

Mechanism of disturbed endothelial cell function on angiogenesis following ischemic brain stroke (Review)

RUI GONG¹, JIN-LANG TAN¹, GANG LIU², XIAO-FANG LIU², LE MA¹ and SHUAI SHI²

¹Department of Rehabilitation of Chinese Medicine, Heilongjiang University of Traditional Chinese Medicine, Harbin, Heilongjiang 150040, P.R. China; ²Department of Acupuncture, Moxibustion and Tuina, The Second Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine, Harbin, Heilongjiang 150001, P.R. China

Received July 16, 2024; Accepted January 10, 2025

DOI: 10.3892/etm.2025.12811

Abstract. The present study focused on the mechanisms of post-ischemic stroke (IS) angiogenesis from the perspective of endothelial cells (ECs) dysfunction. First, it emphasized the importance of hypoxia-inducible factor-1 α in the function of ECs under hypoxic conditions, particularly in promoting angiogenesis and improving cerebral blood supply. Secondly, inflammatory cytokines and adhesion factors (for example, selectins, the immunoglobulin superfamily and integrins) influence the function and angiogenesis of ECs through various mechanisms and signaling pathways following IS. In addition, the effects of oxidative stress on ECs function and angiogenesis were explored, along with the potential of antioxidant strategies to improve EC function and promote angiogenesis. Based on these insights, the present study proposed new therapeutic strategies to ameliorate endothelial dysfunction and promote angiogenesis following IS, including small-molecule drugs targeting specific molecules, gene therapy and traditional Chinese medicine treatments. Finally, the importance of translating these laboratory findings into clinical applications was emphasized, alongside the need for advanced imaging techniques to monitor the dynamic processes of post-IS angiogenesis and evaluate the efficacy of novel therapeutic interventions. These explorations aimed at providing a more comprehensive understanding of EC function and the regulatory mechanisms of a deeper understanding of angiogenesis following IS, offering new intervention strategies for IS treatment.

Contents

1. Introduction
2. EC origin and function
3. EC dysfunction following IS
4. Conclusions

1. Introduction

Ischemic stroke (IS) has amongst the highest morbidity and disability rates worldwide (1). Thrombolytic therapy, which uses a tissue plasminogen activator to restore cerebral blood flow, is currently one of the most effective treatments for IS. However, its application is limited to a small subset of patients, due to the restrictive time window for administration (1,2). During the early stages of IS, collateral circulation is primarily dependent on the reopening of pre-existing vascular networks. In the later stages, this process shifts toward angiogenesis. Research has revealed that angiogenesis typically starts ~3 days post-stroke, and progresses for 21 days or longer (2). Autopsy studies of brain tissue from patients with IS, with varying survival durations, revealed a notable increase in micro-vessel density in the infarcted area compared with the contralateral hemisphere (3). Furthermore, the extent of peri-infarct angiogenesis was found to be positively correlated with survival, survival duration and neurological recovery. These findings suggested that angiogenesis in the infarcted region following IS holds a significant therapeutic potential. In mouse models of middle cerebral artery occlusion (MCAO), proliferating endothelial cells (ECs) have been observed in the ischemic penumbra, contributing to an increase in vascular density (4).

Following IS, EC dysfunction occurs first due to the direct contact between the vascular endothelium and blood in the artery. ECs are located in the innermost layer of the arterial wall, and fluid shear stress in the vessel wall primarily affects ECs. Disturbed blood flow promotes an ECs' response in atherosclerosis, including dysfunction of both contractile and diastolic functions of ECs (5). Ischemic recovery is mediated by neoangiogenesis and requires interactions between ECs and pericytes to form a stable microvascular network (6). The blood-brain barrier (BBB) is primarily constituted

Correspondence to: Professor Shuai Shi, Department of Acupuncture, Moxibustion and Tuina, The Second Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine, 411 Guoge Li Street, Harbin, Heilongjiang 150001, P.R. China
E-mail: 18955386@qq.com

Key words: endothelial cells, angiogenesis, oxidative stress, inflammatory cytokines, adhesion factor, pathway, ischemic stroke

by diverse types of brain ECs. It ensures the transport of specific nutrients from peripheral circulation to the central nervous system (CNS) while preventing the entry of harmful substances. The mechanism through which activation of the Caspase-4/11-GSDMD signaling pathway in brain ECs results in inflammatory disruption of the BBB has been identified (7). A cascade of effects is triggered in ECs following IS, and it is crucial to examine the generation and development of EC dysfunction following IS and to investigate how this dysfunction impacts angiogenesis. The aim of the present review was to summarize and analyze the mechanisms of EC dysfunction post-IS to provide new insights for IS prevention and treatment.

2. EC origin and function

ECs are derived from endothelial progenitor cells (EPCs), which can either distinctly develop into ECs and incorporate into the injured vessel or regulate the injury through paracrine factors acting on surrounding cells and vessels (8).

The differentiation of EPCs into ECs is a multi-step process that can be divided into three distinct phases (9): i) Integrin-mediated adhesion to the extracellular matrix (10); ii) growth factor-driven proliferation and differentiation (11); and iii) the regulation of EC maturation and stabilization by specific transcription factors (12). Beyond their role in differentiating into ECs, EPCs are also known to secrete various paracrine factors, including vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF-1), insulin-like growth factor 1, active monocyte chemoattractant protein 1, macrophage inflammatory protein-1 α and platelet-derived growth factor (13). These secreted factors can interact with multiple cell types to facilitate angiogenesis and promote tissue repair. The origin of ECs and the overall process of angiogenesis are demonstrated in Fig. 1.

3. EC dysfunction following IS

ECs and hypoxia-inducible factor-1 α (HIF-1 α). Under normal oxygen levels, the proline residue of the HIF-1 α protein binds to Von Hippel-Lindau proteins in the presence of oxygen, iron (II) and α -ketoglutarate, leading to its rapid degradation through the ubiquitin-proteasome pathway (14). By contrast, hypoxic conditions prevent the hydroxylation of HIF-1 α , allowing it to accumulate, translocate to the nucleus and bind with HIF-1 β , thereby triggering the transcription of genes related to hypoxia, such as VEGF (15). The brain is highly sensitive to oxygen and nutrient fluctuations, requiring efficient blood supply. To support its metabolic needs, the glucose transporter type 1 (GLUT1) protein is expressed in ECs and neurons, facilitating glucose transport and sustaining glycolytic processes. Following IS, neuronal cells in the ischemic penumbra have an increased demand for nutrients, which cannot be transported without ECs. HIF-1 α activation can maintain redox homeostasis by facilitating glucose transport and glycolysis. Studies have revealed that hypoxic preconditioning helps reduce cortical neuronal loss in rats with traumatic brain injury (16,17). This protective effect is primarily linked to the upregulation of HIF-1 α , which in turn stimulates the expression of GLUT1 and 3, enhancing glucose uptake by neurons. The phosphatidylinositol 3-kinase/protein

