

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Traumatic Penumbra: Opportunities for Neuroprotective and Neurorestorative Processes

---

Andrea Regner, Lindolfo da Silva Meirelles and  
Daniel Simon

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.72156>

---

## Abstract

Traumatic brain injury (TBI) is a major cause of morbidity and mortality worldwide. Understanding the pathophysiology of TBI is crucial for the development of more effective therapeutic strategies. At the moment of the traumatic impact, transfer of kinetic forces causes neurologic damage; this primary injury triggers a secondary wave of biochemical cascades, together with metabolic and cellular changes, called secondary neural injury. These areas of ongoing secondary injury, or areas of “traumatic penumbra,” represent crucial targets for therapeutic interventions. This chapter is focused on the interplay between progression of parenchymal injury and the neuroprotective and neurorestorative processes that are emerging and developing subsequently to traumatic impact. Thus, we emphasized the role of traumatic penumbra in TBI pathogenesis and suggested a crucial contribution of the neurovascular units (NVUs) and paracrine effects of exosomes and miRNAs in promoting neurological recovery.

**Keywords:** traumatic brain injury, traumatic penumbra, neural injury, pericytes, neurovascular unit, neurorestoration

---

## 1. Introduction

Worldwide, injuries account for 15% of the burden of death and disability, while traumatic brain injury (TBI) accounts for up to half of all deaths from trauma [1–6], and often causes severe and long-lasting functional impairment in survivors [7]. TBI affects individuals of all age groups with a bimodal distribution in adolescents and elderly [8, 9], with a major predominance in the male population [10, 11]. Blunt trauma accounts for about 88–95% of TBI cases, whereas the remaining 5–12% of cases are the result of penetrating injuries [12]. Because of the high-impact nature of trauma-inducing accidents, patients commonly suffer

concomitant injuries to multiple body regions and organs, otherwise known as multitrauma or polytrauma, that are capable of modifying the pathobiology and outcomes of TBI [13].

TBI is classified by different methods; in the 1970s, Teasdale and Jennett introduced the Glasgow Coma Scale (GCS) to objectively assess the degree of impaired consciousness [14]. Based on GCS, TBI is classified into mild (GCS score 14–15), moderate (9–13), and severe (3–8) [15]. At present, GCS is the most used method for TBI classification, however, has a number of limitations [15, 16]. A recent study reported that normal GCS did not indicate an absence of head injury, as among patients with GCS 15 in the Emergency Department, 26% had serious/critical TBI [17]. Therefore, stratification of severity and prediction of death and functional outcome is essential for determining treatment strategies and allocation of resources for patients with TBI. Among the most studied predictors of TBI outcome, age is a consistent predictor, as well as GCS scores and pupillary parameters [18]. Recent studies show a series of either tissue-specific or circulating biomarkers that are useful in the clinical status evaluation of these patients [19–22].

Intracranial hypertension is the main cause of death in patients with TBI and contributes to secondary brain injury if not managed correctly [23–25]. Therefore, the management of TBI focuses on the control of intracranial pressure (ICP) and maintenance of adequate cerebral perfusion, oxygenation, and metabolism attempting to limit secondary injury progression [26–28]. Mortality rates have decreased in the last decades, largely due to improvements in trauma systems and supportive critical care [29]. Yet, case fatality rates in severe TBI have not decreased significantly since 1990 [30], remaining with an outstanding mortality, because up to 50% of the patients will still die and nearly all survivors will present some degree of sequelae [3, 4, 6, 31–33]. To the present, regardless of over dozens of phase III clinical trials, there are no specific treatments known to improve TBI outcomes [13]. Hence, TBI is heterogeneous in terms of pathophysiology, clinical presentation, and outcome, with case fatality rates ranging from <1% in mild TBI up to 50% in severe TBI. A key issue in TBI care is the temporal progression of injury cascades and the design of therapeutic approaches to improve functional recovery after TBI.

This chapter is focused on the interplay between progression of parenchymal injury and the neuroprotective and neurorestorative processes that are emerging and developing subsequently to traumatic impact. Thus, we emphasized the role of traumatic penumbra in TBI pathogenesis and suggested a crucial contribution of the neurovascular units (NVUs) and paracrine effects of exosomes and miRNAs in promoting neurological recovery.

## 2. Mechanisms of neural injury in the traumatic penumbra

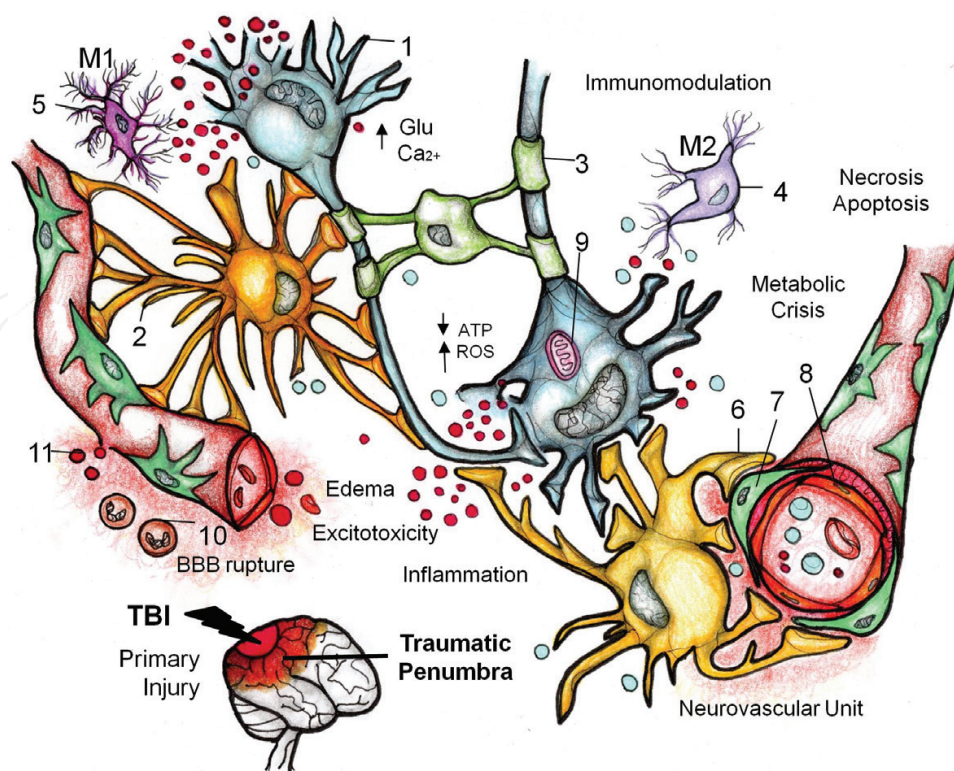
TBI is unique since it results from an external force, which can inflict devastating effects to brain vasculature, neighboring neural tissue, and blood-brain barrier (BBB) [34]. Together, neurons, vascular cells (endothelial cells) and perivascular components of the BBB (astrocytes and pericytes) form the neurovascular unit (NVU). NVU is at the basis of neurovascular coupling, which allows cerebral blood flow to local regulation according to neuronal

activity in specific areas of the brain [34]. TBI may cause mechanical deformation and damage to the entire NVU [20, 35, 36], compromising barrier integrity and leading to dysautoregulation of brain vessels and BBB disruption. In this context, brain edema may occur and result in increased ICP and decreased cerebral perfusion [37]. In fact, compensatory mechanisms are exceeded as brain volume increases due to edema, and ICP rises exponentially and correlates with increased mortality and poor functional outcomes [20, 38–40]. The impact of trauma causes mechanical forces that engender deformation of the brain tissue, resulting in immediate neural damage, called primary injury [40]. This primary injury triggers a secondary wave of biochemical cascades, together with metabolic and cellular changes, occurring within seconds to minutes after the trauma and lasting for days, months or years [40]. The ongoing brain damage characteristic of secondary injury culminates in notable cell death [24, 40, 41]. Typically, initial neuronal death following acute brain injury occurs by necrosis, on a time scale of minutes, then, a second wave of delayed cell death occurs mostly by apoptosis [13, 42–45]. Indeed, this protracted course of cell death following TBI may represent a unique opportunity for therapeutic intervention. Following TBI, brain lesions are not limited to the site of the primary trauma, but expand progressively and centrifugally. Therefore, secondary brain injury develops and progresses in the traumatic penumbra, that is, the potentially salvageable brain tissue surrounding the primary lesion [46, 47]. Indeed, clinical studies have demonstrated that expansion of the penumbra impairs cerebral blood flow and leads to edema and compromised local metabolism, resulting in clinical deterioration [48–50].

The traumatic penumbra is characterized by metabolic changes as a consequence of neural injury progression [51–53] culminating in cell death [26, 54]. In this scenario of metabolic crisis, astrocytes may exert a neuroprotective action supplying substrates of glycogen metabolism for the survival of ischemic neurons and oligodendroglial cells [49]. Thus, astrocytes also play crucial roles in the injury site after TBI, as they exert homeostatic mechanisms critical for maintaining neural circuit function, such as buffering neurotransmitters, modulating extracellular osmolarity, and calibrating neurovascular coupling [55]. Accordingly, astrocytes are thought to exert many beneficial effects post-TBI [56] as providing neurotrophins that support and guide axons in their recovery [57], increasing cell proliferation, and promoting the long-term survival of neurons by inhibiting apoptosis [58, 59]. However, when the presence of astrocytes is too large and they become over activated, they may build a dense physical and chemical barrier surrounding the injury site (glial scar), which encapsulates and isolates the axons. This not only protects the remaining healthy brain from the neurotoxic environment of the injury site but also interferes and prevents the regeneration and repair of the damaged tissue [60, 61].

We will resume some of the phenomena involved in cellular injury in the traumatic penumbra. Particularly, excitotoxicity, oxidative stress, mitochondrial dysfunction, and neuroinflammation are processes that contribute to neurological damage and impairment of neural recovery following TBI (**Figure 1**). In the injured brain, excitotoxicity derives from an acute increase in extracellular glutamate levels due to excessive release from depolarized neurons, leakage from neuronal and glial cells exhibiting damaged membranes, or the extravasation through a disrupted BBB [53, 62–64]. TBI also involves enhanced glutamatergic activity at





**Figure 1.** Schematic representation of mechanisms of neural injury in the traumatic penumbra. The neural tissue disruption of primary injury triggers a cascade of cellular events that result in areas of traumatic penumbra characteristic of secondary injury, leading to necrosis and apoptosis. Secondary injury progression can either evolve to edema that culminates in an uncontrollable increase of intracranial pressure leading to brain death or trigger mechanisms of neural tissue survival and recovery. The first 96 hours after the trauma are critical for the cellular processes involved in ongoing secondary neural injury. Various cellular components are involved in secondary injury progression in the traumatic penumbra: (1) neuron, (2) reactive astrocyte, (3) oligodendrocyte, (4) microglia M2 anti-inflammatory phenotype, (5) microglia M1 pro-inflammatory phenotype, (6) astrocyte endfoot, (7) pericyte, (8) endothelial cells, (9) mitochondria, (10) peripheral immune cells, and (11) signaling molecules. Excitotoxicity is a central mechanism of injury and triggers a cascade of events, such as increase in calcium influx, cellular damage mediated by ROS, and mitochondrial dysfunction resulting in metabolic crisis and culminating in cell death. A pro-inflammatory phase occurs in the first hours and days. In that microenvironment, microglia polarize into a M1 pro-inflammatory phenotype. Reactive astrocytosis occurs and contributes both to injury and neurorestoration. Acutely after TBI, in the neurovascular unit, swelling of perivascular astrocytes occur and the swollen endfeet constrict capillaries, leading to a reduction in oxygen availability. Also, focal microhemorrhages contribute to inflammatory processes. In this scenario, pericytes contribute to alterations in BBB permeability, angiogenesis, clearance of toxic metabolites, and hemodynamic responses. BBB rupture is evident and contributes to inflammation and edema of the neural tissue.

extrasynaptic sites due to failure of glutamate uptake, gliotransmission, reverse operation of the glutamate transporters, increase in presynaptic glutamate release or increase in the number and/or stability of glutamatergic receptors [53, 62, 65, 66]. The increase in glutamate levels occurs several minutes after the primary trauma, peaks in about 10 minutes and stays increased for several days [45, 64]. Excitotoxicity also causes calcium influx and overload [67, 68], resulting in cellular damage due to several mechanisms (i.e. activation of destructive calcium-dependent proteases, oxidative stress, mitochondrial impairment and transition pore formation, and apoptotic events) [51, 53, 62, 69–71]. Noteworthy, the massive influx of calcium causes production of reactive oxygen species (ROS) in mitochondria. The calcium overload leads to swelling and compromised function of mitochondria, instigating impaired

energy metabolism [51, 72]. Conversely, the damaged tissue needs more energy for its repair than under physiological conditions, resulting in what has been termed a “flow/metabolism mismatch,” a factor that aggravates injury in the traumatic penumbra [62, 73]. Furthermore, increased glutamatergic release into the extracellular milieu following injury causes marked increases in glucose use and accumulation of extracellular lactate [53, 74–79]. This deregulated cerebral metabolism leads to decreased ATP production causing the failure of ATP-dependent ion channels and proteins leading to ionic osmotic alterations that result in cell swelling and culminating in necrosis [80]. Mitochondrial dysfunction may be central to the pathophysiology of TBI through metabolic derangements, oxidative stress, and apoptosis. In fact, a recent study showed mitochondrial ultrastructural alterations at progressive distances from the center of the penumbra in tissue samples from TBI patients [81]. In the setting of TBI, the production of ROS is enhanced [82–84], and the neuroprotective systems become overwhelmed and result in oxidative cell damage. Furthermore, ROS can contribute to disruption of the BBB, edema, and neuroinflammation [34].

