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REVIEW PAPER

Long Noncoding RNAs in the Pathophysiology of Ischemic Stroke

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Abstract
Ischemic stroke is an acute brain injury with high mortality and disability rates worldwide. The pathophysiological effects of ischemic stroke are driven by a multitude of complex molecular and cellular interactions that ultimately result in brain damage and neurological dysfunction. The Human Genome Project revealed that the vast majority of the human genome (and mammalian genome in general) is transcribed into noncoding RNAs. These RNAs have several important roles in the molecular biology of the cell. Of these, the long noncoding RNAs are gaining particular importance in stroke biology. High-throughput analysis of gene expression using methodologies such as RNA-seq and microarrays have identified a number of aberrantly expressed lncRNAs in the post-stroke brain and blood in experimental models as well as in clinical samples. These expression changes exhibited distinct temporal and cell-type-dependent patterns. Many of these lncRNAs were shown to modulate molecular pathways that resulted in deleterious as well as neuroprotective outcomes in the post-stroke brain. In this review, we consolidate the latest data from the literature that elucidate the roles and functions of lncRNAs in ischemic stroke. We also summarize clinical studies identifying differential lncRNA expression changes between stroke patients and healthy individuals, and genetic variations in lncRNA loci that are correlated with an increased risk of stroke development.

Keywords Ischemic stroke · lncRNA · Gene expression · Brain · Preclinical · Clinical

Introduction to lncRNAs

Following the completion of the Human Genome Project, it became evident that our genome undergoes pervasive transcription. Only a minor portion (2.94%) of the genome encodes the protein-coding transcriptome (Djebali et al. 2012), whereas the vast majority (75%) is transcribed into noncoding RNAs (ncRNAs) (ENCODE Project Consortium 2012; Ponjavic et al. 2007). This widespread noncoding transcription produces a number of different subtypes of ncRNAs such as microRNA (miRNA), small nucleolar RNA (snoRNA), piwi-interacting RNA (piRNA), long noncoding RNA (lncRNA), and circular RNA (circRNA), to name a few. Of these, the lncRNAs are one of the most widely studied subtypes in stroke.

LncRNAs are broadly classified as noncoding RNAs that are > 200 nucleotides. Although they do not encode proteins, many lncRNAs share features with mRNAs such as transcription by RNA polymerase II, 5′ capping, splicing, and polyadenylation (Cabili et al. 2011; Carninci et al. 2005; Guttman et al. 2009, 2010; Shiekhattar et al. 2012). LncRNAs originate from a wide variety of genomic locations, such as intergenic regions, introns, enhancers, promoters, or even from protein-coding gene loci either with a partial overlap with protein-coding exons on the same strand or from the antisense strand (Ma et al. 2013). The ability of lncRNAs to interact with other RNAs, DNA, and proteins imparts a broad range of functional capabilities such as regulation of chromatin state, scaffolding of regulatory proteins, organization of nuclear architecture, modulation of splicing, and competitive binding and sequestration of miRNAs (also known as 'sponging') (Beltran et al. 2008; Hutchinson et al. 2007; Kopp and Mendell 2018; Rinn et al. 2007; Wang et al. 2008).

In mammals, lncRNAs are most highly expressed in the brain (Derrien et al. 2012; Mercer et al. 2010). They have been implicated in the pathophysiology of a number of neurodevelopmental disorders as well as injuries such as Prader-Willi Syndrome (Powell et al. 2013), Alzheimer's disease (Massone et al. 2011), amyotrophic lateral sclerosis

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(Nishimoto et al. 2013), traumatic brain injury (Zhong et al. 2016), and stroke (Bhattarai et al. 2017; Dharap et al. 2012; Zhang et al. 2016). Here we review the latest discoveries on lncRNA expression, functions, and mechanisms in the post-stroke brain.

