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BIOMEDICAL SCIENCES

MiR-181c-5p ameliorates learning and memory in sleep-deprived mice via HMGB1/TLR4/NF-κB pathway

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Abstract: Sleep deprivation (SD) can lead to cognitive impairment caused by neuroinflammation. MiR-181c-5p/HMGB1 axis plays a part in anti-inflammation effects. However, the mechanism that miR-181c-5p facilitates learning and memory in SD mice remains unclear. So we investigated the role of miR-181c-5p in learning and memory impairment induced by SD. We overexpressed miR-181c-5p in the mice hippocampus by injecting lentivirus vector-miR-181c-5p (LV-miR-181c-5p) particles. Mice were divided into four groups: control (Ctrl), SD, SD + miR-181c-5p and SD + vector. We found that mice in the third group showed ameliorated learning and memory compared with the fourth group. The content of ionized calcium binding adaptor molecule 1 (IBA-1) in the third group was decreased compared with the fourth group. Moreover, the expression levels of HMGB1, TLR4 and p-NF-κB in the hippocampus of overexpressed miR-181c-5p mice were reduced. In total, miR-181c-5p ameliorated learning and memory in SD mice via the HMGB1/TLR4/NF-κB pathway.

Key words: sleep deprivation, miR-181c-5p, learning-memory ability, HMGB1/TLR4/NF-Kb.

INTRODUCTION

With the rapid development of society, people are under increasing pressure in their daily work and life and suffer from sleep deprivation (SD), among which people get less than 4-h sleep a day. Thus, SD has gradually become one of the main problems affecting people's health. Studies indicated that SD led to cognitive impairment, such as impaired learning, attentiveness and different kinds of memories (Nguyen et al. 2013, Patti et al. 2010, Krause et al. 2017). SD is commonly associated with cardiometabolic, neoplastic, autoimmune and neurodegenerative diseases (Garbarino et al. 2021). The high clinical incidence of SD seriously threatened public health (Lowe et al. 2017). Therefore, it is necessary to identify how SD impaired learning and memory and seek a new strategy for treating and preventing disease.

It was reported that microglia were the primary immune cells of the central nervous system (CNS) and took part in CNS homeostasis (Kabba et al. 2018). Dysregulation of microglia could give rise to neurological disease (Nayak et al. 2014). Evidence indicated that postoperative cognitive decline (POCD) had something to do with the inflammatory process of hippocampal microglia (Feng et al. 2017). Inflammation involves the pathogenesis of several neurodegenerative disorders, including Alzheimer's disease (AD) (Li et al. 2017). High Mobility Group Box 1 (HMGB1) protein was secreted by activated monocytes and macrophages and released by mechanically injured and necrotic cells (Muller

et al. 2001, Scaffidi et al. 2002). HMGB1 could activate microglia to increase the contents of inflammatory factors, which promotes the secretion of HMGB1 protein (Wang et al. 2015). Scaffidi et al. (2002) reported that the ability of *Hmgb1*$^{-/-}$ necrotic cells to promote inflammation was significantly decreased (Scaffidi et al. 2002). However, the role of HMGB1 in the neuroinflammation of SD mice remained unclear.

MicroRNAs (miRNAs), a class of 21–24 nucleotides, were small non-coding RNAs abundantly expressed in the CNS (Ruberti et al. 2012). It served as a critical regulatory role in several diseases, including neurodegenerative diseases (Wang et al. 2020). Research in recent years demonstrated that in sporadic AD, the down-regulation of miR-223, miR-137, and miR-181c was related to the progression of AD (Geekiyanage et al. 2012, Zhou et al. 2016, Wei et al. 2020). Besides, the research by Zhang et al. (2012) indicated that miR-181c regulated microglia-mediated neuronal apoptosis by inhibiting tumor necrosis factor-alpha (TNF-α) (Zhang et al. 2012). The miR-181c-5p-HMGB1 could regulate sepsis-induced microglial activation and hippocampal neuronal apoptosis (Li et al. 2021). In addition, the miR-181c-5p/HMGB1 axis may be a crucial pathway of berberine-induced anti-inflammation effects on stroke and may offer a novel method for targeted therapy (Cao et al. 2020). Li et al. (2021) demonstrated that miR-181c-5p could bind to HMGB1 (Li et al. 2021), so we supposed that miR-181c-5p might protect the hippocampus from neurodegenerative diseases by targeting HMGB1.

Research showed that miR-216a-5p could ameliorate learning-memory ability and attenuate the inflammatory response of AD mice by suppressing the HMGB1/NF-κB pathway (Shao 2021a). Thus, the HMGB1/NF-κB pathway was involved in learning, memory capabilities, and inflammatory reaction in AD mice. In addition, the HMGB1/TLR4/NF-κB signaling pathway attenuated excessive activation and neuroinflammation of microglial (Xu et al. 2020). However, the effect of the HMGB1/TLR4/NF-κB pathway on learning and memory deficiency in SD mice was still unknown.

This study attempted to discuss the mechanisms of miR-181c-5p in ameliorating SD mice's cognitive impairment to provide a theoretical basis for treating this disease.

MATERIALS AND METHODS

Animal experiment

Twenty 2-month-old male C57BL/6 mice (Hunan SJA laboratory animal company) were randomly divided into 4 groups: control (Ctrl), SD, SD + miR-181c-5p (injected with LV-miR-181c-5p, purchased from honorgene company) and SD + vector (injected with LV-vector, purchased from honorgene company), 5 mice in each group. Mice in SD, SD + miR-181c-5p and SD + vector groups stay awake for 20 h every day for 21 days. Ctrl mice were kept in the identical conditions as the experimental group but could sleep freely. 14 days later, mice were anesthetized with sodium pentobarbital before immobilization in the stereotaxic apparatus. 2 μL of LV-vector or LV-miR-181c-5p lentiviral particles was injected into the hippocampus at 0.4 μL/min (Shao 2021a, Wu et al. 2019). Animals were evaluated for learning and memory behavior at fixed times after SD. After behavioral testing, mice were sacrificed, and the hippocampus was collected for immunostaining and western blot analysis. All mouse experiments complied with the ARRIVE guidelines and were approved by the Experimental Animal Ethics Committee (Approval ID: SC20220176).

