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Review

Non-coding RNAs in acute ischemic stroke: from brain to periphery

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From the Contents

Abstract

Acute ischemic stroke is a clinical emergency and a condition with high morbidity, mortality, and disability. Accurate predictive, diagnostic, and prognostic biomarkers and effective therapeutic targets for acute ischemic stroke remain undetermined. With innovations in high-throughput gene sequencing analysis, many aberrantly expressed non-coding RNAs (ncRNAs) in the brain and peripheral blood after acute ischemic stroke have been found in clinical samples and experimental models. Differentially expressed ncRNAs in the post-stroke brain were demonstrated to play vital roles in pathological processes, leading to neuroprotection or deterioration, thus ncRNAs can serve as therapeutic targets in acute ischemic stroke. Moreover, distinctly expressed ncRNAs in the peripheral blood can be used as biomarkers for acute ischemic stroke prediction, diagnosis, and prognosis. In particular, ncRNAs in peripheral immune cells were recently shown to be involved in the peripheral and brain immune response after acute ischemic stroke. In this review, we consolidate the latest progress of research into the roles of ncRNAs (microRNAs, long ncRNAs, and circular RNAs) in the pathological processes of acute ischemic stroke–induced brain damage, as well as the potential of these ncRNAs to act as biomarkers for acute ischemic stroke prediction, diagnosis, and prognosis. Findings from this review will provide novel ideas for the clinical application of ncRNAs in acute ischemic stroke.

Key Words: acute ischemic stroke; apoptosis; blood–brain barrier damage; circular RNAs; excitatory toxicity; long non-coding RNAs; microRNAs; neuroinflammation; non-coding RNAs; oxidative stress

Introduction

Acute ischemic stroke (AIS) is a clinical emergency and a condition with high morbidity, mortality, and disability rates (Campbell et al., 2019). Although intravenous thrombolysis and endovascular therapy are recommended as effective methods for early recanalization, the benefits to AIS patients may be limited by the narrow treatment time window and complications such as hemorrhage (Powers et al., 2019). Therefore, clarifying the molecular biological mechanism of AIS is conducive to finding effective targets for prevention and treatment and is the current research focus.

Non-coding RNAs (ncRNAs) refer to RNAs that do not encode proteins. There are about 3 billion bases in the human genome, and approximately three-quarters of the sequences can be transcribed. Less than 3% of sequences ultimately encode proteins, and the remaining transcribed sequences become ncRNAs (ENCODE Project Consortium, 2012). It has been demonstrated that at least 98% of the human genome contains non-coding regions, 80% of which are transcribed into ncRNAs. NcRNAs can be divided into short ncRNAs (< 200 nt) or long ncRNAs (lncRNAs; > 200 nt) according to the number of nucleotides (Djebali et al., 2012) and mainly include microRNA (miRNAs), linear lncRNAs, and circular (circ)RNAs. In the past 20 years, innovations in whole-genome sequencing technology have facilitated a large number of studies on ncRNAs. These have provided evidence that ncRNAs are key regulatory factors required to maintain cell functions, either in the physical or pathological state (Esteller, 2011; Adelman and Egan, 2017; Ruffo et al., 2023; Sherazi et al., 2023; Wang et al., 2023). How to elucidate the pathophysiology and explore prevention and treatment targets of AIS from the perspective of ncRNAs have become hot topics recently.

Because of the ischemia and hypoxia associated with AIS, brain tissues rapidly undergo a series of pathological processes, such as excitatory toxicity, oxidative stress, neuroinflammation, apoptosis, and blood–brain barrier (BBB) damage. These unbalanced pathological reactions also cause secondary damage to brain tissues (Iadecola et al., 2020). An increasing number of studies

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show that ncRNAs regulate these pathological reactions, leading to brain tissue repair or brain damage aggravation. Therefore, ncRNAs are considered potential targets for future AIS treatments (Vemuganti, 2013). In addition, the expression and distribution of ncRNAs in the peripheral circulatory system are altered in AIS, providing a new area for biomarker determination and a potential clinical prognostic model for AIS (Takuma et al., 2017).

In this review, we comprehensively explore the modes of action and the effect of three types of ncRNAs (miRNAs, lncRNAs, and circRNAs) during the pathological processes (in the brain as well as the peripheral systems) of AIS. In particular, we investigate the potential use of ncRNAs as biomarkers in AIS and discuss their potential use in predictions and prognostics. Because of the extensive participation of multiple regulatory pathways before and after AIS, we also touch on the possible therapeutic ncRNA targets in AIS identified in the latest research and hope our work will provide researchers in the field further insights.

Database Search Strategy

In this narrative review, we included studies that explored the modes of action, brain pathology, and potential clinical application of ncRNAs in AIS. The majority of references (~90%) were written in the English language and were full-text articles published between March 1991 and October 2023. The research articles considered in this review related to the following keywords: acute ischemic stroke, noncoding RNAs, short noncoding RNAs, long noncoding RNAs, microRNAs, linear lncRNAs, circRNAs, ischemia, hypoxia, excitatory toxicity, oxidative stress, neuroinflammation, apoptosis, BBB damage, exosome, and antioxidant. Various combinations of the terms were used, and the associated results were electronically retrieved from the PubMed database. Articles were screened by title and abstract, and articles that did not accord with our topic were excluded from further analysis. The timeline of development of non-coding RNAs in acute ischemic stroke is shown in **Figure 1**.

MicroRNAs and Acute Ischemic Stroke

Modes of action of miRNAs

MiRNAs are short endogenous RNAs of 22 nucleotides characterized by abundant types and conserved evolution. MiRNAs are formed through either classical or non-classical pathways, and mature miRNAs are guided by the conformational arrangement of RNA-induced silencing complex proteins towards target mRNAs (Lee et al., 2002; Cifuentes et al., 2010; Xie et al., 2013). Most miRNAs can inhibit the translation or promote the degradation of mRNAs by binding to the 3′-untranslated region (UTR) directly, resulting in the post-transcriptional silencing of mRNAs and thus reducing the expression of target gene proteins (Diederichs and Haber, 2007; Winter et al., 2009). A few miRNAs can enhance the translation of target genes by binding to the 5′-UTR region of target transcripts (Ørom et al., 2008).

From the transcription of miRNA genes to the formation of mature miRNAs, each stage is regulated by a variety of proteins (Ha and Kim, 2014). One miRNA can regulate multiple target genes, and one target gene can be regulated by multiple miRNAs. Therefore, a physiological or pathological phenomenon is the result of the cumulative effect of multiple miRNAs and target genes (Flynt and Lai, 2008).

