ MSCs combined with trans-retinoic acid (RA) mixture	Fluid percussion injury Rat	CD133+ ABCG2+ CXCR4+ MSCs expressed neuronal lineage markers and survived for 1 and 3 month post-transplantation with the potential to reduce cognitive impairment seen in TBI	[51]
Yan et al. 2013	Human amnion-derived mesenchymal stem cells (AMSC)	Controlled TBI impact model a weight-drop device Rat	Transplanted TBI rats demonstrated significant increase in neurological function, brain morphology, and increase in expression of neurotrophic and growth factors, thereafter stimulating endogenous growth factors and promoting neurorehabilitation	[52]
Wallenquist et al. 2012	Neural stem and progenitor cells (NSPC)	Controlled cortical impact (CCI) Mouse	Ibuprofen down-regulated TBI-induced inflammatory response. Interestingly transplanted neuroblast were found near the impacted area and ipsilateral hippocampus suggesting that ibuprofen anti-inflammatory properties is crucial for the survival and differentiation of the grafts	[53]
Shear et al. 2011	Neural stem cells (NSCs)	Controlled cortical impact (CCI) Mouse	NSCs are optimal when used 2–7 days post-TBI. The transplant location plays a key role in cell survival, differentiation, migration, and functional efficacy. NSC also stimulate protective and neurotrophic factors rather than replacing neuronal or glial cells	[54]

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Table 11.1 (continued)

Author	Stem cell type	TBI animal model	Outcomes/results	References
Lee et al. 2013	Neural stem cells (NSCs)	Corticectomy Rat	NSCs improved behaviors and motor evoked potentials when combined with rehabilitation therapy. The groups that received combination therapy and only rehabilitation demonstrated a prolonged effect in expression of the endogenous NSCs	[55]
de Freitas et al. 2012	Bone marrow-derived mesenchymal stem cells (MSCs) or bone marrow mononuclear cells (BMMCs)	Ablation by aspiration Rat	Bone marrow mononuclear (BMMC) cells are more efficient and accessible than MSCs	[56]
Chuang et al. 2012	Secretome from human mesenchymal stem cells	Fluid percussion injury Rat	MSC-derived secretome attenuated motor deficits seen after TBI injury. Markers for apoptosis and neuronal cell loss were also decreased in the secretome-treated animals. Conversely, secretome increased the levels of VEGF positive cells	[57]
Walker et al. 2012	Bone marrow-derived mesenchymal stromal cells (MSCs)	TBI model Rat	Neuroprotection produced by MSC via enhanced M2 cell activation of anti-inflammatory response, thereby reducing exacerbated inflammatory reaction associated TBI	[58]
Tu et al. 2012	Mesenchymal stem cells from umbilical cord (UCSMCs) and temperature-sensitive UCSMCs (tsUCSMCs)	Fluid percussion injury Rat	The combination of hypothermia with UCSMCs, or tsUCSMCs is beneficial in improving motor and cognitive function when used together rather than stem cell therapy alone after TBI injury	[59]

(continued)

Table 11.1 (continued)

Author	Stem cell type	TBI animal model	Outcomes/results	References
Antonucci et al. 2012	Amniotic Fluid-derived Stem cells (AFS)	Controlled cortical impact (CCI) and fluid percussion injury Rat and mouse	AFS cells are good transplant donor cells due to high renewable capacity and have a capacity to effectively differentiate to multiple lineages	[60]
Joo et al. 2012	Neural stem cells (NSCs)	Focused brain irradiation Mouse	NSC supplementation enhanced endogenous neurotrophic factors and was able to differentiate into astrocytes and neurons which migrated to the irradiated areas of the brain	[61]
Shi et al. 2012	Human umbilical cord mesenchymal stem cells (hUC-MSCs) In vitro BDNF blended chitosan scaffolds on neural stem cell	TBI model Rat	BDNF is beneficial in promoting neuronal differentiation of NSC	[62]
Yang et al. 2011	Schwann cells differentiated from adipose-derived stem cells (ADSC-SCs)	Contusion brain injury Rat	Transplantation of ADSC-SCs into rats with contused brain promoted locomotor function and reduced reactive gliosis compared to undifferentiated ADSCs	[63]
Skardelly et al. 2011	Human fetal neural progenitor cell (hfNPC)	Controlled cortical impact (CCI) Rat	MRI analysis showed a smaller lesion size in animals that received the transplants as compared to non-transplanted animals. Histological analysis demonstrated increased levels of angiogenic markers and reduced astroglial reaction at 4 weeks after transplantation	[64]

