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MicroRNA-181b-5p attenuates early postoperative cognitive dysfunction by suppressing hippocampal neuroinflammation in mice

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ABSTRACT

Background: Postoperative cognitive dysfunction (POCD) is a common complication after surgery and its occurrence is associated with increased morbidity and mortality. However, the pathophysiology of this complication remains largely unknown. Efforts to identify causes of POCD have focused on the hippocampal neuroinflammation. Recently, accumulated evidence indicates that NeurimmiRs, a subset of microRNAs (miRNAs), which modulate both neuronal and immune processes, play an important role in neuroinflammation. However, the impact of NeurimmiRs on POCD has not been investigated. We hypothesized that NeurimmiRs is involved in surgery-induced cognitive impairment in adult mice via mediating hippocampal neuroinflammation.

Methods: MicroRNA(miR)-181b-5p was found to be downregulated in the hippocampi of mice with POCD using microRNA array, which was also verified *in vivo* in the mouse model of POCD by Quantitative real-time polymerase chain reaction (qPCR). Subsequently, the expression of miR-181b-5p was measured in lipopolysaccharide (LPS) stimulated BV-2 microglial cells and hippocampal tissues of the mice with POCD. Then, loss of function and overexpression studies were performed by transfection with miR-181b-5p mimic/ inhibitor in cultured BV-2 cell lines and intrahippocampal injection of miR-181b-5p agomir before Surgery/Anesthesia, to identify the role of miR-181b-5p in neuroinflammation and cognitive impairments. QPCR, western blot and ELISA were used to determine the expression of proinflammatory mediators. Immunofluorescence staining was applied to evaluate the activation of microglia. Furthermore, we used bioinformatics analysis and dual-luciferase assay to predict and verify the potential target of miR-181b-5p.

Results: The results indicated that miR-181b-5p mimic could repress the mRNA and protein expression of proinflammatory mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and monocyte chemoattractant protein (MCP)-1 in LPS-stimulated BV-2 microglial cells, while the miR-181b-5p inhibitor induced upregulation of the above-mentioned proinflammatory factors. Further bioinformatics analysis showed that miR-181b-5p was predicted to potentially target the 3′-untranslated region (UTR) of TNF-α, and binding sites of miR-181b-5p in the 3′-UTR of TNF-α were identified by dual-luciferase assay. Importantly, injecting miR-181b-5p agomir into the hippocampus of mice before surgery, ameliorated the hippocampus-dependent memory, and was accompanied by downregulation of proinflammatory factors expression and reduced microglial activation in the hippocampus of POCD mice.

Conclusions: Collectively, these findings suggest that miR-181b-5p attenuates early POCD by suppressing hippocampal neuroinflammation in mice. They also highlight the importance of studying miRNAs in the context of POCD and identify miR-181b-5p as a novel potential therapeutic target for improving POCD.

Abbreviations: POCD, Postoperative cognitive dysfunction; TNF-α, Tumor necrosis factor-α; IL-1β, Interleukin-1β; BBB, Blood-brain barrier; MCP-1, Monocyte chemoattractant protein-1; MiRNAs, MicroRNAs; AD, Alzheimer's disease; IRF4, Interferon regulatory factor 4; CFH, Complement factor-H protein; IL-6, Interleukin-6; MRP8, myeloid-related protein-8; TLR4, Toll like receptor 4; IRAK4, interleukin-1 receptor-associated kinase 4; FCT, Fear conditioning test; OFT, Open-field test; qPCR, Quantitative real-time polymerase chain reaction; NC, Negative control; Iba-1, Ionized calcium binding adaptor molecule-1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide; HMGB-1, High mobility group box-1 protein; PTEN, phosphatase and tensin homologue; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MAPK, mitogen-activated protein kinase

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1. Introduction

Postoperative cognitive dysfunction (POCD), a common postoperative complication [1–3], involves a wide range of cognitive functions including working memory, long term memory, information processing, attention and cognitive flexibility [4], adversely affecting quality of life, social dependence, and mortality [5]. However, the neuropathogenesis and targeted intervention(s) for POCD remain largely to be determined.

Evidence is accumulating for a key role of neuroinflammation, which is secondary to innate immune response to the aseptic trauma of surgery, in the development of POCD [6,7]. Surgical trauma engages the innate immune system to release proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. These cytokines signals can be transmitted to the brain and lead to neuroinflammation through the impaired blood-brain barrier (BBB), direct neural pathways (via primary autonomic afferents), or transport across the BBB [8]. Increased brain proinflammatory cytokines can overactivate microglia, which induces further proinflammatory factors, such as TNF-α, IL-1β, and monocyte chemoattractant protein (MCP)-1 release, in cerebral tissue and fuels a vicious cycle of neuroinflammation [9,10]. Furthermore, overactivated microglia creates a neurotoxic response, causes neuronal injury, and affects neuronal functions essential for learning and memory, leading to cognitive impairment [11].

The hippocampus has considerable importance for cognition and mood, particularly for the formation of new memory [12,13]. In addition, hippocampus-dependent learning and memory is especially vulnerable to inflammatory insults [14]. Accordingly, efforts to identify causes and pathogenesis for POCD have focused on the postoperative hippocampal neuroinflammation. Rodent models suggest that hippocampal neuroinflammation contributes to POCD [15–19]. Our previous study has also suggested that the orthopedic surgery may induce neuroinflammation in the hippocampus and may lead to hippocampus-dependent learning and memory impairment in mice [20].

MicroRNAs (miRNAs) are small non-coding RNAs of 19–25 nucleotides in length, which play a significant role in post-transcriptional gene regulation by binding with their target mRNAs [21,22]. Recent findings have demonstrated their important roles in neuroinflammation. A subset of miRNAs have been demonstrated to modulate both neuronal and immune processes (here termed NeurimmiRs [23]). An accumulating body of evidence suggests a pivotal role of NeurimmiRs in neuroinflammation. For instance, in Alzheimer's disease (AD), in which neuroinflammation is a central component, upregulation of miR-125b was found in the hippocampus and medial frontal gyrus of AD patient [24]. In addition, miR-125b has also been shown to repress the expressions of interferon regulatory factor 4 (IRF4) and complement factor-H protein (CFH), which are factors involved in the proinflammatory response, in human primary astrocytes [25]. Moreover, miR-132 has been demonstrated to negatively regulate the release of IL-1β and interleukin (IL)-6 induced by myeloid-related protein-8 (MRP8), which is an endogenous ligand of Toll like receptor 4 (TLR4), in astrocytes by targeting interleukin-1 receptor- associated kinase 4 (IRAK4) [26]. Furthermore, in prion disease, a uniquely infectious neurodegenerative condition, miR-146a overexpression has been reported in the brain of prion infected mice concurrent with the onset of prion deposition and appearance of activated microglia [27]. Additionally, miR-146a has also been reported to be induced in response to inflammatory cues, such as IL-1β, as a negative-feedback regulator of the human astrocyte-mediated inflammatory response [28]. However, the role of miRNAs in surgery-induced hippocampal neuroinflammation and cognitive decline, has yet to be determined.

Therefore, the aim of the current study was to investigate the role of miRNAs in hippocampal inflammation and POCD. In the present study, we applied microarray analysis to detect the miRNA profile in the hippocampus of the mouse model with POCD. A total of 22 miRNAs were found to be differentially expressed, and among these altered miRNAs, miR-181b-5p had the greatest decrease. Moreover, miR-181b-5p is highly conserved both in humans and in mice, and present at a high level in the mammalian brain [29]. More importantly, miR-181b-5p has been widely reported to participate in the pathogenesis of inflammation and neurological diseases [30–35]. Accordingly, in the current study, we chose miR-181b-5p as the candidate miRNA. We hypothesized that miR-181b-5p would be associated with the hippocampal inflammation and cognitive impairment in mice with POCD. The findings of this investigation may be helpful to promote more studies to investigate the role of NeurimmiRs in the neuropathogenesis of POCD and identify miR-181b-5p as a novel potential therapeutic target for improving POCD.

2. Materials and methods

2.1. Animals

All procedures were approved by the Animal Ethics Committee of Zhongnan Hospital of Wuhan University, Hubei, China, and all experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Efforts were made to minimize the number of animals used. C57BL/6 male mice (The Experimental Animal Center of Hubei province, Wuhan, China) (4-month-old, weighing 20–30 g) were used in this study. All animals were housed five per cage with free access to food and water. The temperature, humidity, and day-night cycle were maintained according to the standards established by the experimental animal laboratory at Zhongnan Hospital of Wuhan University. The mice were acclimatized to the laboratory environment for 1 week before experiments.