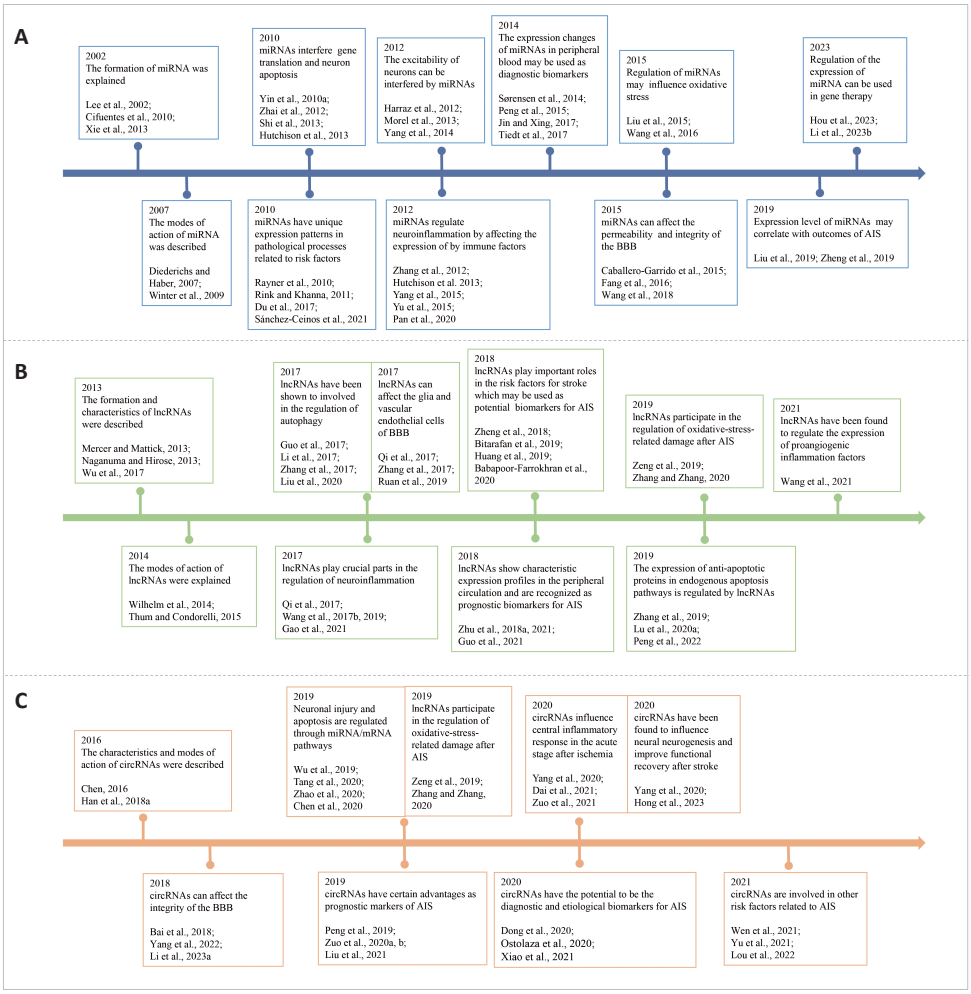

Figure 1 | Timeline of research of non-coding RNAs in acute ischemic stroke.
(A) MicroRNA; (B) long non-codingRNA; (C) circular RNA. AIS: Acute ischemic stroke; BBB: blood–brain barrier; circRNAs: circular RNAs; lnRNAs: linear long ncRNAs; miRNA: microRNA.

MiRNAs and brain pathology of AIS

MiRNAs and excitatory toxicity

Excitatory toxicity is one of the hyperacute pathological reactions of AIS. The expression of receptors and transporters on the surface of neurons can be interfered with or promoted by miRNAs, leading to alterations in neurons excitability and thus affecting the prognosis of AIS. For example, *in vivo* it has been shown that overexpression of miR-223 in the hippocampus reduces the expression of glutamate receptor 2 and N-methyl-D-aspartate receptor subunit 2B, which inhibits the neuronal calcium influx mediated by the N-methyl-D-aspartate receptor and alleviates neuronal excitatory toxicity (Harraz et al., 2012). However, the excitatory toxicity of neurons can also be increased by the overexpression of miRNAs. Yang et al. (2014) showed that miR-107 can inhibit the expression of glutamate transporter-1 protein by binding to glutamate transporter-1 mRNA to increase extracellular glutamate accumulation and aggravate neuronal injury. Further studies found that the communication between neurons and glial cells was promoted through exosome-packaged miRNAs that play an important role in maintaining glutamate homeostasis after ischemic stroke (Lachenal et al., 2011; Morel et al., 2013).

Current studies on the effect of miRNAs in excitatory toxicity have mostly focused on the regulation of the aspartic acid receptor subtypes glutamate receptor 2 and glutamate transporter-1, but whether other molecules and inhibitory neurons are regulated by miRNAs has not been clarified.

MiRNAs and oxidative stress

Oxidative stress is one of the main pathological mechanisms of brain injury after ischemia and is mainly due to the imbalance between oxidation and antioxidants caused by excess intracellular reactive oxygen species (Chen et al., 2011). Hence, the regulation of endogenous oxidase and antioxidant enzyme activities by miRNAs after ischemia may further eliminate or alleviate the damage caused by oxidative stress. Based on this hypothesis, Liu et al. (2015) showed that inhibiting miR-424 expression in the peripheral infarct region of mice resulted in an increase in manganese superoxide dismutase and superoxide dismutase with antioxidant function, as well as a decrease in the formation of reactive oxygen species and lipid peroxidation, ultimately increasing neurovascular integrity and decreasing infarct volume. Moreover, the expression level of nuclear factor-erythroid 2-related factor 2, an important transcription factor of endogenous antioxidants, was significantly upregulated as a result of the downregulation of the expression of miR-424 (Liu et al., 2015). Similarly, it has been shown that miR-93 can directly bind to the 3′-UTR region of nuclear factor-erythroid 2–related factor 2 mRNA to affect superoxide dismutase activity (Wang et al., 2016). These studies suggested that miRNAs play important regulatory roles in maintaining the balance between oxidation and antioxidant activity.

MiRNAs and neuroinflammation

Neuroinflammation is one of the main causes of secondary injury after ischemia and is caused by the activation of microglia, infiltration of the peripheral circulation by immune cells, and activation of various pro-inflammatory cytokines and chemokines (Hallenbeck, 1996; Zheng and Yenari, 2004). MiRNAs regulate neuroinflammation by affecting the expression of cytokines by immune cells. For example, miR-181c directly binds to the 3′-UTR region of tumor necrosis factor-α (TNF-α) mRNA in neuronal cells to inhibit TNF-α protein expression, thereby reducing neuronal injury and inhibiting microglia activation (Zhang et al., 2012). Similarly, Hutchison et al. (2013) found that the expression of anti-inflammatory factor interleukin (IL)-10 in astrocytes *in vitro* was upregulated by the overexpression of miR-181.

The inhibition of the nuclear factor-kappa B (NF-κB) pathway, a classic inflammation pathway, can provide neuroprotection after ischemia (Buchan et al., 2000). There are many miRNAs involved in neuroinflammation through the NF-κB pathway, such as miR-22, and the activity of a coactivator in the NF-κB pathway was inhibited by miR-22 in an oxygen- and glucose-deprivation model (Yu et al., 2015). In microglia, miR-203 inhibits the translation of myeloid differentiation factor 88 mRNA by binding to its 3′-UTR region, thereby preventing NF-κB from entering the nucleus (Yang et al., 2015). Moreover, the expression of microglia-specific protein ionized calcium-binding adaptor molecule-1 is reduced by miR-181a, thus alleviating the nerve injury induced by inflammation (Xu et al., 2015).

In addition to inflammatory factors, adhesion molecules also have crucial functions in the migration, responses, and activation of immune cells, and miRNAs are involved in the regulation of adhesion molecules (Ramiro et al., 2018). Recently, Pan et al. (2020) found that the overexpression of miR-126-3p/miR-5P in vascular endothelial cells suppresses the expression of IL-1β and TNF-α, as well as the vascular cell adhesion molecule-1 and E-selectin, and thereby reduces BBB damage and improves prognosis. Although inhibiting inflammatory factors in brain tissue after AIS can alleviate neurological damage in *in vivo* and *in vitro* models, these results have been seen only in the experimental stage. There are no miRNA anti-inflammatory therapies that have achieved clinical stage development so far. Therefore, whether the miRNAs involved in neuroinflammation are therapeutic targets for AIS remains to be discussed.

MiRNAs and apoptosis

Excitatory toxicity, oxidative stress, and neuroinflammation occur within hours to days after ischemia, accelerating the death of neurons in the ischemic penumbra region (Broughton et al., 2009). Apoptosis, a classic and common form of cell death, can be activated by endogenous and exogenous pathways (Broughton et al., 2009).

In the endogenous apoptosis pathways, anti-apoptotic genes such as B-cell lymphoma-2 (Bcl-2) family genes, are crucial for reducing neuron injury after ischemia (Nakka et al., 2008). Members of the miR-15 family inhibit Bcl-2 translation and promote apoptosis by binding to the 3′-UTR region of Bcl-2 mRNA in vascular endothelial cells and neurons, and inhibiting miR-15 expression can provide neuroprotective effects (Yin et al., 2010b; Shi et al., 2013). Similarly, miR-181a, miR-497, miR-384-5p, and miR-134 all promote neuronal apoptosis by downregulating the protein expression of Bcl-2 (Yin et al., 2010a; Hutchison et al., 2013). Furthermore, antagonists of miR-124 have been reported to increase the expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL, but not the pro-apoptotic proteins Bax and Bad, and thereby reduce the apoptosis of cortical neurons (Bao et al., 2020).