Importantly, neuroinflammation is known to be important for the short- and long-term consequences of TBI [85]. Various factors influence the inflammatory response of the brain to TBI. These factors include activation of resident central nervous system (CNS) immune cells and cerebral infiltration of peripheral immune cells (through a disrupted BBB); these cells mediate inflammatory processes through secretion of a variety of inflammatory cytokines, chemokines, adhesion molecules, ROS, and complement factors [86, 87]. Immediately following injury, the levels of various cytokines change drastically in the brain parenchyma and take approximately 48 hours to return to normal [45]. Accordingly, regional, intrathecal, and systemic concentrations of various inflammatory cytokines (interleukin-1, -1 $\beta$ , -6, -8, -10, -12, and tumor necrosis factor- $\alpha$ ) are altered shortly after TBI in humans and experimental models [88–94]. Even though neuroinflammation is generally considered to have negative effects on the neural tissue, interleukins may actually exert beneficial effects on the injured brain by triggering mechanisms of response to tissue injury. Clearly, the beneficial effects of these cytokines are dependent on their concentrations and the timing/conditions of their expression following TBI [53]. The dual role of these cytokines on TBI is observable during the pro-inflammatory phase (in the first hours and days after TBI) as well as through the reparative phase, which lasts for days to months after TBI [95]. Of these cytokines, IL-1 $\beta$  is of special importance because its action on astrocytes makes them release of matrix metalloproteinases (MMPs) [96] that cause further BBB breakdown by promoting and prolonging neuroinflammation [97]. Modulating these inflammatory cells by changing their phenotype from pro-inflammatory to anti-inflammatory would likely promote therapeutic effects on TBI [42, 59, 98]. Additionally, peripheral injuries of the multi-injured patient may increase circulating levels of many of the inflammatory cytokines worsening TBI outcomes [13, 68].

As the major cellular component of the innate immune system in the central nervous system (CNS) and the first line of defense whenever injury or disease occurs, microglia play a critical role in neuroinflammation through the production of various cytokines, proteases, and ROS [45, 99]. In the injured brain, microglia can produce neuroprotective factors, clear cellular debris, and orchestrate neurorestorative processes that are beneficial for neurological recovery after TBI [100, 101]. Microglia can polarize into distinct phenotypes, depending on

the microenvironment in which they are activated. The macrophage/microglial populations are shown to result in a mix of pro-inflammatory M1 and anti-inflammatory M2 microglia/macrophage populations following TBI [56, 102]. It is thought that M1 microglia/macrophage populations are responsible for the production of oxidative species, increased synthesis of pro-inflammatory cytokines, low levels of anti-inflammatory cytokines, and much of the phagocytic activity. As a result, they may contribute to injury progression. M2 populations on the other hand are believed to play a role in angiogenesis, remodeling of the extracellular matrix, and support regeneration following injury [103]. When appropriately queued, microglia can also release neurotrophins to augment neuronal growth and survival [104]. Deficits in the ability of microglia to perform these functions or to appropriately switch between M1 and M2 phenotypes detrimentally affect brain function [105]. Microglial activation within the injured area is observed within 6–48 hours post injury [99] but evidence has shown that microglia can maintain a primed or pro-inflammatory profile for weeks to months after the acute effects of injury have dissipated [106]. Recently, it was shown that extracellular vesicles may exchange pro-inflammatory molecules between brain immune cells, as well as to the systemic circulation, as pathways of inflammation propagation following TBI [107].

Notwithstanding the previous characterization of the pathophysiologic responses to TBI, these biologic responses occur in individuals who possess biologic differences that can modify their response to injury [53, 108]. Over the last years, evidence has showed that the brain is capable of significant structural and functional repair, plasticity, and regeneration. Approaches for accomplishing this include reawakening the growth potential of the surviving neurons or antagonizing the inhibition of axonal growth and synaptogenesis. Alternatively, cellular replacement is achievable in certain brain regions that possess nascent neural stem cells [25, 43, 109–111]. Thus, the discussed concept of traumatic penumbra imbues the transition between injury and repair at the NVU with profound implications for selecting the appropriate type and timing of neuroprotective interventions [34]. In this scenario, it is instigating to investigate which cellular pathways in the traumatic penumbra could play key roles for neurorestoration and, therefore, represent novel therapeutic opportunities for TBI.

### **2.1. The neurovascular unit in the traumatic penumbra**

NVU comprises vascular cells (endothelial cells), perivascular constituents of the blood-brain barrier (pericytes and astrocytes) and their associated neurons, as well as extracellular matrix components [112]. NVU also includes microglial cells, vascular smooth muscle cells located around blood vessels, specialized cellular compartments such as the endothelial glycocalyx, the endothelial lining of cerebral capillaries, capillary tight junctions, and the capillary basement membrane [113]. Together, the components of NVU detect physiological needs of the neural tissue and respond accordingly to supply these demands [112]. Consequently, under normal conditions, cerebral blood flow is maintained constant despite wide changes in perfusion pressure [114], a phenomenon called autoregulation of cerebral blood flow [115].

Traumatic cerebral vascular injury (TCVI) is a major feature of TBI disease. While the complex molecular and cellular mechanisms responsible for functional deficits after TBI are not fully understood, substantial data indicate that TCVI underlies a significant fraction of TBI-related disability. Therefore, in view of its physiological function, the NVU plays an important role



in the pathogenesis of TBI, whether responding to physical trauma or participating in the cascade of events that leads to secondary injury in the traumatic penumbra [116]. Endothelial cells, for example, respond to hemodynamic forces by releasing factors that promote constriction or dilation. Neurons associated with the neural cerebral vasculature release neurotransmitters (e.g. norepinephrine and serotonin for vasoconstriction, and acetylcholine, substance P, and vasoactive intestinal polypeptide for vasodilation) that diffuse into the tunica media and act on receptors in the smooth muscle cell layer to elicit either vasoconstriction or dilation. Based on local activity and needs, basal forebrain neurons release vasoactive mediators on cortical microvessels and supporting astrocytes to modulate microvascular tone [113]. Consequently, neuronal metabolism and activity are tightly coupled to local cerebral blood flow [117].

Microvascular injury is observed in animal models of TBI, whether the injury is caused by impact acceleration, fluid percussion or controlled cortical impact (CCI). Immediately after TBI, endothelial cells are damaged; subsequently, secondary injury extends to the other components of the NVU; decreased blood flow and focal hypoxia disturb the NVU, and various pathophysiological events, such as BBB disruption, edema, and focal ischemia, take place [118]. The NVU response to these events include the increased production of nitric oxide and consequent increase of blood flow right after TBI followed by a period of decreased production of NO and consequent decrease of blood flow [119]. Another aspect of the response of the NVU to these events is the release of damage-associated molecular patterns that trigger secretion of pro-inflammatory mediators such as tumor necrosis factor, interleukin-6, and interleukin-1 $\beta$  by glial cells [120]. The trade-off to this response consists of unwanted side effects such as BBB disruption, edema, hypoperfusion, and oxidative stress, all of which contribute to increase severity of the secondary injury.

Ultrastructural changes in endothelial cells at acutely injury sites are observable 3 hours after TBI and are still present 1 week later [121]. During this, time swelling of perivascular astrocytes is evident; their swollen endfeet constrict capillaries, which leads to a redistribution of capillary blood flow that can reduce oxygen availability to cerebral tissue even if ischemia is not obvious [122]. Data from experimental TBI models indicate that increased extravasation of the contents of blood vessels through microhemorrhages is evident between 3 and 12 hours after the injury [116]. Most of these focal hemorrhages occur in pericontusional tissue, while some occur within the contusion itself and diffusely throughout the ipsilateral, noncontused cerebral hemisphere; intravascular microthrombi, in turn, peak at 48 hours after TBI but persist for at least 9 days [123]. Focal microhemorrhages are accompanied by activation of microglia, reactive gliosis, and recruitment of macrophages; 3 months after the injury, these microbleed sites are surrounded by glial scars and are characterized by major loss of myelin [124].

Clearly, endothelial cells are not alone in the response to TBI. During necrotic phases, cytokines, such as TNF and IL-1, are released by astrocytes, microglia, endothelial cells, and neurons and contributed to the initiation of neuroinflammation [125]. These cytokines induce microglial activation and expression by endothelial cells of adhesion molecules, such as intercellular adhesion molecule 1, aka CD54 (ICAM-1), vascular cell adhesion protein 1, aka CD106 (VCAM-1), P-selectin (CD62P), and E-selectin (CD62E), which in turn allow attachment of leukocytes (neutrophils and



monocytes) to the endothelium and their passage across the BBB. These events lead to increased production of proinflammatory factors at the injured tissue, and leukocytes start releasing MMPs [125]. These MMPs, which include MMP-2, MMP-3, and MMP-9, degrade extracellular matrix proteins and tight junction proteins that join endothelial cells with each other, which results in increased permeability of the BBB. Not surprisingly, the levels of some of these TBI-associated molecules in the blood, as is the case of MMP-9 [126], have been associated with the outcome of TBI and may become important tools for patient screening at the emergency unit in the future.

## 2.2. Pericytes in the traumatic penumbra

Another cell of the NVU, the pericyte, has been recognized as a component of the BBB more than a century ago [127]. Functions attributed to pericytes in the CNS include regulation of the BBB permeability, angiogenesis, clearance of toxic metabolites, and capillary hemodynamic responses [128]. Through the past two decades, pericytes have been increasingly receiving attention from researchers around the world owing to growing knowledge on their properties, which suggested they could behave as stem or progenitor cells not only in the mesodermal tissues [129–132] but also in the CNS [133]. Indeed, various types of evidence suggest that pericytes behave as mesenchymal stem cells in vivo [134], especially the fact that pericytes isolated through various techniques give rise to cultured cells with mesenchymal stem cell characteristics [135–137]. Experiments in which the progeny of cells expressing certain pericyte markers was genetically labeled indicate that pericytes give rise to differentiated progeny in situ in various tissues [138–142], while a recent fate tracing study indicates that does not happen [143]. Albeit in contrast with previous findings in this area, this latter study confirmed that isolated pericytes give rise to cultures with mesenchymal stem cell characteristics.

Most of the knowledge on mesenchymal stem cells comes from in vitro studies that used cultured cells with mesenchymal stem cell characteristics. The International Society for Cellular Therapy has proposed that these cultured cells be called mesenchymal stromal cells (MSCs) unless they are proved to be stem cells using strict criteria [144], and many studies on this cell population use this terminology, although it may be inaccurate. Even though MSCs, owing to their ability to differentiate into various mature cell types, may be used for tissue engineering, it is their ability to secrete trophic and immunomodulatory molecules [145–147] that render them so interesting for cell therapies. Therefore, the acronym “MSC” has been proposed to be used in reference to these cells, but under the designation of “medicinal signaling cells” [148] or any other that does not include “stem cells” [149].