2.2. Experimental protocol

The first experiment was designed to determine the effects of Surgery/ Anesthesia on cognitive function and hippocampal miRNA expression profiles in mice. Mice were randomly divided into 2 groups: control group and surgery group. The mice in the surgery group were subjected to an intramedullary fixation surgery for tibial fracture under isoflurane anesthesia, while the mice in the control group received 100% oxygen for 20 min without surgery. The mice were trained for fear conditioning 24 h prior to surgery treatments. Fear conditioning test (FCT) was performed on 1, 3, and 7 days after surgery. The open-field test (OFT) was carried out 15 min before the training phase and each test phase of FCT. Within each group, separate cohorts were subjected to assessment at each time point (n = 10 per cohort). The animals were sacrificed immediately after the behavioral assessment on postoperative days 1, 3, and 7. Brains were rapidly harvested and the hippocampus was dissected and stored in RNAlater at −80 °C until assaying for miRNA microarray. Three mice with the most obvious shortening in the freezing time of the context test were chosen from surgery group and their hippocampus were used for microarray analysis to detect the miRNA profile, and three mice were chosen randomly from control group as the control. Quantitative real-time polymerase chain reaction (qPCR) was used for validation of the microarray results.

A subsequent study was conducted to determine the effects of miR-181b-5p on hippocampal neuroinflammation and cognitive behavior in mice. Mice were randomly divided into 4 experimental groups: control group, surgery group, surgery + agomir group, and surgery + agomir control group. Mice in surgery group received surgery under isoflurane anesthesia to determine the contribution of surgical and anesthetics stress to the level of proinflammatory factors in hippocampus. Mice in surgery + agomir group were injected with miR-181b-5p agomir into the hippocampus 48 h prior to Surgery/Anesthesia, and mice in surgery + agomir control group received the treatment of preoperative negative control (mismatched-miR-181b-5p agomir), thus allowing us to investigate the impact of miR-181b-5p agomir on hippocampal

inflammation and cognitive function in mice. Mice were sacrificed at 6, 12, 24 h after surgery and hippocampus were collected for qPCR and ELISA assay (n = 6 per cohort). On postoperative days 1, and 3, mice were anesthetized and transcardially perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde, and then, hippocampal tissues were collected for immunostaining (n = 3 per cohort). Behavioral tests were conducted on 1 day before and 1, 3, 7 days after surgery (n = 10 per cohort).

2.3. Surgical model

Mice received open tibial fracture with intramedullary fixation under isoflurane anesthesia as described in a previous study with modifications [19,36,37]. Specifically, anesthesia was induced with 3.0% isoflurane followed by maintenance with 1.5% isoflurane carried by 100% oxygen. After shaving the mouse, an incision was made lateral to the tibia, and the bone was exposed, after that, a small hole was drilled at the level of the tibial tuberosity, then a 0.38-mm intramedullary fixation pin was introduced into the medullary canal. An osteotomy was created at the junction of the middle and distal thirds of the tibia, and the incision was sutured with 6/0 Vicryl thread. The entire procedure from the induction of anesthesia to the end of surgery lasted about 20 min. A heat pad was used to keep the mouse body temperature between 36 °C and 37°C during the surgery. Following surgery, all mice were recovered in a suitable environment and then returned to home cage. To treat the pain associated with the skin incision, a 2% lidocaine solution was applied locally before the incision, and 1% tetracaine hydrochloride mucilage was applied to the wound twice daily until 3 days after surgery.

2.4. Stereotaxic surgery and intrahippocampal microinjection

For overexpression of miR-181b-5p, mice in surgery + agomir group had the treatments of miR-181b-5p agomir (Guangzhou RiboBio Co., Ltd., Guangzhou, China) hippocampal injection 48 h before Surgery/Anesthesia. And mice in surgery + agomir control group received the same dosage of the negative control (agomir-NC; Guangzhou RiboBio Co., Ltd., Guangzhou, China) preoperatively as a control. Mice were anesthetized with 2% isoflurane in 70% N_2O balance O_2 by facemask and placed in a stereotaxic apparatus. A tiny hole (0.05 mm in diameter) was drilled on the skull surface just above hippocampus. Mice were injected with 2 μl of miR-181b-5p agomir or agomir-NC per side at 0.25 μl/minute, corresponding to a total dose of 1 nmol of miR-181b-5p agomir or agomir-NC, into bilateral hippocampus 48 h prior to Surgery/Anesthesia. Stereotaxic coordinates used for intrahippocampal injections were as follows (from bregma): anteroposterior (AP), −1.9 mm; mediolateral (ML), ± 1.5 mm; dorsoventral (DV), −1.4 mm [38].

2.5. Behavioral tests

The behavioral changes were detected by OFT and FCT before and after surgery. Mice were trained for fear conditioning on 1 day prior to surgery and FCT were performed on 1, 3, and 7 days after surgery [39]. In addition, OFT was carried out 15 min before FCT to evaluate the locomotor activity of the mice. In all tests each apparatus was cleaned with 75% ethanol after each mouse to remove odors.

2.5.1. OFT

The OFT was carried out to assess the locomotor activity of mice. Each mouse was placed individually into the center of an open-field chamber under dim light and it was recorded for a total period of uninterrupted 5 min. The activities were automatically recorded by a video camera connected to the Any-Maze animal tracking system software (Xinruan Information Technology Co. Ltd., Shanghai, China), and movement parameters were calculated by the software. The total distance traveled was used to determine the locomotor activity of the mice.

2.5.2. FCT

FCT can test associative learning and memory [16,17,40,41]. One day prior to the operation, mice were trained for fear conditioning to establish long-term memory. Each mouse was placed in the conditioning chamber and allowed to explore it for 120 s. Mice were then exposed to an auditory cue (70 dB, conditional stimulus) for 20 s, and a contextual interval of 25 s, and finally a 2-seconds electrical foot shock (0.70-mA, unconditional stimulus). The tone and foot-shock pairing was repeated for six times and there was a random interval from 45 to 60 s between two pairs of stimuli.

The test phase of the FCT consists of a context test and a tone test to assess hippocampus-dependent or hippocampus-independent memory respectively [42–45]. During the contextual test, mice were placed back to the same conditioning chamber without any tone or electric shock for 5 min. The tone test was performed 2 h after the contextual test. Mice were placed in a chamber different from the pre-operation training for 5 min. During that period, mice were given sound stimuli (70 dB, 3 min) without any shock stimuli. The Any-Maze animal tracking system software was utilized to record the freezing times.

2.6. Microarray analysis

MiRNA microarray analysis was conducted to explore the differentially expressed miRNAs in hippocampus. The expression of miRNAs contained in the miRBase version 19 was analyzed by microarray using the μParaflo®Microfluidic Biochip Technology of LC Sciences (TX, USA). A total of 4–8 μg of miRNA- enriched RNA was used for miRNA microarray. All microarray reagents and detailed steps used for labeling, hybridization, image acquisition, normalization and data analysis were identical to those reported previously [46]. The signal data was derived by subtracting the background and by normalizing and analyzing the signals employing a locally weighted regression filter. Signal intensities were normalized and transformed by log2. The *t*-test was performed between control and surgery groups, and p-values were calculated. Hierarchical clustering was performed by average linkage and Euclidean distance metric. Finally, the graphic displays were visualized as heat map results of hierarchical clustering.

2.7. RNA extraction, reverse transcription, and qPCR assay

Total RNA was extracted from hippocampal tissues using TRIzol (Invitrogen, CA, USA) in accordance with manufacturer's protocol. Total RNA concentration and purity were detected by a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA). The M−MLV Reverse Transcriptase kit (Invitrogen, CA, USA) was used to synthesize cDNA. According to the manufacturer's instructions, qPCR analysis was performed with SYBR Premix Ex Taq (TaKaRa, Dalian, China) on the StepOne real-time PCR system (Life technologies, CA, USA). The primers are listed in Table 1. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. U6 and GAPDH were used as references.

2.8. ELISA assay

The mice were sacrificed and the hippocampal tissues were harvested for protein content quantification. Levels of TNF-α, IL-1β, and MCP-1 were measured using ELISA Kits (eBioscience, CA, USA) according to the manufacturer's instructions. The concentrations of the cytokines were quantified with reference to the standard curve.

2.9. Immunofluorescence for Iba-1

Morphological changes of microglia were studied by

Table 1
Primers sequence.