Construction of an SD mouse model

The flower pot technique established the SD mouse model. The SD experiment was carried out in a 4.0×10^4 cm^3 water box (50×40×20 cm) containing cylinder-shaped platforms (5 cm in height, 3 cm in diameter, 1 cm above horizontal plane) on which mice were fed and drank freely. The platforms were spaced 4 cm apart, and the mice could move freely. The mice in Rapid Eye Movement (REM) sleep showed decreased muscle tone, causing them to bow their heads to the water and wake up, making them unable to fall asleep. During the experiment, the room temperature was kept at 25°C, and the light was always on. The 20 h sleeping time of mice was deprived per day with 4 h left for rest (8 a.m. to 12 noon).

Morris water maze test (Shao 2021b)

The location-navigation test was used to estimate the ability to learn and memorize. From day 17 to day 21 after the start of SD, mice were put in one quadrant (the quadrants with the platform were excluded), and the escape latencies were written down. When the mice climbed onto the platform, they could rest on it for 20 s. If the mice didn't succeed in swimming to the platform within 120 s, they were laid and held on the platform for 20 s. Under this situation, the escape latency of mice was regarded as 120 s. This training was carried out for 5 days with 4 trials per mouse per day (interval between trials: 20-30 min). During training, escape latencies and the mean swimming velocities (m/s) were analyzed. The platforms were taken away for space exploration testing on the sixth day. The mice were placed in one quadrant (except the aim quadrant), and we recorded the time that mice spent in each quadrant and the times the mouse traversed the initial platform position within 120 s. The timeline of experiments with SD mice was shown in Figure 1.

Hematoxylin-eosin (HE) staining

Paraffin sections from mouse hippocampus were baked at 60°C for 12 h. Then the slices were deparaffinized by xylene treatment for 20 min, total 3 times. Then the sections were put in 100%, 100%, 95%, 85% and 75% ethanol in sequence for 5 min at each level, after which they were soaked in distilled water for 5 min. Subsequently, the slices were stained with HE dye liquor (Abiowell, China) and observed with a microscope (BA210T, Motic, China).

Immunohistochemistry (IHC)

Paraffin sections from mouse hippocampus were deparaffinized and hydrated, as described above. After that, we immersed the sections in 0.01 M citrate buffer (pH 6.0), boiled them in a laboratory electric stove and cooled them to room temperature for heat-recovery

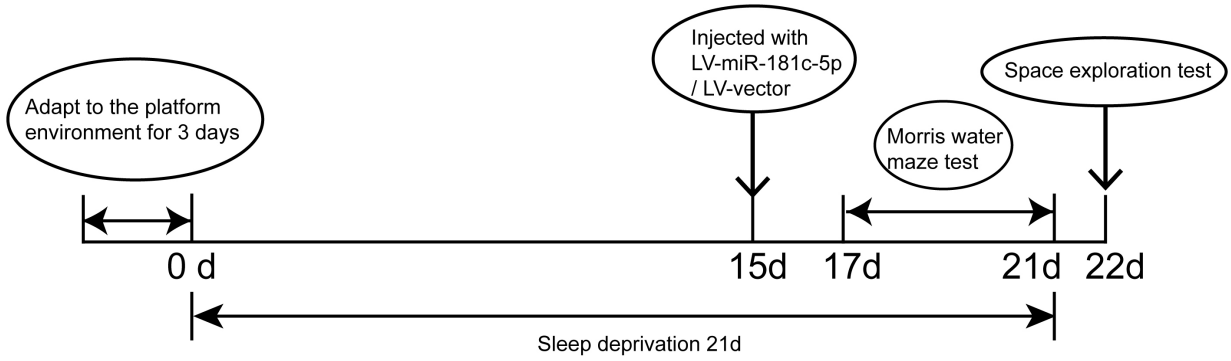

Figure 1. The timeline of experiments with Sleep deprivation (SD) mice.

antigen. Then, the sections were washed with 0.01 M PBS for 3 min, which was repeated 3 times. 1% periodic acid was added for 10 min to deactivate endogenous enzymes, and the slices were rinsed with PBS for 3 min 3 times. Next, sections were incubated overnight at 4°C with primary antibodies: postsynaptic density protein 95 (PSD95) (20665-1-AP, 1:200, Proteintech); synaptophysin (SYN) (20258-1-AP, 1:200, Proteintech); IBA-1 (10904-1-AP, 1:200, Proteintech). Next, the slices were incubated at 37°C for 30 min with secondary antibodies: anti-rabbit, rabbit, and multimeric anti-rabbit IgG-HRP. IHC staining was performed using a DAB substrate kit (ZLI-9018, ZSGB-BIO, China). Slices were then dehydrated with gradient alcohol (60-100%) for 5 min. After being taken out, they lay in xylene for 10 min, sealed with neutral gum and watched under a BA210T microscope.

Nissl staining

Paraffin sections were deparaffinized and hydrated, as mentioned above. The sections were dyed with nissl dye for 0.5 ~ 1 min, washed off the floating color with distilled water and differentiated with a differentiation solution (1% glacial acetic acid). After that, slices were mounted in buffered glycerol and observed under the BA210T microscope.

Dual luciferase assay

The wild-type (HMGB1-WT) or mutant (HMGB1-MUT) sequences of HMGB1 were inserted into the pHG-MirTarget vector to construct reporter plasmids. The miR-181c-5p mimic and miRNA negative control (NC) (GenePharma, shanghai, China) were transfected into 293A cells as needed. The dual luciferase system (E1910, Promega, USA) was applied to survey luciferase activity. The luciferase activities were measured utilizing a GloMax 20/20 detector (Promega, USA).