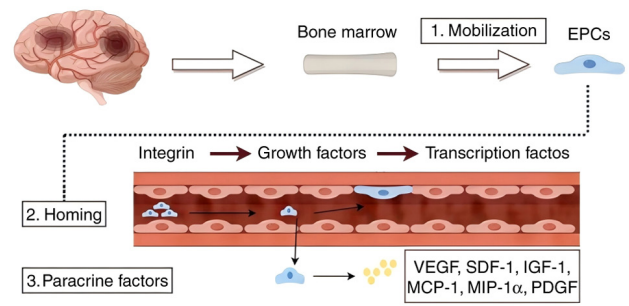


Figure 1. Endothelial cells derived from EPCs. EPCs, endothelial progenitor cells; VEGF, vascular endothelial growth factor; SDF-1, stromal cell derived factor-1; IGF-1, insulin-like growth factor-1; MCP-1/CCL2, active monocyte chemoattractant protein-1; MIP-1 α , macrophage inflammatory protein-1 α ; PDGF, platelet-derived growth factor.

kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway plays a crucial role in vascularization, including the survival, migration and angiogenesis of ECs. That activation of the PI3K/AKT/mTOR pathway further stimulates its downstream target, HIF-1 α , which regulates the expression of VEGF, a key factor that promotes vascularization following IS (18). In addition, receptors such as the epidermal growth factor receptor, fibroblast growth factor receptors and IL-6 receptor exert neuroprotective effects against cerebral ischemia by activating the PI3K/AKT/mTOR pathway, leading to increased levels of HIF-1 α (19). HIF-1 α , in turn, promotes angiogenesis in ischemic tissues by increasing VEGF expression (20). In ECs, the PI3K/AKT pathway is also involved in angiogenesis through its regulation of nitric oxide (NO) signaling, which is modulated by endothelial NO synthase (eNOS). Evidence suggests that NO donors can enhance the transcriptional activity and expression of HIF-1 α , thereby increasing VEGF mRNA levels (21). These findings indicated that HIF-1 α plays a pivotal role in regulating EC dysfunction under oxidative stress following IS, largely through stimulating angiogenesis. The mechanism of HIF-1 α and ECs angiogenesis-related factors is demonstrated in Fig. 2.

ECs and inflammatory cytokines. Pro-inflammatory molecules, such as TNF- α , IL-1 and IL-6, are key contributors to the development of cerebral infarction. Although these factors exacerbate tissue damage mainly by promoting inflammatory responses, they can, in some cases, participate in angiogenesis by modulating angiogenesis-related signaling pathways.

TNF- α . TNF- α is a cytokine that exerts numerous pro-inflammatory effects and is pivotal in both pathological and physiological processes (22). Following IS, TNF- α is activated within 1 h, reaches its peak between 6 and 10 h, and typically diminishes within 1 to 2 days (23). Increased TNF- α exerts both neuroprotective and neurotoxic effects following IS (23). TNF- α exerts its effects by binding to two receptors, TNF receptor 1 (TNFR1) and TNFR2, both of which are involved in hind limb ischemia-induced angiogenesis (24). When TNF- α interacts with TNFR1, it triggers the expression of pro-erythropoietin (EPO) in cerebral ECs by increasing the EPO receptor (EPOR) levels. EPO then promotes angiogenesis by upregulating EPOR, which enhances the activation of

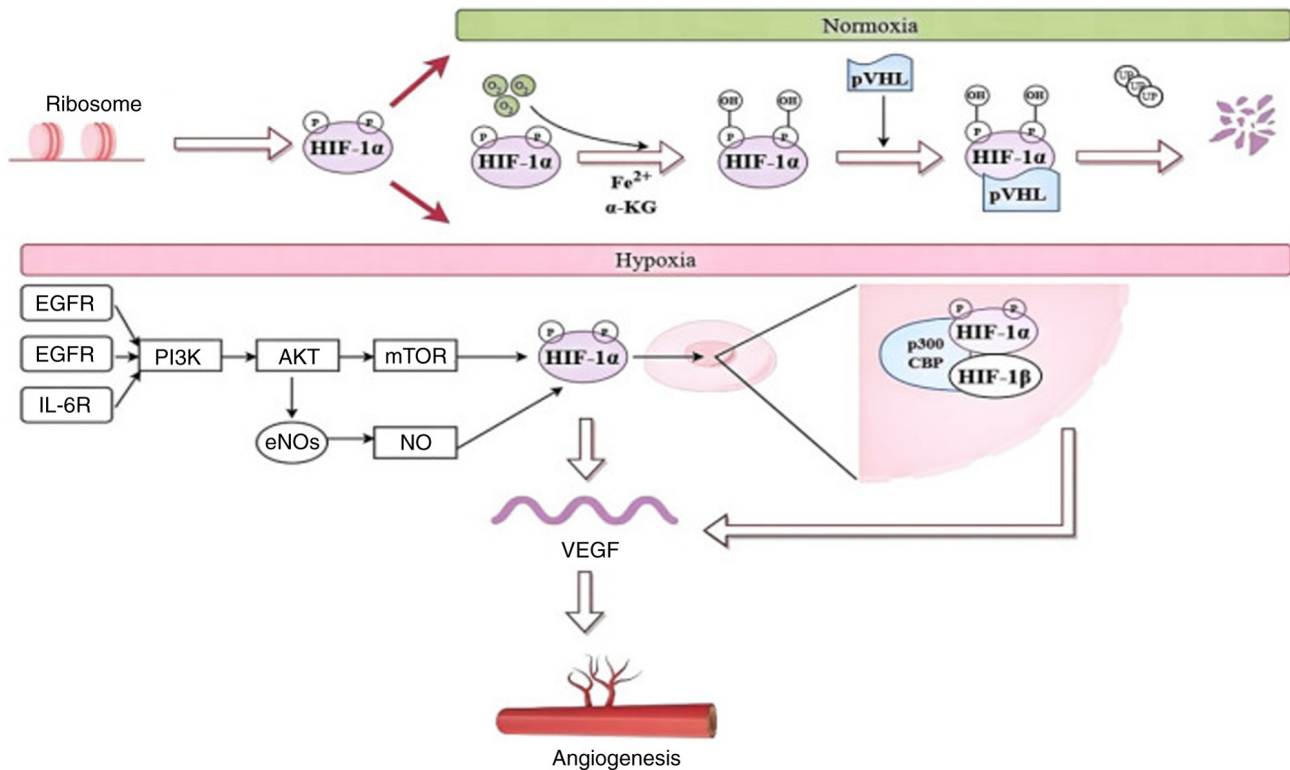


Figure 2. HIF-1 α derived from the ribosomes can be expressed in different ways under normoxic and hypoxic conditions. Arrows indicate signal propagation downstream. HIF-1 α , hypoxia-inducible factor-1 α ; Fe²⁺, ferrous iron; α -KG, α -ketoglutarate; EGFR, epidermal growth factor receptor, FGFR, fibroblast growth factor receptors; IL-6R, interleukin-6 receptor; PI3K/AKT/mTOR, phosphatidylinositol 3/Kinase protein kinase B/mammalian target of rapamycin; NO, nitric oxide; eNOS, endothelial NO synthase; VEGF, vascular endothelial growth factor.

signaling pathways such as VEGF/VEGFR2 and angiopoietin-1/Tie2 (Ang1/Tie2) (25). Furthermore, TNF- α directly influences ECs to regulate angiogenesis via VEGF signaling activation. In addition, TNF activates several signaling pathways, including NF- κ B and AKT. NF- κ B activation by TNF can trigger AKT signaling, and both the NF- κ B and PI3K/AKT pathways are crucial for the upregulation of TNF/TNFR1 in EPOR (26). These findings suggested that TNF- α may play a neuroprotective role by promoting angiogenesis through EPO and its associated signaling mechanisms.

