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REVIEW ARTICLE

Angiogenesis and Blood-Brain Barrier Permeability in Vascular Remodeling after Stroke

Yi Yang[1,*] and Michel T. Torbey[1]

[1]*Department of Neurology, University of New Mexico Health Sciences Center; Albuquerque, New Mexico, 87131, USA*

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Abstract: Angiogenesis, the growth of new blood vessels, is a natural defense mechanism helping to restore oxygen and nutrient supply to the affected brain tissue following an ischemic stroke. By stimulating vessel growth, angiogenesis may stabilize brain perfusion, thereby promoting neuronal survival, brain plasticity, and neurologic recovery. However, therapeutic angiogenesis after stroke faces challenges: new angiogenesis-induced vessels have a higher than normal permeability, and treatment to promote angiogenesis may exacerbate outcomes in stroke patients. The development of therapies requires elucidation of the precise cellular and molecular basis of the disease. Microenvironment homeostasis of the central nervous system is essential for its normal function and is maintained by the blood-brain barrier (BBB). Tight junction proteins (TJP) form the tight junction (TJ) between vascular endothelial cells (ECs) and play a key role in regulating the BBB permeability. We demonstrated that after stroke, new angiogenesis-induced vessels in peri-infarct areas have abnormally high BBB permeability due to a lack of major TJPs in ECs. Therefore, promoting TJ formation and BBB integrity in the new vessels coupled with speedy angiogenesis will provide a promising and safer treatment strategy for improving recovery from stroke. Pericyte is a central neurovascular unite component in vascular barriergenesis and are vital to BBB integrity. We found that pericytes also play a key role in stroke-induced angiogenesis and TJ formation in the newly formed vessels. Based on these findings, in this article, we focus on regulation aspects of the BBB functions and describe cellular and molecular special features of TJ formation with an emphasis on role of pericytes in BBB integrity during angiogenesis after stroke.

Keywords: Cerebral **s**troke, vascular remodeling, angiogenesis, tight junction proteins, blood-brain barrier permeability, barriergenesis.

1. INTRODUCTION

Treatment options for ischemic stroke are very limited since ischemia-induced brain injury is a complex and multi-stage process. New approaches to the treatment and medical care of stroke are urgently needed [1]. Currently, early treatments of patients with acute ischemic stroke aimed to achieve reperfusion include intravenous (IV) and intra-arterial therapies, based on the 2019 AHA/ASA guideline [2]. However, intravenous recombinant tissue plasminogen activator (rtPA) must be given within 4.5 hours after stroke onset, while patients presenting within 0-6 hours of symptom onset (6-24 hours from last known normal for carefully selected patients) have access to mechanical thrombectomy. This limits their use to around 5% of stroke victims; thus, research has focused on novel neurorestorative approaches that could be given beyond the hyperacute phase of stroke, since the time window for therapies aimed at improving stroke recovery is far longer. In addition, treatments to improve the stroke recovery is extremely important to maximize patient's functional independence and quality of life. Neurorestorative events include neurogenesis and angiogenesis. Emerging evidence shows that angiogenesis is a key feature of ischemic stroke recovery and neuronal post-stroke re-organization [3-6]. Angiogenesis induction and new vessel generation contribute to neurorepair processes, including neurogenesis and synaptogenesis [3, 4, 6-8].

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels, which is an important process that occurs during both health and disease. During the development of brain vasculature, blood vessels form *via* two distinct processes: vasculogenesis and angiogenesis [9]. Vasculogenesis involves the proliferation and differentiation of mesoderm-derived angioblasts into endothelial cells (ECs). After the primary vascular plexus is formed by vasculogenesis, a more complex vascular network is established *via* angiogenesis. Like other vascular networks, brain vessels undergo formation, stabilization, branching, pruning and specialization. The vasculatures formed by vasculogenesis and angiogenesis are stabilized *via*

*Address correspondence to this author at the Department of Neurology; University of New Mexico Health Sciences Center; Albuquerque, New Mexico, 87131, USA; Tel: (505) 272-5987; Fax: (505) 272-8306;
E-mail: yyang@salud.unm.edu

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the recruitment of mural cells and generation of the extracellular matrix. They are then fine-tuned in response to environmental cues from neighboring cells before finally acquire featuring suitable for the brain function [9, 10].

After the stroke, ischemic penumbra tissue releases angiogenic factors that induce proliferation of ECs and migration of endothelial progenitor cells for the formation of new blood vessels. Factors released by ECs *in vitro* trigger neural stem cell proliferation [11]. The leading process of the migrating neural progenitor cells (NPCs) is closely associated with blood vessels, suggesting that this interaction provides directional guidance to the NPCs. These findings suggest that blood vessels play an important role as a scaffold for NPCs migration toward the damaged brain region. In addition, evidence showed that between 30 and 90 days of reperfusion, the density of new vessels in the peri-infarct regions regressed significantly [12]. Therapeutic angiogenesis may remain insufficient if it does not prevent the regression of established vessels in the peri-infarct regions [13]; therefore, angiogenesis could be a key therapeutic target for stroke recovery [3].

Nevertheless, current pharmacological and other approaches to enhance angiogenesis may have dual natures since some growth factors involved in post-ischemic angiogenesis are faced with challenges that may have detrimental adverse effects and worsen stroke outcome [1, 14-16]. Ischemia-induced cerebral angiogenesis can be boosted by a huge variety of agents, stem cells, as well as other manipulations in experimental models of rodent stroke. The literature reviewed by Beck *et al* and Font *et al* provides promising evidence supporting stimulating post-ischemic angiogenesis to improve neurological function [1, 14]. They also presented information demonstrating that almost all treatment strategies are not angiogenesis-specific, rather, strategies influence other post-ischemic events too, such as vascular permeability and inflammation, and enhancing angiogenesis, and may have detrimental effects in the brain by increasing blood-brain barrier (BBB) permeability [5, 17]. Increased angiogenic growth factors like vascular endothelial growth factor (VEGF) and its receptors were seen in human tissue after ischemic stroke [18]. Treatment of stroke with VEGF is a double-edged sword due to VEGF-induced new vessels are immature and leaking [19], which might exacerbate edema, for example, a major and often life-threatening complication of various brain injuries [1, 14-16].

The central nervous system (CNS) requires precise control of their bathing microenvironment for optimal function, and an important element in this control is the BBB [20]. The BBB is formed by the endothelial cells lining the brain microvessels, under the inductive influence of neighboring cell types within the neurovascular unit (NVU), the milieu of neurons, astrocytes (AC), pericytes (PC), microglia and other components of the brain parenchyma that communicate with ECs (Fig. **1** [21]). The endothelium forms the major interface between the blood and the CNS; by a combination of low passive permeability and presence of specific transport systems, enzymes and receptors regulate molecular and cellular traffic across the barrier layer. ECs are interconnected by tight junctions (TJ) that reveal a unique morphology and biochemical composition of brain vasculature. Tight junction proteins (TJP) are integral transmembrane proteins that form the TJ strands between ECs. TJPs play an important role in establishing fully-functional BBB barrier function that is critical in the regulation of permeability of brain microvessels. This highlights the significance of translational angiogenesis therapy: facilitation of functional BBB and determination of appropriate points of intervention for functional vascular remodeling during stroke recovery.

2. BBB MATURATION AND MAINTENANCE DURING EMBRYONIC DEVELOPMENT AND ADULTHOOD

2.1. Cellular Barrier-Neurovascular Unit (NVU)

The structural and functional integrity of the brain depends on the delicate balance between substrate delivery through blood flow and energy demands imposed by neural activity. BBB plays a major role in controlling the neuronal microenvironment. Cerebral vessels have extremely specialized characteristics that allow them to form the BBB. ECs, inter-endothelial tight junctions, the basal lamina, perivascular ACs and PCs, and microglia are jointly referred to as the BBB or, more recently, as the NVU (Fig. **1**) [9, 20, 22-25]. CNS ECs have specific transporter and receptor proteins to control entry and exit of metabolites across cells (transcellular transport) and high electrical resistance TJs to limit movement between adjacent cells (paracellular transport); and low levels of transcytotic vesicles compared to peripheral endothelia and an absence of fenestrae (small pores that allow rapid passage of molecules in peripheral endothelial cells) [26]. Around the cerebral ECs is a basal lamina composed primarily of laminin, fibronectin, and heparan sulfate. The basal lamina provides a structural barrier to the extravasation of cellular blood elements and anchors ECs and ACs [27]. Anatomically, most ACs have stellate shapes containing multiple processes. These ACs expand toward neurons and vessels. The processed ends of the ACs, so-called endfeet, contact the vessel wall and form large compartments that enclose most blood vessels of the brain and play a decisive role in the maintenance of the barrier properties of the brain microcapillary ECs. PCs share a basement membrane with ECs and form direct synaptic-like peg-socket focal contacts with endothelium through N-cadherin and connexins (such as connexin 43, CX43), allowing exchanges of ions, metabolites, second messengers, and ribonucleic acids between the two cell types. PCs play important roles in maintaining BBB integrity, aiding in angiogenesis and microvascular stability [28-33]. Low rate of vesicular transport (transcytosis) has been identified as one of the two unique properties of CNS ECs that maintain the restrictive quality of the BBB [34]. Loss of PCs increased transcytosis and enhanced BBB permeability in mouse model of intracerebral hemorrhage [35] and in pericytic-laminin conditional knockout mouse [36]. Microglia, the brain resident immune cells, are activated in response to injury and orchestrate the brain's inflammatory response [37].

2.2 Molecular Barrier-endothelium Tight Junction Proteins

The BBB proper is made up of ECs interconnected by TJs that reveal a unique morphology and biochemical composition of brain vasculature. Cerebrovascular endothelial

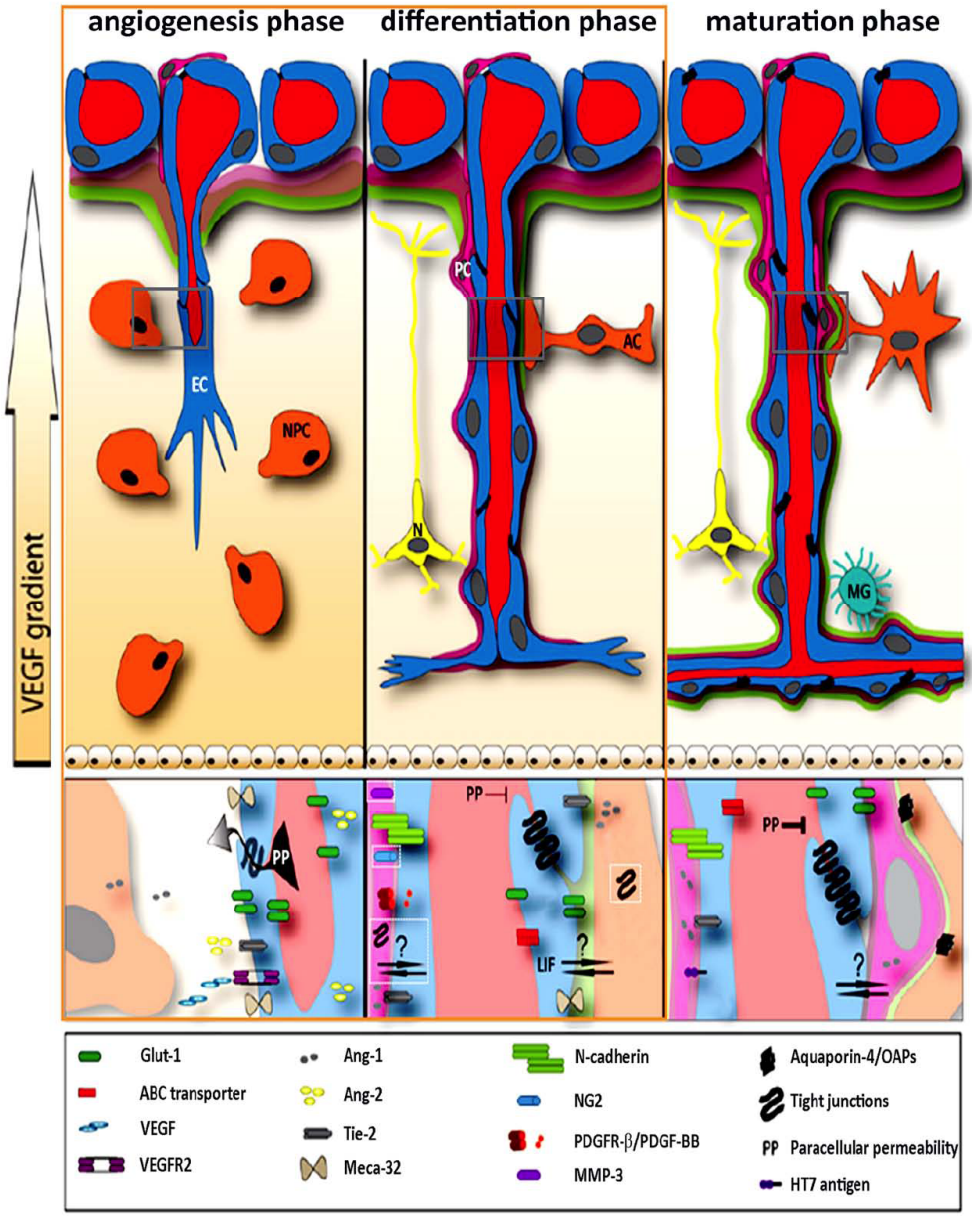


Fig. (1). Differentiation of the blood-brain barrier (BBB). ***Angiogenesis phase***: Vascular sprouts radially invade the embryonic neuroectoderm towards a concentration gradient of VEGF-A, which is produced by neuroectodermal cells located in the ventricular layer. VEGF-A binds to its endothelial receptor, the receptor tyrosine kinase flk-1/KDR/VEGFR2. The EC specific receptor tyrosine kinase Tie-2 and its ligand Ang-1 are involved in angiogenic sprouting early during embryogenesis. The cerebral ECs show Glut-1 evenly distributed and the MECA-32 antigen is highly expressed, contributing to poor barrier characteristics and high paracellular permeability (PP). ***Differentiation phase***: The phenotype of cerebral ECs changes such that they downregulate the expression of the MECA-32 antigen. Glut-1 is now enriched on the abluminal surface of the endothelium. The TJs become complex and thus tight for small polar molecules. Phenotypic changes of ECs are accompanied by their close contact with PCs and astroglial cells. Recruitment of PCs along the differentiating BBB vessels is ensured by several mechanisms. PDGF-BB produced by ECs binds to its receptor PDGFR-β on PCs; N-cadherin enriched at sites of PC-EC contact; Ang-1 expressed by PCs binds to the endothelial receptor tyrosine kinase Tie-2. ECs produce leukemia inhibitory factor (LIF), inducing the maturation of ACs *via* the LIF-Rb. Furthermore, increased oxygen level and EC-derived PDGF-BB lead to an upregulation of SSeCKS in ACs that in turn upregulates Ang-1. ***Note***: During vascular remodeling after stroke, the newly formed vessels in peri-infarct regions demonstrate cellular features as *angiogenesis phase* and *differential phase* as shown above. In the differential phase, the only TJP that formed the TJ strands between ECs is claudin-5, while occludin and ZO-1 are expressed by ACs and PCs. The vascular PCs also express NG2, MMP-3, and other angiogenic factors. The molecular mechanisms involved in crosstalk between ECs, ACs and PCs required for TJ formation and maturation in the newly formed vessels remain under studied. ***Maturation phase***: Despite the fact that the cerebral ECs form the barrier proper, close contact with PCs, ACs and maybe neuronal cells is required for the maintenance of the BBB. The molecular mechanisms involved in this crosstalk required for BBB maintenance in the mature CNS remain unknown to date. Modified from Stefan Liebner *et al*., Int. J. Dev. Biol. 2011, 55, 467-476. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

