Non-Coding RNA in Acute Ischemic Stroke: Mechanisms, Biomarkers and Therapeutic Targets

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Abstract
Non-coding RNAs (ncRNAs) are a class of functional RNAs that regulate gene expression in a post-transcriptional manner. NcRNAs include microRNAs, long non-coding RNAs and circular RNAs. They are highly expressed in the brain and are involved in the regulation of physiological and pathophysiological processes, including cerebral ischemic injury, neurodegeneration, neural development, and plasticity. Stroke is one of the leading causes of death and physical disability worldwide. Acute ischemic stroke (AIS) occurs when brain blood flow stops, and that stoppage results in reduced oxygen and glucose supply to cells in the brain. In this article, we review the latest progress on ncRNAs in relation to their implications in AIS, as well as their potential as diagnostic and prognostic biomarkers. We also review ncRNAs acting as possible therapeutic targets in future precision medicine. Finally, we conclude with a brief discussion of current challenges and future directions for ncRNAs studies in AIS, which may facilitate the translation of ncRNAs research into clinical practice to improve clinical outcome of AIS.

Keywords
Non-coding RNAs, ischemic stroke, cerebral ischemic injury, biomarker, therapeutic target

Introduction
Stroke is one of the leading causes of death and long-term disability, causing a high economic burden to society in both developed and developing countries⁵. Ischemic stroke, which accounts for 80% of all strokes, is the result of cerebral artery occlusion that decreases cerebral blood flow and causes rapid loss of brain functions⁶. The improvements in current treatments for cerebral ischemia are limited by many factors, particularly a narrow therapeutic window and an incomplete understanding of the cellular and molecular changes following acute ischemic stroke (AIS)⁷. Therefore, achieving an understanding of the pathogenesis and underlying mechanisms of cerebral ischemic injury is urgent, as it will help develop novel diagnostic and therapeutic targets for patients with AIS.

Non-coding RNAs (ncRNAs), a class of genetic, epigenetic and translational regulators, consists of microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), each of which play important physiological and pathological roles by controlling transcription and translation⁸–⁶. NcRNAs are abundantly expressed in mammalian brains while recent studies show that cerebral ischemia alters ncRNAs expression profiles⁹–¹¹. A growing number of studies have demonstrated that ncRNAs (especially miRNAs and lncRNAs) play a role in the pathogenic processes related to cerebral ischemia and post-stroke recovery³,¹⁰,¹¹. These pathogenic processes include excitotoxicity, oxidative stress, neuroinflammation, and apoptosis, which can cause secondary brain damage and can impede functional recovery in patients with AIS¹². However, literature on the circRNAs implicated in cerebral ischemic injury remains unknown.

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MiRNAs, small molecules of 21–25 nucleotides in length, are a highly abundant and evolutionarily conservative class of endogenous ncRNAs. They inhibit translation and degrade the respective mRNA through imperfect or near perfect base pairing, mostly to the 3’ untranslated region (UTR) of target mRNAs\textsuperscript{13}. LncRNAs, usually defined as having more than 200 nucleotides, are cell- and tissue-specific. They can be subclassified by their functionality, by the genomic location contained between gene coding regions (long intergenic ncRNAs), or by overlapping cording genes in either sense or anti-sense directions\textsuperscript{14}. LncRNAs function as guides for chromatin-modifying-complexes or transcription factors in the nucleus\textsuperscript{15}. Cytoplasm LncRNAs traditionally regulate the translation of mRNA by controlling mRNA stability or acting as competing endogenous RNA (ceRNA)\textsuperscript{16}. CircRNAs (single-stranded and conserved RNA molecules) are formed by backsplicing of many primary RNA transcripts from which mRNAs are synthetized\textsuperscript{17}. They are extremely stable and are not degraded by RNaseR, owing to the absence of defined 5’ and 3’ ends\textsuperscript{18}. CircRNAs can control gene expression by various mechanisms, including functioning as ceRNA by sponging miRNA, forming ternary complexes with proteins, and encoding proteins\textsuperscript{19–21}.

Advances in preclinical studies have established underlying mechanisms of cerebral ischemic injury resulting from dysregulation of ncRNAs, and have identified potential biomarkers and therapeutic targets to treat cerebral ischemia. However, to date, none of these advances have been successfully translated into clinical practice. The aim of this review is to provide a systemic description of the complex functions of ncRNAs in cerebral ischemia, and how these basic research findings could be translated into clinical practice. We discuss the functions and underlying molecular mechanisms of ncRNAs in AIS, and then explain the roles of ncRNAs as potential biomarkers. Next, we provide examples in which ncRNAs act as therapeutic targets, and then conclude with an outlook about how ncRNAs may impact on the prevention and treatment for AIS in the future.

**Functions and Molecular Mechanisms of ncRNAs in Cerebral Ischemia**

**ncRNAs involved in nuclear factor kappa-B (NF-κB) signaling pathway.** Inflammatory reaction is crucial to the pathogenesis of brain tissue damage in cerebral ischemia. Proinflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alfa (TNF-α) are induced by molecules released from injured tissue, blood vessels, and necrotic cells in ischemic brain injury. This results in inflammation, leading to exacerbation of primary brain damage\textsuperscript{22}. The NF-κB signaling pathway, which regulates the expression of several genes involved in inflammatory responses, is activated by these cytokines\textsuperscript{23}. In non-stimulated conditions, NF-κB is sequestered in the cytoplasm through its interaction with κB inhibitor (IkB). In response to inflammatory signaling, IkB is phosphorylated by the IkB kinase (IKK) complex and then ubiquitylated by β-TRC, leading to its degradation through the proteasome. This contributes to the release of NF-κB. Next, it enters the nucleus and binds to its transcriptional targets including genes that encode pro-inflammatory cytokines, chemokines, and leukocyte adhesion molecules. The expression of inflammation-related signaling pathway molecules is regulated not only by transcription factors, but also by ncRNAs at the post-transcriptional level\textsuperscript{24}.