Even though the question as to whether or not pericytes are able to give rise to mature cell types in situ warrants further experimentation, it is likely that pericytes may still be important for regenerative purposes even if they do not behave as stem cells in the body. Pericytes can give rise to cultured cells able to secrete a wide range of trophic and immunomodulatory molecules; consequently, it is possible that pericytes can secrete these types of molecules in vivo too. When tissue injury occurs, pericytes undergo a process called activation—their gene expression profile changes and they become proliferative. As MSC cultures endowed with the ability of secreting trophic molecules can be derived from prospectively isolated pericytes, it is likely that these MSCs possess characteristics of activated pericytes. An early study has

shown that some pericytes detach from blood vessels and migrate toward the cerebral tissue after CCI in rats [150]. Pericytes have been shown to become activated in a CCI model and progress to a state of *reactive pericytosis* [151] in reference to the well-known reactive gliosis observed in various types of CNS injuries. In that study, the number of pericytes in the pericontusional area decreased drastically after the injury, remained lower than normal up to 3 days after the injury, and doubled 5 days after the injury; additionally, the authors found that these activated pericytes remained limited by an area of reactive gliosis. Pericytes were shown to undergo apoptosis in a cortical organotypic slice culture subjected to hypoxia [152]. More recently, cells with characteristic pericyte markers have been detected and isolated from necrotic cerebral tissue affected by stroke; these cells were able to establish MSC cultures [153]. Human brain pericytes cultured under hypoxic conditions were shown to upregulate the expression of neurotrophin-3, which boosted NGF produced by astrocytes under hypoxia, contributing to a neuroprotective effect [154]. Whereas, noncultured pericytes isolated from human adipose tissue express message not only for neurotrophin-3 but also for other neurotrophic factors, such as NGF, BDNF, GDNF, and persephin [155].

Another important characteristic of pericytes that can be inferred from their relationship to MSCs is the ability to secrete molecules that interfere with the action of immune system cells, blocking inflammation [156]. However, it should be noted that pericytes do not become activated immediately upon TBI, and during the initial stages of the response to this injury, they may contribute to the recruitment of inflammatory cells. Some studies have presented evidence that pericytes may contribute to neuroinflammation owing to their ability to perceive infection-related or pro-inflammatory signals and respond through secretion of chemokines that recruit inflammatory cells [157]. In contrast, cultured pericytes have also been shown to be immunosuppressive, as they can inhibit the proliferation of T cells to the same extent as MSCs isolated through traditional methods [136]. It is likely, therefore, that pericytes display a pro-inflammatory phenotype at the onset of TBI, but become immunosuppressive as they undergo activation, thus contributing to maintenance of a balanced level of inflammation as the response to injury progresses.

Together, the information depicted above indicates that, under injury conditions such as TBI or stroke, a number of pericytes die; whereas, the surviving ones become activated, increase in number, and secrete a number of molecules that exert trophic and immunomodulatory effects on their surroundings, contributing to mitigate tissue damage caused by the insult, as previously suggested [155]. While further experimentation is warranted to gain insight into the details of this process, some questions, such as what is the mode of delivery of these soluble factors, are yet to be elucidated. Not long after the introduction of the concept that MSCs exert their reparative effects by means of paracrine factors, microvesicles were found to work as vehicles for the delivery of trophic molecules secreted by MSCs in acute tubular injury [158]. The same principle could well apply to activated pericytes in TBI, but that requires validation. On the other hand, this proposed action of pericytes during the response to TBI suggests that this process could be explored for the purpose of diagnostics and intervention. On one hand, the detection of pericyte-related molecules in the blood could provide information on the status of the lesion in acute TBI patients. On the other hand, knowledge on the main pericyte-derived molecules involved in trophic support of the surrounding cells in

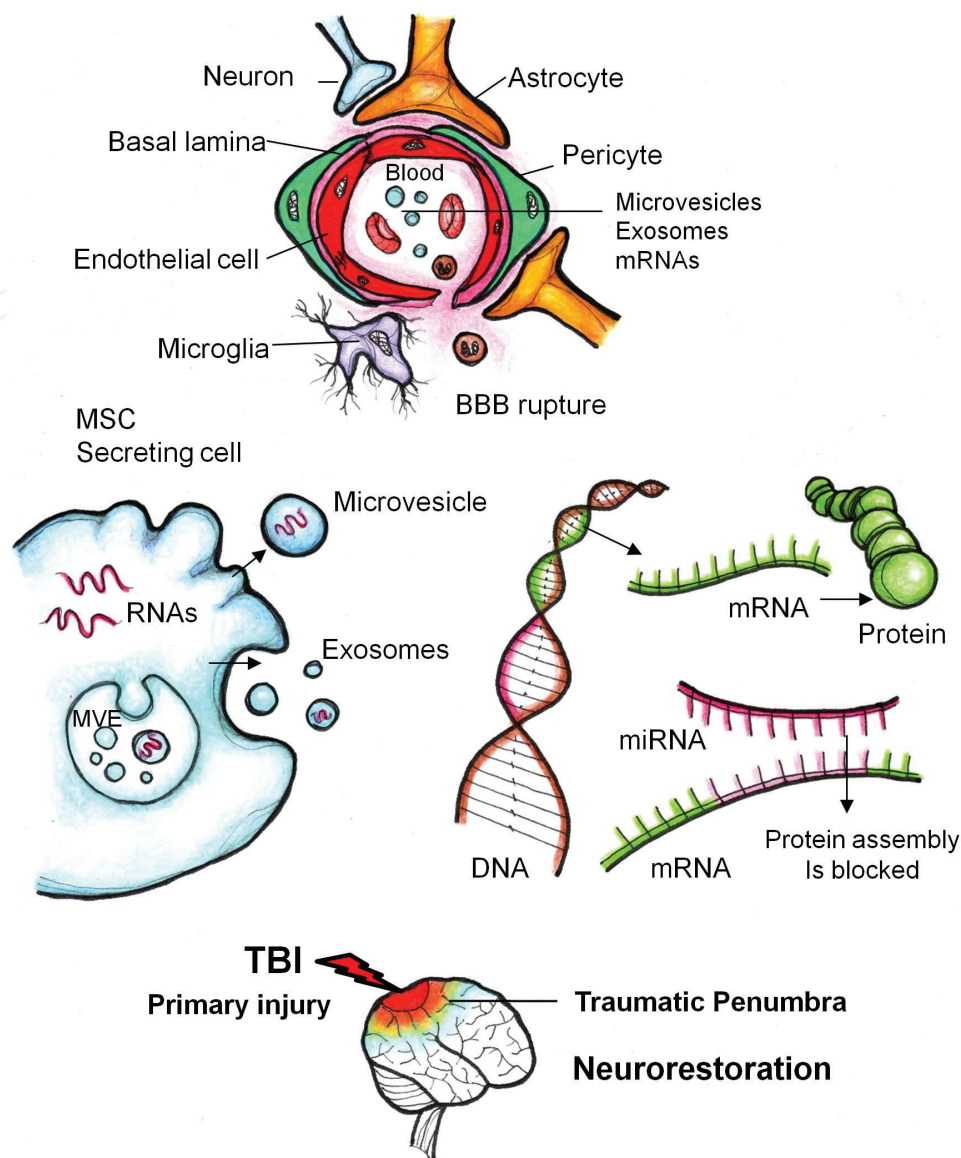
the injured cerebral tissue may allow the development of novel pharmacological approaches to minimize tissue damage during the early stages of TBI. These pharmacological approaches could be further enhanced with the use of microvesicles as delivery vehicles.

### 3. Neuroprotective and neurorestorative processes in the traumatic penumbra

TBI triggers adaptive and maladaptive reactions to injury as damaged tissue attempts to recover [159]. The secondary injury events initiated in the traumatic penumbra lead to cellular dysfunction and death and determine the extent of brain damage. There may be overlapping signals and substrates between the initial trigger of injury and the subsequent endogenous mechanisms of neurorestoration and remodeling (**Figure 2**). The extended nature of these events and the multiplicity of targets offer opportunities for innovative therapeutic interventions [34, 160]. Undeniably, with therapeutic options centered on supportive care, trauma-related mortality and morbidity is an area with unlimited scope for advancement. Therefore, modulating endogenous repair mechanisms through enhancing neurogenesis could be an attractive approach for novel therapies for TBI [161].

Neurogenesis was once thought to be discontinued after brain development in mammals. However, certain areas of the brain retain the ability to generate neurons and glia [162, 163]. In these areas, neural stem cells (NSC) continue the developmental mechanisms to replace and replenish damaged cells. Neurogenic response includes three different phases: proliferation or generation of new cells, migration of new cells to target areas, and differentiation into proper cell types [164]. Many factors may affect adult neurogenesis, such as growth factors, exercise, enriched environment, or stress [161]. Studies have shown that TBI induces an upregulation of neurogenesis in varying types of TBI models [165]. In that sense, strategies such as supplementing varying types of trophic factors (i.e. BDNF, VEGF, S100 $\beta$ ), manipulating transcriptional regulators, or other pharmacological approaches targeting different aspects of the endogenous neurogenic response have shown promising results improving functional recovery following experimental models of TBI [45, 155, 161, 165]. The potential use of cellular therapies to prevent secondary neural injury and promote recovery of injured tissue in trauma is an area of emerging investigation. Preclinical data indicate that restorative therapies targeting multiple parenchymal cells, including cerebral endothelial cells, neural stem/progenitor cells, and oligodendrocyte progenitor cells, enhance TBI-induced angiogenesis, neurogenesis, axonal sprouting, and oligodendrogenesis [45, 161, 166, 167].

Cellular therapies fall into two main categories of cell types: adult multipotent cells and pluripotent embryonic stem cells (ESCs). Adult multipotent cells, such as mesenchymal stem cells, multipotent adult progenitor cells (MAPCs), hematopoietic stem cells (HSCs), and bone marrow mononuclear cells (BMMNCs), have the capacity to generate a limited number of terminally differentiated cell types [168]. Cell-based therapies have been shown to improve outcomes in preclinical studies of trauma-related conditions via several mechanisms, which include: (i) production of soluble factors that regulate the exacerbated cell damage through



**Figure 2.** Schematic representation of neurorestorative mechanisms in the traumatic penumbra. Secondary injury progression in the traumatic penumbra can trigger mechanisms of neural tissue survival and recovery. After TBI, a pro-inflammatory phase occurs in the first hours and days and is followed by a reparative phase lasting from days to months. Secondary injury progression involves a cascade of events that results in cellular damage through diverse signaling pathways. In this scenario, components of the neurovascular unit may orchestrate several neurorestorative mechanisms in the traumatic penumbra. Of particular interest are the neurorestorative mechanisms associated to cellular therapies using mesenchymal stem cells (MSCs). MSCs may exert paracrine effects through secretion of microvesicles and exosomes, evoking endogenous reparative mechanisms, and functional recovery following TBI. Indeed, microvesicles and exosomes can deliver miRNAs to recipient cells, promoting gene regulation and enhancing neuroplasticity.

anti-inflammatory and cell-protective effects (i.e., growth factors, cytokines, microvesicles, exosomes); (ii) replacement of lost cells by differentiating and integrating into the damaged tissue microenvironment; and (iii) stimulation of endogenous regeneration of the injured tissue [167]. A multitude of cell types derived from a variety of tissues are currently under preclinical and clinical investigation for applications in trauma [167]. Multipotent MSCs have shown promise as an effective therapy for brain injuries in experimental models of acute brain



injury [165, 169–172] and potentially in clinical settings [173, 174]. However, previous studies show that only a small proportion of transplanted MSCs actually survive and few MSCs differentiate into neural cells in injured brain tissues. It seems that the predominant mechanisms by which MSCs participate in brain remodeling and functional recovery are likely related to their secretion-based paracrine effect rather than a cell replacement effect [45, 175–178] (Figure 2).

In effect, MSCs secrete or express factors that reach neighboring parenchymal cells either via a paracrine effect or a direct cell-to-cell interaction, or MSCs may induce host cells to secrete bioactive factors, which promote survival and proliferation of the parenchymal cells (brain remodeling) and thereby improve functional recovery [178, 179]. In addition to their soluble factors, therapeutic effects of MSCs may be attributed to their generation and release of exosomes [178]. Exosomes are endosomal origin small membrane vesicles released by almost all cell types and contain not only proteins and lipids but also messenger RNAs and microRNAs (miRNAs) [180]. Recent evidence indicates that exosomes have a crucial role in cell-to-cell communication. In contrast to transplanted exogenous MSCs, nanosized exosomes derived from MSCs do not proliferate and are less immunogenic and easier to store and deliver than MSCs [181–183]. Exosomes generated from MSCs improved functional recovery in rats after TBI [45, 175]. Exosomes play an important role in intercellular communication and are promising therapeutic agents because their complex cargo of proteins and genetic materials has diverse biochemical potential to participate in multiple biochemical and cellular processes, an important attribute in the treatment of complex diseases with multiple secondary injury mechanisms involved [45]. The refinement of MSC therapy from a cell-based therapy to cell-free exosome-based therapy offers several advantages, as it eases the arduous task of preserving cell viability and function, storage, and delivery to patient [175–178]. Further exploring the mechanisms by which the secretion-based paracrine effect of MSCs participates in neurorestoration and functional recovery following TBI is an outstanding opportunity for research. The development of cell-free exosome-based therapies for TBI may allow to deliver targeted regulatory genes (miRNAs) to enhance neuroplasticity and to amplify neurological recovery in TBI.

