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Original Article

MiR-7684-5p leads to surgery-induced cognitive decline in mice probably through the downregulation of SorLA

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Abstract: Background: Postoperative cognitive dysfunction is a postoperative severe complication caused by many factors. However, its specific pathogenesis remains unclear. MicroRNAs (miRNAs), which are involved in the pathogenesis of neurodegenerative diseases, may also affect POCD. Methods: In this research, microarray technology was used to screen 26 miRNAs that had a differential expression in the hippocampus of mouse between the surgery group and control group. The qRT-PCR verification on the hippocampuses of 10 pairs of mouse testifies the high expression of miR-7684-5p in the surgery group (identical with the result of chip). Results: Surgical trauma was found to induce the expression of miR-7684-5p with the accumulation of Aβ in the hippocampus. Furthermore, miR-7684-5p knockdown effectively reduced the levels of Aβ triggered by surgery, and attenuated hippocampal-dependent memory impairment. Moreover, we testify that sorLA is a target gene of miR-7684-5p through bioinformatics prediction and dual-luciferase report gene experiment. Conclusions: Our data indicate that decreased postoperative cognitive function may be caused by the increased generation of Aβ by reducing sorLA expression. Our work implicates miR-7684-5p as a potential biomarker and a novel therapeutic target.

Keywords: Cognitive decline, miR-7684-5p, orthopedic surgery, β-amyloid, sorting protein-related receptor

Introduction

Postoperative cognitive dysfunction (POCD) is a cognitive dysfunction syndrome that occurs following anesthesia and surgery, which likely has myriad causes. POCD can be considered a lack of resilience to perioperative stress, which thereby generates cognitive-related clinical symptoms [1]. Studies on the incidence of POCD can usually be traced to the multi-center clinical study published by the International Study of Postoperative Cognitive Dysfunction (ISPOCD) group in the Lancet in 1998 [2]. Results revealed that seven days following surgery, POCD was present in 25.8% of the investigated elderly patients; while this was present in 9.9% of elderly patients three months after surgery. POCD often leads to delayed post-operative recovery and prolonged hospital stays, and increases medical expenses, especially among elderly patients. However, the specific mechanism of POCD remains unclear, as it involves multiple mechanisms such as β-amyloid (Aβ) deposition, the phosphorylation of tau proteins, immune inflammation, and neuronal apoptosis [3-6]. Therefore, it is of great significance and urgency to define the molecular mechanisms of POCD and develop targeted drugs to achieve early prevention, diagnosis and treatment.

MicroRNAs (miRNAs) are small, non-coding RNA molecules with approximately 22 single-stranded nucleotides, which mediate homologous sequence-dependent gene silencing in cells. Many studies have confirmed that miRNA plays an important role in the development and progression of the neural system, learning and memory, and causes numerous neurological diseases [7-9]. Furthermore, the research revealed the participation of miRNAs in the regulation of synaptic plasticity, study and memory function through the regulation of the morphology of dendrites [10] at the beginning of brain development, the synthesis of synapse local proteins [11], and memory constrain or destabilization upon retrieval, in order to allow new learning or memory updating to occur [12]. However, it remains unclear whether miRNAs

Figure 1. Experimental design. LV+Sur group and NC+Sur group respectively in the 3 days before operation, the use of the stereoscopic direction finder double sided hippocampus to perform a miR-7684-5p reduction lentivirus carrier single injection, orthopedic surgery was conducted three days later, while the control group received no specific treatment. Four groups of mice were trained for the first 1 day, Behavioral tests were performed on the third day following the operation, and hippocampal tissue was harvested following the tests. (FCT: Fear Conditioning Test. OFT: Open Field Test).

also control the occurrence and development of POCD.

Based on the above research, we screen the differential expression of miRNAs in the hippocampus of mouse between the surgery group and control group through a POCD animal model, and selected the hippocampus of 10 pairs of mice for qRT-PCR assay, testifying that miR-7684-5p is higher in the hippocampus of mice in the control group (identical with the result in chip). In addition, we further studied the function and mechanism of miRNA-7684-5p in POCD through advanced technological means such as the dual-Luciferase report gene detection experiment and the construction of the gene knockdown lentiviral vector. This project has important theoretical significance and potential social economic effect. It would bring a brand-new horizon to the research of POCD, deepen the recognition of its pathogenesis, and offer new molecular target points for its prevention and treatment.

Materials and methods

Animals

The animals used in this study were C57BL/6 mice purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All mice were male, aged 12-14 months, and weighed 25-35 g. Mice were placed in a room with a controlled temperature and humidity of 25°C and 55%, respectively. Furthermore, all mice were acclimated to the environment for one week prior to the initiation of the study. The experiment was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" issued by the National Institutes of Health and approved by the Animal Ethics Committee of the Beijing Chao-Yang Hospital, Capital Medical University.

Animal model of POCD

According to a previously described method, the aged mice were anesthetized and underwent orthopedic surgery to establish the POCD model, which was used widely to mimic POCD in elderly human patients [13-15]. The surgical model can be described as follows: A left tibial fracture was performed with an intramedullary nail fixation on the mice under isoflurane. In strict sterile condition, an incision on the upper left hind paw of mouse was performed, the muscle was detached, a 0.5 stainless steel was inserted into the marrow cavity from the upper end of the tibia, the periosteum was stripped, and osteotomy was performed. Then, anesthesia (2% isoflurane mixed with 30% FiO_2 for induction, and 1.5% maintenance, Baxter International Inc., Deerfield, IL, USA) and butorphanol (0.1 mg/kg, subcutaneous injection; Jiangsu Hengrui Medicine Co. Ltd., Lianyungang, China) were administered. Finally, a 5-0 nylon suture was used to close the wounded skin. The supply of isoflurane was immediately removed after the operation, and the animals were placed back in their cage and were allowed to wake up normally. After the operation, a heat pad and a temperature control lamp were used to maintain the body temperature of the mice to approximately 37°C. The study was divided into four groups (n = 10): Ctrl group (without any intervention), Sur group (as mentioned above, conduct orthopedic surgery under isoflurane anesthesia), LV+Sur group (the use of the stereoscopic direction finder double sided hippocampus to perform a miR-7684-5p reduction lentivirus carrier single injection, each side at 2.0 μl, 4 × 10^8 TU/mL, orthopedic surgery was conducted three days later), NC+Sur group (the use of the stereoscopic direction finder double sided hippocampus to perform a single injection of lentivirus negative control vector, with 2.0 μl on each side, ortho-