IL-1. The pro-inflammatory cytokine IL-1 plays a central role in cerebrovascular inflammation following ischemic injury. After cerebral ischemia, microglia, astrocytes and ECs release two isoforms of IL-1, IL-1 α and IL-1 β , which primarily target ECs and astrocytes (27,28). IL-1 stimulates the expression of LG3, a C-terminal fragment of perlecan, in brain cell cultures. Perlecan is a key component of the basement membrane of ECs in mature tissues, and LG3 has been identified as a potentially neuroprotective and pro-angiogenic fragment of the extracellular matrix (29). LG3 can mitigate β -amyloid-induced neuronal toxicity by binding to the α 2 β 1 integrin (30). In addition, IL-1 promotes the expression of pentraxin-3 (PTX3), an acute-phase protein involved in brain repair processes following ischemia. PTX3 is crucial for mechanisms such as neurogenesis and angiogenesis in the post-ischemic brain (31). After 14 days of MCAO, VEGF exerts a potent pro-angiogenic effect through the activation of VEGFR2, which is upregulated after ischemia and reduced in PTX3 knockout mice (32).

The pro-inflammatory actions of IL-1 β are mediated via its receptor, IL-1R1. The inhibition of IL-1 β binding to IL-1R1 can prevent cerebral edema and brain tissue damage in experimental IS models. IL-1 β stimulates the chemokines SDF-1/CXCL-12, which accumulates at the BBB, promoting leukocyte infiltration into the CNS, along with microvascular leakage and cerebral edema (33). Studies on the impact of IL-1 β on trans-endothelial electrical resistance in human cerebral microvasculature have revealed that IL-1 β disrupts the endothelial barrier through the PKC-dependent phosphorylation of PKC- θ and ZO-1 (34). As IL-1 β is a key inflammatory mediator, targeting the inhibition of PKC- θ or ZO-1 phosphorylation offers a potential strategy to prevent BBB disruption during neuroinflammation (35). In addition, macrophage-derived IL-1 β enhances the expression of the pro-angiogenic isoform VEGF-A165a, while suppressing the anti-angiogenic VEGF-A165b through the activation of signal transducer and activator of transcription-3 (STAT-3) and NF- κ B pathways. This shift promotes VEGF expression and contributes to inflammatory vascularization (36).

IL-1 α has been revealed to be significantly more potent than IL-1 β in activating ECs, as evidenced by the increased expression of the pro-angiogenic chemokine CXCL-1. In addition, IL-1 α stimulates a strong, concentration-dependent expression of the angiogenic mediator IL-6 (37). It also promotes EC proliferation, migration and the formation of tubular structures. These responses can be blocked by the IL-1 receptor antagonist. Thus, while IL-1-driven inflammation can exacerbate ischemic injury, it also plays a dual role

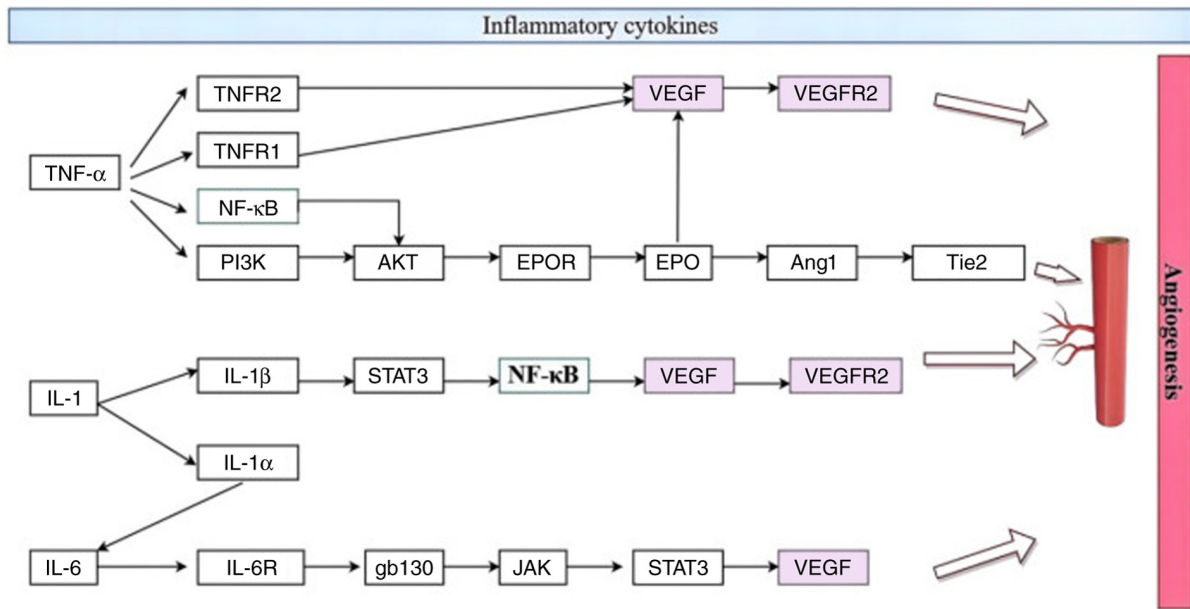


Figure 3. Endothelial cells and inflammatory cytokines. TNFR1/2, TNF receptor 1/2; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; EPO, erythropoietin; EPOR, EPO receptor; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2; Ang1, angiopoietin-1; Tie2, tyrosine kinase with immunoglobulin and EPO receptor domains receptor; STAT3, signal transducer and activator of transcription 3; IL-6R, IL-6 receptor; gp130, glycoprotein 130; JAK, Janus kinase.

by enhancing the brain repair mechanisms and supporting functional recovery.