Category	Sequences
miR-181b-5p	RT:5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACCCACCG-3′
	Forward:5′ -ATTCATTGCTGTCGGTGGGT-3′
	Reverse:5′- CTCAACTGGTGTCGTGGAGTC-3′
TNF-α	Forward:5′- TCCCCAAAGGGATGAGAAGTT-3′
	Reverse:5′- GAGGAGGTTGACTTTCTCCTGG-3′
IL-1β	Forward:5′- GGGCCTCAAAGGAAAGAATCT-3′
	Reverse:5′- GAGGTGCTGATGTACCAGTTGG-3′
MCP-1	Forward:5′- CCCTACTATTCCTGATGGCACT-3′
	Reverse:5′- CTATGAGAAACCCACCACATCTG-3′
U6	Forward:5′-CTCGCTTCGGCAGCACAT-3′
	Reverse:5′-AACGCTTCACGAATTTGCGT-3′
GAPDH	Forward:5′- TGAAGGGTGGAGCCAAAAG-3′
	Reverse:5′- AGTCTTCTGGGTGGCAGTGAT-3′

immunofluorescence staining for ionized calcium binding adaptor molecule(Iba)-1. On postoperative days 1 and 3, mice were anesthetized with isoflurane and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were harvested, postfixed in 4% paraformaldehyde overnight, and transferred to 30% sucrose in PBS at 4 °C, then the hippocampus were cut into 25 μm-thick coronal sections. Iba-1 staining was performed as previously described [47,48]. Briefly, sections were incubated with rabbit anti-Iba-1 (cat. no. 019–19741; 1:200; Wako Pure Chemical Industries, Ltd., Osaka, Japan) primary antibodies overnight at 4 °C, and subsequently incubated with biotinylated goat anti-mouse immunoglobulin (Ig)-G (cat. no. BA-9200; 1:100; Vector Laboratories, Inc., Burlingame, CA, USA) secondary antibodies at room temperature for 2 h in the dark. Lastly, after washing, the fluorescent images were captured by a fluorescence microscope (Olympus Corp., Tokyo, Japan).

2.10. Cell culture, transfection and LPS-treatment

The mouse microglial cell line BV-2 was obtained from China Center for Type Culture Collection, Wuhan, China. These cells were cultured in Alpha Minimal Essential Medium (α-MEM medium; Hyclone, UT, USA) supplemented with 10% fetal bovine serum (FBS; Zhejiang tianhang Biotechnology Co., Ltd., Hangzhou, China) and 1% penicillin/streptomycin (P/S; Beyotime, Shanghai, China) at 37°C in a humidified atmosphere of 5% CO_2. Then the cells were sub-cultured into 24-well plates and maintained until subconfluence.

BV-2 cells were sub-cultured to the confluence of 30–50% in 24-well plates prior to use in the experiments. MiR-181b-5p mimics, miR-181b-5p mimic-negative control (NC), miR-181b-5p inhibitor, or miR-181b-5p inhibitor-NC (Shanghai GenePharma Co., Ltd., Shanghai, China) were mixed with Lipofectamine 2000 Transfection Reagent (Invitrogen, CA, USA), and then added into the culture medium. After transfection for 48 h, transfection efficiency was assessed by detection of Cy3-labeled miRNA. The Cy3 fluorescence signal was observed using an Olympus IX51 inverted microscope (Olympus Corp., Tokyo, Japan), and the cellular uptake efficiency was calculated as the percentage of Cy3-positive cells. All oligonucleotides were transfected at a final concentration of 25 nM.

In our previous study, we used that concentrations of 1 μg/ml of LPS to induce NO and TNF-α production in primary microglia cultures [49]. In the preliminary experiments, we found that LPS at the concentration of 1 μg/ml could also lead to increased mRNA and protein expressions of proinflammatory mediators, such as TNF-α, IL-1β, and MCP-1, in BV-2 cells (Supplemental information Fig. 1B and Supplemental information Fig. 1C). Thus, we stimulated BV-2 cells with 1 μg/ml of LPS. Medium from microglia cultures was collected 48 h after transfection, and then the LPS (1 μg/ml) was added to the wells for 6 h after which the cells were harvested for RNA or protein isolation. In the preliminary experiments, we found that the expression of miR-181b-5p was downregulated significantly by LPS when compared with control conditions (Supplemental information Fig. 1A).

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] (Sigma-Aldrich, MO, USA) was used to examine the effect of transfection and LPS treatments on cell viability, according to the manufacturer's protocol. Reactions were quantified using a Bio-Rad (Hercules, CA, USA) microplate reader with a 540 nm excitation wavelength filter. We found that the viability of BV-2 cells in culture was not influenced by the combined treatments of transfection and LPS (Supplemental information Fig. 2).

2.11. Western blot analysis

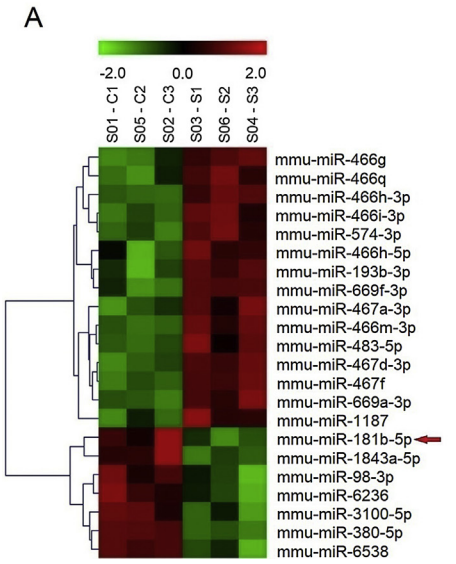
The BV-2 cells were homogenized using ice-cold lysis buffer supplemented with protease inhibitors to extract total proteins. The protein content was quantitated using a BCA assay kit (ASPEN biological, Wuhan, China). Proteins were then electrophoresed on SDS-PAGE and transferred onto PVDF membrane (Millipore, MA, USA). The membranes were blocked using 5% skimmed milk for 1 h at room temperature. After that, the membranes were incubated at 4°C overnight with rabbit anti-human primary antibodies. The membranes were then washed three times (5 min each) with PBST and incubated with anti-rabbit horseradish peroxidase (HRP) conjugated secondary antibody for 30 min at room temperature. After four washes (5 min each) of the membrane with PBST the western blots were developed using chemiluminescence reagents. Finally, the membranes were developed by ECL kits (ASPEN biological, Wuhan, China). GAPDH was used as an internal control.

2.12. Target prediction

Conventional online programs, including TargetScan, miRanda, and PicTar were used to predict targets of miR-181b-5p.

2.13. Dual-luciferase reporter gene assay

According to the results of the bioinformatics prediction, a conservative hsa-miR-181 binding sequence of the 3′untranslated region (UTR) of TNF-α mRNA (wild-type; 5′-UUAUUUAUUUACAGAUGAAUGUA-3′) or a mutant sequence (mutant; 5′-UUAUUUAUUUACAGACACGAAAU-3′) was cloned. Luciferase reporter plasmids were generated by insertion of the wild-type or mutant TNF-α sequences into the multiple cloning site (Mlu I and Hind III) of a pMIR-REPORT Luciferase plasmid (Obio Technology Co., Ltd., Shanghai, China) downstream of the luciferase reporter gene. 293 T cells were transfected with dual luciferase-expressing vectors. The cells were harvested 48 h after the transfection and assayed for luciferase activity using the Dual-Luciferase Reporter Assay System (Promega, WI, USA). Each transfection was repeated twice in triplicate.



B

miRNA	Fold change	*p*
miRNAs upregulated		
miR-669f-3p	5.162±0.246	0.024
miR-466m-3p	5.003±1.577	0.018
miR-466q	4.010±1.307	0.019
miR-467a-3p	3.450±1.285	0.025
miR-466i-3p	3.228±0.868	0.026
miR-467d-3p	3.154±0.503	0.004
miR-574-3p	3.064±0.745	0.008
miR-466h-3p	2.945±0.427	0.007
miR-466g	2.912±0.438	0.010
miR-669a-3p	2.829±0.699	0.022
miR-467f	2.786±0.394	0.002
miR-466h-5p	2.127±0.427	0.043
miR-1187	1.541±0.240	0.032
miR-193b-3p	1.337±0.044	0.041
miR-483-5p	1.341±0.144	0.047
miRNAs downregulated		
miR-1843a-5p	-0.860±0.019	0.044
miR-181b-5p	-1.284±0.128	0.033
miR-6538	-0.522±0.114	0.039
miR-380-5p	-0.450±0.035	0.004
miR-6236	-0.440±0.132	0.040
miR-98-3p	-0.422±0.146	0.043
miR-3100-5p	-0.004±0.005	0.025

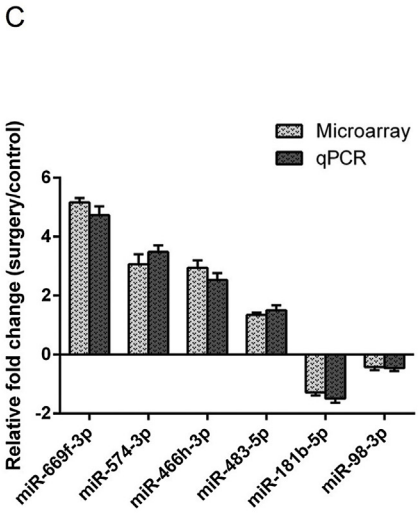


Fig. 1. Effects of Surgery/Anesthesia on hippocampal miRNA expression profiles in mice. (A) Heat map for differentially-expressed miRNAs analysis. Each column represents samples and each row represents miRNAs. Red represents upregulated miRNAs and green stands for downregulated miRNAs. The first three columns represent the expression of miRNAs in the control group, while the last three columns represent the expression of the corresponding miRNAs expression in the surgery group. The heat map for 22/506 detected miRNAs (4.35%) that were differentially expressed. (B) miRNAs differentially-expressed in the hippocampus between the control and surgery group as analyzed by microarray. (C) Comparison of qPCR observations to microarray results by fold-change of 6-selected miRNAs. The fold changes of these miRNAs measured by qPCR were in concordance with the microarray analysis data. The data are plotted as the mean ± standard error of the mean for each group (n = 3 per group). qPCR, Quantitative real-time polymerase chain reaction.