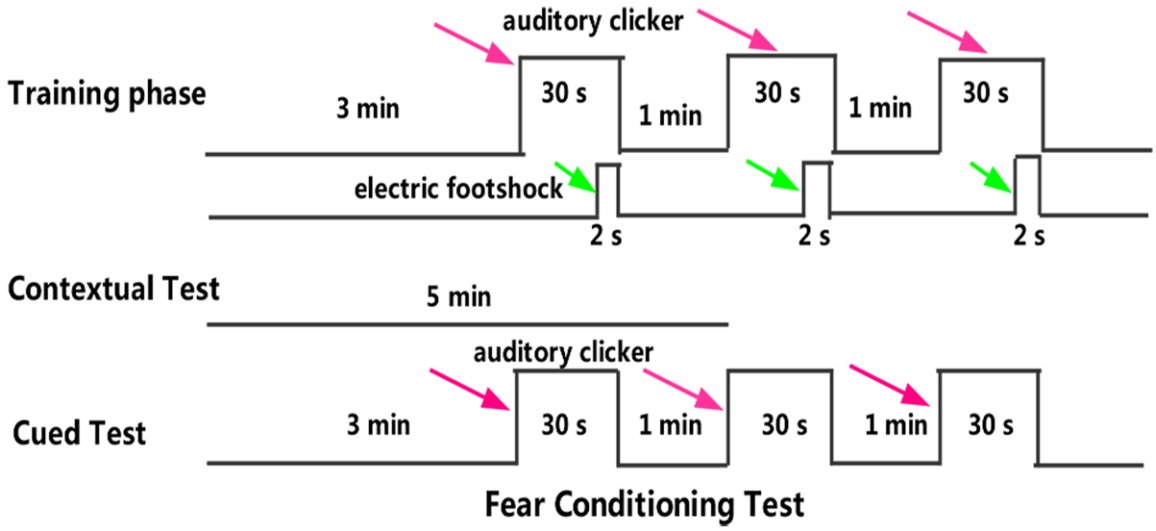

Figure 2. Work flow of the FCT test. The red arrows indicate sound stimuli and the green arrows indicate electric shock stimuli. The four groups were subjected to behavioral training (training phase) one day prior to the operation and behavioral test (the contextual test and the cued test) was performed on the third day following the operation.

pedic surgery was conducted three days later) (research platform, refer to **Figure 1**).

Behavioral tests

Open field test (OFT): OFT was used to evaluate anxiety and locomotor activity in the experimental animals. A mouse was placed directly into the center of the open field (100 × 100 × 48 cm, length × width × height). The movements of the mouse in the open field were recorded by a digital camera during the 5-minute testing session [16]. General locomotion activity (total distance of the grid lines crossed by each mouse), center square duration (the time spent by each mouse in the central square), and rearing (frequency at which each mouse stands on their hind legs in the maze) were counted.

Fear conditioning test (FCT): The purpose of FCT was to investigate the ability of animals to learn and memorize the association between unpleasant experiences and environmental implications [17]. Sound and light are commonly used as conditioning stimuli (CS), while an aversive stimulus (such as an electric shock to the foot) acts as an unconditional stimulus (US). These two types of stimuli appear in pairs during the test. After CS-US training, in addition to the association between the sound and electric shock, animals are able to associate the link between the electric shock and the surrounding environment.

One day prior to the operation, after a 3-minute exploration period, mice were given three pairs of sound stimuli (2000 Hz, 90 Db, and 30 seconds) and electric shock stimuli (1 mA, 2 seconds). Each electric shock stimulus was given at the last two seconds of the corresponding sound stimulus, and both ended at the same time. There was an interval of one minute between two pairs of stimuli.

On the third day following the operation, a contextual test and cued test were performed. During the contextual test, mice were placed in a context that was similar to that of the pre-operation training for a 5-minute observation period without the stimulation of sound and electric shock. During the cued test, two hours after the completion of the contextual test, mice were placed in a context different from that of the pre-operation training (the interior was changed). After a 3-minute exploration period, the mice were given three sound stimuli (2,000 Hz, 90 Db, and 30 seconds) without an electric shock stimulus. The interval between two stimuli was one minute. Image analysis software was utilized to calculate the freezing time of the mice in the contextual test and the cued test (**Figure 2**).

Stereotaxic injection of lentiviral vectors

Mice were anesthetized with chloral hydrate (400 mg/kg, *i.p.*). Holes were drilled above the CA1 field of the hippocampus (injection coordinates relative to the bregma were as follows:

anterior/posterior = -2.0 mm, medial/lateral = ±1.8 mm, ventral = -2.0 mm) using standard stereotaxic procedures (RWB Life Science Co. Ltd., China). The negative control vector or LV vector (2 μL, 4 × 10^8 TU/mL, miR-7684-5p knockdown lentivirus, as well as their negative control lentivirus, were all purchased from GeneChem, Shanghai, China) was bilaterally microinfused into the hippocampus *via* a stainless steel cannula connected to a Hamilton microsyringe (Hamilton, Bonaduz, Switzerland). An infusion pump was maintained at an infusion rate of 0.2 μL/min, and the cannula was left in place for five minutes following completion of the infusion [18, 19].