LncRNA Expression in Ischemic Stroke

LncRNA expression in the post-stroke brain has been studied by several groups using high-throughput methodologies such as microarrays (Dharap et al. 2012; Dykstra-Aiello et al. 2016; Liu et al. 2018) and deep sequencing (Bhattarai et al. 2017; Kim et al. 2018). The altered expression of lncRNAs in the adult cortex during ischemic stroke was first reported in spontaneously hypertensive rats undergoing transient focal ischemia (Dharap et al. 2012). Using microarrays, the expression levels of 8314 lncRNAs were evaluated, of which 443 lncRNAs were differentially altered from 3 to 12 h of reperfusion as compared to sham controls. Several of these stroke-responsive lncRNAs were found to be directly associated with the chromatin-modifying proteins Sin3a and coREST, which are co-repressors of the master neuronal silencer RE-1 Silencing Transcription Factor (REST) (Dharap et al. 2013). Because REST is a known mediator of neuronal cell death in ischemia (Noh et al. 2012), these findings suggested a role for the lncRNAs in REST function. A subsequent study investigating one such lncRNA that was commonly bound to Sin3a and coREST confirmed that it participated in post-stroke neuronal death by modulating REST target genes (Mehta et al. 2015). These early studies provided some of the first evidence of the functional significance of lncRNAs in stroke.

More recently, we reported the first comprehensive genome-wide evaluation of post-stroke lncRNA and mRNA expression in the mouse cortex using deep sequencing (Bhattarai et al. 2017, 2019). Because a single gene can yield multiple transcript isoforms with differing expression patterns and functions, it is important to study gene expression at the isoform level in order to identify the specific isoforms that are differentially altered in response to stroke. Using such isoform-level analyses, we identified 259 lncRNA isoforms at 6 h, 378 lncRNA isoforms at 12 h, and 217 lncRNA isoforms at 24 h of reperfusion that were differentially altered as compared to sham controls. Of these, we found that 213, 322, and 171 isoforms at 6, 12, and 24 h of reperfusion, respectively, were previously unannotated. These novel lncRNAs comprised > 70% of the total altered lncRNAs in our study, thereby marking the discovery of several new stroke-responsive noncoding loci in the adult cerebral cortex. Another recent study using microarray identified 255 lncRNAs that were differentially expressed in the mouse cortex at 48 h of reperfusion as compared to sham controls (Liu et al. 2018). Bioinformatics analysis incorporating the altered mRNAs and lncRNAs in this study suggested possible gene regulatory relationships between the stroke-responsive lncRNAs and mRNAs.

Studies in cerebral blood vessels and circulating blood after stroke have shown that lncRNA expression is also altered in the cerebral microvasculature. Evaluation of gene expression using RNA-seq in mouse primary brain microvascular endothelial cells (BMECs) after oxygen–glucose deprivation (OGD) identified 362 differentially altered lncRNAs as compared to normoxic controls (Zhang et al. 2016). Some of these findings were corroborated in vivo by conducting real-time PCR using microvessels isolated from the mouse cerebral cortex following transient middle cerebral artery occlusion (MCAO) (Zhang et al. 2016). Subsequent studies in endothelial cells in vitro and in vivo showed that several of the ischemia-responsive lncRNAs participate in important post-ischemic processes such as inflammation, cell survival, and angiogenesis (Long et al. 2018; Zhang et al. 2017a; Zhou et al. 2016). In human, multiple studies examining lncRNA expression in the circulating blood of stroke patients versus healthy controls revealed that lncRNAs are specifically and robustly altered in blood cells and plasma, and show multiple differentiating patterns based on sex and reperfusion time (Dykstra-Aiello et al. 2016; Zhu et al. 2019).

Together, these studies demonstrate that ischemic stroke induces widespread changes in the expression of lncRNAs in the brain and blood with distinct expression characteristics that may underlie progression of the ischemic pathophysiology. Current investigations in the field are focused on understanding the roles, mechanisms, and functional significance of these lncRNAs in mediating the post-stroke pathophysiology. In the following sections, we present some of the lncRNAs that have been the subject of multiple investigations in stroke and review the studies that have illuminated their roles and mechanisms in post-stroke inflammation, cell death, brain damage, and neurological dysfunction. A summary of these lncRNAs and their roles in the various pathophysiological processes during stroke is presented in Table 1.

Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1)

MALAT1 is an abundantly expressed and highly conserved lncRNA in mammals. It was originally implicated in tumorigenesis and metastasis in various types of cancers, but it also has important roles in ischemia. Recent studies showed that MALAT1 expression was robustly induced in endothelial cells undergoing OGD in vitro, and this response was recapitulated in the adult cortical microvasculature during transient focal ischemia (Chen et al. 2018a; Zhang et al. 2016). In order to understand

Table 1 Stroke-responsive lncRNAs and their roles in pathophysiological processes following stroke

LncRNA	Inflammation	Apoptosis/cell death	Autophagy	Angiogenesis	References
MALAT1	±	±	±	+	Guo et al. (2017), Li et al. (2017), Ren et al. (2019), Ruan et al. (2019), Wang et al. (2019a, b), Yang et al. (2018a), Zhang et al. (2017b, Zhang et al. 2018)
MEG3	Unknown	+	Unknown	–	Liu et al. (2016, 2017), Yan et al. (2016, 2017)
SNHG12	–	–	Unknown	+	Long et al. (2018), Yin et al. (2019), Zhao et al. (2018)
H19	+	+	+	Unknown	Wang et al. (2017a, b)
ANRIL	+	Unknown	Unknown	+	Zhang et al. (2017), Zhou et al. (2016)
FosDT	Unknown	+	Unknown	Unknown	Mehta et al. (2015)

"+" promotes; "–" inhibits; "±" promotes or inhibits depending on context

ANRIL Antisense Noncoding RNA in the INK4 Locus, *FosDT* Fos downstream transcript, *MEG3* Maternally expressed gene, *MALAT1* Metastasis-associated lung adenocarcinoma transcript 1, *SNHG12* Small nucleolar RNA host gene 12

the significance of MALAT1 in ischemia, several studies used gene perturbation experiments to alter MALAT1 expression in vivo as well as in vitro and investigate its effects on post-ischemic outcomes. MALAT1 knockout mice exhibited larger infarct volumes and severe neurological deficits in response to transient focal ischemia as compared to wild-type controls (Zhang et al. 2017b). Knockdown of MALAT1 expression in BMECs in vitro increased OGD-induced cell death and caspase-3 activity. Further, the loss of MALAT1 resulted in increased expression of pro-inflammatory mediators such as IL-6, MCP-1, E-selectin, and the pro-apoptotic protein Bim in response to the ischemic injury in vitro as well as in vivo, indicating that MALAT1 has anti-inflammatory and anti-apoptotic functions in the brain microvasculature. Consistent with these observations, studies from several other groups have demonstrated roles for MALAT1 in promoting endothelial cell survival, vascular integrity, and angiogenesis in stroke (Li et al. 2017; Ren et al. 2019; Ruan et al. 2019; Wang et al. 2019a, b; Yang et al. 2018a).

Contrary to these findings supporting a beneficial role for MALAT1 in brain endothelial cells, a recent study showed that high expression of MALAT1 resulted in deleterious outcomes in endothelial cells during OGD (Zhang et al. 2018). Silencing MALAT1 reduced endothelial cell apoptosis and increased endothelial cell proliferation during OGD by decreasing the expression of the pro-apoptotic proteins p-53 (phosphorylated form), Bax, and MDM2. Similarly, in a mouse model of stroke silencing MALAT1 resulted in decreased infarct sizes and improved neurological scores by abrogating the MDM2-p53-mediated pathway of cell death (Zhang et al. 2018). A recent study showed that MALAT1 can orchestrate cellular changes by interacting with miRNAs during stroke. In this study, upregulated MALAT1 caused the downregulation of miR-30 via sponging; this resulted in de-repression of the miR-30a targeted gene Beclin-1, which in turn promoted autophagy-induced cell death (Guo et al. 2017). Inhibition of MALAT1 reversed these effects and decreased infarct volumes as well as neurological deficits in mice (Guo et al. 2017).

Together, these studies show that MALAT1 is a key regulator of the post-stroke pathophysiology, but the contexts and conditions under which MALAT1 modulates beneficial or deleterious outcomes, and the mechanisms by which it achieves this, need further clarification.