The emerging evidence suggests that ncRNAs modulates many aspects of the canonical or noncanonical transcription factor NF-κB pathway (Table 1). This pathway is involved in various neurological pathologies underlying cerebral ischemic injury\textsuperscript{25}. MiR-146a plays an important role in inhibiting NF-κB signaling through the targeting of tumor necrosis factor receptor associated factor 6 (TRAF6) and receptor-associated kinase-1 (IRAK1), two adaptor proteins that act upstream of the NF-κB pathway\textsuperscript{26}. In aged-rat stroke models, the downregulation of miR-146a increases the expression of IRAK1, activating NF-κB in cerebral vasculature. This can exacerbate ischemic brain damage; monotherapy of tissue plasminogen activator (tPA) further amplifies these detrimental process; VELCADE, a potent proteasome inhibitor, inactivates NF-κB through the upregulation of miR-146a, resulting in post-stroke neuroprotection in aged rats\textsuperscript{27}. MiR-155, an inflammation-related miRNA, is induced in mouse ischemic cerebral cortices by middle cerebral artery occlusion (MCAO)\textsuperscript{28}. MiR-155 promotes TNF-α and IL-1β expression by upregulating toll-like receptor 4 (TLR4) expression, and downregulating suppressor of cytokine signaling (SOCS1) and myeloid differentiation primary response 88 (MyD88) expression. This leads to the activation of NF-κB signaling in the ischemic cerebral tissue of MCAO mice and oxygen and glucose deprivation/reoxygenation (OGD/R)-treated BV2 cells; acetylbritannilactone exerts potent anti-inflammatory actions by suppressing miR-155 expression\textsuperscript{29}. MiR-9 inhibits the expression of proinflammatory molecules by targeting NF-κB in rat models of MCAO\textsuperscript{30}.

LncRNAs are also involved in the regulation of neuro-inflammation in ischemic brain injury. Several LncRNAs are active in NF-κB signaling pathway in cerebral ischemic injury. LncRNA C2dat1 regulates Calcium/Calmodulin-dependent Protein Kinase II Delta (CaMKIIδ) expression via activation of NF-κB signaling pathway, and promotes neuronal survival in murine models of focal cerebral ischemia\textsuperscript{31}. The overexpression of LncRNA ANRIL activates NF-κB signaling pathway by enhancing phosphorylation of IkB. This upregulates vascular endothelial growth factor (VEGF) and promotes angiogenesis in diabetes mellitus, combined with cerebral infarction in rat models\textsuperscript{32}. The abnormal upregulation of LncRNA Gm4419 causes neuroinflammation injury during OGD/R through IkB phosphorylation and NF-κB activation, which increases the expression of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α in OGD/R-treated microglial cells\textsuperscript{33}.
Table 1. Emerging roles of ncRNAs on neuroinflammation and oxidative stress in AIS.

| NcRNAs       | Targets               | Mechanisms                                      | Function of ncRNAs                          | Reference(s) |
|--------------|-----------------------|-------------------------------------------------|---------------------------------------------|--------------|
| miR-146a     | TRAF6, IRAK1          | Activate NF-κB in cerebral vasculature          | Promote neuroinflammation                   | Zhang et al. |
| miR-155      | TLR4, SOCS1 and MyD88 | Promote TNF-α and IL-1β expression              | Promote neuroinflammation                   | Wen et al.   |
| miR-9        | NF-κB                 | Inhibit NF-κB, p65, TNF-α and IL-1β expression  | Inhibit neuroinflammation                   | Liu et al.   |
| lncRNA ANRIL | VEGF                  | Activation of NF-κB signaling pathway           | Promote neuroinflammation                   | Xu et al.    |
| lncRNA Gm4419| IκB, NF-κB            | Increase the expression of IL-1β, IL-6 and TNF-α| Promote neuroinflammation                   | Zhang et al. |
| miR-23a-3p   | –                     | Reduce NO and 3-NT, increase MnSOD              | Attenuate oxidative stress                  | Wen et al.   |
| miR-424      | Nrf2                  | Decrease LDH leakage and MDA level, promote MnSOD| Inhibit oxidative stress                    | Zhao et al.  |
| miR-106b-5p  | Mcl-1, Bcl-2          | Reduce LDH release and elevate SOD activity, decrease MDA content |                             | Li et al.    |

AIS: acute ischemic stroke, ncRNA, non-coding RNA; miRNA, microRNA; lncRNA, long non-coding RNA; TRAF6, tumor necrosis factor receptor associated factor 6; IRAK1, receptor-associated kinase-1; TLR4, Toll-like receptor 4; SOCS1, suppressor of cytokine signaling 1; MyD88, myeloid differentiation primary response 88; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1β; NF-κB, nuclear factor kappa-B; CaMKIIδ, Calcium/Calmodulin-dependent Protein Kinase II Delta; VEGF, vascular endothelial growth factor; IκB, κB inhibitor; Nrf2, nuclear factor erythroid 2-related factor; LDH, lactate dehydrogenase; NO, nitric oxide; MDA, malondialdehyde; MnSOD, manganese superoxide dismutase; 3-NT, 3-nitrotyrosine; Mcl-1, myeloid cell leukemia-1; Bcl-2, B cell lymphoma-2.

ncRNAs involved in oxidative stress. Oxidative stress, an imbalance between upregulated expression of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and missing antioxidants, contributes to secondary brain damage after cerebral ischemia, via oxidative modifications of proteins, lipids, and DNA. Both ischemia and reperfusion elevate the production of ROS, such as superoxide anion, hydrogen peroxide (H₂O₂), hydroxyl radical, and singlet oxygen. Increased ROS levels injure neurons and glia by promoting mitochondrial dysfunction, the activation of inflammatory signaling, calpain activation, and apoptosis. This determines the infarct volume and the recovery of neurological function following cerebral ischemia. Emerging evidence has indicated that ncRNAs play an important role in regulating the balance between oxidants and antioxidants in experimental stroke models in vivo and in vitro.

