European Review for Medical and Pharmacological Sciences 2020; 24: 12324-12333

LncRNA LncOGD-1006 alleviates OGD-induced ischemic brain injury regulating apoptosis through miR-184-5p/CAAP1 axis

J.-Y. CHEN[1], H. CHEN[2], T. LI[3], L. YANG[4], X.-M. YE[5], W.-Y. GAO[6], S.-P. ZHANG[1], L. ZONG[7]

[1]Department of Neurology, Guangzhou Red Cross Hospital, Medical College, Jinan University, Guangzhou, China
[2]Department of Neurology, Affilicated Jinan Third Hospital of Jining Medical University, Jinan City, Shandong Province, China
[3]Department of Neurology, Xiangyang Central Hospital, Xiangyang City, Hubei, China
[4]Department of Neurology, Chongqing Emergency Medical Center, The Affiliated Central Hospital to Chongqing University, Guangzhou, China
[5]Wound and Scar Treatment Center, Guangzhou Red Cross Hospital,Medical College, Jinan University
[6]Department of Neurology, Wuhan Hankou Hospital, Guangzhou, China
[7]Department of Internal Medicine, Skin Disease and Venereal Disease Prevention and Control Hospital in Zaozhuang City, Guangzhou, China

J.-Y. Chen, H. Chen, and T. Li are co-first authors

Abstract. – OBJECTIVE: This study aimed to explore the effect of long non-coding RNA (LncRNA) LncOGD-1006 to ischemic stroke and the possible mechanism.

MATERIALS AND METHODS: The primary brain microvascular endothelial cells (bEnd.3) of oxygen-glucose deprivation (OGD) was used as a mimic of ischemic stroke *in vitro*.

RESULTS: The results showed that LncOGD-1006 was upregulated in bEnd.3 after OGD-induced.

CONCLUSIONS: LncOGD-1006 might act as a ceRNA to inhibit apoptosis in bEnd.3 cells by targeting miR-184-5p/CAAP1 pathway.

Key Words:
MiRNA-106, Pediatric osteosarcoma, PI3K/AKT signaling pathway.

Introduction

Stroke is one of the main cerebrovascular diseases that cause human death and disability in the world, so as to seriously endanger human health[1]. However, the molecular mechanism of ischemic stroke has not yet been fully understood and the pathogenesis of brain ischemia is still worth exploring. Brain ischemia could be caused by a thrombus, embolus occlusion, or hemorrhage due to rupture of the blood vessels, and then, these might result in ischemia stroke which could further reduce and block the blood supply of brain tissues[2]. A few minutes after the onset of the stroke, the sudden decrease in blood flow lead to irreversible damage and subsequent cell death. Furthermore, apoptosis may be the main reason caused brain microvascular endothelial cells death. Therefore, it is urgent for us to detect the mechanism of neuronal apoptosis, which may help us to find new therapies to alleviate brain damage resulting from ischemic stroke.

Although long non-coding RNAs (LncRNAs), cannot code protein, they play a key role in the regulation in different process of cells, including chromatin modification, transcription, and post-transcriptional processing[3,4]. At present, the researches of LncRNAs mainly focus on tumor and neurodegenerative diseases, but increasing investigations indicate that LncRNAs might have a great impact on the pathogenesis of brain injury. Recently, there are many kinds of literature on stroke response changes in LncRNAs and some researchers have highlighted the importance of LncRNAs in the pathogene-

Corresponding Author: Lan Zong, MD; e-mail: iiorfguj@163.com

sis of ischemic stroke[5-8]. Whereas, the molecular mechanisms of LncRNAs in ischemic stroke is still unclear. In particular, the influence of LncRNAs in cerebrovascular endothelial biology and pathophysiology in ischemic stroke is unknown and worthy of investigation.

In this study, bioinformatics analysis revealed a possible binding site for LncOGD-1006 and miR-184-5p, which suggest that LncOGD-1006 may have a function as a sponge to miR-184-5p. Subsequently, we examined the expression levels of LncOGD-1006 and miR-184-5p in brain microvascular endothelial cells (bEnd.3) after oxygen-glucose deprivation (OGD). The relationship between LncOGD-1006 and miR-184-5p was determined by RNA-RNA pull-down assay and Dual-Luciferase reporter assay. Besides, we found that the conserved anti-apoptotic protein 1 (CAAP1) was a target gene of miR-184-5p. These results suggested the LncOGD-1006 might act as a ceRNA to inhibit apoptosis in bEnd.3 cells by targeting miR-184-5p/ CAAP1 pathway.

Materials and Methods

Materials and Reagents

Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), and Trypsin-EDTA were purchased from HyClone company (South Logan, UT, USA). Cell Counting Kit-8 (CCK-8) was obtained from Dojindo Laboratories (Kumamoto, Japan). TRIzol reagent and Lipofectamine 3000 and pcDNA3.1 purchased from Invitrogen (Carlsbad, CA, USA).

Cell Culture and OGD Treatment

The bEnd.3 brain microvascular endothelial cell line was purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle Medium (DMEM). Oxygen and glucose deprivation was performed in bEnd.3 cells as previously described[9].

Cell Viability Assay

The effect of siRNA on the cell viability was assessed by the Cell Counting Kit-8 (CCK-8). Briefly, the experimental method was described as follows: cells (about 10^4/well) were seeded on 96-well plates for 24 h, and then washed with PBS twice to remove the media. The sample had been incubating at 37°C for 3 h after adding 900 μL serum-free DMEM medium and 100 μL CCK-8 solution. Absorbance at 450 nm was measured using a microplate reader (BioTek, Winooski, VT, USA).

Cell Apoptosis Assay

Apoptosis of bEnd.3 cells were examined by Annexin V-FITC/PI Apoptosis Detection kit (Beyotime, Shanghai, China). The bEnd.3 cells were harvested and washed with PBS twice after intervention as they were seeded on 6-well plates with 6×10^5 cells per well and incubated in 37°C for 24h. Aside from that, these cells were strained by 5 μL Annexin-V FITC and 5 μL PI for 15 min at room temperature in the dark. Finally, the cells were washed using PBS twice and then the apoptotic bEnd.3 cells were analyzed by flow cytometer (BD, Franklin Lake, NJ, USA).

