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Review Article

The Importance of Endothelial Reprogramming in Intracranial Atherosclerosis

Danbi Jo[2], Juhyun Song[1,2]

[1]*Biomedical Science Graduate Program (BMSGP), Chonnam National University*
[2]*Department of Anatomy, Chonnam National University Medical School*

Abstract : Intracranial atherosclerosis (ICAS) develops through complex interactions between multiple cell types, primarily cerebrovascular endothelial cells and neuroglia. The pathophysiology of ICAS involves progressive vascular wall remodeling, characterized by the accumulation of inflammatory cells, activated mesenchymal cells, oxidized lipids, and altered extracellular matrix components within the arterial intima, resulting in atherosclerotic plaque formation. Endothelial dysfunction represents a key initiating mechanism in atherogenesis. Endothelial reprogramming encompasses phenotypic plasticity and functional alterations of endothelial cells, including endothelial-to-mesenchymal transition (EndMT), transcriptional factors, cell signaling, growth factors, miRNAs. In the central nervous system, endothelial cell phenotype is intrinsically linked to blood-brain barrier (BBB) integrity and function. Therapeutic modulation of endothelial reprogramming presents a potential non-pharmacological cellular intervention strategy for ICAS. This review examines the mechanistic roles of endothelial cells in intracranial atherosclerosis and synthesizes current evidence regarding endothelial reprogramming in ICAS pathogenesis.

Keywords : Intracranial atherosclerosis (ICAS), Endothelial-to-mesenchymal cell transition (EndMT), Blood brain barrier (BBB), Endothelial reprogramming

INTRODUCTION

Intracranial atherosclerosis (ICAS) is characterized by pathological arterial wall remodeling, involving progressive plaque accumulation and luminal stenosis within the cerebral vasculature, representing a primary etiology of ischemic stroke [1]. Atherosclerosis is reported to cause cerebral infarction related to ICAS in 20 to 40 out of 100,000 people worldwide [2]. ICAS accounts for 8~10% of all ischemic strokes, with a notably higher prevalence (30~50%) in Asian populations [3]. Multiple risk factors contribute to ICAS pathogenesis, including advanced age, diabetes mellitus, hypertension, hypercholesterolemia, obesity, cardiovascular disease, and metabolic disorders [4-6]. The accumulation of sticky substances in the arteries, called plaque, narrows or occludes the blood vessels, preventing sufficient blood flow to the brain [7,8]. The gradual deposition of atheromatous plaques, comprising lipids, cholesterol, and inflammatory mediators, leads to hemodynamic alterations that significantly increase stroke risk [9,10].

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Correspondence to: Juhyun Song, Ph.D. (Department of Anatomy, Chonnam National University Medical School, Hwasun 58128, Jeollanam-do, Republic of Korea)
E-mail: juhyunsong@chonnam.ac.kr

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Endothelial cells, which compose the inner surface of blood vessels, play an important role in regulating the homeostasis of blood vessel walls [11]. Endothelial dysfunction represents an early pathophysiological marker in atherogenesis [12]. Within the central nervous system, endothelial cells are integral components of the blood-brain barrier (BBB), maintaining selective permeability and protecting neural tissue from potentially harmful blood-borne substances and immune cells [13]. Cerebrovascular endothelial dysfunction promotes atheromatous plaque formation and progression through multiple mechanisms, including inflammatory cascade activation, oxidative stress induction, and BBB disruption, ultimately contributing to stroke pathogenesis and other cerebrovascular disorders [14-16]. A key mechanism of endothelial dysfunction involves endothelial-to-mesenchymal transition (EndMT), wherein endothelial cells undergo phenotypic transformation, acquiring mesenchymal characteristics and functions [17,18]. EndMT facilitates plaque development and inflammatory responses, thereby accelerating atherogenesis [19]. In the cerebral vasculature, EndMT of brain endothelial cells (BECs) compromises BBB integrity, promoting immune cell infiltration and contributing to various neurological pathologies [20,21].