In the exogenous apoptosis pathways, death receptors and ligands on the cell surface, such as the Fas receptor, are important for the activation of apoptosis. A previous study showed that miR-25 inhibits the expression of the Fas receptor protein by binding to the 3′-UTR region of Fas receptor mRNA, thereby reducing apoptosis (Zhang et al., 2016). The cysteine aspartate protease 3 (caspase-3) pathway is considered an interaction point between endogenous and exogenous apoptosis pathways. Research found

that the activation of caspase-3 can be suppressed by miR-99a and miR-let-7c-5p to decrease neuron apoptosis (Ni et al., 2015; Tao et al., 2015b). These studies suggested that miRNAs play vital roles in both endogenous and exogenous apoptosis pathways.

In addition to the apoptotic pathways, other forms of cell death induced by AIS are also regulated by miRNAs, including autophagy, necrotizing apoptosis, and iron death. MiR-30a has shown a pro-apoptotic effect via its binding with beclin-1 mRNA, which is a key molecule of autophagy (Wang et al., 2014). Moreover, miR-207 and miR-352 respectively bind with the 3′-UTR regions of lysosomal associated membrane protein 2 and Hexb mRNA to relieve the autophagy injury induced by ischemia (Tao et al., 2015a). In necrotic apoptosis, miR-223-5p binds to the 3′-UTR region of K^+-dependent Na^+/Ca^{2+} exchanger mRNA to interfere with its translation, whereas an intraventricular injection of an antagonist anti-miR-223-5p increased the expression of K^+-dependent Na^+/Ca^{2+} exchanger, which protects the neurons (Cuomo et al., 2019). In the study of iron death, miR-214 was found to bind with TP53 and transferrin receptor-1 mRNA to regulate iron death in AIS (Lu et al., 2020a).

In general, the post-transcriptional regulation of miRNAs has been involved in various types of cell death after AIS. Compared with apoptosis and autophagy, necrotic apoptosis and iron death in AIS are rarely studied, although the elucidation of cell death mechanisms is conducive to the development of AIS therapy.

MiRNAs and BBB damage

Cerebrovascular edema is the most direct manifestation of BBB damage, which involves abnormal tight-junction proteins and surface receptors on vascular endothelial cells, as well as disorders of matrix metalloproteinases (MMPs) and aquaporins (AQPs) (Michinaga and Koyama, 2015). In a study interested in vascular endothelial cells, expression of the Tie 2 receptor was inhibited by miR-150 via its binding with the 3′-UTR region of Tie 2 mRNA; accordingly, the abundance of the downstream adhesion molecule claudin-5 was decreased, affecting the permeability of the BBB (Fang et al., 2016). MiR-130a and miR-155 can respectively affect the expression of occludin and zonula occludens 1 (ZO-1) to regulate the integrity of the BBB and determine the prognosis of AIS (Caballero-Garrido et al., 2015; Wang et al., 2018).

The basement membrane of BBB is degraded and destroyed by MMPs, especially MMP-9 (Planas et al., 2001; Lee et al., 2004). In a rat cerebral infarction model, miR-21 downregulation reduced the expression of MMP-9; however, the mode of interaction between miR-21 and MMP-9 has not been elucidated (Deng et al., 2013).

As the most abundant AQP in the brain, the expression of AQP4 in astrocyte feet is critical to the maintenance of BBB morphology and function (Manley et al., 2000). In an oxygen- and glucose-deprivation model, miR-145 in astrocytes was shown to directly target the 3′-UTR of AQP4 mRNA and reduce the expression of the AQP4 protein (Zheng et al., 2017). Furthermore, miR-130a was demonstrated to be an inhibitor of the AQP4 M1 transcription promoter in its involvement in BBB regulation (Sepramaniam et al., 2012).

In conclusion, whether BBB injury is directly caused by vascular damage or indirectly caused by the pathological reactions of immune cells induced by ischemia, miRNAs are directly or indirectly involved in the regulation of cerebral edema.

MiRNAs and peripheral biomarkers

MiRNAs as predictive biomarkers for AIS

A systematic analysis published in 2019 revealed that hypertension, diabetes, hyperlipidemia, and atherosclerosis are risk factors for AIS (GBD 2019 Stroke Collaborators, 2021). Moreover, miRNAs have unique expression patterns and play essential roles as regulators in the pathological processes related to these risk factors. The expression of miR-223-3p was decreased in the plasma of diabetic patients, and intracellular miR-223-3p was involved in the pathological processes of diabetes by regulating glucose and lipid metabolism (Sánchez-Ceinos et al., 2021). Likewise, miR-33 was involved in the pathological process of hyperlipidemia by affecting the expression of various lipid transport molecules (Rayner et al., 2010), which provided hints that miRNAs associated with AIS risk factors alone or in combination are potential biomarkers for predicting AIS (Rink and Khanna, 2011).

Currently, the widely used Framingham Stroke risk score, which encompasses the clinical risk factors of stroke, is used to predict the individual probability of cerebrovascular events within 10 years (Wolf et al., 1991). However the score tends to overestimate the incidence probability in the general population (Bineau et al., 2009; McClure et al., 2014). Considering that disorders of vascular endothelial cells are closely related to the future occurrence of cerebrovascular diseases, many researchers believe that the overestimation of incidence probability may be due to the fact that the score fails to take into account the functional state of vascular endothelia from the molecular perspective (Dufouil et al., 2017; Flueckiger et al., 2018). Vascular endothelial function is regulated by miRNAs (Rink and Khanna, 2011), and as a consequence, a model combining miRNAs and clinical stroke risk factors may be a more effective indicator to predict stroke. On the basis of the hypothesis, a recent large cohort study compared models of miRNAs with the models of each clinical risk factor, and the model that combined three miRNAs (miR-1268b, miR-4433b-3p, and miR-6803-5p) with three clinical risk factors (age, alcohol, and systolic blood pressure) performed better at predicting cerebrovascular diseases (Sonoda et al., 2019). Despite the ethnic homogeneity of the population in a study by Sonoda et al. (2019), it provided a reference for subsequent studies on the use of miRNAs as biomarkers to predict AIS in combined molecular and clinical parameter models.

The incidence of ischemic stroke is considered to be hereditary, with a probability of 37.9% (Bevan et al., 2012). Genetic heritability and variability are reflected by single-nucleotide polymorphisms (SNPs), which can be used to predict the occurrence of cerebrovascular diseases. The meta-analysis conducted by Du et al. (2017) showed that SNPs of the miR-149 gene were significantly correlated with the risk of stroke in an East Asian population, suggesting miRNA gene variants have potential use as markers for AIS prediction.

MiRNAs as diagnostic biomarkers for AIS

MiRNAs are widely expressed in peripheral blood and cerebrospinal fluid, so they are feasible biomarkers for the diagnosis of AIS. Additionally, thanks to the convenience of obtaining peripheral blood and detecting dynamic changes to miRNAs, the expression changes of miRNAs in peripheral blood of patients with AIS have been widely investigated. Using sequencing, Tiedt et al. (2017) identified 32 differentially expressed miRNAs for the first time in the plasma of discovery samples of AIS patients and controls. Three stably upregulated miRNAs (miR-125a-5p, miR-125b-5p, and miR-143-3p) were

finally confirmed in a replication cohort, with a sensitivity of 85.6%, specificity of 76.3%, and an area under curve (AUC) of 0.9, as able to predict stroke with a higher sensitivity than the 72.5% of computed tomography. Furthermore, platelets were found to be the main source of the three differentially expressed miRNAs (Sørensen et al., 2014; Peng et al., 2015; Jin and Xing, 2017), which could explain the absence of a correlation between infarct volume and neuron death. In addition, Tan et al. (2009) first revealed the feasibility of using miR-25, miR-125b-2, miR-125b-627, miR-125b-27a, miR-125b-488, and miR-145 as biomarkers in the whole blood of young AIS patients. MiR-145 is also considered a potential marker for AIS diagnosis in serum (Jia et al., 2015). Additionally, the expression of miRNAs, such as miR-4656 and miR-4443, in peripheral blood mononuclear cells (PBMCs) can be dysregulated by AIS. Although these differential miRNAs have not been verified in large samples, they may still provide potential markers for AIS diagnosis (Bam et al., 2018; Li et al., 2020).

In addition to AIS patient screening, miRNAs can also be used to identify AIS from hemorrhagic stroke. A recent study showed that 25 miRNAs derived from peripheral blood exosomes, such as miR-30a-3p, miR-224-5p, and miR-98-3p, clearly differentiated AIS from hemorrhagic stroke, with an AUC of 0.813 (Kalani et al., 2020).