Cell Transfection

MiR-184-5p mimics, NC mimics, miR-184-5p inhibitor, and NC inhibitor were acquired from GenePharma (Shanghai, China), si-LncOGD-1006-1, si-LncOGD-1006-2, and si-NC were purchased from Samgon (Shanghai, China). The bEnd.3 cells were seeded on the 6-well plates with 5×10^5 cells for 24 h and transfected with si-LncOGD-1006-1, si-LncOGD-1006-2, or miR-184-5p mimics, miR-184-5p inhibitor or overexpressed pcDNA3.1(+)-LncOGD-1006 plasmid by Lipofectamine 3000. The si-NC, NC mimics, NC inhibitor, and pcDNA3.1(+) vector were negatively controls, respectively.

Quantitative Real-Time PCR

The total RNA was extracted from bEnd.3 cell lines using TRIzol reagent (Invitrogen, Shanghai, China). The cDNAs were synthesized using First-Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. qRT-PCR was conducted to investigate the relative expression level in a real-time thermal cycler Quantstudio 6 Flex (ABI, Waltham, MA, USA) according to a previous protocol[5]. To be specifically, the procedures of qRT-PCR were as follows: an initial denaturation was set at 94 °C for 3 min. After that, there would be 40 cycles of 94°C for 10 s and 60 °C for 1 min. Also, the melting curve analysis from 65°C to 95°C. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression level of LncOGD-1006, miR-184-5p, and CAAP1. The algorithm of $2^{-\Delta\Delta CT}$ was applied to analyze the expression level[6]. An unpaired

t-test was used to compare the data. The primers were showed in Table I.

Western Blot Assay

The proteins were isolated with an Extraction Kit from Eubiotech Co.Ltd. (Shanghai, China). The 12.5% SDS-PAGE was used in this experiment according to the previous report[7]. After the proteins were transferred into a polyvinylidene fluoride membranes, the membrane was blocked with 5% skimmed milk in TBS, and then, incubated with primary polyclonal antibodies against anti-cleaved-caspase3 (CST, Boston, Mass, USA, 1:1000), anti-BAD (Abcam, London, UK, 1:1000), anti-Bcl-2 (Abcam, London, UK, 1:1000), anti-CAAP1 (Abcam, London, UK, 1:1000), and anti-GAPDH (CST, Boston, MA, USA, 1:1000) at 4°C overnight. After membranes were washed four times with TBS containing 0.05% Tween-20, they were incubated with mouse anti-rabbit secondary antibodies (MR, Shanghai, China) for 2 h. Bound antibodies were visualized with an ECL Western Blotting Reagent Kit.

RNA-Fluorescence In Situ Hybridization

Fluorescence in situ hybridization assay was based on our previous studies[8]. In brief, the bEnd.3 cells were dropped on slides and dehydrated in 70% ethanol overnight at 4°C. Then, the slides were incubated in 2 × SSC buffers (15 mM sodium citrate, 150 mM sodium chloride, pH 7.5) with pH = 7.6 for 30 min at 37°C. The miR-184-5P probe for 5 h at 37°C. Followed by being washed with PBS, the bEnd.3 cells were labeled with 4,6-diamidino-2-phenylindole (DAPI) for 5 min. The images were observed with confocal microscopy.

Table I. The primer sequences for qRT-PCR.

Primers	Sequences (5′-3′)
Lnc-OGD 1006F	ACGTGTCTTGAGATGCCAAA
Lnc-OGD 1006R	TCCTCTCCCTCTTCCTCTCTC
CAAP1F	ATAGAAGGCCCATGCAACGAA
CAAP1R	TCTGACCAGCTTCACTTTGGA
β-actinF	CTCCATCCTGGCCTCGCTGT
β-actinR	GCTGTCACCTTCACCGTTCC
miR-184-5pF	TGGACGGAGAACTGATAAGGGT
REVERSE	CCTTATCAGTTCTCCGTCCATT
U6F	CTCGCT TCGGCAGCACA
U6R	AACGCTTCACGAATTTGCGT

Dual-Luciferase Reporter Assay

The putative binding sites between LncOGD-1006 and miR-184-5P (or miR-184-5P and CAAP1) were predicted using TargetScans (http://www.targetscan.org/vert_72/) analysis. The bEnd.3 cells were co-transfected with wild-type (wt) or point mutant type (mut) pmirGLO-LncOGD-1006 3′ UTR and miR-184-5P mimics (pmirGLO-CAAP1 3′ UTR and miR-184-5P) or NC mimics using Lipofectamine 3000 reagent in compliance to standard procedures. 72 h later, the Luciferase activity assay was measured by a Dual-Luciferase reporter assay kit.

RNA Pull-Down Assay

To further confirm the relationship between LncOGD-1006 with miR-184-5P, bEnd.3 cells were co-transfected miR-184-5P with LncOGD-1006-sense probe and LncOGD-1006-antisense probe (synthesized by Shanghai Gene Pharma Co. Ltd, China) according to the manufacturer's instruction. Cell lysate was collected after transfection 48 hours and with M-280 streptavidin magnetic beads as previously reported[9]. The bound RNAs were analyzed by qRT-PCR.

Statistical Analysis

All experiments in this study were in the triplicate. The results of multiple experiments are presented as the mean ± standard deviation (SD). Statistical comparisons (*p*-values) were calculated using one-way analysis of variance (ANOVA). Probability values of $p<0.05$ were considered indicating a significant difference.

Results

LncOGD-1006 Expression Was Upregulated by in Cellular Model of bEnd.3

According to the previous study, LncOGD-1006 was upregulated in brain microvascular endothelial cells after oxygen-glucose deprivation (OGD)[10]. In the present study, the OGD model was established to identify the role of LncOGD-1006 in bEnd.3 cells. As shown in Figure 1A, the mRNA expression of LncOGD-1006 was upregulated by OGD. Stimulation of bEnd.3 cells with OGD-treatment did show a significant increase in LncOGD-1006 expression from 4 h to 48 h (Figure 1B).

