Knockdown lncRNA Neat1 regulates the activation of microglia and alleviate neuronal apoptosis through AKT/STAT3 pathway after cerebral ischemic reperfusion.

Xunran Ni
Harbin Medical University
Qian Su
Harbin Medical University
Wenbo Xia
Harbin Medical University
Yanli Zhang
Harbin Medical University
Kejuan Jia
Harbin Medical University
Guozhong Li
Harbin Medical University
Zhiqiang Su (suzhiqiang2020@126.com)
Harbin Medical University https://orcid.org/0000-0002-2200-7938

Research

Keywords: Neat1、microglia polarize, cerebral ischemic reperfusion injury, akt, stat3Key words: microglia polarize, cerebral ischemic reperfusion injury, long non-coding RNA, NEAT1

Posted Date: February 8th, 2020

DOI: https://doi.org/10.21203/rs.2.22907/v1

Abstract

Background

Acute cerebral ischemia may cause serious consequences, one of them is brain injury caused by the uncontrolled reperfusion which occurs once the circulation is re-established. Neat1 is one of long non-coding RNA presents important roles in the immune system. However, the potential roles and underlying molecular mechanisms of it in cerebral ischemic reperfusion injury is still unclear.The aim of the present study was to investigate the function of LncRNA Neat1 in cerebral ischemic reperfusion injury and its possible advantage for neure.

Methods

In our study, oxygen glucose deprivation was used in vitro to mimic cerebral I/R injury. CCK8 was used to mesure cell viability and flow cytometry measured cell apoptosis. qRT-pcr was used to measure the phenotypic marker of M1 and M2 microglia, western blot was used to detect the protein expression of AKT/STAT3 pathway.

Results

Knockdown the LncRNA Neat1 alleviated the apoptosis induced by OGD/R and increased the cell viability.We further provided that Neat1 may inhibite microglia polarize to M1 phenotype to reduce the damage of immunoreaction caused after cerebral I/R injury through AKT/STAT3 pathway.

Conclusions

We evaluated the function and mechanism of lncRNA Neat1 in cerebral I/R injury, And LncRNA Neat1 may be a potential target for new therapeutic intervention.

Introduction

Cerebralvescular diseases which have highly disability and mortality rates are becoming a common disease in the elderly[1]. The only way to avoid the permanent injury of cerebral tissue ischemia is to recover the brain bloodstream in the ischemic region of patient as fast as can. Nevertheless, some more sever symptoms will occur after the artery recanalization is called ischemia/reperfusion (I/R) injury[2]. The main pathophysiological characteristics of I/R injury include immunoreaction and apoptosis[3]. However, in clinical, there is no specific medicine to treat I/R injury due to lack of understanding of its mechanisms[4].

Long non-coding RNAs(lncRNAs) are longer than 200 nucleotides RNAs without ability of encoding proteins[5]. Recent years, lncRNAs are proved to participate in various pathological and physiological processes such as apoptosis, cell cycle progression, differentiation and inflammation[6]. Nuclear enriched abundant transcript 1(NEAT1) as one of the lncRNAs, was reported to be involved in different kinds of

diseases[7,8], and was proved to relative with myocardial IR injury[9], and promote activation of inflammasomes in macrophages[10].

Microglia are the main immune cells of the central nervous system (CNS) and exert diverse functions in the pathogenesis of various neurological diseases[11]. Once activated, microglia serves as a double-edged sword during the pathological process of disease in CNS[12]. When acute brain injury occoured, classically activated microglia (M1 phenotype) release some kind of cytokines and exacerbate inflammatory damage[12]. On the other hand, alternative activated microglia (M2 phenotype) provide neuroprotective effects by phagocytosis and removal of cell debris, attenuate local inflammation, and tissue remodeling[13].

Hence, in this experiment, we hypothesized that lncRNA Neat1 will regulate microglia activation and promote neuronal apoptosis after cerebral I/R injury. And that would provide a potential target for new therapeutic intervention of cerebral I/R injury.

Methods

2.1 Cell culture

BV–2 cell was maintained in Minimum Essential Medium, Alpha 1×with Eagle's salts, ribonucleosides, deoxyribonucleosides & L-glutamine (MEM; Corning) supplemented with 10% fetal bovine serum (FBS; HyClone) and 1% Sodium Pyruvate(SP 100mmol Gibco NY USA) and 1% penicillin/streptomycin(PS; 100IU/ml penicillin, 100μg/ml streptomycin). N2a cell was maintained in Dulbecco's Modification of Eagle's Medium (DMEM; Corning) supplemented with 10% fetal bovine serum (FBS; HyClone) and 1% penicillin/streptomycin (PS; 100IU/ml penicillin, 100μg/ml streptomycin). An incubator with 5% CO2 at 37°C was used to incubate all the cells.