Maternally Expressed Gene (MEG3)

MEG3 is a maternally expressed imprinted lncRNA gene. Early studies established MEG3 as a tumor suppressor gene, and loss-of-function of this lncRNA led to various types of cancers (Bao et al. 2018). In cerebral ischemia, MEG3 expression was upregulated in the mouse brain as well as in mouse primary neurons in vitro in response to transient ischemia and was linked to increased cell death (Liu et al. 2016; Yan et al. 2016, 2017). Functional studies showed that MEG3 promoted post-ischemic cell death via multiple mechanisms. In one study, ischemia-induced MEG3 was shown to directly interact with p53 in the mouse brain and this was correlated with increased neuronal death. Disruption of the MEG3-p53 interaction resulted in decreased post-stroke infarct sizes at 24 h and 72 h of reperfusion and reduced neuronal death in the cortex and striatum at 7 days of reperfusion (Yan et al. 2016). These neuroprotective effects upon disruption of the MEG3-p53 interaction were also recapitulated in mouse primary neurons undergoing OGD and 24 h of reoxygenation (Yan et al. 2016). In another study, MEG3 was shown to sponge miR-21 in the mouse brain during transient ischemia, resulting in the downregulation of miR-21. One of the validated targets of miR-21 is the Programmed Cell Death Complex 4 (PDCD4) mRNA, which encodes a pro-apoptotic protein. Upon knocking down MEG3, the brains of the treated mice exhibited an increase in miR-21 expression and a corresponding decrease in PDCD4 protein expression, which was correlated with reduced infarct volumes (at 24 h of reperfusion), decreased

neuronal death (at 3 days of reperfusion), and improved behavioral outcomes (up to 4 weeks of reperfusion) (Yan et al. 2017). Another study reported that MEG3 also functioned as sponge for miR-181b in post-ischemic HT22 cells and in the post-ischemic mouse cortex (Liu et al. 2016). Mechanistic studies in HT22 cells demonstrated that the sequestration and downregulation of miR-181b by MEG3 resulted in translational de-repression of the miR-181b target gene 12/15-lipoxygenase (12/15-LOX), causing the 12/15-LOX protein to be upregulated. The increased levels of 12/15-LOX were correlated with hypoxia-induced apoptosis of the HT22 cells, possibly due to increased oxidative stress (Liu et al. 2016). These observations were recapitulated in mice wherein knockdown of MEG3 resulted in upregulation of miR-181b and downregulation of 12/15-LOX protein in infarcted tissues, and was associated with decreased infarct volumes, decreased edema, and improved neurological function (Liu et al. 2016).

In contrast to the ischemia-induced *upregulation* of MEG3 in the aforementioned mouse studies, a recent study in the adult rat brain reported that the expression of one transcript variant of MEG3 was *downregulated* for up to 3 days after transient ischemia (Liu et al. 2017). Consistent with the mouse studies, though, this downregulation of MEG3 was correlated with improved neurological outcomes after stroke in rat. Further investigations showed that the stroke-responsive downregulation of MEG3 in the rat brain was associated with increased microvessel density in the peri-infarct regions up to 28 days after stroke as compared to controls that overexpressed MEG3. Similarly, in vitro experiments using human dermal microvascular endothelial cells (HMEC-1 cell line) showed that inhibition of MEG3 resulted in increased cell migration and proliferation, capillary-like structure formation, sprouting, and branch points as compared to controls overexpressing MEG3 (Liu et al. 2017). In both the in vivo (rat) and in vitro (human cell line) studies, MEG3 inhibition was associated with activation of the Notch signaling pathway genes NICD, Hes-1 and Hey-1, suggesting that the Notch signaling pathway may be modulated by MEG3 (Liu et al. 2017).

Together, these studies showed that MEG3 exhibits differential expression patterns and diverse mechanisms of action after stroke in a species- and cell-type dependent manner. In spite of these contrasting patterns, it is apparent that the downregulation of MEG3 is strongly linked to post-stroke neuroprotection.