IL-6. IL-6 is a multifunctional cytokine and an important messenger molecule between leukocytes, vascular ECs and thin-walled tissue-resident cells, produced by several types of cells, such as monocytes, macrophages, adipocytes, hematopoietic cells and ECs (38). The function of IL-6 is mediated through a unique receptor system consisting of two functional proteins: An IL-6-specific receptor (IL-6R) and a second glycoprotein, gp130. Signaling through gp130 is mediated by two pathways: The JAK-STAT and the Ras-MAPK pathway (39). In the vasculature, IL-6 can act directly or indirectly on vascular ECs. IL-6 preconditioning induces VEGF secretion from neural stem cells via STAT3, which promotes angiogenesis in the ischemic brain (40). A related study (41) found that two days after mild transient cerebral ischemia, wild-type mice exhibited a significant early increase in IL-6 gene transcription and protein levels in the ischemic brain. This was accompanied by an early upregulation of mRNAs for angiogenesis-related genes, such as VEGFR2 and eNOS. In mice with MCAO, the increase in circulating VEGF was diminished following IL-6 knockdown at 48 h (42). Monocyte-derived IL-6 plays a critical role in programming microglia to repair the damaged brain vasculature. Cerebrovascular repair was absent in IL-6 knockout mice or those lacking microglial IL-6Ra expression, but could be restored through exogenous IL-6 administration (43).

The aforementioned studies suggested that IL-6 promotes angiogenesis following cerebral infarction, thereby providing long-term histological and functional protection. ECs and inflammatory cytokines are shown in Fig. 3.

ECs and adhesion factors. The EC membrane serves as the interface between ECs and the external environment,

facilitating material exchange and signal transmission. The integrity and dynamic changes of the EC membrane significantly influence the activity and role of different adhesion molecules. These molecules, expressed on the ECs membrane, are critical in vascular biology as they regulate blood flow, immune cell migration and inflammatory responses. Specifically, adhesion molecules are vital for leukocyte infiltration into the CNS. The molecular interactions between ECs and circulating leukocytes are primarily mediated by three categories of adhesion molecules: Selectins such as E-, P- and L-selectins, immunoglobulin superfamily members such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and integrins.

Selectins. Selectins are membrane-bound glycoproteins with three main members: E-selectin (CD62E), P-selectin (CD62P) and L-selectin (CD62L) (44). The molecular mechanisms of leukocyte infiltration into ischemic tissues involve these selectins. CD62E, also known as E-selectin, plays a crucial role in the adhesion of leukocytes to ECs, and its involvement in the homing and angiogenesis of EPCs has been experimentally studied. CD62E is synthesized in response to inflammatory stimuli, such as TNF- α and IL-1, and is expressed on the EC membrane within hours of stimulation. CD62P or P-selectin, is found on ECs and platelet granule membranes, and is rapidly expressed on the outer cell membrane upon activation by factors such as thrombin or histamine. CD62L is present on lymphocytes, neutrophils and monocytes, and following cell activation it is cleaved from the membrane through proteolytic processes.

Immunoglobulin superfamily. The immunoglobulin superfamily consists of a group of cell surface and soluble proteins, several of which serve as adhesion molecules. A total of 5 key members of this family include ICAM-1, ICAM-2, VCAM-1, platelet endothelial cell adhesion molecule 1 and mucosal

vascular addressin in cell adhesion molecule 1. Among these, ICAM-1 and VCAM-1 are some of the most extensively studied adhesion molecules in the context of IS. The expression of these EC adhesion molecules is a crucial step in the recruitment of circulating leukocytes to areas of inflammation. Hypoxic conditions and cytokines, such as IL-1 β and TNF- α , significantly enhance the expression of these molecules (45).

The role of ICAM-1 in regulating cerebral leukocyte recruitment during neuroinflammation and ischemia has been clearly established through studies involving ICAM-1 knockout mice and the use of ICAM-1 blocking antibodies (46). *In vivo* research in mice has revealed that TNF- α triggers the upregulation of both ICAM-1 and VCAM-1. The expression levels of these molecules begin to rise between 2 and 5 h after brain injury, peak between 5 and 9 h, and remain elevated above baseline levels for at least 24 h (47). Of note, the increases in ICAM-1 and VCAM-1 expression are dose-dependent, with a significant upregulation at 5 $\mu\text{g}/\text{kg}$ and a maximal increase observed at doses between 10-25 $\mu\text{g}/\text{kg}$.

Correlative studies have revealed that VEGF increases VCAM-1 and ICAM-1 protein levels and promotes leukocyte adhesion in an NF- κB -dependent manner (48). ICAM-1 expression influences VEGF-A-induced eNOS activity and angiogenesis by regulating endothelial glutathione levels (49). Studies also have revealed that both VCAM-1 and integrin $\alpha 4$ (ITGA4) are upregulated by TNF- α in ECs. sVCAM-1-induced angiogenesis is facilitated by the upregulation of VEGF through the p38 MAPK/FAK signaling pathway. The sVCAM-1/ITGA4 pathway may play a role in inflammatory angiogenesis (50).

The aforementioned findings suggested that inflammatory cytokines promote the increase in the expression of ICAM-1 and VCAM-1, which enhances the interaction between ECs and circulating leukocytes, thereby promoting angiogenesis following IS.

Integrins. Integrins are heterodimeric proteins made up of distinct α -subunits paired with common β -subunits. The β -subunits are categorized into three subfamilies: $\beta 1$, $\beta 2$ and $\beta 3$ integrins. Integrins in the $\beta 1$ subfamily play a key role in connecting to the extracellular matrix, binding to collagen, laminin and fibronectin. By contrast, $\beta 2$ integrins are crucial for mediating the adhesion between leukocytes and ECs. $\beta 3$ integrins, also referred to as integrin $\alpha\beta 3$ or cytokines, are involved in blood clot formation and stabilization. These integrins are vital for regulating processes such as hematopoiesis, leukocyte recruitment and inflammatory responses (51).

Brain ECs demonstrate minimal expression of adhesion molecules, which prevents peripheral immune cells from crossing into the CNS (25). $\beta 2$ Integrin, a key integrin subunit, plays a significant role in directing EPCs to areas of active vascular angiogenesis. Known as a leukocyte-specific receptor, $\beta 2$ integrin interacts with various ICAM family members and polysaccharides (52).

The impact of neuroinflammatory mechanisms (whether detrimental or protective) depends on the timing following cerebral ischemia. Inflammation may exacerbate ischemic injury in the early stages of IS, whereas the inflammatory response may protect neurons by promoting neurogenesis, angiogenesis and neuroplasticity in the later stages. Integrins such as $\alpha 5\beta 1$ and $\alpha\beta 3$, play a crucial role in regulating the

processes of angiogenesis and inflammation following cerebral ischemia (53). The Ang1/Tie2 signaling pathway and the integrin $\alpha 5\beta 1$ interact with ECs following IS, promoting angiogenesis (54). VEGF upregulation by $\alpha 5\beta 1$ and $\alpha\beta 3$, and their ligand-endo-ligand proteins in the ischemic penumbra, stimulate EC proliferation. TNF- α or FGF-2-induced angiogenesis is dependent on $\alpha\beta 3$, while VEGF and TGF- α -induced angiogenesis is dependent on $\alpha\beta 5$, suggesting that αv integrins play a key role in potent angiogenesis (55).