11.4 Mechanistic Interpretation of Therapeutic Benefit Involving Stem Cells

It is unclear what brings about the purported benefit from stem cell transplantation. One possibility is the transformation of the transplanted cells into neurons [65]. There appears to be a positive relationship between the degree of behavioral improvement and the number of transplanted cells that stain positive for neuron-specific markers [26]. However, transplanted cells often do not develop normal processes, and thus the benefit may not be mediated only by neuronal circuitry [66].

A second hypothesis that is not mutually exclusive is that the transplanted cells may also assist via differentiation into neuroectodermal-derived cell types other than neurons. MSCs migrate and transform into astrocytes [32]. Hematopoietic cells can differentiate into microglia and macroglia [38]. Bone marrow-derived stem cells may also assist in blood vessel regeneration following brain tissue damage in several ways. The stromal cell-derived factor-1 (SDF-1)/CXCR4 system assists in integration of cells into injured tissue by promoting the adhesion of CXCR4-positive cells onto vascular endothelium [67]. SDF-1 also augments vasculogenesis and neo-vasculogenesis of ischemic tissue by recruitment of EPC [68]. Bone marrow is a source of these endothelial progenitors [69]. Adult bone marrow-derived cells have been shown to participate in angiogenesis by the formation of periendothelial vascular cells [70]. Intravenous administration of MSCs induced angiogenesis in the ischemic boundary zone after stroke [43]. We also observed that crude bone marrow is a source of endothelial cells after experimental stroke [41]. Interestingly TBI induces cell proliferation in the hippocampus and the subventricular zone differentiated into mature neuronal cells 10 days post-TBI [71, 72]. The vasculature in the CNS becomes activated after injury and initiates a self-repair mechanism to combat the compromised site through the activation and mobilization of EPC from bone marrow and peripheral blood. Angiogenesis is believed to be a neuroprotective factor that can rescue nerve cells from secondary cell death injury [73]. Vascular endothelial growth factor (VEGF) induces angiogenesis and mobilizes EPC in diseases associated with blood vessel disorders such as stroke and TBI [74], thereby serves as a crucial growth factor in the creation of new vascular cells for BBB repair. VEGF also stimulates and supports preexisting endothelium-derived angiogenic cells which in synergy all these components are key players for brain repair [75].

Trophic factors produced by the transplanted cells could be a factor. Via this mechanism, bone marrow grafts may assist in restoring brain blood flow and also repairing the BBB [37]. Trophic factors from MSCs may play a role in brain repair itself. Recent evidence suggests that intravenous administration of MSCs increases the expression of nerve growth factor and brain-derived neurotrophic factor following TBI [76]. Understanding the exact mechanism(s) responsible for the therapeutic benefit seen following stem cell transplantation in the CNS is now at a critical junction in view of the planned FDA allowance for limited clinical trials of bone marrow-derived multipotent adult progenitor cells in acute ischemic stroke

[77]. Similarly, insights into the mechanism of action mediating stem cell therapeutic benefits in TBI will aid in optimization of cell dose, route of delivery, and timing of initiation of cell transplantation for clinical applications.

In accordance with the STAIR (Stroke Therapy Academic Industry Roundtable) and STEPS (Stem cell Therapeutics as an Emerging Paradigm for Stroke) criteria, investigations of the mechanism of action mediating experimental therapeutics in stroke are vital for extending their potential clinical utility [78, 79]. A similar call for strict translational guidelines has been advanced for TBI [80], and a set of consensus recommendations has been published to provide standards and best practices for future investigations in testing novel therapeutics in TBI animal models [81].