2.2 Lentiviral vector constructs preparation and infection

Lentivirus expressing siRNA(si-Neat1) and control lentivirus(si-con) were packaged by HanBio (Shanghai, China). The packaged recombinant lentivirus then transfected NEAT1 knockdown cells, and then puromycin was used for selection up to at least 15 days.

2.3 OGD/R

Oxygen glucose deprivation (OGD) in microglia was performed as described previously[14]. Briefly, the original culture medium was removed and replaced with glucose/serum-free DMEM at first. Then, the plates were transferred into an anaerobic chamber for 2 h at 37 °C,

which had already been balanced with 5% CO2 and 95% N2 without oxygen. During the reperfusion stage, the plates were then returned to normal medium in normal incubator for 2–72h

2.4 PCR(Quantitative Real Time PCR)

Total RNA was isolated from the cell using TRIzol (Life Technologies Carlsbad, CA, USA), total RNA of blood was isolated using TRIzol LS Reagent(Life Technologies Carlsbad, CA, USA), cDNA was prepared in 10 μl reaction volume using the ReverTra Ace qPCR RT Kit (TOYOBO CO., OSAKA JAPAN) and real-time PCR analysis run in triplicate using FastStart Universal SYBR Green Master (Rox) (Roche, Mannheim Germany). All the qRT-PCR experiments and data analysis in the present research were performed in accordance with the MIQE guidelines.

2.5 Protein isolation and Western blot assay

Protein samples were isolated from cells and quantified using a bicinchoninic acid (BCA) assay kit (Sigma Aldrich). We used 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to separate the proteins. Proteins were then transferred to a nitrocellulose membrane using an electroblotting technique. The membrane was blocked using a 5% blocking solution (non-fat milk) and then incubated in a blocking buffer with the following primary antibodies overnight at 4°C: Akt, Stat3, P-Akt, P-Stat3, β-actin. The following day, goat secondary antibody conjugated with horseradish peroxidase (HRP) was added to the blocking buffer (1:1,000, non-fat milk), and a chemiluminescence kit (Thermo Fisher Scientific, Shanghai, China) was used to detect the proteins.

2.6 CCK8

N2a cells were plated at a concentration of 20,000 cells/ml in 96-well plates. After exposure experiments, the supernatant of both OGD/R lncRNA Neat1 knockdown BV–2 cells and untreatment lncRNA Neat1 knockdown BV–2 cells were culcured with N2a cells for 24 hr. Then 10 μL CCK–8 reagent (Beyotime, Shanghai, China) were added into each well and incubated for 2 hr at 37°C, a microplate reader (Tecan, SWITZERLAND) was employed to detect the absorbance at 450nm.

2.7 flow cytometry

N2a cells were collected and resuspended in binding buffer. FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen, USA) was used and the cells were stained using FITC-Annexin V and propidium iodide (PI). A BD FACSCcanto flow cytometer was carried out to do apoptosis analysis.

2.8 Statistical analysis

All statistical data were analyzed by GraphPad Prism 7.0(GraphPad Software Inc., San Diego, CA, USA). Statistical comparisons were measured by the two-tailed. Student t-test between two groups and by one-way ANOVA with Dunnett's multiple comparison among more than three groups. Asterisks are used to indicate statistical significance;*, **, ***, and**** indicate $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively.

Results

3.1 Neat1 was upregulated in cerebral ischemia

Quantitative real-time PCR (qRT-PCR) was used to test relative expression of Neat1 to investigate whether Neat1 was related to cerebral ischemic stroke in AIS patients. The basic characteristics of participants are listed in Table 1. In out study, Neat1 was markedly increased in AIS patients' blood compared with controls(Figure 1A). In addition, Neat1 expression was positively correlated with the time of onset of illness(Figure 1B) and the volume of cerebral infarct(Figure 1C). In addition, Neat1 expression was positively correlated with stroke severity, which was evaluated based on National Institute of Health Stroke Scale (NIHSS) scores(Figure 1D) and infarct volume(Figure 1E).

3.2 OGD exposure and preliminary analysis of lncRNA Neat1

We used serum-free and glucose-free medium culcure the BV–2 cells to mimic the cerebral ischemic reperfusion injury. After 2h OGD exposure, we treated cells with different time course of reperfusion. At the same time, we detected the expression of lncRNA Neat1 under the OGD/R exposure. The expression of lncRNA Neat1 was significantly increased and reached the highest changes in the 48h reperfusion (Figure 2A). We used siRNA to knockdown the lncRNA Neat1. As shown in Figure 2B, the expression of lncRNA Neat1 was downregulated.