Adhesion molecules on ECs play a crucial role in maintaining vascular health and function. Targeting these molecules to modulate their expression can reduce inflammation and improve EC function. For example, the development of small-molecule inhibitors targeting ICAM-1 or VCAM-1 may help suppress intravascular inflammation and reduce cerebrovascular lesions (56). The development of therapies that promote EC repair and regeneration may help to improve the function of the vascular endothelium and provide a new direction in the treatment of cerebrovascular diseases. For example, stem cell therapy (57) or gene therapy (58) is used to promote endothelial repair and restore the normal function of blood vessels. The association between ECs and adhesion factors is demonstrated in Fig. 4.

4. Conclusions

Following IS, ECs trigger a cascade of reactions, including oxidative stress and inflammation. Promoting angiogenesis can help alleviate the ischemic and hypoxic conditions in the affected brain tissue.

Next, the clinical translational applications which are related to the aforementioned cytokines will be discussed. For HIF-1 α , related studies have revealed that Dan-Deng-Tong-Nao soft gel capsules promote angiogenesis to safeguard brain tissue against IS and exert beneficial effects in brain microvascular ECs by activating the HIF-1 α /VEGFA/NOTCH1 signaling pathway (57-59). Chinese patent medicines have significant advantages in the long-term treatment of stroke, due to their versatility and multi-target nature (59). Tissue kallikrein (TK) has emerged as a promising neuroprotective agent in IS. Research in preclinical models has revealed that TK supplementation activates the PI3K/AKT signaling pathway by enhancing the expression of bradykinin receptor 2 during the ischemic phase. This activation promotes the nuclear translocation of HIF-1 α , which in turn boosts the expression of VEGF and eNOS, strengthening the neurovascular unit. In addition, TK suppresses the activation of the kallikrein-kinin system triggered by reperfusion injury, effectively reducing inflammation, oxidative stress production and endothelial barrier dysfunction (60). The treatment of endothelial barrier dysfunction by Dan-Deng-Tong-Nao soft gel capsules and TK warrants continued exploration in the future.

Inflammatory cytokines such as TNF, IL-1 and IL-6 play a crucial role in tissue damage during stroke, making them important targets for IS treatment. Studies have revealed that these cytokines significantly contribute to stroke-induced injury (61). Specifically, TNF- α and IL-1 β can trigger the expression of procoagulant molecules, such as tissue factor and plasminogen activator inhibitor-1, by activating the NF- κB and AP-1 signaling pathways (61).

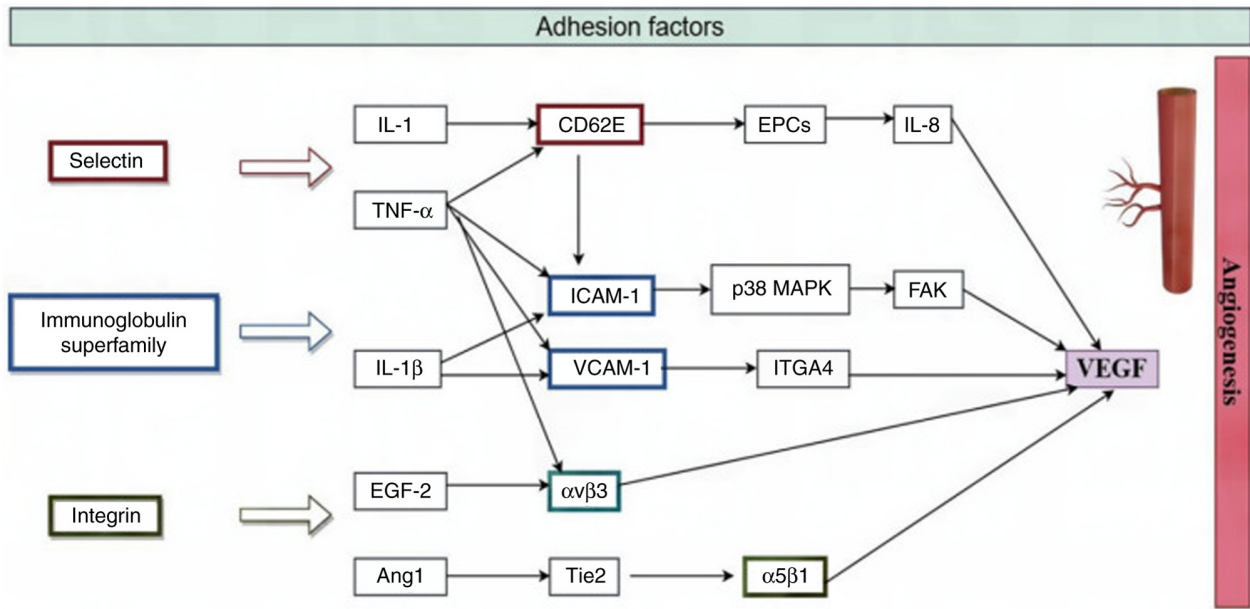


Figure 4. Endothelial cells and adhesion factors. CD62E, e-selectin; EPCs, endothelial progenitor cells; ICAM-1, intercellular adhesion molecule 1; p38 MAPK, p38 mitogen-activated protein kinase; FAK, focal adhesion kinase; VCAM-1, vascular cell adhesion molecule 1; ITGA4, Integrin $\alpha 4$; FGF-2, fibroblast growth factor 2; $\alpha v\beta 3$, Integrin $\alpha v\beta 3$; Ang1, Angiopoietin-1; Tie2, tyrosine kinase with immunoglobulin and EPO receptor domains receptor; $\alpha 5\beta 1$, Integrin $\alpha 5\beta 1$; VEGF, vascular endothelial growth factor.

Hexahydrocurcumin (HHC), a major metabolite of curcumin, has been found to improve hypertensive and vascular remodeling in N ω -Amino-L-arginine methyl ester (L-NAME)-induced rats (62). TGF- β plays a pivotal role in inflammatory responses by promoting fibrogenesis, a key process in vascular remodeling. Matrix metalloproteinases (MMPs), a family of zinc-dependent enzymes, are involved in degrading extracellular matrix components such as collagen and elastin. During vascular formation and remodeling, MMPs act as inflammatory mediators. Elevated levels of TGF- $\beta 1$, MMP-2 and MMP-9 have been observed in L-NAME-induced hypertensive rats, indicating their involvement in the pathogenesis of vascular changes.

HHC treatment has been revealed to reduce the expression of key proteins involved in vascular remodeling, including TGF- $\beta 1$, MMP-9 and collagen type I. In addition, N-myc downstream-regulated gene 1 (NDRG1), a member of the NDRG family, plays a crucial role in cell differentiation, proliferation and stress responses. NDRG1 is a key mediator in regulating endothelial inflammation, thrombotic responses and vascular remodeling. NDRG1 knockdown using lentivirus-based NDRG1 shRNA significantly reduces the expression of cytokines, chemokines and adhesion molecules induced by IL-1 β and TNF- α (63). Given this, the strategic use of HHC at appropriate times, combined with targeted regulation of NDRG1, holds promise as a therapeutic approach for modulating endothelial inflammation, thrombotic responses and angiogenesis in the context of IS.