11.5 BBB Breakdown in CNS Trauma

We again draw from our observations of BBB breakdown in stroke as we advance the hypothesis of BBB compromise in TBI. A closely associated cell death cascade involved in stroke pathogenesis is impairment of the BBB, which further exacerbates brain damage. The central nervous system (CNS) is an immunologically privileged zone, protected from entry of immune cells and serum proteins by the BBB (as well as by the blood–spinal cord barrier and blood–cerebrospinal fluid barrier, but we will focus here on BBB). These CNS barriers control cerebral/spinal cord homeostasis by selective transport of molecules and cells [69–76, 82, 83]. This control is possible due to the unique structure of the microvasculature—in particular capillaries formed by endothelial cells which are connected via adherens and tight junctions [84–86]. Functional integrity of all BBB elements is critical for protection of the CNS from harmful blood substances. Impairment of this cellular machinery may cause BBB breakdown, leading to edema in many cases of brain diseases or injuries, including stroke. Degradation of the extracellular matrix may be concomitant with BBB disruption and tissue softening, leading to more pronounced brain swelling and to severe cerebral edema in stroke patients [87] and other brain disorders such as Alzheimer's disease [88] and multiple sclerosis [89, 90]. Examination of BBB status in stroke reveals evidence of the barrier's altered permeability. Whereas the first phase of stroke is characterized by a surge in tissue Na+ and water content concomitant with an increased pinocytosis and Na+, K+ ATPase activity across the endothelium, the second stage of stroke ensues with BBB breakdown that is associated with infarction of both the parenchyma and the vasculature itself [91]. At this second stage, tissue Na+ level still remains, but the extravasation of serum proteases stands as a likely exacerbating factor [92]. Accumulating evidence implicates serum proteases in degradation of the extracellular matrix metalloproteinases (MMPs), which in turn aggravate BBB disruption and softening of the tissue, eventually manifesting into a well-defined form of brain

swelling [91–93]. Part of the reason for the tPA's limited time window is that the surge in production of free radicals associated with delayed reperfusion brings a second wave of oxidative and nitrative stress that increases the risk of brain hemorrhage and edema [94]. With delayed reperfusion, there is a surge in production of superoxide, NO, and peroxynitrate. Formation of these radicals in the vicinity of blood vessels plays an important role in reperfusion-induced injury. These radicals activate MMPs, which degrade collagen and laminin in the basal lamina, disrupting the integrity of the basement membrane and increasing BBB permeability. Oxidative and nitrative stress also triggers recruitment and migration of neutrophils and other leukocytes to the cerebral vasculature, which release enzymes that further increase basal lamina degradation and vascular permeability. These BBB pathological events can lead to parenchymal hemorrhage, vasogenic brain edema, and neutrophil infiltration into the brain [95]. In the clinic, significant brain edema, such as that seen in malignant MCA infarction, develops in a delayed fashion after large hemispheric strokes and accounts for a high mortality rate (80 % in the case of malignant MCA infarction) [96]. The primary BBB function is controlling CNS homeostasis by selective transport. Substances with molecular weights higher than 400 Da generally cannot cross the BBB by free diffusion. Some molecules cross the barriers via endothelial carrier-mediated or receptor-mediated transporters, see review [69, 70, 82, 97]. It is possible that barrier disruption or dysfunction occurs in stroke, altering CNS homeostasis and allowing entry of harmful molecules from the periphery to the brain [98–100]. Among these injurious molecules are immune/inflammatory factors, such as monocyte/macrophage cells, activated microglia, and reactive astrocytes possibly secreting pro-inflammatory cytokines, which have been detected in stroke patients and animal models [101–103]. Although additional studies are warranted to confirm the BBB status in stroke patients, the above results taken together imply that BBB dysfunction may contribute to stroke pathology. Thus, there could be an impaired endothelium-mediated mechanism in stroke leading to barrier dysfunction.

In the TBI field, 16 patients demonstrated regions with enhanced signals within the brain showing BBB leakage in the cortical regions of at least 15 of the patients [104]. The disrupted BBB regions were surrounding old contusions which suggest that a local trauma had occurred. Models of TBI have been helpful in identifying what occurs to the BBB after impact. After stress from impact the vasculature is a primary target of the injury, leading to leakage of blood-borne proteins and the extravasation of red blood cells [104, 105]. Isolated petechial hemorrhages have also been identified contralaterally to the injury [105]. Along with the extravasation of red blood cells there has been disrupted endothelial lining and endothelial vacuolation, increase intracranial pressure leading to altered cerebral flow and poor neurological outcome due to increase levels of lactate overall causing brain damage and functional deficits [105].