3.3 Function of lncRNA Neat1 in cerebral ischemic reperfusion injury

To investigate the function of lncRNA Neat1 in cerebral ischemic reperfusion injury, CCK–8 results showed that knockdown of lncRNA Neat1 increased the cell viability (Figure 3A). We also performed the flow cytometry to detect the cell apoptosis effect. Knockdown of lncRNA Neat1 significantly reduced the cell apoptosis(Figure 3(B&C)). Thus, our results showed that knockdown of lncRNA Neat1 can protect N2a cells from cerebral ischemic reperfusion injury and lncRNA Neat1 possessed a anti-apoptosis effect.

3.4 LncRNA Neat1 inhibite microglia polarized towards pro-inflammatory (M1) phenotype

We treated Neat1 knockdown BV–2 cells with OGD/R and then detect the mRNA expression of M1 and M2 microglia markers. The M1 markers (CD16, CD32, and CD86) were reduced compared with the control group(Figure 4A,B,C). The M2 markers (BDNF, PDGF, Arg–1) had no obvious changes compared with the control group(Figure 5A, B, C). These data suggested that Neat1 suppress M1 microglial polarization in OGD/R-treated microglial cells.

3.5 LncRNA Neat1 regulate AKT/STAT3 pathway in cerebral ischemic reperfusion injury

Both Akt and Stat3 play important roles in many physiological processes. AKT/STAT3 pathway were detected via western blot. As shown in Figures 6(A, C, E) the phosphorylation of both Akt and Stat3 in Neat1 knockdown group were reduced compared with the NC group after OGD/R. Moreover, the totle proterin expression of Stat3 and AKT were unchanged(Figure6A, B, D). These results indicate that both Akt and Stat3 can be suppressed by Neat1 in BV–2 cells in the setting of OGD/R.

Discussion

Recently, numerous lncRNAs have been proved to participate in cerebral ischemic reperfusion injury[15]. Hence, identification of prognostic lncRNAs for cerebral I/R injury is necessary and important. In this study, we used oxygenglucose deprivation (OGD), an in vitro mimic of ischemic stroke conditions in BV–2 and N2a cells to explore the potential mechanism of cerebral I/F injury, and we found that Neat1 was upregulated in BV–2 cells when OGD/R happened which means that it might play an important role in cerebral I/F injury. In addition, qRT-pcr, western blot, flow cytometry and cell viability were measured to evaluate the function of lncRNA Neat1.

Ischemia-reperfusion may cause apoptosis, autophagy, necrosis and necroptosis[16–18]. If Ischemia-reperfusion injury is severe, cell death may be induced via apoptotic or necrotic Pathways[19]. Our results showed that knockdown of lncRNA Neat1 alleviated the N2a cells apoptosis induced by OGD/R and increased N2a cells viability rate.

For acute ischemic stroke, the AHA/ASA guidelines recommend early revascularization[20]. While at the same time, the following injury which caused by ischemia-reperfusion is a hard problem for physicians to preserve the function of nerve and organ[21]. The most significant task for physicians are control cell damage and protect organ function when cerebral I/R injury happen[21]. The mechanisms of ischemia-reperfusion injury are extremely complicated and diverse, immune response may be one of the most important mechanisms. There is evidence suggest that when the blood supply is re-established after ischemia, inflammation increase[14]. Therefore targeting immune activation will be an emerging therapy in the treatment of ischemia-reperfusion[21]. Microglia are important in the central nervous system which regulate the brain’s immune and inflammatory response following ischemic injury[22]. Most of the time, microglia possess a neuralspecific phenotype and retain a relative quiescent surveillance phenotype for constant monitoring of the brain parenchyma[23–25]. In the process of neurological inflammation, microglial cells perform different functions by polarizing into different phenotypes[26]. It was found in our study that expression of microglia M1 phenotype genes(CD16, CD32, CD86) was decreased in lncRNA Neat1 knockdown group after OGD/R treatment, and at the mean time, the expression of microglia M2 phenotype genes(Arg–1, BDNF, PDGF) was unchaged.

Akt as known as protein kinase B (PKB) is an important factor in cell death and growth[27,28]. Recent evidence suggested that Akt may activate STAT3 as it acts upstream of STAT3[29], and STAT3 perform an important role in proliferation and astrogliosis[28]. AKT/STAT3 is also part of IL–6 pathway which play a crucial part in immunoreaction and may involved in the cerebral I/R injury. Notably, according to our western blot examination results, in the OGD/R group, after knocking down the expression of Neat1, the phosphorylation of both Akt and Stat3 were reduced, nevertheless, total protein expression of Akt and Stat3 remained unchanged. Compared with the OGD/R group, neither Neat1 knowning down group nor NC group have any change in phosphorylation or totle pretein expression of both Akt and Stat3. This suggests that Neat1 may not be connected with the Akt/Stat3 pathway by directly controlling the protein expression levels of the signal molecules.

Microglia which considered as the macrophage in the central nervous system has the same function like mononuclear phagocytes [30]. However, microglia were observed to be more restricted in their capacity to adopt an M2 phenotype and cytokine profile, compared with macrophages[31]. Both macrophages and microglia showed a greater induction of gene expression in response to M1, compared with M2 polarization[30].

