LNC01296 Regulates Apoptosis Genes Birc2 and Bak1 by Targeting miRNA-29c and Participates in Neuroprotection During Cerebral Ischemia/Reperfusion Injury in Rats

Lingjie MENG¹, Yuanpu QI¹, Ke DU¹, Xiaoyu ZHANG²

¹Zhengzhou Railway Vocational and Technical College, Rehabilitation and Treatment Major, Zhengzhou, Henan, China
²Fuzhou Medical College of Nanchang University, Fuzhou, Jiangxi, China

Corresponding author: Lingjie MENG ☉ menglingjiee@126.com

ABSTRACT

AIM: To explore the extent to which LNC01296 inhibits the miRNA-29c expansion genes Birc2 and Bak1 from causing damage induced by brain expansion and reimplantation.

MATERIAL and METHODS: A total of 120 adult male experimental rats were divided to verify the effects of miRNA-29c and LNC01296 on brain expansion/reimplantation injury.

RESULTS: miRNA-29c can inhibit the Birc2/Bak1 pathway and aggravate the brain expansion/reimplantation damage. LNC01296 blocks miRNA-29c from entering the brain to protect it from expansion after reimplantation.

CONCLUSION: Our findings show that LINC01296 can alleviate the injury induced by cerebral ischemia and reimplantation by preventing the inhibitory effect of miR-29c on Birc2 and Bak1. Our research also provides new strategies and goals for the clinical treatment of patients with cerebral ischemia-reperfusion.

KEYWORDS: LNC01296, miRNA-29c, Apoptotic gene, Cerebral ischemia–reperfusion injury, Neuroprotection


INTRODUCTION

Cerebral ischemia–reperfusion injury (CIRI) refers to cerebral ischemia. If blood perfusion in the ischemic area is not restored within a certain period of time, the brain function does not recover. It is further aggravated, causing related functional damage (21). Cerebral ischemia–reperfusion accounts for >80% of ischemic stroke and is also the cause of several stroke-related complications. Therefore, improving the poor prognosis of patients with ischemic stroke and the treatment of recurrent stroke depend on further research in this area and a better understanding of the underlying complex molecular mechanisms regulating the processes (2,20).

In recent years, research interest on long noncoding RNA (lncRNA), a type of noncoding single-stranded RNA with a length of >200 nucleotides (12,13), has increased. Various functions of IncRNA have been identified, including its role in epigenetic changes, transcriptional regulation, post-transcriptional modification, and targeted regulation of microRNAs (miRNA) and proteins resulting in various diseases (5). LINC01296, a newly discovered member of the IncRNA family (7,11), has been found to control the proliferation and apoptosis of the human neuroblastoma cell SK-N-SH by regulating the Wnt/β-catenin signaling pathway, migration, and invasion (23). There are limited studies on the mechanism of CIRI. miRNA-29c is involved in the overall process of focal ischemia in...
The rat brain tissue was sliced, deparaffinized, and

LTerminal Deoxynucleotidyl Transferase dUTP Nick end

Cerebral infarction rate (%) = infarct area/total area × 100%

represents the infarcted brain tissue, and other normal tissue

pictures and analyze the slices for the white area, which

software (National Institutes of Health) was used to obtain

The brain sections were fixed with 4% paraformaldehyde

incubated with TTC solution for 30 minutes. After staining,

minutes. The tissue was then cut along the coronal axis and

phosphate buffered saline (PBS) and kept at −20°C for 10

areas.