Small Nucleolar RNA Host Gene 12 (SNHG12)

SNHG12 is associated with various types of cancers and is known to promote tumorigenesis (Long et al. 2018; Yin et al. 2019). SNHG12 expression is increased after ischemic injury in vitro and in vivo (Long et al. 2018; Yin et al. 2019; Zhao et al. 2018). In mouse BMECs, overexpression of SNHG12 during OGD inhibited the expression of the inflammatory cytokines MCP-1, IL-6, and E-selectin and increased the expression of the pro-angiogenic proteins VEGFA and FGFb, and these gene expression changes were associated with reduced cell death and increased tube formation of the BMECs, indicating a role for SNHG12 in post-ischemic angiogenesis (Long et al. 2018). SNHG12 was shown to achieve these outcomes by targeting miR-199a. This miRNA has previously been shown to inhibit cell proliferation and promote inflammation. In BMECs undergoing OGD, sponging and downregulation of miR-199a by SNHG12 was correlated with reduced cell death and inflammation (Long et al. 2018). In addition to sponging miR-199a, SNHG12 was also shown to sponge and repress miR-150 resulting in the de-repression of its target gene VEGF and promoting capillary-like tube formation in OGD-injured mouse BMECs (Zhao et al. 2018). The extension of this work to a mouse model of stroke showed that the overexpression of SNHG12 in the adult brain using lentiviral vectors resulted in decreased infarct volumes, increased vascular densities in the peri-infarct zone, and improved neurological function in the mice up to 7 days after transient ischemia as compared to sham and empty-vector controls (Zhao et al. 2018). A recent study using mouse primary hippocampal neurons and the mouse neuronal N2a cell line showed that SNHG12 was also upregulated in neuronal cells during ischemia (Yin et al. 2019). Follow-up experiments in N2a cells showed that OGD-induced SNHG12 repressed miR-199a (similar to the aforementioned BMECs), which in turn upregulated the miR-199a target gene SIRT1. This de-repression of SIRT1 was associated with a corresponding increase in p-AMPK protein levels and increased N2a cell survival during ischemia. This suggests that SNHG12 protects against OGD-induced injury by activating the SIRT1-AMPK pathway via miR-199a inhibition in neurons (Yin et al. 2019). In our own RNA-seq evaluation of the post-stroke adult mouse cortex, we did not observe upregulation of SNHG12 at the early time-points of 6 h and 12 h but it was significantly upregulated at 24 h of reperfusion as compared to sham controls (Bhattarai et al. 2017). Because our data was obtained from the whole cortex, the cell-type-specific responses could not be discerned.

H19

H19 is a maternally expressed imprinted gene whose expression is important for early embryonic development, but is generally decreased after birth (Bao et al. 2018). It is increased again in conditions such as cancer, oxidative stress or hypoxia (Bao et al. 2018; Wang et al. 2017). Circulating H19 levels in the blood were higher in stroke patients and in the brain, plasma, and white blood cells of mice undergoing

transient ischemia (Wang 2017a, b). In an experimental mouse model, knockdown of H19 resulted in lower infarct volumes, edema, and neurological deficits after stroke (Wang et al. 2017a, b). This was associated with decreased levels of the pro-inflammatory cytokines TNF-α and IL-1β in the cerebrum and plasma and increased levels of anti-inflammatory mediators TGF-β in the cerebrum and IL-10 in the plasma. In vitro studies using the mouse microglial BV2 cell line revealed that H19 knockdown promoted microglial M1 to M2 polarization (Wang et al. 2017b). Together, these results indicate roles for H19 in mediating inflammatory processes in the brain's innate immune system as well as the peripheral immune system during stroke. However, these results are preliminary and further work needs to be done to establish a functional link between H19 and the immune response in stroke.

H19 has also been reported to promote autophagy and neuronal death in ischemia (Wang et al. 2017a). In the human bone marrow neuroblast-derived SH-SY5Y cell line, OGD significantly induced the expression of H19 as compared to normoxic controls, and this was linked to apoptotic cell death via the activation of autophagy through the DUSP5-ERK1/2 axis (Wang et al. 2017a). Whether this role of H19 and its underlying mechanism extend to the brain has not been tested.