MiRNAs either enhance or attenuate oxidative stress damage in animal models of ischemia/reperfusion (I/R) (Table 1). MiR-23a-3p reduces H₂O₂ induced lactate dehydrogenase (LDH) leakage and production of nitric oxide (NO) and 3-nitrotyrosine (3-NT). It also decreases the expression of caspase-3 in neuro-2a cells. MiR-23a-3p reduces peroxidative production NO and 3-NT, and increases the expression of manganese superoxide dismutase (SOD) through intra-cerebroventricular injection of miR-23a-3p angomir. This leads to dramatic decrease cerebral infarction volume in mouse MCAO models. Similarly, miR-424 inhibits oxidative stress to protect neurons against focal cerebral I/R injury in mice. In vitro miR-424 precludes H₂O₂-induced injury to neurons by decreasing LDH leakage and malondialdehyde (MDA) level. It also promotes manganese SOD activity. Meanwhile, the function of miR-424 suppressing oxidative stress is reversed by nuclear factor erythroid 2-related factor (Nrf2) knockdown and SOD inhibitor treatment. Intra-cerebroventricular injection of miR-424 angomir can reduce cerebral infarction volume and inhibit neuronal apoptosis after I/R, partially through keeping balance between oxidants and anti-oxidants in mouse cortexes. MiR-106b-5p increases in serum of patients with AIS, which is associated with poor prognosis. MiR-106b-5p inhibitor upregulates the myeloid cell leukemia-1 (Mcl-1) and B cell lymphoma-2 (Bcl-2) expression, guarding against glutamate-induced apoptosis and oxidative stress injury, as evidenced by decreased LDH release and elevated SOD activity in PC12 cells. MiR-106b-5p antagonist treatment significantly improves outcome in the rat MCAO models. This includes decreased neurological deficit scores, infarct volumes, and neuronal damage. Furthermore, it significantly decreases MDA content, restores SOD activity, upregulates the expression of Mcl-1 and Bcl-2, and downregulates Bax expression in the rat ischemic cortexes.

Although accumulating evidence has shown miRNAs to be essential mediators for post-transcriptional gene regulating in cerebral ischemia-related oxidative stress injury, the pathogenic mechanisms underlying the effects of lncRNAs and circRNAs in oxidative stress damage following cerebral ischemia have yet to be explored. A series of lncRNAs, such as LincRNA-p21, LincRNA-RoR, LincRNA-JADE, H19, and ANRIL, are involved in oxidative stress, which is associated with DNA damage in cancer. There will be increasing
evidence that both lncRNAs and circRNAs are considered as an important class of ncRNAs in the response to, and control of pathological events leading to oxidative stress injury following ischemic stroke.

**ncRNAs involved in angiogenesis.** Angiogenesis plays an important role in vascular angiogenic remodeling and neurofunctional recovery after stroke. It is controlled by many key angiogenic factors in the brain. Cerebral neovascularization can allow for increased cerebral blood flow, which augments the amount of oxygen and nutrients delivered to ischemic brain tissues. Inducing angiogenesis via various treatments that target angiogenic factors appears to be a useful approach for stroke in experimental study. Accumulating evidence has shown that ncRNAs are essential mediators of both vascular endothelial cell biology and angiogenesis.

VEGF, a classical pro-angiogenic factor, plays a critical role in angiogenesis, and it increases following cerebral ischemia. The administration of vascular endothelial growth factor A (VEGF-A) appears to enhance microvessel density in the ischemic penumbra of animal model, thus promoting post ischemic angiogenesis. Therefore, exploring the mechanisms involved in regulation of VEGF-A after cerebral ischemia is crucial. MiR-140-5p levels are downregulated more than twofold, while the expression of VEGF-A is dramatically increased in rat cerebral tissue following MCAO. Furthermore, MiR-140-5p binds to the 3' UTR of VEGF-A, thus leading to the negative regulation of VEGF-A proteins. In an in vitro model, miR-140-5p exerts an inhibitory effect on angiogenesis after ischemia, partially through targeting VEGF-A. The Notch signaling pathway plays an important part in the progression and homeostasis of the vasculature, as well as in regulating the behavior of endothelial and smooth muscle cells. MiR-137 has been shown to increase the endothelial progenitor cell proliferation and angiogenesis by targeting nuclear receptor subfamily 4 group A member 2 (NR4A2) through the Notch signaling pathway in a mouse model of cerebral ischemia. Similarly, a hypoxia-induced upregulation of miR-210 can activate the Notch signaling pathway, which may enhance angiogenesis after cerebral ischemia. MiR-377, miR-150, miR-107, and miR-210 contribute to angiogenesis after ischemic stroke via targeting VEGF.