TJs join ECs together to form the first barrier that restricts molecules from moving between the blood and the brain [38-40]. The TJPs, critical for the maintenance of the barrier function [41, 42], assemble in the clefts of the cerebral blood vessels to restrict transport across the BBB. Several TJPs have been isolated and cloned. Transmembrane TJPs consist of three integral proteins: claudins, occludin, and junctional adhesion molecules (JAM). Occludin is a ~ 65 kDa phosphoprotein that regulates paracellular permeability [38, 43-46]. Claudins are ~ 22 kDa phosphoproteins that are thought to help maintain high transendothelial electrical resistance. The extracellular loops of occludin, claudins, and JAM originating from neighboring cells form the paracellular barrier of the TJ, which selectively prevents most blood-borne substances from entering the brain. In rodents and adult human brains, claudin-1/3, claudin-5, and occludin have been found to be present in brain endothelial tight junctions forming the BBB. In the brain, claudin-5 is the only EC-specific component of TJ strands [47], appearing to be the most important structural components of BBB TJs [48]. Studies showed controversial roles of claudin-1 in BBB TJ complex organization, helping to form secondary astrocyte/glial barriers to protect the brain from endothelial breakdown [48, 49], or unessential for BBB TJ complex function under physiological conditions [50, 51]. Recent study suggested that claudin-1 results in limited brain endothelial barrier post-stroke recovery, partially due to restricted claudin-5 expression [52]. At the level of the ECs, which are directly in contact with the systemic circulation, the TJPs form a seal that blocks the entry of large proteins and charged molecules [27]. TJs are dynamic structures and TJPs are subject to changes in expression, subcellular location, post-translational modification, and protein-protein interactions under both physiological and pathological conditions [24, 46, 53-60]. Claudins and occludin are close to the blood and junctional adhesion molecules (JAM) and are deeper inside the endothelial cell clefts [38-40, 61, 62]. Zona occludens (ZO)-1 and -3 are cytoplasmic tight junctional accessory proteins, which connect TJs to the actin cytoskeleton [44, 63, 64]. Occludin, claudin-5 and ZO-1, which are the main structural barrier proteins, are considered sensitive indicators of normal and disturbed functional states of the BBB [41]. Disruption of BBB TJ by disease or drugs can lead to impaired BBB function and thus compromise the CNS. Regulation of the three TJPs are essential for the maintenance of the BBB permeability.

2.3. Maturation and Regulation of the BBB Following Angiogenesis

Following angiogenesis of the primary vascular network, brain vessels experience a maturation process, known as barriergenesis, *via* the recruitment of mural cells and the generation of the extracellular matrix, in which the BBB is formed [9, 32, 65, 66]. Several excellent reviews have detailed the cellular and molecular basis of brain angiogenesis and barriergenesis in BBB differentiation and maturation [9, 14, 21, 26, 28, 67]. Fig. **1** modified from the review by Liebner *et al.*, summarized the three phases of BBB conduction and differentiation during development [21] and stroke-induced angiogenesis [68].

ACs have been well documented to induce barrier properties in brain ECs both *in vitro* and *in vivo* [65, 69-71]. ACs are generally thought to be an essential for both the TJ formation and the maintenance of the TJPs in the paracellular spaces between vascular ECs in CNS [22, 72-74]. The period of AC differentiation coincides with that of BBB formation. Differentiating ACs may extend their processes to the vessel wall, thereby sending signals to acquire BBB properties [9, 21]. It is also clear that if brain ECs are cultured *in vitro*, they lose certain BBB characteristics, such as high TEER, the membrane localization of TJPs, and transporter expression; while most BBB characteristics can be regained *via* co-culture with ACs or treatment with AC conditioned medium [9]. Astrocytic laminin polarizes astrocytic endfeet, inhibits PC differentiation, and induces and maintains TJP expression in ECs [75]. However, the role of ACs in BBB development in the immature brain still controversial, for example, the study found that ACs are not present in the developing brain during the time of initial vascularization lends support to a role for ACs in TJ maintenance and not formation [76].

On the other hand, evidence has accumulated to show that PCs have a key role in the development of cerebral vasculature and control key neurovascular functions and neuronal phenotype in brain [9, 32, 70, 77-80]. PCs also play a key role in the cell crosstalk processes during the development of cerebral vasculature and regulation of BBB function in brain diseases [9, 32, 42, 65, 78, 81-83]. PC recruitment is crucial to establishing BBB characteristics. Loss of PCs in platelet-derived growth factor receptor-β (*Pdgfr*β) knockout mice exhibit age-dependent BBB dysfunction resulting from reduced TJ protein expression, whereas young adult mice with hypomorphic alleles of *Pdgf* b display defects in BBB integrity as a result of increased rates of endothelial transcytosis [26, 30, 32]. During development, when ECs invade the central nervous system, PCs are also recruited to the developing vessels. This occurs more than a week before AC generation, suggesting that PCs are critical for BBB integrity [76]. Intriguingly, PCs not only regulate mature BBB integrity, but also function to guide astrocytic foot processes to cerebral vessel walls and mediate the polarization of astrocytic end-feet [26]. In addition, PCs have been shown to decrease with age. A primary loss of PCs may lead to two parallel pathways of neurodegeneration, BBB breakdown and hypoperfusion, which lead to secondary neurodegenerative changes, paralleling an increase in BBB permeability [32].

Studies show that PCs function at the BBB formation and maintenance in several ways: (1) control BBB integrity by regulating the orientation and abundance of endothelial TJPs and AJPs; (2) regulate the stability and architecture of newly formed cerebral microvessels; (3) contribute to secretion and regulate the levels of extracellular matrix proteins forming the basement membrane; (4) regulate capillary diameter and blood flow; and (5) provide clearance and phagocytotic functions in the brain [29, 30, 32, 33, 84, 85].

3. ANGIOGENESIS AFTER STROKE

3.1. Angiogenesis Processes after Stroke

Three processes are implicated in neurorepair: angiogenesis, neurogenesis, and synaptic plasticity. We and others

have demonstrated that some vessels remain in the lesion areas up to 7 days after reperfusion, which provides the cellular basis to trigger angiogenesis [68]. At 3 weeks of reperfusion, we observed that proliferating PCs closely surrounded the increased number of regrowing ECs seen in the peri-infarct areas in a rat model of middle cerebral artery occlusion. These vascular-associated PCs are neural/glial antigen 2 (NG2) and Ki67 positive, suggesting that they are in angiogenic status and contribute to new vessel formation [86-92]. After the stroke, increased vascular remodeling in rat brain is found in the areas of newly-born neuroblasts which migrate from the subventricular zone to the peri-infarcted cortex [93]. ECs are primary effector cells of the angiogenic response after ischemic injury, followed by the PCs and smooth muscle cells. Angiogenesis involves the proliferation of ECs and sprouting of the vessels that eventually increase vascular density [68]. The proliferation of ECs after cerebral ischemia has been extensively demonstrated [6, 8, 88, 94-96]. Studies have shown that stroke-induced active angiogenesis takes place at 3-4 days following the ischemic insult and continues more than 21 days [1, 12, 14, 97, 98]. ECs surrounding the infarcted brain area start to proliferate as early as 12–24 hours following vessel [14, 28, 99-101]. This in turn already leads to an increase of vessels in the peri-infarcted region 3 days following the ischemic injury. A study on temporal angiogenesis and related gene expression in mouse brain demonstrated that vessel proliferation continued more than 21 days following experimental cerebral ischemia [100]. Using human brain samples, studies demonstrated that active angiogenesis takes place at 3–4 days following the ischemic insult [98]. Patients who survived from several days to weeks after cerebral stroke showed a positive correlation between microvessel density and survival [98].

3.2 BBB Permeability of Angiogenic Vessels

Using a 90 min MCAO in spontaneously hypertensive rats (SHRs), we investigated angiogenesis from 24 hours to 3 weeks after reperfusion [68]. An increase of newly formed vessels was observed in the peri-infarct region 3 weeks following the ischemic injury, which is consistent with other reports [14, 99, 100]. We used both MRI and histological techniques to determine functional neurovascular remodeling and BBB integrity (Fig. **2**). MRI T2 and ADC maps showed increased hyperintensity in the infarct area (inf) compared to the peri-infarct regions (Fig. **2A**). The permeability coefficient maps reconstructed from data acquired by dynamic contrast-enhanced MRI (DCEMRI) showed increased BBB transfer rate (Ki) and plasma volume (Vp) in the peri-infarct areas, which was absent in the infarct core [102, 103]. Arterial spin labeling (ASL), used to measure cerebral blood flow (CBF) [104], showed perfusion in the ischemic hemisphere with very low signal in the core infarct area, while the peri-infarct region showed hyperperfusion compared with the contralateral normal hemisphere. RECA1 (a marker of ECs) immunostaining showed increased density of new vessels in the peri-infarct area, corresponding to the elevated BBB transfer rate, plasma volume, and arterial blood perfusion. These data indicated that blood flow returned to the newly formed vessels, suggesting functional vascular remodeling with higher BBB permeability at 3 weeks after stroke.

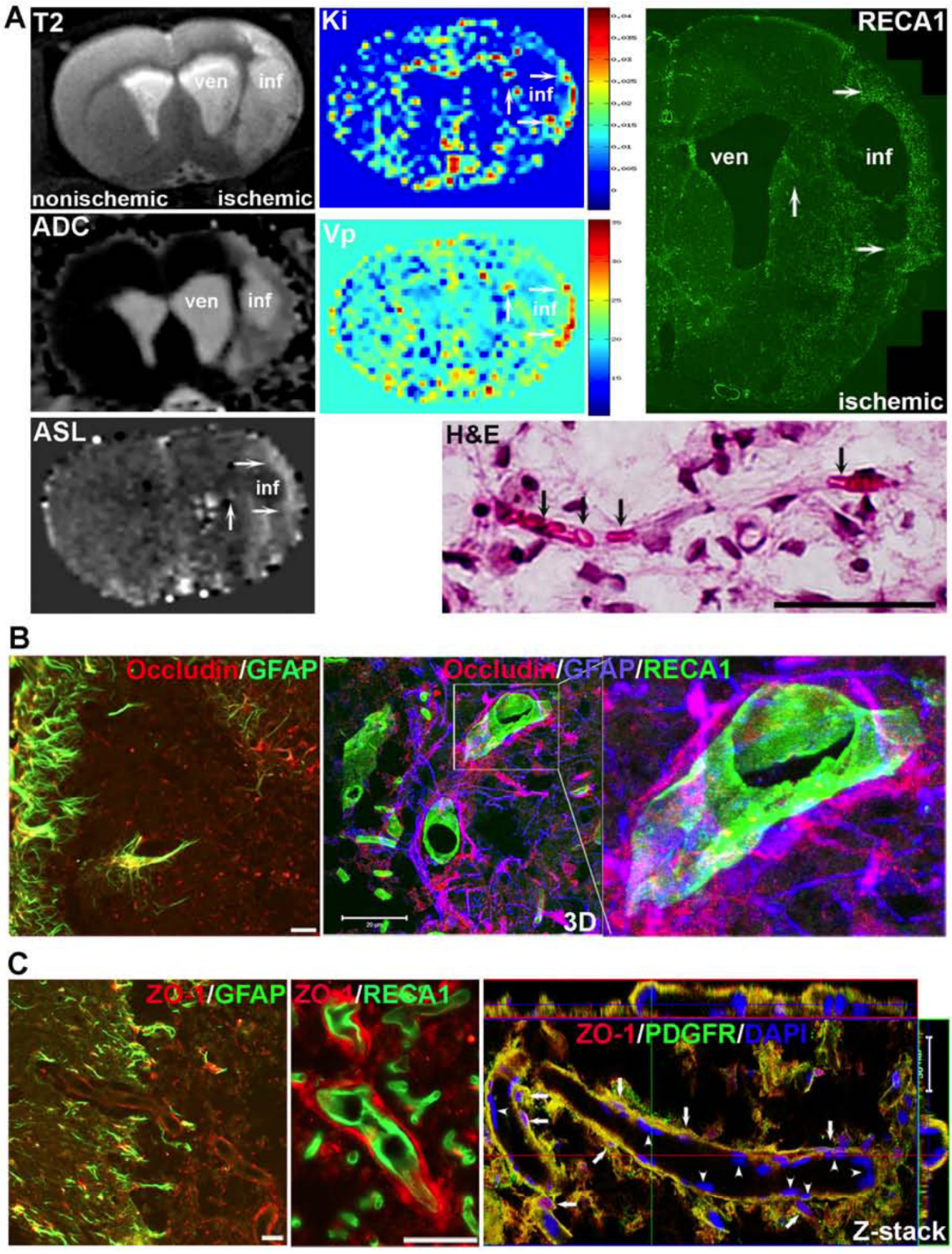
3.3. Tight Junction Formation in Angiogenic Vessels

At 3 weeks after stroke, we observed that TJPs, occludin, claudin-5, and ZO-1, were observed in NVU cells in the peri-infarct regions (Fig. **2B**, **C**, and **D**) [68]. Only claudin-5, which disappeared in BBB from 24 hours to 7 days after stroke, reappeared in the newly formed vascular ECs. The other major TJPs, occludin and ZO-1, were absent from ECs and prominent in the GFAP-positive reactive ACs, which extended processes to closely encapsulate nearby ECs. A striking collection of NG2-positive PCs entirely surrounded vascular ECs and produced ZO-1. The TJPs normally seen within ECs where they form the first barrier to blood-borne substances were expressed by PCs and ACs, which failed to provide a barrier to Evans blue or Gadolinium, which consistent with the MRI data of high BBB permeability. These findings suggest that the TJPs in perivascular cells do not form a functional barrier and that strongly indicates that these new vessels lack barrier properties. Since PCs and ECs have direct synaptic-like peg-socket focal contacts and gap junctions formed with CX43, which allow the exchange of molecules between PCs and ECs [33, 74, 105-107], whether the TJPs move into the ECs or are made de novo will need further study.