Tissue preparation

For western blot analysis, qRT-PCR and enzyme-linked immunosorbent assay, mice were sacrificed under deep anesthesia. The hippocampus was removed, immediately placed in liquid nitrogen, and transferred at -80°C until further examination.

Western blot analysis

The homogenate hippocampus was placed in RIPA buffer solution containing a protease and phosphatase inhibitor, and centrifuged for 25 minutes at 12,000 g under 4°C. Bradford protein was used to measure the reagent box (Beyotime Institute of Biotechnology Co. Ltd., Shanghai, China) and measure the condensation of protein in the supernatant. An equivalent amount of protein sample (20 μg/sample) was denatured for five minutes at 100°C, and transform to polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA) through 8-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, 5% skim milk-Tris-buffered saline (TBS) buffer solution blocking membrane was used for 60 minutes, and was incubated overnight with the following primary antibodies: Monclone antibody Amyloid Precursor Protein (1:500; Cat. no. ab2072; Cambridge, MA, USA); Monclone antibody SorLA (1:1000; Cat. no. ab190684; Cambridge, MA, USA). After washing the membrane three times with TBS containing Tween solution (10 minutes each time, goat anti-rabbit IgG; 1:2,000; Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., Beijing, China), it was incubated with horseradish peroxidase for one hour at room temperature. Treat stripes with intensified chemical light emission detection lotion box (EMD Millipore) were used, and the intensity of each band was quantified by densitometric analysis. The relative expression level was standardized through GAPDH (1:1,000; Cat. no. 5174; Cell Signaling Technology Inc.).

Enzyme-linked immunosorbent assay

The hippocampus was weighed. Then, supersonic treatment was performed in PBS containing a 50-mM protease inhibitor mixture, and centrifuged for 10 minutes at 20,000 g at 4°C. The supernatant liquid was collected, and BCA protein was used to measure the reagent box (Cat. no. 23225; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and quantify the concentration of the sample protein. According to the manufacturer's protocol, the ELISA measurement method was applied to measure the absorbance of $A\beta_{1\text{-}42}$ (Wuxi Donglin Sci & Tech Development Co. Ltd., Jiangsu, China). The intensity of the color was measured at a wavelength of 450 nm using an iMarkmicroplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Quantitative real-time PCR

Total RNA was extracted from hippocampal tissues using TRIzol (Invitrogen, Carlsbad, CA, USA), according to manufacturer's instructions. Subsequently, total RNA was assessed by electrophoresis on denaturing agarose gel and quantified using a NanoDrop spectrophotometer (NanoDrop, USA). After RNA isolation, M-MLV reverse transcription (Promega, Madison, WI, USA) was used to synthesize cDNA. Quantitative PCR analysis and data collection were performed on the ABI 7500 qPCR system (Applied Biosystems, Foster City, CA, USA) using the primer pairs. MiR-7684-5p: 5'TCTGGGAAGCCTGGGCAGCA3'; and the PCR cassettes used (Qiagen, Valencia, CA, USA) contained the reverse primer, in which only the forward primer of the miR-7684-5p was listed.

U6: F, 5'CTCGCTTCGGCAGCACA3'; R, 5'AACGCTTCACGAATTTGCGT3'. For quantitative results, the relative expression level of each gene was calculated by using the $2^{-\Delta\Delta Ct}$ method, and fold changes are shown as mean ± SD in three independent experiments. U6 was used as references.

Microarray experimental procedure

A DNA microarray analysis was conducted to screen the differentially expressed genes from

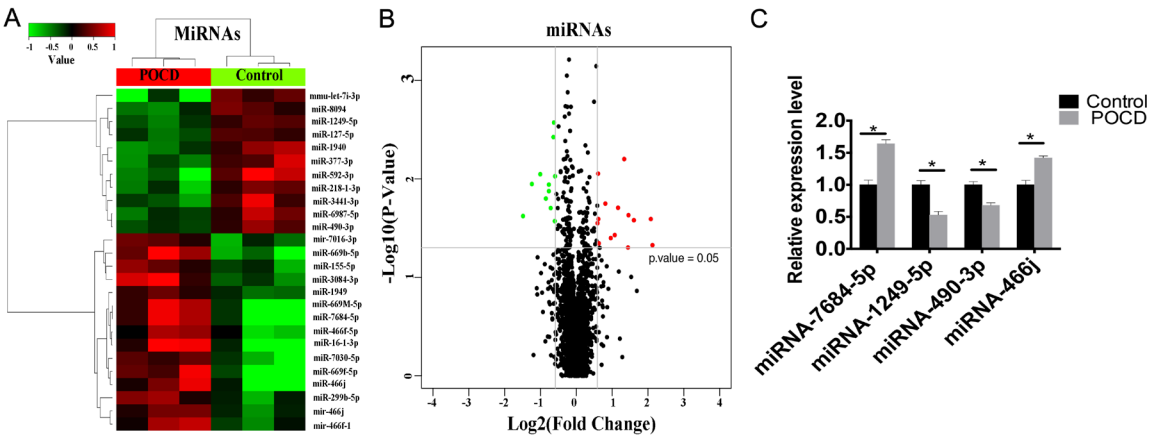

Figure 3. The expression profiles of miRNAs and the expression of significant transcripts validated by quantitative RT-PCR. A. Heat Map showing the expression profiles of miRNAs. The screening criteria were: FC greater than 1.5, P < 0.05 and FDR < 0.05 for miRNAs. The expression values are depicted in line with the color scale. The intensity increases from green to red. Each column represents one sample, and each row indicates a transcript). B. Volcano plot reflecting the number, significance, and reliability of differentially expressed miRNAs. The abscissa is log2 (FC value) and the ordinate is -log10 (*p*-value), Red dots are up-regulated genes, green dots are down-regulated genes, and black dots are genes that displayed no apparent difference between the two groups. C. The relative expression levels of four miRNAs are shown comparing the Ctrl group and Sur group. Data are presented as mean ± SD, (n = 10). *P < 0.05.

the hippocampal tissues (Affymetrix _7G_ miRNA 4.0 Array was used to analyze miRNA profiles). The procedure included the following steps: sample RNA extraction; sample RNA quality analysis; cDNA formation; sense cDNA fragmentation; biotin labeling; chip hybridization; chip elution; chip scanning; signal value detecting, filtering and removing signals that were weaker than that of the background. The data were analyzed through the miRNA QC Tool using the Affymetrix default analysis settings, and quantile as the normalization method. The commonly adopted criteria for screening differential gene expression were a fold change (FC) value greater than 1.5, $P < 0.05$ and FDR < 0.05. A greater FC value indicates greater differentiation between the expression of two samples, and a smaller *P*-value suggests a higher reliability of the analysis results.