As noted above, VEGF is enhanced after injury and stimulates angiogenesis as well therefore is beneficial for newborn neuronal cells and endogenous neurogenesis. In a closed head mice injury model, VEGF was seen to decrease the lesion volume caused by TBI and it also increased the amount of BrdU positive

cells, demonstrating an increase in neurogenesis and gliogenesis after TBI [106]. This study indicates the vascular repair may be beneficial for TBI. Accordingly, we discuss below the potential of stem cell therapy for BBB repair.

11.6 EPC Therapy for BBB Repair in CNS Trauma

In discussing neurovascular repair for TBI, we build upon the more mature field of BBB repair in stroke. EPC, initially described by Asahara et al. [107] are immature endothelial cells that circulate in peripheral blood. In their pioneering study, transplanted EPC, isolated from human blood, were found in the endothelium of newly formed vessels in ischemic regions, indicating that a discrete cell population within the human blood participates in the formation of new vessels after ischemia. Griese et al. also found that grafted EPC populated the endothelium in animals with experimentally induced endothelial damage [108], further advancing the notion that EPC contribute to the repair of damaged endothelium. The dogma that existed until recently is that neovascularization, or formation of new blood vessels, results exclusively from proliferation and migration of preexisting endothelial cells, a process referred as to angiogenesis [109]. Furthermore, vasculogenesis or vascularization, defined as in situ differentiation of vascular endothelial cells from endothelial precursor cells, was thought to occur only in the embryo during vascular development. However, recent evidence has now established that circulating bone marrow-derived EPCs are capable of homing to neovascularization sites, proliferating, and differentiating into endothelial cells [110, 111]. EPCs have been identified mainly in the mononuclear cell fraction of peripheral blood, leukapheresis products, and in umbilical cord blood [107, 112], but can also be harvested from bone marrow. Over the last few years, EPCs have been studied as biomarkers to assess the risk of cardiovascular disease in human subjects. For example, a low EPC count predicts severe functional impairments in several cardiovascular pathologies such as diabetes [113], hypercholesterolemia [114], hypertension [115, 116], scleroderma [117, 118], aging [116, 119], cigarettes smoking [116, 120, 121], and coronary artery disease [84]. In addition, EPCs have been examined as potent donor graft cells for transplantation therapy.

Transplantation of EPCs into ischemic tissues has emerged as a promising approach in the treatment of diseases with blood vessels disorders [122–124]. In mouse models of ischemic injury, EPCs injection led to improved neovascularization in hind limb ischemia [122–124]. Based largely on these laboratory findings suggesting angiogenic and vasculogenic potential of EPCs, clinical studies have been initiated to reveal whether patients with lower EPC numbers are at higher risk for atherosclerotic events, and whether patients with ischemic events may benefit from EPC administration [125].

Clinical studies to date suggest the therapeutic potential of EPC transplantation, although this assumption should be approached with much caution due to being open label trials, observational and/or anecdotal accounts, and limited number of

patients. Ex vivo expanded EPC, isolated from peripheral blood mononuclear cells, can incorporate into the foci of myocardial neovascularization [126, 127], and intracoronary infusion of peripheral blood or bone marrow-derived progenitors in patients with acute myocardial infarction was associated with significant benefits in post-infarction remodeling [128–135]. Still in observational studies in patients with myocardial infarction, higher numbers of EPC correlate with better prognosis, more myocardial salvage [136], viability and perfusion [137], and more collaterals in the ischemic zone [138]. Randomized clinical trials on autologous bone marrow-derived cells are mixed; whereas transplanted coronary artery disease patients display improved left ventricular function at least in the short term [139], transplanted patients with chronic ischemic heart failure exhibit modest to no effects on change in left ventricular function [140].