Enzyme-linked immunosorbent assay (ELISA) and biochemical testing

100 mg hippocampal tissues were cut into small pieces and homogenated with 1 mL of 1×PBS. The mixture was placed at -20°C overnight. After 2 repeated freeze-thaw treatments at 2-8°C, the cell membrane was destroyed, and the supernatant was obtained after centrifuging at 5000g for 5 min. For the detection method, we referred to the kit instructions. Mouse TNF-α ELISA Kit (KE10002), mouse interleukin (IL)-1β ELISA Kit (KE10003) and mouse IL-6 ELISA Kit (KE10007) were ordered from Proteintech (USA). Glutathione peroxidase (GSH-PX) biochemical kit (A005-1), malondialdehyde (MDA) biochemical kit (A003-1) and superoxide dismutase (SOD) biochemical kit (A001-3) were bought from Nanjing Jiancheng Bioengineering Institute.

Protein extraction and western blot

After the mice were anesthetized, hippocampal tissues were quickly separated. Total protein lysates were extracted by homogenization in RIPA supplemented with protease inhibitors. Proteins were electrophoresed on 10% SDS-PAGE gel and transferred to the PVDF membrane. After pre-blocking in 5% skim milk for 90 min at room temperature, TBST were incubated overnight at 4°C with primary antibodies, followed by secondary antibodies at room temperature for 1 h. The membranes were washed three times in TBS+Tween-20 (TBST) and then visualized with enhanced chemiluminescence (ECL) western blot substrate. The antibodies used were as follows: HMGB1 (10829-1-AP, Proteintech, USA); IBA-1 (ab178847, Abcam, UK); TNF-α (17590-1-AP, Proteintech, USA); IL-1β (ab254360, Abcam, UK); TLR4 (19811-1-AP, Proteintech, USA); NF-κB (10745-1-AP, Proteintech, USA); p-NF-κB (ab76302, Abcam, UK); β-actin (66009-1-Ig, Proteintech, USA); HRP goat anti-mouse IgG (SA00001-1,

Proteintech, USA); HRP goat anti-rabbit IgG (SA00001-2, Proteintech, USA).

Statistical analysis

For statistical analysis, the GraphPad Prism8.0 program was utilized. The one-way ANOVA was performed to analyze all data. Statistical significance was marked when $P < 0.05$.

RESULTS

Overexpression of miR-181c-5p ameliorated learning and memory in SD mice

To determine whether miR-181c-5p could ameliorate the capacities to learn and memorize in SD mice, we overexpressed miR-181c-5p by injecting LV-miR-181c-5p lentiviral particles into the hippocampus of mice. Morris water maze test was carried out to measure the learning and memory changes in each group. We evaluated the escape latencies. It was found that during the training period, the latency time of mice in the SD, SD + miR-181c-5p, and SD + vector groups was longer than mice in the Ctrl group. What's more, compared with the SD + vector group, the latency period of mice in the SD + miR-181c-5p group was decreased (Figure 2a). In addition, we also measured the swimming speed to the platform. As Figure 2b showed, the swimming speed in the SD, SD + miR-181c-5p, and SD + vector

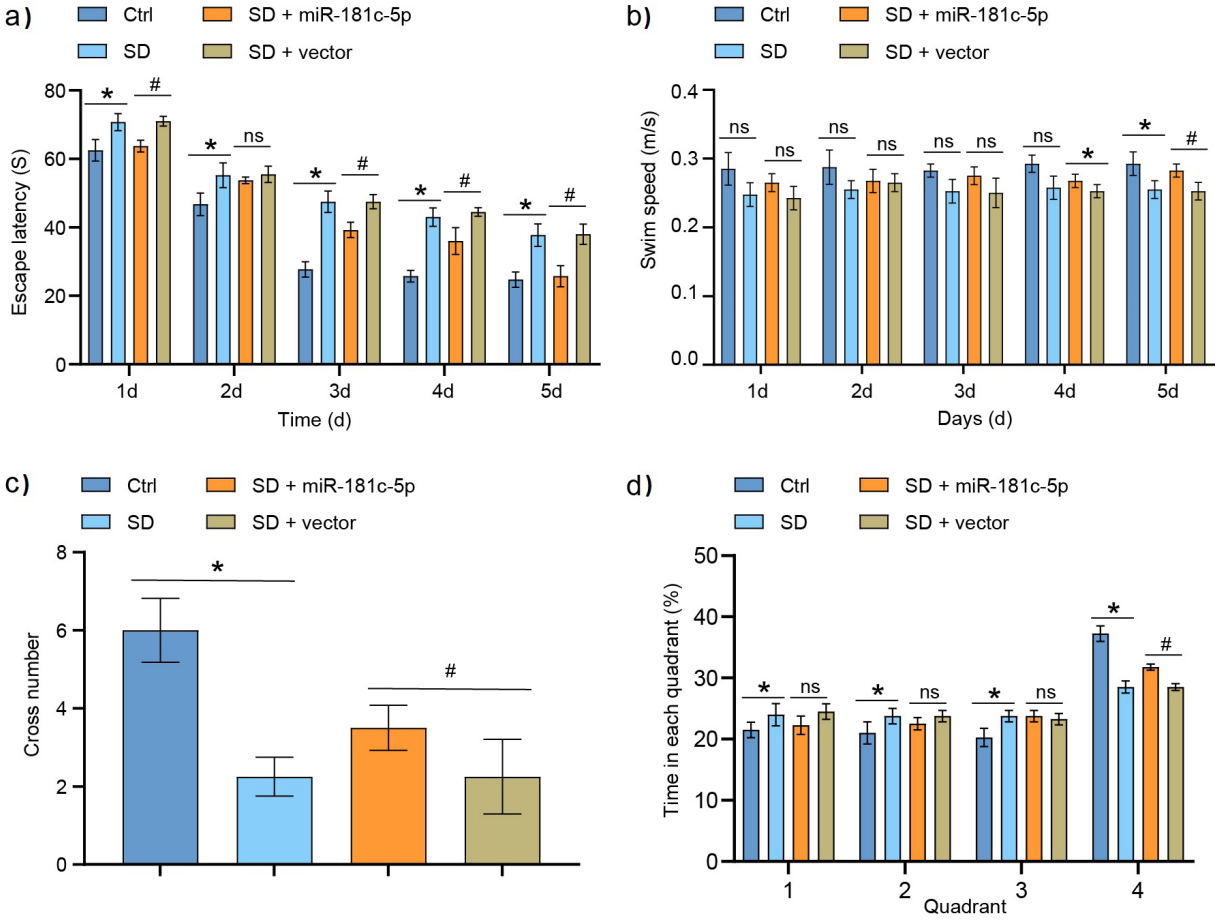

Figure 2. Overexpression of miR-181c-5p could facilitate learning and memory in SD mice. a) Morris water maze test was applied to detect the escape latency period, b) swim speed, c) the times the mouse crossed the original platform position and d) the ratio of time that mice spent traversing each quadrant in the spatial probing. One-way ANOVA. *P<0.05 vs Ctrl. #P<0.05 vs SD + vector. ns means no significance.

