Azhar Int J Pharm Med Sci 2023; Vol 3 (2):1-10
(Review Article)

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

The role of lncRNAs in stroke: MEG3 as a promising candidate

Ola SM. Ali [1], Olfat GA. Shaker [2], Wafaa AH. Sroor [3], and Marwa AA. Abdeen [3, *]

[1] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Girls), Al-Azhar University.
[2] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University.
[3] Division of Hormones Evaluation, Control Division, Egyptian Drug Authority (EDA).
***** Correspondence: abdin_marwa@hotmail.com

Article history: Received 2021-12-30 **Revised 2022-03-21** **Accepted 2022-08-30**

Abstract: Globally, stroke is considered a major cause of incapacity and death. It results in high economic load to peoples in almost all countries. Numerous mechanisms have been anticipated to contribute to the pathophysiology of ischemic stroke, including epigenetic modification and dysregulation of some noncoding RNAs. In the recent years, there has been a great focus on studying long noncoding RNAs (lncRNAs) which have been recognized as possible biomarkers and therapeutic goals to treat ischemia. It was observed that maternally expressed gene 3 (MEG3)- lncRNA perform many functions and is implicated in the pathophysiology and/ or recovery of many diseases. Beside the high expression levels of MEG3 in brain, the upregulation of MEG3 after ischemic stroke was also observed. The current review sets sights on spotlighting the functions of lncRNAs, especially MEG3 in the pathogenesis of stroke as well as figuring out its genetic variants that are associated with various diseases, including stroke risk.

Keywords: Stroke; Pathophysiology; Noncoding RNAs; lncRNA; MEG3.

1. INTRODUCTION

Cardiovascular diseases (CVDs), including stroke, are the chief cause of death worldwide. In 2019, CVDs accounted for almost 18.6 million deaths [1]. Stroke and ischemic heart diseases continued to be the humanity's major murderers, that kept on being the top origins of mortality in the last two decades [2], before the worldwide eruption of Covid-19. Stroke is considered as a candidate of the foremost origins of continuing incapacity and mortality, that results in a serious economic problem to humanities of numerous nations [3]. Many pathophysiologic mechanisms are proposed to be implicated in ischemic stroke. There is evidence that patients with ischemic stroke have had altered and sexually dimorphic long noncoding RNAs (lncRNA) expression compared with normal controls, suggesting the future use of lncRNA as a promising biomarker for stroke risk [4]. Nowadays, comprehending regulatory role of noncoding RNA (ncRNA) has become one of the most vital missions of science [5]. Maternally expressed gene 3 (MEG3), a promising RNA that has been reported to perform many functions, and to be implicated in the pathophysiology and/ or recovery of many diseases [6], was also observed to be upregulated in stroke and contributing to its pathogenesis by various suggested mechanisms [7,8]. Single-nucleotide polymorphisms (SNPs) are variations of DNA as a result of change in nucleotides sequences (A, T, C, or G) by 1% or more in certain populations [9]. Various association studies showed that SNPs have influential functions in principal molecular and hormonal systems, and are associated to several disorders [10]. SNPs could disorder the secondary structure of the lncRNAs, affecting their molecular function and having an effect on their expression pattern [11]. SNPs in MEG3 gene, also, have their contributory roles and many studies proved the association between different genetic variants of MEG3 and several diseases, including ischemic stroke [12–14].

2. STROKE

Stroke is defined as a neural disorder resulting from acute central damage that happens in the brain

and/ or the spinal cord, which arises from vascular origins, including intracerebral hemorrhage, cerebral infarction and subarachnoid hemorrhage (SAH). Stroke is classed into two chief categories: hemorrhagic stroke, that happens due to a brain bleed, or ischemic stroke, that happens due to obstruction in a cerebral blood vessel [15].

Globally, in 2019, there were 6.6 million deaths attributed to CVD, 3.3 million deaths of which were from ischemic stroke, 2.9 million deaths were from intracerebral bleeding, and four tenths were due to SAH [1].

In Egypt, both prevalence and mortality rates for ischemic stroke have shown alarming increase from the year 2016 to the year 2017. Sorrowfully, the age-adjusted ischemic stroke prevalence per one hundred thousand, in 2016 (940.2) has increased in 2017 to become 1374/100 000, whereas the age-adjusted rate of mortality per one hundred thousand for ischemic stroke was 53.8 in 2016 and has become 82 in 2017 [16,17].

Numerous mechanisms have been anticipated to contribute to the pathophysiology of ischemic stroke. One of these mechanisms is the reactive oxygen species (ROS) formation, which is a key machinery for neuronal damage of ischemic, as well as reperfused brain tissue. Brain is explicitly subtle to oxidative damage due to plentiful lipid content and oxygen demand and the chemical reactions that involve glutamate and dopamine oxidation, as well [18].

Another previously suggested mechanism for stroke is the involvement of the well-known tumor suppressor protein p53. p53 is a key regulator of numerous reactions in cellular stress, that contributes to apoptosis of neurons when activated following ischemia in some brain areas [19].