### 3.1. MicroRNAs

Previous studies have demonstrated that TBI induces extensive temporal changes in the expression of brain protein, mRNA and miRNA [184–186]. There has been a growing interest on the role of miRNA in normal CNS development and function, as well as in disease, including TBI, stroke, and neurodegenerative disorders. Mature, functional miRNA sequences are single-stranded RNA molecules composed of 20–25 nucleotides, which regulate gene expression post-transcriptionally through direct effects on 3'-untranslated region (3' UTR) of mRNA, resulting in translation repression or mRNA degradation. One miRNA usually targets more than 100 genes [187]. In turn, a gene may be regulated by multiple miRNAs [188]. It is estimated that over 2000 miRNAs have been involved in the regulation of approximately 30% of the human protein-coding genes [189].

Microarray analyses in animal models of TBI have shown a dynamic temporal regulation of miRNA expression. A report described that a peak of downregulated and upregulated miRNAs was observed after injury in rat cerebral cortex at 24 and 72 hours, respectively [190]. The research also revealed that a large number of miRNA was expressed at four different time points after injury: 136 at 6 hours, 118 at 24 hours, 149 at 48 hours, and 203 at 72 hours. In addition, only miR-21 expression was upregulated within all the four time points post injury, indicating that this miRNA may be involved in the complex process of TBI course. Another study analyzed changes in expression of 444 miRNAs within the hippocampus of rat TBI models at 3 and 24 hours after controlled cortical impact injury [184]. The results showed that 50 miRNAs had decreased expression levels and 35 miRNAs exhibited increased expression levels in the hippocampus after injury. A bioinformatic analysis of the predicted targets of a subset of the miRNAs with altered expression after TBI (miR-107, -130a, -223, -433-3p, -451, and -541) revealed that many of the target genes are involved in biological functions and processes that play a role in TBI pathophysiology, including transcription, proliferation, morphogenesis, and signal transduction. A study of microarray analyses of miRNA expression profile in rat hippocampus found that 10 of 156 reliably detected miRNAs were significantly and consistently altered from 1 hour to 7 days post injury [186]. Bioinformatic and gene ontology analyses revealed 107 putative target genes, as well as several biological processes that might be initiated by the dysregulated miRNAs, that include miR-144, miR-153, and miR-340-5p. Recently, a study analyzed the biological roles of about 600 genes that are targeted by 10 TBI-altered miRNAs [191]. Bioinformatic analysis suggested that neurodegeneration results from a global miRNA-mediated suppression of genes essential for maintaining proteostasis, the competing and integrated biological pathways that control the synthesis, folding, trafficking, and degradation of proteins. Notably, dysregulation of these essential genes would significantly impair synaptic function and functional connectivity of the brain.

MicroRNAs have emerged as novel serum diagnostic biomarkers for various diseases. The use of miRNA as biomarkers of brain injury in the serum or CSF could serve as tools for both diagnosing and stratifying TBI severity. As a biomarker of pathologic process, miRNA have several unique features, including cell-, tissue-, and disease-specific expression patterns [192, 193]. Studies of CSF in a rat model of mild blast TBI found a significant increase in levels of one miRNA, miR-let-7i, as early as 3 hours post injury [194]. Prediction analysis revealed that this miRNA targets TBI-related proteins, such as S100B and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), suggesting a possible role for miR-let-7i in regulating TBI pathology. Studies in patients with TBI have identified other miRNAs that may serve as diagnostic biomarkers for severe (miR-16, -92a, and -765) [195] and mild brain injury (mir143-3p and mir423-3p) [196]. A recent study using a microarray platform identified 14 miRNAs differentially expressed (10 upregulated and 4 downregulated) in CFS of severe TBI patients who remained unconscious for 2 weeks compared with controls [197]. Another study using microarray analyzed the expression of 754 miRNAs in serum of TBI patients with polytrauma aiming to find biomarkers able to discriminate between mild and severe TBI [198]. The analysis revealed two miRNAs (miR-425-5p and miR-502) that were downregulated in mild TBI at early time points and two miRNAs (miR-21 and miR-335)

that were upregulated in severe TBI. Moreover, miR-425-5p and miR-21 were predictors of 6-month outcome, but with differences regarding the timepoint when they were analyzed (miR-425-5p: until 1 hour and also between 4 and 12 hours from injury; miR-21: between 4 and 12 hours from injury). Overall, these studies have shown a potential role of miRNA as TBI biomarkers, but only miR21 has been identified as a candidate in more than one study [198, 199]. Given that pre-clinical optimism in finding good biomarkers in the past has not been successfully translated in clinical settings [200], further evaluation of these miRNAs with larger, multicenter patient cohorts is needed to explore their use as effective biomarkers applied to diagnosis and prognosis of TBI.

Besides the studies that evaluated miRNAs as TBI biomarkers, there was an interest about if miRNAs can be used as therapeutic targets. Hypothermia is a promising treatment for TBI patients because reducing body temperature attenuates neurological damage and improves functional outcomes [201]. A study presented an intervention on a specific miRNA-regulated pathway treating rats with an antagonist of miRNA-29c in an animal model of deep hypothermic circulatory arrest. The results showed that neurologic function, as assessed by vestibulomotor and cognitive performance tests, was improved in the pretreated animals as compared to the placebo group. Studies have shown that some miRNAs that show altered expression after TBI are also temperature sensitive and may be reduced under hypothermic conditions [202]. Since the pathways in which individual miRNAs can act are often numerous [203], further studies are needed to clarify the use of miRNAs in TBI therapy.

The analysis of miRNA in the TBI context may help in understanding the pathophysiology and possible treatments for TBI as it will provide insights into injury-related gene networks. However, the underlying molecular mechanisms of how miRNAs cause neurodegeneration or neurorestoration after TBI remains elusive. Investigating the role of miRNAs in neurological disorders is a new frontier for neurological research.

### 3.2. Extracellular vesicles and exosomes

MSCs have shown promise in the field of regenerative medicine, since exogenously administered MSCs target injured tissue, interact with brain parenchymal cells, and promote neurorestoration and recovery of neurological function after brain injuries [178, 191, 204, 205]. Despite the differentiation capacity of MSCs, the principal mechanism of their therapeutic action seems to be a robust paracrine capacity, related to their soluble factors as well as generation and release of microvesicles and exosomes [178, 205].

Extracellular vesicles (EVs) are membrane bound entities that transmit signals between cells via all cells and are found in all body fluids [206, 207]. The term “EV” includes microvesicles, exosomes, and oncosomes, among other vesicles that may be variously defined by origin, size, and markers [208–210]. EVs interact with target cells by binding to cell surface receptors, transfer of membrane proteins, membrane fusion, endosomal uptake, and cargo extrusion through vesicle-cell channels [206, 211, 212]. The EV protein and RNA compositions generally reflect that of progenitor cells [211]. Their ability to transport molecules and to target specific cell

populations raised possibilities for their development as therapeutic tools [212–214]. MSC-EVs seem to exert positive impacts on tissue-specific stem cells, promote angiogenesis, and suppress oxidative stress and fibrosis, and, noteworthy, may suppress pro-inflammatory responses in brain injury [215, 216]. Indeed, it was shown that MSC-EVs are able to convert M1 into M2 macrophages and, therefore, by switching pro-inflammatory into tolerogenic environments, MSC-EV administration might promote regenerative processes [170, 205, 212, 215, 217–221]. These therapeutic potentials position EVs as highly competitive alternatives to stem cells, as the EVs are likely to be safer than their parental secreting stem cells [212].

Exosomes are endosome-derived small membrane nanosized vesicles (30–100 nm in diameter) generated by almost all cell types and released into extracellular fluids, playing a pivotal role in intercellular communication [178, 215]. MSC is the most prolific exosome producer among the cell types known to produce exosomes [204, 222]. Exosomes contain various molecular constituents including proteins and RNAs from maternal cells. Among these constituents are miRNAs, which play crucial roles in mediating biological function due to their prominent role in gene regulation. Via exosomes, MSCs transfer their therapeutic factors, especially miRNAs, to recipient cells, and thereby modify gene expression [205, 208]. Although all exosomes contain the constitutive array of proteins, lipids, and RNAs, their contents vary in accordance with the cellular origin and the physiological or pathological condition of the cell and of its extracellular environment [204]. Most of the studies have demonstrated that MSC-derived exosomes contain various miRNAs, which participate in the cell-cell communication and alter the fate of recipient cells [204, 223, 224].

Overall, it has been widely accepted that the exosome secretion is an efficient adaptive mechanism since environmental challenges (such as stress conditions) can influence its composition, biogenesis, and secretion [204, 205, 225]. In fact, through preconditioning or genetic manipulation of neural cells, their exosome secretion profile can be modified [205, 215]. Of note, hypoxia and endothelial activation may be reflected in RNA and protein exosome composition [226, 227]. Furthermore, stressed cells that released exosomes conferred resistance against oxidative stress to recipient cells, suggesting that cells modulate intracellular stress situations and modify the surrounding environment via the secretion of exosomes [225, 228]. Also, the MSC exosome profile can be modified by pretreatment. When MSCs were *in vitro* exposed to brain tissue extracted from rats subjected to middle cerebral artery occlusion, the miR-133b levels in the released exosomes from MSCs were significantly increased [229]. Thus, there is a feedback between the MSC and its environment, and through which ischemic conditions will modify the exosome contents, and consequently, the secreted exosomes affect and modify the tissue environment [205, 230]. Regarding the brain, impacts of MSC-EV treatment were mainly studied in models for ischemic stroke and TBI and reduced apoptosis rates in affected brains, while promoted angiogenesis and neurogenesis [175–177, 215, 231–236]. Both systemic pro-inflammatory and neuroinflammatory cues were reduced following MSC-EV treatment [107, 215].

Administration of cell-free exosomes derived from MSCs is sufficient to exert therapeutic effects of intact MSCs after brain injury [176, 231, 232, 234]. The exosomes transfer RNAs and proteins to other cells which then act epigenetically to alter the function of the



recipient cells [175, 178, 205, 215, 225]. Previous studies indicated that MSCs promised to be an effective therapy for brain injury in TBI [175–177, 215, 231–236]. Instead of brain remodeling and functional recovery by cell replacement effects, evidence suggests that the major effects of neurorestoration were due to the paracrine effects of secretion-based factors such as MSCs-derived exosomes that may reduce neuroinflammation, promote neurogenesis and angiogenesis, rescue pattern separation and spatial learning impairments, and improve functional recovery after TBI in animal models [107, 176, 178, 191, 204, 205, 215, 216, 235–237]. In addition, as exosomes contain various miRNAs, which play a key role in modifying the phenotype and/or the physiology and modulating the cellular processes of the recipient cell, and miRNAs such as miR-21 could be potential therapeutic targets for interventions after TBI, the combination of miRNAs and MSC-derived exosomes might be a novel approach for the treatment of TBI [238]. That is, MSCs-derived exosomes that carry and transfer their cargo such as miRNAs to parenchymal cells may mediate brain plasticity and improve functional recovery after TBI [204]. Furthermore, another potential application of brain endothelial-derived eMVs could be as biosignatures for monitoring the health of the BBB in CNS conditions associated with trauma and neuroinflammation [239].

Hence, MSC-derived exosomes play an important role in intercellular communication and have shown promise in the field of regenerative medicine including treatment of TBI. The refinement of MSC therapy from a cell-based therapy to cell-free exosome-based therapy offers several advantages, as it eases the arduous task of preserving cell viability, storage, and delivery to patient [178, 215]. Indeed, due to the nanosize of exosomes, they can across the BBB and present lower risk of vascular occlusion than intact stem cells [204]. Developing a cell-free exosome-based therapy for TBI may open up a variety of means to deliver targeted regulatory genes (miRNAs) to enhance multifaceted aspects of neuroplasticity and neurorestoration in TBI [178, 205].