TTC staining distinguished between the surviving and infarcted

brain tissues after stroke. The brain tissue was washed with

The following primers were used in this study:

miRNA-29c forward primer: ATAGCGGGCCGCAGATCCATGGA

reverse primer: ATACTCGAGGAAGTTAGGAA

LINC01296 forward primer: 5′-AACCTGGCACCAGCCTCACT-3′

reverse primer: 5′-CGGCCAACTTCTTTACCATC-3′

Birc2 forward primer: 5′-CATCTAGCCTTGCTGTTGGA-3′

reverse primer: 5′-CTACTGAGGAGGTGTTGGA-3′

Bak1 forward primer: 5′-CATCCTTACCGCCTTACCC-3′

reverse primer: 5′-CTACCCCAACCCCTTACCC-3′

Statistical Analysis

GraphPad Prism 7.0 (GraphPad Software Company, USA) software was used to analyze the data. The experimental result p<0.05, and the difference between the groups was considered statistically significant. The data were expressed as mean ± standard deviation.
RESULTS

LINC01296 Expression Decreased in the Cerebral Infarction Model Created by Cerebral Ischemia–Reperfusion

To clarify LINC01296’s role in the ischemia–reperfusion injury model, we designed the sham group, MCAO group, MCAO+PBS group, MCAO+pc-DNA-LINC01296 group, MCAO+Sh-LINC01296 group, and the corresponding negative control group. The level of LINC01296 in the mouse brain tissue was measured. The results showed that when compared with the sham group, the level of LINC01296 in the mouse brain tissue of the rats in the MCAO model group was reduced. After the injection of pc-DNA-LINC01296, the expression of LINC01296 increased; however, after the injection of Sh-LINC01296, the expression of LINC01296 decreased (Figure 1).

LINC01296 Reduced Cerebral Infarction Caused by Cerebral Ischemia and Reperfusion

We used TTC staining to determine the infarction rate of the rats in each group to further clarify the role of LINC01296 in the ischemia–reperfusion injury model. We found that the cerebral infarction rate decreased after the injection of pc-DNA-LINC01296 but increased after the injection of Sh-LINC01296 (Figure 2).

TUNEL staining was used to determine the apoptotic rate of the brain cells. The results showed that the injection of Sh-LINC01296 aggravated the apoptosis of the brain cells, while the injection of pc-DNA-LINC01296 alleviated the apoptosis (Figure 3).
Western blot was used to detect the expression of apoptosis-related genes. In the Sh-LINC01296 group, the expressions of Birc2 and BAK1 were observed to be decreased. On the other hand, after the injection of pc-DNA-LINC01296, the expression levels increased (Figure 4).

**LINC01296 Relieved CIRI by Regulating the Apoptotic Genes Birc2 and Bak1**

LINC01296 can target and inhibit the miR-29c expression in endometrial cancer cells. We observed that overexpressed miR-29c can interfere with and inhibit the expression of Birc2 and Bak1, thereby aggravating many types of cell apoptosis and worsening CIRI. The luciferase reporter gene detection method was used in this study to verify the targeting effect of LINC01296 on miR-29c in the PC12 cells. In the LINC01296 and miR-29c analog systems, the fluorescence intensity was low. The targeting effect of miR-29c on Birc2 and Bak1 demonstrated reduced fluorescence intensity in the wild-type miR-29c mimic system.

These results indicate that LINC01296 targets and reduces the expression of miR-29c and that the targeted inhibition of miR-29c ultimately promotes the expressions of Birc2 and Bak1.

**LINC01296 Promoted the Expression of Birc2 and Bak1 by Targeting the miR-29c Pathway and Alleviated CIRI**

RT-PCR was performed to determine the level of miR-29c in the brain of each group of rats to explore the role of LINC01296 in cerebral ischemia–reperfusion-induced injury. We found that after LINC01296 overexpression, the expression of miR-29c decreased compared to that of the MCAO group (Figure 5). miR-29c antagonist was administered to the rats, and TTC staining was used to determine brain cell apoptosis. We observed that miR-29c antagonist inhibited the protective effect of LINC01296 on cerebral ischemia–reperfusion brain...
cells (Figure 6). In addition, we identified that the expressions of Birc2 and Bak1 in the brain tissues were enhanced after miR-29c antagonir intervention (Figure 7). Therefore, we believe that LINC01296 overexpression inhibits the expression of miR-29c, while the reduction of miR-29c levels promotes the expressions of Birc2 and Bak1.