Antisense ncRNA in the INK4 locus (ANRIL), a 3.8-kb lncRNA, is involved in many biological processes including cell growth and proliferation. ANRIL increases VEGF expression and enhances angiogenesis by activating the NF-kB signal pathway in diabetes mellitus combined with cerebral infarction rats. MiR-153-3p levels decrease, whereas hypoxia inducible factor-1z (HIF-1z) and its downstream targets (VEGF-A and Notch1) are upregulated in rat permanent MCAO models. Hypoxia induces the expression of lncRNA HIF1A-AS2 in human umbilical vein endothelial cells (HUVECs). Furthermore, lncRNA HIF1A-AS2 increases HIF-1z expression by sponging miR-153-3p, and HIF1A-AS2 facilitates HUVECs viability, migration ability, and tube formation. Consequently, HIF1A-AS2 promotes angiogenesis in hypoxia through activating HIF-1z/VEGF/Notch1 cascades by sponging miR-153-3p in HUVECs. The expression of lncRNA MEG3 and NOX4 in the rat brain microvascular endothelial cells are increased after OGD/R. The knockdown of MEG3 protects brain microvascular endothelial cells against OGD/R-induced apoptosis by decreasing NOX4 and p53 expression, and reducing intracellular ROS. It also enhances HIF-1a and VEGF expression. Furthermore, p53 promotes NOX4 expression by directly binding in the promoters of NOX4. This result indicates that MEG3 mediates angiogenesis after cerebral infarction via regulating p53/NOX4 axis.

**ncRNAs involved in microglial polarization.** Microglia are brain-resident innate immune cells that function as drivers of the neuroinflammatory response that results in secondary neuronal injury. When cerebral ischemia occurs, microglia are activated to secrete both harmful and neuroprotective cytokines/chemokines. The balance of the two opposite mediators determines the destiny of injured neurons in the focal ischemic brain area. The activated peripheral macrophages are usually divided into classic phenotype and alternative phenotype, also called M1 and M2. Microglia show a similar dynamic phenotypic response to either pathogenic or cytokine stimulation just like peripheral macrophages, ranging from an M1-like pro-inflammatory activation phenotype to the M2-like alternative activation phenotype. M1-like microglia produce pro-inflammatory molecules, such as TNF-a, IL-6, and IL-12, which exacerbate neuronal damage; M2-like microglia secrete anti-inflammatory cytokines that play a role in neuroprotection.

Increasing evidence has shown that miRNAs regulate the inflammatory activation of microglia (Table 2). MiR-424 levels decrease in the plasma of patients with AIS and in the mouse brain tissues at 4, 8, and 24 h after MCAO ischemia. Both cerebral infarction size and brain edema are lessened after overexpression of miR-424 in a mouse MCAO model. Furthermore, in vitro experiments have found that miR-424 suppressed neuronal apoptosis and microglial activation. This study implied that miR-424 protects against ischemic cerebral damage by inhibiting the activation of microglia by post-transcriptional regulating the expression of proteins involved in cell cycle. This includes CCND1, CDC25A, and CDK6. The knockdown of miR-377 promotes angiogenesis and inhibits microglial activation by targeting VEGF and early growth response protein 2 (ERG2), thus alleviating ischemic brain injury in rat MCAO models and microglial cells. In a study, the integrated expression profiles of mRNA and miRNA have been depicted in polarized primary murine microglia through microarrays and bioinformatics analysis. MiR-689, miR-124, and miR-155 were shown as predictors for pro-inflammatory pathways and M1-like activation phenotypes. MiR-711 and miR-145 were found as the predictors mediating anti-inflammatory signals and M2-like activation phenotypes.
Table 2. NcRNAs involved in the inflammatory activation of microglia in AIS.

| NcRNAs | Targets | Mechanisms | Inflammatory activation of microglia | Reference(s) |
|--------|---------|------------|-------------------------------------|--------------|
| miR-424 | CCND1, CDC25A, and CDK6 | Regulate cell cycle | Inhibit | Zhao et al. 60 |
| miR-377 | VEGF and EGR2 | – | Promote | Fan et al. 51 |
| miR-689, miR-124, and miR-155 | – | – | Promote | Freilich et al. 58 |
| miR-771 and miR-145 | – | – | Inhibit | Freilich et al. 58 |
| lncRNA SNHG14 | PLA2G4A | Sponge miR-145-5p | Promote | Qi et al. 61 |
| lncRNA Gm4419 | Ixβ, NF-κB | Increase the expression of IL-1β, IL-6, and TNF-α | Promote | Wen et al. 33 |
| lncRNA H19 | HDAC1 | – | Promote | Wang et al. 62 |

AIS: acute ischemic stroke, ncRNA, non-coding RNA; miRNA, microRNA; lncRNA, long non-coding RNA; CCND1, cyclin D1; CDC25A, cell division cycle 25 homolog A; CDK6, cyclin-dependent kinase 6; VEGF, vascular endothelial growth factor; EGR2, growth response protein 2; PLA2G4A, cytosolic phospholipase A2; HDAC1, histone deacetylase 1.

Table 3. NcRNAs involved in the BBB integrity in AIS.

| NcRNAs | Targets | Mechanisms | BBB integrity | Reference(s) |
|--------|---------|------------|---------------|--------------|
| miR-130a | Homeobox A5 | Induce monolayer permeability | Break | Wang et al. 63 |
| miR-15a | Bcl-2 | Protect endothelial cells | Stable | Yin et al. 64 |
| miR-34a | cytochrome c | Reduce mitochondrial function and disrupt tight junctions | Disrupt | Bukeirat et al. 65 |
| Let-7 and miR-98 | – | Inhibit the production of CCL2 and CCL5 | Protect | Rom et al. 66 |
| miR-155 | – | Inhibit the expression of tight junction proteins | Break | Lopez-Ramirez et al. 67 |
| lncRNA Malat1 | ULK2 | Sponge miR-26b | Stable | Li et al. 68 |
| circRNA DLGAP4 | miR-143 | Regulate endothelial-mesenchymal transition | Preserve | Bai et al. 11 |

AIS: acute ischemic stroke, BBB: blood–brain barrier, ncRNA, non-coding RNA; miRNA, microRNA; lncRNA, long non-coding RNA; circRNA, circular RNA; Bcl-2, B cell lymphoma-2; ULK2, unc51 like kinase 2.