Interestingly, epigenetics represents an innovative field in the study of stroke. Several epigenetic tags may be helpful for predicting the risk of stroke, as well as its consequences and recovery. Because of numerous combinations of interplaying influences, each being has a unique epigenetic code, which may cause varying levels of stroke risk, outcome and recovery [20]. Besides, earlier studies have investigated the fundamental machineries of brain ischemic damage stemming from either up- or down-regulation of some ncRNAs. These ncRNAs have become recognized as possible biological markers or therapeutic goals for the treatment of ischemia [3]. Numerous lncRNAs are localized inside the nucleus, and previous studies have demonstrated that they affect gene expression [21,22] either by stimulation or repression, or by altering chromatin methylation or acetylation [23]. Epigenetic modification of DNA methylation might control inflammatory damage and repair processes during the injury of stroke. This also exposes patients to chronic inflammation which additionally increases the recurrence of stroke [20].

3. NONCODING RNAS (NCRNAS) AND LONG NONCODING RNAS (LNCRNAS)

For many ages it has been thought that most of RNAs in our bodies are messenger RNAs (mRNAs), i.e.: protein coding RNAs. Nonetheless, previous research work has changed this concept, indicating that the majority of RNAs do not code for proteins, and that these ncRNAs can play an important role and even increase our knowledge of human illnesses [5].

Long noncoding RNAs are ncRNAs with the length of 200 nucleotides, or more, that are transcribed independently and do not have recognized protein-coding function [24,25]. Nevertheless, several lncRNAs were observed to code for small peptides in human cells [26,27]. The multiplicity of the non-coding transcriptome is considered as a claim to explain the outstanding phenotypic variations noticed among species having relatively similar protein-coding genes [28]. In fact, several lncRNAs are similar to mRNAs in various aspects. However, the overall characteristics distinguishing mRNAs from lncRNAs do exist, where mRNAs are usually lengthier than lncRNAs, having more, yet shorter, exons and rather higher expression levels [29].

3.1. The Role of ncRNAs and lncRNAs in Ischemic Stroke

Noncoding RNAs are generously expressed in human brain whilst several research works have shown the alteration of their expression in cerebral ischemia [3,4,30,31]. Long non coding RNAs are among numerous molecule types that produce operative changes in ischemic stroke. Though, the research relating lncRNAs to ischemic stroke remains inadequate, and the fundamental mechanisms of regulation for many lncRNAs (that have been realized associated with ischemic stroke) have not been deeply studied yet, these causal mechanisms cannot be overlooked. Moreover, lncRNA, as a vital endogenous regulatory mechanism, is foreseeable to be a novel mode and spotlight for ischemic stroke regulation [32, 33].

After brain ischemia, inflammatory responses are critical to cerebral tissue injury pathogenesis. Following cerebral ischemic injury, molecules released from damaged and necrotic tissues, as well as, from blood vessels, provoke proinflammatory cytokines, like tumor necrosis factor alpha (TNF-α), interleukin-6 and interleukin-1b, causing inflammation and aggravation of initial cerebral injury [34]. Not only do the transcription factors regulate the expression of inflammation-related signaling pathway molecules, but also do the ncRNAs regulate their expression at a significant level [3,35].

Moreover, ncRNAs are implicated in oxidative damage, which in turn participates in consequent cerebral injury following brain ischemia, through oxidative alterations of macromolecules, such as: DNA, proteins, and lipids [18]. Besides, the implication of ncRNAs in angiogenesis plays a vital role in neurofunctional recovery and vascular angiogenic remodeling after ischemic stroke [3,36,37].

The expression levels of numerous lncRNAs were observed to be heightened in animals with brain ischemia or in vitro in oxygen-glucose deprived (OGD) cells, such as: MEG3 [38,39], Fos downstream transcript (FosDT) [40], metastasis-associate lung adenocarcinoma transcript 1 (MALAT1) [41], H19 [42,43] and CaMK2D-associated transcript1 (C2dat1) [44], which have been proposed to enhance apoptosis, inflammation and new blood vessels formation. MEG3, MALAT1 and H19 genes were predicted by Gene Ontology enrichment analysis to exert effects on inflammation, angiogenesis and neurogenesis, via gene regulation mechanisms [23].

FosDT lncRNA gene expression was increased significantly during the acute phase after transient middle cerebral artery occlusion (MCAO) of adult rats. Whereas rats treated with FosDT siRNA developed significantly tinier infarcts and their function recovery was improved, compared to control rats treated with siRNA. This recovery was measured by measuring sensorimotor deficits, motor coordination/motor learning and vestibulomotor function, [40].

MALAT1 was suggested to defend the endothelium of blood vessels of human brain against apoptosis stimulated by Oxygen–glucose deprivation & reoxygenation (OGD-R). This defense was beleived to be by a phosphatidylinositol 3-kinase-dependent survival mechanism, rather than by decreasing the overproduction of ROS induced by OGD-R [45]. While it was found that suppression of H19 lncRNA and autophagy protected OGD/R cells from apoptosis, autophagy activation induced by OGD/R was inhibited by H19 siRNA. Moreover, polymorphism in H19 gene is associated with ischemic stroke increased risk, as revealed from the outcomes obtained from ischemic patients' peripheral blood samples [43].