**DISCUSSION**

As a global public health threat, CIRI has severely affected patients’ quality of life, for example, those with difficulties in memory, learning, speech, and mobility (24,25). Under normal circumstances, the body’s brain tissue can activate endogenous antioxidation and anti-ischemic mechanisms when subjected to harmful stimuli, such as hypoxia and ischemia. These mechanisms can protect the dying nerve cells and promote the recovery of nerve function (6). However, when the body lacks exogenous intervention, the initiation of endogenous neuroprotection is relatively slow. The content of protective media and the duration of action are very much limited; the damage mechanism has an absolute advantage, often surpassing the endogenous neuroprotective effects (8,14). Therefore, it is necessary to apply external environmental stimuli to the body. Through a series of neural reflexes, the brain’s tolerance to noxious stimuli, such as ischemia and hypoxia, can be increased significantly. It can

![Figure 5: Reverse transcription polymerase chain reaction to determine the level of miR-29c in the brain of each group of rats. Sham group: control group, MCAO group: cerebral infarction model caused by cerebral ischemia–reperfusion, MCAO+PBS group: cerebral infarction model caused by cerebral ischemia–reperfusion plus solvent group, MCAO+pc-DNA-LINC01296 group: cerebral infarction model caused by cerebral ischemia–reperfusion + overexpression of LINC01296, MCAO-Sh-N01296 group: cerebral infarction model caused by cerebral ischemia–reperfusion + low expression of LINC01296, *p<0.05 vs. the sham group; #p<0.05 vs. MCAO+pc-DNA3.1 group; and &p<0.05 vs. MCAO+Sh-NC group.](image)

![Figure 6: Triphenyltetrazolium chloride staining for determining the apoptosis of the brain cells. MCAO+PBS group: cerebral infarction model caused by cerebral ischemia–reperfusion plus solvent group, MCAO+pc-DNA-LINC01296 group: cerebral infarction model caused by cerebral ischemia–reperfusion + overexpression of LINC01296, MCAO+miR-29c-antagomir group: cerebral infarction model caused by cerebral ischemia–reperfusion + miR-29c-antagomir. MCAO+pc-DNA-LINC01296+miR-29c-antagomir group: cerebral infarction model caused by cerebral ischemia–reperfusion + miR-29c-antagomir + overexpression of LINC01296. *p<0.05 vs. MCAO+PBS group; #p<0.05 vs. MCAO+pc-DNA3.1 group; and &p<0.05 vs. MCAO + miR-29c-antagomir group.](image)
LINC01296 Participates in Neuroprotection

This study has shown that LINC01296 can target and inhibit the function of miR-29c, which is consistent with the above research results. In addition, the overexpression of LINC01296 eliminated the inhibitory effect of miR-29c and protected the nerve cells in the brain. The overexpression of LINC01296 also resulted in the upregulation of Birc2 and Bak1 levels. These results indicate that LINC01296 can relieve the nervous system damage caused by cerebral ischemia and reperfusion by nullifying the inhibitory effect of miR-29c on Birc2 and Bak1.

CONCLUSION

In summary, our research has demonstrated that LINC01296 can alleviate the injury caused by cerebral ischemia and reperfusion by inhibiting the inhibitory effect of miR-29c on Birc2 and Bak1. Furthermore, our findings have provided new strategies and goals for the clinical treatment of patients with cerebral ischemia–reperfusion. Based on the results, it is evident that miR-29c aggravates CIRI by interfering with Birc2 and Bak1 pathways and that the injury effect is assuaged after the injection of the miR-29c antagonist. The injury effect is enhanced after agonist injection.
AUTHORSHIP CONTRIBUTION

Study conception and design: LM, YQ
Data collection: YQ, KD, XZ
Analysis and interpretation of results: YQ, KD, XZ
Draft manuscript preparation: LM
Critical revision of the article: LM, YQ
All authors (LM, YQ, KD, XZ) reviewed the results and approved the final version of the manuscript.

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