To sum up, our data revealed that NEAT1 can exert neuroprotective effect through inhibit AKT/STAT3 phlogistic pathways, and significantly inhibite the polarization of microglia to M1 phenotype. Whether LncRNA Neat1 can promoted the polarization of microglia to M2 phenotype and the mechanism of it requires further study.

Conclusions

The expression of long non-coding RNA NEAT1 is up-regulated after cerebral ischemic injury. Cerebral ischemia-reperfusion may cause more severe damage than cerebral ischemia. Knockdown of Neat1 significantly reduce microglia polarize towards pro-inflammatory (M1) phenotype and cell apoptosis after cerebral ischemia/reperfusion through inhibiting inflammatory pathways and increase the activity of neuronal cells. The results may provide new strategies of treatment for cerebral ischemia/reperfusion from epigenetic perspective.

Declarations

Abbreviations

CCK8: Cholecystokinin octapeptide; ECL: Enhanced chemiluminescence; FBS: Fetal bovine serum; PBS: Phosphate-buffered saline; qRT-PCR: quantitative Real Time-PCR.

Acknowledgements

NOT APPLICABLE

Authors' contributions

ZS, GL designed this work and provide the fund. XN wrote the paper. QS collected clinical data. XN, WX performed the experiments. YZ and KJ analyzed the data. ZS joined the discussions. All authors have approved the final version of the manuscript and have agreed to be accountable for all aspects of the work regarding questions related to the accuracy or integrity of any part of the work.

Funding

This study was supported by the National Natural Science Foundation (No. 81771289); Postgraduate Research & Practice Innovation Program of Harbin Medical University (No. YJSCX2018–32HYD).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experiments were approved by the Ethics Committee of Harbin Medical University.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

Department of Neurology, The First Affiliated Hospital of Harbin Medical University, 150001, Harbin, PR China.

The Key Laboratory of Myocardial Ischemia, Harbin Medical University, Ministry of Education, 150001, Harbin, PR China.

References

1. FengXua, RongjiMa, GuixingZhang, ShengWang, JiangwenYin, ErqiangWang, ErfengXiong, QingtongZhang, YanLi. Estrogen and propofol combination therapy inhibits endoplasmic reticulum stress and remarkably attenuates cerebral ischemia-reperfusion injury and OGD injury in hippocampus.Biomedicine & Pharmacotherapy 108(2018) 1596–1606.
2. Feng Yu, Wei Xue, Liuyi Dong, Xiangyang Hu, Dake Huang, and Kai Wang.Tetrahydroxystilbene Glucoside Suppresses NAPDH Oxidative Stress to Mitigate Apoptosis and Autophagy Induced by Cerebral Ischemia/Reperfusion Injury in Mice.Evidence-Based Complementary and Alternative Medicine Volume 2019, Article ID 3913981, 9 pages.
3. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, et al. Current mechanistic concepts in ischemia and reperfusion injury. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology 2018;46:1650–1667.
4. Dai-Qiang Liu, Shu-Ping Chen, Jia Sun, Xiao-Mei Wang, Nan Chen, Ya-Qun Zhou, Yu-Ke Tian, Da-Wei Ye.Berberine protects against ischemia-reperfusion injury: a review of evidence from animal models and clinical studies. Pharmacological Research. S1043–6618(19)31349–0
5. Mercer, T. R., Dinger, M. E. & Mattick, J. S. Long non-coding RNAs: insights into functions. Nat. Rev. Genet. 10, 155–159 (2009).

6. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol. 2012;9:703–719.
7. Imamura K, Imamachi N, Akizuki G, et al. Long noncoding RNA NEAT1-dependent SFPQ relocation from promoter region to paraspeckle mediates IL8 expression upon immune stimuli. Mol Cell. 2014;53:393-406.
8. Chen Y, Qiu J, Chen B, et al. Long non-coding RNA NEAT1 plays an important role in sepsis-induced acute kidney injury by targeting miR-204 and modulating the NF-kappaB pathway. Int Immunopharmacol. 2018;59:252-260.
9. Ma, M., Hui, J., Zhang, Q. Y., Zhu, Y., He, Y., & Liu, X. J. (2018). Long non-coding RNA nuclear-enriched abundant transcript 1 inhibition blunts myocardial ischemia reperfusion injury via autophagic flux arrest and apoptosis in streptozotocin-induced diabetic rats. Atherosclerosis, 277, 113–122.
10. Pengfei Zhang, Limian Cao, Rongbin Zhou, Xiaolu Yang,Mian Wu.The lncRNA Neat1 promotes activation of inflammasomes in macrophages.NATURE COMMUNICATIONS (2019) 10:1495.
11. Deczkowska, A., Keren-Shaul, H., Weiner, A., Colonna, M., Schwartz, M., Amit, I., 2018. Disease-Associated Microglia: A Universal Immune Sensor of Neurodegeneration. Cell 173, 1073–1081.
12. Ming Wang, Lin Cheng, Zhong-Liang Chen, Rajneesh Mungur, Shan-Hu Xu, Jiong Wu, Xiao-Li Liu, Shu Wan.Hyperbaric oxygen preconditioning attenuates brain injury after intracerebral hemorrhage by regulating microglia polarization in rats.CNS Neurosci Ther(CNS Neuroscience & Therapeutics) 2019 Aug 14.
13. Hu X, Leak RK, Shi Y, et al. Microglial and macrophage polarization—new prospects for brain repair. Nat Rev Neurol. 2015;11(1):56.
14. Manhua Lv, Y. Liu, J. Zhang, L. Sun. Roles of inflammation response in microglia cell through Toll-like receptors 2/interleukin–23/interleukin–17 pathway in cerebral ischemia/reperfusion injury. Neuroscience. 2011 Mar 10;176:162–72.
15. Zhang J, Yuan L, Zhang X, et al. Altered long non-coding RNA transcriptomic profiles in brain microvascular endothelium after cerebral ischemia [J]. Exp Neurol. 2016;277:162–170.
16. Lopez-Neblina F, Toledo AH, Toledo-Pereyra LH: Molecular biology of apoptosis in ischemia and reperfusion. J Invest Surg 2005;18:335–350.
17. Ling Q, Yu X, Wang T, Wang SG, Ye ZQ, Liu JH: Roles of the Exogenous H2S-Mediated SR-A Signaling Pathway in Renal Ischemia/ Reperfusion Injury in Regulating Endoplasmic Reticulum Stress-Induced Autophagy in a Rat Model. Cell Physiol Biochem 2017;41:2461–2474.
18. Zhu J, Yao K, Wang Q, Guo J, Shi H, Ma L, Liu H, Gao W, Zou Y, Ge J: Ischemic Postconditioning-Regulated miR–499 Protects the Rat Heart Against Ischemia/Reperfusion Injury by Inhibiting Apoptosis through PDCD4. Cell Physiol Biochem 2016;39:2364–2380.
19. Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, Lips DJ, Doevendans PA: Role of apoptosis in reperfusion injury. Cardiovasc Res 2004;61:414–426.
20. William J. Powers, Alejandro A, Rabinstein, Teri Ackerson, Opeolu M. Adeoye, Nicholas C. Bambakidis, Kyra Becker, José Biller, Michael Brown, Bart M. Demaerschalk, Brian Hoh.2018

Guidelines for the Early Management of Patients With Acute Ischemic Stroke. A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association.Stroke. 2018;49:e46–e99.

21. Meng-Yu Wua, Giou-Teng Yiang, Wan-Ting Liao, Andy Po-Yi Tsai, Yeung-Leung Cheng, Pei-Wen Cheng, Chia-Ying Li, Chia-Jung Li. Current Mechanistic Concepts in Ischemia and Reperfusion Injury.Cellular Physiology and Biochemistry.2018;46:1650–1667.
22. Perry, V. H., Nicoll, J. A. R. & Holmes, C. Microglia in neurodegenerative disease. Nature Reviews Neurology 6, 193 (2010).
23. Schmid CD, Melchior B, Masek K, Puntambekar SS, Danielson PE, Lo DD et al. (2009). Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. J Neurochem 109 (Suppl. 1): 117–125.
24. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S et al.(2005) ATP mediates rapid microglial response to local brain injury in vivo. Nature Neurosci 8: 752–758.
25. Nimmerjahn A, Kirchhoff F, Helmchen F (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308: 1314–1318.
26. Lu Huang, Shizheng Wu, Hao Lic, Zhancui Dangd, Yue Wu.Hypoxic preconditioning relieved ischemic cerebral injury by promoting immunomodulation and microglia polarization after middle cerebral artery occlusion in rats.Brain Research 1723(2019) 146338.
27. B Manning, L Cantley. AKT/PKB signaling:navigating downstream. Cell, 2007; 129, 1261–74. doi: 10.1016/j.cell.2007.06.009
28. Inukai, M., Hara, A., Yasui, Y., Kumabe, T., Matsumoto, T., Saegusa, M., 2015. Hypoxiamediated cancer stem cells in pseudopalisades with activation of hypoxia-inducible factor–1α/Akt axis in glioblastoma. Hum. Pathol. 46, 1496–1505.
29. D Malanga, C De Marco, I Guerriero, et al. The Akt1/IL–6/STAT3 pathway regulates growth of lung tumor initiating cells. Oncotarget, 2015; 6, 42667–86.
30. Ruben Orihuela, Christopher A McPherson and Gaylia Jean Harry.REVIEW Microglial M1/M2 polarization and metabolic states. British Journal of Pharmacology. (2016) 173 649–665.
31. Durafourt BA1, Moore CS, Zammit DA, Johnson TA, Zaguia F, Guiot MC et al. (2012). Comparison of polarization properties of human adult microglia and blood-derived macrophages. Glia 60: 717–727.

