

UC Irvine

UC Irvine Previously Published Works

Title

Response of the cerebral vasculature following traumatic brain injury

Permalink

<https://escholarship.org/uc/item/0704x9sv>

Journal

Cerebrovascular and Brain Metabolism Reviews, 37(7)

ISSN

1040-8827

Authors

Salehi, Arjang
Zhang, John H
Obenaus, Andre

Publication Date

2017-07-01

DOI

10.1177/0271678x17701460

Peer reviewed

Response of the cerebral vasculature following traumatic brain injury

Arjang Salehi^{1,2}, John H Zhang^{3,4,5} and Andre Obenaus^{1,2,6}

Abstract

The critical role of the vasculature and its repair in neurological disease states is beginning to emerge particularly for stroke, dementia, epilepsy, Parkinson's disease, tumors and others. However, little attention has been focused on how the cerebral vasculature responds following traumatic brain injury (TBI). TBI often results in significant injury to the vasculature in the brain with subsequent cerebral hypoperfusion, ischemia, hypoxia, hemorrhage, blood–brain barrier disruption and edema. The sequelae that follow TBI result in neurological dysfunction across a host of physiological and psychological domains. Given the importance of restoring vascular function after injury, emerging research has focused on understanding the vascular response after TBI and the key cellular and molecular components of vascular repair. A more complete understanding of vascular repair mechanisms are needed and could lead to development of new vasculogenic therapies, not only for TBI but potentially vascular-related brain injuries. In this review, we delineate the vascular effects of TBI, its temporal response to injury and putative biomarkers for arterial and venous repair in TBI. We highlight several molecular pathways that may play a significant role in vascular repair after brain injury.

Keywords

Angiogenesis, brain trauma, neurovascular unit, regeneration and recovery, vasculogenesis

Received 8 September 2016; Revised 13 February 2017; Accepted 2 March 2017

Introduction

Over the last decade, traumatic brain injury (TBI) research has focused almost exclusively on neuroprotective strategies that reduce neuronal cell loss.¹ Unfortunately, this approach has failed to develop any meaningful therapeutics for clinical treatment. One less explored potential target has been the brain vasculature and its impact on TBI outcomes.^{2–4} A major consequence of TBI is direct damage to the cerebral vasculature. Hemorrhage, edema, blood flow abnormalities, and blood–brain barrier (BBB) disruption are immediate and early events that are commonly observed in patients and in animal models of TBI. These early events are then later followed by hypoperfusion, altered delivery of metabolic substrates, and hypoxic and ischemic tissue damage. The vasculature comprises several key cellular elements termed the expanded neurovascular unit (eNVU) and includes neurons, astrocytes, endothelial cells, pericytes, and smooth muscle cells. The eNVU elements work together to regulate cellular metabolism and activity through local blood flow.⁵ After TBI, secondary

injury mechanisms result in the dysregulation of the eNVU leading to subsequent cellular degeneration in severe injuries to altered function in more mild injuries.^{4,6}

Emerging research now suggests that the injured vasculature attempts to undergo repair.^{7–9} Angiogenesis

¹Cell, Molecular and Developmental Biology Program, University of California, Riverside, CA, USA

²Department of Pediatrics, Loma Linda University, Loma Linda, CA, USA

³Department of Physiology and Pharmacology Loma Linda University School of Medicine, CA, USA

⁴Department of Anesthesiology Loma Linda University School of Medicine, CA, USA

⁵Department of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA, USA

⁶Department of Pediatrics, University of California, Irvine, Irvine, CA, USA

Corresponding author:

Andre Obenaus, Department of Pediatrics, Loma Linda University, Coleman Pavilion, Room A-1120, 11175 Campus Street, Loma Linda, CA 92354, USA.

Email: aobenaus@llu.edu

and vasculogenesis are the two primary processes initiated after TBI. The multiple components of the eNVU are actively involved in this response and are regulated by a myriad of cellular and molecular injury-induced signaling factors and processes. A greater understanding of how these signaling pathways coordinate vascular repair will provide insight into new potential drug targets and open up unexplored avenues for therapeutic intervention. Elucidating these pathways will also have broad implications for a host of other brain injuries that impact the vasculature.

The initial portion of this review will describe the cerebrovascular dysfunctions that occur after TBI. The second half of this review will focus on the vascular response to TBI and important cellular and molecular elements that may be active in the vascular repair process.

Traumatic brain injury

TBI is the most common acquired brain injury worldwide with millions of individuals affected across a broad age spectrum.¹⁰ In the United States, the incidence of TBI is 1.7 million, resulting in 52,000 deaths annually.¹¹ The number of trauma-related deaths is higher in European countries.¹² Along with causing significant mortality, the short and long-term consequences associated with TBI have become more apparent and include cognitive deficits (loss of memory, loss of reasoning) and behavioral abnormalities (anxiety, depression, poor social interactions).¹³ Indeed, it is estimated that 43% of TBI survivors develop subsequent chronic disabilities for which there are no clinically effective treatments to assist in functional recovery after TBI.¹⁴

TBI is a heterogeneous condition with regard to etiology. In general, TBI has been defined as damage to the brain caused by an external impact, such as penetration by an object or blast wave.¹⁵ The primary causes of TBI are sports-related injuries, blast exposures, automobile accidents, and falls. In sports, concussions (or repeated TBIs) have gained increased public awareness and are known to increase the risk of dementia and can lead to chronic traumatic encephalopathy and other cognitive disorders in retired athletes.¹⁶ Blast TBIs are the result of blast pressure waves from explosive devices and are frequently observed in soldiers from Iraq and Afghanistan.¹⁷ Clinical studies usually classify brain injuries based on severity (mild to severe). However, in studies using animal models, the resulting injury can be sub-classified based on the form of injury (open or closed head injury), type of impact (impact or blast), location within the brain (focal or diffuse), and symptoms (sub-concussive or post-concussive).

The severity of the injury is initially scored on the Glasgow Coma Scale, a tool used to evaluate a patient's motor, verbal, and eye opening response. Advances in medical imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI), are used extensively to confirm the existence and severity of injury. TBI is grouped into three severities: mild, moderate, or severe. Mild TBI (mTBI) is the most common and results in no overt changes within the brain. Skull fractures, hemorrhage, and altered brain structure(s) are rarely observed by CT scan and standard MRI following mTBI.¹⁸ mTBI often leaves the patient with temporary symptoms; however, clinical studies along with research in animal models show progressive long-term neuropsychological disruptions.^{19,20} In contrast, moderate and severe TBIs result in visible neuroimaging changes within the brain, including hemorrhage and edema.²¹ Most survivors are left with permanent cognitive and physical impairments.²¹ Severe TBIs require immediate medical attention and often exhibit involvement in multiple brain regions. The long-term pathological consequences in these mild to severe TBI cases appears to be a disruption of the white matter tracts and systemic inflammation.²²

Neuropathology of TBI from the initial primary injury is followed by a secondary injury cascade. Primary injury is the mechanical damage that occurs at the time of injury and results in shearing of neurons, glia, and blood vessels. Primary injury is untreatable and only preventable by limiting exposure to the inciting event and taking safety precautions. Secondary injury, on the other hand, occurs over time and initiates a variety of signaling cascades that alter cell function and/or cause cell death. Some common events include oxidative stress, excitotoxicity, mitochondrial dysfunction, and inflammation.²³ These secondary events can last hours to years after the injury and can contribute to functional deficits and long-lasting disabilities.²⁴ Thus, preventing or mitigating the effects of secondary injury is potentially critical to improving the lives of TBI victims.

Vascular consequences of TBI

Extensive research has been conducted to identify potential therapeutic targets for TBI, but none have successfully been translated to the clinic. One major consequence of TBI that has not been extensively investigated is damage to the cerebral vasculature. The disrupted vasculature participates in the pathogenesis of TBI and can lead to secondary injury in the form of hemorrhage, edema, changes in blood flow, and BBB disruption. A better understanding of how these vascular effects after TBI result in chronic disabilities could lead to the development of improved therapeutics.

Here, we will review different types of cerebrovascular complications that occur after TBI and their impact on brain pathology.

Hemorrhage

Hemorrhage occurs in 46% of all TBIs and is increasingly prevalent in moderate and severe injuries.²⁵ An insult to the brain results in bleeding that can gradually progress over the first 24–48 h. In some cases, the leaking blood collects outside the vessel forming a hematoma. Three distinct types of hemorrhage are reported after TBI and are based on their location in the brain: epidural hematomas (between the dura mater and skull), subdural hematomas (between dura and arachnoid mater), and subarachnoid hemorrhage (between the arachnoid and pia mater). Of the three types, post-traumatic subarachnoid hemorrhage is the most severe and is associated with increased mortality and poor outcomes.²⁶ The effects of hemorrhage result in increased intracranial hypertension, vasospasms, and compression of structures in the brain.²⁷ However, this depends on the amount and degree of bleeding into the parenchyma. While most hemorrhage resolves on its own, microbleeds can still be present causing deposition of hemosiderin that is detectable by MRI.²⁸ Accumulation of ferritin/hemosiderin is known to be toxic to brain cells and can elicit an inflammatory response.²⁹ Interestingly, preclinical studies demonstrated that delayed microbleeds following TBI are associated with white matter damage.^{30,31}

Edema

Cerebral edema, the excess accumulation of fluid within the brain, accounts for 50% of deaths in severe head injuries.³² Pediatric patients are at greater risk of developing edema than adults.³³ After TBI, cerebral edema forms at the lesion and incorporates into the surrounding tissue. Two distinct types have been observed, cytotoxic and vasogenic edema. Cytotoxic edema results in water accumulation in cells and is caused by dysregulation of the sodium and potassium pumps on the cell membrane.³⁴ The retention of water and ions can adversely affect cell function and even lead to apoptosis. The BBB remains intact in cytotoxic edema.³⁴ Vasogenic edema results in water accumulation in the extracellular space and is caused by the disruption of the BBB.³⁴ Vasogenic edema is associated with elevated intracranial pressure, tissue swelling, changes in blood flow, and compression of brain structures.³⁵ Although it has been accepted that cytotoxic edema occurs immediately after injury and leads to vasogenic edema, recent studies have expanded on

this concept and proposed two transitional forms of edema that occur after stroke.^{6,34} In this model, anoxic and ionic edema results in dysregulation of the components of eNVU which leads to increased vascular permeability and development of vasogenic edema.³⁴

Changes in cerebral blood flow

Altered cerebral blood flow (CBF) is one of the most common outcomes of TBI. Both regional and global changes in blood flow are observed but depend on the size, location, and type of tissue injury (i.e. contusion, hematoma, or diffuse edema).³⁶ A majority of TBI patients have significant reductions in CBF within the first 12 h after injury,^{36,37} but this is dependent on the severity of the injury. It has been reported that CBF values can reach ischemic levels falling to 18–20 ml/100 ml/min after severe TBI (normal values in humans are ~45–50 ml/100 ml/min).^{36,38} After this initial period, many patients experience hyperemia (increase in CBF values compared to normal tissue) while others have shown low or normal CBF values.^{39,40} Interestingly, at later times after injury, there is an apparent uncoupling between blood flow and tissue metabolism, with long-term outcomes being vasospasm and hypoperfusion.³⁹ These decrements in CBF can result in ischemic brain injury, which is the leading cause of death after severe TBI.³⁹ Although CBF typically normalizes within several weeks, therapeutic procedures to enhance blood flow acutely are needed and could have significant clinical benefits for TBI patients.⁴¹

Vasospasms

Vasospasms, or the abnormal constriction of blood vessels, can adversely affect functional recovery after TBI. The reported incidence of vasospasms after head injury ranges from 19 to 68%.⁴² While most vasospasms are short-lived, severe episodes can last longer but typically resolve by 14 days in closed head injuries.⁴³ Blast TBI victims have been reported to have a higher frequency of developing vasospasms than other forms of head injuries and can last up to 30 days after injury.⁴⁴ The cause of vasospasms after TBI remains undetermined, but others have suggested that it triggered by hypertension and subarachnoid hemorrhage.⁴⁵ A study by Dore-Duffy indicated that pericytes release endothelin-1, a potent vasoconstrictor molecule, near the blood vessels after TBI.⁴⁶ Vasospasms can reduce cerebral perfusion to regions of the brain, which could lead to ischemic injury and infarction if untreated.⁴⁵ Clinicians undertake extensive efforts to manage vasospasms early in order to avoid long-term injuries.

