The Effect of Methyltransferase NSUN2 in Stroke

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Research Article

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Abstract

Stroke is one of the most important diseases that seriously threaten the health and public health of elderly patients.NSUN2 refers to the predominant methyltransferase for RNA m5C methylation, contributing to increased RNA stability, translocation and translation, and playing an important role in the physiopathology. However, there is insignificant progress on the biological functions and mechanisms of NSUN2 in cerebral ischemia-reperfusion injury. Here, C57BL/6 mice were employed to establish a middle cerebral artery ischemia-reperfusion injury model (MCAO) and found to significantly increase in NSUN2 protein and mRNA expression levels by Western blotting and qRT-PCR. Subsequently, NSUN2 knockout mice were exploited to build the MCAO model. This study reported that knockout of NSUN2 significantly aggravated brain infarct size and behavioral scores, while reducing 7-day postoperative survival and increasing neuronal apoptosis and injury in MCAO mice. According to the investigation of Western blotting results, decreased PI3K/AKT, ICAM-1 and Bcl-2 protein expressions and increased apoptosis-related protein (Caspase-3/Bax) were found. Overall, this study suggested that NSUN2 may affect cerebral ischemia-reperfusion injury via PI3K/AKT signaling channel and ICAM-1 protein regulation of apoptosis.

1. Introduction

Stroke is one of the most important diseases that seriously threaten the health and public health of elderly patients. It has a high morbidity, disability and fatality rate. About 75% of survivors lose the ability to live and work to varying degrees(Jiang et al.,2018). And society brings a heavy burden. Therefore, effective prevention and treatment of stroke has become one of the major issues that need to be resolved urgently in the national major chronic disease and public health health strategy.RNA methylation is a critical epigenetic modification in post-transcriptional modification of RNA, i.e., the frontier and hot spot of research over the past few years. Limited studies have shown that RNA m5C methylation is widespread in cells and exerts vital effects on various physiological and pathological processes, and that the mentioned modifications are critical to mRNA stability, processing, regulation of stem cell function, stress response and genetic messaging(Oerum et al.,2017;Blanco et al.,2016a;Liu et al.,2017).Known mutations in RNA modifying enzymes show significant correlations with human diseases(e.g.,carcinoma, metabolic disease, cardiovascular disease, as well as mitochondria-related defects)(Jonkhout et al.,2017).

NSUN2 refers to a major RNA-modifying methyltransferase. Its mechanism of action includes control of cell division, growth arrest and promotion of premature senescence(Xing et al.,2015;Wang,2016;Cai et al.,2016;Yang et al.,2017).NSUN2 is critical to human health and disease, provided by individuals with NSUN2 loss-of-function mutations showing intellectual disability (ID), distal myopathy, and facial dysmorphism(Khan et al.,2012;Komara et al.,2015;Martinez et al.,2012).The idea that NSUN2 deficiency induces neurological abnormalities was elucidated through studies in Drosophila and mice (e.g., knocking out) Drosophila NSUN2 is capable of impairing short-term memory(Abbasi-Moheb et al.,2012).Mice deficient in the NSUN2 germline exhibit various impairments in locomotion and behavior.

In addition, NSUN2 is highly expressed in mouse embryogenesis and increases in the brain, and mice knocked out of NSUN2 show more pronounced microcephaly and mental retardation as impacted by over cell apoptosis within the prenatal brain and other disorders attributed to impaired neuronal differentiation and development(Blanco et al.,2014b).Thus far, the mechanism of the mentioned defects is impaired translation as impacted by increased tRNA fragmentation after NSUN2 deletion(Flores et al.,2017). For this reason, NSUN2 is hypothesized to be also critical to stroke.The present study directly tested this hypothesis.

This study more specifically investigated whether NSUN2 participates to stroke through mediating the phosphatidylinositol 3-kinase/serine/threonine kinase (PI3K/AKT) signaling channel and intercellular adhesion molecule-1 (ICAM-1) onto endothelial cell to determine the mechanism of action of NSUN2 in stroke. Activation of the PI3K/AKT signaling channel, which is critical to the regulation of various cellular functions, inhibits apoptosis in post-ischemic neurons and astrocytes(Zhou et al.,2020). Apoptosis is a programmed cell apoptosis pattern, which is one of the typical pathological features in ischemic stroke and is regulated by a combination of genes, such as Bcl-2 family, Caspase family, NF-κB family(Li et al.,2021;Capece et al.2020).And ICAM-1 is critical to regulating angiogenesis after cerebral ischemia-reperfusion injury(Luo et al.,2016).

Nevertheless, little progress has been made on the biological functions and mechanisms of NSUN2 in stroke. Therefore, the effect of NSUN2 on stroke and the effects on PI3K/AKT signaling channel, ICAM-1 and apoptosis were explored here.

2. Materials And Methods

2.1 Animals

The Animal Experimentation Ethics Committee of Wenzhou Medical University approved the experiment protocol employed here. The present study was conducted based on the Wenzhou Medical University Animal Experimentation Guidelines. Male C57BL/6(20~25g) provided by Beijing Vitonlivar Laboratory Animal Technology Co.NSUN2 knockout mice were generated by mating conditional knockout mice nsun2Flox/Flox with estrogen-induced ER-Cre instrumental mice, identified by PCR and used for ear tagging.The mice are all male.Mice were housed in 2-5/cages and placed in a controlled environment (12h light/dark cycle; 21±2°C; 60-70% humidity) for 1 week prior to surgery. Animals have free access to standard laboratory food and water. Based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No.8023,revised 1978), all animal tests were carried out. Discarded mice were executed using a carbon dioxide execution chamber.