Endothelial reprogramming, including EndMT, plays a significant role in the pathogenesis of intracranial atherosclerosis. To mitigate its effects, it is crucial to restore endothelial cell function or regulate signaling pathways associated with EndMT. Endothelial reprogramming holds promise as a novel therapeutic target for the prevention, progression inhibition, and treatment of atherosclerosis. In this review, we highlight recent advances in the understanding of endothelial reprogramming as a strategy to address endothelial dysfunction in intracranial atherosclerosis.

ENDOTHELIAL DYSFUNCTION IN INTRACRANIAL ATHEROSCLEROSIS

The endothelium is crucial for maintaining the structural integrity of the vascular wall and plays a key role in the homeostasis of vascular functions, including the prevention of platelet aggregation, inhibition of thrombosis, and regulation of selective permeability [22-24]. Endothelial cells regulate blood flow by modulating vascular tone and selectively controlling the movement of ions, fluids, and other molecules through tight junctions [25]. Physiologically, endothelial cells influence inflammation and the immune response by expressing cell adhesion molecules (CAMs), cytokines, and chemokines [26,27]. However, endothelial dysfunction leads to impaired endothelial cell function, which is associated with a reduction in nitric oxide availability, an imbalance between vasodilation and vasoconstriction, increased production of reactive oxygen species (ROS), and heightened oxidative stress [28-30]. The primary contributors to endothelial dysfunction include diabetes, obesity, hypertension, hyperglycemia, smoking, aging, and metabolic syndrome [31].

In the brain, intracranial endothelial cells, referred to as BECs, are integral components of the BBB. They play a critical role in protecting the brain from harmful external substances, regulating the entry and exit of molecules essential for brain metabolism, and maintaining homeostasis [32-34]. Dysfunction of intracranial endothelial cells leads to blood vessel narrowing, lipid modification, foam cell formation, an exacerbated inflammatory response, and platelet aggregation, which contribute to the formation of atherosclerotic plaques and are considered early indicators of intracranial atherosclerosis [35]. Lipid metabolism plays an essential role in the progression of atherosclerosis [36]. Abnormal lipid levels are observed in atherosclerosis patients, and low-density lipoprotein (LDL) transports lipids through the blood [37]. Under oxidative stress, ROS react with LDL and oxidize to form oxidized LDL (ox-LDL), which is ingested by macrophages to form foam cells [38]. Ox-LDL can promote atherosclerosis pathogenesis through endothelial cell damage, platelet activation, smooth muscle cell proliferation and migration [39-41]. Foam cells can contribute to atherosclerotic plaque formation and cause ischemic stroke. Within endothelial cells, nitric oxide (NO) is a reactive free radical that plays a vital role in maintaining vascular homeostasis by promoting vasodilation, inhibiting platelet aggregation, and exerting anti-inflammatory and antioxidant effects [42-44]. Endothelial dysfunction is associated with a reduction in NO production and bioavailability, leading to an imbalance in vascular homeostasis that promotes thrombosis and inflammation in the vascular wall, which can progress to vascular diseases such as atherosclerosis [45-47]. NO is synthesized by nitric oxide synthase (NOS) enzymes, which exist in three isoforms: endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and inducible nitric oxide synthase (iNOS), each with distinct functions and properties [48]. eNOS plays a crucial role