Little is known about the lncRNAs and circRNAs in inflammatory activation of microglia (Table 2). LncRNA SNHG14 is highly expressed in ischemic brain tissues of mouse MCAO models and BV-2 cells after OGD treatment. LncRNA SNHG14 promotes microglial cells activation by upregulating the expression of cytosolic phospholipase A2 (PLA2G4A) via sponging miR-145-5p, and contributes to an increase in TNF-α and NO production as well as neuronal apoptosis in cerebral infarction. LncRNA Gm4419, which has emerged as a critical mediator in the NF-κB signaling pathway activation via IκBα phosphorylation, is induced during OGD/R injury to microglial cells. It promotes production of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, leading to OGD/R injury as cell apoptosis and death. The knockdown of H19, a classic lncRNA that is highly expressed in the plasma of patients with AIS and mouse MCAO models, blocks OGD/R-induced M1 microglial polarization to decrease the expression of TNF-α and CD11b. It also upregulates the production of human arginase-1 (Arg-1) and CD206. Moreover, H19 knockdown reverses the OGD/R-induced increasing expression of HDAC1 and the decrease of acetyl-histone H3 and acetyl-histone H4. Conversely, the upregulation of HDAC1 eliminates the function of H19 knockdown. H19 promotes neuroinflammation by stimulating histone deacetylase 1 (HDAC1)-dependent M1 microglial polarization.

ncRNAs involved in the blood–brain barrier permeability. The blood–brain barrier (BBB) is composed of cerebral endothelial cells, astrocytes, pericytes, the basement membrane, and tight junctions. Maintaining BBB integrity is crucial for brain homeostasis because it limits entry of circulating leukocytes, blood-borne molecules, and toxic substance into the brain. Brain endothelial cells malfunction after cerebral ischemia contributes to BBB breakdown and enhances its permeability. This promotes neuroinflammation and exacerbates cerebral ischemic damage. NcRNAs are highly expressed in cerebral endothelial cells, where they play a critical role in maintaining the normal functions of cerebral vascular. Dysregulation of ncRNAs activation is significantly related to the pathophysiology of cerebral vascular endothelium in the brain response to ischemic stimulation (Table 3).

MiR-130a predominantly expresses in brain microvascular endothelial cells and increases after I/R in rat MCAO models. The administration of antagonir-130a can
contribute to alleviated brain edema, lower BBB permeability, reduced infarct volume, and improved neurologic function. MiR-130a overexpression increases ischemia-induced monolayer permeability in vitro by targeting Homeobox A5. Therefore, MiR-130a mediates brain ischemia-induced BBB dysfunction by inhibiting Homeobox A5 expression. Peroxisome proliferator-activated receptor delta protects endothelial cells against cerebral ischemia induced-damage through suppressing bcl-2 expression by increasing miR-15a levels in mice. This leads to stable BBB integrity. Overexpression of miR-34a reduces mitochondrial function, consisting of impairing mitochondrial oxidative phosphorylation. It also reduces ATP production by negatively regulating cytochrome c, which increases BBB permeability and disrupts tight junctions ZO-1 in brain microvascular endothelial cells. Let-7 and miR-98 prevent BBB breakdown by inhibiting the production of pro-inflammatory cytokines, including chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-C motif) ligand 5 (CCL5) CCL5, under neuroinflammatory conditions in vitro. MiR-155 negatively regulates BBB function during neuroinflammation by suppressing the expression of proteins related to tight junctions in a post-transcriptional manner. LncRNAs and circRNAs have recently emerged as key regulators of brain disorders. However, the studies of their roles in BBB dysfunction is scarce. LncRNA Malat1 levels increases after I/R or OGD/R treatment, and exerts a protective role in endothelial cells against cerebral ischemic damage. It promotes brain microvascular endothelial cells autophagy and survival by sponging miR-26b and upregulating unc-51 like kinase 2 (ULK2) expression. Therefore, LncRNA Malat1 can prevent BBB disruption by protecting brain microvascular endothelial cells against OGD/R injury. Circular RNA DLGAP4 expression is reduced in the plasma of patients with AIS, and has also been shown to be reduced in a mouse MCAO model. The upregulation of circRNA DLGAP4 improves neurological functions, decreases infarct volumes, and prevents BBB dysfunction in mouse transient MCAO models. It also inhibits endothelial-mesenchymal transition that can induce BBB permeability by regulating tight junction proteins and mesenchymal cell marker expression. Moreover, circRNA DLGAP4 sponges miR-143 and inhibit tight junction proteins expression. These results reveal that circRNA DLGAP4 preserves BBB integrity through negatively regulating endothelial-mesenchymal transition by sponging miR-143 and improves outcome in ischemic stroke.

ncRNAs involved in neuroprotection. Cerebral ischemia triggers changes in the ncRNAs profiles and is a concomitant altered expression of proteins involved in neuroprotection in rodents and humans. Many studies have explored the change of these ncRNAs after stroke and are related to neuroprotection in order to gain new biomarkers and therapeutic targets. Both upregulation and downregulation of a special miRNA exert neuroprotection effects against cerebral ischemic damage, including reduced ischemic infarction, improved neurological deficit and anti-apoptosis of neurons in rodent I/R models. Intravenous injections of miR-155 inhibitor improves blood flow and microvascular integrity, which has promoted neurological function recovery in mouse MCAO models. MiR-134 levels rise in primary culture neuronal cells and mouse brain tissue after OGD/R and MCAO, respectively. The knockdown of miR-134 reduces neurons death and apoptosis via upregulating heat shock protein A12B expression in a post-transcriptional manner. This mediates neuroprotection against OGD/R induced neurons injury. Similarly, the inhibition of miR-181b in mouse brains following cerebral ischemia drives neuroprotection against ischemic damage by negatively regulating heat shock protein A5 and ubiquitin carboxy-terminal hydrolase isozyme L1 protein levels. AntagomiR-613, a specific inhibitor of miR-613, exerts neuroprotective function against OGD/R injury in neuronal cells, probably by upregulating Sphingosine kinase 2 expression.