Studies on adhesion molecules indicate that gingerol, the active compound in fresh ginger, undergoes dehydration to form 6-shogaol in dried ginger rhizomes. 6-Shogaol has been revealed to reduce the levels of cell adhesion molecules, particularly in lipopolysaccharide-activated ECs. It notably decreased the expression of ICAM-1, VCAM-1 and E-selectin

on the endothelial surface in response to TNF stimulation. Furthermore, mRNA analysis demonstrated that higher concentrations of 6-shogaol led to a concentration-dependent reduction in the gene expression of ICAM-1, VCAM-1 and E-selectin. Therefore, identifying components with the opposite effect to gingerol may present a new direction for future clinical research aimed at promoting the regulation of EC adhesion molecules and enhancing angiogenesis following IS (64).

In the present study, from the perspective of EC dysfunction, the angiogenesis pathways associated with ECs following IS were summarized. The exploration of these mechanisms can provide intervention strategies for future treatment following IS. The importance of HIF-1 α in EC function under hypoxic conditions was emphasized, particularly in promoting angiogenesis and improving cerebral blood supply, both of which are crucial in the management and avoidance of cerebral ischemia.

Inflammatory cytokines and adhesion factors affect the function and angiogenesis of ECs after IS through distinct mechanisms and signaling pathways, thereby playing a significant role in the pathological processes of cerebral ischemia. However, there remains much to explore regarding the dysfunction of ECs after IS.

For example, from the perspective of inflammatory factors, it is important to continue investigating the expression patterns and mechanisms of action of inflammatory cytokines, including TNF- α , IL-1 and IL-6, after IS and how they regulate angiogenesis by affecting ECs. This includes exploring how these cytokines activate receptors on ECs and their temporal dynamics during inflammation and repair.

From the perspective of adhesion factors, it is necessary to investigate changes in the expression of adhesion factors, such as selectins, the immunoglobulin superfamily, and integrins,

after IS, and how they influence leukocyte recruitment and EC function. Exploring the specific roles of these molecules in angiogenesis and repair, as well as how they interact with other signaling pathways, is critical.

From the perspective of oxidative stress, the impact of oxidative stress generated by ECs on their function and angiogenesis after IS should be studied. Understanding how ECs respond to oxidative stress and whether antioxidant strategies can improve EC function and promote angiogenesis is essential.

Based on the understanding of these pathways and molecules, novel therapeutic strategies can be developed to ameliorate endothelial dysfunction and promote angiogenesis after IS. These strategies may include small molecule drugs targeting specific molecules, biologics, gene therapy and traditional Chinese medicines.

Further translation into clinical applications will be achieved by translating laboratory discoveries into clinical practice, incorporating multimodal imaging technology, and utilizing advanced imaging techniques to monitor the dynamic process of post-IS angiogenesis. These techniques will also assess the effects of novel therapeutic interventions. This includes analyzing clinical trials and patient samples to validate laboratory results and identify the most promising therapeutic targets. These explorations will result in a deeper understanding of EC function and the regulatory mechanisms of angiogenesis after IS, providing new intervention strategies for the treatment of IS.

Acknowledgements

Not applicable.

Funding

The present study was supported by Heilongjiang Provincial Natural Science Foundation of China (grant no. LH2022H080).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

RG conceived and designed the study. JLT, GL, XFL and LM collected the data and performed the literature search. RG was involved in the writing of the manuscript. SS was responsible for the revision of the manuscript. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Saini V, Guada L and Yavagal DR: Global epidemiology of stroke and access to acute ischemic stroke interventions. *Neurology* 97 (Suppl 2): S6-S16, 2021.
- Zhu L, Wang M, Liu Y, Fu P, Zhang W, Zhang H, Roe AW and Xi W: Single-microvessel occlusion produces lamina-specific microvascular flow vasodynamics and signs of neurodegenerative change. *Cell Rep* 42: 112469, 2023.
- Krupinski J, Kaluza J, Kumar P, Kumar S and Wang JM: Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke* 25: 1794-1798, 1994.
- Kim JJ, Kim DH, Lee JY, Lee BC, Kang I, Kook MG, Kong D, Choi SW, Woo HM, Kim DI and Kang KS: cAMP/EPAC signaling enables ETV2 to induce endothelial cells with high angiogenesis potential. *Mol Ther* 28: 466-478, 2020.
- Tamargo IA, Baek KI, Kim Y, Park C and Jo H: Flow-induced reprogramming of endothelial cells in atherosclerosis. *Nat Rev Cardiol* 20: 738-753, 2023.
- Cartland SP, Patil MS, Kelland E, Le N, Boccanfuso L, Stanley CP, Cholan PM, Dona MI, Patrick R, McGrath J, *et al*: The generation of stable microvessels in ischemia is mediated by endothelial cell derived TRAIL. *Sci Adv* 10: eadn8760, 2024.
- Wei C, Jiang W, Wang R, Zhong H, He H, Gao X, Zhong S, Yu F, Guo Q, Zhang L, *et al*: Brain endothelial GSDMD activation mediates inflammatory BBB breakdown. *Nature* 629: 893-900, 2024.
- Yan F, Liu X, Ding H and Zhang W: Paracrine mechanisms of endothelial progenitor cells in vascular repair. *Acta Histochem* 124: 151833, 2022.
- Oh IY, Yoon CH, Hur J, Kim JH, Kim TY, Lee CS, Park KW, Chae IH, Oh BH, Park YB and Kim HS: Involvement of E-selectin in recruitment of endothelial progenitor cells and angiogenesis in ischemic muscle. *Blood* 110: 3891-3899, 2007.
- Resnikoff HA and Schwarzbauer JE: Increased basal fibronectin is sufficient to promote excess endothelial cell matrix assembly causing localized barrier dysfunction. *Mol Biol Cell* 35: ar120, 2024.
- Hildbrand P, Cirulli V, Prinsen RC, Smith KA, Torbett BE, Salomon DR and Crisa L: The role of angiopoietins in the development of endothelial cells from cord blood CD34+ progenitors. *Blood* 104: 2010-2019, 2004.
- Heinisch PP, Bello C, Emmert MY, Carrel T, Dreßen M, Hörer J, Winkler B and Luedi MM: Endothelial progenitor cells as biomarkers of cardiovascular pathologies: A narrative review. *Cells* 11: 1678, 2022.
- Sultan I, Ramste M, Peletier P, Hemanthakumar KA, Ramanujam D, Tirronen A, von Wright Y, Antila S, Saharinen P, Eklund L, *et al*: Contribution of VEGF-B-Induced endocardial endothelial cell lineage in physiological versus pathological cardiac hypertrophy. *Circ Res* 134: 1465-1482, 2024.
- Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, *et al*: Targeting of HIF- α to the von hippel-lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* 292: 468-472, 2001.
- Cha S, Kim HG, Jang H, Lee J, Chao T, Baek NI, Song IS and Lee YM: Steppogenin suppresses tumor growth and sprouting angiogenesis through inhibition of HIF-1 α in tumors and DLL4 activity in the endothelium. *Phytomedicine* 108: 154513, 2023.
- Qin C, Yang S, Chu YH, Zhang H, Pang XW, Chen L, Zhou LQ, Chen M, Tian DS and Wang W: Signaling pathways involved in ischemic stroke: Molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther* 7: 215, 2022.
- Chen X, Qian W, Zhang Y, Zhao P, Lin X, Yang S, Zhuge Q and Ni H: Ginsenoside CK cooperates with bone mesenchymal stem cells to enhance angiogenesis post-stroke via GLUT1 and HIF-1 α /VEGF pathway. *Phytother Res* 38: 4321-4335, 2024.
- Patra K, Jana S, Sarkar A, Mandal DP and Bhattacharjee S: The inhibition of hypoxia-induced angiogenesis and metastasis by cinnamaldehyde is mediated by decreasing HIF-1 α protein synthesis via PI3K/Akt pathway. *Biofactors* 45: 401-415, 2019.