Construction of reporter plasmids and luciferase assay

In order to construct a reporter plasmid containing the 3'UTR of *SorLA*, 300-bp DNA fragment of the partial 3'UTR of *SorLA* containing the wild-type 3'UTR of *SorLA* or mutant 3'UTR of *SorLA* was chemically synthesized and cloned into the pMIR-REPORT luciferase vector (Ambion) downstream of the luciferase gene, and were named pMIR-SorLA-3'UTR-WT and pMIR-SorLA-3'UTR-MT, respectively. The nucleotide sequences of the constructed plasmids were confirmed by DNA sequencing analysis. All steps of the luciferase reporter assay were according to previously described studies [20, 21]. HEK293T cells (1×10^5) were seeded into 24-well plates, and each was transfected with 0.5 μg of either the pMIR-SorLA-3'UTR-WT vector or pMIR- SorLA-3'UTR-MT vector containing Firefly luciferase, together with 0.2 μg of the pRL-TK vector (Promega) containing Renilla luciferase and 50 nM of miR-7684-5p mimic, miR-7684-5p NC or none using the Lipofectamine 2000 transfection reagent, according to manufacturer's protocol. After 48 hours of transfection, luciferase activity was measured using the dual luciferase reporter assay system (Promega). These results were expressed as relative luciferase activity (Firefly LUC/Renilla LUC).

Statistical analysis

The data were analyzed using Graph Pad PRISM version 6 software (San Diego, CA, USA) and the average values in each experimental group were expressed as means ± standard deviation (SD). Statistical analysis for the behavioral test, western blot and qRT-PCR was performed using one-way analysis of variance, followed by Newman-Keuls post-hoc test, where appropriate. A *P*-value < 0.05 was considered statistically significant.

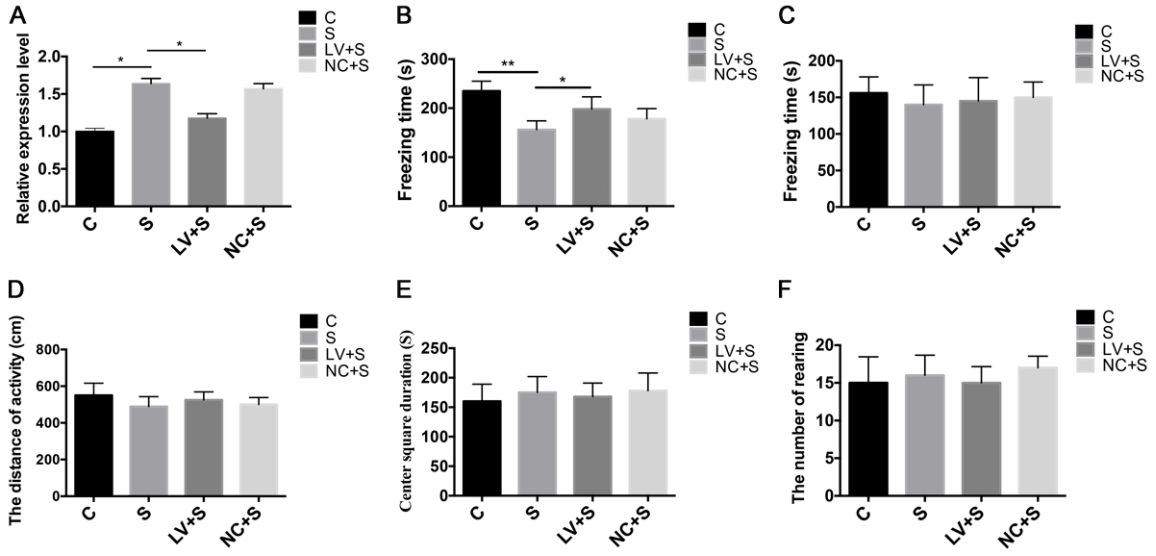

Figure 4. MiR-7684-5p promotes learning and memory impairment induced by orthopedic surgery. (A) On the third day after surgery, The relative expression of miR-7684-5p between the four groups were compared, we use U6 as the reference gene, Data are presented as mean ± SD (n = 10), *P < 0.05; In the OFT, no noticeable difference was detected in general locomotion activity (D), center square duration (E), and rearing (F) between the four groups. Results are expressed as mean ± SD (n = 10); (B) Compared with the control group, the Sur group showed reduced freezing time in the contextual test (P < 0.01); Compared with the Sur group, the freezing time of LV+Sur group was significantly prolonged (P < 0.05). There was no significant difference in the freezing time of NC+Sur group with respect to Sur group (P > 0.05), results are expressed as mean ± SD (n = 10), *P < 0.05, **P < 0.01; (C) No apparent difference was observed in the freezing time between the mice from the four groups in the cued test. Results are expressed as mean ± SD (n = 10).