Peripheral miRNAs from the different sources above have shown good diagnostic value in small cohort studies, although large multicenter studies are needed in the future to verify the reliability of these miRNAs as AIS biomarkers.

MiRNAs as prognostic biomarkers for AIS

In the peripheral blood, miRNAs play a wide range of regulatory roles in the stability of the infarction area after AIS, hence they are potential biomarkers for AIS prognosis. The expression of miR-128 in peripheral lymphocytes was significantly upregulated after AIS, and its expression level was positively correlated with infarct size and poor outcomes (Liu et al., 2019). Furthermore, Zheng et al. (2019) found that the expression of miR-21-5p, miR-206, and miR-3123 associated with MMP9 in plasma could be used to predicted hemorrhagic transformation in patients with cardioembolic stroke, with AUCs of 0.677, 0.687, and 0.661 respectively. Moreover, the outcomes of a small sample-sized study in South Korea suggested that increased expression of miR-17, which is linked to atherosclerosis, is correlated with a reduction in stroke recurrence and survival rate (Kim et al., 2015). In conclusion, peripheral miRNAs all have prediction potential, whether it is the AIS prognostic score, hemorrhagic transformation complication, or stroke recurrence, but their clinical applications need to be further explored. The role of miRNAs in AIS is summarized in **Figure 2**.

Long Noncoding RNAs and Acute Ischemic Stroke

Modes of action of lncRNAs

LncRNAs are classic ncRNAs with a length greater than 200 nucleotides (Djebali et al., 2012). It is believed that most lncRNAs are formed by transcription from corresponding genes in a similar way to mRNA, and a few lncRNAs have their own unique characteristics (Mercer and Mattick, 2013; Naganuma and Hirose, 2013; Wu et al., 2017). The modes of action in lncRNAs can be divided into four categories (Thum and Condorelli, 2015): (1) lncRNAs that serve signal molecules and participate in the conduction of signal pathways, such as the classic competing endogenous RNA (ceRNA) model; (2) lncRNAs that act as decoys and molecular blockers, binding to proteins or mRNA to inhibit transcription or translation and to promote mRNA degradation; (3) lncRNAs that act as molecular chaperons, binding with proteins and guiding the complexes to specific DNA or RNA sequences to activate or inhibit downstream pathways; and (4) lncRNAs that play the role of scaffolding and combine with multiple proteins or RNAs to realize information convergence and integration among different signal pathways. Additionally, a small number of lncRNAs encode peptides, but the function of these peptides in biological activities needs to be further studied (Wilhelm et al., 2014).

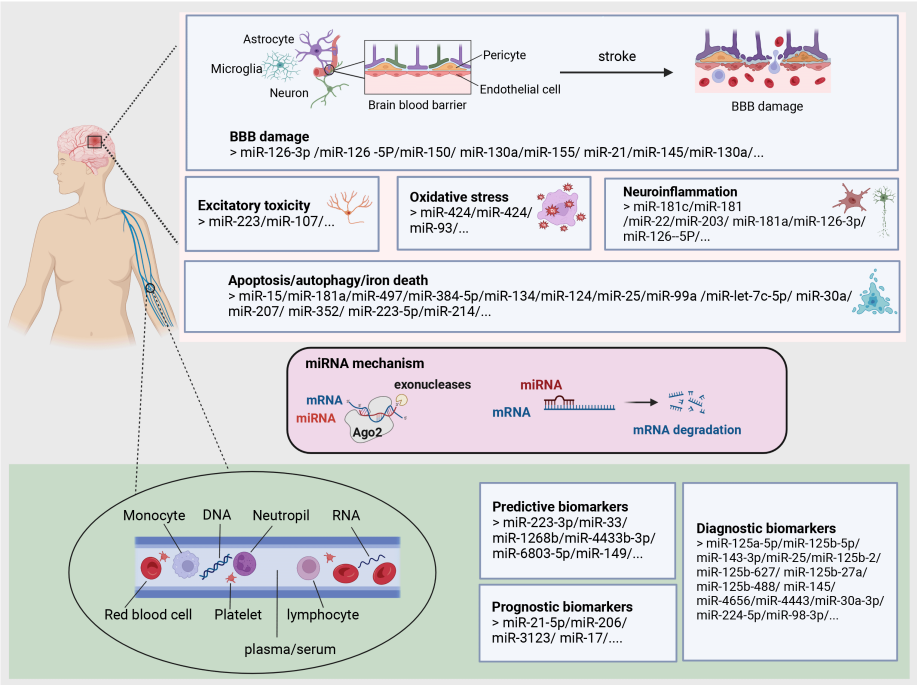

Figure 2 | MicroRNAs and acute ischemic stroke.
Brain tissues in AIS undergo a series of pathological processes. MiRNAs participate in and regulate BBB damage, excitatory toxicity, oxidative stress, neuroinflammation, and death (apoptosis, autophagy, or ferroptosis). The peripheral circulation system also responds to AIS, and the expression and distribution of miRNAs are altered in monocytes, neutrophils, red blood cells, platelets, lymphocytes, plasma, and serum, so they can be used as predictive, diagnostic, and prognostic biomarkers. MiRNAs inhibit the translation or promote the degradation of mRNAs by binding to 3′-UTRs or 5′-UTRs directly. Created with BioRender.com. AIS: Acute ischemic stroke; BBB: blood–brain barrier; miRNA: microRNA; UTR: untranslated region.

LncRNAs and brain pathology of AIS

LncRNAs and oxidative stress

Recent studies have shown that the oxidative-stress-related damage induced by AIS can be regulated using lncRNAs. LncRNA H19 has been demonstrated to competitively bind with miR-148a-3p, increasing the expression of RHO-associated protein kinase 2, which reduces antioxidant levels, and increasing the expression of lipid peroxides to promote neuron oxidative stress (Zeng et al., 2019). Similarly, lncRNA zinc finger antisense 1 has been shown to increase the expression of nitric oxide synthase 3 by competitively binding with miR-582 to inhibit the oxidative stress response and reduce neuronal injury (Zhang and Zhang, 2020).

To date, studies of the regulation mechanism of lncRNAs in AIS oxidative stress have mainly focused on the classical ceRNA mode, namely the lncRNAs/miRNAs/mRNA regulatory network. Only a few lncRNAs have been found to bind with proteins to participate in oxidative stress regulation. For example, lncRNA nuclear-enriched abundant transcript 1 was found to recruit neuro-oncological ventral antigen protein to stabilize the mRNA of mitochondrial fusion protein 2, thereby reducing reactive oxygen species production and alleviating oxidative stress injury (Zhou et al., 2022). Further studies are needed to ascertain if there are other oxidative-stress regulatory methods, such as guide proteins or scaffold proteins, in which lncRNAs participate.

LncRNAs and neuroinflammation

The neuroinflammation induced by AIS is considered a "double-edged sword". In the acute stage, inflammation aggravates secondary injury of brain tissue, while in the recovery stage, inflammatory reactions can clear necrotic tissue. Hence, neuroinflammation is closely related to the prognosis of stroke (Hallenbeck, 1996; Zheng and Yenari, 2004). Recently, a large number of studies have shown that lncRNAs play crucial parts in the regulation of neuroinflammation, especially in the activation of microglia. Qi et al. (2017) found that the activation of microglia after ischemia could be promoted by small nucleolar RNA host gene 14 (*SNHG14*) through the miR-145-5p/phospholipase A2-IVA pathway. Similarly, knockdown of lncRNA H19 weakened deacetylase 1–dependent M1 microglia polarization, reduced pro-inflammatory factor expression, and improved prognosis (Wang et al., 2017b).

Many lncRNAs have been found to be involved in the regulation of neuroinflammation by acting through the NF-κB pathway. In an *in vitro* microglia oxygen-glucose model, the NF-κB pathway was activated by taurine-upregulated gene 1, which targets miR-145a-5p, to reduce microglia transformation from the M1 to M2 type (Wang et al., 2019). In a mouse middle cerebral artery occlusion model, lncRNA Nespas directly bound to transforming growth factor-β-activated kinase 1 mRNA to inhibit the expression of the NF-κB pathway in microglia (Deng et al., 2019). In astrocytes, the activity of the NF-κB pathway was reduced by NF-κB's interaction with lncRNA (Gao et al., 2021). In neurons, lncRNA CAMK2D-associated transcript 1 regulates the expression of NF-κB pathway proteins through calmodulin-dependent protein kinase, therefore affecting the activity of neurons (Xu et al., 2016).