groups decreased compared to that in Ctrl mice. It was worth noting that the swimming speed of mice in the SD + miR-181c-5p was increased than that of the mice in the SD + vector group. We also performed a space exploration test, recording the fraction of time the mice stayed in each quadrant and the frequencies at which the mice traversed the initial platform position within 120 s after the platform was removed. The result indicated that SD mice showed decreased cross number than Ctrl mice. Injection of miR-181c-5p increased the cross number compared with the vector group (Figure 2c). The time of SD + miR-181c-5p mice spent in the first and second quadrants almost as long as Ctrl mice were shorter than that in the SD and SD + vector groups. However, in the fourth quadrant, SD + miR-181c-5p mice spent more time than the SD and SD + vector mice, but less time than the Ctrl mice (Figure 2d). To sum up, the data revealed that miR-181c-5p overexpression could ameliorate learning and memory capacities in SD mice.

Overexpression of miR-181c-5p improved hippocampal neuronal survival and inflammation in SD mice

To observe the effect of miR-181c-5p on mouse hippocampal neurons, we observed the morphological changes in the hippocampus of mice by HE staining. We found that SD mice and SD + vector group mice had significantly reduced numbers of hippocampal neurons, disordered arrangement, cell swelling, and unclear nuclear membrane boundaries compared with Ctrl mice. However, compared with the SD or SD + vector mice, the number of hippocampal neurons in the mice of the miR-181c-5p group was markedly increased, and the cell swelling was reduced. But the number of hippocampal neurons in the mice of the miR-181c-5p group was still not restored to the level of the Ctrl group (Figure 3a). Further, we also noticed the pathological changes of neuronal cells in the CA1 area of the hippocampus of mice by nissl staining. We found abundant neuronal cells in the hippocampus of the Ctrl mice, and the morphology was normal. In contrast, the number of neuronal cells in the SD mice, including the SD and SD + vector groups, was significantly reduced, and many necrotic neurons appeared. Compared with mice in SD and SD + vector groups, the number of neurons in the hippocampus of the miR-181c-5p group increased significantly. There were fewer necrotic neurons (Figure 3b). To assess the degree of oxidative damage in cells, we measured the contents of antioxidant enzymes SOD and GSH-Px in hippocampal tissues. The results showed that SD decreased SOD and GSH-Px levels in the hippocampus compared with Ctrl mice, but overexpressing miR-181c-5p could significantly increase the level of antioxidant enzymes in SD mice. On the other hand, we detected the lipid peroxidation product MDA level to judge the severity of cells attacked by free radicals indirectly. The results showed that MDA levels in the hippocampus of SD mice were significantly increased compared with Ctrl mice. However, compared with SD + vector group, MDA content in SD mice overexpressed miR-181c-5p decreased significantly (Figure 3c). Since inflammation was closely related to cell damage, we also detected the contents of IL-1β, IL-6 and TNF-α. Compared with Ctrl mice, the levels of inflammatory factors in the hippocampus of SD mice were significantly increased. However, compared with the SD + vector group, the contents of inflammatory factors were decreased in SD mice that overexpressed miR-181c-5p (Figure 3d). To clarify whether the pathological damage of the hippocampus in SD mice affects the synaptic transmission, we detected the expressions of synapse-related proteins PSD95 and SYN in the CA1 area of the hippocampus