Table

TABLE 1 Baseline characteristics of the subjects

	AIS(n=64)	Control(n=64)	P
Age (year)	58.85±10.40	58.47±10.11	0.835
Male,n(%)	46(71.9)	47（73.4）	0.843
Smoking, n (%)	25(39.06)	17（26.56）	0.132
Drinking, n (%)	23(35.93)	16（25.00）	0.179
Hypertension, n (%)	32(50.0)	9(14.06）	<0.001*
Diabetes, n (%)	13(20.31)	10（18.75）	0.490
Total cholesterol (mmol/L)	5.15±1.07	5.10±1.14	0.766
Triglyceride (mmol/L)	1.88±1.09	1.57±0.70	0.063

Figures

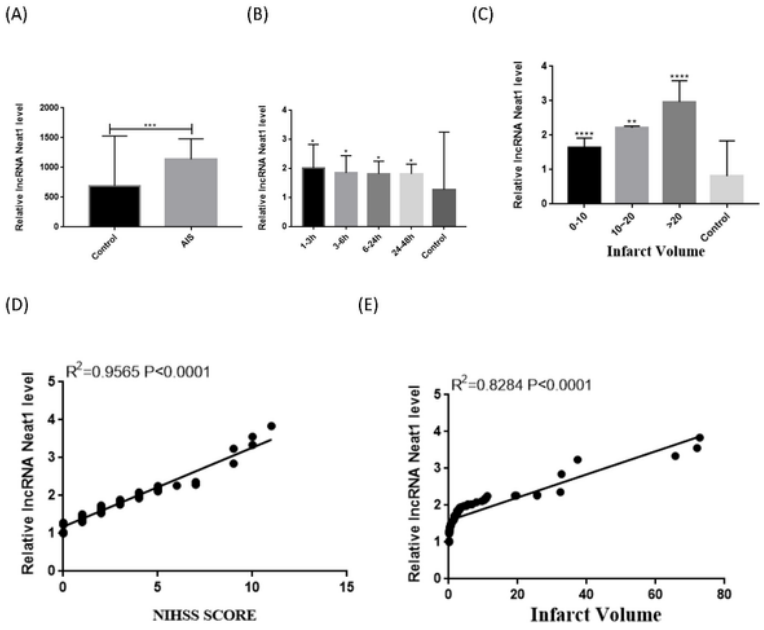

Figure 1 Neat1 was upregulated in cerebral ischemia (A) Expression of Neat1 in blood of AIS patients (n = 64) and healthy controls (n = 64) detected by qRT-PCR. (B) Expression of Neat1 in patients with different onset time compared with healthy controls. (C) Expression of NEAT1 in patients with different infact volume compared with healthy controls. (D) Linear regression analysis was conducted to each individual about Neat1 expression and National Institute of Health Stroke Scale (NIHSS) score. (E) Linear regression analysis was conducted to each individual about Neat1 expression and infarct volume.

Figure 1

Neat1 was upregulated in cerebral ischemia (A) Expression of Neat1 in blood of AIS patients (n = 64) and healthy controls (n = 64) detected by qRT-PCR. (B) Expression of Neat1 in patients with different onset time compared with healthy controls. (C) Expression of NEAT1 in patients with different infact volume compared with healthy controls. (D) Linear regression analysis was conducted to each individual about

Neat1 expression and National Institute of Health Stroke Scale (NIHSS) score. (E) Linear regression analysis was conducted to each individual about Neat1 expression and infarct volume.

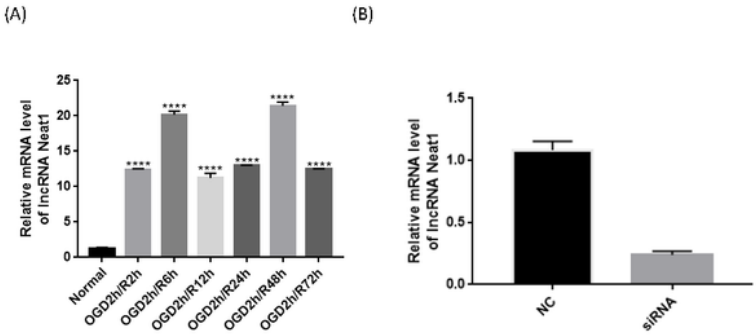

Figure 2. preliminary analysis of lncRNA Neat1. A. The expression of lncRNA Neat1 was increased after the exposure of OGD/R. B. Knockdown of lncRNA Neat1 was verified via real-time PCR.