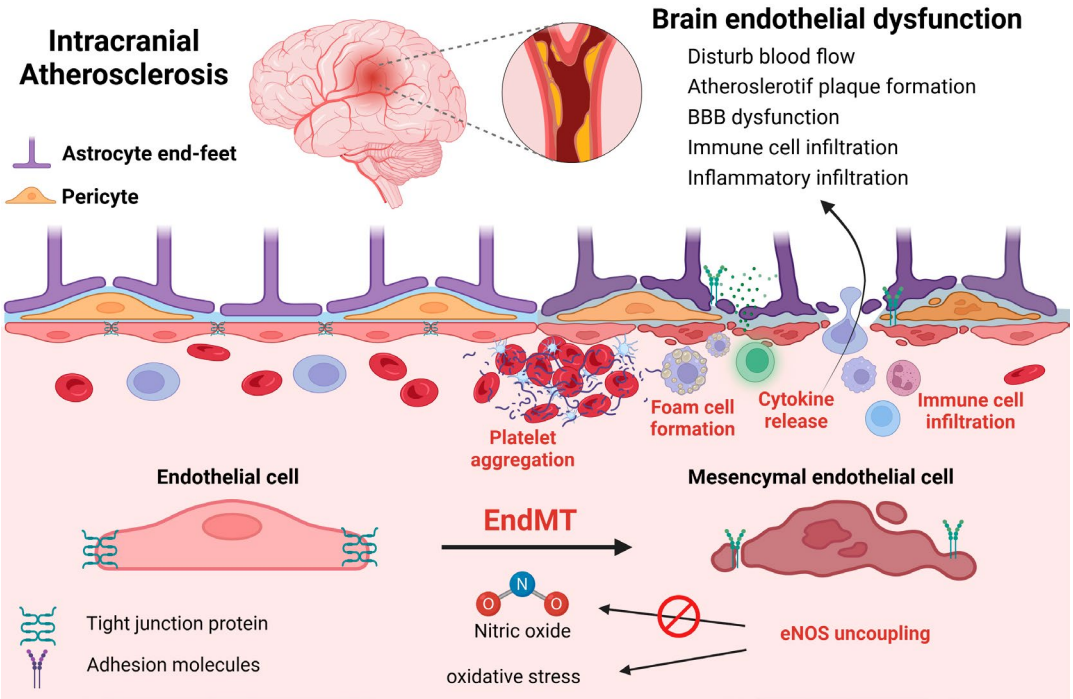

Fig. 1. Endothelial dysfunction in intracranial atherosclerosis. Figure showing the EndMT, the process by which endothelial cells transform into mesenchymal cells. EndMT results in decreased nitric oxide synthesis and increased oxidative stress due to eNOS uncoupling. Endothelial dysfunction leads to disturbance of blood flow, formation of atherosclerotic plaque, BBB dysfunction, immune cell infiltration and inflammatory cytokines and chemokines release.

in maintaining vascular homeostasis, and its deficiency is known to contribute to stroke development by impairing cerebral blood flow, promoting thrombosis, and inducing inflammation [49,50]. eNOS knockout (eNOS −/−) mice exhibit more severe neurological damage following stroke compared to control mice [51]. In the ischemic brain, progenitor cell proliferation and migration in the subventricular zone were reduced, and endothelial cell proliferation and vascular density were reduced at the ischemic border [51]. In eNOS +/− mice, which exhibit partial eNOS deficiency, early thrombotic cerebral infarctions were observed, along with vascular occlusion in the temporoparietal and retrosplenial granular cortex, as well as the hippocampal regions [52]. Aged eNOS +/− mice displayed BBB disruption, amyloid angiopathy, and cognitive decline [52]. eNOS −/− mice subjected to bilateral common carotid artery stenosis (BCAS) exhibited reduced cerebral blood flow, glial activation, heightened inflammatory responses, BBB disruption, brain damage, and impaired cognitive function compared to control mice [53]. eNOS uncoupling occurs when electrons leak from the electron transport chain and are transferred to oxygen molecules, generating superoxide (O2-) instead of NO [54]. Oxidative depletion of the eNOS cofactor tetrahydrobiopterin (BH4) and the depletion of the substrate L-arginine suggest that eNOS uncoupling induces oxidative stress in the brain, contributing to neurological damage [28,54]. These findings highlight NO as a crucial signaling molecule involved in various cerebrovascular diseases by regulating endothelial cell function. Modulating NO activity may offer an effective therapeutic strategy to protect against intracranial atherosclerosis by suppressing inflammatory responses, platelet aggregation, thrombosis, and oxidative damage, while preserving cerebral blood flow (Fig. 1).