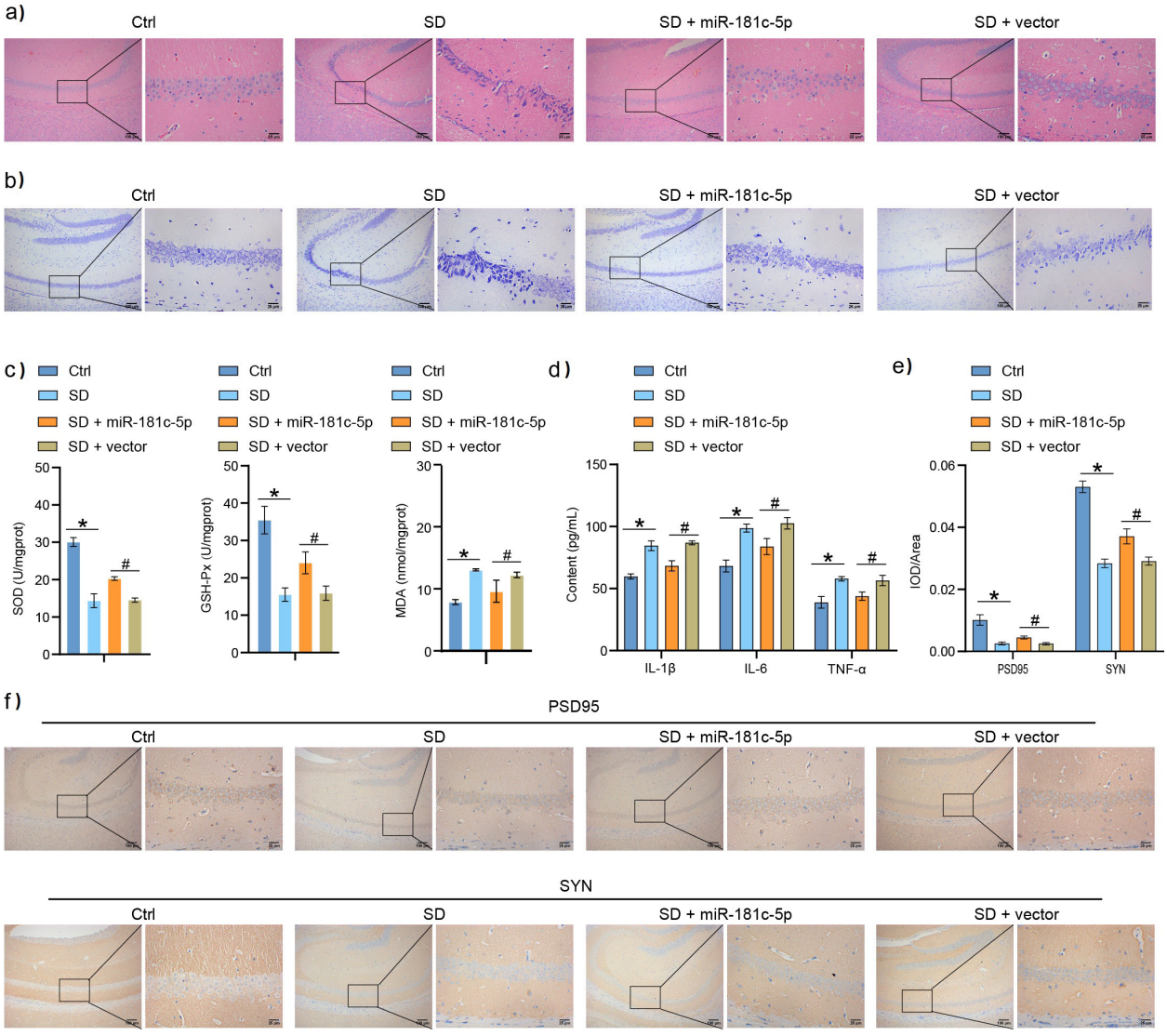

Figure 3. Overexpression of miR-181c-5p could improve hippocampal neuronal survival and inflammation in SD mice. a) HE staining showed damage to the CA1 area of the hippocampus. b) Observation of pathological changes of nissl bodies in the CA1 area of the hippocampus by nissl staining. c) SOD, GSH-Px, and MDA were measured in the hippocampal tissues by a biochemical kit. d) The levels of IL-1β, IL-6 and TNF-α were detected in the hippocampus by ELISA. e-f) Expression of synapse-related PSD95 and SYN in the CA1 area of the hippocampus by IHC. One-way ANOVA. *P<0.05 vs Ctrl. #P<0.05 vs SD + vector.

by IHC. The experimental and modeling data indicated that SD decreased the expression of PSD95 and SYN compared to Ctrl mice. When SD mice overexpressed miR-181c-5p, the expression levels of PSD95 and SYN were significantly up-regulated compared with mice in the SD + vector group (Figure 3e-f). Thus, these data indicated that miR-181c-5p overexpression could improve hippocampal neuronal survival and inflammation in SD mice.

Overexpression of miR-181c-5p inhibited hippocampal microglia activation in SD mice

Given that microglia may be involved in neuroinflammation, we examined the activation status of microglia in the hippocampus of four groups of mice.

The IHC staining indicated that in the CA1 area of the hippocampus, more IBA-1 was found in SD mice than in the Ctrl group. Notably, the content of IBA-1 in the SD + miR-181c-5p was decreased compared with SD + vector group (Figure 4a). Furthermore, a western blot was applied to detect the IBA-1 expression in the hippocampus of the four groups of mice. The same conclusions were drawn (Figure 4b). These results showed overexpression of miR-181c-5p restrained microglia activation in the CA1 area of the hippocampus in SD mice.

miR-181c-5p targeted regulation of HMGB1

It was found that miR-181c-5p had a binding site for HMGB1-3'UTR (Figure 5a). In addition, compared with miRNA NC + HMGB1 WT group, the luciferase activity of miR-181c-5p mimic in the miR-181c-5p + HMGB1 WT group was weaker (Figure 5b). This indicated that miR-181c-5p had a binding site for HMGB1 and could inhibit its transcriptional activity.

Overexpression of miR-181c-5p inhibited HMGB1/TLR4/NF-κB pathway activation in SD mice

To clarify how miR-181c-5p restored SD mice's abilities to learn and memorize, we used western blot to analyze the expression of HMGB1, TLR4 and p-NF-κB proteins. The results showed that SD led to increased HMGB1, TLR4 and p-NF-κB proteins in the hippocampus of mice compared with Ctrl mice. However, compared with SD + vector, the levels of HMGB1, TLR4 and p-NF-κB proteins in SD + miR-181c-5p mice were reduced (Figure 6a-c). These results showed that overexpression of miR-181c-5p inhibited HMGB1/TLR4/NF-κB pathway activation in SD mice. In addition, we also found that overexpression of miR-181c-5p could reduce the levels of TNF-α and IL-1β (Figure 6a-b).