Conversely, the systemic upregulation of some miRNAs also plays a neuroprotective role against I/R damage to the brain by negatively regulating target genes expression. Increasing miR-215 expression not only suppresses cell apoptosis and autophagy in vitro, but also decreases ischemic infarction volume and improves neurological deficits in mouse MCAO models. MiR-215 mimics achieve neuroprotective function against ischemic damage by negatively regulating NF-kB activator 1/IL-17 receptor A signaling. MiR-128-3p can target the 3' UTR of p38α, which is a pro-apoptotic protein and reported to be downregulated 2 h after cerebral ischemia, leading to reduced p38α protein expression. MicroRNA-128-3p protects mice against brain ischemic injury by inhibiting p38α mitogen activated protein kinase activation. Previous studies have indicated that electroacupuncture, fastigial nucleus stimulation, and vagus nerve stimulation promote neuroprotection through induction of the microRNA in cerebral ischemic animal models, respectively. Some molecules, such as cytosine-phosphate-guanine, insulin-like growth factor-1, natural vitamin E α-tocotrienol, and lithium induce neuroprotection in cerebral ischemia by regulating some specific miRNAs.

Disregulation of lncRNAs can result in brain pathologies, including primary brain tumors, neurodegeneration, neuroimmunological disorders, and psychiatric diseases. Alterations of lncRNAs expression profiles has been found in the plasma of patients with AIS and in the brain tissues of mouse MCAO models. LncMalat1, a metastasis-associated lung adenocarcinoma transcript, plays an important anti-apoptotic and anti-inflammatory role in brain microvasculature. It does so by binding to Bim and E-selectin, and inhibiting their expression in mouse brain microvascular endothelial cells inducing by OGD/R and in experimental stroke mouse models. This study suggests that LncMalat1 plays a crucial protective role in ischemic
stroke. LncMalat1 is highly expressed in vitro and in vivo mimics of cerebral ischemia conditions. The knockdown of Malat1 attenuates neuronal cells death through inhibiting beclin1-dependent autophagy by regulating miR-30a expression.

LncRNA-N1LR levels are increased abruptly in focal ischemic cerebral tissues of mice subjected to MCAO. LncRNA-N1LR promotes cell cycle progression and cell proliferation, and also suppresses the apoptosis of N2a cells that are subjected to OGD/R. Furthermore, it reduces neuronal apoptosis and neural cell loss in mouse experimental ischemic models. LncRNA-N1LR enhances neuroprotective roles probably through the inhibition of p53 phosphorylation. Experimental cerebral ischemia induces upregulation of LncRNA MEG3 expression, and recruits p53 into MEG3 complex. LncRNA MEG3 binds with the p53 DNA binding domain (DBD) consisting of amino acids 271–282, and this promotes p53-mediated transactivation and contributes to ischemic neuronal death. Treatment with the membrane-permeable peptide inhibitor Tat-p53-DBD271–282 selectively blocks MEG3-p53 complex, which significantly protects against ischemic neuronal death. LncRNA MEG3 promotes neuronal cells death by interacting with p53 to mediate ischemic damage. Neuron apoptosis is one of main reason for patients with AIS. LncRNA taurine-upregulated gene 1 functions as a neuron apoptosis promoter through increasing Bcl2l11 expression by sponging MiR-9.

**ncRNA biomarkers**

Recent studies have demonstrated altered ncRNAs expression profiles in bodily fluids of AIS patients and rodents cerebral ischemia model. This implies that ncRNAs could be useful biomarkers for diagnosis, disease severity and prognosis in patients with AIS.

**microRNA biomarkers.** Circulating miRNAs have been suggested as potential diagnostic and prognostic biomarkers for AIS, and they may be widely used in future clinical practice (Table 4). A study of plasma samples of patients with AIS identifies that miR-125a-5p, miR-125b-5p, and miR-143-3p are upregulated compared with both healthy control subjects and patients with transient ischemic attack. Furthermore, miR-125b-5p and miR-143-3p return to control levels at day 2, while miR-125a-5p remains elevated at 3 months. These three miRNAs might have clinical utility as early diagnostic markers for AIS. Similarly, another study has shown that the elevated serum expressions of miR-15a, miR-16, and miR-17-5p are correlated with AIS, and that miR-17-5p may be a significant and independent predictor for determining the presence of AIS. MiR-221-3p and miR-382-5p levels were significantly downregulated in a study of the circulating serum of acute stroke patients, and might be usable as potential non-invasive biomarkers for the diagnosis.

Some miRNAs may have value for estimating prognosis in patients with AIS. MiR-150-5p plasma levels are negatively associated with mortality within 90 days, improving risk classification beyond traditional risk factors in acute stroke patients. In patients with AIS, plasma miR-124-3p concentrations are positively related with 3-month mortality. Higher plasma miR-16 concentrations are indicative patient survival, while lower plasma miR-16 concentrations are associated with poor clinical outcome at 3 months. Furthermore, elevated serum miR-132 is a risk biomarker of post-stroke cognitive impairment, and angiogenesis is involved in post-stroke recovery. In order to assess their correlations with risk and severity of acute stroke, a study detects 28 pro-angiogenesis and anti-angiogenesis miRNAs.