2.2 Models and drugs

Anesthesia for each mouse was achieved by intraperitoneally injecting sodium pentobarbital (50 mg/kg, Sigma, USA). Subsequently, the mice under the anesthesia received the ventilation via tracheal intubations for assisting respiration, and the rectal temperature was kept at 37 degrees C with the use of

a heating pad. Under the surgical microscope, the internal carotid artery (ICA), external carotid artery and right carotid artery received the careful separation when the of the neck skin was incised at the median position. A silicone-coated 4-0 monofilament nylon filament received the insertion to the right internal carotid artery and the advancement to the middle cerebral artery beginning, which is occluded. This study employed the filament for holding the filament at the appropriate position. At the end of surgery, the neck wound was closed and the animals were enabled to be recovered based on anaesthetization. The vessels received the removal approximately 10 mm after ligation under isoflurane anesthesia for reperfusion 2 h after ligation. The success of occlusion was judged by the presence of hemiparesis and elevated body temperature to (37.8-38.8°C) prior to reperfusion. In sham-operated animals, the middle cerebral artery was not embolized but the same procedure was performed.

2.3 Survival rate

After establishing the MCAO model, the number of surviving and dead mice was recorded for 7 days.

2.4 Neurological evaluation

Mice were scored neurobehaviorally 24 h after MCAO, and an author blind to the experiment group assessed neurological deficits based on a modified neurobehavioral scoring system. The neurobehavioral score criteria were: 0, no deficit; 1, no voluntary activity with impaired consciousness; 2, falling to the right; 3, circling to the right; 4, failure to extend the right forelimb. After MCAO, the subjects achieving a neurobehavioral score of 0 should be excluded.

2.5 Measurement of infarct size

After 24h of reperfusion, the brain tissue was euthanized with an excess of chloral hydrate, quickly removed, and 2 mm-thick coronal sections (anterior to posterior) were generated and subsequently received the staining by using 2% 2,3,5-triphenyltetrazolium (TTC) solution under the temperature 37°C for 20 min. Subsequently, they received the fixing process by using 4% paraformaldehyde for 24h. The images received the photographing, scanning, and investigation based on Image-Pro Plus 6.0 software system. Infarct volume had the expression of infarcted tissue percentage to overall brain tissues.

2.6 Western blot

At 24h when the reperfusion was completed, this study carried out euthanasia based on the overdose of chloral hydrate, and the infarct-side hippocampus received the rapid separation. The operating process was conducted on a cold surface, and cell metabolism was stopped with liquid nitrogen. Tissue homogenates received the treatment by using 100 RIPA lysis buffer (Beyotime, China), 10 phosphatase inhibitors (Roche, Germany), and 1 PMSF (Beyotime, China). The tissue extract received the thirty minute centrifugating process based on 12,000g at 4℃. This study carried out western blotting by complying with the general procedure. Next, the protein received the transfer in gel into polyvinylidene fluoride membrane (Million, MA, the United States of America) in terms of Western blotting investigation. With the finishing of closure, this study carried out the incubating process for the sample with the use of primary

antibody based on 4℃ through the complete night. The major antibodies: rabbit anti-NSUN2(1:1000, ab259941,abcam), rabbit anti-PI3K(1:1000, AF5112,affinity), rabbit anti-AKT(1:1000, 4685,CST), rabbit anti-P-AKT(1:1000,4060,CST), rabbit anti-ICAM-1(1:1000,ab179707,abcam), rabbit anti-GAPDH antibody (1:5000, AP0063, Bioworld, USA). When the incubation was based on the application of applicable horseradish peroxidase-conjugation secondary antibody, this study carried out the detection for antigen-antibody reacting process by drawing upon Quantity One investigation software (BioRad, San Francisco, CA, USA) as well as optimized chemiluminescence mechanism.

2.7 qRT-PCR investigation

Brain tissue was extracted 24 h after MCAO, the extraction of overall RNA was achieved by adopting Trizol, specific primers were designed, and the produced PCR reaction solution was arranged onto a Realtime PCR instrument for PCR amplification reaction. The reaction conditions are.(30 s under the temperature of 95°C, 5 s under the temperature of 95°C to 30 s under the temperature of 60°C for 39 cycles, 5 s under the temperature of 65°C). The target gene and housekeeping gene of the respective sample received Realtime PCR reactions separately. The primers adopted to detect the transcripts included:Nsun2(sense) 5’GAGAAAGATCGAGCCAACTGTC, (antisense) 5’GTGGGACTTGATGTGGGGAAG.GAPDH (sense) 5’GACATGCCGCCTGGAGAAAC,(antisense) 5’AGCCCAGGATGCCCTTTAGT.

2.8 Immunofluorescence staining

The collection of brain was achieved 24h with the finishing of MCAO, the arrangement within 4% paraformaldehyde (4℃) for 4-6h, the movement into 15% sucrose solution (4℃) in the base and subsequently into 30% sucrose solution (4℃) till sinking. Brains received the embedment process to embedment agent at -20℃ and sectioned with a freezer (section thickness: 8μm).The sections received the freezing process and then were closed for 1 h with a blocking solution prepared from donkey serum, BSA and Triton. After anti-NSUN2 primary antibody, incubate overnight at 4℃. After PBST elution, add fluorescent secondary antibody, incubate and elute, and add DAPI dye to restrain. Leica orthomosaic fluorescence microscopy for observation. The primary antibody below was employed: rabbit anti-NSUN2(1:200,ab259941,abcam).

2.9 HE staining

This study adopted Hematoxylin and eosin (HE) staining for detecting pathological variations within the ischemic penumbra twenty-four h with the finishing of reperfusion. In brief, the mice received the anesthetizing process, and the brain received the fixing through transcardial perfusion by employing 4% paraformaldehyde and 0.9% cold heparinized saline, the paraffin-embeding process, and sectioning process under 4 μm thickness in terms of HE staining, and Nissl staining. Subsequently, the section received the staining by employing HE, and HE-positive cell number within ischemic penumbra received the counting within five distinctive aspects in terms of the respective section within a blinded process under a light microscope (BX51; Olympus, Tokyo, Japan).

2.10 Nissl staining

This study employed Nissl staining for observing neuronal morphologic variations within the ischemic penumbra 24h after reperfusion. The experiment procedure was rigorously used following the producer's Nissl staining tool manual (#G1432, Solarbio). An author kept from the treatment group determined the overall number of Nissl-positive neurons within the penumbra in 5 distinctive view field in terms of the respective section under a light microscopy (BX51; Olympus, Tokyo, Japan).