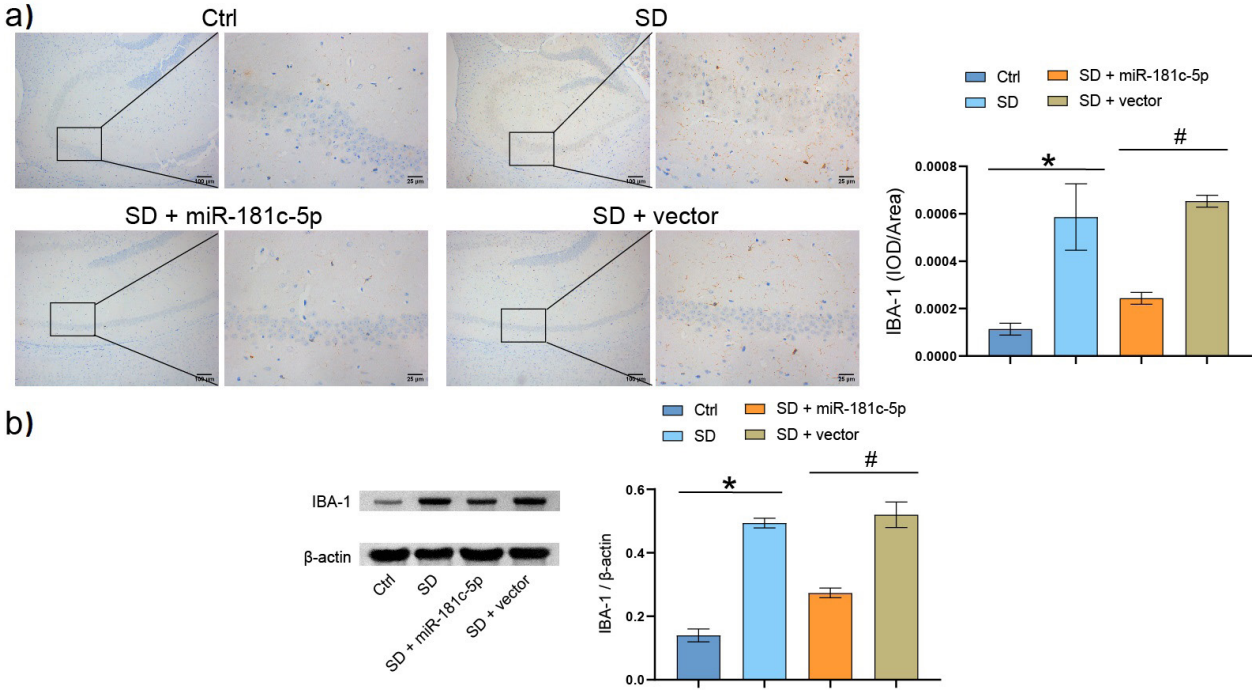

Figure 4. Overexpression of miR-181c-5p inhibited hippocampal microglia activation in SD mice. a) The IBA-1 expression in the CA1 area of the hippocampus was measured by the IHC method. b) Western blot was used to detect the content of IBA-1 in the hippocampal tissues. One-way ANOVA. *P<0.05 vs Ctrl. $^{\#}$P<0.05 vs SD + vector.

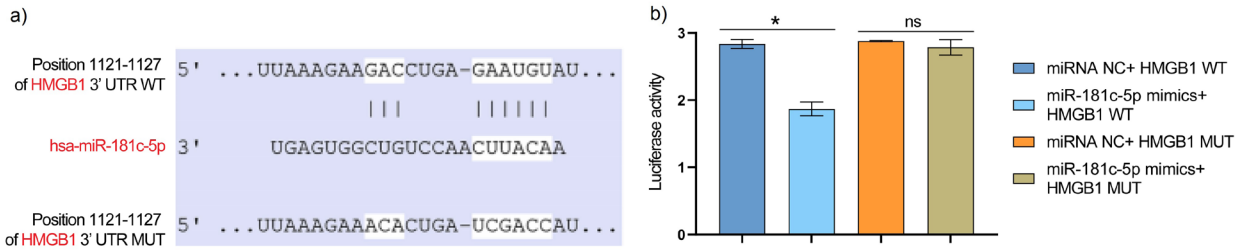

Figure 5. MiR-181c-5p targeted regulation of HMGB1. a) Bioinformatics predicted that miR-181c-5p targeted HMGB1. b) Dual-luciferase results demonstrated that miR-181c-5p inhibited the activity of HMGB1. One-way ANOVA. *P<0.05. ns means no significance.

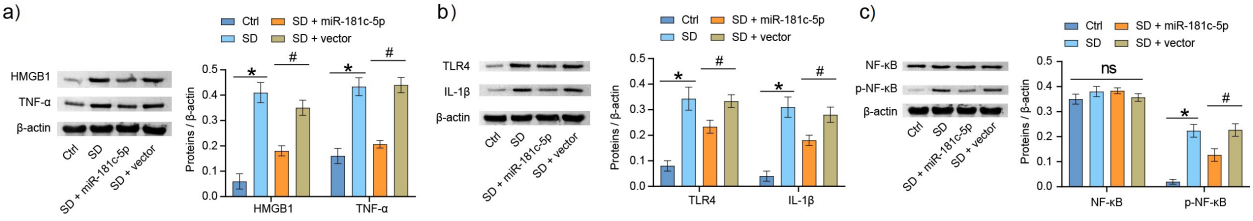

Figure 6. Overexpression of miR-181c-5p inhibited HMGB1/TLR4/NF-κB pathway activation in SD mice. a) Western blot was performed to test the expression levels of proteins in the hippocampus, including HMGB1 and TNF-α, b) TLR4 and IL-1β, c) NF-κB and p-NF-κB. One-way ANOVA. *P<0.05 vs Ctrl. #P<0.05 vs SD+vector. ns means no significance.

DISCUSSION

The rapid development of the social economy leads to the continuous increase of people's mental stress, causing severe SD. It has been widely accepted that SD can degrade cognitive performance (Csipo et al. 2021, Chen et al. 2017, Honn et al. 2019). However, the pathophysiological mechanism of this process has not been completely elucidated. We discovered that miR-181c-5p restored learning and memory in SD mice via the HMGB1/TLR4/NF-κB pathway.

A study by Z. K. et al. demonstrated that the small platform water environment method could simulate SD well (Zhang et al. 2017). Thus, in our study, we used this method to deprive mice of sleeping time. In addition, we performed a morris water maze test to investigate the effect of SD on learning and memory. We found that when the sleeping time of mice was deprived, the ability to learn and memory was significantly damaged. To our surprise, when we overexpressed miR-181c-5p in the hippocampus of mice by injecting LV-miR-181c-5p lentiviral particles, the learning and memory of SD mice were markedly restored. However, the learning and memory in SD + miR-181c-5p group were still impaired compared with Ctrl mice.