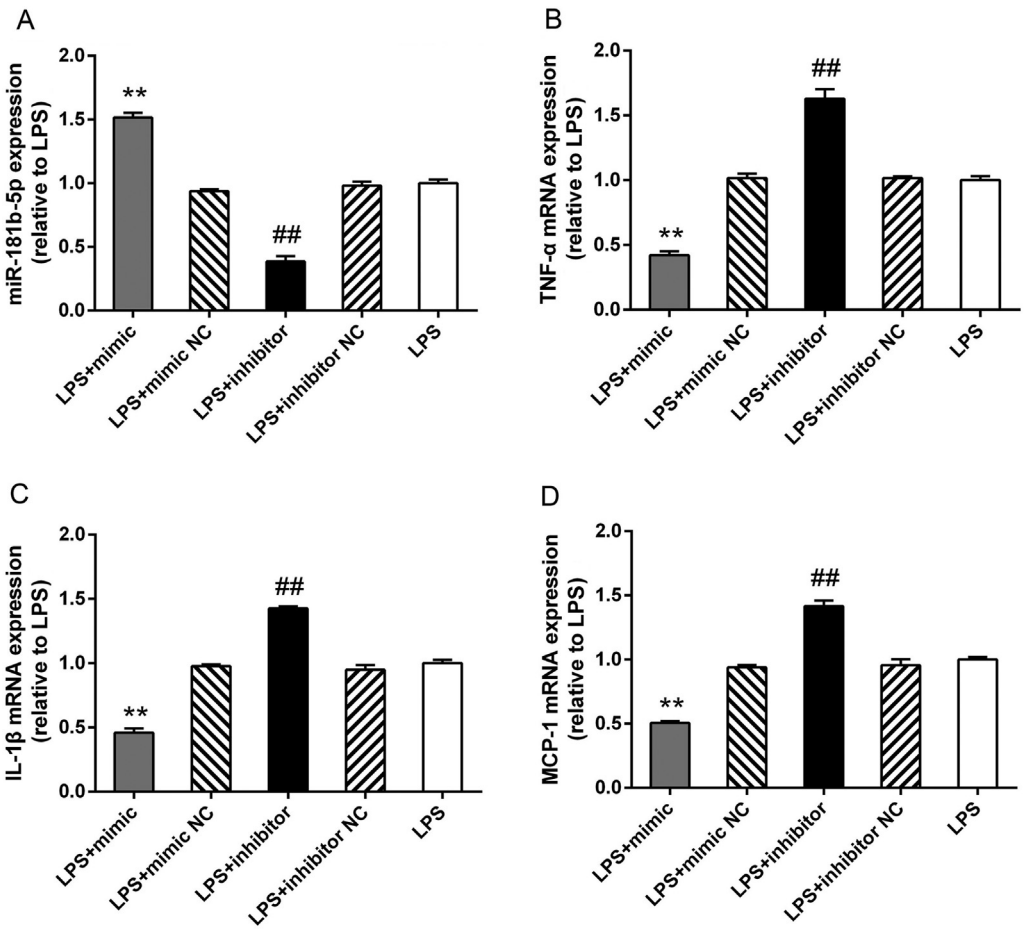


Fig. 2. MiR-181b-5p regulates LPS-induced expression of proinflammatory mediators at the mRNA levels in BV-2 microglial cells. (A) After transfection with miR-181b-5p mimic, inhibitor, mimic negative control, and inhibitor negative control, the LPS was added to stimulate BV-2 microglial cells, and then the expression of miR-181b-5p was measured by qPCR. The expression of miR-181b-5p was significantly enhanced following transfection with mimic, while expression of miR-181b-5p was decreased following transfection with inhibitor. (B-D) Transfection with miR-181b-5p mimic or miR-181b-5p inhibitor changed LPS-induced mRNA expression of proinflammatory factors, such as TNF-α, IL-1β and MCP-1 in BV-2 cells. The mRNA expressions of these proinflammatory factors were downregulated by miR-181b-5p mimic, while miR-181b-5p inhibitor upregulated the mRNA expression of proinflammatory factors. The data are plotted as the mean ± standard error of the mean for each group (n = 6). $^{**}p < 0.01$, compared to LPS + mimic NC; $^{\#\#}p < 0.01$, compared to LPS + inhibitor NC. NC = negative control.

2.14. Statistical analysis

The statistical analysis was performed using SPSS 19.0 statistical software (IBM, Co., Armonk, NY, USA) or GraphPad Prism 6 software for Windows (GraphPad Software, Inc., La Jolla, CA, USA). The normality of data was analyzed by the Shapiro-Wilk test, and the data was found to be normally distributed. The quantitative data are expressed as the mean ± standard error of the mean (SEM), and the error bars indicate the standard error of the mean. Different groups were compared using Student's *t*-test (for two groups) or one-way analysis of variance (for more than two groups), followed by a Student-Newman-Keuls multiple range test for post hoc comparisons. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Altered hippocampal miRNA expression profiles in mice with POCD

The behavior changes of mice were assessed by OFT and FCT and the results indicated that intramedullary fixation surgery for tibial fracture under isoflurane anesthesia could impair the hippocampus-dependent learning and memory (Supplemental information Fig. 3B and C, Supplemental information Fig. 4A), but not the locomotor activity of mice (Supplemental information Fig. 3A), suggesting successful establishment of a mouse model of POCD.

Comparison of differential expression profiles of miRNAs from POCD and control mice revealed 22 miRNAs, of which 15 were upregulated and 7 downregulated (Fig. 1A and Fig. 1B). Among the altered miRNAs, miR-181b-5p had the greatest decrease (fold change = −1.284).

To confirm the accuracy of the microarray analysis, six differentially- expressed miRNAs were selected and qPCR analysis was used to validate the results. From the results of the qPCR, the expression levels of miR-669f-3p, miR-574-3p, miR-466 h-3p and miR-483-5p were identified to be upregulated in the surgery group, while miR-181b-5p and miR-98-3p were downregulated. These data indicated that the fold change of these miRNAs measured by qPCR were in concordance with the microarray analysis data (Fig. 1C).

Given the findings that the miR-181b-5p expression was significantly decreased in the hippocampus of mice with POCD, next, we chose miR-181b-5p as the candidate miRNA, and investigate the role of miR-181b-5p in hippocampal inflammation and cognitive impairment in the setting of surgery.

3.2. MiR-181b-5p regulates LPS-induced expression of proinflammatory mediators in BV-2 microglial cells

The BV-2 microglial cells were successfully transfected with miRNA-cy3 using Lipofectamine 2000 and observed under a fluorescence microscope. The transfection efficiency was found to be around 90% (data not shown, Supplemental information Fig. 5A and B). At the same time, the expression of miR-181b-5p in BV-2 microglial cells was measured by qPCR (Supplemental information Fig. 5C). After transfection with miR-181b-5p mimic, LPS was added to stimulate BV-2 cells, qPCR revealed significant overexpression of miR-181b-5p in LPS + mimic group when compared with LPS group ($p < 0.01$, Fig. 2A). On the other hand, transfection with miR-181b-5p inhibitor before LPS stimulation significantly reduced the expression of miR-181b-5p in BV-2 cell line when compared with LPS group ($p < 0.01$, Fig. 2A).

Transfection with miR-181b-5p mimic or miR-181b-5p inhibitor changed LPS-induced mRNA and protein levels of proinflammatory factors, such as TNF-α, IL-1β and MCP-1 in BV-2 cells. The relative mRNA expressions of these proinflammatory cytokines (TNF-α, IL-1β) and chemokine (MCP-1) were downregulated by miR-181b-5p mimic ($p < 0.01$, Fig. 2B-D), while miR-181b-5p inhibitor upregulated the mRNA expression of proinflammatory cytokines and chemokine ($p < 0.01$, Fig. 2 B-D). The western blot results demonstrated that miR-181b-5p mimic restrained the protein level of the above proinflammatory factors compared to mimic NC group ($p < 0.01$, Fig. 3A-F), whereas miR-181b-5p inhibitor increased the protein expression of these proinflammatory factors compared to inhibitor NC group ($p < 0.01$, Fig. 3A-F). Our finding suggested that miR-181b-5p could regulate LPS-induced expression of proinflammatory mediators in BV-2 microglial cells.