Antisense Noncoding RNA in the INK4 Locus (ANRIL)

ANRIL is an antisense long noncoding RNA encoded from the CDK2A/B locus on chromosome 9p21.3, which is a well-known risk locus for cancer and cardiovascular diseases. ANRIL participates in the regulation of gene expression in *cis* by binding to the polycomb repressive complex (PRC1/2) (Bao et al. 2018; Holdt and Teupser 2018), and in *trans* by binding to PRC-associated transcription factor Yin Yang 1 (YY1) (Zhou et al. 2016). In human endothelial cells, TNF-α induced the expression of ANRIL via NF-κB and this was essential for downstream NF-κB-mediated induction of the IL-6 and IL-8 genes, showing that ANRIL is an important feed-forward component of NF-κB-mediated pro-inflammatory gene induction (Zhou et al. 2016). In stroke, upregulation of ANRIL in a rat model of diabetes mellitus and permanent ischemia was positively correlated with that of VEGF and was associated with increased microvascular density (Zhang et al. 2017a). Consistent with prior work showing a functional link with NF-κB, ANRIL induced the expression of VEGF via the NF-κB pathway (possibly via the VEGF receptor FLT-1) (Zhang et al. 2017a). Administration of an NF-κB pathway inhibitor abrogated the stimulatory effect of ANRIL on VEGF expression and microvascular density. These data suggest that ANRIL may act as a pro-inflammatory and pro-angiogenic factor in stroke.

Fos Downstream Transcript (FosDT)

FosDT is an ischemia-induced lncRNA that originates immediately downstream of the protein-coding Fos gene in the rat genome, with a 105 nucleotide overlap between the 5′ region of FosDT and 3′ region of Fos. In the cortex of adult spontaneously hypertensive rat, FosDT was preferentially bound to the REST co-repressors Sin3a and coREST in response to transient ischemia (Dharap et al. 2013). The REST complex is activated in response to ischemic stroke and mediates the silencing of important neuronal genes, resulting in increased cell death and brain damage (Noh et al. 2012). Due to its close association with Sin3a and coREST, FosDT was hypothesized to participate in REST-mediated gene silencing in stroke. A subsequent study investigating the role of FosDT in the post-stroke rat cortex showed that knockdown of FosDT resulted in the de-repression of the REST target genes GRIA2, NFkB2, and GRIN1, and this was associated with reduced infarct sizes and improved motor function in the treated animals as compared to controls. These results indicate that FosDT is a pro-ischemic lncRNA and its perturbation promotes post-stroke neuroprotection via abrogation of REST-mediated gene silencing (Mehta et al. 2015).

In addition to the lncRNAs discussed above, recent studies have identified further examples such as TUG1, RMST, SNHG14, GAS5, N1LR, and C2dat1 that appear to be involved in the modulation of post-stroke processes. However, due to the limited literature on these lncRNAs in stroke, we have not reviewed them in detail but instead present a summary of their currently known roles in Table 2.

Clinical Relevance of lncRNAs in Stroke

Given the evolving importance of lncRNAs in modulating the post-stroke pathophysiology, a number of clinical studies in recent years have explored lncRNA expression changes and genetic variations in lncRNA genes in stroke patients (Dykstra-Aiello et al. 2016; Guo et al. 2018; Han et al. 2018; Wang et al. 2017a; Zheng et al. 2018; Zhu et al. 2018; Zhu et al. 2019).

Comparisons of lncRNA expression in whole blood samples of male and female stroke patients ($n = 266$) from the United States and Canada with those of vascular risk factor-matched controls revealed that 299 lncRNAs in male and 97 lncRNAs in female were differentially expressed in response to stroke. However, only 6 lncRNAs were common between the sexes, and of these, two showed the same direction of expression (i.e., downregulated in both males and females) whereas the remaining four were altered in the opposite direction in each of the sexes. Some of the differentially expressed lncRNAs originated in the close vicinity of genomic loci of putative risk factors for stroke development

Table 2 A summary of lesser known lncRNAs whose expression is altered after stroke