Brain PCs and ACs play a key role in the development of cerebral vasculature and regulation of BBB function in brain diseases [9, 30, 32, 42, 65, 78, 81, 82, 108], yet, the roles of these NVU cells in neurovascular remodeling during recovery stage after stroke remains obscure. We found that PCs and ACs act spatiotemporally contributing to extraendothelial TJP formation in BBB restoration during recovery at 3 weeks after stroke [68]. The contributions of ACs and PCs to BBB restoration in stroke-induced angiogenesis during recovery are still under studied [107, 109].

3.4. Microglia Activation and Angiogenesis

Microglia are the resident macrophages that control the immune response in the brain. One striking feature of microglial cells is their rapid activation in response to minor pathological alterations/infections in the CNS [110, 111], which orchestrates the brain's inflammatory response [37]. Microglia can assume different activated phenotypes depending on the activating stimulus [112, 113]. Microglia rapidly develop a pro-inflammatory phenotype in response to acute brain injury; meanwhile, activation of microglia also present reparative and anti-inflammatory roles through a regulatory/homeostatic phenotype, which facilitates recovery of injury [114-117]. The pro-inflammatory response includes that microglia become activated, obtain an amoeboid morphology, and release inflammatory cytokines. In the regulatory/homeostatic phase, microglia have an enhanced capacity for phagocytosis and produce anti-inflammatory mediators, which help to terminate the inflammatory response and promote tissue repair and remodeling. During the recovery of stroke, microglia in anti-inflammatory phenotypes play a key role in the promotion of neurovascular remodeling through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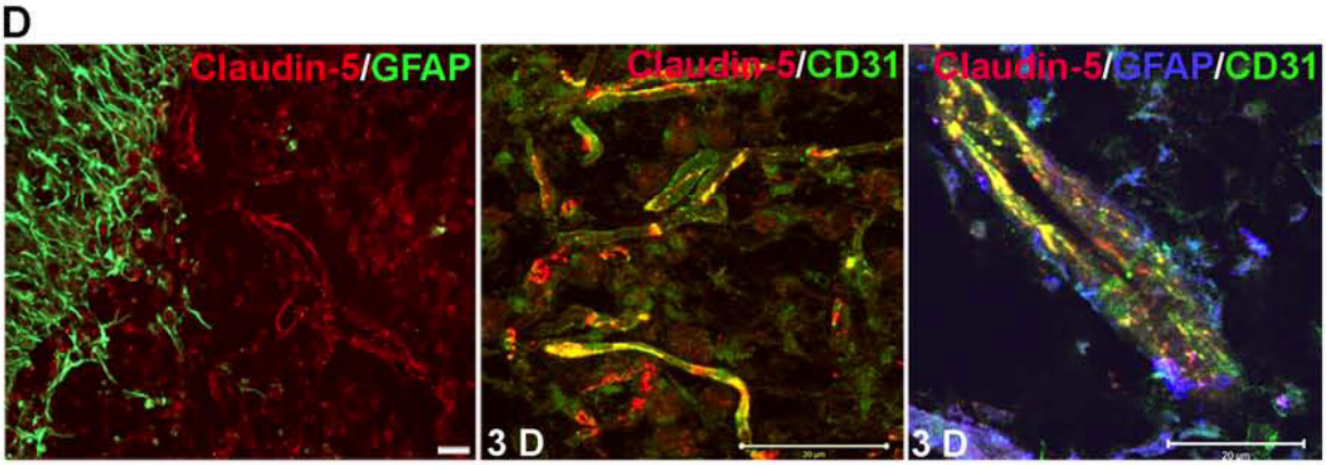

Fig. (2). Blood flow, BBB permeability, and expression of TJPs in the new vessels within peri-infarct regions 3 weeks of reperfusion in spontaneously hypertensive rat subjected to transient middle cerebral artery occlusion. (**A**) Hyperintensive areas in the anatomical T2 image and ADC map show the lesion extent and tissue ischemia. ven: Ventricle; inf : Core infarct area. Color-coded permeability coefficient maps reconstructed from DCEMRI data demonstrate the regions of high (red) and low (blue) permeability. The parametric image K_i map represents BBB transfer rate. The parametric image V_p map represents plasma volume. Elevated values of K_i and V_p are located in the vicinity of core infarct area (arrows). There are no signals of K_i and V_p in the core infarct area. The color scales used for the permeability and plasma volume signal intensity. ASL map shows higher CBF in peri-infarct areas (arrows). RECA1 immunostaining shows the increased density of new vessels in the peri-infarct area (arrows), corresponding to the elevated BBB transfer rate, plasma volume and blood perfusion (arrows). H&E staining shows red blood cells located inside of the new microvessels (arrows). (**B**) Left panel: double-immunostaining of occludin (red) with astrocytes (GFAP, green) shows that occludin was co-localized with reactive astrocytes adjacent to or within the peri-infarct region. Middle and right panels: triple-immunostaining of occludin (red) with ACs (blue) and ECs (green). The 3D confocal images demonstrate that ACs expressing occludin (shown in purple when co-localized) with end-feet closely surrounding vessels. (**C**) Double immunostaining of ZO-1 with astrocytes, endothelial cells, and PCs (PDGFR), respectively. ZO-1 co-localized with reactive astrocytes (GFAP) but not with ECs (RECA1). Z-stack confocal image shows PCs (green) surrounding vessels express ZO-1 (arrows). Arrowheads indicate DAPI-stained endothelium. (**D**) Double immunostaining shows no co-localization (left panel) between ACs (green) and claudin-5 (red). The 3D confocal image (middle panel) demonstrates that claudin-5 was co-localized with ECs (CD31, green) of microvessels within the peri-infarct region. Confocal image (right panel) of triple-immunostaining shows that ACs (blue) surround a vessel with claudin-5 in endothelial cells (green). Scale bars=20 or 50 μm. Cited from Yi Yang *et al*., Journal of Cerebral Blood Flow & Metabolism (2013), 1104-1114. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

the release of growth-related proteins and cytokines from peripheral and resident immune cells [37, 112, 113, 117, 118]. We also found that in the peri-infarct areas a new population of active regulatory-phenotype microglia expressing TGF-β and IL-10 is involved in the promotion of TJP expression and BBB integrity at 4 weeks after stroke [119].

4. POTENTIAL MOLECULAR AND CELLULAR TARGETS TO FACILITATE BBB MATURATION AFTER STROKE.

Cerebral ischemia-induced angiogenesis is a complex and dynamic process involving the different vascular components such as ECs, vascular ACs and PCs, fibroblasts, smooth muscle cells, and the extracellular matrix [120-122]. Understanding the cellular and molecular mechanisms regulating TJ formation and BBB integrity during angiogenesis after stroke will be key to the understanding of angiogenesis and is crucial for our knowledge of effective treatment of stroke. A detailed analysis of the contribution of the different vascular components in the angiogenesis, especially in BBB restoration, will therefore be valuable for therapeutic angiogenesis in stroke.

4.1. The BBB Functions Depend on the Crosstalk between PCs and ECs

The cell-cell signaling in the NVU is known to have key roles in how the brain responds to ischemic injury after stroke. Brain PCs and ACs play a key role in the development of cerebral vasculature and regulation of BBB function in brain diseases [9, 30, 32, 42, 65, 78, 81, 82]. The roles of these NVU cells in neurovascular remodeling during the recovery stage after stroke remains obscure. We found that the proliferating vascular PCs participate in the neovascularization in the peri-infarct areas after stroke by expressing TJPs, VEGF, and matrix metalloprotease (MMP)-3. There is an overexpression of NG2 in vascular-associated PCs at 3 weeks after stroke in rat, which is spatiotemporally expressed with MMPs, VEGF, and TJPs [68]. Along with its ability to reveal the activated/angiogenic state of microvessels, the PC-derived NG2 is an important factor in promoting EC migration and morphogenesis during the early stages of neo-vascularization [123]. Pathologic angiogenesis can be reduced by targeting PCs *via* the NG2 proteoglycan. In NG2 knock-out mice, proliferation of both PCs and ECs in retina in response to hypoxia is significantly reduced [92]. There

are several possible mechanisms by which NG2 regulates angiogenesis: NG2 has been shown to bind directly to basic fibroblast growth factor and platelet-derived growth factor AA (PDGF-AA), and may enhance interaction of these growth factors with cell surface receptors [123].

4.2 NG2-Dependent Crosstalk between PCs and ECs

NG2 is a prominent component of activated PCs, not ECs, in both normal and pathological microvessels [124]. The NG2 proteoglycan is expressed by microvascular PCs in newly formed blood vessels. Studies on cancer angiogenesis have demonstrated that NG2 plays important roles in PC recruitment and interaction with ECs during microvessel development [92, 123, 125-128]. Altered interactions between these key microvessel components lead to deficits in both PC and EC maturation [129] and these changes at the cellular and structural levels result in decreased tumor vessel patency and increased tumor vessel leakiness. Recently, using Cre/lox technology created $NG2^{fl/fl}$:pdgfrβ-Cre mice and NG2 siRNA in human microvascular PCs [124] to specifically ablate NG2 in PCs demonstrated a deficit of tumor blood vessel structure and function and reduction of endothelial junctions, which increased endothelial permeability *in vivo* and *in vitro*. These findings suggest that NG2-positive PCs may play a critical role during the formation of TJ in ECs during vascular remodeling [68, 86, 91]. Treatments targeting on the NG2-PCs pathway may provide angiogenic therapeutic strategies to promote BBB maturation in the new vessels and improve stroke outcome.

The 3-hydroxy-3- methylglutaryl coenzyme A reductase inhibitors (statins) are potent inhibitors of cholesterol biosynthesis used to treat hypercholesterolemia and prevent recurrent stroke. The five statins commonly used in clinical practice are atorvastatin, fluvastatin, lovastatin, rosuvastatin, and simvastatin [130, 131]. Beyond their lipid-lowering effects, increasing evidence indicates that statins, particularly atorvastatin, are neuroprotective in several brain injuries, including stroke [132-136]. Patients who took statins prior to the onset of stroke demonstrated significantly decreased mortality and improved outcome [83, 137]. Statins promote angiogenesis in ischemic stroke. The effect of statins on the induction of angiogenesis is dose dependent and biphasic, a pro-angiogenic effect at low doses and anti-angiogenic and pro-apoptotic effect at high doses [1, 17, 138, 139]. They are relatively safe, orally available agents that may acquire novel therapeutic indications through their angiogenic modulating effects [1, 17, 130, 140-142]. Recent preclinical and clinical trial studies show that atorvastatin blunts cerebral cavernous angioma (CA) lesion development, hemorrhage, and rebleeding in stabilizing CAs after a symptomatic hemorrhage, through inhibiting RhoA kinase [143]. Statins at higher concentrations have been demonstrated to up-regulating endothelial NO synthase (eNOS) gene expression and direct activation of eNOS in endothelial cells [133, 136]. The low dose-dependent proangiogenic effects of atorvastatin correlated with the activation of the Pl3k–Akt pathway [144]. Statins increase brain EC expression of Gas6/Axl and thereby activate the PI3K–AKT pathway, which regulates endothelial cell survival, proliferation and migration, and increases angiogenesiss [145-148]. Statins also increase vascular stabilization and decrease BBB permeability after stroke [149]. The signaling pathways that NG2 regulates the interaction between PC and EC are involved in α3β1 integrin mediated PI3K/AKT and Ras/ERK1/2 in EC survival and proliferation [123, 124, 128]. Very interesting, the dose-dependent proangiogenic effects of atorvastatin correlated with the activation of the Pl3k–Akt pathway [144], suggesting a role of the NG2 signaling pathway in promoting BBB maturation in the new vessels during stroke-induced angiogenesis in brain. This suggested that a therapeutic potential of treatment with statins during stroke recovery could stabilize the newly formed vessels and facilitate maturation of BBB in stroke recovery.

4.3. Sphingosine 1 Phosphate (S1P) Signaling Regulates BBB Integrity

S1P is a bioactive sphingolipid that, acting through its five G-protein coupled S1P-receptors ($S1PR_{1\text{-}5}$), modulates a large diversity of biological mechanisms (cell proliferation, survival, cytoskeletal reorganization, migration)[150, 151], including BBB integrity [152, 153]. Of the five S1P receptors, $S1PR_{1,2,3}$ are expressed in ECs, $S1PR_1$ is expressed in ACs and $S1PR_3$ in PCs [154, 155], the cell types that are the major cellular barrier components of the BBB. S1P induces changes in BBB function mediated through both $S1PR_1$ and $S1PR_2$ [156]. $S1PR_1$ signaling plays important roles in modulating vascular barrier function, vascular tone, and the regulation of lymphocyte trafficking [157, 158]. $S1PR_1$ signaling in brain vascular ECs is also needed for TJ complex assembly and the normal function of the BBB [154, 159]. Activation of $S1PR_1$ was found to increase endothelial barrier integrity by modulating EC cytoskeletal forces [154, 160] *via* the PI3K/Akt/Rac signaling pathway [161-164]. $S1PR_1$ promotes the expression of TJPs and AJPs in ECs and ACs, enhances BBB integrity [151], reduces vascular leakage, limits leukocyte infiltration, and inhibits astrogliosis [154, 160, 165, 166]. $S1PR_3$ cooperates with $S1PR_1$ in stimulating migration of EC progenitors and EC proliferation, thereby contributing to vasculogenesis and angiogenesis [167]. $S1PR_2$ plays pivotal roles in CNS autoimmunity, cell differentiation, and enhances BBB permeability and leukocyte entry [156, 168, 169] *via* Rho/ROCK pathways and the inhibition of $S1PR_1$ [161, 163, 170]. $S1PR_2$ activity impairs remyelination, and enhances BBB leakage and demyelination in animal models of multiple sclerosis [171]. $S1PR_3$ cooperates with $S1P_1$ in stimulating the migration of EC progenitors and EC proliferation, thereby contributing to vasculogenesis and angiogenesis [154, 172]. SEW2871 is a selective agonist for $S1PR_1$ [173, 174], therapeutic manipulation of the S1P pathway using SEW2871 has shown promise in preclinical models of Alzheimer's disease, traumatic, and acute stroke brain injury[160, 175, 176]. Therapy for CNS diseases [175, 177-180] now includes the use of FDA approved fingolimod to target the $S1PR_{1,3\text{-}5}$ pathway for multiple sclerosis treatment [175, 181]. Pilot clinical trials examining the efficacy and safety of fingolimod in patients showed that fingolimod enhances the efficacy of alteplase administration in acute ischemic stroke without serious adverse events recorded [182, 183]. Targeting endothelial S1P-S1PR signaling pathway during stroke recovery may promote vascular TJ formation and BBB integrity in angiogenic vessels.