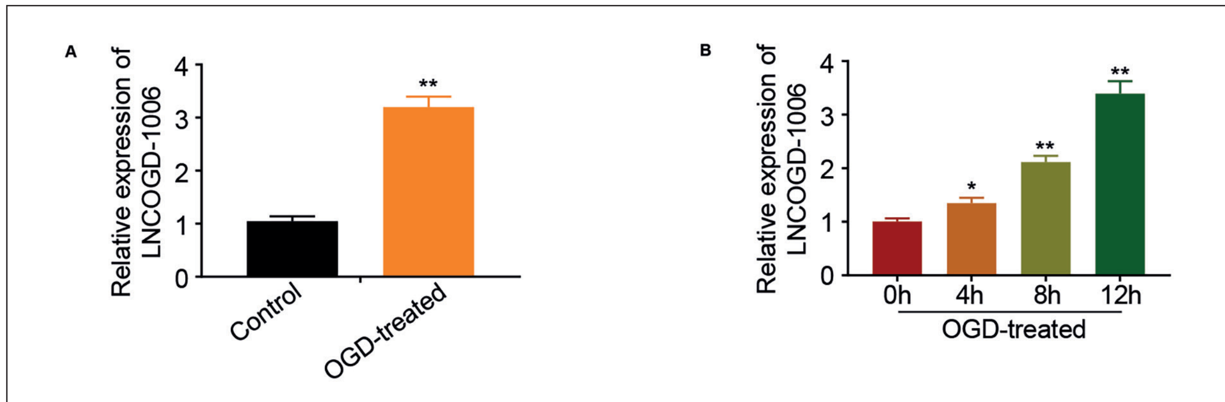

Figure 1. Preliminary analysis of LncOGD-1006. The expression of LncOGD-1006 was increased while OGD-induced (**A**). The LncOGD-1006 expression level was upregulated with time development of OGD treatment (**B**).

Knockdown of LncOGD-1006 Promotes Cell Apoptosis

Based on the result above, LncOGD-1006 was assumed to play an important role in OGD-induced ischemic stroke. To investigate the role of LNCOGD in ischemic stroke, we designed two pairs of si-LNCODGs (si-LncOGD-1006-1 and si-LncOGD-1006-2) to knockdown LncOGD-1006 in bEnd.3 cells. The results showed that the expression of LncOGD-1006 down-regulated by si-LncOGD-1006-1 and si-LncOGD-1006-2 (Figure 2A). The viability of OGD-induced bEnd.3 cells was decreased after tranfecting two si-LNCODGs (Figure 2B). Specifically, apoptosis rate was difference between the two interfered groups and the control group, and the bEnd.3 cells apoptosis rate was significantly increased by si-LncOGD-1006-1 and

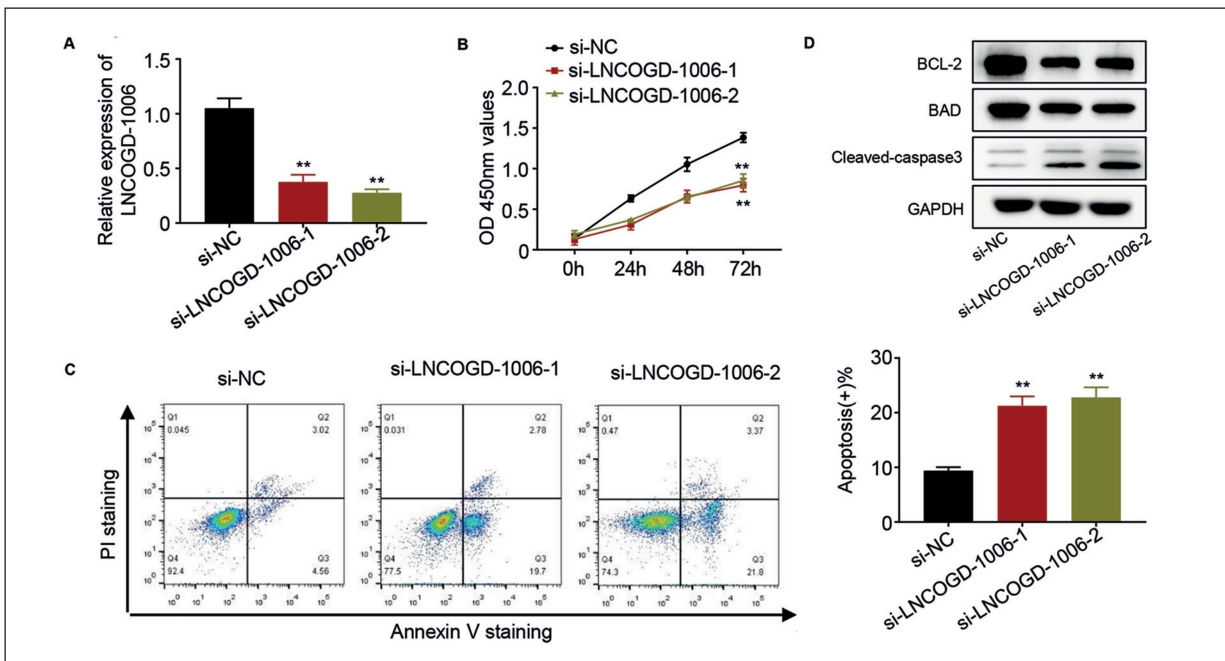

Figure 2. Knockdown of LncOGD-1006 suppress bEnd.3 apoptosis. Knockdown of LncOGD-1006 was verified via RT-PCR (**A**). Cell viability was increased with LncOGD-1006 interfered (**B**). The bEnd.3 apoptosis rate was increased analyzed by annexin V-FITC/PI Apoptosis detection kit (**C**). Apoptosis-associated proteins were detected by WB (**D**).

si-LncOGD-1006-2 (Figure 2C). Further analysis showed that the expression of BCL-2 and BAD proteins were downregulated after knocking down LncOGD-1006, but cleaved-caspase3 protein was upregulated (Figure 2D). Overall, these results revealed that inhibition of LncOGD-1006 promote the apoptosis in OGD-induced bEnd.3 cells.