2.11 Tunel staining

The mouse brain tissue was paraffin-embedded, 4μm slices were fixed with paraformaldehyde, cleaned two times with PBS repeatedly, and stained with Tunel by complying with the Roche Tunel Fluorescent Staining Kit, and restrained with DAPI.A leica ortho-fluorescence microscope was applied for observation. The nuclei were green for positive cells.

2.12 Statistical investigation

Data have the expression of mean±standard deviations, except for infarct size and survival, denoted as percentages. Statistical investigation was conducted with GraphPad Prism 8.0 (GraphPad, San Diego, CA). The Kolmogorov-Smirnov test was performed to perform the normal distribution test. One-way ANOVA (One-way ANOVA) was applied for the infarct area determination, Western blotting assay, with Bonferroni correction followed in terms of post hoc t-test. Wilcoxon signed rank sum test (Wilcox test) was employed for comparing neurological deficit score. Non-linear regression (nonlinear regression) was adopted to compare survival rates. This study gave statistical importance as $P<0.05$.

3. Results

3.1 NSUN2 expression increases in mouse brain ischemia-reperfusion injury

Fig.1 Western blotting and qRT-PCR analyses showed that NSUN2 protein (Fig.1A) and mRNA (Fig.1B) expression noticeably increased in the ischemic half dark zone region of the brain at 24 h after ischemia-reperfusion injury in the model group of mice compared with the sham-operated group. To gain insights into the variations in NSUN2 expression in mouse brain ischemia-reperfusion injury, we used immunofluorescence labeling of NSUN2 and found that the number of NSUN2 expression and fluorescence intensity increased noticeably and localized in the nucleus (Fig.1C).

3.2 NSUN2 knockdown exacerbates ischemia-reperfusion injury in mouse brain

Fig.2 To further clarify the effect of NSUN2 within cerebral ischemia-reperfusion injury of mice, we used Nsun2-KO mice to establish the MCAO model. In contrast to the MCAO group, neurological function

scores increased within the MCAO-KO group, thereby demonstrating a further decrease in neuromotor function (Fig.2A). In addition, the 7-day postoperative survival rate of mice in the MCAO-KO group significantly decreased (Fig.2B). TTC staining for measuring brain injury within three groups(Fig.2C). Fig. 2C illustrates that the MCAO-KO group had 44.20 ± 1.028% infarct volume. HE, Nissl and Tunel staining for assessing survival and neuronal damage within ischemic semidark zone after MCAO injury (Fig.2D/E). According to Fig. 2D and 2E, cells positive after HE staining (HE+) achieved intact nucleolus, compact structure, and clear outline. Cells positive after Nissl staining (Nissl+) achieved intact neuron as well as flush cell body, whereas neuron with injury achieved shrunken cell body followed by pyknotic nuclei and Shrunken. Furthermore, according to Tunel staining, in the MCAO-KO group, the percentage of TUNEL-positive cell within the cerebral cortex further increased.

3.3 NUSN2 knockdown inhibits the expression of PI3K/AKT signaling channel and ICAM-1 in the ischemic half dark zone of mouse brain

Figure 3 Next, we examined the effects of NUSN2 knockdown on PI3 K/AKT signaling channel and ICAM-1 expression in mice with cerebral ischemia-reperfusion injury. Western blotting results showed that the PI3K/P-AKT and ICAM-1 protein contents were significantly decreased in the MCAO-KO group compared with the MCAO group at 24h after cerebral ischemia-reperfusion injury in the ischemic half dark zone region of the cerebral cortex, indicating that PI3K/AKT signaling channel activity as well as ICAM-1 protein expression were significantly inhibited after NUSN2 knockdown(Fig. 3A).

3.4 NUSN2 knockdown promotes apoptosis in the ischemic hemispheric zone of mouse brain

Figure 4 Tunel staining results (Fig. 2E) showed that apoptosis was increased. We then measured apoptosis-related proteins(clea-caspase3/caspase3/bcl-2/bax) in the ischemic hemispheric zone of the cerebral cortex 24 h after MCAO mapping by Western blotting. The results of this study showed that the ratio of apoptotic proteins clea-caspase3/caspase3 and bcl-2/bax noticeably increased in the MCAO-KO group compared with the MCAO group, indicating an increase in apoptosis in the ischemic semidark zone of the cerebral cortex 24h after MCAO modeling(Fig. 4A).

4. Discussion

This study initially investigated the effect of NSUN2 in cerebral ischemia-reperfusion injury. We used a mouse MCAO/R model to analyze the expression pattern and function of NSUN2, as well as the effects and potential mechanisms of cerebral ischemia-reperfusion injury by knocking down NSUN2.

NSUN2 refers to a member of the NOL1/ NOP2/SUN domain-containing protein family, primarily localized in the nucleus. NSun2 was reported to be able to modify some non-coding small RNAs besides tRNA.

NSun2 forms covalent intermediates with the cytosine of the target RNA through the cysteine in the molecule in order to activate the spent methylated pyrimidine loop, allowing SAM to make nucleophilic attack to pyrimidine C5, thereby forming m5C modification(Khoddami and Cairns,2013).Neurological phenotypes are often the primary manifestation of mutations affecting the tRNA regulome. The brain's translational machinery bears promise for psychiatric disease treatment(Aguilar-Valles et al.,2021;Fine et al.,2019).NSUN2 is a vital epitranscriptome regulator in mature neurons, and its expression and activity in mature neurons is critical for complex behaviors including synaptic transmission and emotional memory. Deficiency of neuronal NSUN2 can cause impaired neurotransmitter transduction and synaptic signaling causing emotional behavioral variations(Blaze et al.,2021).NSUN2 expression is significantly reduced in equine tissues of Alzheimer's patients, thereby decreasing tRNA methylation and promoting the development of Alzheimer's disease(Wu et al.,2021). As revealed from the results here, NSUN2 knockdown significantly up-regulated neurobehavioral scores, brain infarct size and neuronal death, while decreasing survival in a mouse MCAO/R model. For this reason, this study hypothesized that NSUN2 is critical to cerebral ischemia-reperfusion injury in mice.