**Table 4.** NcRNA biomarkers for AIS and their proposed clinical applications in humans.

| NcRNAs             | Alteration | Sample source       | Proposed clinical application       | Reference(s)             |
|--------------------|------------|---------------------|------------------------------------|--------------------------|
| miR-125a-5p        | Up         | Plasma              | Diagnosis                          | Tiedt et al.             |
| miR-143-3p         | Up         | Plasma              | Diagnosis                          | Tiedt et al.             |
| miR-125b-5p        | Up         | Plasma              | Diagnosis                          | Tiedt et al.             |
| miR-17-5p          | Up         | Serum               | Diagnosis                          | Wu et al.                |
| miR-382-5p         | Down       | Serum               | Diagnosis                          | Wang et al.              |
| miR-221-3p         | Down       | Serum               | Diagnosis                          | Wang et al.              |
| miR-150-3p         | Down       | Plasma              | Prognosis                          | Scherrer et al.          |
| miR-124-3p         | Up         | Plasma              | Prognosis                          | Rainer et al.            |
| miR-16             | Down       | Plasma              | Prognosis                          | Rainer et al.            |
| miR-132            | Up         | Plasma              | Prognosis                          | Huang et al.             |
| miR-223            | Up         | Whole blood         | Prognosis                          | Chen et al.              |
| miR-29b            | Down       | Whole blood cells   | Disease severity                   | Wang et al.              |
| IncRNA NR_002332   | Up         | Whole blood         | –                                  | Dykstra-Aiello et al.    |
| IncRNA AJ131606    | Up         | Whole blood         | –                                  | Dykstra-Aiello et al.    |
| IncRNA f57-2       | Down       | Whole blood         | –                                  | Dykstra-Aiello et al.    |
| IncRNA C10         | Down       | Whole blood         | –                                  | Dykstra-Aiello et al.    |
| IncRNA MIAT        | Up         | Whole blood         | Disease severity prognosis         | Zhu et al.               |

AIS: acute ischemic stroke, ncRNA, non-coding RNA; miRNA, microRNA; lncRNA, long non-coding RNA.
in plasma of patients with AIS compared with healthy controls. This indicates that circulating plasma miR-126, miR-130a, miR-222, miR-218, and miR-185 could serve as promising and independent biomarkers for risk of acute stroke, and miR-126, miR-378, miR-222, miR-101, miR-218, and miR-206 could be used for disease severity estimation of AIS. The brain-enriched miRNAs of MiR-9-5p, miR-9-3p, miR-124-3p, and miR-128-3p are higher than the controls in cerebrospinal fluid from patients with a larger infarct volume.

Circulating exosomal miR-223 from blood samples of patients with AIS is significantly higher than in the controls. Upregulation of exosomal miR-223 is associated with the occurrence of AIS, severity of stroke, and poor prognosis. One study has demonstrated that miR-150 polymorphisms may be used as novel biomarkers for predicting the risk of AIS. The expression of miR-29b is decreased in the white blood cells of humans and mice after cerebral ischemia, and negatively related to severity of stroke and brain infarct volume. Furthermore, the overexpression of miR-29b reduces BBB dysfunction through targeting of AQP-4.

**LncRNAs biomarkers.** Like that of miRNAs, expression of LncRNAs also changes in peripheral blood after AIS (Table 4). LncRNA NR_002332 and LncRNA AJ131606 are upregulated while LncRNA C10 and LncRNA f57-2 are downregulated in a time-dependent manner in whole blood samples from patients with ischemic stroke. LncRNA MIAT levels are significantly increased in blood samples of acute stroke patients and are correlated with the severity of stroke, high-sensitivity C-reactive protein, infarct volume, and 3-month poststroke outcome. The overexpression of LncRNA MIAT indicates poor prognosis. This LncRNA may be used as an independent prognostic marker of functional outcome and survival time for patients with ischemic stroke. In mouse primary brain microvascular endothelial cells after OGD, LncRNA expression signatures are detected by RNA sequencing technology. A total of 147 upregulated LncRNAs and 70 downregulated LncRNAs are found. LncRNA Shg12, LncRNA Malat1, and LncRNA-OGD 1006 are the most highly upregulated LncRNAs, whereas LncRNA 281008D09Rik, LncRNA Peg13, and LncRNA-OGD 3916 are the most highly downregulated LncRNAs.

**Therapeutic targets.** There are several common problems in AIS treatment, including the availability of only two approved approaches, short therapeutic window, and secondary brain damage. Furthermore, mechanical thrombectomy and intravenous thrombolysis has deleterious effects in some patients, such as neurotoxicity, ischemic reperfusion injury, and hemorrhagic transformation. Identifying new targets and improving the prognosis for patients with AIS is dependent on a deeper understanding of mechanisms of this disease. Recent studies have demonstrated that ncRNAs play a crucial role in the pathophysiological processes of neuroinflammation, oxidative stress, excitotoxicity, and apoptosis, thus contributing to cerebral ischemic injury. Drugs derived from ncRNAs have been shown to penetrate BBB and exert neuroprotection against brain ischemic injury in experimental animal stroke models. Therefore, treatments which target ncRNAs or make use of ncRNA molecules might help to improve prognosis of AIS (Table 5).