Results

Differentially expressed miRNA profiles and validation of deregulated miRNAs

The microarray method was used to screen differentially expressed genes in the hippocampal tissues of three pairs of mice (from the Ctrl and Sur groups). The number of differentially expressed miRNAs was 26 (including 15 upregulated miRNAs and 11 downregulated miRNAs, **Figure 3A**). The FC for the upregulation of miR-7684-5p was greater than 4. In addition, utilizing the *P*-value of the paired sample t-test and FC values, a volcano plot was constructed to demonstrate the significant differences between the data of the two groups (**Figure 3B**).

In this study, we selected four miRNAs (from the hippocampal tissues of 10 pairs of mice) to conduct a qRT-PCR analysis. Results revealed that miR-7684-5p and miR-466j were upregulated, while miR-1249-5p and miR-490-3p were downregulated (**Figure 3C**). Results of the qRT-PCR analyses were consistent with the results of the microarray analyses, confirming the reliability of the microarray results.

MiR-7684-5p promotes learning and memory impairment induced by orthopedic surgery

In order to further test the function of miR-7684-5p in the surgery-induced study memory function decrease, we constructed the miR-7684-5p knockdown lentiviral vector. This was performed on the brain through a stereoscopic position finder, and LV vector (2 μL) was bilaterally microinfused into the hippocampus *via* a stainless steel cannula connected to a Hamilton microsyringe. The contamination effect of miR-7684-5p knockdown in the lentiviral vector in hippocampus of mice was confirmed. On the third day after surgery, orthopedic surgery induced the upregulation of miR-7684-5p ($P < 0.05$). Compared with the Sur group, the LV+Sur group was able to apparently reduce the relative expression quantity of miR-7684-5p ($P < 0.05$). In addition, the relative expression quantity of miR-7684-5p in the NC+Sur group has no clear difference compared to the Sur group ($P > 0.05$) (**Figure 4A**).

It was further confirmed that the function of miR-7684-5p in surgery induced study memory function decrease through behavioristics. Behavioral training was performed to small

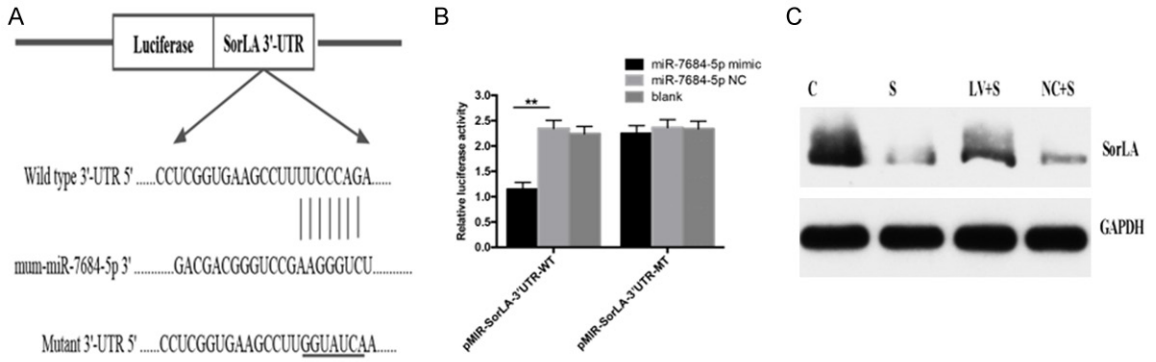

Figure 5. MiR-7684-5p negatively regulated the expression of SorLA. A. Sketch of the construction of pMIR-SorLA-3'UTR-WT or pMIR-SorLA-3'UTR-MT vectors. The mutant binding site is underlined. B. miR-7684-5p inhibited the luciferase activity of wild-type SorLA 3'UTR but not mutant SorLA 3'UTR. C. SorLA protein levels of hippocampal tissues were measured by western blot on 3rd day after orthopedic surgery. The results are means of three independent experiments ± SD. (**P < 0.01).

mice the day before the surgery, and a behavioristics test was conducted on the third day after surgery. The following were discovered: no apparent difference was detected between the four groups of mice in terms of general locomotion activity, center square duration, and rearing in the OFT (**Figure 4D-F**). In the contextual test, the freezing time of mice in the Sur group was significantly shorter than that in mice in the Ctrl group ($P < 0.01$). Compared to the Sur group, the freezing time in the LV+Sur group was clearly prolonged ($P < 0.05$). The freezing time in the NC+Sur group compared to the Sur group had no clear difference ($P > 0.05$) (**Figure 4B**). Furthermore, there was no significant difference between the freezing times of these four groups in the cued test (**Figure 4C**).

MiR-7684-5p negatively regulated the expression of SorLA

An integrated approach was employed to investigate the mechanism by which miR-7684-5p promotes learning and memory impairment induced by orthopedic surgery. Three databases, including TargetScan, PicTar and miRanda, were employed for this purpose. *SorLA* was found among the potential targets of miR-7684-5p combinationally predicted by these three databases. In order to further verify whether miR-7684-5p binds directly to the 3'UTR of *SorLA*, vectors were constructed containing wild-type or mutant 3'UTR of *SorLA* directly fused to the downstream of the Firefly luciferase gene (**Figure 5A**). The wild-type or mutant vector was cotransfected into HEK293T cells with miR-7684-5p mimic, miR-7684-5p NC, or none. Transfection efficiency was normalized by cotransfection with the Renilla reporter vector. As shown in **Figure 5B**, miR-7684-5p inhibited the luciferase activity of wild-type *SorLA* 3'UTR, but not mutant *SorLA* 3'UTR; suggesting that miR-7684-5p could directly bind to the 3'UTR of *SorLA*.

Moreover, the effect of miR-7684-5p on the expression of SorLA protein in mice transfected with the LV vector was evaluated. Through research, compared to the Ctrl group, it was found that SorLA protein expression quantity clearly drops on the third day after surgery ($P < 0.01$). Compared to the Sur group, SorLA protein expression quantity clearly increases ($P < 0.01$). SorLA protein expression quantity in the NC+Sur group has no clear difference, compared to that in the Sur group ($P > 0.05$), as shown in **Figure 5C**. Western blot results confirm the findings obtained from the dual luciferase report assay. Taken together, these findings indicate that SorLA is a direct downstream target for miR-7684-5p in mice.