In addition to glial and neuronal cells, lncRNAs also take part in the regulation of vascular endothelial cell inflammation. Zhang et al. (2017) showed that, in an oxygen- and glucose-deprivation of vascular endothelial cell model, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) directly bound to e-selectin mRNA and inhibited inflammation to decrease the infarct volume. All of these findings suggested that the lncRNA MALAT1 in vascular endothelial cells is involved in the regulation of neuroinflammation (Zhang et al., 2017).

Additionally, with the release of brain inflammatory factors and chemokines after AIS, peripheral immune cells are recruited to the infarct area to participate in neuroinflammation (Kim et al., 2016). In macrophages derived from monocytes, knockdown of the lncRNA Maclpil promoted the inflammatory polarization of macrophages by inhibiting the expression of lymphocyte cytoplasmic protein-1 (Wang et al., 2020b), which suggested that lncRNAs play important roles in the recruitment of peripheral immune cells to the brain, especially T cells, B cells, and natural killer cells. However, there have been few studies reported on this aspect, thus further in-depth research would be conducive to systematically elucidating the mechanisms of neuroinflammation after AIS.

LncRNAs and apoptosis

After AIS, neurons in both infarction area and ischemic penumbra die over time. Apoptosis is the main form of neuron death after AIS and may provide new intervention targets for saving neurons and alleviating ischemic injury (Broughton et al., 2009; Zheng et al., 2023).

The expression of anti-apoptotic proteins in endogenous apoptosis pathways is regulated by lncRNAs. For example, lncRNA FOXD3-AS1 promotes neuron apoptosis by increasing the expression of the pro-apoptotic protein BCL2L13 by binding to miR-765 (Lu et al., 2020b). Similarly, lncRNA SNHG6 reduces the binding of miR-181C-5p to the 3′-UTR of Bim mRNA via the classical ceRNA network (Zhang et al., 2019). Moreover, the activity of caspase 3 protein is enhanced by lncRNA SNHG6 through the miR-181C-5p/Bim pathway (Zhang et al., 2019). A recent study found that knockdown of lncRNA CEBPA-AS1 reduced the expression of endogenous apoptotic proteins Bax, Bcl-2, and caspase 9 through the miR-455/G protein-coupled estrogen receptor regulatory network in a neuronal oxygen- and glucose-deprivation model, as well as the expression of exogenous apoptosis-related proteins caspase 8 and caspase 3 (Peng et al., 2022). More studies on the function of lncRNAs in exogenous apoptosis pathways, especially the regulation of death receptors, are needed. In addition, lncRNA MEG3 has been shown to play a regulatory role in neuronal apoptosis through Wnt/β-catenin and miR-21/programmed cell death 4 regulatory networks, respectively (Yan et al., 2017). While lncRNA MEG3 has been found to directly bind with p53 DNA, enhancing p53-mediated trans-activation and promoting neuronal apoptosis (Yan et al., 2016).

LncRNAs have been shown to involved in the regulation of autophagy in addition to apoptosis. LncRNA MALAT1, one of the lncRNAs most increased in expression after ischemia and hypoxia, was found to not only directly bind to the mRNA of pro-apoptotic protein Bim to inhibit the apoptosis of vascular endothelial cells but also participate in autophagy regulation through various ceRNA mode pathways (Zhang et al., 2017). In neurons, lncRNA MALAT1 was found to promote neuronal autophagy through the miR-30a/beclin1 pathway (Guo et al., 2017). In brain microvascular endothelial cells, lncRNA MALAT1 promotes autophagy through the miR-26b/unc-51 like kinase 2 pathway (Li et al., 2017). A recent study of umbilical vein endothelial cells *in vitro* showed that lncRNA MALAT1 promotes autophagy through miR-19b-3p/hypoxia inducible factor-1α (Liu et al., 2020). Similarly, lncRNA KCNQ1OT1, MEG3, and H19 also participate in

the regulation of autophagy through the ceRNA network (Wang et al., 2017a; Yu et al., 2019; Luo et al., 2020).

In conclusion, lncRNAs play vital roles in the regulation of cell apoptosis and autophagy, with an emphasis on the ceRNA regulatory network mechanism. Further research is needed focusing on whether lncRNAs are involved in other cell death pathways, such as necrotizing apoptosis and iron death, or if they can act through pathways other than the ceRNA mode.

LncRNAs and BBB damage

Glial cells and vascular endothelial cells constitute the main structural and supporting components of BBB, and any factor that can affect BBB structure and function may cause damage (Michinaga and Koyama, 2015; Yue and Hoi, 2023). Qi et al. (2017) and Zhang et al. (2017) have shown that lncRNAs are involved in the inflammation and apoptosis of glia and vascular endothelial cells. For example, lncRNA SNHG14 promotes microglia activation, and lncRNA MALAT1 inhibits the inflammation and apoptosis of cerebrovascular endothelial cells (Qi et al., 2017; Zhang et al., 2017). Therefore, lncRNAs can directly or indirectly participate in the BBB injury process after AIS by regulating inflammatory activation or the apoptosis of vascular endothelial cells.

In addition, lncRNA MALAT1 was found to affect the expression of the tight-junction proteins claudin-5, occludin, and ZO-1 through the cAMP-responsive element-binding protein/peroxisome proliferator-activated receptor-γ coactivator-1α/peroxisome proliferator-activated receptor-γ pathway to maintain the tightness of the BBB structure (Ruan et al., 2019). LncRNA MALAT1 increases AQP4 protein expression through the miR-145/AQP4 pathway in astrocytes, leading to an increase in BBB permeability and aggravating ischemic injury (Wang et al., 2020a). Other studies have shown that lncRNA MALAT1 and lncRNA SNHG15 increase the expression of MMPs in ischemic tissues or cells, thus affecting the integrity of the BBB (Shi et al., 2019; Wen et al., 2020).

The above studies indicated that lncRNAs participate in the regulation of BBB injury after AIS through multiple pathways. It is worth noting that one lncRNA can be involved in multiple pathological processes of AIS. For example, the lncRNA MALAT1 can be involved in inflammation, apoptosis, autophagy, and BBB injury through multiple pathways (Ahmad et al., 2023). In addition, one pathological process can be regulated by multiple lncRNAs. Studying these regulatory networks is conducive to understanding the rapid response of brain tissue after ischemia and to the search for therapeutic intervention targets.

LncRNAs and peripheral biomarkers

LncRNAs as predictive biomarkers for AIS

Previous studies have demonstrated that lncRNAs play important roles in the risk factors for stroke, such as atrial fibrillation and atherosclerosis (Babapoor-Farrokhran et al., 2020; Fasolo et al., 2021). Reportedly, the expression of lncRNA H19, one of the earliest discovered lncRNAs, was elevated in the PBMCs of patients with atherosclerosis and may be a biomarker for this condition (Bitarafan et al., 2019; Huang et al., 2019). Current studies may focus on finding key lncRNAs from the perspective of a single risk factor for stroke. In the future, lncRNAs related to multiple risk factors and combinations of different lncRNAs may be vital for accurate AIS prediction.

LncRNA gene–related SNPs are closely related to AIS. Zheng et al. (2018) revealed that the expression of the lncRNA growth arrest specific 5 (GAS5) was significantly increased in the peripheral blood of AIS patients. Moreover, loss of an allele of RS145204276 was closely correlated to an increased risk of AIS, the mechanisms of which may be the enhanced transcriptional activity of lncRNA GAS5 and increased expression of lncRNA GAS5. Among the northern Chinese population, polymorphisms of the lncRNA H19 rs217727 gene were found to be associated with the risk of AIS, and compared with the CC+CT genotype, the TT genotype's risk of small-vessel ischemic stroke was 1.941 times higher (Zhu et al., 2018b). Although lncRNA H19 gene polymorphism is not considered to be associated with AIS risk, among the southern Chinese population, the rs4929984 genotype was associated with systolic blood pressure in AIS patients, and rs217727 was significantly related to coagulation function and cysteine metabolism. These studies suggested that SNPs of lncRNAs genes are closely related to known AIS risk factors and can be used as one of the indicators to predict the occurrence of AIS.