4. MATERNALLY EXPRESSED GENE 3 (MEG3)

In 2000, MEG3 gene was characterized for the first time by **Miyoshi et al.** using gene trapping technique. It corresponds to the mouse gene trap locus 2 (Gtl2) gene. [46]. The length of MEG3 is approximately 1.6 kilo-bases, occupying their position on chromosome 14q32.3 in humans [47]. Human chromosome 14q32.3 exerts critical functions in differentiation of cells and development of tissues. It comprises an imprinted region, in which MEG3 gene is located, encompassing paternally expressed genes (PEGs), namely, DIO3, DLK1 and RTL1, beside maternally expressed genes (MEGs), namely, MEG3,8 and 9, and numerous huge gatherings of microRNAs [48–50].

At the beginning, the roles of either MEG3 or Gtl2 was unidentified because they do not encompass substantial open reading frame (ORF) [47]. Afterwards, it was established that the ORFs that are encoded by MEG3 transcripts are not needed for MEG3 function, nevertheless, the MEG3 RNA folding structure is crucial to its function, reinforcing the perception that MEG3 acts as an ncRNA [51].

MEG3 is vastly expressed in many tissues, including the placenta, brain, pituitary, and adrenal glands. The transcripts of MEG3 are also detected in the liver, ovaries, testes, spleen, pancreas, and mammary gland [6,52]. Furthermore, MEG3 was also observed to be differentially expressed in neurons [53,54].

Notwithstanding of the existence of a CCAAT-box and TATA-box in its promoter, as well as the presence of a poly(A) tails in its RNA transcripts, which makes it a target gene of RNA polymerase II, MEG3 gene does not encode for proteins [55]. While transcription of MEG3 gene is required for its functions, mutant MEG3 made from cDNA missing the translatable ORF still retains MEG3 full functions [51].

Interestingly, MEG3 lncRNA is encoded by both the paternally imprinted gene DLK1 as well as the maternally imprinted gene, MEG3 form the footprint [8]. It is worth mentioning that genomic imprinting is a distinctive epigenetic incident that mainly shows itself in mammals' placenta, leading to

parent-of-origin explicit differential expression of paternally, as well as maternally inherited alleles [56].

MEG3 comprises ten exons. Using varying exons in the core of the RNA, a single-copy gene of MEG3 is transcribed into different isoforms of its transcripts by alternative splicing. Each MEG3 isoform comprises the shared exons: 1-3 and 8-10, whereas each of them holds a dissimilar blend of exons from four to seven [57]. Twelve MEG3 complementary DNA isoforms were characterized using sequence analysis [55,58]. Figure (1) presents schematic illustration of the locus (DLK1–MEG3) on chromosome 14q32.3 and the 10 exons comprised by MEG3 gene.

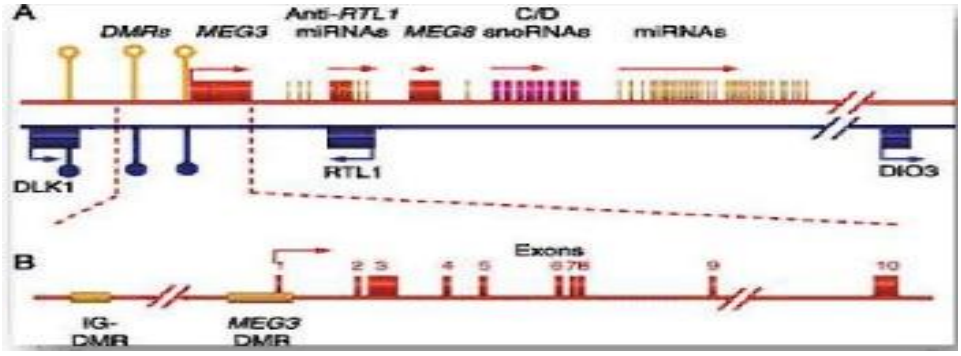

Figure 1. (A) Schematic illustration of a part of human chromosome 14 showing the locus (DLK1–MEG3); (B) MEG3 gene including 10 exons 52.

To perform its function, MEG3 (and its isoforms), as a big RNA molecule encompassing almost 1700 nucleotides, have to form a dense folding structure [55]. Upon analyzing the potential folding structures of MEG3 RNA isoforms using mfold program, each isoform was observed to comprise 3 conserved motifs (M1- M3). Moreover, the expression of each MEG3 isoform is parallel to its stability [55].

MEG3 was reported to perform many functions, and to be implicated in the pathophysiology and/ or recovery of many diseases, including tumors, where it enhances tumor suppression, for example in adenomas of the pituitary gland. In humans, it was found that MEG3 gene expression is totally inhibited in most of pituitary gland adenomas. Furthermore, MEG3a isoform has practically proven a powerful capacity to hinder the proliferation of numerous human cancers, in vitro [6].

Consequently, MEG3 is suggested to be a potential tumor suppressor gene implicated in etiology, development and chemosensitivity of many tumors such as meningioma [57,58] and cervical cancer [31]. Furthermore, a significant downregulation of MEG3 gene was observed in glioma cells. Whereas MEG3, when overexpressed, significantly repressed the proliferation at the same time as stimulating organized cell death and autophagy of glioma tissues [59].

Besides, the action of MEG3 as a tumor suppressor was found to be facilitated via p53-dependant and independent routes [51]. It was demonstrated that every MEG3 isoform was capable of stimulating p53-mediated transactivation, and to suppress DNA synthesis in human colon cancer cell line [55]. However, MEG3 was able also to suppress cell proliferation in the absence of p53 [51].