BBB disruption

BBB disruption following TBI has been well-established. The most common technique to assess BBB disruption in humans is by cerebrospinal fluid/serum albumin quotient, which measures albumin in cerebral spinal fluid in reference to blood serum. Ho et al.⁴⁷ in a retrospective cohort study of patients with severe non-penetrating TBI found that 44% of patients experience BBB disruption which was associated with poor long-term outcome. BBB disruption typically returns to normal by several weeks, but in some patients, it has been reported to last months and years after mild injuries.⁴⁸ The BBB regulates the movement of substrates between the blood and central nervous system (CNS) and is critical in maintaining cellular homeostasis. This selective barrier is composed of tightly connected endothelial cells together with astrocytes, neurons, and pericytes (forming the eNVU). The endothelial tight junction proteins that seal the BBB become disrupted after TBI, either by the contusion and/or increased intracranial pressure.⁴⁹ Neutrophils also exacerbate BBB disruption by secretion of proteases and cytokines.⁵⁰ The disruption of BBB leads to the release of harmful molecules into the local injury microenvironment such as iron, reactive oxygen species, and blood toxins.⁵¹ Such molecules can augment inflammation and increase the risk of edema and hypertension.⁴⁷

Coagulopathy

Altered coagulation profiles after TBI have been reported in numerous studies.^{52–54} Harhangi et al.⁵² in a systematic review of 82 studies confirmed that coagulopathies were present in TBI patients with a prevalence of 33% and that there was a significant correlation between mortality and poor outcomes. One key finding of most studies is that the severity of the TBI is related to the degree of coagulopathy which is in turn related to outcomes and mortality. Further, the time course of the coagulation abnormalities is also dependent upon the severity of injury.⁵⁵ The coagulation cascade appears to be dramatically altered after moderate and severe TBIs, initiated in large part by the release of tissue factor (TF) into the circulation leading to depletion of coagulation factors (extrinsic pathway), decreased platelets, altered BBB and leading to increases in circulating microparticles.^{56–58} Hyperfibrinolysis, with increases in d-Dimer, has also been proposed to be a marker of outcomes via a potential cascade, wherein TF can increase activation of the extrinsic pathway.⁵⁹ Alternatively, low platelet levels may also contribute to increased hemorrhagic evolution particularly early after hospitalization.⁶⁰ The hemostasis mechanisms that can be impacted by TBI are clearly varied but ultimately lead to inflammation, hyperfibrinolysis, endothelial dysfunction, and platelet

abnormalities. Thus, it is clear that early coagulation mechanisms play a significant role in TBI outcomes but more research is warranted on improved biomarkers and therapeutic interventions.^{61,62}

Chronic inflammation

While the inflammatory response is initiated minutes after injury and protects against pathogens, eliminates necrotic cells, and promotes tissue repair, it has become apparent that chronic inflammation is associated with CNS damage.^{63,64} Microglia cells, the resident macrophages in the brain, have drawn much attention. Microglia are rapidly activated in response to injury and work to limit the extent of tissue damage.⁶³ However, persistent microglial activation is a key contributor to secondary brain injury. Several studies have shown that microglia can become activated for weeks to years after brain injury.^{65–67} Assessment of postmortem tissue of TBI patients who died months after the injury revealed increased activation of microglia in the white matter tracts.⁶⁷ Microglia can become over-active in response to danger-associated molecular pattern factors that are released by damaged cells.⁶⁸ As a result, over-active microglia produce high levels of inflammatory cytokines and cytotoxic factors that cause further neurodegeneration.⁶⁹ Additionally, the sustained release of these factors disrupts the BBB, resulting in the transit of inflammatory factors and peripheral immune cells into the brain thereby further increasing the inflammatory response.⁵¹

Cerebral vasculature injury: Repair OR pathology?

While the effects of vascular injury in acquired neurological injury, such as stroke, have been extensively evaluated, our understanding of mechanisms underlying repair is poorly understood. Several studies have suggested that the injured vasculature appears to initiate repair in the course of two to three weeks.^{9,70} Using a novel vessel painting technique to document the acute changes to the cerebral blood vessels after TBI, we observed dramatic alterations in vascular parameters not only at the injury site but also in the contralateral brain in a rodent model of TBI.⁷¹ Extending these early studies, we observed that moderate TBI which results in gross vascular injury is subsequently followed by repair over the course of two weeks (unpublished results, Figure 1). Smaller vessels, such as capillaries appear to recover over a similar time frame. Morgan et al.⁷ using a rodent acceleration impact model observed premature capillary structures at two days post injury. Lateral fluid percussion injury resulted in an early loss of capillaries which slowly increased

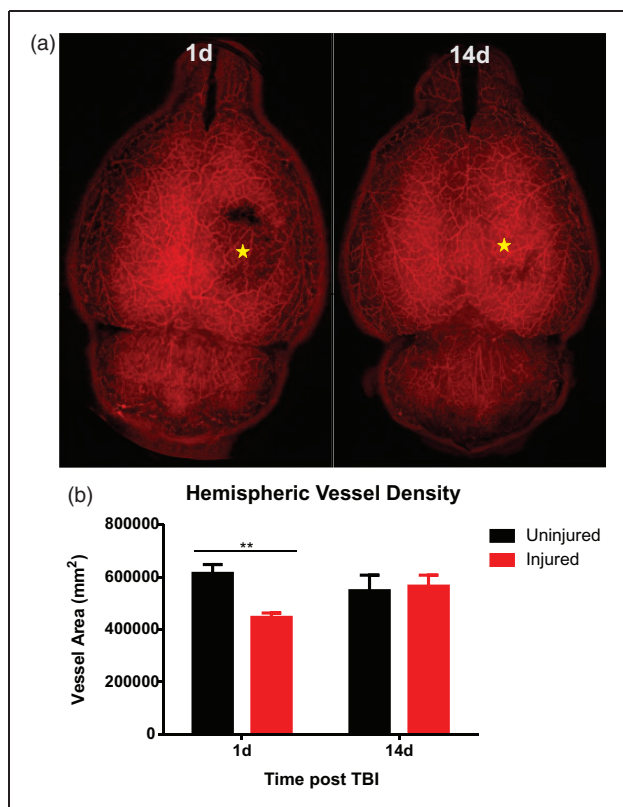


Figure 1. TBI results in vascular injury with subsequent repair. (a) We induced a moderate controlled cortical impact (CCI) in the right hemisphere of adult rats ($n = 5-6/\text{group}$). Animals underwent vessel painting to stain the cerebral vasculature at 1 and 14 days after injury. Our vessel painting technique is performed by injecting Dil (lipophilic 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine Perchlorate) solution into the left ventricle followed by cardiac perfusion and fixation. Whole brain axial images were obtained using wide-field fluorescent microscopy (representative images are shown). Moderate CCI elicits a loss of vasculature that extends beyond the injury site. Over the ensuing 14 days, there is increased vascular density at the injury site. Injury site (star). (b) We performed a classical vascular analysis to quantify the features of labeled vessels in the injured and uninjured hemisphere. Vascular analysis of the injured hemisphere revealed a reduction in vessel density at 1 day compared to the uninjured hemisphere (t -test, $**p < 0.01$). Vessel density was restored and returned to baseline levels by 14 days (t -test, $p = 0.82$). All error bars are presented as standard error of mean.

and reached baseline levels by 14 days.⁷² This study also revealed widespread hypoperfusion throughout the injured brain, with CBF values showing no correlation with vessel density at 14 days. Interestingly despite restoration of capillary structure, hypoperfusion was still present, suggesting that structural repair does not implicitly result in improved function. Similarly, Jia et al.⁷³ using an optical micro-angiography technique found that a penetrating brain injury results in a sudden

loss of blood flow at the lesion site and is gradually reestablished by two to three weeks.⁷³ Therefore, there is evidence that indicates that vascular impairments are immediate but repair, albeit delayed, does occur after TBI.

It is clear that the repair process is influenced by the severity of the injury. Park et al.⁹ explored vascular loss and recovery using a fluid percussion injury model. Their study utilized two injury severities, a moderate (2 atm) and a severe (3 atm) injury and evaluated the vasculature at 1 and 14 days post injury. Both severities resulted in significant vascular injury at one day post injury compared with sham animals. Only moderately injured animals showed improvements by 14 days after injury, where microvascular density in the injured cortex recovered to near-sham levels. The repaired vasculature of severely injured animals was abnormal, with an increased number of irregular and tortuous vessels. These animals also had a significant reduction in microvascular density. Their study highlights that vessel repair can be abnormal and may contribute to the pathological outcomes after brain injuries. It remains to be seen how vascular repair is affected following mTBI.

Other studies have noted significant alterations to the newly formed blood vessels. While morphological changes to the cerebral vessels are apparent early after TBI,^{74,75} it appears that these deficits do not fully recover and can persist for weeks and months after the initial injury. Electron microscopy showed aberrations to the vessel walls, ridged elastic lamina, swelled and apoptotic endothelial cells, and degraded extracellular matrix.⁷⁶⁻⁷⁸ Both large and small vessels appear to be affected. In a fluid percussion injury model, injured rats had fragmented capillaries and reduced capillary diameter at 30 days post injury.⁷⁰ Gama Sosa et al.⁷⁹ utilized a blast model of mTBI and observed a unique vascular pathology that led to chronic changes in the vessels six months after injury. These malformed vessels likely increase the risk of delayed hemorrhage, hypoperfusion, and other secondary effects.^{31,80} Also, an outcome of poor vascular repair when coupled with cerebrovascular dysfunction can lead to further exacerbation of tissue injury. Thus, damaged and abnormal repair of the vasculature may be an important contributing factor to poor outcomes often observed after TBI in humans and in rodent models.

New vessel formation following injury

The formation of new blood vessels (or neovascularization) after acquired brain injury has increasingly gained interest. It has been well-documented that after TBI, two primary mechanisms, angiogenesis and vasculogenesis occur (Figure 2). While both angiogenesis and

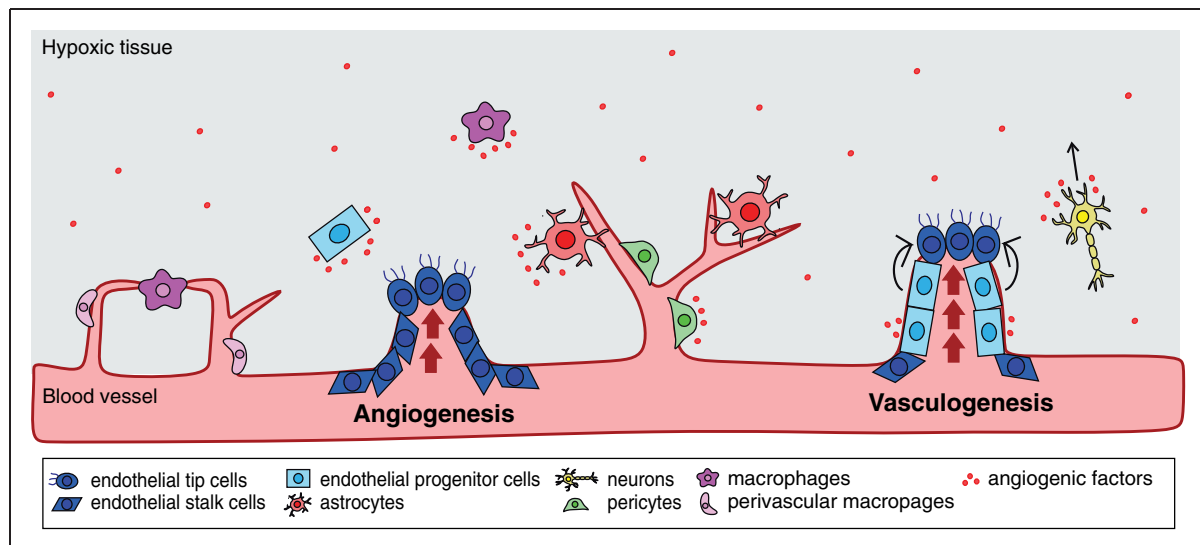


Figure 2. New vessel formation after TBI. Brain trauma leads to damage to the cerebral vasculature with subsequent repair via angiogenic and vasculogenic processes. Mature endothelial cells, in particular endothelial tip and stalk cells, are involved in various steps of angiogenesis. Tip cells (blue oval shape) are found at the end of the vessel sprout and they use their filipodia to guide the sprout toward the angiogenic growth factor source, while stalk cells (blue trapezoid shape) are found inside the vessel lumen and they proliferate to extend the sprout. Circulating endothelial progenitor cells (light blue rectangular shape) play a key role in postnatal vasculogenesis but also contribute to angiogenesis. Endothelial progenitor cells are recruited from the bone marrow that then incorporate into the damaged vessels and differentiate into endothelial cells. Endothelial progenitor cells also are important for the release of angiogenic factors, which regulate angiogenesis. Other cell types support or augment angiogenesis and vasculogenesis. Astrocytes (red), pericytes (green), and perivascular macrophages (light purple globular shape) cooperate with the newly formed vessel and promote vessel stabilization. Neuronal cells (yellow) are associated with the blood vessels and release angiogenic factors that may guide the vessel sprout toward the injury site. Macrophages (dark purple star shape) have been shown to adhere and ligate ruptured vessels in the brain. Additionally, a majority of these cells release angiogenic growth factors into the hypoxic tissue.

vasculogenesis are active during embryonic development and lead to the formation of a primitive vascular network, they also play a role in promoting vascular repair in the adult after injury. These complex but distinct processes play an important role in repairing the injured vasculature after TBI.