#### 4. Conclusions and perspectives

Despite the burden of the morbimortality of neurotrauma, currently, there are no single agent treatments known to improve TBI outcomes. Furthermore, the diverse etiology and complicated pathogenesis of TBI make it difficult for clinical diagnosis and prognosis of outcome. Since TBI acutely triggers adaptive and maladaptive reactions to injury while damaged tissue attempts to recover, understanding the mechanisms of neural injury and neurorestoration is crucial for the development of novel therapeutic approaches. The secondary injury events initiated in the traumatic penumbra lead to cellular dysfunction and death and determine the extent of brain damage. Nevertheless, recent evidence shows that response to injury may also trigger neurorestoration. In the above context, microvesicles and exosomes secreted by MSCs may induce intrinsic repair mechanisms that sustain post-traumatic recovery. Indeed, evidence shows that cell-free, exosome-based therapies for TBI may deliver molecules that regulate gene expressions to enhance neuroplasticity and neurorestoration following TBI.

## Acknowledgements

The research in the authors' laboratories has been funded by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

## Author details

Andrea Regner<sup>1,2\*</sup>, Lindolfo da Silva Meirelles<sup>1,2</sup> and Daniel Simon<sup>1,2</sup>

\*Address all correspondence to: [regner@uol.com.br](mailto:regner@uol.com.br)

1 School of Medicine, Lutheran University of Brazil, Canoas, RS, Brazil

2 Graduate Program in Cellular and Molecular Biology Applied to Health (PPGBioSaúde), Lutheran University of Brazil, Canoas, RS, Brazil

## References

- [1] Manley GT, Maas AI. Traumatic brain injury: An international knowledge-based approach. *Journal of the American Medical Association*. 2013;**310**(5):473-474
- [2] Menon DK, Schwab K, Wright DW, Maas AI. Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward common data elements for research on traumatic brain injury and psychological health. Position statement: Definition of traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*. 2010;**91**(11):1637-1640
- [3] Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: A brief overview. *The Journal of Head Trauma Rehabilitation*. 2006;**21**(5):375-378
- [4] Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurology*. 2008;**7**(8):728-741
- [5] Masel BE, DeWitt DS. Traumatic brain injury: A disease process, not an event. *Journal of Neurotrauma*. 2010;**27**(8):1529-1540
- [6] Faul M, Xu L, Wald MM, Coronado V. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths, 2002-2006. Atlanta, Georgia: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control; 2010
- [7] Finfer SR, Cohen J. Severe traumatic brain injury. *Resuscitation*. 2001;**48**(1):77-90
- [8] Shivaji T, Lee A, Dougall N, McMillan T, Stark C. The epidemiology of hospital treated traumatic brain injury in Scotland. *BMC Neurology*. 2014;**14**:2

- [9] Lawrence T, Helmy A, Bouamra O, Woodford M, Lecky F, Hutchinson PJ. Traumatic brain injury in England and Wales: Prospective audit of epidemiology, complications and standardised mortality. *BMJ Open*. 2016;**6**(11):e012197
- [10] Tran TM, Fuller AT, Kiryabwire J, Mukasa J, Muhumuza M, Ssenyojo H, et al. Distribution and characteristics of severe traumatic brain injury at Mulago National Referral Hospital in Uganda. *World Neurosurgery*. 2015;**83**(3):269-277
- [11] Song SY, Lee SK, Eom KS, Investigators K. Analysis of mortality and epidemiology in 2617 cases of traumatic brain injury: Korean Neuro-Trauma Data Bank System 2010-2014. *Journal of Korean Neurosurgical Association*. 2016;**59**(5):485-491
- [12] Santiago LA, Oh BC, Dash PK, Holcomb JB, Wade CE. A clinical comparison of penetrating and blunt traumatic brain injuries. *Brain Injury*. 2012;**26**(2):107-125
- [13] McDonald SJ, Sun M, Agoston DV, Shultz SR. The effect of concomitant peripheral injury on traumatic brain injury pathobiology and outcome. *Journal of Neuroinflammation*. 2016;**13**(1):90
- [14] Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. *Lancet*. 1974;**2**(7872):81-84
- [15] Chieriegato A, Martino C, Pransani V, Nori G, Russo E, Noto A, et al. Classification of a traumatic brain injury: The Glasgow coma scale is not enough. *Acta Anaesthesiologica Scandinavica*. 2010;**54**(6):696-702
- [16] McMillan T, Wilson L, Ponsford J, Levin H, Teasdale G, Bond M. The Glasgow outcome scale – 40 years of application and refinement. *Nature Reviews. Neurology*. 2016;**12**(8):477-485
- [17] Savitsky B, Givon A, Rozenfeld M, Radomislensky I, Peleg K. Traumatic brain injury: It is all about definition. *Brain Injury*. 2016;**30**(10):1194-1200
- [18] Llompart-Pou JA, Chico-Fernandez M, Sanchez-Casado M, Alberdi-Odrizola F, Guerrero-Lopez F, Mayor-Garcia MD, et al. Age-related injury patterns in Spanish trauma ICU patients. Results from the RETRAUCI. *Injury*. 2016;**47**(Suppl 3):S61-S5
- [19] Papurica M, Rogobete AF, Sandesc D, Dumache R, Cradigati CA, Sarandan M, et al. Advances in biomarkers in critical ill polytrauma patients. *Clinical Laboratory*. 2016;**62**(6):977-986
- [20] Kawata K, Liu CY, Merkel SF, Ramirez SH, Tierney RT, Langford D. Blood biomarkers for brain injury: What are we measuring? *Neuroscience and Biobehavioral Reviews*. 2016;**68**:460-473
- [21] da Rocha AB, Schneider RF, de Freitas GR, Andre C, Grivicich I, Zanoni C, et al. Role of serum S100B as a predictive marker of fatal outcome following isolated severe head injury or multitrauma in males. *Clinical Chemistry and Laboratory Medicine*. 2006;**44**(10):1234-1242

- [22] Regner A, Kaufman M, Friedman G, Chemale I. Increased serum S100beta protein concentrations following severe head injury in humans: A biochemical marker of brain death? *Neuroreport*. 2001;**12**(4):691-694
- [23] Moscote-Salazar LR, MR A, Alvis-Miranda HR, Calderon-Miranda W, Alcala-Cerra G, Blancas Rivera MA, et al. Severe cranioencephalic trauma: Prehospital care, surgical management and multimodal monitoring. *Bulletin of Emergency and Trauma*. 2016;**4**(1):8-23
- [24] Kinoshita K. Traumatic brain injury: Pathophysiology for neurocritical care. *Journal of Intensive Care*. 2016;**4**:29
- [25] Hawryluk GW, Bullock MR. Past, present, and future of traumatic brain injury research. *Neurosurgery Clinics of North America*. 2016;**27**(4):375-396
- [26] Rosenfeld JV, Maas AI, Bragge P, Morganti-Kossmann MC, Manley GT, Gruen RL. Early management of severe traumatic brain injury. *Lancet*. 2012;**380**(9847):1088-1098
- [27] Brain Trauma F, American Association of Neurological Surgeons, Congress of Neurological Surgeons. Guidelines for the management of severe traumatic brain injury. *Journal of Neurotrauma* 2007;**24**(Suppl 1):S1-106
- [28] Adams H, Kolias AG, Hutchinson PJ. The role of surgical intervention in traumatic brain injury. *Neurosurgery Clinics of North America*. 2016;**27**(4):519-528
- [29] Grande PO. Critical evaluation of the Lund concept for treatment of severe traumatic head injury, 25 years after its introduction. *Frontiers in Neurology*. 2017;**8**:315
- [30] Andriessen TM, Horn J, Franschman G, van der Naalt J, Haitsma I, Jacobs B, et al. Epidemiology, severity classification, and outcome of moderate and severe traumatic brain injury: A prospective multicenter study. *Journal of Neurotrauma*. 2011;**28**(10): 2019-2031
- [31] Agrawal D, Ahmed S, Khan S, Gupta D, Sinha S, Satyarthee GD. Outcome in 2068 patients of head injury: Experience at a level 1 trauma centre in India. *Asian Journal of Neurosurgery*. 2016;**11**(2):143-145
- [32] Moore L, Evans D, Hameed SM, Yanchar NL, Stelfox HT, Simons R, et al. Mortality in Canadian trauma systems: A multicenter cohort study. *Annals of Surgery*. 2017;**265**(1): 212-217
- [33] Tagliaferri F, Compagnone C, Korsic M, Servadei F, Kraus JA. Systematic review of brain injury epidemiology in Europe. *Acta Neurochirurgica*. 2006;**148**(3):255-268
- [34] Logsdon AF, Lucke-Wold BP, Turner RC, Huber JD, Rosen CL, Simpkins JW. Role of microvascular disruption in brain damage from traumatic brain injury. *Comprehensive Physiology*. 2015;**5**(3):1147-1160
- [35] Rodriguez-Baeza A, Reina-de la Torre F, Poca A, Marti M, Garnacho A. Morphological features in human cortical brain microvessels after head injury: A three-dimensional and



- immunocytochemical study. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* 2003;**273**(1):583-593
- [36] Vajtr D, Benada O, Kukacka J, Prusa R, Houstava L, Toupalik P, et al. Correlation of ultrastructural changes of endothelial cells and astrocytes occurring during blood brain barrier damage after traumatic brain injury with biochemical markers of BBB leakage and inflammatory response. *Physiological Research*. 2009;**58**(2):263-268
- [37] da Fonseca AC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, et al. The impact of microglial activation on blood-brain barrier in brain diseases. *Frontiers in Cellular Neuroscience*. 2014;**8**:362
- [38] Winkler EA, Minter D, Yue JK, Manley GT. Cerebral Edema in traumatic brain injury: Pathophysiology and prospective therapeutic targets. *Neurosurgery Clinics of North America*. 2016;**27**(4):473-488
- [39] Marmarou A. A review of progress in understanding the pathophysiology and treatment of brain edema. *Neurosurgical Focus*. 2007;**22**(5):E1
- [40] McKee AC, Daneshvar DH. The neuropathology of traumatic brain injury. *Handbook of Clinical Neurology*. 2015;**127**:45-66
- [41] Ghajar J. Traumatic brain injury. *Lancet*. 2000;**356**(9233):923-929
- [42] Krishnamurthy K, Laskowitz DT. Cellular and molecular mechanisms of secondary neuronal injury following traumatic brain injury. In: Laskowitz D, Grant G, editors. *Translational Research in Traumatic Brain Injury*. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 5
- [43] Plummer S, Van den Heuvel C, Thornton E, Corrigan F, Cappai R. The neuroprotective properties of the amyloid precursor protein following traumatic brain injury. *Aging & Disease*. 2016;**7**(2):163-179
- [44] Zhang X, Chen Y, Jenkins LW, Kochanek PM, Clark RS. Bench-to-bedside review: Apoptosis/programmed cell death triggered by traumatic brain injury. *Critical Care*. 2005;**9**(1):66-75
- [45] Aertker BM, Bedi S, Cox CS Jr. Strategies for CNS repair following TBI. *Experimental Neurology*. 2016;**275**(Pt 3):411-426
- [46] Stoffel M, Eriskat J, Plesnila M, Aggarwal N, Baethmann A. The penumbra zone of a traumatic cortical lesion: A microdialysis study of excitatory amino acid release. *Acta Neurochirurgica. Supplement*. 1997;**70**:91-93
- [47] Harish G, Mahadevan A, Pruthi N, Sreenivasamurthy SK, Puttamalles V, Keshava Prasad TS, et al. Characterization of traumatic brain injury in human brains reveals distinct cellular and molecular changes in contusion and pericontusion. *Journal of Neurochemistry*. 2015;**134**(1):156-172