4.4. Microglial Alternative Activation Involves in BBB Integrity in Stroke

Ischemic stroke induces a cascade of metabolic and inflammatory consequences that extend from the core of the infarct into the penumbra. Activated microglia have been the target of experimental and clinical studies focusing on neuroinflammation after focal cerebral ischemia [184, 185]. Neuroinflammation accompanies microglial immunophenotype changes over time from pro-inflammatory to regulatory/homeostatic (anti-inflammatory) after ischemic stroke, with one phenotype predominating over another in a time-dependent manner [112, 117, 186]. The pro-inflammatory response of microglia/macrophage includes increased expression of the cytokines TNF-α and IL-1β, while in the regulatory/homeostatic phase, expression switches to the anti-inflammatory or reparative cytokines, such as IL-10 and TGF-β, and their co-stimulatory proteins [37, 114, 187]. The location of the microglia (core vs. penumbra) with respect to the infarct is the critical determinant of that phenotype [117]. After 90 minutes of transient ischemia, activated microglia increased in number from 3.5 to 7 days in the peri-infarct [188]. Studies indicated that the peri-infarct is dominated by proinflammatory, proliferating, and activated microglia that increase in number over the first week after ischemia [139, 189-192]. Importantly, these spontaneous repair-related molecular and cellular changes in brain after stroke can be influenced by several factors including drug treatment [193, 194]. Activated microglia can be seen in ischemic hemispheres as early as 24 h reperfusion and reach a peak at 1 week that extends to 4 weeks [119]. At 4 weeks after stroke, the active microglia surrounding and within the peri-infarct areas expressed both pro-inflammatory factors (TNF-α and IL-1β) and anti-inflammatory factors (TGF-β and IL-10) [119, 195]. TGF-β signaling pathway has been demonstrated to involve in the regulation of BBB functional integrity and TJP expression during inflammation, and may lower the BBB permeability [58, 196-198]. We found that treatment with minocycline at an early stage of stroke promotes increased expression of TGF-β and IL-10, and facilitates the shifting of microglia functional activity from pro-Inflammation to anti-inflammation during recovery [119]. Besides the active microglia, the proliferating microglia-like pericytes, closely surrounding angiogenic vessels in the peri-infarct areas, also expressed TGF-β. Importantly, an enhanced restructuring of the BBB TJPs by minocycline provide functionality to the BBB despite an immature endothelium during neurovascular remodeling during stroke recovery [119]. This suggests a novel treatment potential for facilitating neurological recovery by influencing spontaneous repair-related alteration of microglia activation in the brain after stroke with medicine exposure.

CONCLUSION AND FUTURE DIRECTIONS

In summary, clinical outcomes of ischemic cerebral stroke are poor, and treatment for recovery improvement is of utmost importance. Angiogenesis and re-vascularization are the main repair processes following stroke. Promising evidence suggests that stimulating post-ischemic angiogenesis can improve neurological function [1, 14]. Induction of functional angiogenesis in the brain after stroke poses a number of particular challenges, including (a) functional BBB barriergenesis; (b) correct timing of the use of therapeutic agents; and (c) localization of the pro-angiogenic signal to areas of injury. To take advantage of angiogenesis as a therapeutic concept for stroke treatment, the knowledge of the precise molecular and cellular mechanisms is vital. It is necessary to define and optimize restorative therapies by characterizing the cellular and molecular mechanisms through which angiogenesis occurs and the functional BBB forms in response to spontaneous and therapy-induced vascular remodeling. TJPs play an important role in allowing these newly formed vessels to establish fully-functional BBB and promoting BBB restoration coupled with speedy angiogenesis, which may improve functional outcome and recovery following a stroke injury. Studies examining whether mature BBB is restored in stroke-induced re-vasculature and determining appropriate points of intervention for the facilitation of functional BBB restoration will provide critical preclinical information that addresses the major clinical challenge to accelerate cerebral angiogenesis without exacerbating brain edema and inflammation.

CONSENT FOR PUBLICATION

Not applicable.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Font, M.A.; Arboix, A.; Krupinski, J. Angiogenesis, neurogenesis and neuroplasticity in ischemic stroke. *Curr. Cardiol. Rev.,* **2010**, *6*(3), 238-244. http://dx.doi.org/10.2174/157340310791658802 PMID: 21804783

[2] Powers, W.J.; Rabinstein, A.A.; Ackerson, T.; Adeoye, O.M.; Bambakidis, N.C.; Becker, K.; Biller, J.; Brown, M.; Demaerschalk, B.M.; Hoh, B.; Jauch, E.C.; Kidwell, C.S.; Leslie-Mazwi, T.M.; Ovbiagele, B.; Scott, P.A.; Sheth, K.N.; Southerland, A.M.; Summers, D.V.; Tirschwell, D.L. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke,* **2019**, *50*(12), e344-e418. http://dx.doi.org/10.1161/STR.0000000000000211 PMID: 31662037

[3] Hermann, D.M.; Zechariah, A. Implications of vascular endothelial growth factor for postischemic neurovascular remodeling. *J. Cereb. Blood Flow Metab.,* **2009**, *29*(10), 1620-1643. http://dx.doi.org/10.1038/jcbfm.2009.100 PMID: 19654590

[4] Snapyan, M.; Lemasson, M.; Brill, M.S.; Blais, M.; Massouh, M.; Ninkovic, J.; Gravel, C.; Berthod, F.; Götz, M.; Barker, P.A.; Parent, A.; Saghatelyan, A. Vasculature guides migrating neuronal precursors in the adult mammalian forebrain *via* brain-derived neurotrophic factor signaling. *J. Neurosci.,* **2009**, *29*(13), 4172-4188.

http://dx.doi.org/10.1523/JNEUROSCI.4956-08.2009 PMID: 19339612

[5] Lin, T.N.; Sun, S.W.; Cheung, W.M.; Li, F.; Chang, C. Dynamic changes in cerebral blood flow and angiogenesis after transient focal cerebral ischemia in rats. Evaluation with serial magnetic resonance imaging. *Stroke,* **2002**, *33*(12), 2985-2991. http://dx.doi.org/10.1161/01.STR.0000037675.97888.9D PMID: 12468801

[6] Teng, H.; Zhang, Z.G.; Wang, L.; Zhang, R.L.; Zhang, L.; Morris, D.; Gregg, S.R.; Wu, Z.; Jiang, A.; Lu, M.; Zlokovic, B.V.; Chopp, M. Coupling of angiogenesis and neurogenesis in cultured endothelial cells and neural progenitor cells after stroke. *J. Cereb. Blood Flow Metab.,* **2008**, *28*(4), 764-771. http://dx.doi.org/10.1038/sj.jcbfm.9600573 PMID: 17971789

[7] Brea, D.; Sobrino, T.; Ramos-Cabrer, P.; Castillo, J. [Reorganisation of the cerebral vasculature following ischaemia]. *Rev. Neurol.,* **2009**, *49*(12), 645-654. PMID: 20013717

[8] Nakagomi, N.; Nakagomi, T.; Kubo, S.; Nakano-Doi, A.; Saino, O.; Takata, M.; Yoshikawa, H.; Stern, D.M.; Matsuyama, T.; Taguchi, A. Endothelial cells support survival, proliferation, and neuronal differentiation of transplanted adult ischemia-induced neural stem/progenitor cells after cerebral infarction. *Stem Cells,* **2009**, *27*(9), 2185-2195. http://dx.doi.org/10.1002/stem.161 PMID: 19557831

[9] Lee, H.S.; Han, J.; Bai, H.J.; Kim, K.W. Brain angiogenesis in developmental and pathological processes: regulation, molecular and cellular communication at the neurovascular interface. *FEBS J.,* **2009**, *276*(17), 4622-4635. http://dx.doi.org/10.1111/j.1742-4658.2009.07174.x PMID: 19664072

[10] Jain, R.K. Molecular regulation of vessel maturation. *Nat. Med.,* **2003**, *9*(6), 685-693. http://dx.doi.org/10.1038/nm0603-685 PMID: 12778167

[11] Shen, Q.; Goderie, S.K.; Jin, L.; Karanth, N.; Sun, Y.; Abramova, N.; Vincent, P.; Pumiglia, K.; Temple, S. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science,* **2004**, *304*(5675), 1338-1340. http://dx.doi.org/10.1126/science.1095505 PMID: 15060285

[12] Yu, S.W.; Friedman, B.; Cheng, Q.; Lyden, P.D. Stroke-evoked angiogenesis results in a transient population of microvessels. *J. Cereb. Blood Flow Metab.,* **2007**, *27*(4), 755-763. http://dx.doi.org/10.1038/sj.jcbfm.9600378 PMID: 16883352

[13] Carmeliet, P. Fibroblast growth factor-1 stimulates branching and survival of myocardial arteries: a goal for therapeutic angiogenesis? *Circ. Res.,* **2000**, *87*(3), 176-178. http://dx.doi.org/10.1161/01.RES.87.3.176 PMID: 10926865

[14] Beck, H.; Plate, K.H. Angiogenesis after cerebral ischemia. *Acta Neuropathol.,* **2009**, *117*(5), 481-496. http://dx.doi.org/10.1007/s00401-009-0483-6 PMID: 19142647

[15] Zhang, Z.G.; Zhang, L.; Jiang, Q.; Zhang, R.; Davies, K.; Powers, C.; Bruggen, Nv.; Chopp, M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J. Clin. Invest.,* **2000**, *106*(7), 829-838. http://dx.doi.org/10.1172/JCI9369 PMID: 11018070

[16] Zhang, Z.G.; Zhang, L.; Tsang, W.; Soltanian-Zadeh, H.; Morris, D.; Zhang, R.; Goussev, A.; Powers, C.; Yeich, T.; Chopp, M. 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*(4), 379-392. http://dx.doi.org/10.1097/00004647-200204000-00002 PMID: 11919509

[17] Chen, J.; Chopp, M. Neurorestorative treatment of stroke: cell and pharmacological approaches. *NeuroRx,* **2006**, *3*(4), 466-473. http://dx.doi.org/10.1016/j.nurx.2006.07.007 PMID: 17012060

[18] Issa, R.; Krupinski, J.; Bujny, T.; Kumar, S.; Kaluza, J.; Kumar, P. Vascular endothelial growth factor and its receptor, KDR, in human brain tissue after ischemic stroke. *Lab. Invest.,* **1999**, *79*(4), 417-425. PMID: 10211994

[19] Shen, F.; Walker, E.J.; Jiang, L.; Degos, V.; Li, J.; Sun, B.; Heriyanto, F.; Young, W.L.; Su, H. Coexpression of angiopoietin-1 with VEGF increases the structural integrity of the blood-brain barrier and reduces atrophy volume. *J. Cereb. Blood Flow Metab.,* **2011**, *31*(12), 2343-2351. http://dx.doi.org/10.1038/jcbfm.2011.97 PMID: 21772310

[20] Abbott, N.J. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *J. Inherit. Metab. Dis.,* **2013**, *36*(3), 437-449. http://dx.doi.org/10.1007/s10545-013-9608-0 PMID: 23609350

[21] Liebner, S.; Czupalla, C.J.; Wolburg, H. Current concepts of blood-brain barrier development. *Int. J. Dev. Biol.,* **2011**, *55*(4-5), 467-476. http://dx.doi.org/10.1387/ijdb.103224sl PMID: 21769778

[22] Abbott, N.J. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J. Anat.,* **2002**, *200*(6), 629-638. http://dx.doi.org/10.1046/j.1469-7580.2002.00064.x PMID: 12162730

[23] Abbott, N.J.; Friedman, A. Overview and introduction: the blood-brain barrier in health and disease. *Epilepsia,* **2012**, *53*(Suppl. 6), 1-6. http://dx.doi.org/10.1111/j.1528-1167.2012.03696.x PMID: 23134489

[24] Yang, Y.; Estrada, E.Y.; Thompson, J.F.; Liu, W.; Rosenberg, G.A. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. *J. Cereb. Blood Flow Metab.,* **2007**, *27*(4), 697-709. http://dx.doi.org/10.1038/sj.jcbfm.9600375 PMID: 16850029

[25] Yang, Y.; Rosenberg, G.A. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. *Stroke,* **2011**, *42*(11), 3323-3328. http://dx.doi.org/10.1161/STROKEAHA.110.608257 PMID: 21940972

[26] Keaney, J.; Campbell, M. The dynamic blood-brain barrier. *FEBS J.,* **2015**, *282*(21), 4067-4079. http://dx.doi.org/10.1111/febs.13412 PMID: 26277326

[27] Rosenberg, G.A.; Yang, Y. Vasogenic edema due to tight junction disruption by matrix metalloproteinases in cerebral ischemia. *Neurosurg. Focus,* **2007**, *22*(5), E4. http://dx.doi.org/10.3171/foc.2007.22.5.5 PMID: 17613235

[28] Zhao, Z.; Nelson, A.R.; Betsholtz, C.; Zlokovic, B.V. Establishment and Dysfunction of the Blood-Brain Barrier. *Cell,* **2015**, *163*(5), 1064-1078. http://dx.doi.org/10.1016/j.cell.2015.10.067 PMID: 26590417

[29] Armulik, A.; Genové, G.; Betsholtz, C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev. Cell,* **2011**, *21*(2), 193-215. http://dx.doi.org/10.1016/j.devcel.2011.07.001 PMID: 21839917

[30] Armulik, A.; Genové, G.; Mäe, M.; Nisancioglu, M.H.; Wallgard, E.; Niaudet, C.; He, L.; Norlin, J.; Lindblom, P.; Strittmatter, K.; Johansson, B.R.; Betsholtz, C. Pericytes regulate the blood-brain barrier. *Nature,* **2010**, *468*(7323), 557-561. http://dx.doi.org/10.1038/nature09522 PMID: 20944627

[31] Armulik, A.; Mäe, M.; Betsholtz, C. Pericytes and the blood-brain barrier: recent advances and implications for the delivery of CNS therapy. *Ther. Deliv.,* **2011**, *2*(4), 419-422. http://dx.doi.org/10.4155/tde.11.23 PMID: 22826851

[32] Bell, R.D.; Winkler, E.A.; Sagare, A.P.; Singh, I.; LaRue, B.; Deane, R.; Zlokovic, B.V. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron,* **2010**, *68*(3), 409-427. http://dx.doi.org/10.1016/j.neuron.2010.09.043 PMID: 21040844

[33] Winkler, E.A.; Bell, R.D.; Zlokovic, B.V. Central nervous system pericytes in health and disease. *Nat. Neurosci.,* **2011**, *14*(11), 1398-1405. http://dx.doi.org/10.1038/nn.2946 PMID: 22030551

[34] Andreone, B.J. Blood-Brain Barrier Permeability Is Regulated by Lipid Transport-Dependent Suppression of Caveolae-Mediated Transcytosis. *Neuron,* **2017**, *94*, 581-594 e5. http://dx.doi.org/10.1016/j.neuron.2017.03.043

[35] Gautam, J.; Xu, L.; Nirwane, A.; Nguyen, B.; Yao, Y. Loss of mural cell-derived laminin aggravates hemorrhagic brain injury. *J. Neuroinflammation,* **2020**, *17*(1), 103. http://dx.doi.org/10.1186/s12974-020-01788-3 PMID: 32252790