Overexpression of LncOGD-1006 Inhibits Apoptosis

In order to further clarify the possible mechanism of LncOGD-1006 in OGD-induced ischemic stroke, we constructed pcDNA3.1(+)-LncOGD-1006 plasmid, and then, transfected this plasmid into bEnd.3 cells. The results can be seen in Figure 3A as the expression of LncOGD-1006 was significantly upregulated. The viability of bEnd.3 cells transfected with pcDNA3.1(+)-LncOGD-1006 plasmid was significantly increased (Figure 3B). Moreover, apoptosis rate was clearly decreased between LncOGD-1006 overexpression group with control group (pcDNA3.1(+) group) (Figure 3C). To further analyze apoptosis mechanism, BCL-2, BAD and cleaved-caspase3 proteins were detected by Western blot. The results showed that expression of BCL-2 and BAD proteins were upregulated, while cleaved-caspase3 protein was downregulated (Figure 3D).

LncOGD-1006 Is One of Targets for MiR-184-5p

To explore the detailed mechanism of LncOGD-1006 in OGD-induced ischemic stroke. Firstly, nuclear and cytoplasm were separated and analyzed, the results demonstrated that the expression of LncOGD-1006 was significantly higher in cytoplasm than that in nuclear (Figure 4A). Secondly, we investigated the distribution of LncOGD-1006 on bEnd.3 cells by RNA-FISH. As shown in Figure 4B, LncOGD-1006 was mainly expressed in the cytoplasm in bEnd.3 cells. The expression of candidate target miR-184-5p of LncOGD-1006 was assessed by miRanda analysis (Figure 4C). By comparing binding sites of LncOGD-1006 and miR-184-5p, the results depicted that LncOGD-1006 and miR-184-5p had an overlapping sequence on 5′-UTR of miR-184-5p region. RNA-pulldown proved that LncOGD-1006-sense probes might be enriched in miR-184-5p comparing with the LncOGD-1006-antisense probe (Figure 4D). After that, Luciferase reporter assays suggested that the miR-184-5p mimic

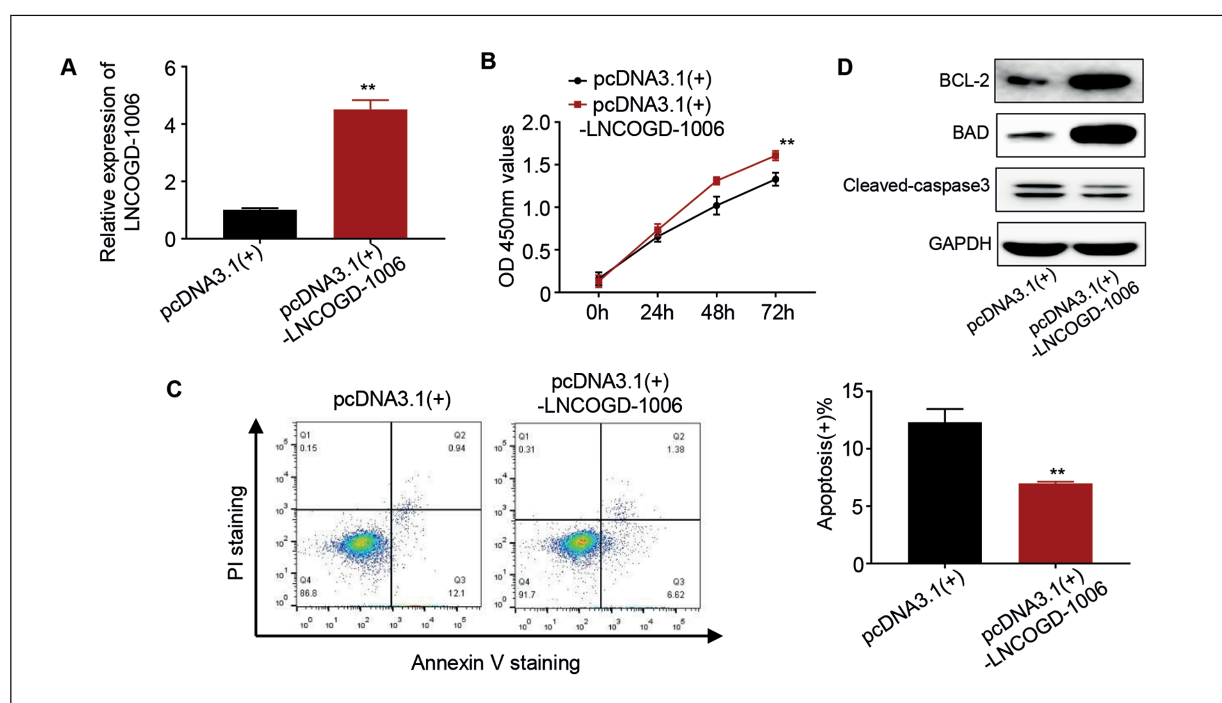

Figure 3. Overexpression of LncOGD-1006 activate bEnd.3 apoptosis. Overexpression of LncOGD-1006 was verified *via* RT-PCR (**A**). Cell viability was decreased with LncOGD-1006 overexpressed (**B**). The bEnd.3 apoptosis rate was decreased analyzed by annexin V-FITC/PI Apoptosis detection kit (**C**). Apoptosis-associated proteins were detected by WB (**D**).