- [48] Newcombe VF, Williams GB, Outtrim JG, Chatfield D, Gulia Abate M, Geeraerts T, et al. Microstructural basis of contusion expansion in traumatic brain injury: Insights from diffusion tensor imaging. *Journal of Cerebral Blood Flow and Metabolism*. 2013; **33**(6):855-862
- [49] Wu HM, Huang SC, Vespa P, Hovda DA, Bergsneider M. Redefining the pericontusional penumbra following traumatic brain injury: Evidence of deteriorating metabolic derangements based on positron emission tomography. *Journal of Neurotrauma*. 2013;**30**(5):352-360
- [50] Sheriff FG, Hinson HE. Pathophysiology and clinical management of moderate and severe traumatic brain injury in the ICU. *Seminars in Neurology*. 2015;**35**(1):42-49
- [51] Algattas H, Huang JH. Traumatic brain injury pathophysiology and treatments: Early, intermediate, and late phases post-injury. *International Journal of Molecular Sciences*. 2013;**15**(1):309-341
- [52] Buitrago Blanco MM, Prashant GN, Vespa PM. Cerebral metabolism and the role of glucose control in acute traumatic brain injury. *Neurosurgery Clinics of North America*. 2016;**27**(4):453-463
- [53] McGinn MJ, Povlishock JT. Pathophysiology of traumatic brain injury. *Neurosurgery Clinics of North America*. 2016;**27**(4):397-407
- [54] Ding K, Wang H, Wu Y, Zhang L, Xu J, Li T, et al. Rapamycin protects against apoptotic neuronal death and improves neurologic function after traumatic brain injury in mice via modulation of the mTOR-p53-Bax axis. *The Journal of Surgical Research*. 2015;**194**(1):239-247
- [55] Burda JE, Bernstein AM, Sofroniew MV. Astrocyte roles in traumatic brain injury. *Experimental Neurology*. 2016;**275**(Pt 3):305-315
- [56] Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: Opportunities for therapeutic intervention. *Brain, Behavior, and Immunity*. 2012;**26**(8):1191-1201
- [57] Baez E, Echeverria V, Cabezas R, Avila-Rodriguez M, Garcia-Segura LM, Barreto GE. Protection by neuroglobin expression in brain pathologies. *Frontiers in Neurology*. 2016; **7**:146
- [58] Zhao Z, Alam S, Oppenheim RW, Prevett DM, Evenson A, Parsadanian A. Overexpression of glial cell line-derived neurotrophic factor in the CNS rescues motoneurons from programmed cell death and promotes their long-term survival following axotomy. *Experimental Neurology*. 2004;**190**(2):356-372
- [59] Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, et al. Neuroinflammatory responses to traumatic brain injury: Etiology, clinical consequences, and therapeutic opportunities. *Neuropsychiatric Disease and Treatment*. 2015;**11**:97-106

- [60] Ziebell JM, Morganti-Kossmann MC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics*. 2010;7(1):22-30
- [61] Castejon OJ. Biopathology of astrocytes in human traumatic and complicated brain injuries. Review and hypothesis. *Folia Neuropathologica*. 2015;53(3):173-192
- [62] Cheng G, Kong RH, Zhang LM, Zhang JN. Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies. *British Journal of Pharmacology*. 2012;167(4):699-719
- [63] Regner A, Alves LB, Chemale I, Costa MS, Friedman G, Achaval M, et al. Neurochemical characterization of traumatic brain injury in humans. *Journal of Neurotrauma*. 2001;18(8):783-792
- [64] Bullock R, Zauner A, Woodward JJ, Myseros J, Choi SC, Ward JD, et al. Factors affecting excitatory amino acid release following severe human head injury. *Journal of Neurosurgery*. 1998;89(4):507-518
- [65] Yi JH, Pow DV, Hazell AS. Early loss of the glutamate transporter splice-variant GLT-1v in rat cerebral cortex following lateral fluid-percussion injury. *Glia*. 2005;49(1):121-133
- [66] Parsons MP, Raymond LA. Extrasynaptic NMDA. receptor involvement in central nervous system disorders. *Neuron*. 2014;82(2):279-293
- [67] Vink R, Nimmo AJ. Novel therapies in development for the treatment of traumatic brain injury. *Expert Opinion on Investigational Drugs*. 2002;11(10):1375-1386
- [68] Hofman M, Koopmans G, Kobbe P, Poeze M, Andruszkow H, Brink PR, et al. Improved fracture healing in patients with concomitant traumatic brain injury: Proven or not? *Mediators of Inflammation*. 2015;2015:204842
- [69] Saatman KE, Creed J, Raghupathi R. Calpain as a therapeutic target in traumatic brain injury. *Neurotherapeutics*. 2010;7(1):31-42
- [70] Weber JT. Altered calcium signaling following traumatic brain injury. *Frontiers in Pharmacology*. 2012;3:60
- [71] Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: Identifying novel targets for neuroprotection. *Progress in Neurobiology*. 2014;115:157-188
- [72] Maciel EN, Vercesi AE, Castilho RF. Oxidative stress in Ca(2+)-induced membrane permeability transition in brain mitochondria. *Journal of Neurochemistry*. 2001;79(6):1237-1245
- [73] Rockswold SB, Rockswold GL, Defillo A. Hyperbaric oxygen in traumatic brain injury. *Neurological Research*. 2007;29(2):162-172
- [74] Kawamata T, Katayama Y, Hovda DA, Yoshino A, Becker DP. Administration of excitatory amino acid antagonists via microdialysis attenuates the increase in glucose utilization seen following concussive brain injury. *Journal of Cerebral Blood Flow and Metabolism*. 1992;12(1):12-24

- [75] Bergsneider M, Hovda DA, Shalmon E, Kelly DF, Vespa PM, Martin NA, et al. Cerebral hyperglycolysis following severe traumatic brain injury in humans: A positron emission tomography study. *Journal of Neurosurgery*. 1997;**86**(2):241-251
- [76] Bergsneider M, Hovda DA, Lee SM, Kelly DF, McArthur DL, Vespa PM, et al. Dissociation of cerebral glucose metabolism and level of consciousness during the period of metabolic depression following human traumatic brain injury. *Journal of Neurotrauma*. 2000;**17**(5):389-401
- [77] Bergsneider M, Hovda DA, McArthur DL, Etchepare M, Huang SC, Sehati N, et al. Metabolic recovery following human traumatic brain injury based on FDG-PET: Time course and relationship to neurological disability. *The Journal of Head Trauma Rehabilitation*. 2001;**16**(2):135-148
- [78] Wu HM, Huang SC, Hattori N, Glenn TC, Vespa PM, Hovda DA, et al. Subcortical white matter metabolic changes remote from focal hemorrhagic lesions suggest diffuse injury after human traumatic brain injury. *Neurosurgery*. 2004;**55**(6):1306-1315 discussion 16-7
- [79] Yoshino A, Hovda DA, Kawamata T, Katayama Y, Becker DP. Dynamic changes in local cerebral glucose utilization following cerebral conclusion in rats: Evidence of a hyper- and subsequent hypometabolic state. *Brain Research*. 1991;**561**(1):106-119
- [80] Werner C, Engelhard K. Pathophysiology of traumatic brain injury. *British Journal of Anaesthesia*. 2007;**99**(1):4-9
- [81] Balan IS, Saladino AJ, Aarabi B, Castellani RJ, Wade C, Stein DM, et al. Cellular alterations in human traumatic brain injury: Changes in mitochondrial morphology reflect regional levels of injury severity. *Journal of Neurotrauma*. 2013;**30**(5):367-381
- [82] Lewen A, Matz P, Chan PH. Free radical pathways in CNS injury. *Journal of Neurotrauma*. 2000;**17**(10):871-890
- [83] Braughler JM, Hall ED. Involvement of lipid peroxidation in CNS injury. *Journal of Neurotrauma*. 1992;**9**(Suppl 1):S1-S7
- [84] Halestrap AP, Woodfield KY, Connern CP. Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. *The Journal of Biological Chemistry*. 1997;**272**(6):3346-3354
- [85] Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature*. 2015;**523**(7560):337-341
- [86] Rock KL, Kono H. The inflammatory response to cell death. *Annual Review of Pathology*. 2008;**3**:99-126
- [87] Mathew P, Graham DI, Bullock R, Maxwell W, McCulloch J, Teasdale G. Focal brain injury: Histological evidence of delayed inflammatory response in a new rodent model of focal cortical injury. *Acta Neurochirurgica. Supplementum (Wien)*. 1994;**60**:428-430



- [88] Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: A double-edged sword. *Current Opinion in Critical Care*. 2002;**8**(2):101-105
- [89] Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: Relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *Journal of Neuroimmunology*. 1999;**101**(2):211-221
- [90] Fassbender K, Schneider S, Bertsch T, Schlueter D, Fatar M, Ragoschke A, et al. Temporal profile of release of interleukin-1beta in neurotrauma. *Neuroscience Letters*. 2000;**284**(3):135-138
- [91] Maier B, Schwerdtfeger K, Mautes A, Holanda M, Muller M, Steudel WI, et al. Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. *Shock*. 2001;**15**(6):421-426
- [92] Lenzlinger PM, Morganti-Kossmann MC, Laurer HL, McIntosh TK. The duality of the inflammatory response to traumatic brain injury. *Molecular Neurobiology*. 2001;**24**(1-3): 169-181
- [93] Ferreira LC, Regner A, Miotto KD, Moura S, Ikuta N, Vargas AE, et al. Increased levels of interleukin-6, -8 and -10 are associated with fatal outcome following severe traumatic brain injury. *Brain Injury*. 2014;**28**(10):1311-1316
- [94] Frugier T, Morganti-Kossmann MC, O'Reilly D, McLean CA. In situ detection of inflammatory mediators in post mortem human brain tissue after traumatic injury. *Journal of Neurotrauma*. 2010;**27**(3):497-507
- [95] Schmidt OI, Heyde CE, Ertel W, Stahel PF. Closed head injury – An inflammatory disease? *Brain Research. Brain Research Reviews*. 2005;**48**(2):388-399
- [96] Ralay Ranaivo H, Zurich SM, Choi N, Hodge JN, Wainwright MS. Mild stretch-induced injury increases susceptibility to interleukin-1beta-induced release of matrix metalloproteinase-9 from astrocytes. *Journal of Neurotrauma*. 2011;**28**(9):1757-1766
- [97] Roberts DJ, Jenne CN, Leger C, Kramer AH, Gallagher CN, Todd S, et al. Association between the cerebral inflammatory and matrix metalloproteinase responses after severe traumatic brain injury in humans. *Journal of Neurotrauma*. 2013;**30**(20):1727-1736
- [98] Hanrahan F, Campbell M. Neuroinflammation. In: Laskowitz D, Grant G, editors. *Translational Research in Traumatic Brain Injury*. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 6
- [99] Toklu HZ, Tumer N. Oxidative stress, brain edema, blood-brain barrier permeability, and autonomic dysfunction from traumatic brain injury. In: Kobeissy FH, editor. *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects*. Boca Raton (FL): CRC Press/Taylor & Francis; 2015. Chapter 5

- [100] Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. *Experimental Neurology*. 2016;**275**(Pt 3):316-327
- [101] Xu H, Wang Z, Li J, Wu H, Peng Y, Fan L, et al. The polarization states of microglia in TBI: A new paradigm for pharmacological intervention. *Neural Plasticity*. 2017;**2017**: 5405104
- [102] Hernandez-Ontiveros DG, Tajiri N, Acosta S, Giunta B, Tan J, Borlongan CV. Microglia activation as a biomarker for traumatic brain injury. *Frontiers in Neurology*. 2013;**4**:30
- [103] Chhor V, Le Charpentier T, Lebon S, Ore MV, Celador IL, Josserand J, et al. Characterization of phenotype markers and neurotoxic potential of polarised primary microglia in vitro. *Brain, Behavior, and Immunity*. 2013;**32**:70-85
- [104] Parekkadan B, Berdichevsky Y, Irimia D, Leeder A, Yarmush G, Toner M, et al. Cell-cell interaction modulates neuroectodermal specification of embryonic stem cells. *Neuroscience Letters*. 2008;**438**(2):190-195
- [105] Chiu CC, Liao YE, Yang LY, Wang JY, Tweedie D, Karnati HK, et al. Neuroinflammation in animal models of traumatic brain injury. *Journal of Neuroscience Methods*. 2016; **272**:38-49
- [106] Witcher KG, Eiferman DS, Godbout JP. Priming the inflammatory pump of the CNS after traumatic brain injury. *Trends in Neurosciences*. 2015;**38**(10):609-620
- [107] Kumar A, Stoica BA, Loane DJ, Yang M, Abulwerdi G, Khan N, et al. Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. *Journal of Neuroinflammation*. 2017;**14**(1):47
- [108] Lingsma HF, Yue JK, Maas AI, Steyerberg EW, Manley GT, Investigators T-T. Outcome prediction after mild and complicated mild traumatic brain injury: External validation of existing models and identification of new predictors using the TRACK-TBI pilot study. *Journal of Neurotrauma*. 2015;**32**(2):83-94
- [109] Hall ED. Translational principles of neuroprotective and neurorestorative therapy testing in animal models of traumatic brain injury. In: Laskowitz D, Grant G, editors. *Translational Research in Traumatic Brain Injury*. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 11
- [110] Kochanek PM, Jackson TC, Ferguson NM, Carlson SW, Simon DW, Brockman EC, et al. Emerging therapies in traumatic brain injury. *Seminars in Neurology*. 2015;**35**(1):83-100
- [111] Jablonska A, Lukomska B. Stroke induced brain changes: Implications for stem cell transplantation. *Acta Neurobiologiae Experimentalis (Wars)*. 2011;**71**(1):74-85
- [112] Muoio V, Persson PB, Sendeski MM. The neurovascular unit – Concept review. *Acta Physiologica (Oxford, England)*. 2014;**210**(4):790-798
- [113] Stanimirovic DB, Friedman A. Pathophysiology of the neurovascular unit: Disease cause or consequence? *Journal of Cerebral Blood Flow and Metabolism*. 2012;**32**(7):1207-1221