[36] Gautam, J.; Cao, Y.; Yao, Y. Pericytic Laminin Maintains Blood-Brain Barrier Integrity in an Age-Dependent Manner. *Transl. Stroke Res.,* **2020**, *11*(2), 228-242. http://dx.doi.org/10.1007/s12975-019-00709-8 PMID: 31292838

[37] Colton, C.A. Heterogeneity of microglial activation in the innate immune response in the brain. *J. Neuroimmune Pharmacol.,* **2009**, *4*(4), 399-418. http://dx.doi.org/10.1007/s11481-009-9164-4 PMID: 19655259

[38] Ballabh, P.; Braun, A.; Nedergaard, M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol. Dis.,* **2004**, *16*(1), 1-13. http://dx.doi.org/10.1016/j.nbd.2003.12.016 PMID: 15207256

[39] Brightman, M.W.; Reese, T.S. Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.,* **1969**, *40*(3), 648-677. http://dx.doi.org/10.1083/jcb.40.3.648 PMID: 5765759

[40] Liebner, S.; Kniesel, U.; Kalbacher, H.; Wolburg, H. Correlation of tight junction morphology with the expression of tight junction proteins in blood-brain barrier endothelial cells. *Eur. J. Cell Biol.,* **2000**, *79*(10), 707-717. http://dx.doi.org/10.1078/0171-9335-00101 PMID: 11089919

[41] Willis, C.L.; Leach, L.; Clarke, G.J.; Nolan, C.C.; Ray, D.E. Reversible disruption of tight junction complexes in the rat blood-brain barrier, following transitory focal astrocyte loss. *Glia,* **2004**, *48*(1), 1-13. http://dx.doi.org/10.1002/glia.20049 PMID: 15326610

[42] Zlokovic, B.V. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron,* **2008**, *57*(2), 178-201. http://dx.doi.org/10.1016/j.neuron.2008.01.003 PMID: 18215617

[43] Furuse, M.; Hirase, T.; Itoh, M.; Nagafuchi, A.; Yonemura, S.; Tsukita, S.; Tsukita, S. Occludin: a novel integral membrane protein localizing at tight junctions. *J. Cell Biol.,* **1993**, *123*(6 Pt 2), 1777-1788. http://dx.doi.org/10.1083/jcb.123.6.1777 PMID: 8276896

[44] Hirase, T.; Staddon, J.M.; Saitou, M.; Ando-Akatsuka, Y.; Itoh, M.; Furuse, M.; Fujimoto, K.; Tsukita, S.; Rubin, L.L. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J. Cell Sci.,* **1997**, *110*(Pt 14), 1603-1613. PMID: 9247194

[45] McCaffrey, G.; Seelbach, M.J.; Staatz, W.D.; Nametz, N.; Quigley, C.; Campos, C.R.; Brooks, T.A.; Davis, T.P. Occludin oligomeric assembly at tight junctions of the blood-brain barrier is disrupted by peripheral inflammatory hyperalgesia. *J. Neurochem.,* **2008**, *106*(6), 2395-2409. http://dx.doi.org/10.1111/j.1471-4159.2008.05582.x PMID: 18647175

[46] McCaffrey, G.; Willis, C.L.; Staatz, W.D.; Nametz, N.; Quigley, C.A.; Hom, S.; Lochhead, J.J.; Davis, T.P. Occludin oligomeric assemblies at tight junctions of the blood-brain barrier are altered by hypoxia and reoxygenation stress. *J. Neurochem.,* **2009**, *110*(1), 58-71. http://dx.doi.org/10.1111/j.1471-4159.2009.06113.x PMID: 19457074

[47] Morita, K.; Sasaki, H.; Furuse, M.; Tsukita, S. Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. *J. Cell Biol.,* **1999**, *147*(1), 185-194. http://dx.doi.org/10.1083/jcb.147.1.185 PMID: 10508865

[48] Liebner, S.; Fischmann, A.; Rascher, G.; Duffner, F.; Grote, E.H.; Kalbacher, H.; Wolburg, H. Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. *Acta Neuropathol.,* **2000**, *100*(3), 323-331. http://dx.doi.org/10.1007/s004010000180 PMID: 10965803

[49] Pfeiffer, F.; Schäfer, J.; Lyck, R.; Makrides, V.; Brunner, S.; Schaeren-Wiemers, N.; Deutsch, U.; Engelhardt, B. Claudin-1 induced sealing of blood-brain barrier tight junctions ameliorates chronic experimental autoimmune encephalomyelitis. *Acta Neuropathol.,* **2011**, *122*(5), 601-614. http://dx.doi.org/10.1007/s00401-011-0883-2 PMID: 21983942

[50] Nitta, T.; Hata, M.; Gotoh, S.; Seo, Y.; Sasaki, H.; Hashimoto, N.; Furuse, M.; Tsukita, S. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J. Cell Biol.,* **2003**, *161*(3), 653-660. http://dx.doi.org/10.1083/jcb.200302070 PMID: 12743111

[51] Ohtsuki, S.; Sato, S.; Yamaguchi, H.; Kamoi, M.; Asashima, T.; Terasaki, T. Exogenous expression of claudin-5 induces barrier properties in cultured rat brain capillary endothelial cells. *J. Cell. Physiol.,* **2007**, *210*(1), 81-86. http://dx.doi.org/10.1002/jcp.20823 PMID: 16998798

[52] Sladojevic, N.; Stamatovic, S.M.; Johnson, A.M.; Choi, J.; Hu, A.; Dithmer, S.; Blasig, I.E.; Keep, R.F.; Andjelkovic, A.V. Claudin-1-Dependent Destabilization of the Blood-Brain Barrier in Chronic Stroke. *J. Neurosci.,* **2019**, *39*(4), 743-757. http://dx.doi.org/10.1523/JNEUROSCI.1432-18.2018 PMID: 30504279

[53] Huber, J.D.; Egleton, R.D.; Davis, T.P. Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends Neurosci.,* **2001**, *24*(12), 719-725. http://dx.doi.org/10.1016/S0166-2236(00)02004-X PMID: 11718877

[54] Huber, J.D.; Hau, V.S.; Borg, L.; Campos, C.R.; Egleton, R.D.; Davis, T.P. Blood-brain barrier tight junctions are altered during a 72-h exposure to lambda-carrageenan-induced inflammatory pain. *Am. J. Physiol. Heart Circ. Physiol.,* **2002**, *283*(4), H1531-H1537. http://dx.doi.org/10.1152/ajpheart.00027.2002 PMID: 12234806

[55] Lochhead, J.J.; McCaffrey, G.; Quigley, C.E.; Finch, J.; DeMarco, K.M.; Nametz, N.; Davis, T.P. Oxidative stress increases blood-brain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation. *J. Cereb. Blood Flow Metab.,* **2010**, *30*(9), 1625-1636. http://dx.doi.org/10.1038/jcbfm.2010.29 PMID: 20234382

[56] Lochhead, J.J.; McCaffrey, G.; Sanchez-Covarrubias, L.; Finch, J.D.; Demarco, K.M.; Quigley, C.E.; Davis, T.P.; Ronaldson, P.T. Tempol modulates changes in xenobiotic permeability and occludin oligomeric assemblies at the blood-brain barrier during inflammatory pain. *Am. J. Physiol. Heart Circ. Physiol.,* **2012**, *302*(3), H582-H593. http://dx.doi.org/10.1152/ajpheart.00889.2011 PMID: 22081706

[57] Mark, K.S.; Davis, T.P. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am. J. Physiol. Heart Circ. Physiol.,* **2002**, *282*(4), H1485-H1494. http://dx.doi.org/10.1152/ajpheart.00645.2001 PMID: 11893586

[58] Ronaldson, P.T.; Demarco, K.M.; Sanchez-Covarrubias, L.; Solinsky, C.M.; Davis, T.P. Transforming growth factor-beta signaling alters substrate permeability and tight junction protein expression at the blood-brain barrier during inflammatory pain. *J. Cereb. Blood Flow Metab.,* **2009**, *29*(6), 1084-1098. http://dx.doi.org/10.1038/jcbfm.2009.32 PMID: 19319146

[59] Luh, C.; Feiler, S.; Frauenknecht, K.; Meyer, S.; Lubomirov, L.T.; Neulen, A.; Thal, S.C. The Contractile Apparatus Is Essential for the Integrity of the Blood-Brain Barrier After Experimental Subarachnoid Hemorrhage. *Transl. Stroke Res.,* **2019**, *10*(5), 534-545. http://dx.doi.org/10.1007/s12975-018-0677-0 PMID: 30467816

[60] Burek, M.; König, A.; Lang, M.; Fiedler, J.; Oerter, S.; Roewer, N.; Bohnert, M.; Thal, S.C.; Blecharz-Lang, K.G.; Woitzik, J.; Thum, T.; Förster, C.Y. Hypoxia-Induced MicroRNA-212/132 Alter Blood-Brain Barrier Integrity Through Inhibition of Tight Junction-Associated Proteins in Human and Mouse Brain Microvascular Endothelial Cells. *Transl. Stroke Res.,* **2019**, *10*(6), 672-683. http://dx.doi.org/10.1007/s12975-018-0683-2 PMID: 30617994

[61] Wolburg, H.; Wolburg-Buchholz, K.; Liebner, S.; Engelhardt, B. Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse. *Neurosci. Lett.,* **2001**, *307*(2), 77-80. http://dx.doi.org/10.1016/S0304-3940(01)01927-9 PMID: 11427304

[62] Lippoldt, A.; Kniesel, U.; Liebner, S.; Kalbacher, H.; Kirsch, T.; Wolburg, H.; Haller, H. Structural alterations of tight junctions are associated with loss of polarity in stroke-prone spontaneously hypertensive rat blood-brain barrier endothelial cells. *Brain Res.,* **2000**, *885*(2), 251-261. http://dx.doi.org/10.1016/S0006-8993(00)02954-1 PMID: 11102579

[63] Hirase, T.; Kawashima, S.; Wong, E.Y.; Ueyama, T.; Rikitake, Y.; Tsukita, S.; Yokoyama, M.; Staddon, J.M. Regulation of tight junction permeability and occludin phosphorylation by Rhoa-p160ROCK-dependent and -independent mechanisms. *J. Biol. Chem.,* **2001**, *276*(13), 10423-10431. http://dx.doi.org/10.1074/jbc.M007136200 PMID: 11139571

[64] Hawkins, B.T.; Davis, T.P. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol. Rev.,* **2005**, *57*(2), 173-185. http://dx.doi.org/10.1124/pr.57.2.4 PMID: 15914466

[65] Al Ahmad, A.; Taboada, C.B.; Gassmann, M.; Ogunshola, O.O. Astrocytes and pericytes differentially modulate blood-brain barrier

characteristics during development and hypoxic insult. *J. Cereb. Blood Flow Metab.,* **2011**, *31*(2), 693-705. http://dx.doi.org/10.1038/jcbfm.2010.148 PMID: 20827262

[66] Liebner, S.; Plate, K.H. Differentiation of the brain vasculature: the answer came blowing by the Wnt. *J. Angiogenes. Res.,* **2010**, *2*, 1-10. http://dx.doi.org/10.1186/2040-2384-2-1 PMID: 20150991

[67] Engelhardt, B.; Liebner, S. Novel insights into the development and maintenance of the blood-brain barrier. *Cell Tissue Res.,* **2014**, *355*(3), 687-699. http://dx.doi.org/10.1007/s00441-014-1811-2 PMID: 24590145

[68] Yang, Y.; Thompson, J.F.; Taheri, S.; Salayandia, V.M.; McAvoy, T.A.; Hill, J.W.; Yang, Y.; Estrada, E.Y.; Rosenberg, G.A. Early inhibition of MMP activity in ischemic rat brain promotes expression of tight junction proteins and angiogenesis during recovery. *J. Cereb. Blood Flow Metab.,* **2013**, *33*(7), 1104-1114. http://dx.doi.org/10.1038/jcbfm.2013.56 PMID: 23571276

[69] Brillault, J.; Berezowski, V.; Cecchelli, R.; Dehouck, M.P. Intercommunications between brain capillary endothelial cells and glial cells increase the transcellular permeability of the blood-brain barrier during ischaemia. *J. Neurochem.,* **2002**, *83*(4), 807-817. http://dx.doi.org/10.1046/j.1471-4159.2002.01186.x PMID: 12421352

[70] Morizawa, Y.M.; Hirayama, Y.; Ohno, N.; Shibata, S.; Shigetomi, E.; Sui, Y.; Nabekura, J.; Sato, K.; Okajima, F.; Takebayashi, H.; Okano, H.; Koizumi, S. Reactive astrocytes function as phagocytes after brain ischemia *via* ABCA1-mediated pathway. *Nat. Commun.,* **2017**, *8*(1), 28. http://dx.doi.org/10.1038/s41467-017-00037-1 PMID: 28642575

[71] Choi, Y.K.; Kim, K.W. AKAP12 in astrocytes induces barrier functions in human endothelial cells through protein kinase Czeta. *FEBS J.,* **2008**, *275*(9), 2338-2353. http://dx.doi.org/10.1111/j.1742-4658.2008.06387.x PMID: 18397319

[72] Tao-Cheng, J.H.; Nagy, Z.; Brightman, M.W. Tight junctions of brain endothelium *in vitro* are enhanced by astroglia. *J. Neurosci.,* **1987**, *7*(10), 3293-3299. http://dx.doi.org/10.1523/JNEUROSCI.07-10-03293.1987 PMID: 3668629

[73] Haseloff, R.F.; Blasig, I.E.; Bauer, H.C.; Bauer, H. In search of the astrocytic factor(s) modulating blood-brain barrier functions in brain capillary endothelial cells *in vitro*. *Cell. Mol. Neurobiol.,* **2005**, *25*(1), 25-39. http://dx.doi.org/10.1007/s10571-004-1375-x PMID: 15962507

[74] Abbott, N.J.; Rönnbäck, L.; Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.,* **2006**, *7*(1), 41-53. http://dx.doi.org/10.1038/nrn1824 PMID: 16371949

[75] Yao, Y.; Chen, Z.L.; Norris, E.H.; Strickland, S. Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nat. Commun.,* **2014**, *5*, 3413. http://dx.doi.org/10.1038/ncomms4413 PMID: 24583950

[76] Daneman, R.; Zhou, L.; Kebede, A.A.; Barres, B.A. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature,* **2010**, *468*(7323), 562-566. http://dx.doi.org/10.1038/nature09513 PMID: 20944625

[77] Bonkowski, D.; Katyshev, V.; Balabanov, R.D.; Borisov, A.; Dore-Duffy, P. The CNS microvascular pericyte: pericyte-astrocyte crosstalk in the regulation of tissue survival. *Fluids Barriers CNS,* **2011**, *8*(1), 8. http://dx.doi.org/10.1186/2045-8118-8-8 PMID: 21349156