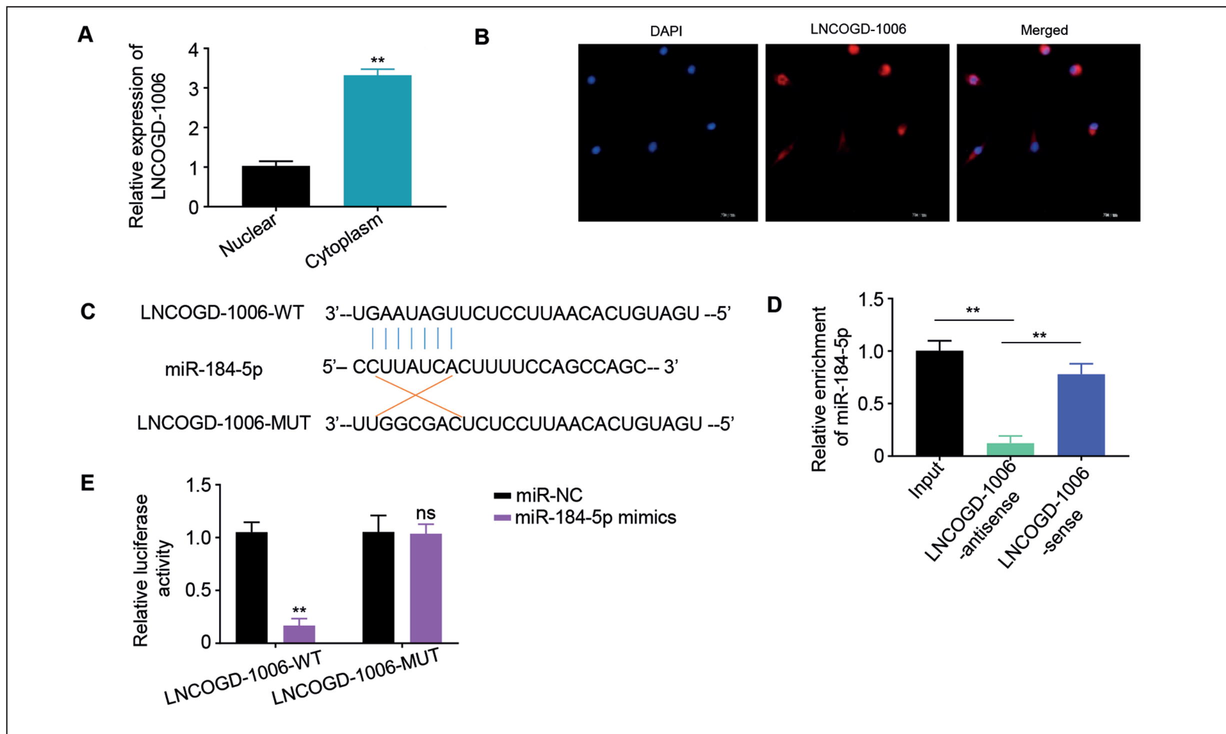

Figure 4. The relationship between LncOGD-1006 with miR-184-5p. LncOGD-1006 was located on cytoplasm via RT-PCR (**A**). LncOGD-1006 was mainly expressed in the cytoplasm detected by Immunofluorescence. Images magnification ×40 (**B**). The potential binding sequence between LncOGD-1006 and miR-184-5p (**C**). RNA-pulldown proved that LncOGD-1006-sense probes may be enriched miR-184-5p (**D**). The miR-184-5p decreased LncOGD-1006 mimic luciferase activities through Luciferase reporter assays (**E**).

significantly decreased Luciferase activities of pmirGLO-LncOGD-1006-WT reporter, but not of pmirGLO-LncOGD-1006-MUT reporter (Figure.4E).

LncOGD-1006 Functions as a ceRNA for MiR-184-5p

The next question was about miR-184-5p. We suspected that the miR-184-5p expression was affected by LncOGD-1006. Afterward, miR-184-5p was downregulated as expected when LncOGD-1006 was overexpressed by transfecting pcDNA3.1(+)-LncOGD-1006 plasmid (Figure 5A). Moreover, miR-184-5p was upregulated when LncOGD-1006 was knocked down by si-LncOGD-1006s (Figure 5B). Further analysis showed that the apoptosis rate was greatly decreased while bEnd.3 cells transfected to pcDNA3.1(+)-LncOGD-1006 + scramble, but the apoptosis rate was recovered by pre-treated with miR-184-5p mimics (Figure.5C). One of the mechanisms of cytoplasmic LncRNAs as competitive endogenous RNAs (ceRNAs), which sponge various miRNAs to dampen their regulatory effects on target mRNAs[11]. It suggested that LncOGD-1006 should be a ceRNA for miR-184-5p to regulate bEnd.3 cells apoptosis.

LncOGD-1006 Functions as a Sponge RNA for MiR-184-5p to Facilitate CAAP1 Expression

We hypothesized that LncOGD-1006 affected one (some) target protein expression through miR-184-5p. In order to confirm the molecular mechanism, bioinformatic analysis demonstrated that there was an overlapping area between miR-184-5p and CAAP1-WT, but not CAAP1-MUT (Figure 6A). Next, the relative expression of CAAP1 was upregulated while miR-184-5p was blocked by miR-184-5p inhibitor1 or inhibitor2 (Figure 6B). Likewise, the CAAP1 expression was significantly downregulated by transfecting miR-184-5p mimics (Figure 6C). Meanwhile, Luciferase reporter assays presented that the miR-184-5p mimic significantly reduced the Luciferase activities of pmir-