Hippocampus is a neural structure that is especially vulnerable to SD. The research found that the hippocampus was involved in forming and ameliorating learning and memory and strengthening declarative memory (Hainmueller & Bartos 2018, Lisman et al. 2017, Qiu et al. 2021). Therefore, we wondered whether SD would impair hippocampal neuronal survival and whether the overexpression of miR-181c-5p could rescue this injury. Just as we suspected, morphological changes verified by HE staining showed that compared with Ctrl mice, SD mice had significantly reduced numbers of hippocampal neurons, disordered arrangement, cell swelling, and unclear nuclear membrane boundaries. Moreover, the number of hippocampal neurons in the mice of the SD+miR-181c-5p group was rising, and the

cell swelling was reduced. On the other hand, nissl staining also indicated pathological lesions of nissl bodies in the hippocampus, and the overexpression of miR-181c-5p reversed the injury. Thus, we drew the conclusion that overexpressed miR-181c-5p improved hippocampal neuronal survival. As inflammation and oxidative stress played an important role in cognitive impairment caused by sleep deprivation (Qiu et al. 2021, Farajdokht et al. 2021), we evaluated the inflammation and oxidative stress level. We found that neuroinflammation and oxidative stress increased in SD mice, and overexpression of miR-181c-5p could alleviate these changes. SD substantially inhibited the synthesis of proteins involved in hippocampal neuroplasticity and impaired the growth and development of nervous tissue in the hippocampus (Fernandes et al. 2015). To clarify whether the pathological damage of the hippocampus in SD mice affects the synaptic transmission, we detected the expressions of synapse-related proteins PSD95 and SYN in the hippocampus by IHC. The result showed that SD could impair synaptic plasticity in the hippocampus. The data above suggested that overexpressed miR-181c-5p improved hippocampal neuronal survival and inflammation in SD mice.

Recent studies have shown that microglia can regulate the liveness of nerves and accelerate learning (Parkhurst et al. 2013, Zhan et al. 2014). Neuro-immune responses, named as "neuroinflammation", were guided by four primary features: the activation of microglial, the increase of cytokines and chemokines, aggregation of peripheral immune cells and the damage of local tissue (Estes & McAllister 2014, O'Callaghan et al. 2008). In the current study, we examined the activation status of microglia in the hippocampus of four groups of mice. IBA-1 was the potential marker for microglia activation, so we detected the IBA-1 expression in the hippocampus and found that SD could promote microglial activation. In addition, overexpression of miR-181c-5p inhibited hippocampal microglia activation in SD mice.

It was reported that miR-181c-5p could bind to HMGB1-3'UTR and inhibit its transcriptional activity (Li et al. 2021), which was also demonstrated in the current study. The data we acquired indicated that miR-181c-5p bound to HMGB1 inhibited its transcriptional activity. Research showed that miR-216a-5p could ameliorate the ability to learn and memorize and attenuate the inflammatory response of AD mice by inhibiting the HMGB1/NF-κB pathway (Shao 2021a). In addition, the HMGB1/TLR4/NF-κB signaling pathway attenuated excessive microglial activation and neuroinflammation (Xu et al. 2020). However, the role of the HMGB1/TLR4/NF-κB signaling pathway in learning and memory deficiency in SD mice was still unknown. Here, we analyzed the expression levels of HMGB1, TLR4 and p-NF-κB proteins. The results indicated that SD increased HMGB1, TLR4 and p-NF-κB proteins in the hippocampus. While compared with SD + vector group, the levels of HMGB1, TLR4 and p-NF-κB proteins in the hippocampus of overexpressed miR-181c-5p mice were reduced. Moreover, the TNF-α and IL-1β were also inhibited in the SD + miR-181c-5p group. These results showed that overexpression of miR-181c-5p inhibited HMGB1/TLR4/NF-κB pathway activation in SD mice.Our research found that miR-181c-5p ameliorated learning and memory in SD mice via the HMGB1/TLR4/NF-κB pathway. The miR-181c-5p could be a therapeutic target for cognitive dysfunction induced by SD.

Acknowledgments

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REFERENCES

CAO DW, LIU MM, DUAN R, TAO YF, ZHOU JS, FANG WR, ZHU JR, NIU L & SUN JG. 2020. The lncRNA Malat1 functions as a ceRNA to contribute to berberine-mediated inhibition of HMGB1 by sponging miR-181c-5p in poststroke inflammation. Acta Pharmacol Sin 41: 22-33.

CHEN J, LIANG J, LIN X, ZHANG YANG, ZHANG YAN, LU L & SHI J. 2017. Sleep Deprivation Promotes Habitual Control over Goal-Directed Control: Behavioral and Neuroimaging Evidence. J Neurosci 37: 11979-11992.

CSIPO T ET AL. 2021. Sleep deprivation impairs cognitive performance, alters task-associated cerebral blood flow and decreases cortical neurovascular coupling-related hemodynamic responses. Sci Rep 11: 20994.

ESTES ML & MCALLISTER AK. 2014. Alterations in immune cells and mediators in the brain: it's not always neuroinflammation! Brain Pathol 24: 623-630.

FARAJDOKHT F, VATANDOUST SM, HOSSEINI L, FEKRI K, RAHIGH AGHSAN S, MAJDI A, SADIGH-ETEGHAD S & MAHMOUDI J. 2021. Sericin protects against acute sleep deprivation-induced memory impairment via enhancement of hippocampal synaptic protein levels and inhibition of oxidative stress and neuroinflammation in mice. Brain Res Bull 174: 203-211.

FENG X, VALDEARCOS M, UCHIDA Y, LUTRIN D, MAZE M & KOLIWAD SK. 2017. Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. JCI Insight 2: e91229.

FERNANDES C, ROCHA NB, ROCHA S, HERRERA-SOLIS A, SALAS-PACHECO J, GARCIA-GARCIA F, MURILLO-RODRIGUEZ E, YUAN TF, MACHADO S & ARIAS-CARRION O. 2015. Detrimental role of prolonged sleep deprivation on adult neurogenesis. Front Cell Neurosci 9: 140.

GARBARINO S, LANTERI P, BRAGAZZI NL, MAGNAVITA N & SCODITTI E. 2021. Role of sleep deprivation in immune-related disease risk and outcomes. Commun Biol 4: 1304.

GEEKIYANAGE H, JICHA GA, NELSON PT & CHAN C. 2012. Blood serum miRNA: non-invasive biomarkers for Alzheimer's disease. Exp Neurol 235: 491-496.

HAINMUELLER T & BARTOS M. 2018. Parallel emergence of stable and dynamic memory engrams in the hippocampus. Nature 558: 292-296.

HONN KA, HINSON JM, WHITNEY P & VAN DONGEN HPA. 2019. Cognitive flexibility: A distinct element of performance impairment due to sleep deprivation. Accid Anal Prev 126: 191-197.