MiR-7684-5p enhances surgery-induced β-amyloid accumulation in the hippocampus that may through the downregulation of SorLA

It was confirmed that SorLA is the target gene of miR-7684-5p, but the SorLA gene can regulate the metabolic pathway of (Amyloid precursor protein, APP). Therefore, the present study examined the effect of miR-7684-5p on the levels of APP and $A\beta_{1-42}$. It was found that, com-

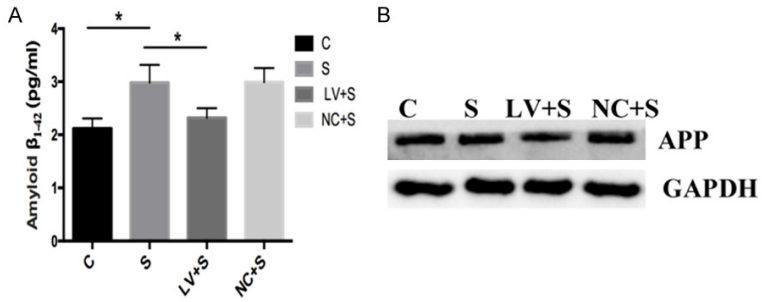

Figure 6. Effects of miR-7684-5p on the levels of APP and $A\beta_{1-42}$ in the hippocampus of aged mice. A. The expression of $A\beta_{1-42}$ was determined using an enzyme-linked immunosorbent assay on 3rd day after surgery. It was found that, compared with the Ctrl group, the Sur group can clearly reduce SorLA protein content ($P < 0.01$) and increase the generation of $A\beta_{1-42}$ ($P < 0.05$). Compared to the Sur group, the LV+Sur group can clearly increase SorLA protein content three days after surgery ($P < 0.05$) and reduce the generation of $A\beta_{1-42}$ ($P < 0.05$). The sorLA protein expression quantity in the NC+Sur group and the generation of $A\beta_{1-42}$ had no clear difference compared to that in the Sur group ($P > 0.05$). Results are expressed as mean ± SD (n = 10).

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Discussion

As an increasing number of elderly patients undergo surgery and anesthesia each year, optimizing postoperative cognitive function and preventing/treating POCD are major public health issues. Therefore, exploring the mechanism of postoperative cognitive function is of great significance for improving the prognosis of patients and identifying therapeutic targets for restorating cognitive function. In the present study, we focused on the functions of a newly identified miRNA in the central nervous system, miR-7684-5p, in response to surgical intervention. We found that miR-7684-5p expression was correlated with the accumulation of Aβ in the hippocampus and hippocampal-dependent memory impairment. By constructing a knockdown lentiviral vector, the interference of the expression of miR-7684-5p reduced the generation of Aβ and improved study and memory function. In this study, the newly identified miRNA will be beneficial for improving the understanding of the mechanisms in the pathogenesis of POCD.

In clinical practice, the incidence of POCD is relatively higher among patients of orthopedic surgery, especially among elderly patients. Thus, in this study, we employed the animal model of POCD for orthopedic surgery developed by Professor Terrando [15]. Then, on the third day after surgery, the hippocampal tissues of mice from the Ctrl group and Sur group were collected. The FC for the upregulation of miR-7684-5p was greater than 4. Microarray screening was conducted to identify differentially expressed genes. Twenty-six miRNAs were identified including 15 upregulated miRNAs and 11 downregulated miRNAs. Moreover, we performed qRT-PCR verifications on the hippocampus of 10 pairs of mice to verify the high expression of miR-7684-5p in the Sur group. These qRT-PCR results were consistent with the microarray analysis results.

Next, we injected in the stereospecific hippocampus, through constructing the miR-7684-5p knockdown lentiviral vector, testifying further the function of miR-7684-5p in surgery-induced hippocampal-dependent memory impairment. In this research, OFT was used to evaluate anxiety and locomotor activity in experimental animals. On the third day after surgery, the following were observed: no apparent difference was detected between the four groups of mice in terms of general locomotion activity, center square duration, and rearing in the OFT; testifying no anxiety and movement dysfunction in mice after orthopedic surgery. Moreover, we made further evaluations on the cognitive function of mice through FCT. Several studies in animals have shown that the hippocampus is critical in FCT, and FCT assessment has become a common method for investigating hippocampal-dependent associative mem-

ory in models of POCD [22, 23]. In previous studies, surgical trauma has been found to be associated with impaired cognitive function [24, 25]. In the present study, it was found that orthopedic surgery induced similar effects in the contextual test. Compared with the Ctrl group, mice in the Sur group had shorter freezing times on day three following surgery, which indicated that surgery led to hippocampal-dependent memory impairment. However, compared to the Sur group, freezing time was significantly longer in the LV+Sur group, indicating that knockdown miR-7684-5p can reverse the surgery induced drop in study memory function.

It has been well-documented that miRNAs alter target gene expression at the post-transcriptional level [26-28]. The function and mechanism of miR-7684-5p in surgery induced study memory function decrease. Furthermore, we have confirmed that miR-7684-5p negatively regulated the expression of SorLA protein in aged mice through western blot. We made an attempt to identify the potential target genes of miR-7684-5p. Three databases, including TargetScan, PicTar and miRanda, were employed for this purpose. *SorLA* was found to be among the potential targets of miR-7684-5p combinatorically predicted by these three databases. In order to further verify whether miR-7684-5p binds directly to 3'UTR of *SorLA*, we constructed vectors containing wild-type or mutant 3'UTR of *SorLA* directly fused to the downstream of the Firefly luciferase gene. We found that miR-7684-5p inhibited the luciferase activity of wild-type SorLA 3'UTR, but not mutant SorLA 3'UTR, suggesting that miR-7684-5p could directly bind to the 3'UTR of *SorLA*. Therefore, SorLA was predicted to be a direct target of miR-7684-5p by bioinformatics analysis, which was confirmed with dual luciferase report assay and western blot.