CHANGED ENDOTHELIAL CELL IDENTITY IN INTRACRANIAL ATHEROSCLEROSIS

EndMT is a biological process in which endothelial cells acquire the functional and morphological characteristics of

mesenchymal cells, such as enhanced motility and contractility. Recent studies have linked EndMT to dysfunction of BECs [20,55]. In the brain, BEC dysfunction is associated with various neurological disorders, including multiple sclerosis, autoimmune encephalitis, and cerebral cavernous malformations, which result from BBB breakdown [56,57]. Intracranial atherosclerosis damages BECs, promotes plaque formation in blood vessels, and exacerbates BBB disruption, ischemic stroke, vascular dementia, and cognitive impairment [58-60]. During EndMT, BECs downregulate the expression of proteins that are critical for the structure and function of tight junctions, including claudin-5, claudin-11, claudin-25, occludin, zonula occludens-1 (ZO-1), ZO-2, junctional adhesion molecules (JAM-A, JAM-B, JAM-C), and adherens junction proteins such as α-catenin, β-catenin, platelet endothelial cell adhesion molecule-1 (PECAM-1), and vascular endothelial cadherin (VE-cadherin), thereby impairing the maintenance of barrier integrity [61,62]. Additionally, EndMT promotes BBB permeability by upregulating the expression of adhesion molecules and matrix metalloproteinases, including vascular cell adhesion molecule-1 (VCAM-1), matrix metalloproteinase-3 (MMP3), MMP9, intracellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1) [63,64].

EndMT induced by inflammatory responses is regulated by several signaling pathways, including tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), WNT, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), NOTCH, and BMPR2-JNK pathways [65-67]. TGF-β, a well-known inflammatory cytokine, is a key factor in inducing EndMT, which plays a critical role in acute vascular diseases and fibrosis [68]. In endothelial cells, TGF-β activation is mediated through type I receptors, specifically activin receptor-like kinases 1 and 5 (ALK1 and ALK5) [69]. Signal transduction from the TGF-β receptor complex to the nucleus is facilitated by Smad transcriptional regulators [70]. Smad2/3 are phosphorylated by ALK5, form a complex with Smad4, translocate to the nucleus, and regulate gene expression to promote EndMT [71]. Furthermore, inhibition of signal transducer and activator of transcription 3 (STAT3), a transcriptional activator implicated in fibrosis in endothelial cells, has been shown to induce EndMT via the TGF-β1/ALK5/Smad4 signaling pathway [72]. In brain endothelial cells (BECs), activation of SNAI1 and TAK1, which are regulators of EndMT, modulates the TGF-β1 and interleukin 1-beta (IL-1β) signaling pathways, both of which are involved in EndMT and BBB dysfunction [55]. FGF is crucial for endothelial cell functions, including proliferation, migration, maintenance of vascular integrity, and regulation of cell identity [73]. FGF and TGF-β exert opposing effects on endothelial cell function [74]. The FGF receptor 1 (FGFR1)/MAP4K4 pathway induces TGF-β/Smad signaling and inhibits EndMT in endothelial cells, playing an important role in mitigating fibrotic vascular diseases associated with EndMT [75]. FGF2 controls TGF β1 signaling by regulating the expression of TGF β receptor complex (ALK5, TGFBR2, SARA) [76]. FGF2 modulates TGF-β1 signaling by regulating the expression of the TGF-β receptor complex (ALK5, TGFBR2, SARA) [76]. VEGF, a key regulator of endothelial cell proliferation, differentiation, vascular permeability, and angiogenesis [77] may act as one of the most effective inhibitors of EndMT. VEGF exhibits an antifibrotic effect by inhibiting EndMT through suppression of TGF-β signaling and Smad2 activity [78].