Angiogenesis

Angiogenesis is the formation of new blood vessels from an existing vasculature. New vessels form by sprouting from the main vessels (sprouting angiogenesis) or splitting in two daughter vessels (intussuscepted angiogenesis). Sprouting angiogenesis is commonly observed after injury and is mediated by resident endothelial cells (ECs), which line the luminal surface of the blood vessels forming the endothelium.⁸¹ As previously mentioned, new vessel sprouts are detected at a relatively short time after injury with many studies reporting increased capillary density after TBI,^{7,9,72,82} where new capillaries appear to migrate from the perilesional tissue into the injury site (unpublished results).

Tissue hypoxia, or low tissue oxygenation, is one of the primary triggers of angiogenesis. Clinical studies have reported the presence of hypoxia after TBI,

which is typically caused by cerebral hypoperfusion and ensuing ischemia.^{37,83} Experimental TBI animal models revealed an up-regulation of hypoxia-driven molecules in the injured brain. One key reporter molecule is hypoxia-inducible factor-1 (HIF-1 α),^{9,84} which has been associated with vascular repair in stroke and other disease models. Low oxygen inhibits the degradation of HIF-1 α where it accumulates and binds to hypoxia-inducible factor-1 β . This transcription factor promotes expression of a number of angiogenic growth factors.⁸⁵ These factors constitute a class of signaling molecules that are involved in vascular repair. For example, vascular endothelial growth factor (VEGF) exhibits robust expression in the latter stages of experimental and clinical TBI.^{86,87} Other factors are also up-regulated and contribute to vascular repair (a more thorough discussion of angiogenic factors will be described later in this review).

Sprouting angiogenesis is an important repair process that requires the coordinated interactions of ECs. The endothelium is activated in response to brain trauma.⁸⁸ The hypoxic tissue causes release of angiogenic growth factors into the nearby tissue.⁸⁵ These factors signal to the nearby vessels to detach the pericytes and degrade the local extracellular

matrix.⁸⁵ ECs form vessel sprouts and then migrate and proliferate in the direction of the hypoxic tissue.⁸⁹ To facilitate this process, ECs specialize into distinct phenotypes known as endothelial tip and stalk cells: tip cells migrate and lead the vessel sprout, while neighboring stalk cells proliferate and form the vessel lumen.⁸¹ Initially, an immature and unstable vascular structure is formed. Following recruitment of the pericytes, this immature vessel is remodeled to form a stable, functioning vessel.⁸⁵

Vasculogenesis

Vasculogenesis is the de novo formation of new blood vessels. The term vasculogenesis has been used in various injury models and refers to a subset of bone marrow-derived cells with vasculogenic potential. Increasing evidence suggests that endothelial progenitor cells (EPCs) play an important role in postnatal vasculogenesis.⁹⁰ These CD34+ cells originate from the bone marrow and circulate through the vasculature in response to injury. Many TBI studies have detected increased numbers of EPCs in the blood as well as blood vessels at the injury site.^{91–93}

The initiator(s) of vasculogenesis has been a subject of debate, but many speculate that it is triggered in a hypoxic-dependent fashion. A prime example where this is known to occur is in tumor biology, in which many tumors create a hypoxic environment that leads to vascularization. In hypoxic tumors, HIF-1 α mobilizes EPCs to induce vascular growth.⁹⁴ Stromal-derived factor-1 α (SDF-1 α), a chemo-attractant factor that is regulated by HIF-1 α , helps recruit EPCs to the tumor site.⁹⁵ Similar to tumors, tissue hypoxia is evident at the TBI lesion site and leads to the expression of angiogenic growth factors including SDF-1 α .⁸⁵ These factors enter the blood circulation and signal to the bone marrow to mobilize EPCs. Serum VEGF and angiopoietin-1 (Ang-1) levels show a significant correlation with circulating EPCs in patients with severe TBIs suggesting that these growth factors may mediate recruitment of EPCs into the blood stream.⁹¹ Thus, hypoxia is likely the driving force for vasculogenesis after moderate and severe TBI.

Unlike angiogenesis, vasculogenesis is initiated in the bone marrow. EPCs remain adhered to the marrow stromal cells under physiological conditions. After injury, EPCs become activated where they are released from the stromal cells and enter the blood stream.⁹⁴ In TBI patients, EPC levels decrease at one to two days post injury and rapidly increase and peak by five to seven days after injury.^{91,92,96} Within the blood, EPCs mature and acquire similar phenotypes as ECs.⁹⁷ EPCs are known to home to sites of vascular injury.⁹⁴ Guo et al.⁹³ detected CD34+ EPCs around the lesion at one day after TBI which peaked at seven days post injury.

The levels of EPCs correlated with angiogenesis after TBI. The function of EPCs has been much debated, but many studies suggest that EPCs play a dual structural and instructive role in vasculogenesis. EPCs can incorporate into the injured vessels and differentiate into new ECs.^{7,93} Additionally, others have shown that EPCs act as supporting cells that release trophic factors to the injured vessels.^{98,99} These secreted factors induce angiogenesis by promoting proliferation and migration of ECs.⁹⁹

Supportive role of the eNVU

Various cells types participate in angiogenesis and vasculogenesis after injury. Although EPCs and ECs are considered the two primary players in these repair processes, the other components of the eNVU, such as astrocytes, pericytes, and neurons, also possess angiogenic functions and have been largely overlooked in TBI repair. Additionally, macrophages and perivascular macrophages are thought to play a significant role in the vascular response to injury (Table 1).

Astrocytes

Astrocyte activation after injury has been well described and are known to accumulate around a lesion, in a process known as astrogliosis. These cells perform numerous functions, some of which are related to vascular repair. Reactive astrocytes are known to secrete trophic factors that promote angiogenesis and BBB repair. Astrocytes are an important source of angiogenic

Table 1. Active cell types in vascular repair.

Cells	Functions	References
Endothelial cells	Proliferation and migration to form new vessels	81a
Endothelial progenitor cells	Mobilization from the bone marrow, homing to sites of vascular injury, and in situ differentiation/trophic support	91–93,96
Astrocytes	Trophic support, vessel stabilization	104,106,129
Pericytes	Triggering angiogenesis, trophic support, vessel stabilization	109,112a
Neurons	Trophic support, possible role in vessel guidance	115,116a
Macrophages	Trophic support, vessel adhesion and ligation	82,124
Perivascular macrophages	Vessel stabilization	126

^aPapers covers general aspects of vascular repair.

growth factors after TBI, including VEGF and SDF-1 α ^{82,100} High mobility group box 1 (HMGB-1), a danger-associated molecular pattern molecule, has been suggested to have beneficial effects in the chronic stages after brain injury. HMGB-1 mediates crosstalk between astrocytes and ECs and EPCs after injury and can lead to neurovascular remodeling.^{101–103} Additionally, astrocytes can produce tenascin, thrombospondin, and other proteins found in the extracellular matrix.¹⁰⁴

Astrocytes also participate in glial scarring. Although much of the focus has been placed on its inhibition related to axonal regeneration after injury,¹⁰⁵ glial scarring may play a beneficial role in vascular repair. During scarring, reactive astrocytes have close interactions with peri-lesional vessels.¹⁰⁶ These intimate interactions with blood vessels are evident at seven to sixty days post injury, which coincides with the formation of the new vasculature. Astrocytes are integral components of the BBB and are essential for normal BBB function.¹⁰⁷ Thus, these astrocyte-vessel interactions may function in stabilizing the blood vessels and restoring blood flow to the injured tissue.

Pericytes

Accumulating evidence suggests that pericytes are important players in vascular repair. Pericytes, or mural cells, cover the surface of the micro-vessels where they reside within the basement membrane in direct contact with the endothelium.¹⁰⁸ The pericyte population declines in the first few days after controlled cortical impact (CCI).¹⁰⁹ Additionally, a majority of pericytes are observed leaving the blood vessels after weight drop impact. Their detachment from the vessels facilitates a critical step for angiogenesis,⁴⁶ as pericytes are required to destabilize and detach from the vessel wall which allows ECs to form vessel sprouts. A number of angiogenic factors are responsible for their detachment from the vessels.¹¹⁰

At later stages of injury, pericytes become reactive and dramatically increase around the lesion, in a process known as pericytosis. When active, pericytes secrete angiogenic growth factors that mediate EC activity and vascular integrity.¹⁰⁸ Ang-1 is one such factor and has been shown to induce expression of the tight junction protein occludin.¹¹¹ Along with providing trophic support, active pericytes make close interactions with newly formed vessels.¹¹² Pericyte recruitment is critical for the latter stages of angiogenesis and contributes to stabilizing the new vessel.¹¹²

Neurons

While neurons are essential for functional recovery after injury, there is some evidence that they may also

participate in vascular repair. Following brain injury, increased neurogenesis is observed in the sub-ventricular zone,¹¹³ in which new neurons are localized to the peri-lesional vessels.¹¹⁴ Administration of neural progenitor cells after TBI results in a transient increase in angiogenesis adjacent to the lesion border.¹¹⁵ Neural stem and progenitor cells are known to secrete trophic factors into the injury microenvironment, including a number of angiogenic growth factors.¹¹⁶ Interestingly, however, one study showed that neurons limit angiogenesis by binding and sequestering angiogenic factors in the retina.¹¹⁷ Although this work was specific to retinal neurons, it is plausible that cortical neurons act in a similar manner and may guide angiogenic branching toward the injury site. Thus, this raises the possibility that neuronal cells may be important in regulating post-injury angiogenesis.

Macrophages

Macrophages, a monocyte-derived cell that is part of the inflammatory response, have been implicated in vascular repair. Activated macrophages are grouped into two forms: classically activated, anti-angiogenic macrophages (M1) or alternatively activated, pro-angiogenic macrophages (M2 including the M2a and M2c subsets).¹¹⁸ However, primary human M1 macrophages have been shown to secrete pro-angiogenic molecules *in vitro*.¹¹⁹ After CCI, macrophages are broadly activated adopting both an M1 and M2 phenotype,^{118,120} but appear to be a shift to M1 phenotype at later time points.¹²¹ Macrophages can express angiogenic growth factors and matrix remodeling proteases after TBI, including VEGF, FGF, and matrix metalloproteinase-9 (MMP-9).^{82,122} In fact, they contribute significantly to late increases in VEGF levels.⁸² Additionally, macrophages may mediate vascular pruning through secretion of pro-apoptotic factors.¹²³ Along with providing trophic support, these cells physically interact with the sprouting vessels. Liu et al.¹²⁴ demonstrated that macrophages promote repair of cerebrovascular ruptures in a zebrafish model. Macrophages were seen adhering to the ends of the ECs and ligating the ruptured vessels. Also, during development, macrophages mediate fusion between endothelial tip cells leading to vessel growth and anastomoses.¹²⁵

Perivascular macrophages

Unlike peripheral macrophages that infiltrate the brain parenchyma, perivascular macrophages reside in the perivascular space and are closely associated with vessel walls.¹²⁶ After weight drop injury, there was a significant increase in the number of CD163+

perivascular macrophages between two and four days post injury, which was mainly localized to the lesion site.¹²⁷ The extent to which perivascular macrophages mediate vascular repair after TBI is presently unknown, but are likely to have similar functions as peripheral macrophages. Perivascular M2 macrophages have been implicated in tumor angiogenesis and depletion of these cells attenuates vascularization and tumor growth.¹²⁸ Additionally, perivascular macrophages are critical in maintaining vascular integrity under physiological conditions.¹²⁶ M2 macrophages, but not M1 macrophages, have a significant impact on barrier integrity which is likely due to strong interactions with the endothelium.¹²⁶ Thus, perivascular macrophages may function in stabilizing the new vessels after trauma.

Injury-induced signaling factors involved in vascular repair

Numerous signaling molecules, including growth factors, cytokines, and chemokines, are up-regulated after TBI and have well-defined functions. Here, we review several important extracellular factors that are known to directly affect the vasculature and discuss their respective roles in the vascular repair process (Table 2). We propose a molecular mechanism in which these signaling factors interact and lead to vascular repair (Figure 3). Other factors, such as pro-inflammatory cytokines, may indirectly affect vascular repair and will not be described here but have been reviewed extensively previously.¹²⁹

VEGF

VEGF is a key regulator of angiogenesis and modulating its levels has a significant effect on vessel formation.⁸⁶ Krum et al.⁸⁷ utilized a specific neutralizing antibody to inhibit endogenous VEGF and tested its effect on tissue repair after brain infusion injury.