SorLA is a member of the protein receptor family within the range of VPS10P, a group of type 1 transmembrane receptors that traffic target proteins among the Golgi apparatus, cell surface and endosomes in various mammalian cell types. The expression of SorLA is mainly in nerve cells of the cortex, hippocampus, and cerebellum [29, 30]. Many studies have shown that SorLA can regulate the metabolic pathway of APP [31-33]. In the process of the newly synthesized APP passing the Golgi's apparatus and reaching the plasmalemma, some are cracked into the sAPPα. The untracked APP goes from plasmalemma into the late endosome through a swallowing effect, and is cracked into sAPPβ and Aβ. SorLA acts as a sorter. Moreover, some studies have confirmed that the receptor traps APP in the Golgi, reducing the amount of precursors that reach the cell surface for processing. In addition, SorLA may also shuttle APP from early endosomes back to the Golgi, further reducing the extent of Aβ production in late endosomes. The correlations between cognitive decline and the pathological markers of AD, including the production of $A\beta_{1-42}$ and the hyperphosphorylation of tau protein in the hippocampus of mice following major surgery [34, 35]. Based on this, the present study analyzed the expression of $A\beta_{1-42}$ in the hippocampus of aged mice. We discover that compared to the Ctrl group, the Sur group was able to clearly reduce SorLA protein content, and increase the generation of $A\beta_{1-42}$. Compared to the Sur group, the LV+Sur group was able to clearly increase SorLA protein content on the third day after surgery, and reduce the generation of $A\beta_{1-42}$. Therefore, we conjecture that miR-7684-5p enhances surgery-induced β-amyloid accumulation in the hippocampus possibly through downregulating SorLA.

Although the present study demonstrated that surgical injury upregulated the expression of miR-7684-5p, and the induced β-amyloid accumulation promotes learning and memory impairment, unanswered questions still remain. First, we only observed the expression of miR-7684-5p the third day after surgery, as well as relevant changes of mouse behavioristics, transcription level and protein level, with no consecutive observation followed up. It was not determined whether these changes still existed seven days after surgery. Next, we testified that miR-7684-5p can downregulate SorLA, and that miR-7684-5p can promote the generation of $A\beta_{1-42}$. Various relevant studies concerning AD have also shown that the reduced expression of SorLA accelerated the generation of $A\beta_{1-42}$, but we were not able to verify the relation between SorLA expression and $A\beta_{1-42}$ generation. That is to say, it remains unknown if the decreased expression of SorLA promotes the increased generation of $A\beta_{1-42}$ in POCD research. Therefore, we only conjecture that the miR-7684-5p mediated decreased cognitive function after surgery may have been caused

by increased $A\beta_{1-42}$ generation brought by downregulation of SorLA expression.

In conclusion, the present study demonstrated that miR-7684-5p expression was correlated with the accumulation of Aβ in the hippocampus and hippocampal-dependent memory impairment. Interfering with the expression of miR-7684-5p can reduce the generation of Aβ and improve study and memory function. Moreover, we testify that SorLA is the target gene of miR-7684-5p. These findings indicate that decreased postoperative cognitive function may be caused by the increased generation of $A\beta_{1-42}$ through the reduction in sorLA expression.

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Disclosure of conflict of interest

None.

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References

[1] Berger M, Nadler JW, Browndyke J, Terrando N, Ponnusamy V, Cohen HJ, Whitson HE, Mathew JP. Postoperative cognitive dysfunction: minding the gaps in our knowledge of a common postoperative complication in the elderly. Anesthesiol Clin 2015; 33: 517-50.

[2] Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, Rabbitt P, Jolles J, Larsen K, Hanning CD, Langeron O, Johnson T, Lauven PM, Kristensen PA, Biedler A, van Beem H, Fraidakis O, Silverstein JH, Beneken JE, Gravenstein JS. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Postoperative Cognitive Dysfunction. Lancet 1998; 351: 857-861.

[3] Xu J, Yue Y, Zhang R, Zuo P, Yang N, Ji C, Liu W, Wang Y, Wang H, Wu A, Liu Y. Aggravation effect of isoflurane on apoptosis and Tau hyperphosphorylation in Aβ25-35 induced PC12 cells. Cell Mol Neurobiol 2012; 32: 1343-1351.

[4] Hu N, Guo D, Wang H, Xie K, Wang C, Li Y, Wang C, Wang C, Yu Y, Wang G. Involvement of the blood-brain barrier opening in cognitive decline in aged rats following orthopedic surgery and high concentration of sevoflurane inhalation. Brain Res 2014; 1551: 13-24.

[5] Terrando N, Eriksson LI, Ryu JK, Yang T, Monaco C, Feldmann M, Jonsson Fagerlund M, Charo IF, Akassoglou K, Maze M. Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol 2013; 70: 986-95.

[6] Vacas S, Degos V, Tracey KJ, Maze M. High-mobility group box 1 protein initiates postoperative cognitive decline by engaging bone marrow-derived macrophages. Anesthesiology 2014; 120: 1160-7.

[7] Edbauer D, Neilson JR, Foster KA, Wang CF, Seeburg DP, Batterton MN, Tada T, Dolan BM, Sharp PA, Sheng M. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron 2010; 65: 373-84.

[8] Rajasethupathy P, Fiumara F, Sheridan R, Betel D, Puthanveettil SV, Russo JJ, Sander C, Tuschl T, Kandel E. Characterization of small RNAs in Aplysia reveals a role for miR-124 in constraining synaptic plasticity through CREB. Neuron 2009; 63: 803-17.