[78] Dore-Duffy, P.; LaManna, J.C. Physiologic angiodynamics in the brain. *Antioxid. Redox Signal.,* **2007**, *9*(9), 1363-1371. http://dx.doi.org/10.1089/ars.2007.1713 PMID: 17627476

[79] Cui, L.; Zhang, X.; Yang, R.; Wang, L.; Liu, L.; Li, M.; Du, W. Neuroprotection of early and short-time applying atorvastatin in the acute phase of cerebral ischemia: down-regulated 12/15-LOX, p38MAPK and cPLA2 expression, ameliorated BBB permeability. *Brain Res.,* **2010**, *1325*, 164-173. http://dx.doi.org/10.1016/j.brainres.2010.02.036 PMID: 20167207

[80] Lindahl, P.; Johansson, B.R.; Levéen, P.; Betsholtz, C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science,* **1997**, *277*(5323), 242-245. http://dx.doi.org/10.1126/science.277.5323.242 PMID: 9211853

[81] Wang, Y.L.; Hui, Y.N.; Guo, B.; Ma, J.X. Strengthening tight junctions of retinal microvascular endothelial cells by pericytes under normoxia and hypoxia involving angiopoietin-1 signal way. *Eye (Lond.),* **2007**, *21*(12), 1501-1510. http://dx.doi.org/10.1038/sj.eye.6702716 PMID: 17332770

[82] Zacharek, A.; Chen, J.; Cui, X.; Li, A.; Li, Y.; Roberts, C.; Feng, Y.; Gao, Q.; Chopp, M. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies angiogenesis and vascular stabilization after stroke. *J. Cereb. Blood Flow Metab.,* **2007**, *27*(10), 1684-1691. http://dx.doi.org/10.1038/sj.jcbfm.9600475 PMID: 17356562

[83] Aslanyan, S.; Weir, C.J.; McInnes, G.T.; Reid, J.L.; Walters, M.R.; Lees, K.R. Statin administration prior to ischaemic stroke onset and survival: exploratory evidence from matched treatment-control study. *Eur. J. Neurol.,* **2005**, *12*(7), 493-498. http://dx.doi.org/10.1111/j.1468-1331.2005.01049.x PMID: 15958087

[84] Quaegebeur, A.; Segura, I.; Carmeliet, P. Pericytes: blood-brain barrier safeguards against neurodegeneration? *Neuron,* **2010**, *68*(3), 321-323. http://dx.doi.org/10.1016/j.neuron.2010.10.024 PMID: 21040834

[85] Gautam, J.; Yao, Y. Roles of Pericytes in Stroke Pathogenesis. *Cell Transplant.,* **2018**, *27*(12), 1798-1808. http://dx.doi.org/10.1177/0963689718768455 PMID: 29845887

[86] Ozerdem, U.; Grako, K.A.; Dahlin-Huppe, K.; Monosov, E.; Stallcup, W.B. NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev. Dyn.,* **2001**, *222*(2), 218-227. http://dx.doi.org/10.1002/dvdy.1200 PMID: 11668599

[87] Hughes, S.; Chan-Ling, T. Characterization of smooth muscle cell and pericyte differentiation in the rat retina *in vivo*. *Invest. Ophthalmol. Vis. Sci.,* **2004**, *45*(8), 2795-2806. http://dx.doi.org/10.1167/iovs.03-1312 PMID: 15277506

[88] Roitbak, T.; Li, L.; Cunningham, L.A. Neural stem/progenitor cells promote endothelial cell morphogenesis and protect endothelial cells against ischemia *via* HIF-1alpha-regulated VEGF signaling. *J. Cereb. Blood Flow Metab.,* **2008**, *28*(9), 1530-1542. http://dx.doi.org/10.1038/jcbfm.2008.38 PMID: 18478024

[89] Li, L.; Harms, K.M.; Ventura, P.B.; Lagace, D.C.; Eisch, A.J.; Cunningham, L.A. Focal cerebral ischemia induces a multilineage cytogenic response from adult subventricular zone that is predominantly gliogenic. *Glia,* **2010**, *58*(13), 1610-1619. http://dx.doi.org/10.1002/glia.21033 PMID: 20578055

[90] Kim, S.S.; Yoo, S.W.; Park, T.S.; Ahn, S.C.; Jeong, H.S.; Kim, J.W.; Chang, D.Y.; Cho, K.G.; Kim, S.U.; Huh, Y.; Lee, J.E.; Lee, S.Y.; Lee, Y.D.; Suh-Kim, H. Neural induction with neurogenin1 increases the therapeutic effects of mesenchymal stem cells in the ischemic brain. *Stem Cells,* **2008**, *26*(9), 2217-2228. http://dx.doi.org/10.1634/stemcells.2008-0108 PMID: 18617687

[91] Virgintino, D.; Girolamo, F.; Errede, M.; Capobianco, C.; Robertson, D.; Stallcup, W.B.; Perris, R.; Roncali, L. An intimate interplay between precocious, migrating pericytes and endothelial cells governs human fetal brain angiogenesis. *Angiogenesis,* **2007**, *10*(1), 35-45. http://dx.doi.org/10.1007/s10456-006-9061-x PMID: 17225955

[92] Ozerdem, U.; Stallcup, W.B. Pathological angiogenesis is reduced by targeting pericytes *via* the NG2 proteoglycan. *Angiogenesis,* **2004**, *7*(3), 269-276. http://dx.doi.org/10.1007/s10456-004-4182-6 PMID: 15609081

[93] Tsai, P.T.; Ohab, J.J.; Kertesz, N.; Groszer, M.; Matter, C.; Gao, J.; Liu, X.; Wu, H.; Carmichael, S.T. A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. *J. Neurosci.,* **2006**, *26*(4), 1269-1274. http://dx.doi.org/10.1523/JNEUROSCI.4480-05.2006 PMID: 16436614

[94] Du, Y.; Shi, L.; Li, J.; Xiong, J.; Li, B.; Fan, X. Angiogenesis and improved cerebral blood flow in the ischemic boundary area were detected after electroacupuncture treatment to rats with ischemic stroke. *Neurol. Res.,* **2011**, *33*(1), 101-107. http://dx.doi.org/10.1179/016164110X12714125204317 PMID: 20546685

[95] Kielczewski, J.L.; Jarajapu, Y.P.; McFarland, E.L.; Cai, J.; Afzal, A.; Li Calzi, S.; Chang, K.H.; Lydic, T.; Shaw, L.C.; Busik, J.; Hughes, J.; Cardounel, A.J.; Wilson, K.; Lyons, T.J.; Boulton, M.E.; Mames, R.N.; Chan-Ling, T.; Grant, M.B. Insulin-like growth factor binding protein-3 mediates vascular repair by enhancing nitric oxide generation. *Circ. Res.,* **2009**, *105*(9), 897-905.

http://dx.doi.org/10.1161/CIRCRESAHA.109.199059 PMID: 19762684

[96] Gotts, J.E.; Chesselet, M.F. Vascular changes in the subventricular zone after distal cortical lesions. *Exp. Neurol.,* **2005**, *194*(1), 139-150. http://dx.doi.org/10.1016/j.expneurol.2005.02.001 PMID: 15899251

[97] Krupinski, J.; Stroemer, P.; Slevin, M.; Marti, E.; Kumar, P.; Rubio, F. Three-dimensional structure and survival of newly formed blood vessels after focal cerebral ischemia. *Neuroreport,* **2003**, *14*(8), 1171-1176. http://dx.doi.org/10.1097/00001756-200306110-00014 PMID: 12821803

[98] Krupinski, J.; Kaluza, J.; Kumar, P.; Kumar, S.; Wang, J.M. Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke,* **1994**, *25*(9), 1794-1798. http://dx.doi.org/10.1161/01.STR.25.9.1794 PMID: 7521076

[99] Beck, H.; Acker, T.; Wiessner, C.; Allegrini, P.R.; Plate, K.H. Expression of angiopoietin-1, angiopoietin-2, and tie receptors after middle cerebral artery occlusion in the rat. *Am. J. Pathol.,* **2000**, *157*(5), 1473-1483. http://dx.doi.org/10.1016/S0002-9440(10)64786-4 PMID: 11073808

[100] Hayashi, T.; Noshita, N.; Sugawara, T.; Chan, P.H. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. *J. Cereb. Blood Flow Metab.,* **2003**, *23*(2), 166-180. http://dx.doi.org/10.1097/01.WCB.0000041283.53351.CB PMID: 12571448

[101] Marti, H.J.; Bernaudin, M.; Bellail, A.; Schoch, H.; Euler, M.; Petit, E.; Risau, W. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. *Am. J. Pathol.,* **2000**, *156*(3), 965-976. http://dx.doi.org/10.1016/S0002-9440(10)64964-4 PMID: 10702412

[102] Candelario-Jalil, E.; Taheri, S.; Yang, Y.; Sood, R.; Grossetete, M.; Estrada, E.Y.; Fiebich, B.L.; Rosenberg, G.A. Cyclooxygenase inhibition limits blood-brain barrier disruption following intracerebral injection of tumor necrosis factor-alpha in the rat. *J. Pharmacol. Exp. Ther.,* **2007**, *323*(2), 488-498. http://dx.doi.org/10.1124/jpet.107.127035 PMID: 17704356

[103] Taheri, S.; Candelario-Jalil, E.; Estrada, E.Y.; Rosenberg, G.A. Spatiotemporal correlations between blood-brain barrier permeability and apparent diffusion coefficient in a rat model of ischemic stroke. *PLoS One,* **2009**, *4*(8), e6597. http://dx.doi.org/10.1371/journal.pone.0006597 PMID: 19668371

[104] Tanaka, Y.; Nagaoka, T.; Nair, G.; Ohno, K.; Duong, T.Q. Arterial spin labeling and dynamic susceptibility contrast CBF MRI in postischemic hyperperfusion, hypercapnia, and after mannitol injection. *J. Cereb. Blood Flow Metab.,* **2011**, *31*(6), 1403-1411. http://dx.doi.org/10.1038/jcbfm.2010.228 PMID: 21179070

[105] Alonso, A.; Reinz, E.; Jenne, J.W.; Fatar, M.; Schmidt-Glenewinkel, H.; Hennerici, M.G.; Meairs, S. Reorganization of gap junctions after focused ultrasound blood-brain barrier opening in the rat brain. *J. Cereb. Blood Flow Metab.,* **2010**, *30*(7), 1394-1402. http://dx.doi.org/10.1038/jcbfm.2010.41 PMID: 20332798

[106] Ezan, P.; André, P.; Cisternino, S.; Saubaméa, B.; Boulay, A.C.; Doutremer, S.; Thomas, M.A.; Quenech'du, N.; Giaume, C.; Cohen-Salmon, M. Deletion of astroglial connexins weakens the blood-brain barrier. *J. Cereb. Blood Flow Metab.,* **2012**, *32*(8), 1457-1467. http://dx.doi.org/10.1038/jcbfm.2012.45 PMID: 22472609

[107] ElAli, A.; Thériault, P.; Rivest, S. The role of pericytes in neurovascular unit remodeling in brain disorders. *Int. J. Mol. Sci.,* **2014**, *15*(4), 6453-6474. http://dx.doi.org/10.3390/ijms15046453 PMID: 24743889

[108] Zhang, Y.; Zhang, X.; Wei, Q.; Leng, S.; Li, C.; Han, B.; Bai, Y.; Zhang, H.; Yao, H. Activation of Sigma-1 Receptor Enhanced Pericyte Survival *via* the Interplay Between Apoptosis and Autophagy: Implications for Blood-Brain Barrier Integrity in Stroke. *Transl. Stroke Res.,* **2020**, *11*(2), 267-287. http://dx.doi.org/10.1007/s12975-019-00711-0 PMID: 31290080

[109] Quaegebeur, A.; Lange, C.; Carmeliet, P. The neurovascular link in health and disease: molecular mechanisms and therapeutic implications. *Neuron,* **2011**, *71*(3), 406-424. http://dx.doi.org/10.1016/j.neuron.2011.07.013 PMID: 21835339

[110] Pan, J.; Konstas, A.A.; Bateman, B.; Ortolano, G.A.; Pile-Spellman, J. Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. *Neuroradiology,* **2007**, *49*(2), 93-102. http://dx.doi.org/10.1007/s00234-006-0183-z PMID: 17177065

[111] Kastrup, A.; Engelhorn, T.; Beaulieu, C.; de Crespigny, A.; Moseley, M.E. Dynamics of cerebral injury, perfusion, and blood-brain barrier changes after temporary and permanent middle cerebral artery occlusion in the rat. *J. Neurol. Sci.,* **1999**, *166*(2), 91-99. http://dx.doi.org/10.1016/S0022-510X(99)00121-5 PMID: 10475101

[112] Lampron, A.; Elali, A.; Rivest, S. Innate immunity in the CNS: redefining the relationship between the CNS and Its environment. *Neuron,* **2013**, *78*(2), 214-232. http://dx.doi.org/10.1016/j.neuron.2013.04.005 PMID: 23622060

[113] Gordon, S.; Martinez, F.O. Alternative activation of macrophages: mechanism and functions. *Immunity,* **2010**, *32*(5), 593-604. http://dx.doi.org/10.1016/j.immuni.2010.05.007 PMID: 20510870

[114] Bai, Q.; Xue, M.; Yong, V.W. Microglia and macrophage phenotypes in intracerebral haemorrhage injury: therapeutic opportunities. *Brain,* **2020**, *143*(5), 1297-1314. http://dx.doi.org/10.1093/brain/awz393 PMID: 31919518

[115] Pimenova, A.A.; Marcora, E.; Goate, A.M. A Tale of Two Genes: Microglial Apoe and Trem2. *Immunity,* **2017**, *47*(3), 398-400. http://dx.doi.org/10.1016/j.immuni.2017.08.015 PMID: 28930654

[116] Krasemann, S. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity,* **2017**, *47*, 566-581 e9.