Neutralizing VEGF levels resulted in a significant reduction in vessel proliferation and an increase in vessel degeneration. Administration of VEGF has emerged as a potential treatment for vascular injuries. Several studies have shown that VEGF treatment enhances angiogenesis in TBI,^{130,131} ischemia,^{132,133} and stroke.¹³⁴ VEGF signaling is mediated through Flt-1 (VEGF-R1) and Flk-1 (VEGF-R2) that are expressed on ECs.¹³⁵ Flk-1 is the predominate receptor and is associated with angiogenesis.¹³⁵ Binding of VEGF to Flk-1 (and neuropilin-1 co-receptor) induces down-stream signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphoinositide 3' kinase (PI3K).¹³⁶ Together these pathways promote EC proliferation, migration, and survival, which are indispensable for angiogenesis.¹³⁶ These effects are prevalent in the chronic stages of TBI, where VEGF levels are increased around the injury site.⁸²

VEGF has been shown to increase EPC expression and induce vessel formation after hind limb and myocardial ischemia as well as physiological conditions, confirming its role in postnatal vasculogenesis.⁹⁰ As we have stated earlier in this review, much of the work on VEGF-mediated vasculogenesis was performed in tumors and its role in TBI is limited to correlative studies in patients.^{91,137} VEGF regulates EPC activity in a similar manner as ECs. In EPCs, VEGF binds to Flk-1 and leads to their mobilization and release from the bone marrow and induces differentiation.⁹⁴ The effects are mediated by the PI3K pathway which leads to expression of nitric oxide (NO; a potent vasodilator) and MMP-9.¹³⁸

Fibroblast growth factor 2

Similar to VEGF, fibroblast growth factor 2 (FGF2) is a potent mitogen and stimulator of angiogenesis. Given its angiogenic effects, FGF2 administration has been utilized in several brain injury models. FGF2 treatment after closed head injury has been shown to increase

Table 2. Role of extracellular factors in modulating vascular response.

Factors	Functions	References
VEGF	Promotes angiogenesis and vasculogenesis	87,91,130,131,205,206
FGF2	Promotes angiogenesis and maintains BBB integrity	131,141,207
SDF-1 α	Attractant for ECs, EPCs, and other vascular cells	143,144,208,209
MMP-9	Extracellular matrix degradation and expression of cell activating cytokines	151,152
Ang-1	Maintains and stabilizes blood vessels; associated with vasculogenesis	91,156
EPO	Contributes to angiogenesis and vasculogenesis	159,164,210
vWF	Controls hemostasis and inhibits angiogenesis	170,171

VEGF: vascular endothelial growth factor; FGF2: fibroblast growth factor 2; SDF-1 α : stromal-derived factor-1 α ; MMP-9: matrix metalloproteinase-9; ANG-1: angiopoietin-1; EPO: erythropoietin; vWF: von Willebrand factor

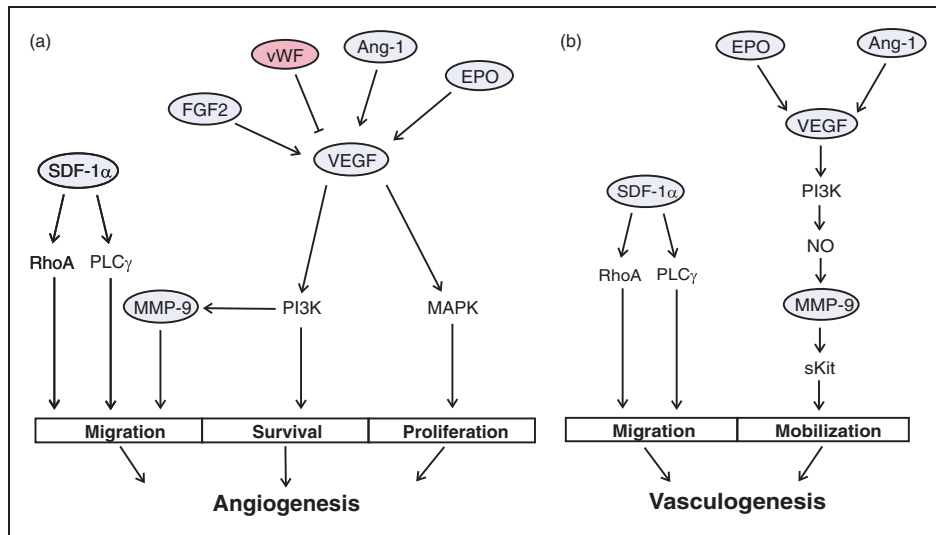


Figure 3. Possible interactions between signaling molecules. (a) The interplay between these angiogenic factors is critical in understanding the molecular mechanism(s) of vascular repair. After trauma, active cells release angiogenic factors into the injured tissue. VEGF is the key inducer of angiogenesis, which activates PI3K and MAPK pathway leading to EC survival and proliferation. FGF2, Ang-1, and EPO are also up-regulated at distinct times after TBI and these factors signal through VEGF signaling, either by increasing expression of VEGF and/or initiating down-stream PI3K and MAPK pathways. Along with pro-angiogenic factors, anti-angiogenic factors, including vWF, have been shown to inhibit angiogenesis. Migratory processes are controlled by SDF-1 α , which activates down-stream PLC γ and RhoA pathway. MMP-9, a target of the PI3K pathway, is pivotal for EC migration. (b) Angiogenic factors are released into the blood stream which can activate cells from bone marrow. Serum VEGF, EPO, and Ang-1 are elevated after TBI and have been associated with postnatal vasculogenesis. Similar to angiogenesis, vasculogenesis is mediated by the VEGF signaling pathway. VEGF causes activation of the PI3K pathway and leads to the up-regulation of NO and MMP-9. MMP-9 converts the Kit ligand to its active, soluble form (sKit). sKit is responsible for mobilizing EPCs from the bone marrow. Activated EPCs then migrate to sites of injury in a process known as chemotaxis. SDF-1 α activates down-stream PLC γ and RhoA pathway to induce EPC migration.

vessel density compared with non-treated animals.¹³¹ However, animals treated with VEGF alone showed higher vessel growth than FGF2. ECs express both FGF receptors (FGFR-1 and FGFR-2) and are sensitive to the FGF family. Binding of FGF2 to its receptors leads to activation of proliferative, migratory, and survival processes.¹³⁹ MAPK, PI3K, and Phospholipase C gamma (PLC γ) pathways have been linked with these important angiogenic processes.¹³⁹ Alternatively, Kano et al.¹⁴⁰ have proposed a mechanism involving FGF2 induction of VEGF with FGF2 signaling acting in conjunction with VEGF signaling to promote angiogenesis.

FGF2 is critical in maintaining vascular integrity. Wang et al.¹⁴¹ showed that FGF2 treatment after CCI preserved BBB integrity and improved neurological function. The authors found that FGF2 activates the PI3K pathway and leads to up-regulation of tight junction proteins (claudin-5 and occludin) and inhibits RhoA, an antagonist of BBB formation. Rac-1, a down-stream effector of the PI3K, is responsible for FGF-2 protective effects of the BBB.¹⁴¹ Similar findings were observed in a model of intracerebral hemorrhage, wherein FGF2 treatment reduced BBB damage, cerebral edema, and improved outcomes.¹⁴²

Stromal cell-derived factor 1 alpha

Stromal cell-derived factor 1 alpha (SDF-1 α) is an important chemokine for various cell types. Recent studies found that SDF-1 α attracts CD34-expressing cells from the bone marrow, which include EPCs and ECs. Inhibition of endogenous SDF-1 α after TBI reduces the number of CD34+ cells, which coincides with decreased vessel density around the injury site.¹⁴³ Further evidence suggests that exogenous SDF-1 α has angiogenic benefits. Administration of SDF-1 α after fluid percussion and weight drop injury enhances vessel formation, reduces BBB damage, and improves neurological outcomes.^{143,144} The most extensively studied SDF-1 α receptor is CXCR4, which is expressed on EPCs, ECs, and smooth muscle cells.¹⁴⁵ Following injury, neural stem/progenitor cells and other damaged cells release SDF-1 α into the injury microenvironment. ECs are known to up-regulate SDF-1 α expression when exposed to hypoxia.¹⁴⁵ SDF-1 α binding to CXCR4 activates PLC γ and Rho pathways to promote a chemotactic response.¹⁴⁶ As a result, CD34-expressing cells are activated and migrate toward the SDF-1 α gradient.¹⁴⁷ Along with recruiting cells, SDF-1 α has been

shown to directly regulate EC and EPC function via the PI3K and MAPK pathway.¹⁴⁶

MMP-9

MMP-9 is a protease enzyme that is necessary for initiating angiogenesis.¹⁴⁸ This enzyme is expressed by ECs and functions by degrading the extracellular matrix, which provides for sufficient space for EC migration and the formation of new vessel lumens.¹⁴⁹ Some matrix proteins that are degraded by MMP-9 include collagen, laminin, thrombospondin, and fibronectin.¹⁵⁰ Additionally, it is critical in the processing and activation of growth factors thereby affecting the levels of pro- and anti-angiogenic factors in the tissue.¹⁵⁰ For example, MMP-9 can cleave and release activated VEGF in tumors cells.¹⁵⁰

MMP-9 also mediates vasculogenesis by affecting EPC activity. In animal models of TBI, MMP-9 levels increase and localize to EPCs and cortical vessels around the lesion.¹⁵¹ Serum MMP-9 levels correlate with increases in EPC levels in clinical TBI.¹³⁷ Huang et al.¹⁵² showed that MMP-9-deficient mice have reduced vascularization after ischemic injury resulting from reduced EPC expression and stem cell activating cytokines such as Kit ligand. These cytokines promote activation of stem cells into the bone marrow and have been implicated in hematopoiesis. EPCs are normally quiescent and remain attached to the stromal cells of bone marrow mediated by membrane bound Kit ligand (inactive form).¹⁵³ After injury, bone marrow cells activate the PI3K pathway to increase production of NO which in turn up-regulates MMP-9.¹⁵⁴ MMP-9 converts the inactive Kit ligand to its active soluble form, sKit.¹⁵³ Consequently, sKitL disrupts EPC adhesion to the bone marrow allowing for their mobilization and release into the blood circulation.

Ang-1

Ang-1 promotes vessel stabilization in the latter stages of vascular repair. Because of its angiogenic effects, delivery of exogenous Ang-1 may have significant benefits if administered early. Pharmaceutical compounds, such as simvastatin, can increase Ang-1 expression and are known to improve vascular function in stroke models.¹⁵⁵ Under physiological conditions, cerebral vessels express high levels of Ang-1. After cryogenic injury, there is a marked decrease in Ang-1 in the acute stages.¹⁵⁶ This is accompanied by increases in Ang-2, which is an Ang-1 antagonist and is associated with BBB breakdown. At two to six days post injury, Ang-1 levels slowly increase which correlates with

angiogenesis.¹⁵⁶ Ang-1 is primarily produced by pericytes and directly interacts with ECs.¹⁵⁷ Both Ang-1 and Ang-2 signal through the Tie-2 receptor, with Ang-1 acting as an agonist and Ang-2 as an antagonist.¹⁵⁷ The balance between Ang-1 and Ang-2 determines its signaling. Ang-1/Tie-2 signaling promotes EC proliferation, migration, and survival, in part through activation of the VEGF pathway.¹⁵⁷

Ang-1 has also been linked to vasculogenesis. Serum Ang-1 and EPCs showed similar expression profiles in patients with severe TBIs, suggesting a possible role in mobilizing EPCs from the bone marrow.⁹¹ The mechanism is not completely understood, but many suggest that Ang-1 stimulates vasculogenesis through the VEGF pathway. Activation of the PI3K pathway is critical and leads to the up-regulation of NO, MMP-9, and sKit.¹⁵³

Erythropoietin

Erythropoietin (EPO), a well-recognized hematopoietic cytokine, can act as a growth factor in different tissues.¹⁵⁸ Although EPO controls erythropoiesis (production of red blood cells), emerging evidence suggests that EPO contributes to angiogenesis. EPO treatment has shown promising results in animal models of TBI. Delayed EPO treatment after CCI increases vascularization around the injury site and improves vascular function in a dose-dependent manner.¹⁵⁹ EPO treatment after CCI resulted in enhanced CBF induced by L-arginase administration, likely by stimulating production of NO.¹⁶⁰ Although EPO is mostly produced in the kidney, increased expression of EPO and its receptor (EPO-R) are evident in ECs, neurons, and astrocytes after injury.¹⁶¹ EPO expression is regulated by HIF-1 α .¹⁴³ In ECs, EPO/EPO-R signaling stimulates VEGF expression which in turn activates VEGF signaling.¹⁶² Neural progenitor cells are also affected and may interact with ECs to promote angiogenesis.¹⁶³ In addition, EPO can activate the PI3K pathway independent of VEGF.¹⁶²