Next, the potential mechanisms of NSUN2 in cerebral ischemia-reperfusion injury was further explored. In this study, PI3K/P-AKT, ICAM-1 and bcl-2 levels were suggested to decrease significantly as well as clea-caspase3, and bax levels increased noticeably in the brain tissue of NSUN2 knockout mice model by WB investigation. The PI3K/AKT signaling channel is critical to regulating various different cellular functions (e.g., metabolism, growth, proliferation, survival, transcription and protein synthesis)(Manning and Toker,2017). After the occurrence of cerebral ischemia, the body can release some substances that can activate tyrosine kinase receptors (e.g., nerve cell growth factor and integrins) which can activate PI3K/AKT signal channel after phosphorylation of PI3K and AKT, and the effector molecules are then capable of exerting anti-apoptotic effects via apoptotic proteins (e.g., BCL-2 family proteins and Caspase family proteins) and apoptotic genes; also It can regulate mitochondria, endoplasmic reticulum, etc., thus exerting anti-apoptotic effects(Okoreeh et al.,2017;Zheng et al.,2019). Bax and Bcl-2 pertain to the Bcl-2 family; Bcl-2 refers to an anti-apoptotic protein, and Bax is a pro-apoptotic protein. Cysteine proteases are a family of cysteine proteases, i.e., vital mediators of apoptosis and neurodegeneration in neuronal cells. Cleavage Caspase-3 cleavage is activated by I/R, thereby causing DNA breakage and mitochondrial dysfunction and then increasing cell apoptosis(Yasuda et al.,2014).In MCAO model mice, olivetin exerts anti-apoptotic effects by increasing Bcl-2 expression and decreasing Bax expression(Yu et al.,2016).In analytical studies of gastrointestinal tumors, the PI3K/Akt signaling channel was found to be the most important channel affected by m5c methylation, and GO investigation of differential proteins in the PI3K/Akt signaling channel revealed that NSUN2 is critical to both regulation of protein binding and signaling(Xiang et al.2020).

As a transport channel for blood supplying the whole body with energy, the number and function of blood vessels closely affect the function of organs and even the entire organism. The regeneration and repair of blood vessels directly affect the prognosis of cerebral ischemia-reperfusion injury(Kanazawa et al.,2019). RNA methylation, a vital channel to regulate gene expression, is capable of facilitating vascular regeneration and repair via various mechanisms(Zaitseva et al.,2019). ICAM-1 mediates cell-to-cell or cell-

to-matrix contact and binding, thereby participating in cell signaling and activation, with low expression at rest and increased expression in response to stimuli (e.g., inflammatory mediators and oxidative stress)(Lawson and Wolf,2009). Moreover, it has been demonstrated that ICAM-1 is significantly correlated with cerebral infarction(Deddens et al.,2017). The study indicated that m5C methyltransferase NSun2 up-regulates the expression of cell adhesion molecule ICAM-1 at the translational level through mRNA methylation, thereby promoting vascular repair through adhesion of leukocytes to vascular endothelial cells. As revealed from the results here, NSUN2 knockdown significantly inhibits the expression of PI3K/p-Akt/ICAM-1 protein in the mouse MCAO/R model.

The shortcoming of this study is that the mechanism of the effect of NSUN2 on PI3K/p-Akt/ICAM-1 protein was not further explored.

In brief, the results of this study demonstrated that NSUN2 is critical to stroke in mice, and NSUN2 knockdown significantly increases stroke injury and decreases survival in mice, probably attributed to increased apoptosis attributed to NSUN2 knockdown, thereby inhibiting PI3K/Akt signaling channel and ICAM-1 expression.This study will expand the new mechanism of stroke, provide a scientific theoretical basis for finding new therapeutic targets for stroke injury, and play an important role in reducing diseases in elderly patients and reducing public health pressure.

Declarations

Data Availability Statements

All data generated or analysed during this study are included in this published article(and its supplementary information files).

Ethics declarations

Ethics approval

The study was approved by the Animal Experimentation Ethics Committee of Wenzhou Medical University (approval number wydw2021–0120).

Consent to participate

All participants provided their written informed consent to participate in this study. Content and procedures of written information consent were inspected thoroughly within the ethics approval procedure.

Consent for Publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article(and its supplementary information files).

Competing of Interests

The authors declare no competing interests.

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Authors' contributions

Conceptualization,Xuqing Ni and Xia Liu;methodology, Xuqing Ni and Xinyu Yao; software, Shan Li; validation, Yurun Zhu,Ye Zhu and Yunchang Mo; formal analysis and investigation, Xia Liu and Yurun Zhu;data curation,Ye Zhu and Yunchang Mo;writing—original draft preparation, Xuqing Ni; writing—review and editing, Qinxue Dai; supervision and funding acquisition, Qinxue Dai and Junlu Wang.

References

1. Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, et al. (2012). Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet, 90: 847-55.doi: 10.1016/j.ajhg.2012.03.021.
2. Aguilar-Valles A, De Gregorio D, Matta-Camacho E, Eslamizade MJ, Khlaifia A, Skaleka A, et al. (2021). Antidepressant actions of ketamine engage cell-specific translation via eIF4E. Nature, 590: 315-9.doi: 10.1038/s41586-020-03047-0.
3. Blanco S, Bandiera R, Popis M, Hussain S, Lombard P, Aleksic J, et al. (2016). Stem cell function and stress response are controlled by protein synthesis. Nature, 534: 335-40. doi: 10.1038/nature18282.
4. Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, et al. (2014). Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. EMBO J, 33: 2020-39.doi: 10.15252/embj.201489282.
5. Blaze J, Navickas A, Phillips HL, Heissel S, Plaza-Jennings A, Miglani S, et al. (2021). Neuronal Nsun2 deficiency produces tRNA epitranscriptomic alterations and proteomic shifts impacting synaptic signaling and behavior. Nat Commun, 12: 4913.doi: 10.1038/s41467-021-24969-x.
6. Cai X, Hu Y, Tang H, Hu H, Pang L, Xing J, et al. (2016). RNA methyltransferase NSUN2 promotes stress-induced HUVEC senescence. Oncotarget, 7: 19099-110.doi:10.18632/oncotarget.8087.