Similar randomized trials of autologous bone marrow-derived cells have been carried out in patients with peripheral artery disease and showed improved endothelium-dependent vasodilation [141], ankle brachial index, rest pain, and pain-free walking time [142], but the degree of functional recovery was not as robust as seen in animal models. Clearly, these results are obtained from autologous bone marrow-derived cells, which are heterogenous with scarce number of EPCs, thus may not closely approximate EPC endpoints. For clinical application of EPC in neurovascular disease, the available studies are much more limited with only three observational studies in patients with stroke. In 25 patients with an ischemic stroke, CD34+ cells peaked 7 days after stroke but generally reverted to baseline after 30 days [143]. Interestingly, higher CD34+ cell levels at 30 days related to higher numbers of infarcts on magnetic resonance imaging and also to cerebrovascular function as measured with positron emission tomography scanning (cerebral metabolic rate of oxygen and cerebral blood flow). On the other hand, decreased numbers of clusters of rapidly adhering cells were seen after stroke and in "stable cerebrovascular disease," compared to controls free of vascular disease [144]. Higher age and the presence of cerebrovascular disease in general independently related to lower EPC numbers. The discrepancies in the results of these studies may be due to mismatched controls for age of patients and/or the lack of methodological design for testing specific hypotheses on the causal role of EPC in cerebrovascular disease [144]. Although the primary mitigating mechanisms underlying stroke pathogenesis and its abrogation by cell therapy are still uncertain, there is substantial evidence implicating immunological attack upon the brain and/or its vasculature; widespread inflammatory reactions in stroke may trigger a cascade of events which alter the integrity of the BBB, resulting in migration of leukocytes into the CNS. Leukocyte transmigration across the BBB during stroke immune/inflammatory processes could influence inter-endothelial junctional complex function leading to vascular endothelium damage and BBB breakdown. Equally a key component to our mechanism-based thesis is that disruption or dysfunction of the BBB, preceding entry of harmful substances into the brain parenchyma, could be a key initial factor in stroke pathogenesis. Thus, restoration of barrier integrity may have a critical role in preventing stroke progression. Our studies have begun to

address these questions, particularly, whether endothelial cell replacement can restore structural and functional properties of the BBB after stroke. Results of this study will provide the basis for pursuing cell therapy both for non-tPA and tPA-treated ischemic stroke patients, as well as for patients with neurodegenerative disorders characterized by BBB dysfunction.

As we extend EPC therapy for BBB repair in TBI, we apply similar concepts of transplanting exogenous EPC for TBI, stimulating endogenous EPC in TBI, and augmenting blood flow, angiogenesis and/or vasculogenesis in TBI using drugs. A soluble factor known as tissue inhibitor of matrix metalloproteinase-3 (TIMP3) is produced by MSCs and has been demonstrated to mediate the beneficial effects of MSCs on endothelial function including the structural and functional restoration of a compromised BBB caused by TBI [145]. Following transplantation of MSCs, TIMP3 upregulated and attenuated the TBI-associated BBB permeability after TBI; blocking TIMP3 expression led to a compromised BBB [145]. Repairing the BBB by the transplantation of exogenous EPCs has also been explored, taking advantage of EPCs' capacity to migrate to the site of injury and contribute to the regeneration of vascular tissue by releasing angiogenic factors and creating structural components of capillaries. In addition, BBB repair can benefit from transplantation of stem cell progenitors and growth factors that are released by the grafted cells to the host microenvironment facilitating BBB repair and maintenance [146].

11.7 Conclusion

The recognition that BBB breakdown closely accompanies CNS trauma warrants therapies designed to arrest this BBB dysfunction. Currently, much of the therapy implemented for CNS trauma does not consider the capacity of BBB damage after injury. It is our contention that if EPC transplantation promotes restoration of the vascular endothelium, the clinical effects could be far reaching and substantially help a large population of patients that may be excluded from the current therapeutic window of neuroprotection for TBI. Although a plethora of accumulating stem cell research is quickly translating into clinical trials, it is important to gain insights into the mechanisms of action, which will aid in optimizing the safety and efficacy of these stem cells in CNS trauma. TBI is a public health problem that afflicts children and adults, and in the last decade is rampant to our military soldiers. Almost half a million of visits yearly to the emergency wards are related to TBI. The need for better understanding of cell death pathways associated with TBI, especially secondary cell loss, is crucial to developing an effective treatment. Here, we advance the notion that a treatment regimen directed at attenuating TBI deficits should consider the pivotal role of BBB repair in order to maintain CNS homeostasis and enhance neuronal regeneration. Structurally and functionally restoring the BBB in an acute, sub-acute, and even chronic phases of injury setting may afford therapeutic benefits against TBI. A regenerative mechanism involving the repair of the damaged BBB by EPC is key to the successful outcome of cell therapy

in CNS trauma. Cell therapy tailored at EPC recruitment and/or directed secretion of EPC-soluble factors into the traumatized brain stands as a potent strategy for BBB repair in TBI.

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