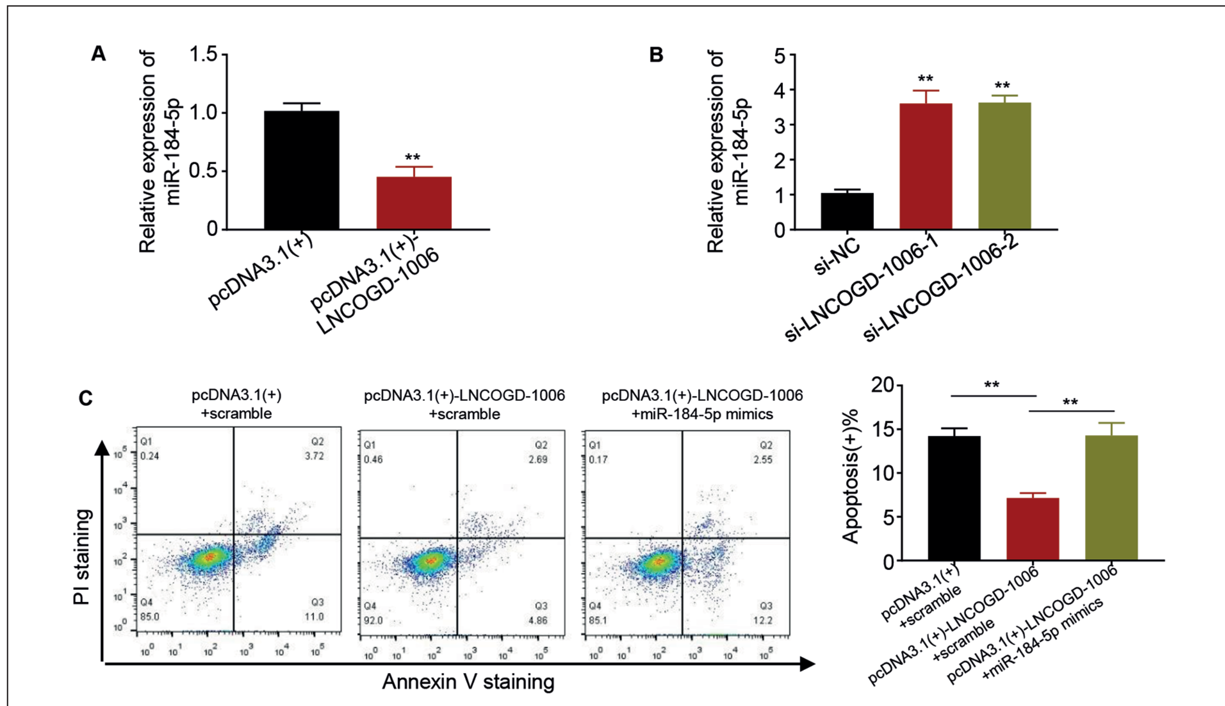

Figure 5. LncOGD-1006 negatively regulate miR-184-5p. The expression level of miR-184-5P was measured by RT-PCR with LncOGD-1006 overexpressed (**A**) or interfered (**B**). the apoptosis rate was detected with LncOGD-1006 overexpressing or blocked by miR-184-5p mimics (**C**).

GLO-CAAP1-WT reporter, but not those of pmirGLO-CAAP1-MUT reporter (Figure 6D). Moreover, CAAP1 was significantly downregulated while LncOGD-1006 was blocked by transfecting si-LncOGD-1006-1. On the other hand, it was upregulated while pretreated with miR-184-5p inhibitor (Figure 6E). However, CAAP1 could be downregulated while pretreated with miR-184-5p mimics (Figure 6F) and was significantly upregulated while LncOGD-1006 was over-expressed by transfecting pcDNA3.1(+)-LncOGD-1006 plasmid. Besides, the results also showed that CAAP1 was increased by OGD challenging (Figure 6G). After that, apoptosis rate was detected by Annexin V-FITC/PI apoptosis detection kit while CAAP1 was knocked down by transfecting si-CAAP1-1 and si-CAAP1-2. As shown in Figure 6H, the si-CAAP1-1 and si-CAAP1-2 groups reported significantly more decreased trends than control group to further analyze apoptosis mechanism, apoptosis-associated protein was analyzed by WB, showing that expression of BCL-2 and BAD proteins were up-regulated while cleaved-caspase3 protein was down-regulated (Figure 6I).

Discussion

In previous studies, there are many LncRNAs identified in brain ischemic stroke using RNA-Seq analysis[9,12-16]. In the present research, we detected the function and explored the possible mechanism of LncOGD-1006 in brain ischemic stroke. The results detected that bEnd.3 responded to ischemic attack with decreased LncOGD-1006 expression, demonstrating a potential functional role of LncOGD-1006 in the pathogenesis of ischemic stroke. The expression or dysfunction of LncRNA is closely related to the occurrence of human diseases, including cancer, neurodegenerative diseases, and many other serious diseases strongly affecting human health[17-20]. In this study, we found that the expression level of LncOGD-1006 in bEnd.3 cells for 12h on oxygen-glucose deprivation model in *vitro*. CCK-8, Annexin V-FITC and apoptosis-associated proteins were also detected to assess the function of LncOGD-1006. The results showed that the apoptosis rates and cell death rates were significantly increased with knockdown of LncOGD-1006, and decreased with overexpression of LncOGD-1006. We further evaluated the role and mechanism of LncOGD-1006/miR-184-