7. Capece D, Verzella D, Di Francesco B, Alesse E, Franzoso G, Zazzeroni F (2020). NF-κB and mitochondria cross paths in cancer: mitochondrial metabolism and beyond. Semin Cell Dev Biol, 98: 118-28.doi: 10.1016/j.semcdb.2019.05.021.
8. Deddens LH, van Tilborg G, van der Marel K, Hunt H, van der Toorn A, Viergever MA, et al. (2017). In Vivo Molecular MRI of ICAM-1 Expression on Endothelium and Leukocytes from Subacute to Chronic Stages After Experimental Stroke. Transl Stroke Res, 8: 440-8.doi: 10.1007/s12975-017-0536-4.
9. Fine AS, Nemeth CL, Kaufman ML, Fatemi A (2019). Mitochondrial aminoacyl-tRNA synthetase disorders: an emerging group of developmental disorders of myelination. J Neurodev Disord, 11: 29.doi: 10.1186/s11689-019-9292-y.
10. Flores JV, Cordero-Espinoza L, Oeztuerk-Winder F, Andersson-Rolf A, Selmi T, Blanco S, et al. (2017). Cytosine-5 RNA Methylation Regulates Neural Stem Cell Differentiation and Motility. Stem Cell Reports, 8: 112-24.doi: 10.1016/j.stemcr.2016.11.014.
11. Jiang J,Dai J,Cui H(2018).Vitexin reverses the autophagy dysfunction to attenuate MCAO-induced cerebral ischemic stroke via mTOR/Ulk1 pathway. Biomed Pharmacother, 99:583-590.doi:10.1016/j.biopha.2018.01.067.
12. Jonkhout N, Tran J, Smith MA, Schonrock N, Mattick JS, Novoa EM (2017). The RNA modification landscape in human disease. RNA, 23: 1754-69.doi: 10.1261/rna.063503.117.
13. Kanazawa M, Takahashi T, Ishikawa M, Onodera O, Shimohata T, Del Zoppo GJ (2019). Angiogenesis in the ischemic core: A potential treatment target. J Cereb Blood Flow Metab, 39: 753-69.doi: 10.1177/0271678X19834158.
14. Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V, et al. (2012). Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. Am J Hum Genet, 90: 856-63.doi: 10.1016/j.ajhg.2012.03.023.
15. Khoddami V, Cairns BR (2013). Identification of direct targets and modified bases of RNA cytosine methyltransferases. Nat Biotechnol, 31: 458-64.doi: 10.1038/nbt.2566.
16. Komara M, Al-Shamsi AM, Ben-Salem S, Ali BR, Al-Gazali L (2015). A Novel Single-Nucleotide Deletion (c.1020delA) in NSUN2 Causes Intellectual Disability in an Emirati Child. J Mol Neurosci, 57: 393-9.doi: 10.1007/s12031-015-0592-8.
17. Lawson C, Wolf S (2009). ICAM-1 signaling in endothelial cells. Pharmacol Rep, 61: 22-32.doi: 10.1016/s1734-1140(09)70004-0.
18. Li Z, Xiao G, Wang H, He S, Zhu Y (2021). A preparation of Ginkgo biloba L. leaves extract inhibits the apoptosis of hippocampal neurons in post-stroke mice via regulating the expression of Bax/Bcl-2 and Caspase-3. J Ethnopharmacol, 280: 114481.doi: 10.1016/j.jep.2021.114481.
19. Liu RJ, Long T, Li J, Li H, Wang ED (2017). Structural basis for substrate binding and catalytic mechanism of a human RNA:m5C methyltransferase NSun6. Nucleic Acids Res, 45: 6684-97.doi: 10.1093/nar/gkx473.
20. Luo Y, Feng J, Xu Q, Wang W, Wang X (2016). NSun2 Deficiency Protects Endothelium From Inflammation via mRNA Methylation of ICAM-1. Circ Res, 118: 944-56.doi:

10.1161/CIRCRESAHA.115.307674.

21. Manning BD, Toker A (2017). AKT/PKB Signaling: Navigating the Network. Cell, 169: 381-405.doi: 10.1016/j.cell.2017.04.001.
22. Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, et al. (2012). Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. J Med Genet, 49: 380-5.doi: 10.1136/jmedgenet-2011-100686.
23. Oerum S, Dégut C, Barraud P, Tisné C (2017). m1A Post-Transcriptional Modification in tRNAs. Biomolecules, 7 .doi: 10.3390/biom7010020.
24. Okoreeh AK, Bake S, Sohrabji F (2017). Astrocyte-specific insulin-like growth factor-1 gene transfer in aging female rats improves stroke outcomes. Glia, 65: 1043-58.doi: 10.1002/glia.23142.
25. Wang W (2016). mRNA methylation by NSUN2 in cell proliferation. Wiley Interdiscip Rev RNA, 7: 838-42.doi: 10.1002/wrna.1380.
26. Wu W, Lee I, Spratt H, Fang X, Bao X (2021). tRNA-Derived Fragments in Alzheimer's Disease: Implications for New Disease Biomarkers and Neuropathological Mechanisms. J Alzheimers Dis, 79: 793-806.doi: 10.3233/JAD-200917.
27. Xiang S, Ma Y, Shen J, Zhao Y, Wu X, Li M, et al. (2020). m(5)C RNA Methylation Primarily Affects the ErbB and PI3K-Akt Signaling Pathways in Gastrointestinal Cancer. Front Mol Biosci, 7: 599340.doi: 10.3389/fmolb.2020.599340.
28. Xing J, Yi J, Cai X, Tang H, Liu Z, Zhang X, et al. (2015). NSun2 Promotes Cell Growth via Elevating Cyclin-Dependent Kinase 1 Translation. Mol Cell Biol, 35: 4043-52.doi: 10.1128/MCB.00742-15.
29. Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, et al. (2017). 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. Cell Res, 27: 606-25.doi: 10.1038/cr.2017.55.
30. Yasuda N, Ishii T, Oyama D, Fukuta T, Agato Y, Sato A, et al. (2014). Neuroprotective effect of nobiletin on cerebral ischemia-reperfusion injury in transient middle cerebral artery-occluded rats. Brain Res, 1559: 46-54.doi: 10.1016/j.brainres.2014.02.007.
31. Yu H, Liu P, Tang H, Jing J, Lv X, Chen L, et al. (2016). Oleuropein, a natural extract from plants, offers neuroprotection in focal cerebral ischemia/reperfusion injury in mice. Eur J Pharmacol, 775: 113-9.doi: 10.1016/j.ejphar.2016.02.027.
32. Zaitseva TS, Alcazar C, Zamani M, Hou L, Sawamura S, Yakubov E, et al. (2019). Aligned Nanofibrillar Scaffolds for Controlled Delivery of Modified mRNA. Tissue Eng Part A, 25: 121-30.doi: 10.1016/s1734-1140(09)70004-0.
33. Zheng T, Shi Y, Zhang J, Peng J, Zhang X, Chen K, et al. (2019). MiR-130a exerts neuroprotective effects against ischemic stroke through PTEN/PI3K/AKT pathway. Biomed Pharmacother, 117: 109117.doi: 10.1016/j.biopha.2019.109117.
34. Zhou Z, Dun L, Wei B, Gan Y, Liao Z, Lin X, et al. (2020). Musk Ketone Induces Neural Stem Cell Proliferation and Differentiation in Cerebral Ischemia via Activation of the PI3K/Akt Signaling Pathway. Neuroscience, 435: 1-9.doi: 10.1016/j.neuroscience.2020.02.031.