4.1. MEG3 and stroke

MEG3 also supports apoptosis of cells in ischemic stroke. It practically intermingles with p53 facilitating ischemic injury [7]. MEG3 upregulation has been observed in acute ischemic stroke in OGD/R models or transient MCAO animal models by numerous authors [38,39,60–62].

Interestingly, MEG3 knockdown is protective against brain injury caused by ischemia. Besides, the knockdown of MEG3 also enhances the performance of neurons by acting as competitive endogenous RNA (ceRNAs) that struggles to bind directly to miR-21, that facilitates the ischemic death of neurons instead of programmed cell death 4 (PDCD4) mRNA [61]. MEG3-lncRNA also competitively inhibited miR-181b, which inhibits 12/15 lipoxygenase action in MCAO-induced infarction of brain neurons in mouse [60]. Moreover, MEG3 knockdown was found to hinder inflammatory reactions and cell death stimulated by OGD/R [38].

Another study revealed the relationship between MEG3 lncRNA and another miRNA, miR-424-5p, and their involvement in ischemic stroke, and showed an inverse relationship between them. Where, Semaphorin 3A (Sema3A) and MEG3

have shown overexpression in ischemic stroke samples, while the expression of miR-424-5p was lowered. MEG3 enhanced ischemic stroke progression via repressing miR-424-5p, which targeted Sema3A gene. The later encodes for vital protein for normal neuronal pattern development and is able to stimulate the mitogen activated protein kinase (MAPK) signaling pathway which contributes to the pathophysiology and development of ischemic stroke [39].

In a previous research work, OGD treatment was observed to remarkably increase the expression of Bax, cleaved caspase-3 and MEG3, besides, it boosted human brain microvascular endothelial cells (hBMECs) apoptosis, whereas small interfering MEG3 (si-MEG3) inhibited those actions. These findings were supported by the same authors in a further in vivo study which revealed the significant elevation of the expression of MEG3 in a period of forty-eight hours of acute ischemic stroke. Moreover, those patients whose blood samples contained greater levels of MEG3 exhibited comparatively poor prognosis, suggesting the role of MEG3 as prognostic marker for deteriorating consequences and death in ischemic stroke [62].

Moreover, MEG3 has also been reported to affect hemorrhagic stroke, where MEG3- lncRNA was significantly highly expressed in SAH patients than in normal controls. This was positively proportional to the severity of SAH. While, MEG3 was overexpressed, many consequences were observed: activity of neurons was reduced, whereas some apoptotic mediators' expression levels were elevated (such as: cleaved Caspase-3 and p53), hence, apoptosis was augmented [63].

On the contrary, **Liu et al.** have demonstrated the downregulation of MEG3 after transient MCAO in rats. The findings of **Liu et al.** proposed that MEG3 downregulation improved cerebral ischemic injuries and augmented blood vessels formation and growth after stroke. In addition, while preventing MEG3 gene expression stimulated endothelial cells to migrate and proliferate, and hence, stimulated the growth of cerebral vessels, MEG3 overexpression caused the reverse effect [8]. Table (1) summarizes literature reported dysregulation of MEG3 gene associated with different stroke subtypes.

Table 1. Literature reported dysregulation of MEG3 gene expression in stroke.

Disease	MEG3 Expression levels	In:	Authors (year)
Ischemic stroke	upregulated	MCAO mice, and in vitro: in OGD-cultured HT22 cell	X. Liu et al. (2016) [60]
Ischemic stroke	upregulated	MCAO adult mice	Yan et al. (2016) [7]
Ischemic stroke	upregulated	MCAO mice, and in vitro: N2a cell OGD/R model	Yan et al. (2017) [61]
Ischemic stroke	downregulated	MCAO model in rat, and in vitro: HMEC-1 cells	J. Liu et al. (2017) [8]
Subarachnoid hemorrhage (SAH)	upregulated	pre-chiasmatic SAH model in SD rats, and SAH patients	Z. Liang et al. (2018) [63]
Ischemic stroke	upregulated	MCAO rat model, and in vitro: OGD/R-treated neurocytes	J. Liang et al. (2020) [38]
Ischemic stroke	upregulated	ischemic stroke model in mice, and in vitro in (hBMECs)	Wang et al. (2020) [64]
Ischemic stroke	upregulated	MCAO mice, and in vitro: OGD/R-treated neurocytes	Xiang et al. (2020) [39]
Ischemic stroke	upregulated	OGD/R-treated neural stem cells	Zhao et al. (2021) [65]
Ischemic stroke combined with hyperglycemia (diabetic brain ischemic injury)	upregulated	OGD in rat brain microvascular endothelial cells (RBMVECs), combined with hyperglycemia	Chen et al. 2021 [66]
Ischemic stroke	upregulated	cerebral ischemia/ reperfusion mice, and OGD/R-treated HT22 cells	Li et al. 2022 [67]

4.2. Association between single nucleotide polymorphisms (SNPs) in MEG3 and several diseases

Over the last decades, there has been a great focus in the literature on finding the association between various MEG3 SNPs and several diseases. As an example: MEG3 (rs941576) SNP, which is located in the imprinted region of the human chromosome 14q32.2 (comprising DLK1, a functional gene for type one diabetes mellitus, T1D), was robustly reported to be associated with T1D inherited susceptibility, from the paternal allele transmission route [12]. Furthermore, AA genotype of the SNP MEG3 rs7158663 was observed to be associated with the risk of diabetes mellitus type two [68].