EndMT in BECs is a process that contributes to atherosclerosis and BBB dysfunction by increasing BBB permeability, facilitating the filtration of immune substances, and promoting plaque formation [20]. Several studies have sought to elucidate the relationship between changes in junctional proteins in BECs and their impact on BBB permeability, atherosclerosis, and BBB breakdown. Claudin-5 (CLDN5), a critical tight junction protein, plays a vital role in BBB integrity, and its aberrant expression has been linked to neurodegenerative disorders [79]. One study demonstrated that CLDN5 deletion in the mouse brain resulted in increased intercellular leakage of molecules from the blood into the brain [80]. In adult mice, decreased CLDN5 expression selectively enhanced brain leakage depending on the size of the molecule and induced inflammatory responses in endothelial and glial cells lacking CLDN5 [80]. The regulation of BBB and neuronal function following ischemic stroke has been studied using occludin-deficient mice [62]. In a thrombosis mouse model, decreased expression of occludin was observed in BECs at ischemic lesions, leading to increased BBB permeability, accelerated brain tissue damage, and reduced angiogenesis following cerebral infarction, compared to control mice. Alongside the reduction in occludin expression after stroke, decreased levels of CLDN5 and ZO-1 were also noted. It remains unclear whether the absence of occludin directly reduced CLDN5 and ZO-1 expression or whether this effect was secondary to BBB disruption following stroke [62]. Nevertheless, occludin plays a

crucial role in maintaining BBB integrity in BECs and may present a potential therapeutic target for ischemic stroke.

Additionally, inflammatory responses not only affect the expression of junctional proteins but also alter the inflammatory phenotype of BECs. Inflammatory activation of BECs has been shown to increase the expression of adhesion molecules such as VCAM-1, ICAM-1, PECAM-1, and other adhesion molecules [64,81]. These changes play a significant role in the recruitment and migration of leukocytes to the brain, contributing to brain atherosclerosis [82,83]. Furthermore, BECs respond to inflammatory cytokines, including IL-1β, interleukin-6 (IL-6), and TNF-α [84] as well as chemokines such as C-C motif chemokine ligand 2 (CCL2, MCP-1), CCL3, CCL5, C-X-C motif chemokine ligand 8 (CXCL8), CXCL10, and CXCL12, which recruit leukocytes into the brain [85,86].

THERAPEUTIC STRATEGIES RELATED TO ENDOTHELIAL REPROGRAMMING IN INTRACRANIAL ATHEROSCLEROSIS

Endothelial reprogramming encompasses various phenotypic transitions of endothelial cells, such as EndMT. While current therapeutic approaches for intracranial atherosclerosis include lipid-lowering agents, antiplatelet medications, antihypertensive drugs, and anti-inflammatory compounds [87-89], these interventions have limited efficacy in disease reversal as they neither eliminate existing atherosclerotic lesions nor completely halt disease progression. EndMT has been implicated in the progression of atherosclerosis, and studies suggest that inhibiting EndMT can mitigate the development of the disease. Specifically, TGF-β, a key mediator of EndMT, plays a crucial role in atherosclerosis pathogenesis. Therefore, targeting TGF-β signaling may significantly reduce both EndMT and atherosclerosis. TGF-β modulates endothelial cell function through the regulation of two active signaling pathways mediated by its receptors: the ALK5-Smad2/3 pathway and the ALK1-Smad1/5 pathway. TGF-β receptor activation induces the ALK1-Smad1/5 pathway and concurrently inhibits the ALK5-Smad2/3 pathway, thereby suppressing EndMT [90]. FGF signaling is critical for maintaining vascular integrity and the expression of vascular endothelial growth factor receptor 2 (VEGFR2) [91]. Inhibition of FGF signaling promotes TGF-β signaling and accelerates EndMT [91]. Conversely, FGF-1 exerts its effects by reducing TGF-β signaling and inhibiting EndMT through the MAPK/ERK signaling pathway [92]. The transcription factor ETS1 is a key regulator of EndMT, which