Figures

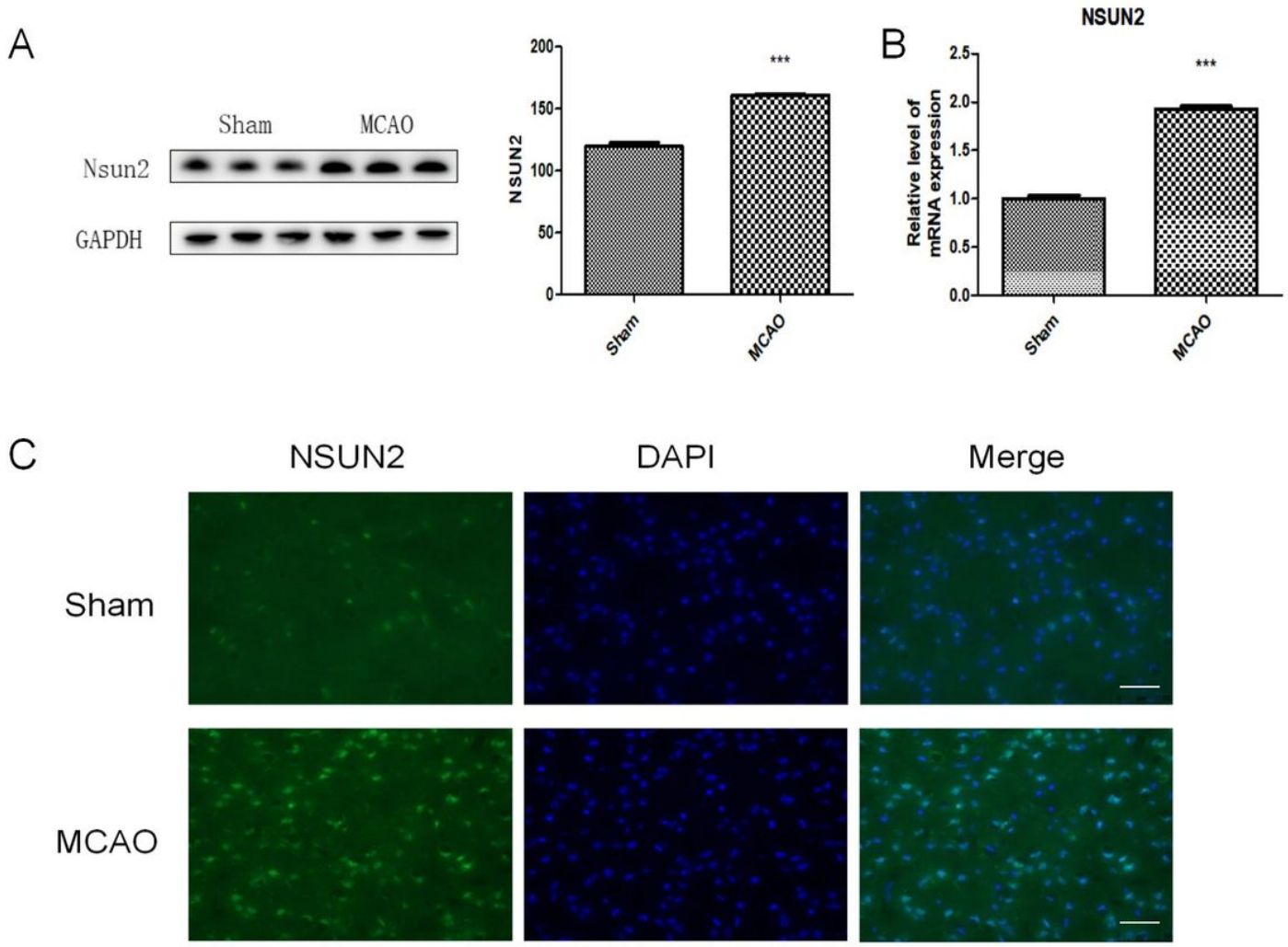

Figure 1

Expression of NSUN2 in the ischemic semidark zone of the cerebral cortex 24 h with the finishing of cerebral ischemia-reperfusion injury.□A□Western blotting detection of NSUN2 protein expressions within the ischemic semidark zone pertaining to cerebral cortex 24 h after cerebral ischemia-reperfusion injury, n=6.□B□Expression of NSUN2 mRNA in the ischemic semidark zone of the cerebral cortex 24 h with the finishing of cerebral ischemia-reperfusion injury was detected by performing qRT-PCR, n=6.□C□ Immunofluorescence staining investigation, NSUN2 in green, nuclei in blue, n=6. Scale bars:10μm. Data have the presentation of mean±SD. ***P < 0.05 VS the sham group.