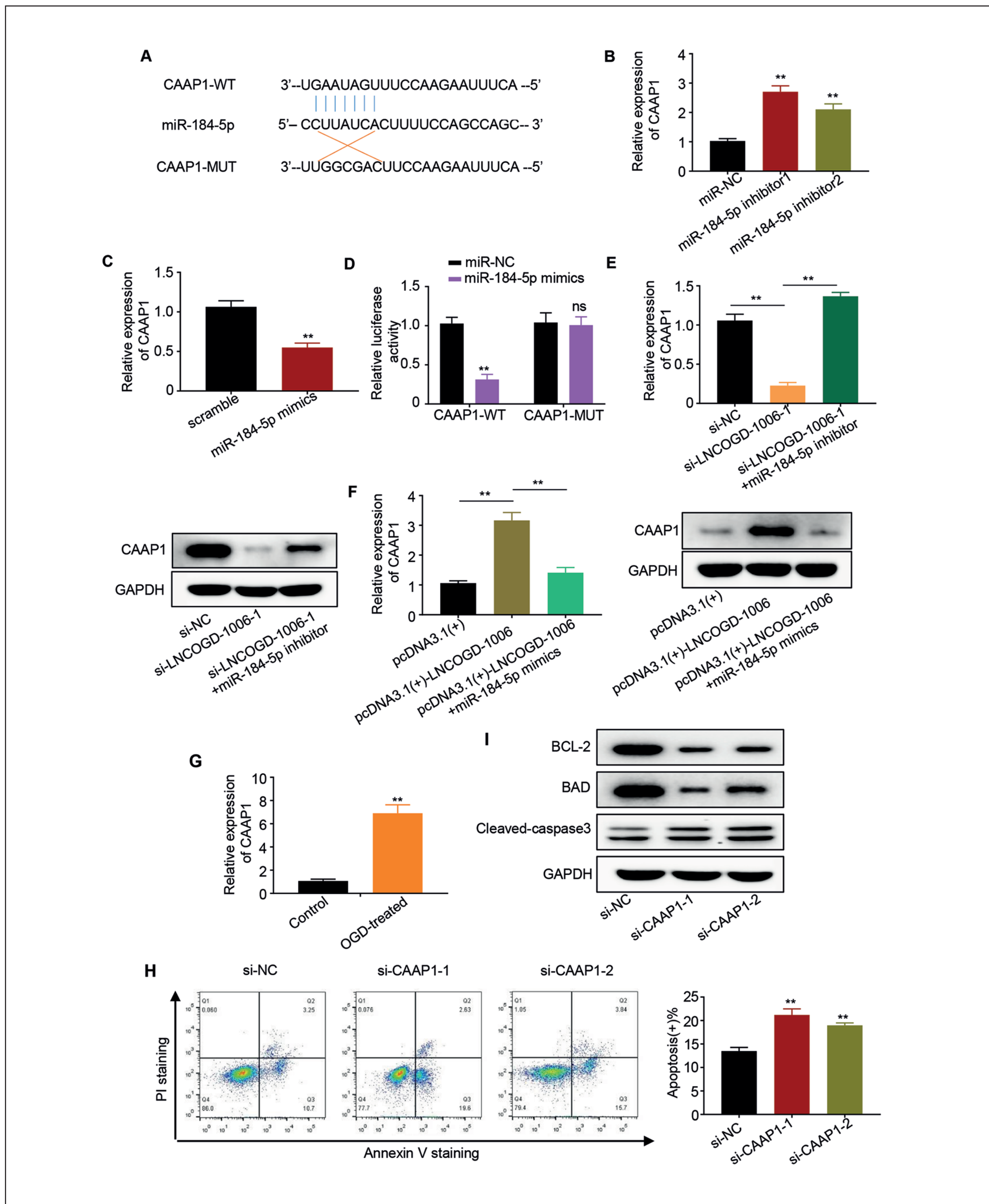

Figure 6. LncOGD-1006/miR-184-5p/CAAP1 axis in OGD injury. The potential binding sequence between miR-184-5p and CAAP1 (**A**). The expression level of CAAP1 was measured by RT-PCR with miR-184-5P interfered (**B**) or overexpressed (**C**). The CAAP1 decreased miR-184-5p mimic luciferase activities through luciferase reporter assays (**D**). The expression of CAAP1 was detected by RT-PCR or WB with si-LncOGD-1006-1 interfered and blocked by miR-184-5p inhibitor (**E**) or overexpressed and blocked by miR-184-5p mimics (**F**). the mRNA relative expression of CAAP1 was up-regulated via RT-PCR on OGD-induced (**G**). The bEnd.3 apoptosis rate was decreased analyzed by annexin V-FITC/PI Apoptosis detection kit (**H**). Apoptosis-associated proteins were detected by WB (**I**).

5p/CAAP1 axis in ischemic stroke. It may play a potential role in therapeutic intervention of ischemic stroke.

MicroRNA has been applied in many different biological processes, including cell cycle[21], translation regulation[22], methylation[23], ubiquitination[24], and apoptosis[25], creating great achievements[26,27]. Our results showed that LncOGD-1006 located on the membrane and targeted to miR-184-5p. In the meanwhile, we also found that the knockdown of LncOGD-1006 could increase the expression level of miR-184-5p, while overexpression of LncOGD-1006 could decrease the miR-184-5p, suggesting LncOGD-1006 had a negative regulation on expression of miR-184-5p. We also proved that apoptosis rate was blocked by miR-184-5p mimics in LncOGD-1006 interfered group. It suggested that there is endogenous competition for bEnd.3 apoptosis between LncOGD-1006 with miR-184-5p.

To further certify the role of LncOGD-1006 in ischemic stroke, the expression of CAAP1 was identified to be upregulated in miR-184-5p knockdown groups and downregulated in miR-184-5p mimics groups. The expression of CAAP1 was blocked by miR-184-5p and LncOGD-1006 recovered the effect of CAAP1 on bEnd.3 cells. In addition, the effect of CAAP1 on bEnd.3 apoptosis significantly increased by knockdown CAAP1. The data are in accordance with our hypothesis and it might be tested that LncOGD-1006 could attenuate bEnd.3 apoptosis by making miR-184-5p a sponge and inhibiting its function of antagonizing CAAP1.

Conclusions

We demonstrated a new mechanism for LncOGD-1006 as a ceRNA targeting miR-184-5p and CAAP1 in cerebral ischemic stroke. We performed a series of comprehensive experiments to explore the association between LncOGD-1006 and brain ischemic injury, and finally found that LncOGD-1006 antagonized miR-184-5p by sponging miR-184-5p who could inhibit the CAAP1 transcription and deteriorate brain ischemic injury. The current research may provide a new intervention target for inhibiting or reversing ischemic stroke injuries.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

The present study was funded by Natural Science Foundation of Guangdong Province (2015A030313737), Guangdong Science and Technology Planning Project (2014A020212652) and Xiangyang Key Science and Technology Project ([2019]-5-24).

Authors' Contribution

JC acquired and analyzed the data, and wrote the main manuscript text. LY contributed to the data analysis and data interpretation. XY designed the study, interpreted the data, and provided archive data. WG contributed to the design of the study and data acquisition, SZ edited the manuscript. LZ prepared the manuscript. All authors read and approved the final manuscript.