Figure 2

preliminary analysis of lncRNA Neat1. A. The expression of lncRNA Neat1 was increased after the exposure of OGD/R. B. Knockdown of lncRNA Neat1 was verified via real-time PCR.

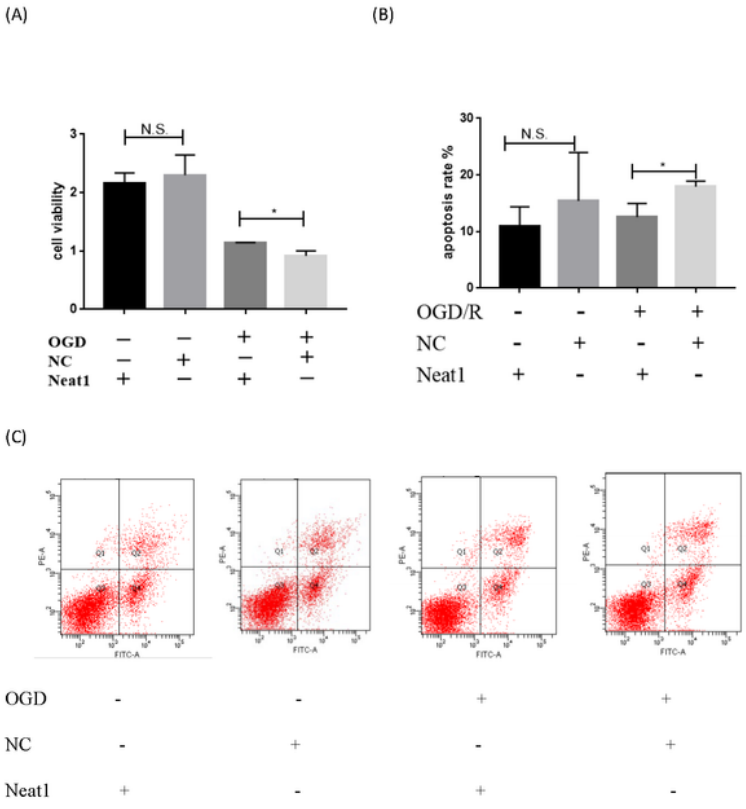

Figure 3. Function of lncRNA Neat1 in cerebral ischemic reperfusion injury A. Cell viability revealed that knockdown of lncRNA Neat1 significantly increased the cell viability. B and C. Knockdown of lncRNA Neat1 significantly reduced the apoptosis cells.

Figure 3

Function of lncRNA Neat1 in cerebral ischemic reperfusion injury A. Cell viability revealed that knockdown of lncRNA Neat1 significantly increased the cell viability. B and C. Knockdown of lncRNA Neat1 significantly reduced the apoptosis cells.

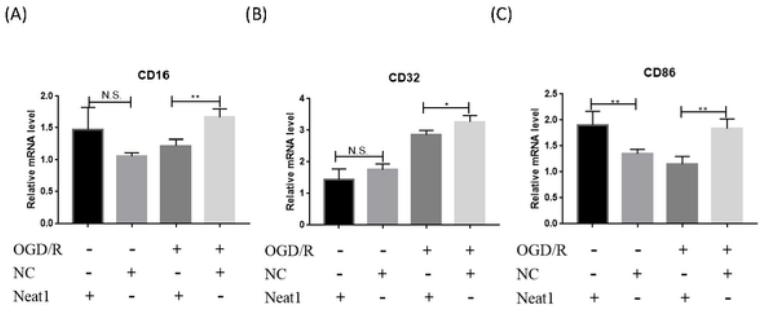

Figure 4. Neat1 inhibite OGD/R-induced M1 polarization in BV2 cells.Determined by qRT-PCR the mRNA expression levels of M1 markers (A) CD16, (B) CD32, (C) CD86.

Figure 4

Neat1 inhibite OGD/R-induced M1 polarization in BV2 cells.Determined by qRT-PCR the mRNA expression levels of M1 markers (A) CD16, (B) CD32, (C) CD86.

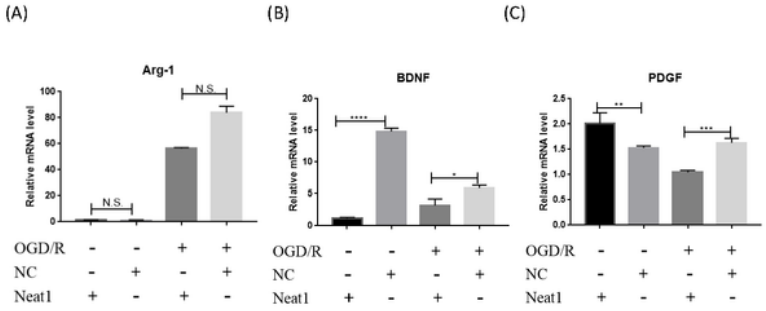

Figure 5. Neat1 promote OGD/R-induced M2 polarization in BV2 cells.Determined by qRT-PCR the mRNA expression levels of M2 markers (A) IL-4 (B) IL-10