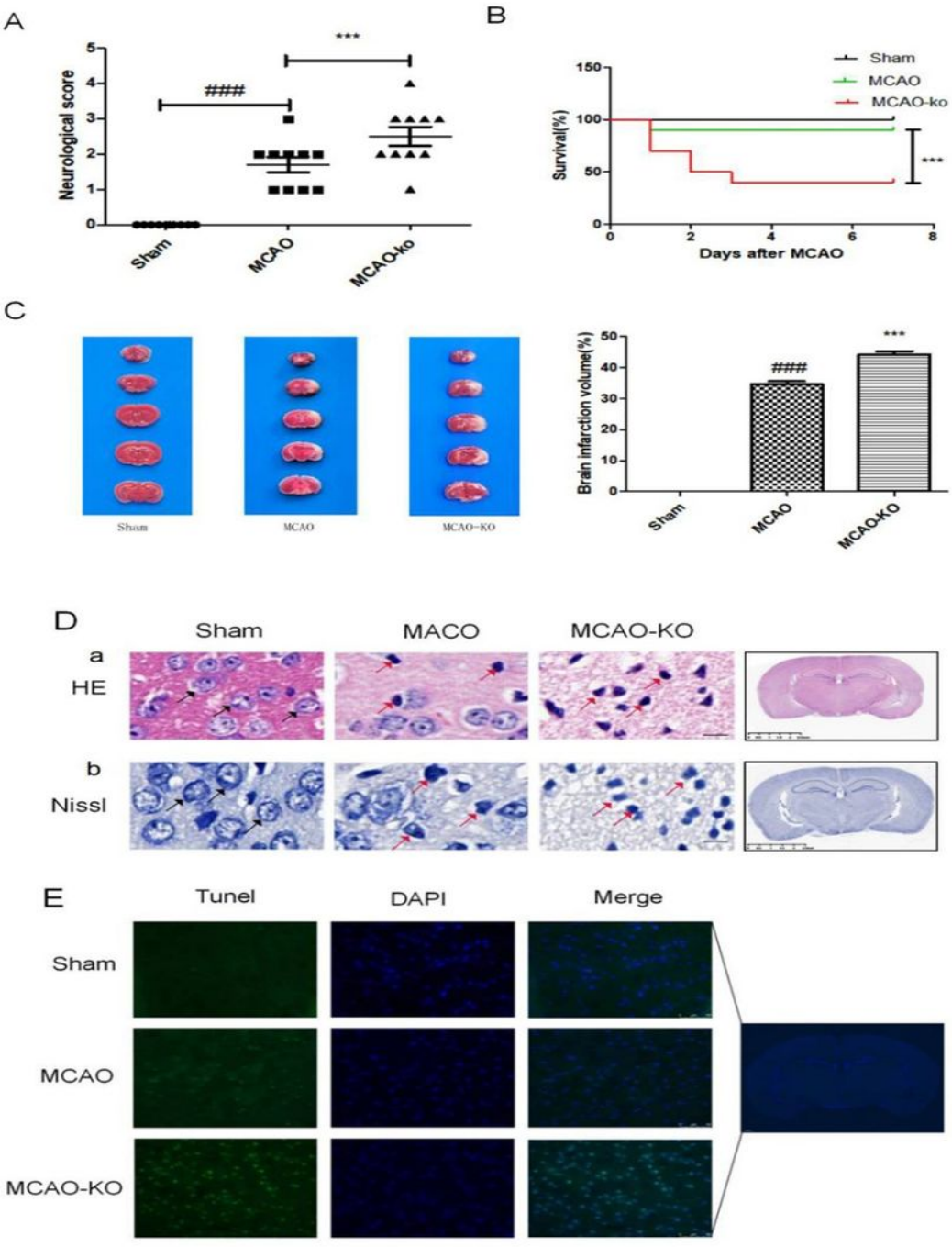

Figure 2

NSUN2 knockdown exacerbates brain ischemia-reperfusion injury in mice.(A) Neurobehavioral scores of the three groups of mice at 24 h postoperatively, n=10.(B)7-day postoperative survival rate in three groups of mice, n=10.(C)TTC staining results, red indicates normal tissue and white indicates infarcted tissue, n=5.(Da)HE staining indicating cell morphologic variations in the ischemic penumbra 24h after reperfusion. Scale bars=10μm. Black arrow represents the clear cell outline and compact structure. Red

arrow represents the cell with sparse arrangement, cell outline showed fuzziness, and structure was disordered, n=5.(Db)Nissl staining indicating morphology-related neuronal variations in the ischemic penumbra 24h after reperfusion. Scale bars=10μm. Black arrow represents the intact neuron as well as flush cell body. Red arrow represents the injured neuron covering shrunken cell body followed by shrunken and pyknotic nuclei, n=5.(E)Tunel staining indicating neuronal death within the ischemic penumbra 24h after reperfusion. Scale bars=10μm. Tunel vesicles are green and nuclei are blue, n=5.Data are expressed as mean±SD. ***P< 0.05 VS the MCAO group.###P < 0.05 VS the Sham group.