- [114] Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiological Reviews*. 1959;**39**(2):183-238
- [115] Harper AM. Autoregulation of cerebral blood flow: Influence of the arterial blood pressure on the blood flow through the cerebral cortex. *Journal of Neurology, Neurosurgery, and Psychiatry*. 1966;**29**(5):398-403
- [116] Kenney K, Amyot F, Haber M, Pronger A, Bogoslovsky T, Moore C, et al. Cerebral vascular injury in traumatic brain injury. *Experimental Neurology*. 2016;**275**(Pt 3):353-366
- [117] Villringer A, Dirnagl U. Coupling of brain activity and cerebral blood flow: Basis of functional neuroimaging. *Cerebrovascular and Brain Metabolism Reviews*. 1995;**7**(3):240-276
- [118] Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nature Reviews. Neurology*. 2010;**6**(7):393-403
- [119] Cherian L, Hlatky R, Robertson CS. Nitric oxide in traumatic brain injury. *Brain Pathology*. 2004;**14**(2):195-201
- [120] Simon DW, McGeachy MJ, Bayir H, Clark RSB, Loane DJ, Kochanek PM. The far-reaching scope of neuroinflammation after traumatic brain injury. *Nature Reviews. Neurology*. 2017;**13**(9):572
- [121] Maxwell WL, Irvine A, Adams JH, Graham DI, Gennarelli TA. Response of cerebral microvasculature to brain injury. *The Journal of Pathology*. 1988;**155**(4):327-335
- [122] Ostergaard L, Engedal TS, Aamand R, Mikkelsen R, Iversen NK, Anzabi M, et al. Capillary transit time heterogeneity and flow-metabolism coupling after traumatic brain injury. *Journal of Cerebral Blood Flow and Metabolism*. 2014;**34**(10):1585-1598
- [123] Stein SC, Chen XH, Sinson GP, Smith DH. Intravascular coagulation: A major secondary insult in nonfatal traumatic brain injury. *Journal of Neurosurgery*. 2002;**97**(6):1373-1377
- [124] Glushakova OY, Johnson D, Hayes RL. Delayed increases in microvascular pathology after experimental traumatic brain injury are associated with prolonged inflammation, blood-brain barrier disruption, and progressive white matter damage. *Journal of Neurotrauma*. 2014;**31**(13):1180-1193
- [125] Jullienne A, Badaut J. Molecular contributions to neurovascular unit dysfunctions after brain injuries: Lessons for target-specific drug development. *Future Neurology*. 2013;**8**(6):677-689
- [126] Simon D, Evaldt J, Nabinger DD, Fontana MF, Klein MG, do Amaral Gomes J, et al. Plasma matrix metalloproteinase-9 levels predict intensive care unit mortality early after severe traumatic brain injury. *Brain Injury*. 2017;**31**(3):390-395
- [127] Sa-Pereira I, Brites D, Brito MA. Neurovascular unit: A focus on pericytes. *Molecular Neurobiology*. 2012;**45**(2):327-347

- [128] Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nature Neuroscience*. 2016;**19**(6):771-783
- [129] Bianco P, Cossu G. Uno, nessuno e centomila: Searching for the identity of mesodermal progenitors. *Experimental Cell Research*. 1999;**251**(2):257-263
- [130] Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: Nature, biology, and potential applications. *Stem Cells*. 2001;**19**(3):180-192
- [131] da Silva Meirelles L, Caplan AI, Nardi NB. Search of the in vivo identity of mesenchymal stem cells. *Stem Cells*. 2008;**26**(9):2287-2299
- [132] da Silva Meirelles L, de Deus Wagatsuma VM, Malta TM, Bonini Palma PV, Araujo AG, Panepucci RA, et al. The gene expression profile of non-cultured, highly purified human adipose tissue pericytes: Transcriptomic evidence that pericytes are stem cells in human adipose tissue. *Experimental Cell Research*. 2016;**349**(2):239-254
- [133] Dore-Duffy P, Katychhev A, Wang X, Van Buren ECNS. microvascular pericytes exhibit multipotential stem cell activity. *Journal of Cerebral Blood Flow and Metabolism*. 2006;**26**(5):613-624
- [134] da Silva Meirelles L, Bellagamba BC, Camassola M, Nardi NB. Mesenchymal stem cells and their relationship to pericytes. *Front Biosci (Landmark Ed)*. 2016;**21**:130-156
- [135] Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. 2008;**3**(3):301-313
- [136] da Silva Meirelles L, Malta TM, de Deus Wagatsuma VM, Palma PV, Araujo AG, Ribeiro Malmegrim KC, et al. Cultured human adipose tissue pericytes and mesenchymal stromal cells display a very similar gene expression profile. *Stem Cells and Development*. 2015;**24**(23):2822-2840
- [137] da Silva Meirelles L, Malta TM, Panepucci RA, da Silva Jr WA. Transcriptomic comparisons between cultured human adipose tissue-derived pericytes and mesenchymal stromal cells. *Genomics Data* 2016;**7**:20-25
- [138] Dellavalle A, Maroli G, Covarello D, Azzoni E, Innocenzi A, Perani L, et al. Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. *Nature Communications*. 2011;**2**:499
- [139] Feng J, Mantesso A, De Bari C, Nishiyama A, Sharpe PT. Dual origin of mesenchymal stem cells contributing to organ growth and repair. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**(16):6503-6508
- [140] Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *The American Journal of Pathology*. 2010;**176**(1):85-97



- [141] Maes C, Kobayashi T, Selig MK, Torrekens S, Roth SI, Mackem S, et al. Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. *Developmental Cell*. 2010;**19**(2):329-344
- [142] Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, et al. White fat progenitor cells reside in the adipose vasculature. *Science*. 2008;**322**(5901):583-586
- [143] Guimaraes-Camboa N, Cattaneo P, Sun Y, Moore-Morris T, Gu Y, Dalton ND, et al. Pericytes of multiple organs do not behave as mesenchymal stem cells in vivo. *Cell Stem Cell*. 2017;**20**(3):345-359
- [144] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for cellular therapy position statement. *Cytotherapy*. 2006;**8**(4):315-317
- [145] Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *Journal of Cellular Biochemistry*. 2006;**98**(5):1076-1084
- [146] Meirelles Lda S, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine & Growth Factor Reviews*. 2009;**20**(5-6):419-427
- [147] Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *Journal of Cellular Physiology*. 2007;**213**(2):341-347
- [148] Caplan AI. What's in a name? *Tissue Engineering. Part A*. 2010;**16**(8):2415-2417
- [149] Caplan AI. Mesenchymal stem cells: Time to change the name. *Stem Cells Translational Medicine*. 2017;**6**(6):1445-1451
- [150] Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, Rafols JA. Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvascular Research*. 2000;**60**(1):55-69
- [151] Zehendner CM, Sebastiani A, Hugonnet A, Bischoff F, Luhmann HJ, Thal SC. Traumatic brain injury results in rapid pericyte loss followed by reactive pericytosis in the cerebral cortex. *Scientific Reports*. 2015;**5**:13497
- [152] Zehendner CM, Wedler HE, Luhmann HJA. Novel in vitro model to study pericytes in the neurovascular unit of the developing cortex. *PLoS One*. 2013;**8**(11):e81637
- [153] Tatebayashi K, Tanaka Y, Nakano-Doi A, Sakuma R, Kamachi S, Shirakawa M, et al. Identification of multipotent stem cells in human brain tissue following stroke. *Stem Cells and Development*. 2017;**26**(11):787-797
- [154] Ishitsuka K, Ago T, Arimura K, Nakamura K, Tokami H, Makihara N, et al. Neurotrophin production in brain pericytes during hypoxia: A role of pericytes for neuroprotection. *Microvascular Research*. 2012;**83**(3):352-359

- [155] da Silva Meirelles L, Simon D, Regner A. Neurotrauma: The crosstalk between neurotrophins and inflammation in the acutely injured brain. *International Journal of Molecular Sciences*. 2017;**18**(5):1082
- [156] Caplan AI, Sorrell JM. The MSC curtain that stops the immune system. *Immunology Letters*. 2015;**168**(2):136-139
- [157] Rustenhoven J, Jansson D, Smyth LC, Dragunow M, Brain Pericytes A. Mediators of neuroinflammation. *Trends in Pharmacological Sciences*. 2017;**38**(3):291-304
- [158] Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *Journal of the American Society of Nephrology*. 2009;**20**(5):1053-1067
- [159] Choi YK, Maki T, Mandeville ET, Koh SH, Hayakawa K, Arai K, et al. Dual effects of carbon monoxide on pericytes and neurogenesis in traumatic brain injury. *Nature Medicine*. 2016;**22**(11):1335-1341
- [160] Tu Y, Chen C, Sun HT, Cheng SX, Liu XZ, Qu Y, et al. Combination of temperature-sensitive stem cells and mild hypothermia: A new potential therapy for severe traumatic brain injury. *Journal of Neurotrauma*. 2012;**29**(14):2393-2403
- [161] Patel K, Sun D. Strategies targeting endogenous neurogenic cell response to improve recovery following traumatic brain injury. *Brain Res*. 2016;**1640**(Pt A):104-113
- [162] Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proceedings of the National Academy of Sciences of the United States of America*. 1993;**90**(5):2074-2077
- [163] Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J. Multipotent progenitor cells in the adult dentate gyrus. *Journal of Neurobiology*. 1998;**36**(2):249-266
- [164] Hallbergson AF, Gnatenco C, Peterson DA. Neurogenesis and brain injury: Managing a renewable resource for repair. *The Journal of Clinical Investigation*. 2003;**112**(8):1128-1133
- [165] Rolfe A, Sun D. Stem cell therapy in brain trauma: implications for repair and regeneration of injured brain in experimental TBI models. In: Kobeissy FH, editor. *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects*. Boca Raton (FL): CRC Press/Taylor & Francis; 2015. Chapter 42
- [166] Xiong Y, Qu C, Mahmood A, Liu Z, Ning R, Li Y, et al. Delayed transplantation of human marrow stromal cell-seeded scaffolds increases transcallosal neural fiber length, angiogenesis, and hippocampal neuronal survival and improves functional outcome after traumatic brain injury in rats. *Brain Research*. 2009;**1263**:183-191
- [167] Pati S, Rasmussen TE. Cellular therapies in trauma and critical care medicine: Looking towards the future. *PLoS Medicine*. 2017;**14**(7):e1002343

- [168] Pati S, Pilia M, Grimsley JM, Karanikas AT, Oyeniyi B, Holcomb JB, et al. Cellular therapies in trauma and critical care medicine: Forging new Frontiers. *Shock*. 2015;**44**(6): 505-523
- [169] Chen Q, Long Y, Yuan X, Zou L, Sun J, Chen S, et al. Protective effects of bone marrow stromal cell transplantation in injured rodent brain: Synthesis of neurotrophic factors. *Journal of Neuroscience Research*. 2005;**80**(5):611-619
- [170] Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurology*. 2002;**1**(2):92-100
- [171] Mahmood A, Lu D, Chopp M. Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. *Neurosurgery*. 2004;**55**(5): 1185-1193
- [172] Mahmood A, Lu D, Chopp M. Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. *Journal of Neurotrauma*. 2004;**21**(1):33-39
- [173] Doeppner TR, Hermann DM. Stem cell-based treatments against stroke: Observations from human proof-of-concept studies and considerations regarding clinical applicability. *Frontiers in Cellular Neuroscience*. 2014;**8**:357
- [174] Cox Jr CS, Baumgartner JE, Harting MT, Worth LL, Walker PA, Shah SK, et al. Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. *Neurosurgery* 2011;**68**(3):588-600
- [175] Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, et al. Effect of exosomes derived from multipotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *Journal of Neurosurgery*. 2015;**122**(4):856-867
- [176] Zhang Y, Chopp M, Zhang ZG, Katakowski M, Xin H, Qu C, et al. Systemic administration of cell-free exosomes generated by human bone marrow derived mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury. *Neurochemistry International*. 2017;**111**:69-81
- [177] Kim DK, Nishida H, An SY, Shetty AK, Bartosh TJ, Prockop DJ. Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(1):170-175
- [178] Xiong Y, Mahmood A, Chopp M. Emerging potential of exosomes for treatment of traumatic brain injury. *Neural Regeneration Research*. 2017;**12**(1):19-22
- [179] Marsh SE, Blurton-Jones M. Neural stem cell therapy for neurodegenerative disorders: The role of neurotrophic support. *Neurochemistry International*. 2017;**106**:94-100
- [180] Barteneva NS, Fasler-Kan E, Bernimoulin M, Stern JN, Ponomarev ED, Duckett L, et al. Circulating microparticles: Square the circle. *BMC Cell Biology*. 2013;**14**:23