Additionally, several SNPs in MEG3 genes were associated with breast cancer susceptibility [69]. SNPs detected in MEG3 gene exhibited differential imprinting in different tumor subtypes, compared to normal tissue samples. In a clinical trial of neoadjuvant chemotherapy for breast cancer, the genotype AG+GG of the dominant model of MEG3 (rs941576) as well as, the genotype TC+CC of the dominant model of MEG3 (rs10132552), showed significant association with better survival (without disease) for patients with breast cancer [70].

A recent study made to assess the association between MEG3 and the risk of breast cancer in fibroadenoma patients concluded that rs7158663 AA variant is significantly associated with susceptibility to breast cancer in controls as well as patients with fibroadenoma, compared to GG variant [71]. MEG3 polymorphisms were, also, studied in association with other cancers like: colorectal cancer and neuroblastoma [72,73]. MEG3 rs7158663 AA genetic variant was also significantly associated with the risk of colorectal cancer, in comparison with GG variant [72].

Furtheremore, it was found that both MEG3 SNPs (rs7158663 and rs3087918) mounted the possibility of acute myeloid leukemia, proposing a possible function of MEG3 in its pathogenesis [74]. Whereas, MEG3 rs7158663 A allele was reported to be significantly associated with gastric cancer risk [75]. Moreover, MEG3 polymorphisms also have shown associations with different diseases, other than tumors. MEG3 rs941576 A/G genetic variation, for example, was shown to be in association with rheumatoid arthritis aggravation in Egyptians [76]. Whereas, the AA genotype of MEG3 rs7158663 significantly increased osteoarthritis risk in chinese subjects by about two folds more than GG. This increased risk was shown in the dominant model, as well as the recessive model. The risk of osteoarthritis of the allele A was, also, greater than G allele by about one and half times [64].

4.3. Association between MEG3 polymorphisms and stroke

Nowadays, despite the great focus in the literature on finding the association between various MEG3 gene SNPs and several diseases, up till now, to the best of our knowledge, there has not been so much research work on investigating MEG3 SNPs that are associated with stroke. So far, there has been two published studies to find association of some MEG3 SNPs with ischemic stroke susceptibility of Han people [13] and Egyptians [14].

Firstly, a previous association study was conducted to evaluate the probable association of MEG3 genetic polymorphisms, namely, rs4081134 and rs7158663, and miR-181b SNP (rs322931) with the susceptibility to ischemic stroke in Chinese. Combined analyses in this study unveiled that [MEG3 (rs7158663 AG/AA) + miR-181b (rs322931 CT/TT)] and [MEG3 (rs7158663 GG) + miR-181b (rs322931 CT/TT)] heightened the susceptibility to ischemic stroke, in comparison with (rs7158663 GG+ rs322931 CC). Whereas rs4081134 genotypes exhibited no significant association with ischemic stroke risk [13].

Secondly, in a recent previous research work, our team demonstrated that MEG3 rs941576 genetic variant, alone or combined with rs7158663, is associated with acute ischemic stroke risk, advocating that rs941576 may have a role in acute ischemic stroke pathogenesis and may be helpful in its prognosis, later on [14].

5. CONCLUSIONS

The current review investigated the importance and the alarming increase of the incidence and prevalence of stroke, especially ischemic stroke, that urged scientists and health care providers to seek for novel scopes for its prognosis, treatment, and prevention. Recently, lncRNAs provide a state-of-the-art field to study human diseases, including cerebral ischemia, and the interrelated factors contributing to their risks, etiologies, pathophysiology, and complications. In this review article we have, also, reviewed several previous studies that investigated causal mechanisms of

ischemic brain damage following up- and down-regulation of some lncRNAs, especially MEG3. However, the basic mechanisms for MEG3 and some other lncRNAs which have been observed to be associated with ischemic stroke, has not been deeply researched yet. We finally conclude that further extensive and intensive research works needed to be made on MEG3 gene and its genetic variants as encouraging potential biological markers and even therapeutic goals for ischemic stroke treatment.

Funding: This research received no external funding.

Conflicts of Interest: All authors declare no conflicts of interest.

Author Contribution: All authors contributed significantly in writing, revising and editing the manuscript.