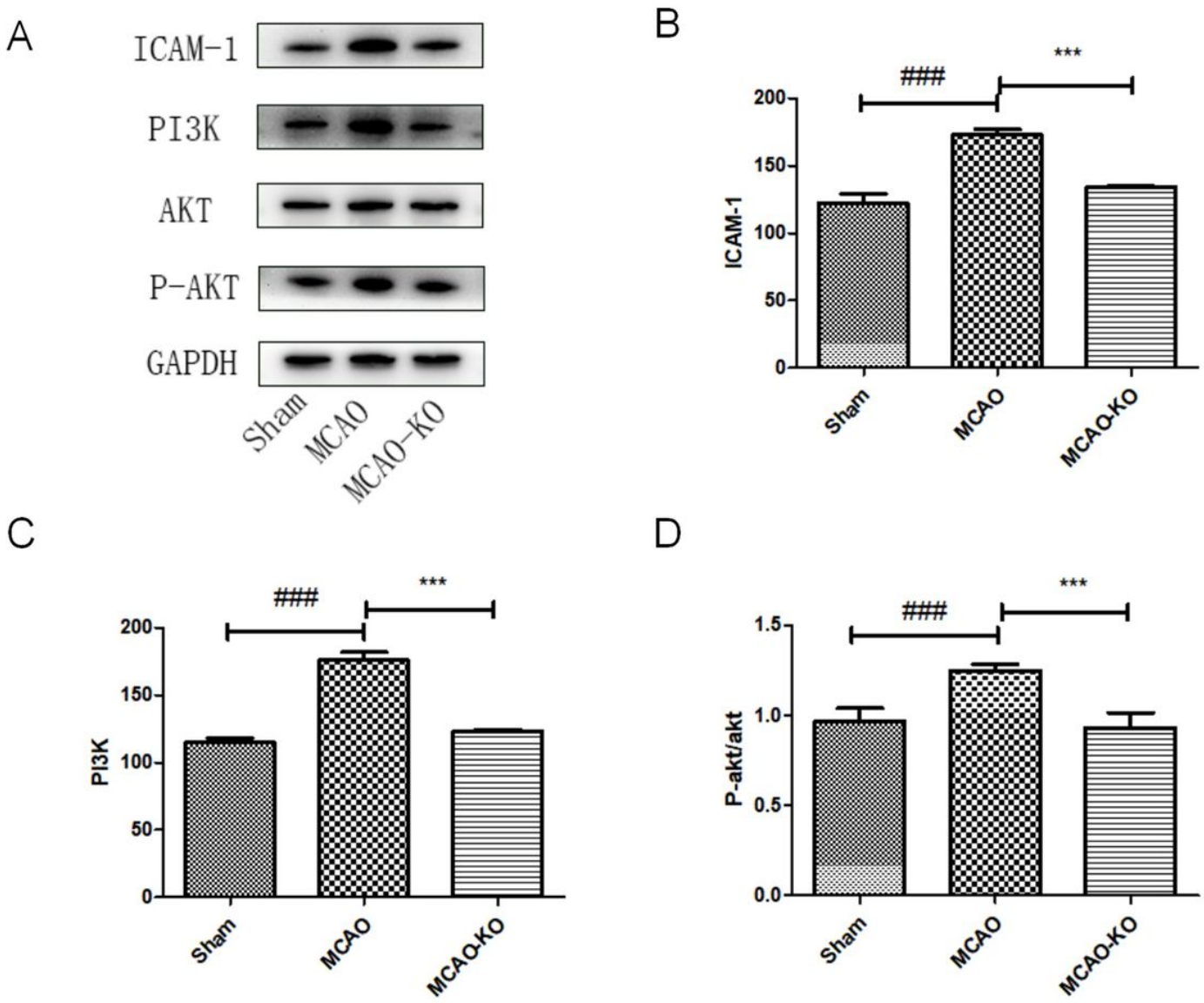

Figure 3

NUSN2 knockdown inhibits the expression of PI3K/AKT signaling channel and ICAM-1 in the ischemic half dark band region of mouse brain.(A) Western blotting detection of PI3K, AKT, P-AKT and ICAM-1 protein expression in the ischemic semidark zone of the cerebral cortex 24h after cerebral ischemia-reperfusion injury, n=6.(B/C/D) Histogram of the expression content of PI3K, AKT, P-AKT and ICAM-1

proteins. Data are expressed as mean±SD. ***P< 0.05 VS the MCAO group.###P< 0.05 VS the Sham group.

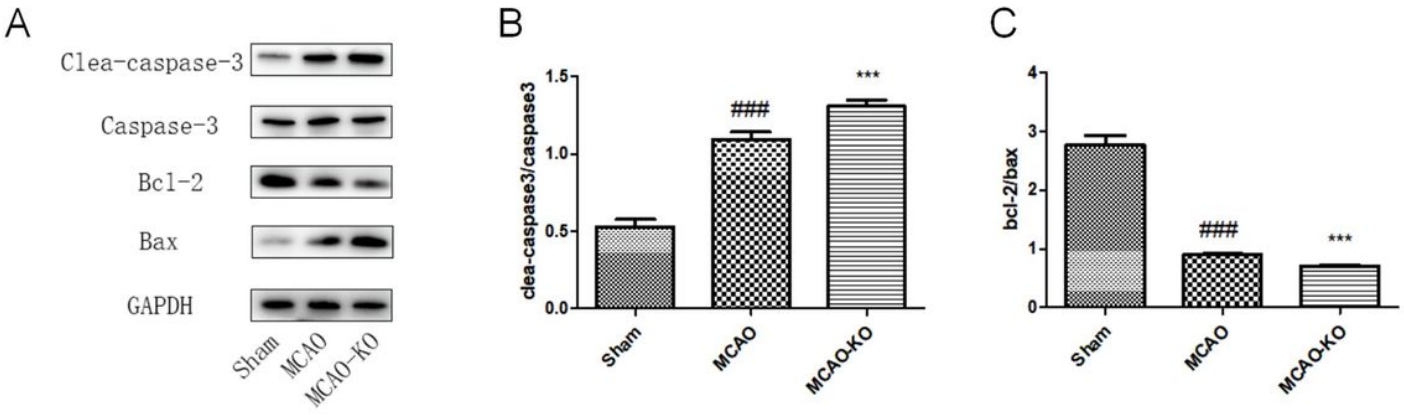

Figure 4

NUSN2 knockdown promotes apoptosis in the ischemic hemispheric zone of mouse brain.(A) Western blotting detection of clea-caspase3,caspase3,bcl-2 and bax protein expression in the ischemic semidark zone of the cerebral cortex 24h after cerebral ischemia-reperfusion injury n=6.(B/C) Histogram of the expression content of clea-caspase3/caspase3 and bcl-2/bax proteins. Data are expressed as mean±SD. ***P< 0.05 VS the MCAO group.###P< 0.05 VS the Sham group.

Supplementary Files

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