[117] Taylor, R.A.; Sansing, L.H. Microglial responses after ischemic stroke and intracerebral hemorrhage. *Clin. Dev. Immunol.,* **2013**, *2013*, 746068. http://dx.doi.org/10.1155/2013/746068 PMID: 24223607

[118] Giunti, D.; Parodi, B.; Cordano, C.; Uccelli, A.; Kerlero de Rosbo, N. Can we switch microglia's phenotype to foster neuroprotection? Focus on multiple sclerosis. *Immunology,* **2014**, *141*(3), 328-339. http://dx.doi.org/10.1111/imm.12177 PMID: 24116890

[119] Yang, Y.; Salayandia, V.M.; Thompson, J.F.; Yang, L.Y.; Estrada, E.Y.; Yang, Y. Attenuation of acute stroke injury in rat brain by minocycline promotes blood-brain barrier remodeling and alternative microglia/macrophage activation during recovery. *J. Neuroinflammation,* **2015**, *12*, 26. http://dx.doi.org/10.1186/s12974-015-0245-4 PMID: 25889169

[120] Carmeliet, P.; Jain, R.K. Angiogenesis in cancer and other diseases. *Nature,* **2000**, *407*(6801), 249-257. http://dx.doi.org/10.1038/35025220 PMID: 11001068

[121] del Zoppo, G.J. Stroke and neurovascular protection. *N. Engl. J. Med.,* **2006**, *354*(6), 553-555. http://dx.doi.org/10.1056/NEJMp058312 PMID: 16467542

[122] Fan, Y.; Yang, G.Y. Therapeutic angiogenesis for brain ischemia: a brief review. *J. Neuroimmune Pharmacol.,* **2007**, *2*(3), 284-289. http://dx.doi.org/10.1007/s11481-007-9073-3 PMID: 18040863

[123] Fukushi, J.; Makagiansar, I.T.; Stallcup, W.B. NG2 proteoglycan promotes endothelial cell motility and angiogenesis *via* engagement of galectin-3 and alpha3beta1 integrin. *Mol. Biol. Cell,* **2004**, *15*(8), 3580-3590. http://dx.doi.org/10.1091/mbc.e04-03-0236 PMID: 15181153

[124] You, W.K.; Yotsumoto, F.; Sakimura, K.; Adams, R.H.; Stallcup, W.B. NG2 proteoglycan promotes tumor vascularization *via* integrin-dependent effects on pericyte function. *Angiogenesis,* **2014**, *17*(1), 61-76. http://dx.doi.org/10.1007/s10456-013-9378-1 PMID: 23925489

[125] Ozerdem, U.; Monosov, E.; Stallcup, W.B. NG2 proteoglycan expression by pericytes in pathological microvasculature. *Microvasc. Res.,* **2002**, *63*(1), 129-134. http://dx.doi.org/10.1006/mvre.2001.2376 PMID: 11749079

[126] Gibby, K.; You, W.K.; Kadoya, K.; Helgadottir, H.; Young, L.J.; Ellies, L.G.; Chang, Y.; Cardiff, R.D.; Stallcup, W.B. Early vascular deficits are correlated with delayed mammary tumorigenesis in the MMTV-PyMT transgenic mouse following genetic ablation of the NG2 proteoglycan. *Breast Cancer Res.,* **2012**, *14*(2), R67. http://dx.doi.org/10.1186/bcr3174 PMID: 22531600

[127] Huang, F.J.; You, W.K.; Bonaldo, P.; Seyfried, T.N.; Pasquale, E.B.; Stallcup, W.B. Pericyte deficiencies lead to aberrant tumor

vascularizaton in the brain of the NG2 null mouse. *Dev. Biol.,* **2010**, *344*(2), 1035-1046.
http://dx.doi.org/10.1016/j.ydbio.2010.06.023 PMID: 20599895

[128] Stallcup, W.B.; Huang, F.J. A role for the NG2 proteoglycan in glioma progression. *Cell Adhes. Migr.,* **2008**, *2*(3), 192-201.
http://dx.doi.org/10.4161/cam.2.3.6279 PMID: 19262111

[129] You, W.K.; Bonaldo, P.; Stallcup, W.B. Collagen VI ablation retards brain tumor progression due to deficits in assembly of the vascular basal lamina. *Am. J. Pathol.,* **2012**, *180*(3), 1145-1158.
http://dx.doi.org/10.1016/j.ajpath.2011.11.006 PMID: 22200614

[130] Elewa, H.F.; El-Remessy, A.B.; Somanath, P.R.; Fagan, S.C. Diverse effects of statins on angiogenesis: new therapeutic avenues. *Pharmacotherapy,* **2010**, *30*(2), 169-176.
http://dx.doi.org/10.1592/phco.30.2.169 PMID: 20099991

[131] Schachter, M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam. Clin. Pharmacol.,* **2005**, *19*(1), 117-125.
http://dx.doi.org/10.1111/j.1472-8206.2004.00299.x PMID: 15660968

[132] Funck, V.R.; de Oliveira, C.V.; Pereira, L.M.; Rambo, L.M.; Ribeiro, L.R.; Royes, L.F.; Ferreira, J.; Guerra, G.P.; Furian, A.F.; Oliveira, M.S.; Mallmann, C.A.; de Mello, C.F.; Oliveira, M.S. Differential effects of atorvastatin treatment and withdrawal on pentylenetetrazol-induced seizures. *Epilepsia,* **2011**, *52*(11), 2094-2104.
http://dx.doi.org/10.1111/j.1528-1167.2011.03261.x PMID: 21906051

[133] Kuhlmann, C.R.; Lessmann, V.; Luhmann, H.J. Fluvastatin stabilizes the blood-brain barrier *in vitro* by nitric oxide-dependent dephosphorylation of myosin light chains. *Neuropharmacology,* **2006**, *51*(4), 907-913.
http://dx.doi.org/10.1016/j.neuropharm.2006.06.004 PMID: 16872642

[134] Tousoulis, D.; Oikonomou, E.; Siasos, G.; Chrysohoou, C.; Zaromitidou, M.; Kioufis, S.; Maniatis, K.; Dilaveris, P.; Miliou, A.; Michalea, S.; Papavassiliou, A.G.; Stefanadis, C. Dose-dependent effects of short term atorvastatin treatment on arterial wall properties and on indices of left ventricular remodeling in ischemic heart failure. *Atherosclerosis,* **2013**, *227*(2), 367-372.
http://dx.doi.org/10.1016/j.atherosclerosis.2013.01.015 PMID: 23433403

[135] Ifergan, I.; Wosik, K.; Cayrol, R.; Kébir, H.; Auger, C.; Bernard, M.; Bouthillier, A.; Moumdjian, R.; Duquette, P.; Prat, A. Statins reduce human blood-brain barrier permeability and restrict leukocyte migration: relevance to multiple sclerosis. *Ann. Neurol.,* **2006**, *60*(1), 45-55.
http://dx.doi.org/10.1002/ana.20875 PMID: 16729291

[136] Jungner, M.; Lundblad, C.; Bentzer, P. Rosuvastatin in experimental brain trauma: improved capillary patency but no effect on edema or cerebral blood flow. *Microvasc. Res.,* **2013**, *88*, 48-55.
http://dx.doi.org/10.1016/j.mvr.2013.03.004 PMID: 23538316

[137] Elkind, M.S.; Flint, A.C.; Sciacca, R.R.; Sacco, R.L. Lipid-lowering agent use at ischemic stroke onset is associated with decreased mortality. *Neurology,* **2005**, *65*(2), 253-258.
http://dx.doi.org/10.1212/01.WNL.0000171746.63844.6a PMID: 16043795

[138] Garjani, A.; Rezazadeh, H.; Andalib, S.; Ziaee, M.; Doustar, Y.; Soraya, H.; Garjani, M.; Khorrami, A.; Asadpoor, M.; Maleki-Dizaji, N. Ambivalent effects of atorvastatin on angiogenesis, epidermal cell proliferation and tumorgenesis in animal models. *Iran. Biomed. J.,* **2012**, *16*(2), 59-67.
PMID: 22801278

[139] Camnitz, W.; Burdick, M.D.; Strieter, R.M.; Mehrad, B.; Keeley, E.C. Dose-dependent Effect of Statin Therapy on Circulating CXCL12 Levels in Patients with Hyperlipidemia. *Clin. Transl. Med.,* **2012**, *1*(1), 23.
http://dx.doi.org/10.1186/2001-1326-1-23 PMID: 23369699

[140] Chen, J.; Zhang, Z.G.; Li, Y.; Wang, Y.; Wang, L.; Jiang, H.; Zhang, C.; Lu, M.; Katakowski, M.; Feldkamp, C.S.; Chopp, M. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. *Ann. Neurol.,* **2003**, *53*(6), 743-751.
http://dx.doi.org/10.1002/ana.10555 PMID: 12783420

[141] Moonis, M.; Kane, K.; Schwiderski, U.; Sandage, B.W.; Fisher, M. HMG-CoA reductase inhibitors improve acute ischemic stroke outcome. *Stroke,* **2005**, *36*(6), 1298-1300.
http://dx.doi.org/10.1161/01.STR.0000165920.67784.58 PMID: 15879346

[142] Kalayci, R.; Kaya, M.; Elmas, I.; Arican, N.; Ahishali, B.; Uzun, H.; Bilgic, B.; Kucuk, M.; Kudat, H. Effects of atorvastatin on blood-brain barrier permeability during L-NAME hypertension followed by angiotensin-II in rats. *Brain Res.,* **2005**, *1042*(2), 184-193.
http://dx.doi.org/10.1016/j.brainres.2005.02.044 PMID: 15854590

[143] Polster, S.P. Atorvastatin Treatment of Cavernous Angiomas with Symptomatic Hemorrhage Exploratory Proof of Concept. (AT CASH EPOC) Trial. *Neurosurgery,* **2018**, *85*(6), 843-853.
PMID: 30476251

[144] Urbich, C.; Dernbach, E.; Zeiher, A.M.; Dimmeler, S. Double-edged role of statins in angiogenesis signaling. *Circ. Res.,* **2002**, *90*(6), 737-744.
http://dx.doi.org/10.1161/01.RES.0000014081.30867.F8 PMID: 11934843

[145] Walter, D.H.; Zeiher, A.M.; Dimmeler, S. Effects of statins on endothelium and their contribution to neovascularization by mobilization of endothelial progenitor cells. *Coron. Artery Dis.,* **2004**, *15*(5), 235-242.
http://dx.doi.org/10.1097/01.mca.0000131572.14521.8a PMID: 15238818

[146] Son, B.K.; Kozaki, K.; Iijima, K.; Eto, M.; Nakano, T.; Akishita, M.; Ouchi, Y. Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells. *Eur. J. Pharmacol.,* **2007**, *556*(1-3), 1-8.
http://dx.doi.org/10.1016/j.ejphar.2006.09.070 PMID: 17196959

[147] Melaragno, M.G.; Cavet, M.E.; Yan, C.; Tai, L.K.; Jin, Z.G.; Haendeler, J.; Berk, B.C. Gas6 inhibits apoptosis in vascular smooth muscle: role of Axl kinase and Akt. *J. Mol. Cell. Cardiol.,* **2004**, *37*(4), 881-887.
http://dx.doi.org/10.1016/j.yjmcc.2004.06.018 PMID: 15380678

[148] Melaragno, M.G.; Fridell, Y.W.; Berk, B.C. The Gas6/Axl system: a novel regulator of vascular cell function. *Trends Cardiovasc. Med.,* **1999**, *9*(8), 250-253.
http://dx.doi.org/10.1016/S1050-1738(00)00027-X PMID: 11094334

[149] Mooradian, A.D.; Haas, M.J.; Batejko, O.; Hovsepyan, M.; Feman, S.S. Statins ameliorate endothelial barrier permeability changes in the cerebral tissue of streptozotocin-induced diabetic rats. *Diabetes,* **2005**, *54*(10), 2977-2982.
http://dx.doi.org/10.2337/diabetes.54.10.2977 PMID: 16186401

[150] Proia, R.L.; Hla, T. Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy. *J. Clin. Invest.,* **2015**, *125*(4), 1379-1387.
http://dx.doi.org/10.1172/JCI76369 PMID: 25831442

[151] Spampinato, S.F.; Obermeier, B.; Cotleur, A.; Love, A.; Takeshita, Y.; Sano, Y.; Kanda, T.; Ransohoff, R.M. Sphingosine 1 Phosphate at the Blood Brain Barrier: Can the Modulation of S1P Receptor 1 Influence the Response of Endothelial Cells and Astrocytes to Inflammatory Stimuli? *PLoS One,* **2015**, *10*(7), e0133392.
http://dx.doi.org/10.1371/journal.pone.0133392 PMID: 26197437

[152] Wacker, B.K.; Freie, A.B.; Perfater, J.L.; Gidday, J.M. Junctional protein regulation by sphingosine kinase 2 contributes to blood-brain barrier protection in hypoxic preconditioning-induced cerebral ischemic tolerance. *J. Cereb. Blood Flow Metab.,* **2012**, *32*(6), 1014-1023.
http://dx.doi.org/10.1038/jcbfm.2012.3 PMID: 22314269

[153] Wacker, B.K.; Park, T.S.; Gidday, J.M. Hypoxic preconditioning-induced cerebral ischemic tolerance: role of microvascular sphingosine kinase 2. *Stroke,* **2009**, *40*(10), 3342-3348.
http://dx.doi.org/10.1161/STROKEAHA.109.560714 PMID: 19644058

[154] Prager, B.; Spampinato, S.F.; Ransohoff, R.M. Sphingosine 1-phosphate signaling at the blood-brain barrier. *Trends Mol. Med.,* **2015**, *21*(6), 354-363.
http://dx.doi.org/10.1016/j.molmed.2015.03.006 PMID: 25939882

[155] Vanlandewijck, M.; He, L.; Mäe, M.A.; Andrae, J.; Ando, K.; Del Gaudio, F.; Nahar, K.; Lebouvier, T.; Laviña, B.; Gouveia, L.; Sun, Y.; Raschperger, E.; Räsänen, M.; Zarb, Y.; Mochizuki, N.; Keller, A.; Lendahl, U.; Betsholtz, C. A molecular atlas of cell types and zonation in the brain vasculature. *Nature,* **2018**, *554*(7693), 475-480.

http://dx.doi.org/10.1038/nature25739 PMID: 29443965

[156] Wiltshire, R.; Nelson, V.; Kho, D.T.; Angel, C.E.; O'Carroll, S.J.; Graham, E.S. Regulation of human cerebro-microvascular endothelial baso-lateral adhesion and barrier function by S1P through dual involvement of S1P1 and S1P2 receptors. *Sci. Rep.,* **2016**, *6*, 19814.
http://dx.doi.org/10.1038/srep19814 PMID: 26813587

[157] Cuttler, A.S.; LeClair, R.J.; Stohn, J.P.; Wang, Q.; Sorenson, C.M.; Liaw, L.; Lindner, V. Characterization of Pdgfrb-Cre transgenic mice reveals reduction of ROSA26 reporter activity in remodeling arteries. *Genesis,* **2011**, *49*(8), 673-680.
http://dx.doi.org/10.1002/dvg.20769 PMID: 21557454

[158] Gerhardt, H.; Betsholtz, C. Endothelial-pericyte interactions in angiogenesis. *Cell Tissue Res.,* **2003**, *314*(1), 15-23.
http://dx.doi.org/10.1007/s00441-003-0745-x PMID: 12883993

[159] McVerry, B.J.; Garcia, J.G. Endothelial cell barrier regulation by sphingosine 1-phosphate. *J. Cell. Biochem.,* **2004**, *92*(6), 1075-1085.
http://dx.doi.org/10.1002/jcb.20088 PMID: 15258893