KABBA JA, XU Y, CHRISTIAN H, RUAN W, CHENAI K, XIANG Y, ZHANG L, SAAVEDRA JM & PANG T. 2018. Microglia: Housekeeper of the Central Nervous System. Cell Mol Neurobiol 38: 53-71.

KRAUSE AJ, SIMON EB, MANDER BA, GREER SM, SALETIN JM, GOLDSTEIN-PIEKARSKI AN & WALKER MP. 2017. The sleep-deprived human brain. Nat Rev Neurosci 18: 404-418.

LI L, XU D, LIN J, ZHANG D, WANG G, SUI L, DING H & DU J. 2017. Coenzyme Q10 attenuated beta-amyloid25-35-induced inflammatory responses in PC12 cells through regulation of the NF-kappaB signaling pathway. Brain Res Bull 131: 192-198.

LI M, HU J, PENG Y, LI J & REN R. 2021. CircPTK2-miR-181c-5p-HMGB1: a new regulatory pathway for microglia activation and hippocampal neuronal apoptosis induced by sepsis. Mol Med 27: 45.

LISMAN J, BUZSAKI G, EICHENBAUM H, NADEL L, RANGANATH C & REDISH AD. 2017. Viewpoints: how the hippocampus contributes to memory, navigation and cognition. Nat Neurosci 20: 1434-1447.

LOWE CJ, SAFATI A & HALL PA. 2017. The neurocognitive consequences of sleep restriction: A meta-analytic review. Neurosci Biobehav Rev 80: 586-604.

MULLER S, SCAFFIDI P, DEGRYSE B, BONALDI T, RONFANI L, AGRESTI A, BELTRAME M & BIANCHI ME. 2001. New EMBO members' review: the double life of HMGB1 chromatin protein: architectural factor and extracellular signal. EMBO J 20: 4337-4340.

NAYAK D, ROTH TL & MCGAVERN DB. 2014. Microglia development and function. Annu Rev Immunol 32: 367-402.

NGUYEN ND, TUCKER MA, STICKGOLD R & WAMSLEY EJ. 2013. Overnight Sleep Enhances Hippocampus-Dependent Aspects of Spatial Memory. Sleep 36: 1051-1057.

O'CALLAGHAN JP, SRIRAM K & MILLER DB. 2008. Defining "neuroinflammation". Ann N Y Acad Sci 1139: 318-330.

PARKHURST CN, YANG G, NINAN I, SAVAS JN, YATES JR 3RD, LAFAILLE JJ, HEMPSTEAD BL, LITTMAN DR & GAN WB. 2013. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155: 1596-1609.

PATTI CL, ZANIN KA, SANDAY L, KAMEDA SR, FERNANDES-SANTOS L, FERNANDES HA, ANDERSEN ML, TUFIK S & FRUSSA-FILHO R. 2010. Effects of sleep deprivation on memory in mice: role of state-dependent learning. Sleep 33: 1669-1679.

QIU C, WANG M, YU W, RONG Z, ZHENG HS, SUN T, LIU SB, ZHAO MG & WU YM. 2021. Activation of the Hippocampal LXRbeta Improves Sleep-Deprived Cognitive Impairment by Inhibiting Neuroinflammation. Mol Neurobiol 58: 5272-5288.

RUBERTI F, BARBATO C & COGONI C. 2012. Targeting microRNAs in neurons: tools and perspectives. Exp Neurol 235: 419-426.

SCAFFIDI P, MISTELI T & BIANCHI ME. 2002. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418: 191-195.

SHAO P. 2021a. MiR-216a-5p ameliorates learning-memory deficits and neuroinflammatory response of Alzheimer's disease mice via regulation of HMGB1/NF-kappaB signaling. Brain Res 1766: 147511.

SHAO P. 2021b. MiR-216a-5p ameliorates learning-memory deficits and neuroinflammatory response of Alzheimer's disease mice via regulation of HMGB1/NF-κB signaling. Brain Res 1766: 147511.

WANG FC, PEI JX, ZHU J, ZHOU NJ, LIU DS, XIONG HF, LIU XQ, LIN DJ & XIE Y. 2015. Overexpression of HMGB1 A-box reduced lipopolysaccharide-induced intestinal inflammation via HMGB1/TLR4 signaling in vitro. World J Gastroenterol 21: 7764-7776.

WANG X, ZHOU Y, GAO Q, PING D, WANG Y, WU W, LIN X, FANG Y, ZHANG J & SHAO A. 2020. The Role of Exosomal microRNAs and Oxidative Stress in Neurodegenerative Diseases. Oxid Med Cell Longev 2020: 3232869.

WEI H, XU Y, CHEN Q, CHEN H, ZHU X & LI Y. 2020. Mesenchymal stem cell-derived exosomal miR-223 regulates neuronal cell apoptosis. Cell Death Dis 11: 290.

WU Q, YUAN X, BAI J, HAN R, LI Z, ZHANG H & XIU R. 2019. MicroRNA-181a protects against pericyte apoptosis via directly targeting FOXO1: implication for ameliorated cognitive deficits in APP/PS1 mice. Aging (Albany NY) 11: 6120-6133.

XU X ET AL. 2020. Arctigenin protects against depression by inhibiting microglial activation and neuroinflammation via HMGB1/TLR4/NF-kappaB and TNF-alpha/TNFR1/NF-kappaB pathways. Br J Pharmacol 177: 5224-5245.

ZHANG K, LI YJ, FENG D, ZHANG P, WANG YT, LI X, LIU SB, WU YM & ZHAO MG. 2017. Imbalance between TNFalpha and progranulin contributes to memory impairment and anxiety in sleep-deprived mice. Sci Rep 7: 43594.

ZHANG L, DONG LY, LI YJ, HONG Z & WEI WS. 2012. The microRNA miR-181c controls microglia-mediated neuronal apoptosis by suppressing tumor necrosis factor. J Neuroinflammation 9: 211.

ZHAN Y ET AL. 2014. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 17: 400-406.

ZHOU H, ZHANG R, LU K, YU W, XIE B, CUI D, JIANG L, ZHANG Q & XU S. 2016. Deregulation of miRNA-181c potentially contributes to the pathogenesis of AD by targeting collapsin response mediator protein 2 in mice. J Neurol Sci 367: 3-10.

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