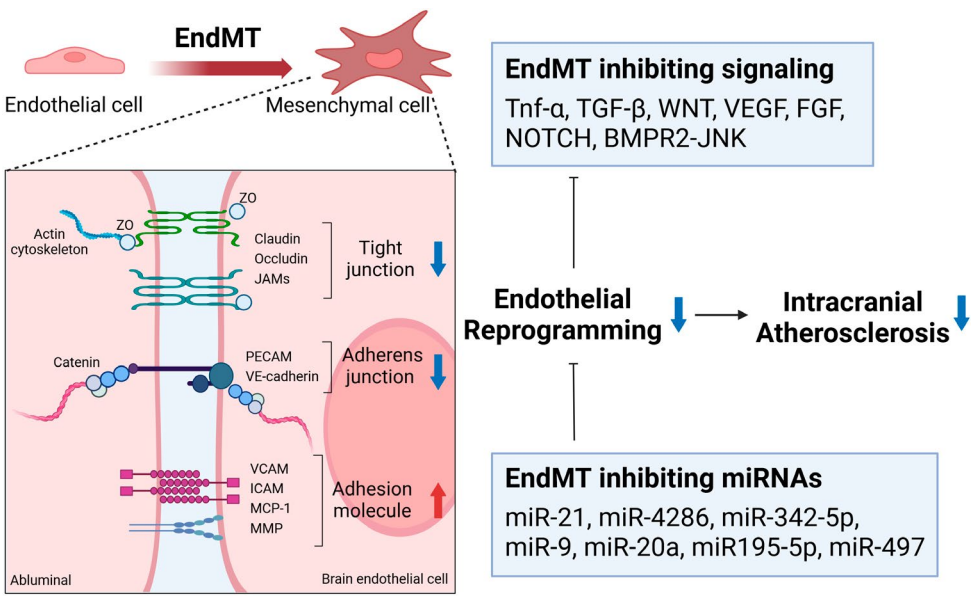

Fig. 2. Simplified scheme representing endothelial reprogramming in intracranial atherosclerosis. Schematic representation of tight junction (Claudin, occluding, JAMs), adherens junction (PECAM, VE-cadherin) and adhesion molecules (VCAM, ICAM, MCP-1, MMP) in the brain endothelial dysfunction. Inhibition of signaling and miRNAs that suppress endothelial reprogramming such as EndMT may reduce the progression of intracranial atherosclerosis.

is closely associated with BBB dysfunction. Under inflammatory conditions, inhibition of ETS1 exacerbates EndMT in central nervous system (CNS) endothelial cells [20]. Notably, upregulation of ETS1 in bEnd.3 cells treated with IL-1β, an inflammatory cytokine, has been shown to inhibit EndMT and improve the function of BECs [93]. Furthermore, the expression of PIM1, a gene involved in atherosclerotic plaque formation [94], is upregulated in response to atherosclerosis. In human umbilical vein endothelial cells (HUVECs) subjected to EndMT, inhibition of PIM1 reduced mesenchymal marker expression and restored endothelial marker expression. Moreover, PIM1 knockdown in a mouse model of atherosclerotic plaques inhibited plaque progression by reducing the inflammatory response in endothelial cells and suppressing EndMT [94].