The angiogenic effects of EPO are partly the result of vasculogenesis. Wang et al.¹⁶⁴ studied the effects of recombinant human EPO treatment in a rat fluid percussion injury model. Human EPO treated animals showed significantly higher EPC levels after TBI, with increased numbers of CD31+ and CD34+ cells found around the lesion. Treated animals also showed improved spatial learning and memory. Similarly, EPO has been shown to increase EPC expression and enhance angiogenesis after myocardial infarction and stroke.^{165,166} EPO is thought to stimulate vasculogenesis through VEGF signaling and the down-stream PI3K pathway, increasing the expression of NO and MMP-9.¹⁶²

von Willebrand factor

von Willebrand factor (vWF) is a glycoprotein that is increased in response to TBI, with elevated serum levels an indicator of poor outcomes.¹⁶⁷ vWF is well-recognized for its role in blood coagulation by promoting platelet adhesion to sites of damaged vessels and aggregation to form the hemostatic plug.^{168–170} It is also known to complex with coagulation Factor VIII. This protein is normally present in its inactive form when bound to vWF, but is activated and released following blood vessel damage.¹⁶⁹ Upon release, Factor VIII interacts with other coagulation factors to promote blood clotting. After vascular injury, the endothelium rapidly secretes vWF into the plasma and basement membrane.¹⁶⁸ vWF binds to collagen near sites of vessel damage and acts as a bridging molecule that interacts with circulating platelets. vWF interaction with the platelet receptor (GPIb-IX) leads to platelet activation and integrin-dependent adhesion to the vessel wall.¹⁶⁸

Unlike most growth factors, vWF has been shown to inhibit angiogenesis. Loss of vWF results in increased vessel formation in vitro and in vivo.¹⁷¹ vWF modulates VEGF-R2 dependent angiogenesis by either interacting with integrin $\alpha\beta3$ on the cell surface and/or affecting storage of Ang-2 within Weibel-Palade bodies.¹⁷² Thus, vWF likely controls premature vessel formation by countering angiogenic factors. Additionally, staining for vWF is routinely used to detect for activated vessels that are subjected to high levels of pro-angiogenic factors.¹⁷³

WNT/ β -catenin cascade: A novel pathway for vascular repair

Given the need to develop new therapies, it is important to identify other signaling mechanisms that may be activated after TBI. One such potential mechanism is the Wnt/ β -catenin (or canonical) cascade, which has been a major focus of research for CNS injuries and diseases.¹⁷⁴ Numerous studies reported activation of Wnt/ β -catenin signaling after TBI.^{175–178} White et al.¹⁷⁵ showed that Wnt/ β -catenin signaling is enhanced in glial cells after CCI, which coincides with astrogliosis. Other studies have revealed that Wnt/ β -catenin signaling regulates expression of key survival factors that affect neurogenesis.^{176,178} Thus, it is clear that Wnt/ β -catenin signaling and its downstream elements participate in regenerative processes after brain injury.

There is strong evidence in support of the Wnt/ β -catenin pathway in vascular repair. This pathway plays a major role during embryonic vascular development and controls many aspects of vascular repair

processes including angiogenesis,¹⁷⁹ BBB formation,¹⁸⁰ vascular remodeling, sprouting,¹⁸¹ and vascular stability.¹⁷⁹ During development, Wnt/ β -catenin signaling works in conjunction with Notch signaling leading to the formation of nascent blood vessels.¹⁸² Notch activation results in the expression of arterial genes leading to arterial differentiation, whereas Notch inhibition results in the expression of venous genes leading to venous differentiation.¹⁸² Along with vascular development, Wnt/ β -catenin signaling has angiogenic functions in the adult. Many angiogenic factors are regulated by Wnt/ β -catenin signaling in cancer, including VEGF, MMP-9, and FGF2.¹⁸³ Based on its importance during development and post injury, we strongly advocate further research of the Wnt/ β -catenin pathway and its effects on vascular repair is warranted (Figure 4).

Distinct response in stroke

Unlike TBI, the cerebral vasculature responds differently after stroke. Ischemic stroke is the result of impeded blood flow due to an occlusion of a vessel.¹⁷⁴

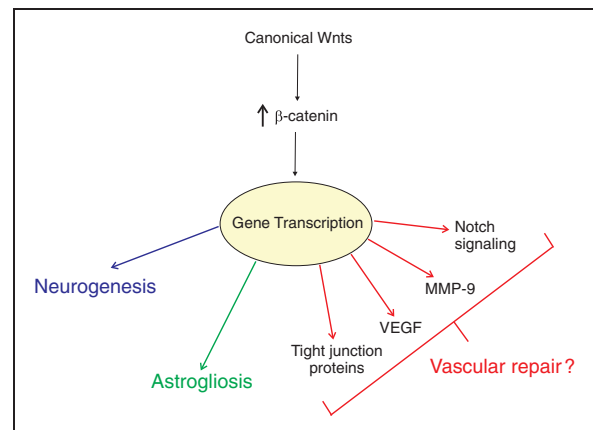


Figure 4. Wnt/ β -catenin signaling in TBI. The Wnt/ β -catenin pathway is tightly regulated by soluble Wnt factors. In the presence of these factors, β -catenin escapes degradation where it accumulates and promotes transcription of Wnt target genes. The role of Wnt/ β -catenin signaling after TBI is not completely understood, but many suggest that it is involved in regenerative processes such as astrogliosis and neurogenesis. It is unclear at the present times what role Wnt/ β -catenin signaling plays in vascular repair, but there is data in support for it. During vascular development, Wnt/ β -catenin signaling is required for the development of CNS blood vessels and works in conjunction with Notch signaling in arterial-venous specification. Additionally, many angiogenic growth factors (VEGF), metalloproteases (MMP-9), and tight junction proteins (Claudin 3 and ZO-1) are both directly and indirectly modulated by the Wnt signaling cascade. Thus, Wnt/ β -catenin pathway and its associated molecules may be involved in repairing the injured vasculature after TBI.

The resulting infarct region is divided into the ischemic core and penumbra, tissue surrounding the core.¹⁸⁴ While the core experiences the greatest degree of tissue injury, the penumbra experiences limited blood flow allowing cells to survive.¹⁸⁵ Acutely after stroke, reduced blood flow activates signaling cascades that lead to vessel collateralization, or arteriogenesis.¹⁸⁶ This process is responsible for bypassing occluded vessels and increasing blood flow to the ischemic tissue. At one day after stroke, increased numbers of anastomosing microvessels are evident in the penumbra.¹⁸⁷ Arteriogenesis is initiated by fluid shear stress and leads to the activation of the endothelium (unlike angiogenesis that is hypoxia-driven).¹⁸⁵ Immune cells, such as monocytes and macrophages, are recruited where they release angiogenic growth factors and cytokines that promote EC and smooth muscle cell migration and proliferation and ultimately leading to the growth of new collateral arteries.¹⁸⁵ While arteriogenesis plays a key role in maintaining blood flow after ischemic stroke, there is no evidence of such a mechanism occurring after TBI.

Angiogenesis is thought to be initiated in the chronic phases of stroke. Post-mortem tissue of patients with ischemic stroke has revealed activated endothelial cells and increased microvessel density in the penumbra.¹⁸⁸ However, age plays a significant factor in post-ischemic angiogenesis.¹⁸⁹ Experimental studies using MRI suggest that the late phases of increased blood flow are the result of angiogenesis.¹⁸⁶ Thus, it appears that TBI and stroke both undergo a similar angiogenic process at these late time points.

Therapeutic perspective

Treatment strategies that target the cerebral vasculature are desperately needed for TBI and other vascular-related brain injuries, such as ischemic stroke, subarachnoid hemorrhage, intracerebral hemorrhage, to name a few. In the last decade, TBI research has primarily focused on the delivery of angiogenic proteins. This approach enhances vascular function in animal models of TBI, but is hampered by various complications that limit its effectiveness for translation to the clinic. Common issues include loss of protein activity, degradation, off-target effects, and ineffective concentrations at the injured tissue. Recently, a greater focus has been placed on delivery devices that increase efficacy and minimize side effects. Degradable hydrogels are used in research and can be designed to deliver multiple angiogenic factors in a controlled and efficient manner, but these are only effective for superficial TBI lesions. Furthermore, these devices can be expanded to administer drugs, living cells, and other bioactive agents. For more information, readers are encouraged to read recent reviews.^{89,190,191}

A majority of the signaling factors discussed above can have detrimental effects after injury. VEGF is a prime example of how one factor can have dual functions. Although VEGF induces angiogenesis, it also enhances vessel permeability in the injured tissue.¹⁹² Studies have demonstrated that VEGF treatment can lead to increased cerebral edema, but this is dependent on the timing of the treatment.^{134,193} Delayed administration has been shown to be the most efficacious in terms of vascularization.^{70,130} Furthermore, co-application of VEGF and other angiogenic factors may help in reducing deleterious effects.¹⁹⁴ Another consideration is the quality of the newly formed vessels. VEGF-induced vessels are inherently unstable and leaky as they develop, which can be attributed to a failure to express specific BBB proteins.¹⁹⁵ Thus, VEGF and other candidate proteins must be carefully selected when used for any future therapeutics.

Cell-based therapies have emerged as a possible treatment option for TBI. Recently, a great deal of attention has been placed on adult mesenchymal stem cells (MSCs). These cells are isolated from the bone marrow, have low antigenicity, and express high levels of growth factors and cytokines.¹⁹⁶ Administration of MSCs after experimental TBI ameliorates functional deficits and improves neurological outcome.^{197–199} In concert with neurogenesis, MSCs enhance angiogenesis and vasculogenesis.^{200–202} Interestingly, Zhang et al.²⁰³ revealed that delivery of MSC-derived exosomes after CCI increases the number of nascent ECs and neurons in the injured tissue thereby improving outcomes. Exosomes are micro-vesicles that have RNA and protein as cargo and could be an intriguing cell-free treatment for TBI. Based on these promising preclinical studies, clinical trials are underway to test the safety and efficacy of autologous MSC treatment in TBI patients.²⁰⁴

Conclusion

TBI is a major clinical problem that is associated with long-term morbidity among survivors. While the underlying mechanism(s) that lead to functional decline after TBI remains poorly understood, emerging research suggests that the damaged cerebral vasculature participates in the development of chronic disabilities. Ameliorating vascular dysfunction remains a key priority in the clinic, but therapeutic options are limited. Moving forward, it is critical to understand the mechanisms in which these cellular and molecular components may affect vascular repair. The eNVU has been understudied in its role in vascular repair and thus requires more attention. Targeting the PI3K and MAPK pathways may be effective therapeutic options but other upstream molecular targets need to be investigated to enhance

vascular repair. A greater understanding of the vascular response to TBI and molecular signals that mediate vascular repair can lead to the development of new vasculogenic therapies. Most importantly, improvements in vascular function would limit secondary injuries leading to improved outcomes for patients with TBI.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: These studies were supported by an NIH Program Project grant from National Institute of Neurological Disorders and Stroke (1P01NS082184, Project 3).