[160] Tsai, H.C.; Han, M.H. Sphingosine-1-Phosphate (S1P) and S1P Signaling Pathway: Therapeutic Targets in Autoimmunity and Inflammation. *Drugs,* **2016**, *76*(11), 1067-1079.
http://dx.doi.org/10.1007/s40265-016-0603-2 PMID: 27318702

[161] Blaho, V.A.; Hla, T. An update on the biology of sphingosine 1-phosphate receptors. *J. Lipid Res.,* **2014**, *55*(8), 1596-1608.
http://dx.doi.org/10.1194/jlr.R046300 PMID: 24459205

[162] Chae, S.S.; Proia, R.L.; Hla, T. Constitutive expression of the S1P1 receptor in adult tissues. *Prostaglandins Other Lipid Mediat.,* **2004**, *73*(1-2), 141-150.
http://dx.doi.org/10.1016/j.prostaglandins.2004.01.006 PMID: 15165038

[163] Mendelson, K.; Evans, T.; Hla, T. Sphingosine 1-phosphate signalling. *Development,* **2014**, *141*(1), 5-9.
http://dx.doi.org/10.1242/dev.094805 PMID: 24346695

[164] Xiong, Y.; Hla, T. S1P control of endothelial integrity. *Curr. Top. Microbiol. Immunol.,* **2014**, *378*, 85-105.
http://dx.doi.org/10.1007/978-3-319-05879-5_4 PMID: 24728594

[165] Eresch, J.; Stumpf, M.; Koch, A.; Vutukuri, R.; Ferreirós, N.; Schreiber, Y.; Schröder, K.; Devraj, K.; Popp, R.; Huwiler, A.; Hattenbach, L.O.; Pfeilschifter, J.; Pfeilschifter, W. Sphingosine Kinase 2 Modulates Retinal Neovascularization in the Mouse Model of Oxygen-Induced Retinopathy. *Invest. Ophthalmol. Vis. Sci.,* **2018**, *59*(2), 653-661.
http://dx.doi.org/10.1167/iovs.17-22544 PMID: 29392309

[166] Vutukuri, R.; Brunkhorst, R.; Kestner, R.I.; Hansen, L.; Bouzas, N.F.; Pfeilschifter, J.; Devraj, K.; Pfeilschifter, W. Alteration of sphingolipid metabolism as a putative mechanism underlying LPS-induced BBB disruption. *J. Neurochem.,* **2018**, *144*(2), 172-185.
http://dx.doi.org/10.1111/jnc.14236 PMID: 29023711

[167] Takuwa, Y.; Du, W.; Qi, X.; Okamoto, Y.; Takuwa, N.; Yoshioka, K. Roles of sphingosine-1-phosphate signaling in angiogenesis. *World J. Biol. Chem.,* **2010**, *1*(10), 298-306.
http://dx.doi.org/10.4331/wjbc.v1.i10.298 PMID: 21537463

[168] Blankenbach, K.V.; Schwalm, S.; Pfeilschifter, J.; Meyer Zu Heringdorf, D. Sphingosine-1-Phosphate Receptor-2 Antagonists: Therapeutic Potential and Potential Risks. *Front. Pharmacol.,* **2016**, *7*, 167.
http://dx.doi.org/10.3389/fphar.2016.00167 PMID: 27445808

[169] Park, S.J.; Im, D.S. Sphingosine 1-Phosphate Receptor Modulators and Drug Discovery. *Biomol. Ther. (Seoul),* **2017**, *25*(1), 80-90.
http://dx.doi.org/10.4062/biomolther.2016.160 PMID: 28035084

[170] Sanchez, T.; Skoura, A.; Wu, M.T.; Casserly, B.; Harrington, E.O.; Hla, T. Induction of vascular permeability by the sphingosine-1-phosphate receptor-2 (S1P2R) and its downstream effectors ROCK and PTEN. *Arterioscler. Thromb. Vasc. Biol.,* **2007**, *27*(6), 1312-1318.
http://dx.doi.org/10.1161/ATVBAHA.107.143735 PMID: 17431187

[171] Wu, S.; Thornhill, R.E.; Chen, S.; Rammo, W.; Mikulis, D.J.; Kassner, A. Relative recirculation: a fast, model-free surrogate for the measurement of blood-brain barrier permeability and the prediction of hemorrhagic transformation in acute ischemic stroke. *Invest. Radiol.,* **2009**, *44*(10), 662-668.
http://dx.doi.org/10.1097/RLI.0b013e3181ae9c40 PMID: 19724234

[172] Sun, L.; Zhou, W.; Mueller, C.; Sommer, C.; Heiland, S.; Bauer, A.T.; Marti, H.H.; Veltkamp, R. Oxygen therapy reduces secondary hemorrhage after thrombolysis in thromboembolic cerebral ischemia. *J. Cereb. Blood Flow Metab.,* **2010**, *30*(9), 1651-1660.
http://dx.doi.org/10.1038/jcbfm.2010.50 PMID: 20424638

[173] O'Sullivan, C.; Dev, K.K. The structure and function of the S1P1 receptor. *Trends Pharmacol. Sci.,* **2013**, *34*(7), 401-412.
http://dx.doi.org/10.1016/j.tips.2013.05.002 PMID: 23763867

[174] Sanna, M.G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M.Y.; Peterson, M.S.; Webb, B.; Lefebvre, S.; Chun, J.; Gray, N.; Rosen, H. Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. *J. Biol. Chem.,* **2004**, *279*(14), 13839-13848.
http://dx.doi.org/10.1074/jbc.M311743200 PMID: 14732717

[175] O'Sullivan, S.; Dev, K.K. Sphingosine-1-phosphate receptor therapies: Advances in clinical trials for CNS-related diseases. *Neuropharmacology,* **2017**, *113*(Pt B), 597-607.
http://dx.doi.org/10.1016/j.neuropharm.2016.11.006 PMID: 27825807

[176] Asle-Rousta, M.; Oryan, S.; Ahmadiani, A.; Rahnema, M. Activation of sphingosine 1-phosphate receptor-1 by SEW2871 improves cognitive function in Alzheimer's disease model rats. *EXCLI J.,* **2013**, *12*, 449-461.
PMID: 26417237

[177] Aoki, M.; Aoki, H.; Ramanathan, R.; Hait, N.C.; Takabe, K. Sphingosine-1-Phosphate Signaling in Immune Cells and Inflammation: Roles and Therapeutic Potential. *Mediators Inflamm.,* **2016**, *2016*, 8606878.
PMID: 26966342

[178] Brinkmann, V. FTY720 (fingolimod) in Multiple Sclerosis: therapeutic effects in the immune and the central nervous system. *Br. J. Pharmacol.,* **2009**, *158*(5), 1173-1182.
http://dx.doi.org/10.1111/j.1476-5381.2009.00451.x PMID: 19814729

[179] Quancard, J.; Bollbuck, B.; Janser, P.; Angst, D.; Berst, F.; Buehlmayer, P.; Streiff, M.; Beerli, C.; Brinkmann, V.; Guerini, D.; Smith, P.A.; Seabrook, T.J.; Traebert, M.; Seuwen, K.; Hersperger, R.; Bruns, C.; Bassilana, F.; Bigaud, M. A potent and selective S1P(1) antagonist with efficacy in experimental autoimmune encephalomyelitis. *Chem. Biol.,* **2012**, *19*(9), 1142-1151.
http://dx.doi.org/10.1016/j.chembiol.2012.07.016 PMID: 22999882

[180] Garris, C.S.; Blaho, V.A.; Hla, T.; Han, M.H. Sphingosine-1-phosphate receptor 1 signalling in T cells: trafficking and beyond. *Immunology,* **2014**, *142*(3), 347-353.
http://dx.doi.org/10.1111/imm.12272 PMID: 24597601

[181] Groves, A.; Kihara, Y.; Chun, J. Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. *J. Neurol. Sci.,* **2013**, *328*(1-2), 9-18.
http://dx.doi.org/10.1016/j.jns.2013.02.011 PMID: 23518370

[182] Zhu, Z.; Fu, Y.; Tian, D.; Sun, N.; Han, W.; Chang, G.; Dong, Y.; Xu, X.; Liu, Q.; Huang, D.; Shi, F.D. Combination of the Immune Modulator Fingolimod With Alteplase in Acute Ischemic Stroke: A Pilot Trial. *Circulation,* **2015**, *132*(12), 1104-1112.
http://dx.doi.org/10.1161/CIRCULATIONAHA.115.016371 PMID: 26202811

[183] Tian, D.C.; Shi, K.; Zhu, Z.; Yao, J.; Yang, X.; Su, L.; Zhang, S.; Zhang, M.; Gonzales, R.J.; Liu, Q.; Huang, D.; Waters, M.F.; Sheth, K.N.; Ducruet, A.F.; Fu, Y.; Lou, M.; Shi, F.D. Fingolimod enhances the efficacy of delayed alteplase administration in acute ischemic stroke by promoting anterograde reperfusion and retrograde collateral flow. *Ann. Neurol.,* **2018**, *84*(5), 717-728.
http://dx.doi.org/10.1002/ana.25352 PMID: 30295338

[184] Emsley, H.C.; Tyrrell, P.J. Inflammation and infection in clinical stroke. *J. Cereb. Blood Flow Metab.,* **2002**, *22*(12), 1399-1419.
http://dx.doi.org/10.1097/01.WCB.0000037880.62590.28 PMID: 12468886

[185] Jacobs, A.H.; Tavitian, B. INMiND consortium. Noninvasive molecular imaging of neuroinflammation. *J. Cereb. Blood Flow Metab.,* **2012**, *32*(7), 1393-1415.
http://dx.doi.org/10.1038/jcbfm.2012.53 PMID: 22549622

[186] Jin, R.; Yang, G.; Li, G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. *J. Leukoc. Biol.,* **2010**, *87*(5), 779-789.
http://dx.doi.org/10.1189/jlb.1109766 PMID: 20130219

[187] Schilling, M.; Besselmann, M.; Müller, M.; Strecker, J.K.; Ringelstein, E.B.; Kiefer, R. Predominant phagocytic activity of resident

microglia over hematogenous macrophages following transient focal cerebral ischemia: an investigation using green fluorescent protein transgenic bone marrow chimeric mice. *Exp. Neurol.,* **2005**, *196*(2), 290-297.
http://dx.doi.org/10.1016/j.expneurol.2005.08.004 PMID: 16153641

[188] Ito, D.; Tanaka, K.; Suzuki, S.; Dembo, T.; Fukuuchi, Y. Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. *Stroke,* **2001**, *32*(5), 1208-1215.
http://dx.doi.org/10.1161/01.STR.32.5.1208 PMID: 11340235

[189] Oliveira, G.B.; Fontes, Ede.A., Jr; de Carvalho, S.; da Silva, J.B.; Fernandes, L.M.; Oliveira, M.C.; Prediger, R.D.; Gomes-Leal, W.; Lima, R.R.; Maia, C.S. Minocycline mitigates motor impairments and cortical neuronal loss induced by focal ischemia in rats chronically exposed to ethanol during adolescence. *Brain Res.,* **2014**, *1561*, 23-34.
http://dx.doi.org/10.1016/j.brainres.2014.03.005 PMID: 24637259

[190] Lartey, F.M.; Ahn, G.O.; Ali, R.; Rosenblum, S.; Miao, Z.; Arksey, N.; Shen, B.; Colomer, M.V.; Rafat, M.; Liu, H.; Alejandre-Alcazar, M.A.; Chen, J.W.; Palmer, T.; Chin, F.T.; Guzman, R.; Loo, B.W., Jr; Graves, E. The relationship between serial [(18) F]PBR06 PET imaging of microglial activation and motor function following stroke in mice. *Mol. Imaging Biol.,* **2014**, *16*(6), 821-829.
http://dx.doi.org/10.1007/s11307-014-0745-0 PMID: 24865401

[191] Schroeter, M.; Dennin, M.A.; Walberer, M.; Backes, H.; Neumaier, B.; Fink, G.R.; Graf, R. Neuroinflammation extends brain tissue at risk to vital peri-infarct tissue: a double tracer [11C]PK11195- and [18F]FDG-PET study. *J. Cereb. Blood Flow Metab.,* **2009**, *29*(6), 1216-1225.
http://dx.doi.org/10.1038/jcbfm.2009.36 PMID: 19352400

[192] Bang, O.Y.; Buck, B.H.; Saver, J.L.; Alger, J.R.; Yoon, S.R.; Starkman, S.; Ovbiagele, B.; Kim, D.; Ali, L.K.; Sanossian, N.; Jahan, R.; Duckwiler, G.R.; Viñuela, F.; Salamon, N.; Villablanca, J.P.; Liebeskind, D.S. Prediction of hemorrhagic transformation after recanalization therapy using T2*-permeability magnetic resonance imaging. *Ann. Neurol.,* **2007**, *62*(2), 170-176.
http://dx.doi.org/10.1002/ana.21174 PMID: 17683090

[193] Cramer, S.C. Repairing the human brain after stroke. II. Restorative therapies. *Ann. Neurol.,* **2008**, *63*(5), 549-560.
http://dx.doi.org/10.1002/ana.21412 PMID: 18481291

[194] Cramer, S.C. Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. *Ann. Neurol.,* **2008**, *63*(3), 272-287.
http://dx.doi.org/10.1002/ana.21393 PMID: 18383072

[195] Iadecola, C.; Anrather, J. The immunology of stroke: from mechanisms to translation. *Nat. Med.,* **2011**, *17*(7), 796-808.
http://dx.doi.org/10.1038/nm.2399 PMID: 21738161

[196] Garcia, C.M.; Darland, D.C.; Massingham, L.J.; D'Amore, P.A. Endothelial cell-astrocyte interactions and TGF beta are required for induction of blood-neural barrier properties. *Brain Res. Dev. Brain Res.,* **2004**, *152*(1), 25-38.
http://dx.doi.org/10.1016/j.devbrainres.2004.05.008 PMID: 15283992

[197] Dohgu, S.; Yamauchi, A.; Takata, F.; Naito, M.; Tsuruo, T.; Higuchi, S.; Sawada, Y.; Kataoka, Y. Transforming growth factor-beta1 upregulates the tight junction and P-glycoprotein of brain microvascular endothelial cells. *Cell. Mol. Neurobiol.,* **2004**, *24*(3), 491-497.
http://dx.doi.org/10.1023/B:CEMN.0000022776.47302.ce PMID: 15206827

[198] Nishioku, T.; Dohgu, S.; Takata, F.; Eto, T.; Ishikawa, N.; Kodama, K.B.; Nakagawa, S.; Yamauchi, A.; Kataoka, Y. Detachment of brain pericytes from the basal lamina is involved in disruption of the blood-brain barrier caused by lipopolysaccharide-induced sepsis in mice. *Cell. Mol. Neurobiol.,* **2009**, *29*(3), 309-316.
http://dx.doi.org/10.1007/s10571-008-9322-x PMID: 18987969