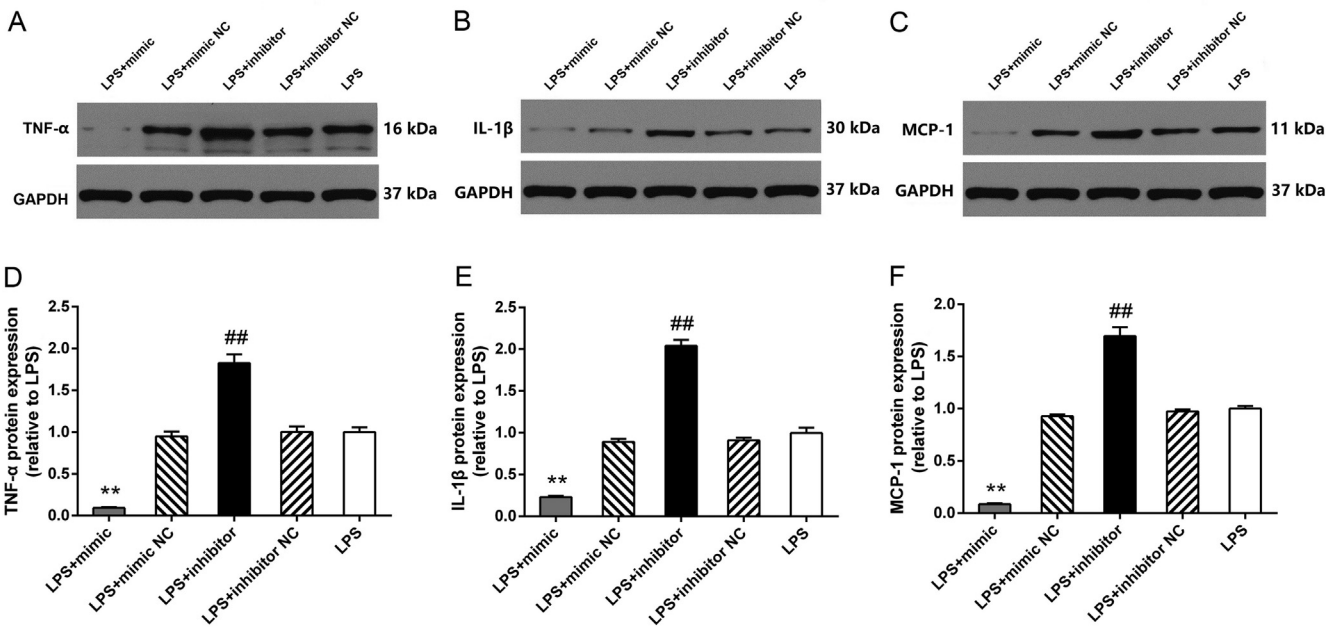


Fig. 3. MiR-181b-5p regulates LPS-induced expression of proinflammatory mediators at the protein levels in BV-2 microglial cells. The protein level of TNF-α (A,D), IL-1β (B,E) and MCP-1 (C,F) were restrained by miR-181b-5p mimic, as compared to mimic NC group, while miR-181b-5p inhibitor increased the protein expression of these proinflammatory factors, as compared to inhibitor NC group. The data are plotted as the mean ± standard error of the mean for each group (n = 6). $^{**}p < 0.01$, compared to LPS + mimic NC; $^{\#\#}p < 0.01$, compared to LPS + inhibitor NC. NC = negative control.

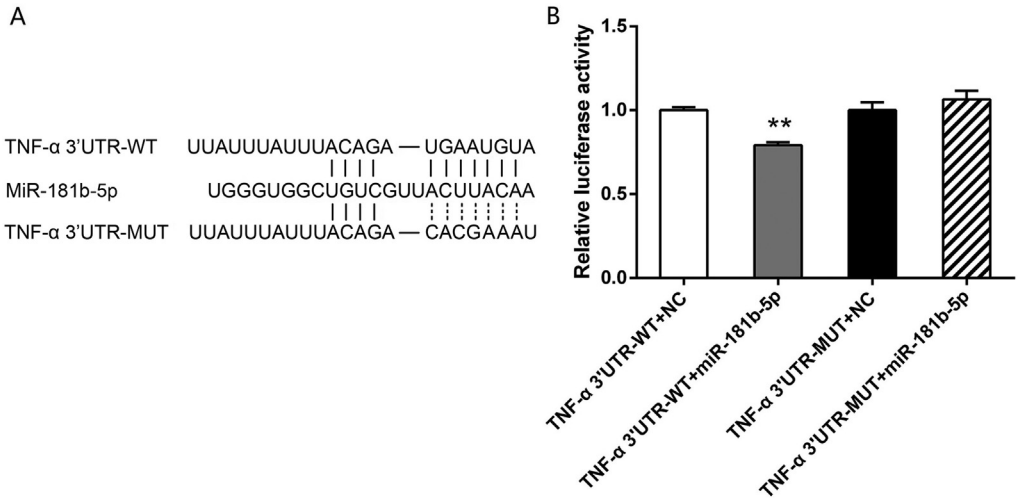


Fig. 4. MiR-181b-5p can direct regulate the expression of TNF-α via binding to its 3′UTR. (A) Mutation of the putative miR-181b-5p binding sites in the 3′UTR of TNF-α mRNA. (B) The dual-luciferase assay showed that miR-181b-5p significantly reduced the luciferase activity of plasmids containing the wildtype 3′UTR region of TNF-α mRNA. The data are plotted as the mean ± standard error of the mean for each group (n = 6). $^{**}p < 0.01$, compared to TNF-α mRNA 3′UTR-WT + NC group. WT = wildtype, NC = negative control.

3.3. MiR-181b-5p can direct regulate the expression of TNF-α via binding to its 3′UTR

Target prediction algorithms such as miRanda, TargetScan and Pictar predicted that miR-181b-5p is a putative regulator of proinflammatory cytokine TNF-α. To investigate the potential interaction between miR-181b-5p and TNF-α, we constructed a pMIR-Report luciferase reporter vector containing the 3′UTR of TNF-α mRNA (pMir-TNF-α 3′UTR-WT) and a mutant pMIR-Report luciferase reporter vector by mutating the miR-181b-5p binding sites within the 3′UTR of TNF-α mRNA (pMir-TNF-α 3′UTR-MUT) (Fig. 4A). pMir-TNF-α 3′UTR-WT and pMir-TNF-α 3′UTR-MUT were transfected into 293 T cells , along with a miR-181b-5p mimic or scrambled mimic (mimic negative control). The dual-luciferase analysis demonstrated that overexpression of miR-181b-5p significantly decreased the luciferase activity of pMir-TNF-α 3′UTR-WT in 293 T cells at 48 h, compared to cells transfected with the wildtype TNF-α 3′UTR and scrambled miR-181b-5p mimic ($p < 0.01$, Fig. 4B). However, the miR-181b-5p mimic did not have a significant effect on the luciferase activity of pMir-TNF-α 3′UTR-MUT ($p > 0.05$, Fig. 4B). These data directly supported the notion that miR-181b-5p can direct regulate the expression of TNF-α via binding to its 3′UTR.

3.4. Effects of miR-181b-5p overexpression on hippocampal inflammation in mice with POCD

To further assess the effects of miR-181b-5p overexpression on hippocampal inflammation in a mouse model of POCD, we enhanced miR-181b-5p expression *in vivo* by intrahippocampal microinjection of miR-181b-5p agomir before Surgery/ Anesthesia. Given the previous findings that TNF-α and IL-1β levels in the hippocampus increase progressively within 24 h following surgery [17,19], in the current study, the hippocampal tissues were collected at 6, 12, and 24 h after surgery.

As can be seen in Fig. 5A-5C, the expression of miR-181b-5p in the hippocampus of mice in surgery group decreased at 6, 12 and 24 h after surgery, as compared to the control condition in mice ($p < 0.05$, Fig. 5A-C). While compared to the surgery + agomir control group, the hippocampal expression of miR-181b-5p of the mice in surgery + agomir group increased at 6, 12 and 24 h after surgery ($p < 0.05$, Fig. 5A-C).

We also detected the protein expressions of TNF-α, IL-1β and MCP-1 in the hippocampus of the mice at 6, 12 and 24 h after surgery by ELISA, and found that Surgery/Anesthesia increased the levels of TNF-α, IL-1β and MCP-1 in the hippocampus of mice, as compared to the control condition in mice at 6, 12 and 24 h after surgery ($p < 0.05$, Fig. 5D-L). Moreover, pretreatment of miR-181b-5p agomir before Surgery/Anesthesia decreased the TNF-α, IL-1β and MCP-1 levels in the hippocampus of mice at 6, 12 and 24 h after surgery, as compared to the preoperative agomir control treatment ($p < 0.05$, Fig. 5D-L).