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

- Kabadi SV and Faden AI. Neuroprotective strategies for traumatic brain injury: Improving clinical translation. *Int J Mol Sci* 2014; 15: 1216–1236.
- Logsdon AF, Lucke-Wold BP, Turner RC, et al. Role of microvascular disruption in brain damage from traumatic brain injury. *Compr Physiol* 2015; 5: 1147–1160.
- Kenney K, Amyot F, Haber M, et al. Cerebral vascular injury in traumatic brain injury. *Exp Neurol* 2016; 275(Pt 3): 353–366.
- Jullienne A, Obenaus A, Ichkova A, et al. Chronic cerebrovascular dysfunction after traumatic brain injury. *J Neurosci Res* 2016; 94: 609–622.
- Zhang JH, Badaut J, Tang J, et al. The vascular neural network – A new paradigm in stroke pathophysiology. *Nat Rev Neurol* 2012; 8: 711–716.
- Jullienne A and Badaut J. Molecular contributions to neurovascular unit dysfunctions after brain injuries: Lessons for target-specific drug development. *Future Neurol* 2013; 8: 677–689.
- Morgan R, Kreipke CW, Roberts G, et al. Neovascularization following traumatic brain injury: Possible evidence for both angiogenesis and vasculogenesis. *Neurol Res* 2007; 29: 375–381.
- Frontczak-Baniewicz M and Walski M. New vessel formation after surgical brain injury in the rat's cerebral cortex I. Formation of the blood vessels proximally to the surgical injury. *Acta Neurobiol Exp* 2003; 63: 65–75.
- Park E, Bell JD, Siddiq IP, et al. An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: A role for hypoxia-inducible factors in traumatic brain injury. *J Cereb Blood Flow Metab* 2009; 29: 575–584.
- Rosenbaum BP, Kelly ML, Kshetry VR, et al. Neurologic disorders, in-hospital deaths, and years of potential life lost in the USA, 1988–2011. *J Clin Neurosci* 2014; 21: 1874–1880.
- Corrigan JD, Selassie AW and Orman JA. The epidemiology of traumatic brain injury. *J Head Trauma Rehabil* 2010; 25: 72–80.
- Maas AI, Menon DK, Steyerberg EW, et al. Collaborative European neurotrauma effectiveness research in traumatic brain injury (CENTER-TBI): A prospective longitudinal observational study. *Neurosurgery* 2015; 76: 67–80.
- Vaishnavi S, Rao V and Fann JR. Neuropsychiatric problems after traumatic brain injury: Unraveling the silent epidemic. *Psychosomatics* 2009; 50: 198–205.
- Selassie AW, Zaloshnja E, Langlois JA, et al. Incidence of long-term disability following traumatic brain injury hospitalization, United States, 2003. *J Head Trauma Rehabil* 2008; 23: 123–131.
- Johnson VE, Meaney DF, Cullen DK, et al. Animal models of traumatic brain injury. *Handb Clin Neurol* 2015; 127: 115–128.
- Guskiewicz KM, Marshall SW, Bailes J, et al. Association between recurrent concussion and late-life cognitive impairment in retired professional football players. *Neurosurgery* 2005; 57: 719–726; discussion 26.
- Yeh DD and Schecter WP. Primary blast injuries – An updated concise review. *World J Surg* 2012; 36: 966–972.
- Benson RR, Gattu R, Sewick B, et al. Detection of hemorrhagic and axonal pathology in mild traumatic brain injury using advanced MRI: Implications for neurorehabilitation. *Neurorehabilitation* 2012; 31: 261–279.
- Konrad C, Geburek AJ, Rist F, et al. Long-term cognitive and emotional consequences of mild traumatic brain injury. *Psychol Med* 2011; 41: 1197–1211.
- Obenaus A. *Traumatic brain injury. Encyclopedia of mental health*. 2nd ed. Oxford: Elsevier, 2015, pp.329–340.
- Maas AI, Stocchetti N and Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurol* 2008; 7: 728–741.
- Spitz G, Alway YJ, Gould K, et al. Disrupted white matter microstructure and mood disorders following traumatic brain injury. *J Neurotrauma* 2016; 34: 807–815.
- Lozano D, Gonzales-Portillo GS, Acosta S, et al. Neuroinflammatory responses to traumatic brain injury: Etiology, clinical consequences, and therapeutic opportunities. *Neuropsychiatr Dis Treat* 2015; 11: 97–106.
- Pop V and Badaut J. A neurovascular perspective for long-term changes after brain trauma. *Transl Stroke Res* 2011; 2: 533–545.
- Perel P, Roberts I, Bouamra O, et al. Intracranial bleeding in patients with traumatic brain injury: A prognostic study. *BMC Emerg Med* 2009; 9: 15.
- Hochstadter E, Stewart TC, Alharfi IM, et al. Subarachnoid hemorrhage prevalence and its association with short-term outcome in pediatric severe traumatic brain injury. *Neurocrit Care* 2014; 21: 505–513.
- Faust K, Horn P, Schneider UC and Vajkoczy P. Blood pressure changes after aneurysmal subarachnoid hemorrhage and their relationship to cerebral vasospasm and clinical outcome. *Clin Neurol Neurosurg* 2014; 125: 36–40.
- Park JH, Park SW, Kang SH, et al. Detection of traumatic cerebral microbleeds by susceptibility-weighted

- image of MRI. *J Korean Neurosurg Soc* 2009; 46: 365–369.
29. Gaasch JA, Lockman PR, Geldenhuys WJ, et al. Brain iron toxicity: differential responses of astrocytes, neurons, and endothelial cells. *Neurochem Res* 2007; 32: 1196–1208.
30. Glushakova OY, Johnson D and 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. *J Neurotrauma* 2014; 31: 1180–1193.
31. Donovan V, Bianchi A, Hartman R, et al. Computational analysis reveals increased blood deposition following repeated mild traumatic brain injury. *Neuroimage Clin* 2012; 1: 18–28.
32. Marmarou A. Pathophysiology of traumatic brain edema: Current concepts. *Acta Neurochir Suppl* 2003; 86: 7–10.
33. Ewing-Cobbs L, Prasad M, Kramer L, et al. Acute neuro-radiologic findings in young children with inflicted or noninflicted traumatic brain injury. *Childs Nerv Syst* 2000; 16: 25–33; discussion 4.
34. Badaut J, Ashwal S and Obenaus A. Aquaporins in cerebrovascular disease: A target for treatment of brain edema? *Cerebrovasc Dis* 2011; 31: 521–531.
35. Nirula R, Millar D, Greene T, et al. Decompressive craniectomy or medical management for refractory intracranial hypertension: An AAST-MIT propensity score analysis. *J Trauma Acute Care Surg* 2014; 76: 944–952; discussion 52–55.
36. Bouma GJ, Muizelaar JP, Stringer WA, et al. Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. *J Neurosurg* 1992; 77: 360–368.
37. Soustiel JF, Glenn TC, Shik V, et al. Monitoring of cerebral blood flow and metabolism in traumatic brain injury. *J Neurotrauma* 2005; 22: 955–965.
38. Bouma GJ, Muizelaar JP, Choi SC, et al. Cerebral circulation and metabolism after severe traumatic brain injury: The elusive role of ischemia. *J Neurosurg* 1991; 75: 685–693.
39. Ostergaard L, Engedal TS, Aamand R, et al. Capillary transit time heterogeneity and flow-metabolism coupling after traumatic brain injury. *J Cereb Blood Flow Metab* 2014; 34: 1585–198.
40. Doshi H, Wiseman N, Liu J, et al. Cerebral hemodynamic changes of mild traumatic brain injury at the acute stage. *PLoS One* 2015; 10: e0118061.
41. Inoue Y, Shiozaki T, Tasaki O, et al. Changes in cerebral blood flow from the acute to the chronic phase of severe head injury. *J Neurotrauma* 2005; 22: 1411–148.
42. Kramer DR, Winer JL, Pease BA, et al. Cerebral vasospasm in traumatic brain injury. *Neurol Res Int* 2013; 2013: 415813.
43. Oertel M, Boscardin WJ, Obrist WD, et al. Posttraumatic vasospasm: The epidemiology, severity, and time course of an underestimated phenomenon: A prospective study performed in 299 patients. *J Neurosurg* 2005; 103: 812–824.
44. Alford PW, Dabiri BE, Goss JA, et al. Blast-induced phenotypic switching in cerebral vasospasm. *Proc Natl Acad Sci U S A* 2011; 108: 12705–12710.
45. Izzy S and Muehlschlegel S. Cerebral vasospasm after aneurysmal subarachnoid hemorrhage and traumatic brain injury. *Curr Treat Options Neurol* 2014; 16: 278.
46. Dore-Duffy P, Owen C, Balabanov R, et al. Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvasc Res* 2000; 60: 55–69.
47. Ho KM, Honeybul S, Yip CB, et al. Prognostic significance of blood-brain barrier disruption in patients with severe nonpenetrating traumatic brain injury requiring decompressive craniectomy. *J Neurosurg* 2014; 121: 674–679.
48. Tomkins O, Shelef I, Kaizerman I, et al. Blood-brain barrier disruption in post-traumatic epilepsy. *J Neurol Neurosurg Psychiatry* 2008; 79: 774–777.
49. Yeoh S, Bell ED and Monson KL. Distribution of blood-brain barrier disruption in primary blast injury. *Ann Biomed Eng* 2013; 41: 2206–2214.
50. Kolaczowska E and Kuberski P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 2013; 13: 159–175.
51. Abdul-Muneer PM, Chandra N and Haorah J. Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. *Mol Neurobiol* 2015; 51: 966–979.
52. Harhangi BS, Kompanje EJ, Leebeek FW, et al. Coagulation disorders after traumatic brain injury. *Acta Neurochir* 2008; 150: 165–175; discussion 75.
53. Yuan Q, Sun YR, Wu X, et al. Coagulopathy in Traumatic brain injury and its correlation with progressive hemorrhagic injury: A systematic review and meta-analysis. *J Neurotrauma* 2016; 33: 1279–1291.
54. de Oliveira Manoel AL, Neto AC, Veigas PV, et al. Traumatic brain injury associated coagulopathy. *Neurocrit Care* 2015; 22: 34–44.
55. Lustenberger T, Talving P, Kobayashi L, et al. Time course of coagulopathy in isolated severe traumatic brain injury. *Injury* 2010; 41: 924–928.
56. Midura EF, Jernigan PL, Kuethe JW, et al. Microparticles impact coagulation after traumatic brain injury. *J Surg Res* 2015; 197: 25–31.
57. Ploplis VA, Donahue DL, Sandoval-Cooper MJ, et al. Systemic platelet dysfunction is the result of local dysregulated coagulation and platelet activation in the brain in a rat model of isolated traumatic brain injury. *J Neurotrauma* 2014; 31: 1672–1675.
58. Laroche M, Kutcher ME, Huang MC, et al. Coagulopathy after traumatic brain injury. *Neurosurgery* 2012; 70: 1334–1345.
59. Nakae R, Takayama Y, Kuwamoto K, et al. Time course of coagulation and fibrinolytic parameters in patients with traumatic brain injury. *J Neurotrauma* 2016; 33: 688–695.
60. Schnuriger B, Inaba K, Abdelsayed GA, et al. The impact of platelets on the progression of traumatic intracranial hemorrhage. *J Trauma* 2010; 68: 881–885.
61. Lorente L. New prognostic biomarkers in patients with traumatic brain injury. *Arch Trauma Res* 2015; 4: e30165.