LncRNAs as diagnostic biomarkers for AIS

LncRNAs are highly expressed in peripheral blood as long-strand RNA. Dykstra-Aiello et al. (2016) first discovered that lncRNAs in the peripheral blood of AIS patients have unique expression characteristics. A total of 299 lncRNAs were differentially expressed in male and 96 in female AIS patients . The expression of lncRNA H19 was found to be increased in the plasma of AIS patients and had a diagnostic sensitivity of 80.6%, specificity of 92.0%, and an AUC of 0.910 for diagnosing AIS (Wang et al., 2017b). Deng et al. (2018) detected three stably differentially expressed lncRNAs (linc-DHFRL1-4, SNHG15, and linc-FAM98A-3) in AIS patients that had a diagnostic sensitivity of 85.7%, specificity of 78.2%, and an AUC of 0.879, superior to the diagnostic efficacy of brain-derived neurotrophic factor (AUC = 0.789) and neuron-specific enolase (AUC = 0.709). These three lncRNAs have also been found to differentiate effectively between AIS and transient ischemic attack, with a diagnostic sensitivity of 85.7%, specificity of 63.6%, and an AUC of 0.847. Moreover, linc-DHFRL1-4 can be used to distinguish transient ischemic attack patients from the normal population (Deng et al., 2018).

The above studies indicated that lncRNAs as biomarkers have a promising application in AIS diagnosis. However, their expression characteristics in the AIS hyperacute phase (less than 4.5 hours) and their diagnostic efficacy compared to computed tomography and magnetic resonance imaging remain to be further defined.

LncRNAs as prognostic biomarkers for AIS

LncRNAs not only play a broad range of regulatory roles in the pathological processes of AIS but also show characteristic expression profiles in the peripheral circulation over time and are recognized as potential prognostic biomarkers for AIS. Zhu et al. (2018a) showed that the expression of lncRNA MIAT was significantly increased in the peripheral leukocytes of AIS patients and is related to poor prognosis and mortality at 3 months, which indicated that it could be used as a diagnostic biomarker for AIS. A recent study found that the lncRNA MEG3 RS4081134 AA genotype is significantly correlated with AIS recurrence and is an independent risk factor for AIS recurrence (Zhu et al., 2021). In addition, SNPs of the lncRNA GAS6-AS1 gene (rs1803628, rs9604573, and rs7140110) were associated with an increased risk of hemorrhagic transformation in AIS patients after intravenous thrombolysis (Guo et al., 2021).

These studies suggested that AIS biomarkers can be found by detecting the expression levels of lncRNAs or exploring lncRNA genotypes and gene regulation. Elucidating the molecular mechanism of these lncRNAs in the brain and peripheral of AIS

will aid in identifying AIS biomarkers with diagnostic specificity. The role of lncRNAs in AIS is summarized in **Figure 3**.

Circular RNAs and Acute Ischemic Stroke

Modes of action of circRNAs

CircRNAs are single-chain ncRNAs with covalently closed-loop structures formed by back-splicing precursor mRNAs (Chen, 2016). Currently, circRNAs can be divided into three types according to the position of circRNAs formed by precursor mRNA cyclization: exonic circRNAs, circular intronic RNAs, and exon-intron circRNAs (Han et al., 2018a). The modes of action of circRNAs can be divided into four categories (Chen, 2016; Han et al., 2018a): (1) CircRNAs that regulate gene transcription or alternative splicing, binding to small nuclear ribonucleoprotein or RNA polymerase II to regulate gene transcription, and compete with parental genes for splicing. (2) CircRNAs that interact with RNA-binding proteins (RBPs) to affect the localization of RBPs. These circRNAs can competitively bind to RBPs in place of mRNAs, thus affecting the translation of mRNAs. They can also bind to RBPs, affecting their function. (3) In ceRNA networks, circRNAs can function as a "sponge" for miRNAs, binding to miRNAs at matched sequences and preventing them from binding to the 3′-UTR regions of target mRNAs. (4) Some circRNAs with encoding functions can be translated into peptides by ribosomes.

CircRNAs and brain pathology of AIS

CircRNAs and neuroinflammation

The activation of glial cells and infiltration of peripheral immune cells are vital pathologic processes of central inflammation in the acute stage after ischemia. Yang et al. (2020) found that overexpression of circRNA SCMH1 (circSCMH1) by exosomes modified with rabies virus glycoprotein inhibited the activation of microglia; reduced the expression of pro-inflammatory factors, such as TNF-α, IL-1β, and IL-6, in the infarct area; and restrained the infiltration of the central system by peripheral lymphocytes, B cells, and mononuclear macrophages, thus alleviating the central inflammatory response and improving prognosis. In addition, evidence gathered through sequencing and bioinformatics analyses indicated circSCMH1 may play a neuroprotective role by binding with methyl-CpG binding protein 2. It is worth mentioning that this research not only explored the role of circSCMH1 in rodent mice but also verified that the overexpression of circSCMH1 promotes neurological recovery after AIS in rhesus monkeys (Yang et al., 2020). Additionally, in the acute phase of AIS, knocking down the expression of peripheral circRNA CDC14A (circCDC14A) led to an increase in the proportion of N2 neutrophils infiltrating the brain and inhibited the activation of astrocytes in the ischemic penumbra, thereby alleviating brain injury (Zuo et al., 2021).

Regarding the inflammatory regulation of neurons after AIS, a recent study has shown that the binding of the circRNA circHECTD1 with miR-133b competitively reduced the binding of miR-133b to the 3′-UTR regions of TNF-receptor-associated factor 3 (TRAF3) and increased the expression of TRAF3, thereby activating the NF-κB pathway and aggravating neuroinflammatory injury (Dai et al., 2021). The studies described above all indicated that circRNAs are involved in the regulation of neuroinflammation after AIS.

CircRNAs and apoptosis

After ischemia, the severity of neuron injury is most closely related to prognosis. Current studies found that neuronal injury and apoptosis are regulated through miRNA/mRNA pathways. Wu et al. (2019) found that knockdown of circRNA TLK1 (circTLK1) reduced the expression of the apoptotic proteins Bax and pro-caspase-3 and increased the expression of the anti-apoptotic protein Bcl-XL in the brain, as well as increasing the number of neurons and improving neural function scores. Further mechanistic investigations showed that circTLK1 exacerbated neuron injury through a miR-335-3p/2,3,7,8-tetrachlorodibenzo-p-dioxin9-inducible polymerase pathway (Wu et al., 2019). Moreover, circ_016719 and circ_0072309 were found to participate in the regulation of apoptosis through miR-29c/Rac-MAPK kinase 6 and miR-100/mammalian target of rapamycin pathways, respectively (Tang et al., 2020; Zhao et al., 2020).

CircRNAs have additionally been proven to be involved in autophagy regulation. CircHECTD1 was found to regulate the expression of the autophagy-related protein microtubule-associated protein 1 light chain 3beta in astrocytes through miR-100/TCDD inducible poly[ADP-ribose] polymerase, thereby activating astrocytes to aggravate ischemic injury (Han et al., 2018b). Furthermore, the circRNA circSHOC2 contained in exosomes derived from astrocytes was also found to regulate the expression of beclin-1, Bax, and Bcl-2 in neurons through the miR-7670-3p/silent-mating-type information regulation 1 pathway, thereby reducing neuronal injury (Chen et al., 2020). These studies suggested that the mechanisms involved in the regulation of autophagy interact with apoptosis, and a single molecule, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin9-inducible poly polymerase and circSHOC2, can regulate both processes simultaneously.