Microglial activation is the key for the hippocampal neuroinflammation in POCD [50]. Compared to the mice in the control group, the hippocampal expression of Iba-1 (marker of activated microglia/ macrophages [51]) in the mice of the surgery group was increased at 24 and 72 h after surgery ($p < 0.05$, Fig. 6). Additionally, pretreatment of miR-181b-5p agomir before Surgery/Anesthesia reduced Iba-1 expression at 24 and 72 h after surgery, as compared to the preoperative agomir control treatment ($p < 0.05$, Fig. 6). Collectively, these findings demonstrated that miR-181b-5p overexpression suppressed the hippocampal inflammation in mice with POCD.

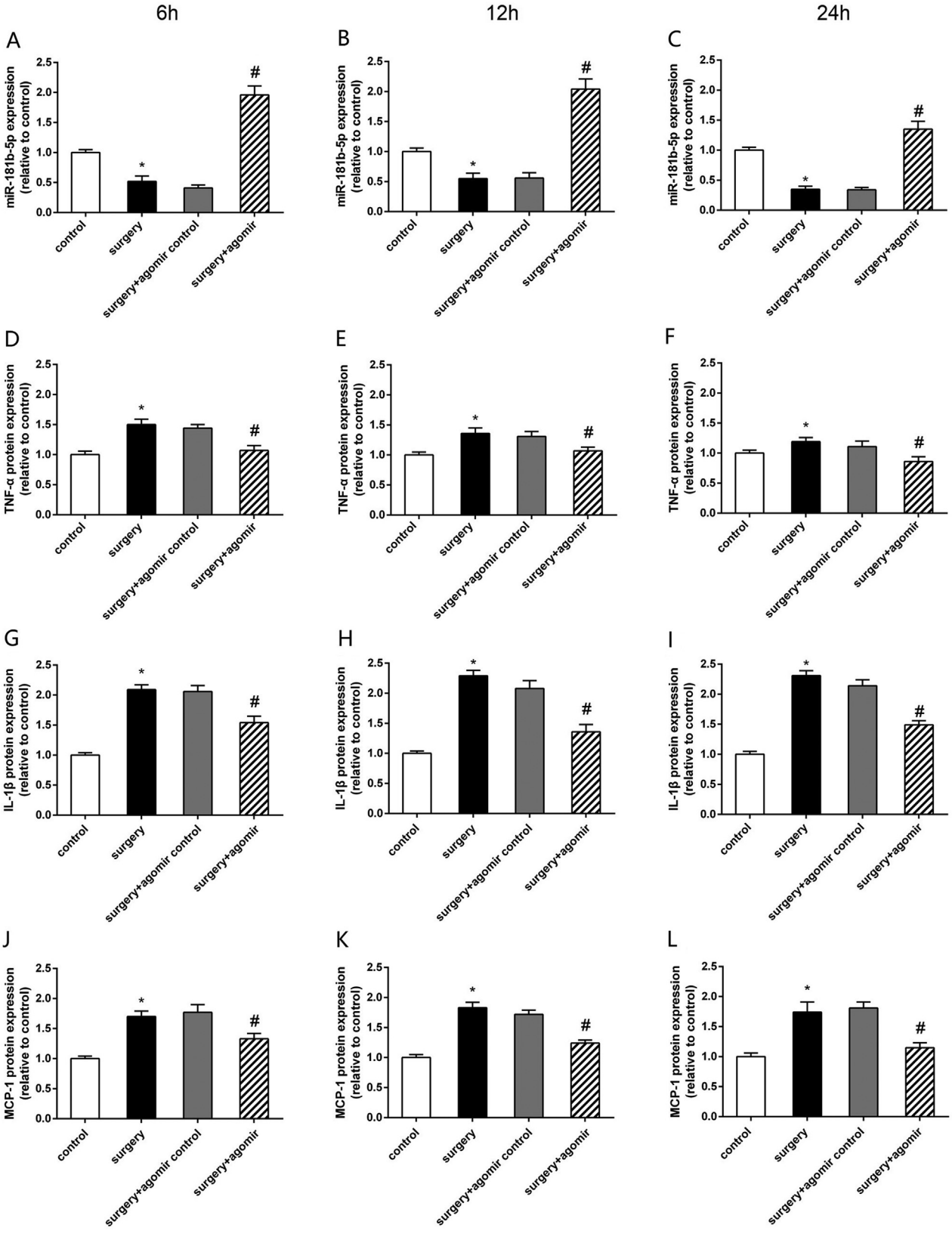
3.5. Effects of miR-181b-5p overexpression on cognitive behavior in the OFT and FCT in mice with POCD

In the OFT, there were no significant differences in total distance traveled by the mice between surgery + agomir control group and surgery + agomir group at all the postoperative time points ($p > 0.05$, Supplemental information Fig. 6A- D). These data suggested that the locomotor activity of mice was not affected by preoperative miR-181b-5p agomir treatment.

In the FCT, the freezing time in the training phase of fear conditioning showed no significant differences between the groups (Supplemental information Fig. 4B), indicating that the baseline learning and memory abilities of the groups were equal. Then, in the context test of FCT, we found that Surgery/ Anesthesia decreased the freezing time on postoperative days 1, 3 and 7, as compared to the control condition in mice ($p < 0.05$, Fig. 7A-C). Importantly, preoperative miR-181b-5p agomir treatment increased the freezing time in the context test on postoperative days 1, 3 and 7, as compared to miR-181b-5p agomir control pretreatment ($p < 0.05$, Fig. 7A-C), indicating that overexpression of miR-181b-5p may attenuate the impairment of hippocampus-dependent learning and memory in mice with POCD. In the tone test of FCT, there was no significant difference in the freezing time among four groups ($p > 0.05$, Fig. 7D-F), which suggested that hippocampus-independent memory was not damaged in the current model.

4. Discussion

The goal of the current study was to assess the role of miR-181b-5p in Surgery/ Anesthesia-induced hippocampal neuroinflammation and cognitive impairment in mice. We demonstrated that miR-181b-5p expression was significantly decreased in the hippocampus of mice with



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POCD. Moreover, miR-181b-5p could regulate LPS-induced expression of proinflammatory mediators in BV-2 microglial cells, and directly target TNF-α via binding to its 3′UTR. Importantly, miR-181b-5p overexpression in the hippocampus attenuates early postoperative cognitive impairment by suppressing hippocampal neuroinflammation in a mouse model of POCD. These findings suggested that miR-181b-5p might be associated with the Surgery/Anesthesia- induced hippocampal neuroinflammation and early cognitive impairment in mice. To our

Fig. 5. Effects of miR-181b-5p overexpression on hippocampal inflammation in mice with POCD. Surgery resulted in an increase in the expression of proinflammatory factors in the hippocampus, while miR-181b-5p agomir pretreatment alleviated the surgery-induced upregulation of proinflammatory factors in the hippocampus. (A-C) The expression of miR-181b-5p in the hippocampus of mice was detected by qPCR. MiR-181b-5p expression in the hippocampus of mice in surgery group decreased at 6, 12 and 24 h after surgery, as compared to the control condition in mice. While compared to the surgery + agomir control group, the hippocampal expression of miR-181b-5p of the mice in surgery + agomir group increased at 6, 12 and 24 h after surgery. (D-L) The protein expression of TNF-α, IL-1β and MCP-1 in the hippocampus of the mice at 6, 12 and 24 h after surgery was measured by ELISA. Surgery/Anesthesia increased the levels of TNF-α, IL-1β and MCP-1 in the hippocampus of mice, as compare to the control condition in mice at 6, 12 and 24 h after surgery. Pretreatment of miR-181b-5p agomir before Surgery/Anesthesia decreased the TNF-α, IL-1β and MCP-1 levels in the hippocampus of mice at 6, 12 and 24 h after surgery, as compared to the preoperative agomir control treatment. The data are plotted as the mean ± standard error of the mean for each group (n = 6 per cohort). $^{*}p < 0.05$, compared to control; $^{\#}p < 0.05$, compared to surgery + agomir control group.

knowledge, this is the first evaluation of the influence of miR-181b-5p on neuroinflammation and cognitive decline in the setting of surgery.