62. Shen X, Dutcher SK, Palmer J, et al. A systematic review of the benefits and risks of anticoagulation following traumatic brain injury. *J Head Trauma Rehabil* 2015; 30: E29–E37.
63. Kumar A and Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. *Brain Behav Immun* 2012; 26: 1191–1201.
64. Hinson HE, Rowell S and Schreiber M. Clinical evidence of inflammation driving secondary brain injury: A systematic review. *J Trauma Acute Care Surg* 2015; 78: 184–191.
65. Gentleman SM, Leclercq PD, Moyes L, et al. Long-term intracerebral inflammatory response after traumatic brain injury. *Forensic Sci Int* 2004; 146: 97–104.
66. Johnson VE, Stewart JE, Begbie FD, et al. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain* 2013; 136: 28–42.
67. Smith C, Gentleman SM, Leclercq PD, et al. The neuroinflammatory response in humans after traumatic brain injury. *Neuropathol Appl Neurobiol* 2013; 39: 654–666.
68. Block ML, Zecca L and Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 2007; 8: 57–69.
69. Hernandez-Ontiveros DG, Tajiri N, Acosta S, et al. Microglia activation as a biomarker for traumatic brain injury. *Front Neurol* 2013; 4: 30.
70. Siddiq I, Park E, Liu E, et al. Treatment of traumatic brain injury using zinc-finger protein gene therapy targeting VEGF-A. *J Neurotrauma* 2012; 29: 2647–2659.
71. Obenaus A, Ng M, Orantes A, et al. Traumatic brain injury results in acute rarefaction of the vascular network. *Sci Rep* 2017; 7: 239.
72. Hayward NM, Tuunanen PI, Immonen R, et al. Magnetic resonance imaging of regional hemodynamic and cerebrovascular recovery after lateral fluid-percussion brain injury in rats. *J Cereb Blood Flow Metab* 2011; 31: 166–177.
73. Jia Y, Grafe MR, Gruber A, et al. In vivo optical imaging of revascularization after brain trauma in mice. *Microvasc Res* 2011; 81: 73–80.
74. Rodriguez-Baeza A, Reina-de la Torre F, Poca A, et al. Morphological features in human cortical brain microvessels after head injury: A three-dimensional and immunocytochemical study. *Anat Rec Discov Mol Cell Evol Bio* 2003; 273: 583–593.
75. Rafols JA, Kreipke CW and Petrov T. Alterations in cerebral cortex microvessels and the microcirculation in a rat model of traumatic brain injury: A correlative EM and laser Doppler flowmetry study. *Neurol Res* 2007; 29: 339–347.
76. Castejon OJ. Ultrastructural alterations of human cortical capillary basement membrane in human brain oedema. *Folia Neuropathol* 2014; 52: 10–21.
77. Danaila L, Popescu I, Pais V, et al. Apoptosis, paraptosis, necrosis, and cell regeneration in posttraumatic cerebral arteries. *Chirurgia* 2013; 108: 319–324.
78. Jullienne A, Roberts JM, Pop V, et al. Juvenile traumatic brain injury induces long-term perivascular matrix changes alongside amyloid-beta accumulation. *J Cereb Blood Flow Metab* 2014; 34: 1637–1645.
79. Gama Sosa MA, De Gasperi R, Janssen PL, et al. Selective vulnerability of the cerebral vasculature to blast injury in a rat model of mild traumatic brain injury. *Acta Neuropathol Commun* 2014; 2: 67.
80. Lynch CE, Crynen G, Ferguson S, et al. Chronic cerebrovascular abnormalities in a mouse model of repetitive mild traumatic brain injury. *Brain Injury* 2016; 30: 1414–1427.
81. Carmeliet P and Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 2011; 473: 298–307.
82. Skold MK, von Gertten C, Sandberg-Nordqvist AC, et al. VEGF and VEGF receptor expression after experimental brain contusion in rat. *J Neurotrauma* 2005; 22: 353–367.
83. Gajavelli S, Kentaro S, Diaz J, et al. Glucose and oxygen metabolism after penetrating ballistic-like brain injury. *J Cereb Blood Flow Metab* 2015; 35: 773–780.
84. Anderson J, Sandhir R, Hamilton ES, et al. Impaired expression of neuroprotective molecules in the HIF-1 α pathway following traumatic brain injury in aged mice. *J Neurotrauma* 2009; 26: 1557–1566.
85. Hirota K and Semenza GL. Regulation of angiogenesis by hypoxia-inducible factor 1. *Crit Rev Oncol Hematol* 2006; 59: 15–26.
86. Krum JM, Mani N and Rosenstein JM. Angiogenic and astroglial responses to vascular endothelial growth factor administration in adult rat brain. *Neuroscience* 2002; 110: 589–604.
87. Krum JM and Khaibullina A. Inhibition of endogenous VEGF impedes revascularization and astroglial proliferation: Roles for VEGF in brain repair. *Exp Neurol* 2003; 181: 241–257.
88. Balabanov R, Goldman H, Murphy S, et al. Endothelial cell activation following moderate traumatic brain injury. *Neurol Res* 2001; 23: 175–182.
89. Van Hove AH and Benoit DS. Depot-based delivery systems for pro-angiogenic peptides: A review. *Front Bioeng Biotechnol* 2015; 3: 102.
90. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999; 85: 221–228.
91. Gong D, Zhang S, Liu L, et al. Dynamic changes of vascular endothelial growth factor and angiopoietin-1 in association with circulating endothelial progenitor cells after severe traumatic brain injury. *J Trauma* 2011; 70: 1480–1484.
92. Liu L, Wei H, Chen F, et al. Endothelial progenitor cells correlate with clinical outcome of traumatic brain injury. *Crit Care Med* 2011; 39: 1760–1765.
93. Guo X, Liu L, Zhang M, et al. Correlation of CD34+ cells with tissue angiogenesis after traumatic brain injury in a rat model. *J Neurotrauma* 2009; 26: 1337–1344.
94. Roncalli JG, Tongers J, Renault MA, et al. Endothelial progenitor cells in regenerative medicine and cancer: A decade of research. *Trends Biotechnol* 2008; 26: 276–283.
95. Du R, Lu KV, Petritsch C, et al. HIF1 α induces the recruitment of bone marrow-derived vascular

- modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell* 2008; 13: 206–220.
96. Liu L, Liu H, Jiao J, et al. Changes in circulating human endothelial progenitor cells after brain injury. *J Neurotrauma* 2007; 24: 936–943.
 97. Reale A, Melaccio A, Lamanuzzi A, et al. Functional and biological role of endothelial precursor cells in tumour progression: A new potential therapeutic target in haematological malignancies. *Stem Cells Int* 2016; 2016: 7954580.
 98. Ziegelhoeffer T, Fernandez B, Kostin S, et al. Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res* 2004; 94: 230–238.
 99. Urbich C, Aicher A, Heeschen C, et al. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol* 2005; 39: 733–742.
 100. Miller JT, Bartley JH, Wimborne HJ, et al. The neuroblast and angioblast chemotactic factor SDF-1 (CXCL12) expression is briefly up regulated by reactive astrocytes in brain following neonatal hypoxic-ischemic injury. *BMC Neurosci* 2005; 6: 63.
 101. Treutiger CJ, Mullins GE, Johansson AS, et al. High mobility group 1 B-box mediates activation of human endothelium. *J Intern Med* 2003; 254: 375–385.
 102. Hayakawa K, Pham LD, Katusic ZS, et al. Astrocytic high-mobility group box 1 promotes endothelial progenitor cell-mediated neurovascular remodeling during stroke recovery. *Proc Natl Acad Sci U S A* 2012; 109: 7505–7510.
 103. Hayakawa K, Miyamoto N, Seo JH, et al. High-mobility group box 1 from reactive astrocytes enhances the accumulation of endothelial progenitor cells in damaged white matter. *J Neurochem* 2013; 125: 273–280.
 104. Jones EV and Bouvier DS. Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease. *Neural Plast* 2014; 2014: 321209.
 105. Ribotta MG, Menet V and Privat A. Glial scar and axonal regeneration in the CNS: Lessons from GFAP and vimentin transgenic mice. *Acta Neurochir Suppl* 2004; 89: 87–92.
 106. Villapol S, Byrnes KR and Symes AJ. Temporal dynamics of cerebral blood flow, cortical damage, apoptosis, astrocyte-vasculature interaction and astrogliosis in the pericontusional region after traumatic brain injury. *Front Neurol* 2014; 5: 82.
 107. Abbott NJ, Ronnback L and Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 2006; 7: 41–53.
 108. Raza A, Franklin MJ and Dudek AZ. Pericytes and vessel maturation during tumor angiogenesis and metastasis. *Am J Hematol* 2010; 85: 593–598.
 109. Zehendner CM, Sebastiani A, Hugonnet A, et al. Traumatic brain injury results in rapid pericyte loss followed by reactive pericytosis in the cerebral cortex. *Sci Rep* 2015; 5: 13497.
 110. Yemisci M, Gursoy-Ozdemir Y, Vural A, et al. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nat Med* 2009; 15: 1031–1037.
 111. Hori S, Ohtsuki S, Hosoya K, et al. A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *J Neurochem* 2004; 89: 503–513.
 112. Bergers G and Song S. The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol* 2005; 7: 452–464.
 113. Parent JM, Vexler ZS, Gong C, et al. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann Neurol* 2002; 52: 802–813.
 114. Kojima T, Hirota Y, Ema M, et al. Subventricular zone-derived neural progenitor cells migrate along a blood vessel scaffold toward the post-stroke striatum. *Stem Cells* 2010; 28: 545–554.
 115. Skardelly M, Gaber K, Burdack S, et al. Long-term benefit of human fetal neuronal progenitor cell transplantation in a clinically adapted model after traumatic brain injury. *J Neurotrauma* 2011; 28: 401–414.
 116. Schiera G, Proia P, Alberti C, et al. Neurons produce FGF2 and VEGF and secrete them at least in part by shedding extracellular vesicles. *J Cell Mol Med* 2007; 11: 1384–1394.
 117. Okabe K, Kobayashi S, Yamada T, et al. Neurons limit angiogenesis by titrating VEGF in retina. *Cell* 2014; 159: 584–596.
 118. Hsieh CL, Kim CC, Ryba BE, et al. Traumatic brain injury induces macrophage subsets in the brain. *Eur J Immunol* 2013; 43: 2010–2022.
 119. Spiller KL, Anfang RR, Spiller KJ, et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds. *Biomaterials* 2014; 35: 4477–4488.
 120. Kim CC, Nakamura MC and Hsieh CL. Brain trauma elicits non-canonical macrophage activation states. *J Neuroinflammation* 2016; 13: 117.
 121. Kumar A, Alvarez-Croda DM, Stoica BA, et al. Microglial/macrophage polarization dynamics following traumatic brain injury. *J Neurotrauma* 2016; 33: 1732–1750.
 122. Frautschy SA, Walicke PA and Baird A. Localization of basic fibroblast growth factor and its mRNA after CNS injury. *Brain Res* 1991; 553: 291–299.
 123. Lobov IB, Rao S, Carroll TJ, et al. WNT7b mediates macrophage-induced programmed cell death in patterning of the vasculature. *Nature* 2005; 437: 417–421.
 124. Liu C, Wu C, Yang Q, et al. Macrophages mediate the repair of brain vascular rupture through direct physical adhesion and mechanical traction. *Immunity* 2016; 44: 1162–1176.
 125. Fantin A, Vieira JM, Gestri G, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood* 2010; 116: 829–840.
 126. He H, Mack JJ, Guc E, et al. Perivascular macrophages limit permeability. *Arterioscler Thromb Vasc Biol* 2016; 36: 2203–2212.
 127. Zhang Z, Zhang ZY, Wu Y, et al. Lesional accumulation of CD163+ macrophages/microglia in rat traumatic brain injury. *Brain Res* 2012; 1461: 102–110.

128. Lin EY, Li JF, Gnatovskiy L, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006; 66: 11238–11246.
129. Addington CP, Roussas A, Dutta D, et al. Endogenous repair signaling after brain injury and complementary bioengineering approaches to enhance neural regeneration. *Biomark Insights* 2015; 10: 43–60.
130. Thau-Zuchman O, Shohami E, Alexandrovich AG, et al. Subacute treatment with vascular endothelial growth factor after traumatic brain injury increases angiogenesis and gliogenesis. *Neuroscience* 2012; 202: 334–341.
131. Thau-Zuchman O, Shohami E, Alexandrovich AG, et al. Combination of vascular endothelial and fibroblast growth factor 2 for induction of neurogenesis and angiogenesis after traumatic brain injury. *J Mol Neurosci* 2012; 47: 166–172.
132. Zhang ZG, Zhang L, Tsang W, et al. Correlation of VEGF and angiopoietin expression with disruption of blood-brain barrier and angiogenesis after focal cerebral ischemia. *J Cereb Blood Flow Metab* 2002; 22: 379–392.
133. Zhang A, Liang L, Niu H, et al. Protective effects of VEGF treatment on focal cerebral ischemia in rats. *Mol Med Rep* 2012; 6: 1315–1318.
134. Dzierko M, Derugin N, Wendland MF, et al. Delayed VEGF treatment enhances angiogenesis and recovery after neonatal focal rodent stroke. *Transl Stroke Res* 2013; 4: 189–200.
135. Shibuya M and Claesson-Welsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp Cell Res* 2006; 312: 549–560.
136. Koch S and Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. *Cold Spring Harb Perspect Med* 2012; 2: a006502.
137. Gong D, Hao M, Liu L, et al. Prognostic relevance of circulating endothelial progenitor cells for severe traumatic brain injury. *Brain Injury* 2012; 26: 291–297.
138. Li W, Wang H, Kuang CY, et al. An essential role for the Id1/PI3K/Akt/NFkB/survivin signalling pathway in promoting the proliferation of endothelial progenitor cells in vitro. *Mol Cell Biochem* 2012; 363: 135–145.
139. Goetz R and Mohammadi M. Exploring mechanisms of FGF signalling through the lens of structural biology. *Nat Rev Mol Cell Biol* 2013; 14: 166–180.
140. Kano MR, Morishita Y, Iwata C, et al. VEGF-A and FGF-2 synergistically promote neoangiogenesis through enhancement of endogenous PDGF-B-PDGFRbeta signaling. *J Cell Sci* 2005; 118: 3759–3768.
141. Wang ZG, Cheng Y, Yu XC, et al. bFGF protects against blood-brain barrier damage through junction protein regulation via pi3k-akt-racl pathway following traumatic brain injury. *Mol Neurobiol* 2015; 53: 7298–7311.
142. Huang B, Krafft PR, Ma Q, et al. Fibroblast growth factors preserve blood-brain barrier integrity through RhoA inhibition after intracerebral hemorrhage in mice. *Neurobiol Dis* 2012; 46: 204–214.
143. Li S, Wei M, Zhou Z, et al. SDF-1alpha induces angiogenesis after traumatic brain injury. *Brain Res* 2012; 1444: 76–86.
144. Sun W, Liu J, Huan Y, et al. Intracranial injection of recombinant stromal-derived factor-1 alpha (SDF-1alpha) attenuates traumatic brain injury in rats. *Inflamm Res* 2014; 63: 287–297.
145. Cencioni C, Capogrossi MC and Napolitano M. The SDF-1/CXCR4 axis in stem cell preconditioning. *Cardiovasc Res* 2012; 94: 400–407.
146. Wu Y and Yoder A. Chemokine coreceptor signaling in HIV-1 infection and pathogenesis. *PLoS Pathog* 2009; 5: e1000520.
147. Rosenkranz K, Kumbruch S, Lebermann K, et al. The chemokine SDF-1/CXCL12 contributes to the ‘homing’ of umbilical cord blood cells to a hypoxic-ischemic lesion in the rat brain. *J Neurosci Res* 2010; 88: 1223–1233.
148. Egeblad M and Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002; 2: 161–174.
149. van Hinsbergh VW and Koolwijk P. Endothelial sprouting and angiogenesis: Matrix metalloproteinases in the lead. *Cardiovasc Res* 2008; 78: 203–212.
150. Lee S, Jilani SM, Nikolova GV, et al. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. *J Cell Biol* 2005; 169: 681–691.
151. Frontczak-Baniewicz M, Walski M, et al. MMP2 and MMP9 in immature endothelial cells following surgical injury of rat cerebral cortex – A preliminary study. *Folia Neuropathol* 2009; 47: 338–346.
152. Huang PH, Chen YH, Wang CH, et al. Matrix metalloproteinase-9 is essential for ischemia-induced neovascularization by modulating bone marrow-derived endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2009; 29: 1179–1184.
153. Heissig B, Hattori K, Dias S, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 2002; 109: 625–637.
154. Gurbel PA, Roe MT, Jakubowski JA, et al. Translational platelet research in patients with coronary artery disease: What are the major knowledge gaps? *Thromb Haemost* 2012; 108: 12–20.
155. Chen J, Cui X, Zacharek A, et al. Increasing Ang1/Tie2 expression by simvastatin treatment induces vascular stabilization and neuroblast migration after stroke. *J Cell Mol Med* 2009; 13: 1348–1357.
156. Nourhaghighi N, Teichert-Kuliszewska K, Davis J, et al. Altered expression of angiopoietins during blood-brain barrier breakdown and angiogenesis. *Lab Invest* 2003; 83: 1211–1222.
157. Echavarría R and Hussain SN. Regulation of angiopoietin-1/Tie-2 receptor signaling in endothelial cells by dual-specificity phosphatases 1, 4, and 5. *J Am Heart Assoc* 2013; 2: e000571.
158. Genc S, Koroglu TF and Genc K. Erythropoietin and the nervous system. *Brain Res* 2004; 1000: 19–31.
159. Meng Y, Xiong Y, Mahmood A, et al. Dose-dependent neurorestorative effects of delayed treatment of traumatic brain injury with recombinant human erythropoietin in rats. *J Neurosurg* 2011; 115: 550–560.