- [181] Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Research*. 2010;**4**(3):214-222
- [182] Zhang B, Yin Y, Lai RC, Lim SK. Immunotherapeutic potential of extracellular vesicles. *Frontiers in Immunology*. 2014;**5**:518
- [183] Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. *Seminars in Cell & Developmental Biology*. 2015;**40**:82-88
- [184] Redell JB, Liu Y, Dash PK. Traumatic brain injury alters expression of hippocampal microRNAs: Potential regulators of multiple pathophysiological processes. *Journal of Neuroscience Research*. 2009;**87**(6):1435-1448
- [185] Hu Z, Yu D, Almeida-Suhett C, Tu K, Marini AM, Eiden L, et al. Expression of miRNAs and their cooperative regulation of the pathophysiology in traumatic brain injury. *PLoS One*. 2012;**7**(6):e39357
- [186] Liu L, Sun T, Liu Z, Chen X, Zhao L, Qu G, et al. Traumatic brain injury dysregulates microRNAs to modulate cell signaling in rat hippocampus. *PLoS One*. 2014;**9**(8):e103948
- [187] Lu J, Clark AG. Impact of microRNA regulation on variation in human gene expression. *Genome Research*. 2012;**22**(7):1243-1254
- [188] John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. *PLoS Biology*. 2004;**2**(11):e363
- [189] Hammond SM. An overview of microRNAs. *Advanced Drug Delivery Reviews*. 2015;**87**:3-14
- [190] Lei P, Li Y, Chen X, Yang S, Zhang J. Microarray based analysis of microRNA expression in rat cerebral cortex after traumatic brain injury. *Brain Research*. 2009;**1284**:191-201
- [191] Boone DK, Weisz HA, Bi M, Falduto MT, Torres KEO, Willey HE, et al. Evidence linking microRNA suppression of essential prosurvival genes with hippocampal cell death after traumatic brain injury. *Scientific Reports*. 2017;**7**(1):6645
- [192] Ye Y, Perez-Polo JR, Qian J, Birnbaum Y. The role of microRNA in modulating myocardial ischemia-reperfusion injury. *Physiological Genomics*. 2011;**43**(10):534-542
- [193] Bartels CL, Tsongalis GJ. MicroRNAs: Novel biomarkers for human cancer. *Clinical Chemistry*. 2009;**55**(4):623-631
- [194] Balakathiresan N, Bhomia M, Chandran R, Chavko M, McCarron RM, Maheshwari RK. MicroRNA let-7i is a promising serum biomarker for blast-induced traumatic brain injury. *Journal of Neurotrauma*. 2012;**29**(7):1379-1387
- [195] Redell JB, Moore AN, Ward 3rd NH, Hergenroeder GW, Dash PK. Human traumatic brain injury alters plasma microRNA levels. *Journal of Neurotrauma* 2010;**27**(12):2147-2156
- [196] Mitra B, Rau TF, Surendran N, Brennan JH, Thaveenthiran P, Sorich E, et al. Plasma micro-RNA biomarkers for diagnosis and prognosis after traumatic brain injury: A pilot study. *Journal of Clinical Neuroscience*. 2017;**38**:37-42



- [197] You WD, Tang QL, Wang L, Lei J, Feng JF, Mao Q, et al. Alteration of microRNA expression in cerebrospinal fluid of unconscious patients after traumatic brain injury and a bioinformatic analysis of related single nucleotide polymorphisms. *Chinese Journal of Traumatology*. 2016;**19**(1):11-15
- [198] Di Pietro V, Ragusa M, Davies D, Su Z, Hazeldine J, Lazzarino G, et al. MicroRNAs as novel biomarkers for the diagnosis and prognosis of mild and severe traumatic brain injury. *Journal of Neurotrauma*. 2017;**34**(11):1948-1956
- [199] Redell JB, Zhao J, Dash PK. Altered expression of miRNA-21 and its targets in the hippocampus after traumatic brain injury. *Journal of Neuroscience Research*. 2011;**89**(2):212-221
- [200] Wong VS, Langley B. Epigenetic changes following traumatic brain injury and their implications for outcome, recovery and therapy. *Neuroscience Letters*. 2016;**625**:26-33
- [201] Choi HA, Badjatia N, Mayer SA. Hypothermia for acute brain injury--mechanisms and practical aspects. *Nature Reviews. Neurology*. 2012;**8**(4):214-222
- [202] Truettner JS, Alonso OF, Bramlett HM, Dietrich WD. Therapeutic hypothermia alters microRNA responses to traumatic brain injury in rats. *Journal of Cerebral Blood Flow and Metabolism* 2011;**31**(9):1897-1907
- [203] Kurlansky P. MicroRNAs: Panacea or Pandora's box? *The Journal of Thoracic and Cardiovascular Surgery*. 2015;**150**(2):407-408
- [204] Yang Y, Ye Y, Su X, He J, Bai W, He X. MSCs-derived exosomes and neuroinflammation, neurogenesis and therapy of traumatic brain injury. *Frontiers in Cellular Neuroscience*. 2017;**11**:55
- [205] Xin H, Li Y, Chopp M. Exosomes/miRNAs as mediating cell-based therapy of stroke. *Frontiers in Cellular Neuroscience*. 2014;**8**:377
- [206] Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borrás FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. *Journal of Extracellular Vesicles*. 2015;**4**:27066
- [207] Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *The Journal of Cell Biology*. 2013;**200**(4):373-383
- [208] Cocucci E, Meldolesi J. Ectosomes. *Current Biology*. 2011;**21**(23):R940-R941
- [209] Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials – An ISEV position paper. *Journal of Extracellular Vesicles*. 2015;**4**:30087
- [210] Tkach M, Thery C. Communication by extracellular vesicles: Where we are and where we need to go. *Cell*. 2016;**164**(6):1226-1232
- [211] Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(8):E968-E977

- [212] Reiner AT, Witwer KW, van Balkom BWM, de Beer J, Brodie C, Corteling RL, et al. Concise review: Developing best-practice models for the therapeutic use of extracellular vesicles. *Stem Cells Translational Medicine*. 2017;**6**(8):1730-1739
- [213] Biancone L, Bruno S, Deregibus MC, Tetta C, Camussi G. Therapeutic potential of mesenchymal stem cell-derived microvesicles. *Nephrology, Dialysis, Transplantation*. 2012;**27**(8):3037-3042
- [214] Fais S, O'Driscoll L, Borrás FE, Buzas E, Camussi G, Cappello F, et al. Evidence-based clinical use of Nanoscale extracellular vesicles in Nanomedicine. *ACS Nano*. 2016;**10**(4):3886-3899
- [215] Borger V, Bremer M, Ferrer-Tur R, Gockeln L, Stambouli O, Becic A, et al. Mesenchymal stem/stromal cell-derived extracellular vesicles and their potential as novel Immunomodulatory therapeutic agents. *International Journal of Molecular Sciences*. 2017;**18**(7):1450
- [216] Bruno S, Deregibus MC, Camussi G. The secretome of mesenchymal stromal cells: Role of extracellular vesicles in immunomodulation. *Immunology Letters*. 2015;**168**(2):154-158
- [217] Chen X, Li Y, Wang L, Katakowski M, Zhang L, Chen J, et al. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. *Neuropathology*. 2002;**22**(4):275-279
- [218] Zhang ZG, Chopp M. Neurorestorative therapies for stroke: Underlying mechanisms and translation to the clinic. *Lancet Neurology*. 2009;**8**(5):491-500
- [219] Wei GJ, An G, Shi ZW, Wang KF, Guan Y, Wang YS, et al. Suppression of MicroRNA-383 enhances therapeutic potential of human bone-marrow-derived mesenchymal stem cells in treating spinal cord injury via GDNF. *Cellular Physiology and Biochemistry*. 2017;**41**(4):1435-1444
- [220] Zhang ZG, Chopp M. Exosomes in stroke pathogenesis and therapy. *The Journal of Clinical Investigation*. 2016;**126**(4):1190-1197
- [221] Hasan A, Deeb G, Rahal R, Atwi K, Mondello S, Marei HE, et al. Mesenchymal stem cells in the treatment of traumatic brain injury. *Frontiers in Neurology*. 2017;**8**:28
- [222] Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, et al. Mesenchymal stem cell: An efficient mass producer of exosomes for drug delivery. *Advanced Drug Delivery Reviews*. 2013;**65**(3):336-341
- [223] Koh W, Sheng CT, Tan B, Lee QY, Kuznetsov V, Kiang LS, et al. Analysis of deep sequencing microRNA expression profile from human embryonic stem cells derived mesenchymal stem cells reveals possible role of let-7 microRNA family in downstream targeting of hepatic nuclear factor 4 alpha. *BMC Genomics*. 2010;**11**(Suppl 1):S6
- [224] Huang JH, Yin XM, Xu Y, Xu CC, Lin X, Ye FB, et al. Systemic administration of exosomes released from mesenchymal stromal cells attenuates apoptosis, inflammation, and promotes angiogenesis after spinal cord injury in rats. *Journal of Neurotrauma*. 2017

- [225] Kassis H, Shehadah A, Chopp M, Zhang ZG. Epigenetics in stroke recovery. *Genes* (Basel). 2017;**8**(3):89
- [226] de Jong OG, Verhaar MC, Chen Y, Vader P, Gremmels H, Posthuma G, et al. Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. *Journal of Extracellular Vesicles*. 2012;**1**. DOI: 10.3402/jev.v1i0.18396
- [227] Yoon JH, Kim J, Kim KL, Kim DH, Jung SJ, Lee H, et al. Proteomic analysis of hypoxia-induced U373MG glioma secretome reveals novel hypoxia-dependent migration factors. *Proteomics*. 2014;**14**(12):1494-1502
- [228] Eldh M, Ekstrom K, Valadi H, Sjostrand M, Olsson B, Jernas M, et al. Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle RNA. *PLoS One*. 2010;**5**(12):e15353
- [229] Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, et al. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells*. 2012;**30**(7):1556-1564
- [230] Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. *PLoS One*. 2014;**9**(2):e88685
- [231] Doeppner TR, Herz J, Gorgens A, Schlechter J, Ludwig AK, Radtke S, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent Postischemic immunosuppression. *Stem Cells Translational Medicine*. 2015;**4**(10):1131-1143
- [232] Chen KH, Chen CH, Wallace CG, Yuen CM, Kao GS, Chen YL, et al. Intravenous administration of xenogenic adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes markedly reduced brain infarct volume and preserved neurological function in rat after acute ischemic stroke. *Oncotarget*. 2016;**7**(46):74537-74556
- [233] Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, et al. MicroRNA cluster miR-17-92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. *Stroke*. 2017;**48**(3):747-753
- [234] Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, et al. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells*. 2013;**31**(12):2737-2746
- [235] Xin H, Wang F, Li Y, Lu QE, Cheung WL, Zhang Y, et al. Secondary release of exosomes from astrocytes contributes to the increase in neural plasticity and improvement of functional recovery after stroke in rats treated with exosomes harvested from MicroRNA 133b-overexpressing multipotent mesenchymal stromal cells. *Cell Transplantation*. 2017;**26**(2):243-257
- [236] Drommelschmidt K, Serdar M, Bendix I, Herz J, Bertling F, Prager S, et al. Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury. *Brain, Behavior, and Immunity*. 2017;**60**:220-232

- [237] Ennour-Idrissi K, Maunsell E, Diorio C. Telomere length and breast cancer prognosis: A systematic review. *Cancer Epidemiology, Biomarkers & Prevention*. 2017;**26**(1):3-10
- [238] Sandhir R, Gregory E, Berman NE. Differential response of miRNA-21 and its targets after traumatic brain injury in aging mice. *Neurochemistry International*. 2014;**78**:117-121
- [239] Andrews AM, Lutton EM, Merkel SF, Razmpour R, Ramirez SH. Mechanical injury induces brain endothelial-derived microvesicle release: Implications for cerebral vascular injury during traumatic brain injury. *Frontiers in Cellular Neuroscience*. 2016;**10**:43