The miR-181 family of miRNAs, including four isoforms, such as miR-181a, miR-181b, miR-181c and miR-181d, with their mature sequences named miR-181a-5p, miR-181b-5p, miR-181c-5p and miR-181d-5p [52], are present at high levels in the mature brain of mammalian [31,53]. As reported earlier [54], in normal brain miR-181a was present at the highest level, with miR-181b about 10%, miR-181c about 34%, and miR-181d about 8% of miR-181a levels. MiR-181 was classified as a “NeurimmiR” that functions in the cross-talk between neuronal and immune systems [23]. Currently, most studies are focused on its functions, such as the regulation of hematopoietic stem cells differentiation [53,55], glioma cells proliferation [56–58], and axon regeneration [35]. However, the immune functions of this miRNA in the CNS have not been completely illustrated. Recently, miR-181b-5p has been widely reported to control excessive inflammation, especially in neurological tissue [30,31]. For instance, in 2013, Hutchison and coworkers [31] found that the inflammatory stimulus, the TLR4 receptor ligand LPS, decreased the levels of the miR-181b, miR-181c and miR-181d in the cerebral cortex of mice *in vivo* and in cultured astrocytes. In addition, miR-181 knockdown significantly enhanced the LPS induced production of multiple proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, as well as high mobility group box-1 protein (HMGB-1) in LPS stimulated astrocytes. Furthermore, Sun and colleagues [32,33] reported that the miR-181 family plays an essential role in endothelial inflammation via regulating critical signaling pathways, for example, miR-181b could target the critical protein of NF-κB nuclear translocation— importin-α3, in *in vitro* and *in vivo* models of vascular endothelium. Of note, miR-181b was also reported to be associated with many neurological diseases. For example, miR-181b was observed to be downregulated in glioblastoma cells [34]. Additionally, miR-181b expression was decreased in the ischemic penumbra in the rat model of focal cerebral ischemic [35]. In the current study, overexpression of miR-181b-5p decreased the production of proinflammatory factors, TNF-α, IL-1β and MCP-1 in the hippocampus of mice with POCD *in vivo* and in cultured microglial cells with LPS treatment. These findings are consistent with the results of Hutchison et al. In the light of the important role of miR-181b in the pathogenesis of inflammation and neurological diseases, we chose miR-181b-5p as the candidate miRNA, and investigate the role of miR-181b-5p in hippocampal inflammation and cognitive impairment in the setting of surgery.

Neuroinflammation has been associated with cognitive impairment and stands out among the mechanisms underlying POCD [20]. Preclinical investigations have shown that hippocampal neuroinflammation contributes to postoperative memory dysfunction because (1) hippocampal areas are known to be involved in memory tasks [59]; (2) hippocampal neuroinflammation profile correlates with the level of memory dysfunction [16,17]; and (3) hippocampal neuroinflammation leads to long-term potentiation disruption [60,61]. Recent years, there is a growing appreciation of the key role of the TNF-α in the hippocampal inflammation and cognitive decline, resulting from surgical trauma. For instance, Ma and colleagues have reported the crucial role of TNF-α mediated signaling pathway in the development of POCD and neuroinflammation. They found that the laparotomy could result in cognitive impairment in aged rats, accompany with significant upregulation of proinflammatory cytokines, including TNF-α, IL-1β, IL-4 and IL-6 in the hippocampus of rats, while intracisternal administration of R-7050, the TNF-α receptor antagonist, significantly attenuated the laparotomy-induced cognitive decline of the aged rats, and inhibited the activation of the downstream NF-κB and MAPK signaling pathways, with a resulting decrease in the production of above mentioned proinflammatory cytokines in the hippocampal tissue of the aged rats following laparotomy [62]. Additionally, TNF-α also exerts neuromodulatory functions, in particular in regulating microglia and astrocytes activation in the brain [63]. For example, TNF-α have been demonstrated to mediate the induction of IL-6 release from rat brain pericytes and astrocyte primary cultures, and then facilitate activation of BV-2 microglia [64]. Importantly, the pivotal role of TNF-α in inducing postoperative hippocampal inflammation and cognitive decline, have been demonstrated by Terrando et al, using a mouse model of orthopedic surgery, which has been adopted to establish the mouse model of POCD in our current study. They found that TNF-α acts upstream of IL-1β and initiates the peripheral cytokine cascade leading to cognitive decline. Moreover, preoperative administration of anti-TNF-α antibody attenuated the surgery-induced upregulation of hippocampal IL-1β, significantly reduced microgliosis after surgery and ameliorated hippocampal-dependent memory impairment [17]. Additionally, TNF-α could induce MCP-1 expression in spinal cord astrocytes [65]. In the present study, TNF-α was predicted to be a direct target of miR-181b-5p by bioinformatics methods, and was validated by dual-luciferase assay. Considering the validation results and the important role of TNF-α in mediating neuroinflammation and POCD, miR-181b-5p-mediated control of TNF-α might play an important role in the reduction of neuroinflammation.

However, the contribution of miR-181b-mediated control of TNF-α in protecting from neuroinflammation might not be crucial. MiR-181b has been shown to target also other important proinflammatory factors. For example, Sun and colleagues have reported that miR-181b could inhibit importin-α3 expression, thereby inhibit downstream NF-κB nuclear translocation [33]. NF-κB is widely known as one of the transcription factors critical for LPS-induced TNF-α expression [66,67] and is involved in IL-1β-induced MCP-1 expression [68]. Moreover, miR-181b could suppress the expression of PTEN, which was reported as the negative regulator of the PI3K-Akt pathway [69,70]. Activation of the PI3K-Akt pathway limits LPS-induced TNF-α gene expression [71]. In addition, the PI3K-Akt pathway could also inhibit the MAPK signaling pathway activated by LPS [71,72]. MAPKs could regulate not only TNF-α expression [73], but also LPS-induced expression of MCP-1 and IL-1β [74,75]. Collectively, miR-181b could impact proinflammatory signaling upstream of TNF-α, IL-1β and MCP-1 on multiple steps. Therefore, although the downregulation of TNF-α expression induced by miR-181b-5p plays an important role in the reduction of neuroinflammation, it could be more likely that the global impact of miR-181b-5p on proinflammatory factors, not only TNF-α, but also IL-1β and MCP-1, alleviated early cognitive impairment in mice. However, whether the regulation role of miR-181b-5p on the expression of IL-1β and MCP-1 is direct or indirect remains unclear, future investigations should look into the specific mechanisms.

There were some potential limitations of our study. First, in the present study, we only employed 4-month-old male mice, but not the