160. Cherian L, Goodman JC and Robertson C. Neuroprotection with erythropoietin administration following controlled cortical impact injury in rats. *J Pharmacol Exp Ther* 2007; 322: 789–794.
161. Tsai PT, Ohab JJ, Kertesz N, et al. A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. *J Neurosci* 2006; 26: 1269–1274.
162. Sautina L, Sautin Y, Beem E, et al. Induction of nitric oxide by erythropoietin is mediated by the {beta} common receptor and requires interaction with VEGF receptor 2. *Blood* 2010; 115: 896–905.
163. Wang L, Chopp M, Gregg SR, et al. Neural progenitor cells treated with EPO induce angiogenesis through the production of VEGF. *J Cereb Blood Flow Metab* 2008; 28: 1361–1368.
164. Wang L, Wang X, Su H, et al. Recombinant human erythropoietin improves the neurofunctional recovery of rats following traumatic brain injury via an increase in circulating endothelial progenitor cells. *Transl Stroke Res* 2015; 6: 50–59.
165. Hirata A, Minamino T, Asanuma H, et al. Erythropoietin enhances neovascularization of ischemic myocardium and improves left ventricular dysfunction after myocardial infarction in dogs. *J Am Coll Cardiol* 2006; 48: 176–184.
166. Yip HK, Tsai TH, Lin HS, et al. Effect of erythropoietin on level of circulating endothelial progenitor cells and outcome in patients after acute ischemic stroke. *Crit Care* 2011; 15: R40.
167. Yokota H, Naoe Y, Nakabayashi M, et al. Cerebral endothelial injury in severe head injury: The significance of measurements of serum thrombomodulin and the von Willebrand factor. *J Neurotrauma* 2002; 19: 1007–1015.
168. Rauch A, Wohner N, Christophe OD, et al. On the versatility of von Willebrand factor. *Mediterr J Hematol Infect Dis* 2013; 5: e2013046.
169. Lenting PJ, Casari C, Christophe OD, et al. von Willebrand factor: The old, the new and the unknown. *J Thromb Haemost* 2012; 10: 2428–2437.
170. Zhang J, Jiang R, Liu L, et al. Traumatic brain injury-associated coagulopathy. *J Neurotrauma* 2012; 29: 2597–2605.
171. Starke RD, Ferraro F, Paschalaki KE, et al. Endothelial von Willebrand factor regulates angiogenesis. *Blood* 2011; 117: 1071–1080.
172. Randi AM, Laffan MA and Starke RD. Von Willebrand factor, angiodyplasia and angiogenesis. *Mediterr J Hematol Infect Dis* 2013; 5: e2013060.
173. Wagner DD and Frenette PS. The vessel wall and its interactions. *Blood* 2008; 111: 5271–5281.
174. Lambert C, Cisternas P and Inestrosa NC. Role of Wnt signaling in central nervous system injury. *Mol Neurobiol* 2015; 53: 2297–2311.
175. White BD, Nathe RJ, Maris DO, et al. Beta-catenin signaling increases in proliferating NG2+ progenitors and astrocytes during post-traumatic gliogenesis in the adult brain. *Stem Cells* 2010; 28: 297–307.
176. Zhang L, Yan R, Zhang Q, et al. Survivin, a key component of the Wnt/beta-catenin signaling pathway, contributes to traumatic brain injury-induced adult neurogenesis in the mouse dentate gyrus. *Int J Mol Med* 2013; 32: 867–875.
177. Shapira M, Licht A, Milman A, et al. Role of glycogen synthase kinase-3beta in early depressive behavior induced by mild traumatic brain injury. *Mol Cell Neurosci* 2007; 34: 571–577.
178. Wu X, Mao H, Liu J, et al. Dynamic change of SGK expression and its role in neuron apoptosis after traumatic brain injury. *Int J Clin Exp Pathol* 2013; 6: 1282–1293.
179. Birdsey GM, Shah AV, Dufton N, et al. The endothelial transcription factor ERG promotes vascular stability and growth through Wnt/beta-catenin signaling. *Dev Cell* 2015; 32: 82–96.
180. Zhou Y, Wang Y, Tischfield M, et al. Canonical WNT signaling components in vascular development and barrier formation. *J Clin Invest* 2014; 124: 3825–3846.
181. Corada M, Nyqvist D, Orsenigo F, et al. The Wnt/beta-catenin pathway modulates vascular remodeling and specification by upregulating Dll4/Notch signaling. *Dev Cell* 2010; 18: 938–949.
182. Corada M, Morini MF and Dejana E. Signaling pathways in the specification of arteries and veins. *Arterioscler Thromb Vasc Biol* 2014; 34: 2372–2377.
183. Qu B, Liu BR, Du YJ, et al. Wnt/beta-catenin signaling pathway may regulate the expression of angiogenic growth factors in hepatocellular carcinoma. *Oncol Lett* 2014; 7: 1175–1178.
184. Ghosh N, Yuan X, Turenius CI, et al. Automated core-panumbra quantification in neonatal ischemic brain injury. *J Cereb Blood Flow Metab* 2012; 32: 2161–2170.
185. Liu J, Wang Y, Akamatsu Y, et al. Vascular remodeling after ischemic stroke: Mechanisms and therapeutic potentials. *Prog Neurobiol* 2014; 115: 138–156.
186. Lin CY, Chang C, Cheung WM, et al. Dynamic changes in vascular permeability, cerebral blood volume, vascular density, and size after transient focal cerebral ischemia in rats: Evaluation with contrast-enhanced magnetic resonance imaging. *J Cereb Blood Flow Metab* 2008; 28: 1491–1501.
187. Krupinski J, Stroemer P, Slevin M, et al. Three-dimensional structure and survival of newly formed blood vessels after focal cerebral ischemia. *Neuroreport* 2003; 14: 1171–1176.
188. Krupinski J, Kaluza J, Kumar P, et al. Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke* 1994; 25: 1794–1798.
189. Petcu EB, Smith RA, Miroiu RI, et al. Angiogenesis in old-aged subjects after ischemic stroke: A cautionary note for investigators. *J Angiogenesis Res* 2010; 2: 26.
190. Overstreet DJ, Dutta D, Stabenfeldt SE, et al. Injectable hydrogels. *J Polym Sci Part B* 2012; 50: 881–903.
191. Atta S, Khaliq S, Islam A, et al. Injectable biopolymer based hydrogels for drug delivery applications. *Int J Biol Macromol* 2015; 80: 240–245.
192. Argaw AT, Asp L, Zhang J, et al. Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *J Clin Invest* 2012; 122: 2454–2468.

193. Zhang ZG, Zhang L, Jiang Q, et al. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J Clin Invest* 2000; 106: 829–838.
194. Valable S, Montaner J, Bellail A, et al. VEGF-induced BBB permeability is associated with an MMP-9 activity increase in cerebral ischemia: Both effects decreased by Ang-1. *J Cereb Blood Flow Metab* 2005; 25: 1491–1504.
195. Schoch HJ, Fischer S and Marti HH. Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain. *Brain* 2002; 125: 2549–2557.
196. Vaquero J and Zurita M. Functional recovery after severe CNS trauma: Current perspectives for cell therapy with bone marrow stromal cells. *Prog Neurobiol* 2011; 93: 341–349.
197. Mahmood A, Lu D, Wang L, et al. Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery* 2001; 49: 1196–1203; discussion 203–204.
198. Mahmood A, Lu D, Yi L, et al. Intracranial bone marrow transplantation after traumatic brain injury improving functional outcome in adult rats. *J Neurosurg* 2001; 94: 589–595.
199. Mahmood A, Lu D, Qu C, et al. Long-term recovery after bone marrow stromal cell treatment of traumatic brain injury in rats. *J Neurosurg* 2006; 104: 272–277.
200. Jiang J, Bu X, Liu M, et al. Transplantation of autologous bone marrow-derived mesenchymal stem cells for traumatic brain injury. *Neural Regen Res* 2012; 7: 46–53.
201. Hu W, Liu J, Jiang J, et al. [Effect of bone marrow mesenchymal stem cells on angiogenesis in rats after brain injury]. *Zhong Nan da Xue Xue Bao Yi Xue Ban = J Central South Univer Med Sci* 2016; 41: 489–495.
202. Wang Z, Wang Y, Wang Z, et al. Engineered mesenchymal stem cells with enhanced tropism and paracrine secretion of cytokines and growth factors to treat traumatic brain injury. *Stem Cells* 2015; 33: 456–467.
203. Zhang Y, Chopp M, Meng Y, et al. Effect of exosomes derived from multipotential mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *J Neurosurg* 2015; 122: 856–867.
204. Zhang ZX, Guan LX, Zhang K, et al. A combined procedure to deliver autologous mesenchymal stromal cells to patients with traumatic brain injury. *Cytotherapy* 2008; 10: 134–139.
205. SimWu H, Jiang H, Lu D, et al. Induction of angiogenesis and modulation of vascular endothelial growth factor receptor-2 by simvastatin after traumatic brain injury. *Neurosurgery* 2011; 68: 1363–1371; discussion 71.
206. Krum JM, Mani N and Rosenstein JM. Roles of the endogenous VEGF receptors flt-1 and flk-1 in astroglial and vascular remodeling after brain injury. *Exp Neurol* 2008; 212: 108–117.
207. Seghezzi G, Patel S, Ren CJ, et al. Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: An autocrine mechanism contributing to angiogenesis. *J Cell Biol* 1998; 141: 1659–1673.
208. Salvucci O, Yao L, Villalba S, et al. Regulation of endothelial cell branching morphogenesis by endogenous chemokine stromal-derived factor-1. *Blood* 2002; 99: 2703–2711.
209. Yamaguchi J, Kusano KF, Masuo O, et al. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 2003; 107: 1322–1328.
210. Xiong Y, Zhang Y, Mahmood A, et al. Erythropoietin mediates neurobehavioral recovery and neurovascular remodeling following traumatic brain injury in rats by increasing expression of vascular endothelial growth factor. *Transl Stroke Res* 2011; 2: 619–632.