19. Chen X, Fu K, Lai Y, Dong C, Chen Z, Huang Y, Li G, Jiang R, Wu H, Wang A, *et al*: Tetrahydropalmitine: Orchestrating survival-Regulating autophagy and apoptosis via the PI3K/AKT/mTOR pathway in perforator flaps. *Biomed Pharmacother* 169: 115887, 2023.
20. Amin N, Chen S, Ren Q, Tan X, Botchway BOA, Hu Z, Chen F, Ye S, Du X, Chen Z and Fang M: Hypoxia inducible factor-1 α attenuates ischemic brain damage by modulating inflammatory response and glial activity. *Cells* 10: 1359, 2021.
21. Jeong H, Choi D, Oh Y, Heo J and Hong J: A Nanocoating co-localizing nitric oxide and growth factor onto individual endothelial cells reveals synergistic effects on angiogenesis. *Adv Health Mater* 11: e2102095, 2022.
22. Wu DM, Liu JP, Liu J, Ge WH, Wu SZ, Zeng CJ, Liang J, Liu K, Lin Q, Hong XW, *et al*: Immune pathway activation in neurons triggers neural damage after stroke. *Cell Rep* 42: 113368, 2023.
23. Di Santo C, La Russa D, Greco R, Persico A, Zanaboni AM, Bagetta G and Amantea D: Characterization of the involvement of tumour necrosis factor (TNF)- α -stimulated gene 6 (TSG-6) in ischemic brain injury caused by middle cerebral artery occlusion in mouse. *Int J Mol Sci* 24: 5800, 2023.
24. Nouri Barkestani M, Shamdani S, Afshar Bakshloo M, Arouche N, Bamba B, Uzan G and Naserian S: TNF α priming through its interaction with TNFR2 enhances endothelial progenitor cell immunosuppressive effect: new hope for their widespread clinical application. *Cell Commun Signal* 19: 1, 2021
25. Zhao ZA, Yan L, Wen J, Satyanarayanan SK, Yu F, Lu J, Liu YU and Su H: Cellular and molecular mechanisms in vascular repair after traumatic brain injury: A narrative review. *Burns Trauma* 11: tkad033, 2023.
26. Yu Z, Witman N, Wang W, Li D, Yan B, Deng M, Wang X, Wang H, Zhou G, Liu W, *et al*: Cell-mediated delivery of VEGF modified mRNA enhances blood vessel regeneration and ameliorates murine critical limb ischemia. *J Control Release* 310: 103-114, 2019.
27. Luís JP, Simões CJV and Brito RMM: The therapeutic prospects of targeting IL-1R1 for the modulation of neuroinflammation in central nervous system disorders. *Int J Mol Sci* 23: 1731, 2022.
28. Riddle RB, Jennbacken K, Hansson KM and Harper MT: Endothelial inflammation and neutrophil transmigration are modulated by extracellular matrix composition in an inflammation-on-a-chip model. *Sci Rep* 12: 6855, 2022.
29. Saini MG and Bix GJ: Oxygen-glucose deprivation (OGD) and interleukin-1 (IL-1) differentially modulate cathepsin B/L mediated generation of neuro protective perlecan LG3 by neurons. *Brain Res* 1438: 65-74, 2012.
30. Parham CL, Shaw C, Auckland LD, Dickeson SK, Griswold-Prenner I and Bix G: Perlecan Domain V inhibits amyloid- β induced activation of the α 2 β 1 integrin-mediated neurotoxic signaling cascade. *J Alzheimers Dis* 54: 1629-1647, 2016.
31. Rajkovic I, Wong R, Lemarchand E, Rivers-Auty J, Rajkovic O, Garlanda C, Allan SM and Pinteaux E: Pentraxin 3 promotes long-term cerebral blood flow recovery, angiogenesis, and neuronal survival after stroke. *J Mol Med (Berl)* 96: 1319-1332, 2018.
32. Bayat M, Tabrizi R, Saied Salehi M, Karimi N, Rahimi M, Hooshmandi E, Razavi Moosavi N, Fadakar N and Borhani-Haghighi A: Association of long non-coding RNA malat1 with serum levels of interleukin-1 B and vitamin D in patients with ischemic stroke. *Galen Med J* 12: 1-10, 2023.
33. Takata F, Nakagawa S, Matsumoto J and Dohgu S: Blood-brain barrier dysfunction amplifies the development of neuroinflammation: understanding of cellular events in brain microvascular endothelial cells for prevention and treatment of BBB dysfunction. *Front Cell Neurosci* 15: 661838, 2021.
34. Mantsounga CS, Lee C, Neverson J, Sharma S, Healy A, Berus JM, Parry C, Ceneri NM, López-Giráldez F, Chun HJ, *et al*: Macrophage IL-1 β promotes arteriogenesis by autocrine STAT3- and NF- κ B-mediated transcription of pro-angiogenic VEGF-A. *Cell Rep* 38: 110309, 2022.
35. Yamasaki Y, Matsuura N, Shozuhara H, Onodera H, Itoyama Y and Kogure K: Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke* 26: 676-80; discussion 681, 1995.
36. Matys P, Mirończuk A, Starosz A, Grubczak K, Kochanowicz J, Kułakowska A and Kapica-Topczewska K: Expanding role of interleukin-1 family cytokines in acute ischemic stroke. *Int J Mol Sci* 25: 10515, 2024.
37. Scheller J, Ettich J, Wittich C, Pudewell S, Floss DM and Rafii P: Exploring the landscape of synthetic IL-6-type cytokines. *FEBS J* 291: 2030-2050, 2024.
38. Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, Matsusaka T, Yoshida K, Sudo T, Naruto M and Kishimoto T: Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell* 77: 63-71, 1994.
39. Sakata H, Narasimhan P, Niizuma K, Maier CM, Wakai T and Chan PH: Interleukin 6-preconditioned neural stem cells reduce ischaemic injury in stroke mice. *Brain* 135: 3298-3310, 2012.
40. Monsour M, Croci DM, Agazzi S and Borlongan CV: Contemplating IL-6, a double-edged sword cytokine: Which side to use for stroke pathology. *CNS Neurosci Ther* 29: 493-497, 2023.
41. Cohen T, Nahari D, Cerem LW, Neufeld G and Levi BZ: Interleukin 6 induces the expression of vascular endothelial growth factor. *J Biol Chem* 271: 736-741, 1996.
42. Ley K: The role of selectins in inflammation and disease. *Trends Mol Med* 9: 263-268, 2003.
43. Choi BR, Johnson KR, Maric D and McGavern DB: Monocyte-derived IL-6 programs microglia to rebuild damaged brain vasculature. *Nat Immunol* 24: 1110-1123, 2023.
44. Frijns CJ and Kappelle LJ: Inflammatory cell adhesion molecules in ischemic cerebrovascular disease. *Stroke* 33: 2115-2122, 2002.
45. Cheng D, Wang Y, Li J, Yao Y, Zhang S and Wang Y: Transcriptomic analysis identifies the S100 calcium-binding protein β subunit (S100B) and intercellular adhesion molecule-1 (ICAM-1) as potential diagnostic biomarkers for acute cerebral infarction. *Genes Dis* 11: 46-48, 2023.
46. Henninger DD, Panés J, Eppihimer M, Russell J, Gerritsen M, Anderson DC and Granger DN: Cytokine-induced VCAM-1 and ICAM-1 expression in different organs of the mouse. *J Immunol* 158: 1825-1832, 1997.
47. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS and Koh GY: Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J Biol Chem* 276: 7614-7620, 2001.
48. Rüegg C, Postigo AA, Sikorski EE, Butcher EC, Pytela R and Erle DJ: Role of integrin alpha 4 beta 7/alpha 4 beta P in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J Cell Biol* 117: 179-189, 1992.
49. Langston W, Chidlow JH Jr, Booth BA, Barlow SC, Lefer DJ, Patel RP and Kevil CG: Regulation of endothelial glutathione by ICAM-1 governs VEGF-A-mediated eNOS activity and angiogenesis. *Free Radic Biol Med* 42: 720-729, 2007.
50. Nakao S, Kuwano T, Ishibashi T, Kuwano M and Ono M: Synergistic effect of TNF- α in soluble VCAM-1-induced angiogenesis through alpha 4 integrins. *J Immunol* 170: 5704-5711, 2003.
51. Ibli SI, Hu J, Looso M, Weigert A, Ratiu C, Wittig J, Drekolia MK, Tombar L, Randriamboavonjy V, Leisegang MS, *et al*: Mapping the endothelial cell S-sulphydrylome highlights the crucial role of integrin sulfhydrylation in vascular function. *Circulation* 143: 935-948, 2021.
52. Bi JJ and Yi L: Effects of integrins and integrin α v β 3 inhibitor on angiogenesis in cerebral ischemic stroke. *J Huazhong Univ Sci Technolog Med Sci* 34: 299-305, 2014.
53. Huang Q, Chen B, Wang F, Huang H, Milner R and Li L: The temporal expression patterns of fibronectin and its receptors- α 5 β 1 and α v β 3 integrins on blood vessels after cerebral ischemia. *Restor Neurol Neurosci* 33: 493-507, 2015.
54. Pang D, Wang L, Dong J, Lai X, Huang Q, Milner R and Li L: Integrin α 5 β 1-Ang1/Tie2 receptor cross-talk regulates brain endothelial cell responses following cerebral ischemia. *Exp Mol Med* 50: 1-12, 2018.
55. Zhang X, Wang L, Han Z, Dong J, Pang D, Fu Y and Li L: KLF4 alleviates cerebral vascular injury by ameliorating vascular endothelial inflammation and regulating tight junction protein expression following ischemic stroke. *J Neuroinflammation* 17: 107, 2020.
56. Jeucken KCM, van Rooijen CCN, Kan YY, Kocken LA, Jongejan A, van Steen ACI, van Buul JD, Olsson HK, van Hamburg JP and Tas SW: Differential contribution of NF- κ B signaling pathways to CD4+ Memory T cell induced activation of endothelial cells. *Front Immunol* 13: 860327, 2022.
57. Xie Y, Sun Y, Liu Y, Zhao J, Liu Q, Xu J, Qin Y, He R, Yuan F, Wu T, *et al*: Targeted delivery of RGD-CD146⁺CD271⁺ human umbilical cord mesenchymal stem cell-derived exosomes promotes blood-spinal cord barrier repair after spinal cord injury. *ACS Nano* 17: 18008-18024, 2023.