LncRNA	Function	Pathway	Cell/animal model	References
GAS5	Neural death	Sponges miR-137 and derepresses Notch1 thereby promoting neuronal death	Mouse brain and mouse primary brain neuronal culture	Chen et al. (2018b)
RMST	Neuronal damage	Knockdown improved cell viability (in vitro), reduced brain infarct and neurological deficit (in vivo)	Mouse brain and primary hippocampal neuronal culture	Hou and Cheng (2018)
SNHG14	Inflammation	Sponges miR-145-5p and derepresses PLA2G4A expression thereby promoting microglial activation	Mouse brain and BV2 cells	Qi et al. (2017)
TUG1	Neuronal damage	Sponges miR-9 thereby increasing the expression of miR-9 target gene Bcl2l11, encoding a pro-apoptotic protein	Rat brain, rat primary cortical neuronal culture and SH-SY5Y cell culture	Chen et al. (2017)
N1LR	Neuro-protection	Prevents apoptosis by inactivating p53	Mouse brain and N2a cells	Wu et al. (2017)
C2dat1	Neuro-protection	CAMK2D mediated activation of NF-κB pathway and increased expression genes like Bcl-xl (anti-apoptotic protein)	Mouse brain and N2a cells	Xu et al. (2016)

C2dat1 CaMK2D-associated transcript 1, *GAS5* growth arrest specific 5, *RMST* Rhabdomyosarcoma 2-Associated Transcript, *SNHG14* Small Nucleolar RNA Host Gene 14, *TUG1* Taurine Upregulated Gene 1

(Dykstra-Aiello et al. 2016). Another study evaluated the differential expression of lncRNAs in acute (24 h) and subacute phases (7 days) in the peripheral blood mononuclear cells of stroke patients versus vascular risk factor-matched healthy controls in a Chinese population using RNA-seq (Zhu et al. 2019). A total of 3009 lncRNAs at 24 h and 2034 lncRNAs on day 7 were differentially altered after stroke, providing further evidence that lncRNAs exhibit discrete expression profiles at various time-points after stroke versus healthy controls. A limitation of this particular study was its very small sample size of $n = 5$ per group.

A study on 126 acute ischemic stroke patients (within 24 h of the onset of stroke symptoms) and 125 controls from China reported that ANRIL expression was negatively correlated with stroke severity, i.e., decreased levels of ANRIL were associated with greater severity of stroke. This study found that decreased levels of ANRIL in plasma were associated with increased levels of the pro-inflammatory cytokines TNF-α, IL-6, and hs-CRP, and decreased levels of the anti-inflammatory cytokine IL-10 (Feng et al. 2019). Interestingly, in vitro gain/loss-of-function studies on ANRIL showed that the expression of ANRIL was positively correlated with that of the gene Caspase Recruitment Domain Family Member 8 (CARD8) (Bai et al. 2014). CARD8 is an adaptor protein that inhibits the NF-κB pathway, but an SNP in the CARD8 locus (rs2043211) results in a functionally compromised protein that can no longer inhibit NF-κB (Bagnall et al. 2008). An analysis in Northern and Central Chinese populations independently revealed that the CARD8 SNP rs2043211 was associated with increased risk of ischemic stroke (Bai et al. 2014), suggesting that the CARD8 SNP may contribute to the increased risk of stroke through the NF-κB pathway. Similarly, the correlation between downregulated ANRIL and worsened stroke outcomes in patients from the Feng et al. study may be mediated by the NF-κB pathway via a decrease in CARD8 expression. Further investigations on this connection between ANRIL, CARD8, and the NF-κB pathway may elucidate whether there is a cause-effect relationship between ANRIL expression, CARD8 downregulation, and the severity of stroke. Contrary to the above observations, however, another study conducted on the Chinese Han population showed increased ANRIL expression in the peripheral blood samples of stroke patients as compared to those from healthy controls, positively linking ANRIL expression to the manifestation of stroke (Yang et al. 2018b). However, one caveat of this study is that although the overall sample size for this study was 550 ischemic stroke patients, only 71 patients were selected for the evaluation of ANRIL expression using real-time PCR. The selection criteria for these 71 patients or the exclusion criteria for the remaining 479 patients were not presented. Hence, it is unclear whether the increased ANRIL expression was limited to a particular subset of ischemic stroke patients or was perhaps more widely applicable to the entire cohort of patients in this study. Additionally, this study did not include data on the stroke timeline, severity or comorbidities that may help explain the differences in post-stroke ANRIL expression as compared to the Feng et al, study, which was conducted in acute ischemic stroke patients with varying severities within 24 h of the onset of stroke symptoms. To clarify the factors underlying these differences, additional studies on stroke patient populations need to be conducted and such studies would benefit from increased sample sizes as well as a nested study design taking into account the covariates associated with the various ischemic stroke subtypes and comorbidities.