Figure 5

Neat1 promote OGD/R-induced M2 polarization in BV2 cells.Determined by qRT-PCR the mRNA expression levels of M2 markers (A) IL-4 (B) IL-10

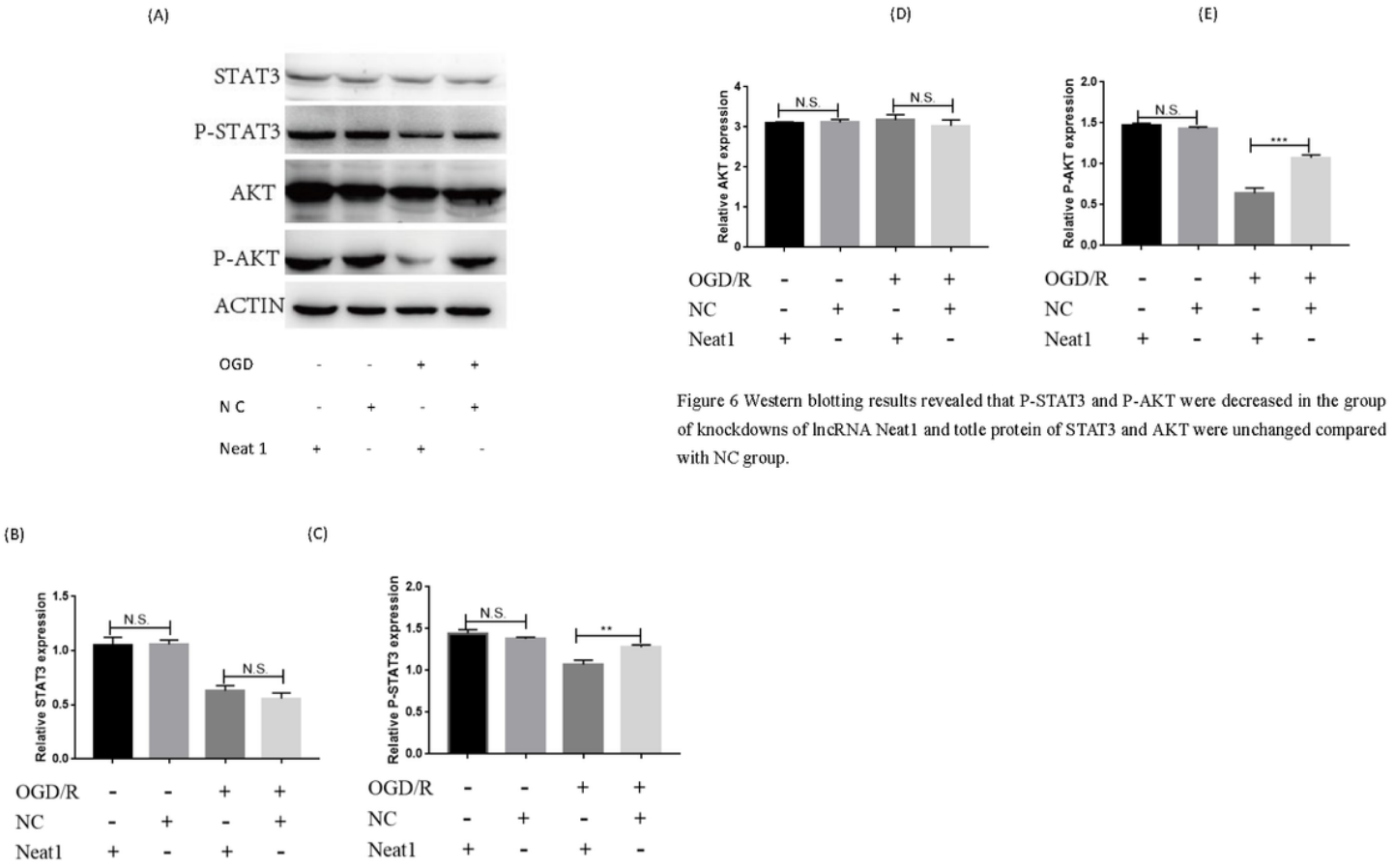

Figure 6 Western blotting results revealed that P-STAT3 and P-AKT were decreased in the group of knockdowns of lncRNA Neat1 and totle protein of STAT3 and AKT were unchanged compared with NC group.

Figure 6

Western blotting results revealed that P-STAT3 and P-AKT were decreased in the group of knockdowns of lncRNA Neat1 and totle protein of STAT3 and AKT were unchanged compared with NC group.