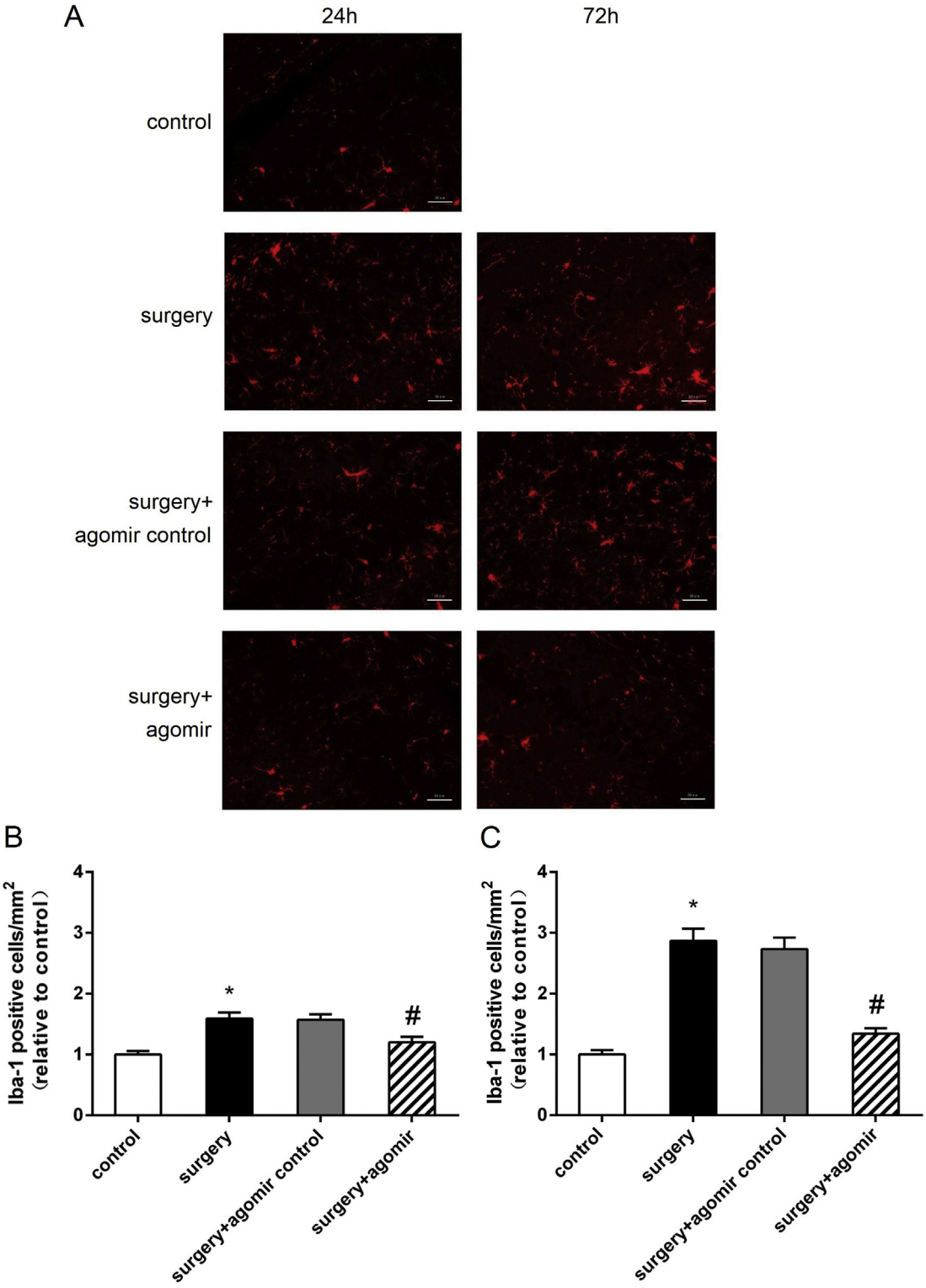

Fig. 6. Effect of miR-181b-5p on hippocampal microglial activation in mice with POCD. Surgery resulted in an increased expression of Iba-1 in the hippocampus of mice, while miR-181b-5p agomir pretreatment alleviated the surgery-induced upregulation of Iba-1 in the hippocampus. (A) Representative immunofluorescence images show the expression of Iba-1 (*red pixels*) in the hippocampus of mice at 24 and 72 h after surgery. Scale bar: 50 μm. Quantitative analyses of the immunofluorescence images at 24 (B) and 72 h (C) after surgery. The data are plotted as the mean ± standard error of the mean for each group (n = 3 per cohort). $^{*}p < 0.05$, compared to control; $^{\#}p < 0.05$, compared to surgery + agomir control group.

aged mice. However, the current study is a pilot study to assess the role of miR-181b-5p in the development of POCD. Future research would include the comparison of the effects of miR-181b-5p on surgery-induced hippocampal inflammation and cognitive behavior changes in mice of different ages and sexes. Second, we just assessed the effects of surgery plus anesthesia on the hippocampal inflammation. Surgery plus anesthesia could have different effects on the miR-181b-5p in different regions of the brain, such as, amygdala, and medial prefrontal cortex.

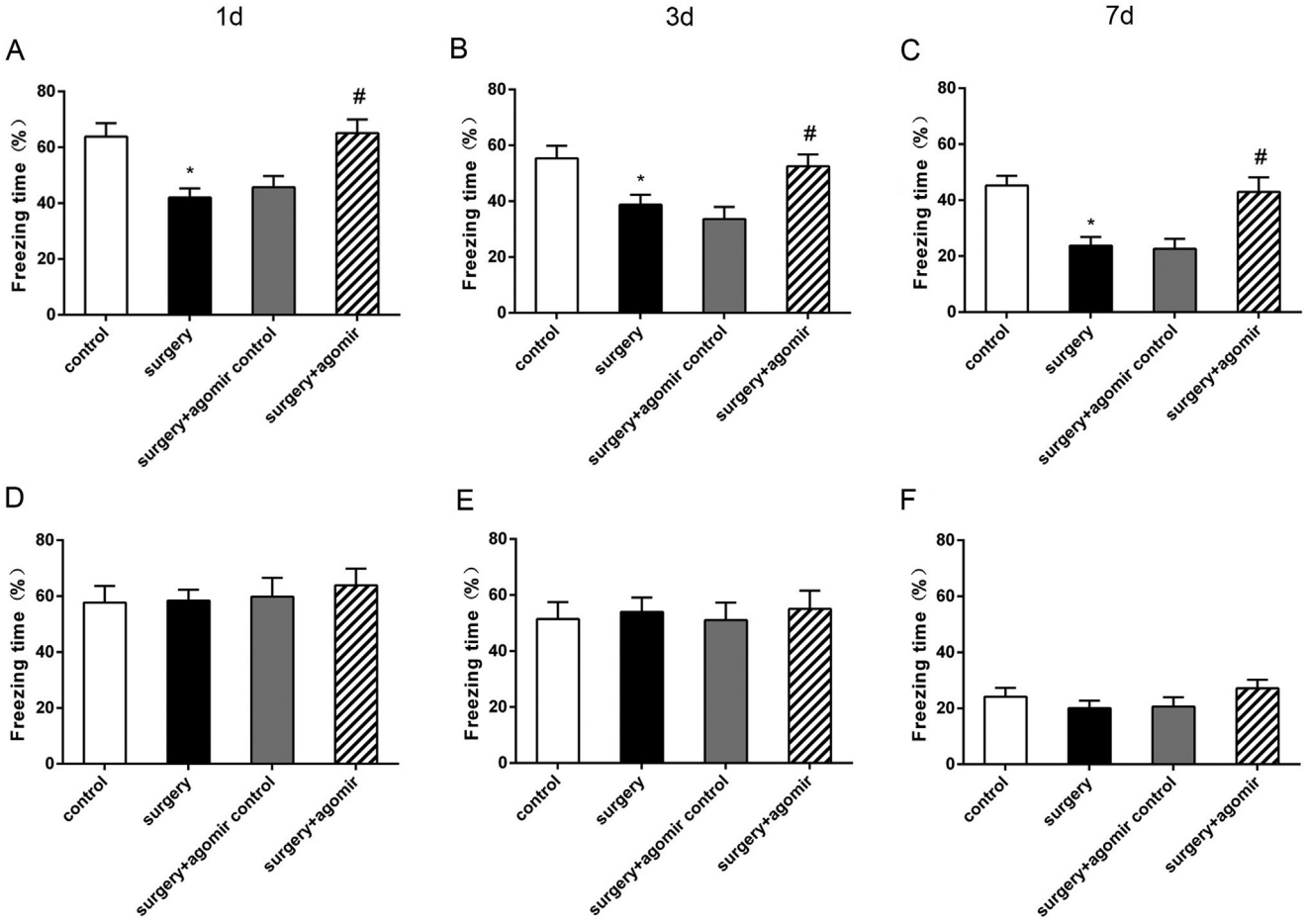

Fig. 7. Effects of miR-181b-5p overexpression on cognitive behavior in the fear conditioning test in mice with POCD. Surgery impaired hippocampal-dependent memory, but not hippocampal-independent memory. MiR-181b-5p pretreatment alleviated postoperative hippocampal-dependent memory impairment. (A-C) In the context test of FCT, Surgery/ Anesthesia decreased the freezing time on postoperative days 1, 3 and 7, as compared to the control condition in mice. Preoperative miR-181b-5p agomir treatment increased the freezing time in the context test on postoperative days 1, 3 and 7, as compared to miR-181b-5p agomir control pretreatment. (D-F) In the tone test of FCT, there was no statistical difference in the freezing time among four groups. The data are plotted as the mean ± standard error of the mean for each group (n = 10 per cohort). $^{*}p < 0.05$, compared to control; $^{\#}p < 0.05$, compared to surgery + agomir control group.

Future investigations should look into the potential effects of surgery plus anesthesia, on the levels of miR-181b-5p in other regions of the brain. Third, in the study, we focused only in the early postoperative cognitive function, further studies on the long-term cognitive function are needed. Fourth, surgery plus anesthesia may have different impacts on the neuroinflammation and cognitive function. The current studies just determined the combined effects of surgery plus anesthesia on hippocampal inflammation and cognitive behavior changes in mice. Future studies will include assessments of the effects of anesthesia or surgery alone on the hippocampal inflammation and cognitive behavior changes in mice. Fifth, we validated TNF-α mRNA as a direct target of miR-181b-5p in the present study, but whether the regulation role of miR-181b-5p on the expression of the other two proinflammatory factors— IL-1β and MCP-1, is direct or indirect remains unclear, future investigations should look into the specific mechanisms. Finally, in the present study, we just detected the expression of miR-181b-5p in the hippocampus of mice, and did not detect its expression in neurons and glial cells respectively. As miR-181b was reported to be expressed in both neurons and neuroglia [76,77], future research will investigate the expression of miR-181b-5p in neurons and glial cells of mouse hippocampus respectively by double immunofluorescence staining.

5. Conclusion

In summary, this study demonstrates that miR-181b-5p may negatively regulate the development of POCD, via suppressing hippocampal neuroinflammation, providing new insights for identifying miR-181b-5p as a novel potential therapeutic target for improving POCD.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

All relevant data are within the paper.

Author contributions

YL and XX designed and performed the experiment, collected and analyzed the data, prepared the manuscript. RD involved in preparing the animal models and participated in interpreting the results. LS

contributed to behavioral testing. ZZ involved in biochemical analysis. LC participated in statistical analysis. MP contributed to study concept and design, secured funding for the project, prepared and critically revised the manuscript. All authors reviewed the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.04.005.

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