References

1) Claiborne SJ, Shanthi M, Mathers CD. Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. Lancet Neurol 2009; 8: 345-354.
2) Li LP, David W, Wong KSL, Wang YJ. Stroke and stroke care in China: huge burden, significant workload, and a national priority. Stroke 2011; 42: 3651-3654.
3) Radak D, Katsiki N, Resanovic I, Jovanovic A, Sudar-Milovanovic E, Zafirovic S, Mousa SA, Isenovic ER. Apoptosis and acute brain ischemia in ischemic stroke. Curr Vasc Pharmacol 2017; 15: 115-122.
4) Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 2013; 20: 300-307.
5) Chen Y, Zhou J. LncRNAs: macromolecules with big roles in neurobiology and neurological diseases. Metab Brain Dis 2017; 32: 281-291.
6) Yan H, Rao J, Yuan J, Gao L, Huang W, Zhao L, Ren J. 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: 3211.
7) Wang H, Liao S, Li H, Chen Y Yu J. Long Non-coding RNA TUG1 sponges mir-145a-5p to regulate microglial polarization after oxygen-glucose deprivation. Front Mol Neurosci 2019; 12: 215.
8) Guo D, Ma J, Yan L, Li T, Li Z, Han X Shui S. Down-regulation of Lncrna MALAT1 attenuates neuronal cell death through suppressing Beclin1-dependent autophagy by regulating Mir-30a in cerebral ischemic stroke. Cell Physiol Biochem 2017; 43: 182-194.
9) Zhang J, Yuan L, Zhang X, Hamblin MH, Zhu T, Meng F, Li Y, Chen YE, Yin KJ. Altered long non-coding RNA transcriptomic profiles in brain microvascular endothelium after cerebral ischemia. Exp Neurol 2016; 277: 162-170.

10) Li XC, Zhou J, Zhou JF, Wang Y, Ma H, Wang Y, Zhao S, Fang WH. SpBark suppresses bacterial infection by mediating hemocyte phagocytosis in an invertebrate model, Scylla paramamosain. Front Immunol 2019; 10: 1992.
11) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
12) Zhou J, Zhao S, Fang WH, Zhou JF, Zhang JX, Ma H, Lan JF, Li XC. Newly identified invertebrate-type lysozyme (Sp lys-i) in mud crab (Scylla paramamosain) exhibiting muramidase-deficient antimicrobial activity. Dev Comp Immunol 2017; 74: 154-166.
13) Gong Y, Ju C, Zhang X. Shrimp miR-1000 functions in antiviral immunity by simultaneously triggering the degradation of two viral mRNAs. Front Immunol 2018; 9: 2999.
14) Xiao Z, Qiu Y, Lin Y, Medina R, Zhuang S, Rosenblum JS, Cui J, Li Z, Zhang X. Blocking lncRNA H19-miR-19a-Id2 axis attenuates hypoxia/ischemia induced neuronal injury. Aging 2019; 11: 3585-3600.
15) Yang XZ, Cheng TT, He QJ, Lei ZY, Chi J, Tang Z, Liao QX, Zhang H, Zeng LS. LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/beta-catenin pathway. Mol Cancer 2018; 17: 126.
16) Guo X, Yang J, Liang B, Shen T, Yan Y, Huang S, Zhou J, Huang J, Gu L. Identification of novel LncRNA biomarkers and construction of LncRNA-related networks in Han Chinese patients with ischemic stroke. Cell Physiol Biochem 2018; 50: 2157-2175.
17) Mishra S, Verma SS, Rai V, Awasthee N, Chava S, Hui KM, Kumar AP, Challagundla KB, Sethi G. Long non-coding RNAs are emerging targets of phytochemicals for cancer and other chronic diseases. Cellular and Molecular Life Sciences 2019; 76: 1947-1966.
18) Zhu L, Yang N, Chen J, Zeng T, Yan S, Liu Y, Yu G, Chen Q, Du G, Pan W, Li X, Zhou H, Huang A, Tang H. LINC00052 upregulates EPB41L3 to inhibit migration and invasion of hepatocellular carcinoma by binding miR-452-5p. Oncotarget 2017; 8: 63724-63737.
19) He B, Bai Y, Kang W, Zhang X, Jiang X. LncRNA SNHG5 regulates imatinib resistance in chronic myeloid leukemia via acting as a CeRNA against MiR-205-5p. Am J Cancer Res 2017; 7: 1704-1713.
20) Zhang X, Liu Z, Shu Q, Yuan S, Xing Z, Song J. LncRNA SNHG6 functions as a ceRNA to regulate neuronal cell apoptosis by modulating miR-181c-5p/BIM signalling in ischaemic stroke. J Cell Mol Med 2019; 23: 6120-6130.
21) Ke S, Li RC, Lu J, Meng FK, Feng YK and Fang MH. MicroRNA-192 regulates cell proliferation and cell cycle transition in acute myeloid leukemia via interaction with CCNT2. Int J Hematol 2017; 106: 258-265.
22) Zhang T, Liu C, Chi L. Suppression of miR-10a-5p in bone marrow mesenchymal stem cells enhances the therapeutic effect on spinal cord injury via BDNF. Neurosci Lett 2019; 714: 134562.
23) Hu T, Chong Y, Cai B, Liu Y, Lu S, Cowell JK. DNA methyltransferase 1-mediated CpG methylation of the miR-150-5p promoter contributes to fibroblast growth factor receptor 1-driven leukemogenesis. J Biol Chem 2019; 294: 18122-18130.
24) Li S, Wang L, Fu B, Berman MA, Diallo A, Dorf ME: TRIM65 regulates microRNA activity by ubiquitination of TNRC6. P Natl A Sci 2014; 111: 6970-6975.
25) Liu S, Wang H, Mu J, Wang H, Peng Y, Li Q, Mao D, Guo L. MiRNA-211 triggers an autophagy-dependent apoptosis in cervical cancer cells: regulation of Bcl-2. Arch Pharmacol 2020; 393: 359-370.
26) Li P, Teng F, GAO F, Zhang M, Wu J, Zhang C. Identification of circulating microRNAs as potential biomarkers for detecting acute ischemic stroke. CelL Mol Neurobiol 2015; 35: 433-447.
27) Liu Y, Ma Y, Zhang B, Wang SX, Wang XM, Yu JM. Genetic polymorphisms in pre-microRNAs and risk of ischemic stroke in a Chinese population. J Mol Neurosci 2014; 52: 473-480.