MiRNAs might be potential targets because they are enriched in brain and are capable of regulating the expression of potentially deleterious genes in post-transcriptional manner after ischemic stroke. Meanwhile, miRNAs control the specific proteins expression that result in the neuroprotection and angiogenesis that promote recovery and repair mechanisms in acute stroke patients. Exogeneous miR-1906 uptake by astrocytes, microglia, and neurons is visualized by Cy3 labeling, which reduces infarct volumes and improves functional outcomes in mouse tMCAO models. MiR-1906 inhibits neuroinflammation after ischemic stroke by targeting TLR4. Intravenously administered miR-17-92 cluster-enriched exosomes harvested from mesenchymal stem cells (MSCs) transfected with an miR-17-92 cluster plasmid promote neural plasticity and functional recovery after stroke through activating PI3K/PKB/mechanistic target of rapamycin/GSK3β pathway by targeting phosphatase and tensin homolog in rats.

The expression of miR-122 in blood decreases in patients and rats following AIS. Intravenous injection of miR-122 mimic reduces neurological deficits and brain infarction, and maintains vessel integrity after MCAO via suppressing target gene expression in blood leukocytes, such as VCAM1, Nos2, and Pla2g2a. Among current studies involved in targeted miRNA therapy, most focus on inhibition detrimental miRNAs by administrating miRNA antagonon or using drugs. Genetic deletion of the miR-15a/16-1 cluster or intravenous delivery of miR-15a/16 -1 antagonon significantly ameliorates cerebral ischemic damage through both upregulation of antiapoptotic proteins (Bel-2 and Bel-w) and inhibition of proinflammatory molecules (IL-6, MCAF, VCAM1, and TNF-α). The administration of miR-383 antagonon attenuates focal ischemic brain injury via directly downregulating peroxisome proliferator-activated receptor gamma expression. Treatment with miR-93 antagonon decreases cerebral infarction volume, neural apoptosis and severity of stroke via activation of Nrf2/HO-1 antioxidant pathway. A study has shown that miR-106b-5p antagonon significantly protects brain tissues against I/R injury by inhibiting apoptosis and oxidative stress in rat MCAO models. Intravenous injections of a specific miR-155 inhibitor decreases brain tissue damage, supports brain microvasculature, and promotes neurofunctional recovery through maintaining the integrity of tight junction proteins by targeting Rheb in mouse MCAO models. The administration of miR-181 antagonon via intra-cerebroventricular and intravenous routes alleviates cerebral ischemic injury and improves long-term behavioral recovery by inhibition of neuroinflammation and upregulation of anti-apoptotic proteins in mice.
after focal cerebral ischemia. Interestingly, inhibition of miR-30a reduces cerebral ischemic injury through enhancing beclin 1-mediated autophagy.

Cumulative studies have indicated that lncRNAs play critical roles in the development and progression of ischemic stroke. Although there has been no lncRNAs-based research on target therapy for ischemic stroke, lncRNAs that participate in the pathologic processes of ischemic stroke, might be useful as therapeutic targets.

Although the potential of ncRNAs in AIS therapy could lead to exciting future directions, many challenges remain. Such challenges are similar to those associated with ncRNAs-based treatments in cancer, including rapid degradation and clearance, poor cellular uptake, off-target effects, and immunogenicity. Off target effects cannot be neglected, because they are the main reason for side effects and poor healing efficacy in miRNA-based therapy; however, to date there is no research related to off target effects of miRNAs-based treatments in AIS. To solve this difficult problem, comprehensive research is needed in the future.

Future directions and conclusions

NeRNAs contribute to cerebral ischemic injury by regulating NFκ-B pathway activation, oxidative stress, microglial polarization, neuronal apoptosis, and BBB permeability. On the contrary, ncRNAs promote functional recovery by enhancing angiogenesis, neurogenesis, and neuroprotection. Much research to date has been done on miRNAs, whereas the role of lncRNAs and circRNAs in ischemic stroke remains largely unknown. Further research will likely unearth these ncRNAs, which is also beneficial in helping us to better understand the mechanisms underlying brain ischemic damage. Most existing research linking ncRNAs to ischemic stroke has been conducted with rodent models of MCAO and in vitro OGD/R models. These experimental models will continue to be useful in defining the role of ncRNAs in AIS, either by using genetic manipulation or through the use of ncRNA antagonomers or agomirs.

An increasing number of case-control studies have demonstrated that alterations in ncRNAs expression can be detected in serum, plasmas, and throughout the bloodstream of patients with ischemic stroke. Many ncRNAs have significantly high disease specificity. Furthermore, many ncRNAs are associated with several clinicopathological parameters of AIS, such as infarct volume, brain edema, severity of stroke, and clinical outcome. Therefore, in the future, ncRNAs may act as non-invasive biomarkers of diagnosis and prognosis for patients with AIS.
Although there has been rapid progress in miRNA biopharmaceutical research, ncRNAs-based treatments have not been used in clinical practice. It has been shown that miRNA mimics and inhibitors can penetrate BBB and protect against brain ischemic injury in animal models with AIS. Exosomes, liposomes and lentivirus might be used for carrying ncRNAs-based drugs and take them to the cerebral infarction area. Hence, ncRNAs-derived therapy may be a novel adjunctive therapeutic strategy to improve outcome after intravenous thrombolysis and endovascular mechanical reperfusion for AIS.

**Author Contribution**
Z-SS participated in the design of the present study. All authors participated in the interpretation and collection of the data. S-WW wrote the initial manuscript. Z-SS revised the manuscript. All authors critically reviewed and edited the manuscript and approved the final version.

**Declaration of Conflicting Interests**
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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![Figure 1. NcRNAs involved in ischemic brain injury.](image)

**Figure 1.** NcRNAs involved in ischemic brain injury. NcRNAs play a role in pathogenic processes related to ischemic cerebral injury. These pathogenic processes include the activation of NF-κB signaling pathway, oxidative stress, microglia activation and BBB breakdown. NF-κB, nuclear factor kappa-B; BBB, blood–brain barrier.
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