REFERENCES

1. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. on behalf of the American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2021 update: a report from the American Heart Association. Circulation. 2021;143:e254–e743.
2. World Health Organization. Global Health Estimates 2018: Disease burden by Cause, Sex, by Country and Region, 2000-2016. World Heal Organ. 2018;
3. Wang SW, Liu Z, Shi ZS. Non-Coding RNA in Acute Ischemic Stroke: Mechanisms, Biomarkers and Therapeutic Targets. Cell Transplant. 2018;27(12):1763–77.
4. Dykstra-Aiello C, Jickling GC, Ander BP, Shroff N, Zhan X, Liu D, et al. Altered Expression of Long Noncoding RNAs in Blood after Ischemic Stroke and Proximity to Putative Stroke Risk Loci. Stroke. 2016;47(12):2896–903.
5. Diamantopoulos MA, Tsiakanikas P, Scorilas A. Non-coding RNAs: the riddle of the transcriptome and their perspectives in cancer. Ann Transl Med. 2018;6(12):241–241.
6. Zhang X, Zhou Y, Mehta KR, Danila DC, Scolavino S, Johnson SR, et al. A Pituitary-Derived MEG3 Isoform Functions as a Growth Suppressor in Tumor Cells. J Clin Endocrinol Metab. 2003;88(11):5119–26.
7. Yan H, Yuan J, Gao L, Rao J, Hu J. Long noncoding RNA MEG3 activation of p53 mediates ischemic neuronal death in stroke. Neuroscience. 2016;337:191–9.
8. Liu J, Li Q, Zhang K shan, Hu B, Niu X, Zhou S min, et al. Downregulation of the Long Non-Coding RNA Meg3 Promotes Angiogenesis After Ischemic Brain Injury by Activating Notch Signaling. Mol Neurobiol. 2017;54(10):8179–90.
9. Chu YH, Hsieh MJ, Chiou HL, Liou YS, Yang CC, Yang SF, et al. MicroRNA gene polymorphisms and environmental factors increase patient susceptibility to hepatocellular carcinoma. PLoS One. 2014;9(2).
10. Stanzione R, Cotugno M, Bianchi F, Marchitti S, Forte M, Volpe M, et al. Pathogenesis of ischemic stroke: Role of epigenetic mechanisms. Genes. 2020;11(1).
11. Sabarinathan R, Tafer H, Seemann SE, Hofacker IL, Stadler PF, Gorodkin J. The RNAsnp web server: predicting SNP effects on local RNA secondary structure. Nucleic Acids Res. 2013;41(Web Server issue):475–9.
12. Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG. The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. Nat Genet. 2010;42(1):68–71.
13. Han X, Zheng Z, Wang C, Wang L. Association between MEG3/miR-181b polymorphisms and risk of ischemic stroke. Lipids Health Dis. 2018;17(1):1–8.
14. Shaker O, Sroor W, Ali O, Soliman H, Abdeen M. Association between MEG3 polymorphisms (rs941576 and rs7158663) and risk of acute ischemic stroke in Egyptian patients. Gene Reports. 2021 Jul 22;24:101286.
15. Parmar P. Stroke: Classification and diagnosis. Clin Pharm. 2018;10(1).
16. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics—2020 update: A report from the

American Heart Association. Circulation. 2020;E139–E596.
17. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation. 2019;139:e56–528.
18. Guldiken B, Demir M, Guldiken S, Turgut N, Turgut B, Tugrul A. Oxidative Stress and Total Antioxidant Capacity in Diabetic and Nondiabetic Acute Ischemic Stroke Patients. Clin Appl Thromb. 2009;15(6):695–700.
19. Hong LZ, Zhao XY, Zhang HL. P53-mediated neuronal cell death in ischemic brain injury. Neurosci Bull. 2010 Jun;26(3):232–40.
20. Ng GYQ, Lim YA, Sobey CG, Dheen T, Fann DYW, Arumugam T V. Epigenetic regulation of inflammation in stroke. Ther Adv Neurol Disord. 2018 Jan 1;11:1–30.
21. Cheng J, Kapranov P, Drenkow J, Dike S, Brubaker S, Patel S, et al. Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. Science (80-). 2005 May 20;308(5725):1149–54.
22. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, et al. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res. 2012 Sep;22(9):1775–89.
23. Bao MH, Szeto V, Yang BB, Zhu SZ, Sun HS, Feng ZP. Long non-coding RNAs in ischemic stroke review-article. Cell Death Dis. 2018;9(3):281.
24. Ulitsky I. Evolution to the rescue: Using comparative genomics to understand long non-coding RNAs. Nat Rev Genet. 2016;17(10):601–14.
25. Batista PJ, Chang HY. Long noncoding RNAs: Cellular address codes in development and disease. Cell. 2013 Mar 14;152(6):1298–307.
26. Wilhelm M, Schlegl J, Hahne H, Gholami AM, Lieberenz M, Savitski MM, et al. Mass-spectrometry-based draft of the human proteome. Nature. 2014 May 28;509(7502):582–7.
27. Thum T, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. Circ Res. 2015 Feb 13;116(4):751–62.
28. Jarroux J, Morillon A, Pinskaya M. Long Non Coding RNA Biology. Adv Exp Med Biol. 2017; 1008:1–46.
29. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet. 2016;17(1):47–62.
30. Dharap A, Nakka VP, Vemuganti R. Effect of focal ischemia on long noncoding RNAs. Stroke. 2012;43(10):2800–2.
31. Zhang J, Yao T, Wang Y, Yu J, Liu Y, Lin Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. Cancer Biol Ther. 2016;17(1):104–13. doi: 10.1080/15384047.2015.1108496.
32. Ren W, Yang X. Pathophysiology of long non-coding RNAs in ischemic stroke. Front Mol Neurosci. 2018 Mar 29;11(96):1–6.
33. Bhattarai S, Pontarelli F, Prendergast E, Dharap A. Discovery of novel stroke-responsive lncRNAs in the mouse cortex using genome-wide RNA-seq. Neurobiol Dis. 2017;108:204–12.
34. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol. 2010;87(5):779–89.
35. Cheng HS, Njock MS, Khyzha N, Dang LT, Fish JE. Noncoding RNAs regulate NF-κB signaling to modulate blood vessel inflammation. Front Genet. 2014;5(DEC):1–14.
36. Yin K-J, Hamblin M, Chen Y. Angiogenesis-regulating microRNAs and Ischemic Stroke. Curr Vasc Pharmacol. 2015 Jul 10;13(3):352–65.
37. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol. 2009 May 1;8(5):491–500.
38. Liang J, Wang Q, Li JQ, Guo T, Yu D. Long non-coding RNA MEG3 promotes cerebral ischemia-reperfusion injury through increasing pyroptosis by targeting miR-485/AIM2 axis. Exp Neurol. 2020 Mar 1;325.
39. Xiang Y, Zhang Y, Xia Y, Zhao H, Liu A, Chen Y. LncRNA MEG3 targeting miR-424-5p via MAPK signaling pathway mediates neuronal apoptosis in ischemic stroke. Aging. 2020;12(4):3156–74.
40. Mehta SL, Kim T, Vemuganti R. Long noncoding RNA fosdt promotes ischemic