CircRNAs and BBB damage

Inflammatory mediators in the brain induced by ischemia can lead to changes in the gene expression of vascular endothelial cells, resulting in endothelial-mesenchymal transition, which causes structural and functional disorders of the BBB (Potenta et al., 2008). Bai et al. (2018) found that the circHECTD1 pathway can increase the expression of tight-junction proteins claudin-5, occludin, and ZO-1 in vascular endothelial cells and reduce the expression of collagen I and collagen III in mesenchymal cells, thus inhibiting endothelial–mesenchymal transition and maintaining the integrity of the BBB. Another study showed that circFoxO3 reduces ischemia-induced BBB injury by activating the autophagy of vascular endothelial cells, thereby increasing the expression of the BBB junction proteins claudin-5 and ZO-1, as well as major facilitator superfamily domain containing 2A, a BBB lipid transporter protein (Yang et al., 2022). Recently, Li et al. (2023a) demonstrated that circSCMH1 in endothelial cells enhances vascular repair via obesity-associated protein (FTO)-regulated N6-methyladenosine (m6A) methylation after a stroke. Hence, circRNAs participate in the regulation of BBB injury after ischemia through multiple molecular mechanisms.

CircRNAs and peripheral biomarkers

CircRNAs as predictive biomarkers for AIS

Considering rupture of the carotid plaque is part of AIS etiology, Wen et al. (2021) found higher circRNA-0006896 expression levels in the serum exosomes of patients with unstable fragile plaques that were positively correlated with low-density lipoprotein cholesterol. Through further molecular mechanistic investigations, they found that the circRNA-0006896/miR-1264/DNA methyltransferase 1 regulatory network in endothelial cells plays an important role in maintaining plaque stability (Wen et al., 2021). Moreover, circ_0030042 was also found to bind with endogenous eukaryotic initiation factor 4A-III through "sponge" adsorption, inhibiting the endothelial autophagy induced by oxidized low-density lipoprotein cholesterol and stabilizing

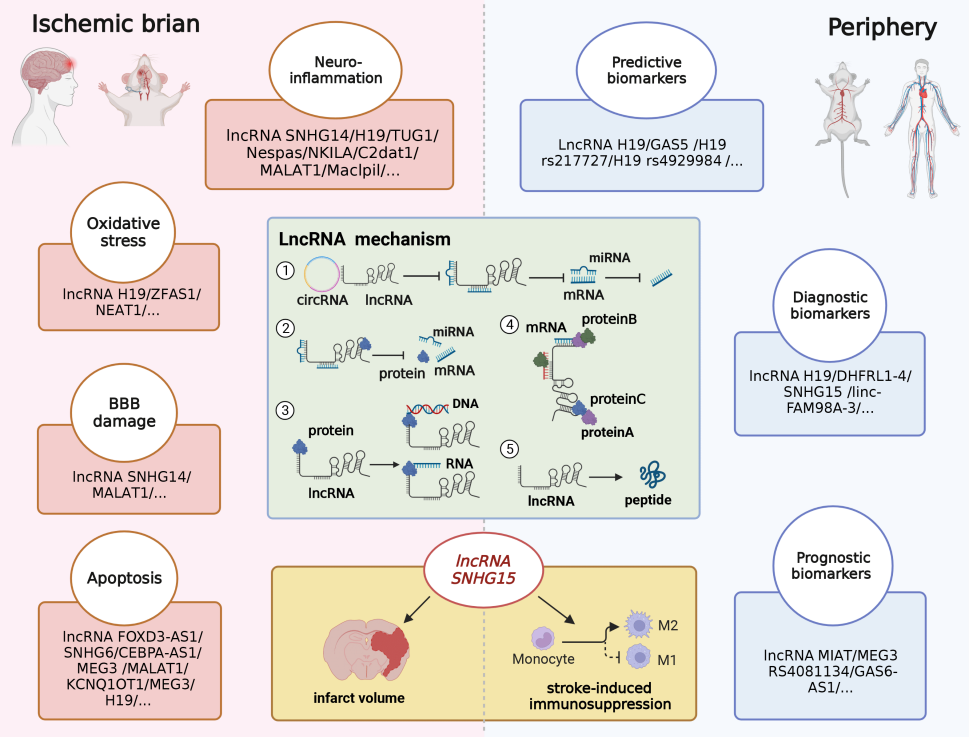

Figure 3 | Long noncoding RNAs and acute ischemic stroke.
LncRNAs regulate neuroinflammation, oxidative stress, BBB damage, and apoptosis in the AIS brain. Differentially expressed lncRNAs can serve as predictive, diagnostic, and prognostic biomarkers for AIS. The lncRNA SNHG15, especially, is upregulated in monocytes after AIS, is involved in stroke-induced immunosuppression by promoting M2 macrophage polarization, and increases the infarct volume. The modes of action of lncRNAs are (1) the classic competing endogenous RNA model; (2) lncRNAs acting as decoys and molecular blockers; (3) lncRNAs acting as molecular chaperons that bind with proteins and guide them to specific DNA or RNA sequences; (3) lncRNAs playing the role of scaffolding; (5) lncRNAs that encode peptides. Created with BioRender.com. AIS: Acute ischemic stroke; BBB: blood–brain barrier damage; lnRNAs: linear long non-coding RNAs.

atherosclerotic plaques (Yu et al., 2021). This provided hints that circRNAs may be used early screening tools for unstable plaques. In addition, circRNAs are involved in other risk factors related to AIS, such as deep vein thrombosis (Lou et al., 2022).

CircRNAs as diagnostic biomarkers for AIS

CircRNAs are adequately expressed in the peripheral blood. A recent study found that four upregulated and four downregulated circRNAs in the PBMCs of AIS patients might be used as biomarkers for the diagnosis of AIS (Dong et al., 2020). Zuo et al. (2020b) found for the first time that circCDC14A, circRNA PDS5B (circPDS5B), and circRNA FUNDC1 (circFUNDC1) were stably and differentially expressed in the plasma of AIS patients, and the specificity of the three circRNAs in diagnosing AIS was 91%, the sensitivity was 71.5%, and the AUC was 0.875. In another study, circRNAs in peripheral blood were sequenced and verified in eight atherosclerotic, 14 cardiogenic, and 8 unexplained AIS patients, and the results showed that the expression of circRNA_102488 was significantly different between atherosclerotic and cardiogenic AIS patients (Ostolaza et al., 2020). Similarly, circ_0043837 and circ_0001801 in plasma exosomes were also found to be potential diagnostic markers for atherosclerotic AIS patients, as well as predictive indicators of plaque rupture (Xiao et al., 2021). These studies suggest that circRNAs have the potential to be the diagnostic and etiological biomarkers for AIS.

CircRNAs as prognostic biomarkers for AIS

CircRNAs are formed by conserved properties and sequences and have a closed-loop structure, which is more stable than a linear one; thus circRNAs have certain advantages as prognostic markers of AIS (Chen, 2016). The AUCs of circCDC14A, circPDS5B, and circFUNDC1 in the plasma of AIS patients for predicting the prognosis of the mRS score at 3 months was 0.960. Further analysis demonstrated that the expression of circFUNDC1 was significantly increased in AIS patients with stroke-associated infections, with a specificity of 61.90%, sensitivity of 69.23%, and an AUC of 0.6612 in predicting stroke-associated infection (Zuo et al., 2020a, b). Peng et al. (2019) found that expression levels of circHECTD1 in PBMCs were positively correlated with the National Institutes of Health Stroke Scale scores of AIS patients, and the specificity, sensitivity, and AUC for predicting stroke recurrence were 54.5%, 71.4%, and 0.694. In addition, although the circSTAT3 rs2293152 GG genotype related to inflammation was not associated with recurrent stroke, it was strongly associated with poor outcomes at 3 months (Liu et al., 2021).

Generally, the current research suggests that circRNAs have certain potential as peripheral biomarkers for AIS. However, compared to the total number of circRNAs, the number that have been reported to be involved in AIS is still relatively limited, and the exploration of molecular mechanisms is still in the preliminary stage. Further research on the pathogenesis and pathological processes that involve circRNAs is needed to find specific diagnostic and therapeutic targets for AIS. The roles of circRNAs in AIS are summarized in **Figure 4**.

Non-Coding RNAs as Therapeutic Targets in Acute Ischemic Stroke

Many ncRNAs, including miRNAs, lncRNAs, and circRNAs, have emerged as key genetic or epigenetic regulators in transcription and translation. Based on the ongoing demonstrations of how ncRNAs participate in stroke pathogenesis, there is an increasing need to develop therapeutic ncRNA targets in AIS.