[9] Bicker S, Lackinger M, Schratt G. MicroRNA-132, -134, and -138: a microRNA troika rules in neuronal dendrites. Cell Mol Life Sci 2014; 71: 3987-4005.

[10] Edbauer D, Neilson JR, Foster KA, Wang CF, Seeburg DP, Batterton MN, Tada T, Dolan BM, Sharp PA, Sheng M. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron 2010; 65: 373-84.

[11] Sajikumar S, Korte M. Metaplasticity governs compartmentalization of synaptic tagging and capture through brain-derived neurotrophic factor (BDNF) and protein kinase Mzeta (PKMzeta). Proc Natl Acad Sci U S A 2011; 108: 2551-6.

[12] Nudelman AS, DiRocco DP, Lambert TJ, Garelick MG, Le J, Nathanson NM, Storm DR. Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. Hippocampus 2010; 20: 492-8.

[13] Vizcaychipi MP, Xu L, Barreto GE, Ma D, Maze M, Giffard RG. Heat shock protein 72 overex-

pression prevents early postoperative memory decline after orthopedic surgery under general anesthesia in mice. Anesthesiology 2011; 114: 891-900.
[14] Zhang MD, Barde S, Yang T, Lei B, Eriksson LI, Mathew JP, Andreska T, Akassoglou K, Harkany T, Hökfelt TG, Terrando N. Orthopedic surgery modulates neuropeptides and BDNF expression at the spinal and hippocampal levels. Proc Natl Acad Sci U S A 2016; 113: E6686-E6695.
[15] Terrando N, Gómez-Galán M, Yang T, Carlström M, Gustavsson D, Harding RE, Lindskog M, Eriksson LI. Aspirin-triggered resolvin D1 prevents surgery-induced cognitive decline. FASEB J 2013; 27: 3564-71.
[16] Terrando N, Yang T, Wang X, Fang J, Cao M, Andersson U, Erlandsson HH, Ouyang W, Tong J. Systemic HMGB1 neutralization prevents postoperative neurocognitive dysfunction in aged rats. Front Immunol 2016; 7: 441.
[17] Zhang C, Zhang Y, Shen Y, Zhao G, Xie Z, Dong Y. Anesthesia/surgery induces cognitive impairment only in female Alzheimer's disease transgenic mice. J Alzheimers Dis 2017; 57: 505-518.
[18] Barbato C, Pezzola S, Caggiano C, Antonelli M, Frisone P, Ciotti MT, Ruberti F. A lentiviral sponge for miR-101 regulates RanBP9 expression and amyloid precursor protein metabolism in hippocampal neurons. Front Cell Neurosci 2014; 13: 28-37.
[19] Katsouri L, Lim YM, Blondrath K, Eleftheriadou I, Lombardero L, Birch AM, Mirzaei N, Irvine EE, Mazarakis ND, Sastre M. PPARγ-coactivator-1α gene transfer reduces neuronal loss and amyloid-β generation by reducing β-secretase in an Alzheimer's disease model. Proc Natl Acad Sci U S A 2016; 113: 12292-12297.
[20] Shi XB, Xue L, Yang J, Ma AH, Zhao J, Xu M, Tepper CG, Evans CP, Kung HJ, deVere White RW. An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. Proc Natl Acad Sci U S A 2007; 104: 19983-19988.
[21] Guan Y, Yao H, Zheng Z, Qiu G, Sun K. MiR-125b targets BCL3 and suppresses ovarian cancer proliferation. Int J Cancer 2010; 128: 2274-2283.
[22] Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A 2004; 101: 14515-20.
[23] Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci 2013; 14: 417-28.
[24] Cibelli M, Fidalgo AR, Terrando N, Ma D, Monaco C, Feldmann M, Takata M, Lever IJ, Nanchahal J, Fanselow MS, Maze M. Role of interleukin-1 beta in postoperative cognitive dysfunction. Ann Neurol 2010; 68: 360-8.
[25] Cao L, Li L, Lin D, Zuo Z. Isoflurane induces learning impairment that is mediated by interleukin 1β in rodents. PLoS One 2012; 7: e51431.
[26] Wang W, Kwon EJ, Tsai LH. MicroRNAs in learning, memory, and neurological diseases. Learn Mem 2012; 19: 359-68.
[27] Shen J, Hung MC. Signaling-mediated regulation of MicroRNA processing. Cancer Res 2015; 75: 783-91.
[28] Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013; 14: 475-88.
[29] Schmidt V, Subkhangulova A, Willnow TE. Sorting receptor SORLA: cellular mechanisms and implications for disease. Cell Mol Life Sci 2017; 74: 1475-1483.
[30] Schmidt V, Willnow TE. Protein sorting gone wrong--VPS10P domain receptors in cardiovascular and metabolic diseases. Atherosclerosis 2016; 245: 194-9.
[31] Willnow TE, Andersen OM. Sorting receptor SORLA--a trafficking path to avoid Alzheimer disease. J Cell Sci 2013; 126: 2751-60.
[32] Andersen OM, Schmidt V, Spoelgen R, Gliemann J, Behlke J, Galatis D, McKinstry WJ, Parker MW, Masters CL, Hyman BT, Cappai R, Willnow TE. Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11. Biochemistry 2006; 45: 2618-28.
[33] Dodson SE, Andersen OM, Karmali V, Fritz JJ, Cheng D, Peng J, Levey AI, Willnow TE, Lah JJ. Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. J Neurosci 2008; 28: 12877-86.
[34] Wan Y, Xu J, Meng F, Bao Y, Ge Y, Lobo N, Vizcaychipi MP, Zhang D, Gentleman SM, Maze M, Ma D. Cognitive decline following major surgery is associated with gliosis, β-amyloid accumulation, and τ phosphorylation in old mice. Crit Care Med 2010; 38: 2190-8.
[35] McManus RM, Heneka MT. Role of neuroinflammation in neurodegeneration: new insights. Alzheimers Res Ther 2017; 9: 14.