brain injury by interacting with REST-associated chromatin-modifying proteins. J Neurosci. 2015 Dec 16;35(50):16443–9.

41. Zhang J, Yuan L, Zhang X, Hamblin MH, Zhu T, Meng F, et al. Altered long non-coding RNA transcriptomic profiles in brain microvascular endothelium after cerebral ischemia. Exp Neurol. 2016 Mar 1;277(January):162–70.
42. Wang J, Zhao H, Fan Z, Li G, Ma Q, Tao Z, et al. Long Noncoding RNA H19 Promotes Neuroinflammation in Ischemic Stroke by Driving Histone Deacetylase 1-Dependent M1 Microglial Polarization. Stroke. 2017 Aug 1;48(8):2211–21.
43. Wang J, Cao B, Han D, Sun M, Feng J. Long non-coding RNA H19 induces cerebral ischemia reperfusion injury via activation of autophagy. Aging Dis. 2017;8(1):71–84.
44. Xu Q, Deng F, Xing Z, Wu Z, Cen B, Xu S, et al. Long non-coding RNA C2dat1 regulates CaMKIIδ expression to promote neuronal survival through the NF-κB signaling pathway following cerebral ischemia. Cell Death Dis. 2016;7(3):1–11.
45. Xin JW, Jiang YG. Long noncoding RNA MALAT1 inhibits apoptosis induced by oxygen-glucose deprivation and reoxygenation in human brain microvascular endothelial cells. Exp Ther Med. 2017 Apr 1;13(4):1225–34.
46. Miyoshi N, Wagatsuma H, Wakana S, Shiroishi T, Nomura M, Aisaka K, et al. Identification of an imprinted gene, Meg3/Gtl2 and its human homologue MEG3, first mapped on mouse distal chromosome 12 and human chromosome 14q. Genes to Cells. 2000;5(3):211–20.
47. Wylie AA, Murphy SK, Orton TC, Jirtle RL. Novel Imprinted DLK1/GTL2 Domain on Human Chromosome 14 Contains Motifs that Mimic Those Implicated in IGF2/H19 Regulation. Genome Res. 2000;10:1711–8.
48. Kagami M, Sekita Y, Nishimura G, Irie M, Kato F, Okada M, et al. Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes. Nat Genet. 2008 Jan 6;40(2):237–42.
49. Howard M, Charalambous M. Molecular basis of imprinting disorders affecting chromosome 14: Lessons from murine models. Vol. 149, Reproduction. BioScientifica Ltd.; 2015. p. R237–49.
50. Chen Y, Gao H, Liu Q, Xie W, Li B, Cheng S, et al. Functional characterization of DLK1/MEG3 locus on chromosome 14q32.2 reveals the differentiation of pituitary neuroendocrine tumors. Aging. 2020 Jan 15;13(1):1422–39.
51. Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, et al. Activation of p53 by MEG3 non-coding RNA. J Biol Chem. 2007;282(34):24731–42.
52. Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: A tumor suppressor. J Mol Endocrinol. 2012;48(3):45–53.
53. 53. Spaethling JM, Na YJ, Lee J, Ulyanova A V., Baltuch GH, Bell TJ, et al. Primary Cell Culture of Live Neurosurgically Resected Aged Adult Human Brain Cells and Single Cell Transcriptomics. Cell Rep. 2017;18:791–803.
54. Alessio E, Bonadio RS, Buson L, Chemello F, Cagnin S. A single cell but many different transcripts: A journey into the world of long non-coding RNAs. International Journal of Molecular Sciences. 2020;21: 302-31.
55. Zhang X, Rice K, Wang Y, Chen W, Zhong Y, Nakayama Y, et al. Maternally expressed gene 3 (MEG3) noncoding ribonucleic acid: Isoform structure, expression, and functions. Endocrinology. 2010;151(3):939–47.
56. Xu W, Dai M, Li F, Liu A. Genomic imprinting, methylation and parent-of-origin effects in reciprocal hybrid endosperm of castor bean. Nucleic Acids Res. 2014;42(11):6987–98.
57. Balik V, Srovnal J, Sulla I, Kalita O, Foltanova T, Vaverka M, et al. MEG3: A novel long noncoding potentially tumour-suppressing RNA in meningiomas. J Neurooncol. 2013;112(1):1–8.
58. Zhang X, Gejman R, Mahta A, Zhong Y, Rice KA, Zhou Y, et al. Maternally expressed gene 3, an imprinted noncoding RNA gene, is associated with meningioma pathogenesis and progression. Cancer Res. 2010;70(6):2350–8.