58. Lin Y, Gil CH, Banno K, Yokoyama M, Wingo M, Go E, Prasain N, Liu Y, Hato T, Naito H, *et al*: ABCG2-expressing clonal repopulating endothelial cells serve to form and maintain blood vessels. *Circulation* 150: 451-465, 2024.
59. Wang L, Li J, Wang Y, Ge C, Huang Q, Li L, Wang N, Chen Y, Zhou X, Chang D, *et al*: Dan-Deng-Tong-Nao softgel capsule promotes angiogenesis of cerebral microvasculature to protect cerebral ischemia reperfusion injury via activating HIF-1 α -VEGFA-Notch1 signaling pathway. *Phytomedicine* 118: 154966, 2023.
60. Ran X, Xu T, Ruan H, Wang X and Zhang Q: Tissue Kallikrein supplementation in ischemic phase protects the neurovascular unit and attenuates reperfusion-induced injury in ischemic stroke. *Pharmacol Res* 209: 107435, 2024.
61. Peiretti F, Alessi MC, Henry M, Anfosso F, Juhan-Vague I and Nalbone G: Intracellular calcium mobilization suppresses the TNF-alpha-stimulated synthesis of PAI-1 in human endothelial cells. Indications that calcium acts at a translational level. *Arterioscler Thromb Vasc Biol* 17: 1550-1560, 1997.
62. Panthiya L, Tocharus J, Onsa-Ard A, Chaichompoo W, Suksamrarn A and Tocharus C: Hexahydrocurcumin ameliorates hypertensive and vascular remodeling in L-NAME-induced rats. *Biochim Biophys Acta Mol Basis Dis* 1868: 166317, 2022.
63. Zhang G, Qin Q, Zhang C, Sun X, Kazama K, Yi B, Cheng F, Guo ZF and Sun J: NDRG1 signaling is essential for endothelial inflammation and vascular remodeling. *Circ Res* 132: 306-319, 2023.
64. Bischoff-Kont I, Primke T, Niebergall LS, Zech T and Fürst R: Ginger constituent 6-shogaol inhibits inflammation- and angiogenesis-related cell functions in primary human endothelial cells. *Front Pharmacol* 13: 844767, 2022.



Copyright © 2025 Gong et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.