Recently, it was revealed that miR-188-5p silencing or miR-212-5p agomir mimic significantly rescued infarct volume and restored motor function in rats (Hou et al., 2023; Li et al., 2023b). Additionally, lncRNA X inactive specific transcript (lncRNA XIST) has been found to regulate the expression of proangiogenic and anti-inflammation factors by targeting miR-92a, and silencing lncRNA XIST impaired angiogenesis and exacerbated cerebral vascular injury in mice (Wang et al., 2021). CircRNAs have also been found to influence neural neurogenesis and improve functional recovery after stroke. Umbilical cord

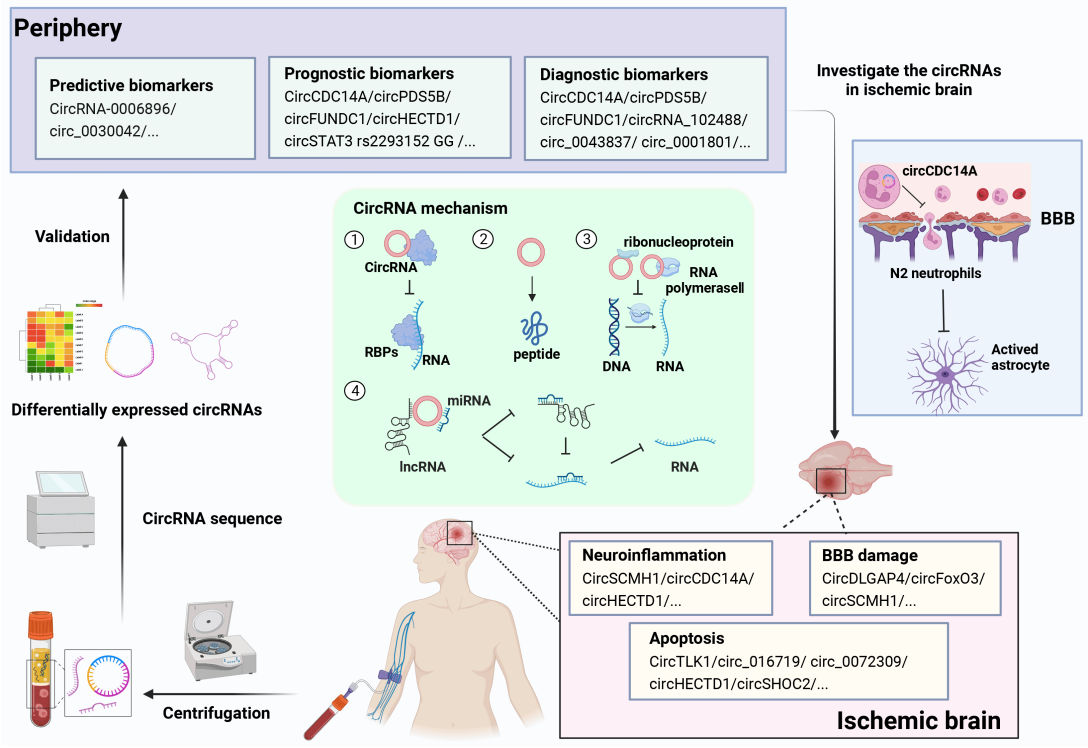

Figure 4 | Circular RNAs and acute ischemic stroke.
The venous blood of AIS patients and healthy controls was collected and centrifuged to obtain RNA from monocytes, neutrophils, red blood cells, platelets, lymphocytes, plasma, and serum. Then the RNA samples were sequenced to find the differentially expressed circRNAs. These circRNAs were validated with larger samples to evaluate their efficacy as predictive, diagnostic, and prognostic biomarkers for AIS. Furthermore, the expression levels of circRNAs were investigated in the ischemic brain to explore the molecular mechanisms of their involvement in neuroinflammation, BBB damage, and apoptosis. In particular, circCDC14A peripherally modulates N2 neutrophil polarization to active astrocytes in the brain. The modes of action of circRNAs: (1) binding to RBPs with mRNAs; (2) translation into peptides; (2) binding to small nuclear ribonucleoproteins or RNA polymerase II to regulate gene transcription; and (4) functioning as miRNA sponges, binding to miRNAs at matched sequences, and preventing miRNAs from binding to the 3′-UTR regions of target mRNAs. Created with BioRender.com. AIS: Acute ischemic stroke; BBB: blood–brain barrier; circRNAs: circular RNAs; RBPs: RNA-binding proteins; UTR: untranslated region.

mesenchymal stem cell–derived exosomes or engineered rabies virus glycoprotein–modified extracellular vesicles are considered effective delivery systems for ischemic stroke drugs (Yang et al., 2020; Hong et al., 2023). By targeting miR-494, umbilical cord mesenchymal stem cell–derived exosomal circRNA BBS2 activates ferroptosis inhibitory gene solute carrier family 7 member 11 signaling to inhibit ferroptosis and relieve ischemic stroke (Hong et al., 2023). Engineered rabies virus glycoprotein-circSCMH1 extracellular vesicles were shown to regulate vascular repair and functional recovery following stroke through the FTO-dependent m6A modification of lipid phosphate phosphatase 3 (Yang et al., 2020; Li et al., 2023a). The protective effects have been demonstrated in nonhuman primate ischemic stroke models, taking circRNAs a step closer to being realized as treatment methods for clinical use.

These studies suggested ncRNAs have a promising therapeutic utility in stroke management, and further research is needed to validate their potential clinical applications.

Conclusion and Future Insights

As transcriptional products, ncRNAs can respond rapidly to changes in the early stages of disease and can be targets for early diagnosis and intervention. In this review, we summarized the progress of research on the potential clinical application of ncRNAs in AIS, from the brain to the periphery. In the central nervous system, the regulatory mechanisms of ncRNAs that effect the pathological processes of AIS and the feasibility of targeting ncRNAs for AIS treatment are two major directions of current research. The expression levels of ncRNAs and the feasibility of using ncRNAs as predictive, diagnostic, and prognostic AIS biomarkers for the peripheral nervous system are new research orientations.

AIS is known to be a systemic disease, and immune responses occur both in the brain and in the peripheral nervous system (Wu et al., 2022). Components of the nervous and immune systems, including the hypothalamic pituitary axis, sympathetic adrenal medullary axis, vagus nerve, and blood stream, are considered to be bridges transducing the bidirectional communication between the brain and peripheral nervous system (Meisel et al., 2005). AIS induces immune deficiency syndrome and the breakdown of immunological barriers in the periphery, promoting the infection and dysfunction of peripheral organs. Ultimately, signals are fed back to the brain to form a vicious circle that worsens the outcomes (Meisel et al., 2005). Recent research has also focused on the functions and molecular mechanisms of ncRNAs in peripheral immune cells and stroke-induced immunodepression (Li et al., 2020; Sun et al., 2022). Moreover, it has been found that the distinct expression of ncRNAs can affect the infiltration of the brain by peripheral immune cells (Zuo et al., 2021). Many ncRNAs have been found to be differentially expressed in peripheral blood after AIS by sequencing, but the function of these ncRNAs in the peripheral immune system, especially in relation to post-stroke pneumonia, and the molecular mechanisms involved in the crosstalk between brain and periphery remain to be illustrated.

A limitation of this review was that the studies collated were mainly fundamental research, and there are limited randomized controlled trial data. Moreover, our study only investigated the role of three ncRNAs (miRNAs, lncRNAs, and circRNAs) in AIS, and information on other ncRNAs, such as small nuclear RNAs and small nucleolar RNAs, needs to be further summarized. To date, no ncRNA treatments have been developed for use in clinical practice, the possible reasons for which include the different inclusion and exclusion criteria of recruitment, differences in study time points in the acute stage, and the various samples employed (Tiedt and Dichgans, 2018). However, the overexpression or knockdown of ncRNAs have been shown to protect against brain ischemic injury and improve outcomes in animal models (Ni et al., 2015; Yang et al., 2020; Sun et al., 2022). Moreover, ncRNA delivery vectors, such as exosomes and lentivirus, have been demonstrated to successfully carry ncRNAs to the target ischemic area (Wu et al., 2019; Yang et al., 2020). With the further development of materials technology in the future, ncRNA-based drug-delivery systems will become safer, more accurate, and more effectively able to cross the BBB to target specific tissues or cells. Hence, ncRNAs offer a new toolset for the prediction and diagnosis of AIS, and their mechanisms of action and roles in AIS are worthy of further exploration.

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