Using genome-wide-association studies, single nucleotide polymorphisms (SNPs) identified in lncRNA loci have been correlated with an increased risk of development of stroke. Two SNP variants (rs2383207 and rs1333049) within the ANRIL locus were correlated with an increased risk of stroke development in males but not in females (Yang et al. 2018b). SNP genotyping of the genes for the lncRNAs H19 in blood samples of 567 ischemic stroke patients and 552 control subjects revealed that the SNP rs217727 in the H19 locus was associated with increased susceptibility to the development of ischemic stroke in the Chinese Han population (Zhu et al. 2018). Similarly, SNP genotyping of the MEG3 and miR-181b loci in blood samples of ischemic stroke patients and healthy controls in a Chinese population demonstrated that the SNP rs322931 in miR-181b alone or in association with two SNPs (rs7158663 and rs4081134) in the MEG3 locus increased the risk of ischemic stroke (Han et al. 2018). Another study on blood samples of ischemic stroke patients and controls in the Chinese population reported that deletion polymorphism rs145204276 in the promoter region of the GAS5 lncRNA gene is associated with an increased risk of developing ischemic stroke (Zheng et al. 2018).

Overall, the identification of key genetic variants in lncRNA loci and/or differential circulating expression patterns of lncRNAs between stroke patients and healthy controls introduces the possibility that evaluation of lncRNA expression or lncRNA gene loci could be a useful clinical tool for assessing the risk of development of stroke or perhaps even for the diagnosis of stroke in the future.

Therapeutic Potential and Future Perspectives

Given the high prevalence of stroke worldwide and a lack of therapeutic interventions other than rtPA therapy based primarily on thrombolysis, there is an urgent need to develop novel treatment strategies to curb the ischemic attack and minimize post-stroke brain damage. A major advantage of RNA is that it can be fairly easily manipulated using small molecule chemistry or other approaches such as ribozymes, aptamers, and antisense oligos (ASOs). Numerous RNA-based therapeutics have been under clinical investigation for diseases ranging from genetic disorders to viral infections to various cancers (Burnett and Rossi 2012). In 2017, the FDA approved an RNA-targeting ASO therapy called 'Spinraza' (nusinersen) as the first and only treatment for spinal muscular atrophy (SMA). SMA occurs due to deficiency in survival motor neuron protein due to genetic mutations. This ASO selectively targets the survival motor neuron pre-mRNA and alters its splicing such that there is increased production of full-length functional protein resulting in neuronal rescue and amelioration of neurodegeneration in patients with SMA. In 2018, the FDA approved the first RNAi therapy for the treatment of peripheral nerve disease caused by hereditary transthyretin-mediated amyloidosis caused due to accumulation of transthyretin deposits in tissues. Called 'Patisiran', this RNAi drug is administered intravenously to target the transthyretin mRNA to block the production of transthyretin protein, which in turn enables the clearance of transthyretin amyloid deposits in peripheral tissues and helps restore tissue function. Such therapeutic breakthroughs make a compelling case to investigate the druggable transcriptome in cerebral ischemia so that it may be harnessed for potential therapies against brain damage.

The preclinical and clinical studies presented in this review illuminate the potential of lncRNAs as novel therapeutic targets in stroke, or even as clinical biomarkers of stroke. Although we now know much more about lncRNA functions in stroke as compared to just a few years ago, there is a lot more that needs to be learned. With continued research in this area and the advancement of our knowledge of lncRNAs and their roles in mediating the post-stroke pathophysiology, we may be able to leverage this information to accelerate the development of novel pharmacological therapies against stroke.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Research Involving Human and Animal Participants This article does not contain any studies with human participants or animals performed by any of the authors.

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