Numerous microRNAs (miRNAs) are known to regulate endothelial cell proliferation, migration, and the maintenance of vascular integrity by modulating transcription factors or participating in signaling pathways associated with EndMT [95]. The inhibition of EndMT-related signaling via miRNAs plays a pivotal role in preventing EndMT [96]. In endothelial cells, TGF-β enhances the expression of miR-21, which subsequently induces EndMT. Blocking miR-21 has been shown to attenuate TGF-β-induced EndMT through the PTEN/AKT signaling pathway [97]. In addition, miR-4286 expression is downregulated in endothelial cell dysfunction induced by (ox-LDL) [98]. Overexpression of miR-4286 diminishes TGF-β1/Smad3 signaling, thereby alleviating endothelial cell damage [98]. The combination of FGF2 reduction and TGF-β1 activation decreases miR-20a expression and activates Smad2/3, which increases EndMT [76]. Therefore, miR-20a expression can inhibit TGF-β1 signaling by decreasing ALK5, TGFBR2 and SARA [76]. Inhibition of miR-9 increases MALAT1 expression and increases sensitivity to TGF-β and NF-κB signaling, thereby activating inflammatory responses [99]. Inhibition of miR-9 can suppress glucose-induced EndMT by regulating TGFBR2, NF-κB and MALAT1 signaling [99]. Inhibition of miR-195-5p can block EndMT by inhibiting TGF-β1/Smad signaling [100]. Increased miR-497 targeting ROCK1 and ROCK2 reduced TGF- β2-stimulated α-SMA and Snail levels [101]. Therefore, regulation of miR-497/ROCK signaling may attenuate EndMT [101] (Fig. 2). BBB dysfunction is a critical hallmark of intracranial atherosclerosis. Understanding the molecular mechanisms underlying BEC dysfunction during the pathological progression of intracranial atherosclerosis could provide insights for developing potential therapeutic strategies aimed at restoring BBB function and mitigating the effects of intracranial atherosclerosis (Fig. 2).

CONCLUSION

In conclusion, this review examines the mechanistic relationship between endothelial reprogramming and intracranial atherosclerosis. Brain endothelial cells constitute a critical component of the BBB, regulating selective molecular transport between the circulation and central nervous system. EndMT promotes atherosclerosis progression through dual mechanisms: disruption of brain endothelial cell function and enhancement of atherosclerotic plaque formation. Thus, therapeutic strategies targeting EndMT may represent a promising approach for attenuating atherosclerosis development. Preclinical studies have demonstrated that modulation of EndMT-associated molecular pathways can mitigate various pathophysiological manifestations of atherosclerosis. However, comprehensive elucidation of the precise mechanisms governing endothelial cell reprogramming remains incomplete. Further investigations utilizing both in vivo models and cellular systems are essential to delineate these regulatory pathways and develop targeted therapeutic interventions.

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ABBREVIATIONS

Intracranial atherosclerosis (ICAS)
blood-brain barrier (BBB)
endothelial-to-mesenchymal transition (EndMT)
brain endothelial cells (BECs)
cell adhesion molecules (CAMs)
reactive oxygen species (ROS)
low-density lipoprotein (LDL)
oxidized low-density lipoprotein (ox-LDL)
nitric oxide (NO)
nitric oxide synthase (NOS)

endothelial nitric oxide synthase (eNOS)
neuronal nitric oxide synthase (nNOS)
inducible nitric oxide synthase (iNOS)
bilateral common carotid artery stenosis (BCAS)
zonula occludens-1 (ZO-1)
junctional adhesion molecules (JAM-A, JAM-B, JAM-C)
platelet endothelial cell adhesion molecule-1 (PECAM-1)
vascular cell adhesion molecule-1 (VCAM-1)
matrix metalloproteinase-3 (MMP3)
intracellular adhesion molecule-1 (ICAM-1)
monocyte chemoattractant protein-1 (MCP-1)
tumor necrosis factor-alpha (TNF-α)
transforming growth factor-beta (TGF-β)
vascular endothelial growth factor (VEGF)
fibroblast growth factor (FGF)
activin receptor-like kinases 1 and 5 (ALK1 and ALK5)
signal transducer and activator of transcription 3 (STAT3)
interleukin 1-beta (IL-1β)
FGF receptor 1 (FGFR1)
Claudin-5 (CLDN5)
interleukin-6 (IL-6)
C-C motif chemokine ligand 2 (CCL2)
C-X-C motif chemokine ligand 8 (CXCL8)
vascular endothelial growth factor receptor 2 (VEGFR2)
central nervous system (CNS)
human umbilical vein endothelial cells (HUVECs)
microRNAs (miRNAs)
transforming growth factor beta receptor 2 (TGFBR2)
Smad anchor for receptor activation (SARA)
Metastasis associated lung adenocarcinoma transcript 1 (MALAT1)
Rho-associated kinase 1 and 2 (ROCK1 and ROCK2)

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