59. Zhao H, Wang X, Feng X, Li X, Pan L, Liu J, et al. Long non-coding RNA MEG3 regulates proliferation, apoptosis, and autophagy and is associated with prognosis in glioma. J Neurooncol. 2018 Nov 15;140(2):281–8.
60. Liu X, Hou L, Huang W, Gao Y, Lv X, Tang J. The mechanism of long non-coding RNA MEG3 for neurons apoptosis caused by hypoxia: Mediated by miR-181b-12/15-LOX signaling pathway. Front Cell Neurosci. 2016 Sep 2;10.
61. Yan H, Rao J, Yuan J, Gao L, Huang W, Zhao L, et al. Long non-coding RNA MEG3 functions as a competing endogenous RNA to regulate ischemic neuronal death by targeting miR-21/PDCD4 signaling pathway. Cell Death Dis. 2017;8(12):1–15.
62. Wang M, Chen W, Geng Y, Xu C, Tao X, Zhang Y. Long non-coding RNA MEG3 promotes apoptosis of vascular cells and is associated with poor prognosis in ischemic stroke. J Atheroscler Thromb. 2020;27(7):718–26.
63. Liang Z, Chi YJ, Lin GQ, Xiao LF, Su GL, Yang LM. LncRNA MEG3 participates in neuronal cell injury induced by subarachnoid hemorrhage via inhibiting the Pi3k/Akt pathway. Eur Rev Med Pharmacol Sci. 2018;22(9):2824–31.
64. Wang H, Li J, Cheng Y, Yao J. Association of Long-Chain Noncoding RNA H19 and MEG3 Gene Polymorphisms and Their Interaction with Risk of Osteoarthritis in a Chinese Han Population. Genet Test Mol Biomarkers. 2020;24(6):328–337.
65. Zhao F, Xing Y, Jiang P, Hu L, Deng S. LncRNA MEG3 inhibits the proliferation of neural stem cells after ischemic stroke via the miR-493-5P/MIF axis. Biochem Biophys Res Commun. 2021 Sep 3;568:186–92.
66. Chen C, Huang Y, Xia P, Zhang F, Li L, Wang E, et al. Long noncoding RNA Meg3 mediates ferroptosis induced by oxygen and glucose deprivation combined with hyperglycemia in rat brain microvascular endothelial cells, through modulating the p53/GPX4 axis. 2021;65(3):3224.
67. Li TH, Sun HW, Song LJ, Yang B, Zhang P, Yan DM, et al. Long non-coding RNA MEG3 regulates autophagy after cerebral ischemia/reperfusion injury. Neural Regen Res. 2022 Apr 1;17(4):824–31.
68. Ghaedi H, Zare A, Omrani MD, Doustimotlagh AH, Meshkani R, Alipoor S, et al. Genetic variants in long noncoding RNA H19 and MEG3 confer risk of type 2 diabetes in an Iranian population. Gene. 2018;675:265–271.
69. Goovaerts T, Steyaert S, Vandenbussche CA, Galle J, Thas O, Van Criekinge W, et al. A comprehensive overview of genomic imprinting in breast and its deregulation in cancer. Nat Commun. 2018;9(1):1–14.
70. Bayarmaa B, Wu Z, Peng J, Wang Y, Xu S, Yan T, et al. Association of LncRNA MEG3 polymorphisms with efficacy of neoadjuvant chemotherapy in breast cancer. BMC Cancer. 2019;19:877.
71. Ali MA, Shaker OG, Alazrak M, Abdelhafez MN, Khalefa AA, Hemeda NF, et al. Association analyses of a genetic variant in long non-coding RNA MEG3 with breast cancer susceptibility and serum MEG3 expression level in the Egyptian population. Cancer Biomarkers. 2020;28(1):49–63.
72. Cao X, Zhuang S, Hu Y, Xi L, Deng L, Sheng H, et al. Associations between polymorphisms of long non-coding RNA MEG3 and risk of colorectal cancer in Chinese. Oncotarget. 2016;7(14):19053–9. doi: 10.18632/oncotarget.7764.
73. Zhuo ZJ, Zhang R, Zhang J, Zhu J, Yang T, Zou Y, et al. Associations between lncRNA MEG3 polymorphisms and neuroblastoma risk in Chinese children. Aging. 2018;10(3):481–91.
74. Mazraeh SA, Gharesouran J, Ghafouri-Fard S, Ketab FNG, Hosseinzadeh H, Moradi M, et al. Association between WT1 and MEG3 polymorphisms and risk of acute myeloid leukemia. Meta Gene. 2020;23.
75. Kong X, Yang S, Liu C, Tang H, Chen Y, Zhang X, et al. Relationship between MEG3 gene polymorphism and risk of gastric cancer in Chinese population with high incidence of gastric cancer. Biosci Rep. 2020;40(11).
76. Wahba AS, Ibrahim ME, Mesbah NM, Saleh SM, Abo-elmatty DM, Mehanna ET. Long non-coding RNA MEG3 and its genetic variant rs941576 are associated with rheumatoid arthritis pathogenesis in Egyptian patients. Arch Physiol Biochem. 2020;