RESEARCH PAPER

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The effect of miR-155-5p on long-term memory of sleep-deprived mice through the BDNF/NF-κB pathway

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Sleep deprivation (SD) is a prevalent sleep issue in modern society that significantly impairs neurological function and quality of life in affected individuals. This study seeks to investigate the involvement of the miR-155-5p/BDNF axis in SD mice, aiming to establish a theoretical foundation for potential treatment strategies. Male C57BL/6 mice were utilized in the construction of a SD model using the flower pot technique. HT22 cells were selected for cellular experiments. The Morris water maze was employed to assess the learning and memory capabilities of the mice. HE staining was utilized to observe pathological changes in hippocampal tissue. Levels of IL-1β, IL-6, and TNF-α were analyzed using ELISA. The expression level of miR-155-5p was quantified *via* RT-qPCR. The binding between miR-155-5p and brain-derived neurotrophic factor (BDNF) was confirmed through a dual-luciferase reporter assay. Apoptosis of hippocampal neurons was assessed using TUNEL. Western blot analysis was conducted to evaluate the expression levels of BDNF, p65, and p-p65. The Morris water maze test revealed that the mice exhibited prolonged escape latency, decreased swimming velocity, and reduced time spent in the target platform quadrant, which are indicative of a successful construction of the SD model. The observed cognitive deficits in the mice were associated with SD-induced damage to the hippocampal tissue, leading to increased levels of miR-155-5p and decreased levels of BDNF. miR-155-5p was found to directly bind to BDNF, thereby suppressing its mRNA and protein expression. The upregulation of BDNF effectively mitigated hippocampal damage by attenuating cell apoptosis and reducing inflammation levels in SD mice. Additionally, the BDNF/NF-κB pathway was found to be suppressed in SD mice through the downregulation of miR-155-5p. Therefore, the silencing of miR-155-5p inhibited the activation of the NF-κB pathway by upregulating BDNF, which improved long-term memory and reduced neuronal damage in SD mice.

Key words: miR-155-5p; BDNF/NF-κB pathway; sleep deprivation; cognitive function

INTRODUCTION

Sleep is a natural and reversible physiological state marked by a decreased response to external stimuli (Brianza-Padilla et al., 2018). Sleep deprivation (SD) occurs when individuals are unable to obtain normal and sufficient sleep due to various internal and external factors, resulting in either a decrease in total sleep time or disrupted sleep patterns (Wright et al., 2015). SD can have serious impacts on cognitive and emotional function and can affect the brain in various ways, such as inhibiting the growth of hippocampal neuronal cells (Guzmán-Marín et al., 2003), and increasing inflammatory levels (Irwin et al., 2010). Numerous studies have demonstrated that SD can impair learning, memory, and other cognitive abilities (Killgore, 2010), although the exact underlying mechanism remains unclear. Therefore, there is a significant challenge in investigating the mechanisms behind the cognitive dysfunction induced by SD.

MiRNA is a single-stranded noncoding RNA that has been discovered in recent years (Huntzinger & Izaur-

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ralde, 2011). It has the ability to bind to mRNA, thereby inhibiting its translation and expression (Huntzinger & Izaurralde, 2011). Research has shown that many miRNAs are linked to the regulation of sleep (Goodwin et al., 2018). Some miRNAs exhibit different expression levels in the sleep and awake states (Davis et al., 2007), as well as in various brain regions such as the cortex, hippocampus, and hypothalamus (Davis et al., 2012). For example, miR-155-5p, a serum miRNA, is highly expressed during SD (Haider et al., 2014). Studies have indicated that SD can reduce the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus (Mohammadipoor-Ghasemabad et al., 2019). BDNF, the most abundant neurotrophic factor (Huang & Reichardt, 2001), plays a crucial role in regulating synaptic homeostasis and plasticity, which are essential for learning and memory (Caviedes et al., 2017). Decreased BDNF levels in the hippocampus have been associated with reduced neuron proliferation (Cieślik et al., 2011), and the development of major depression (Nestler et al., 2002). Conversely, studies have suggested that increasing BDNF levels can be beneficial for improving cognitive function (Looti Bashiyan et al., 2021). However, the specific mechanism by which miR-155-5p regulates cognitive function in SD mice through BDNF remains unclear.

Sleep can play a role in supporting and repairing the immune system, helping individuals defend against infection and inflammatory damage (Garbarino et al., 2021). When SD occurs, there are changes in the distribution of immune cells and the release of inflammatory factors, leading to sustained activation of the inflammatory response (Irwin, 2019). Numerous studies have demonstrated that SD activates astrocytes and microglia in the central nervous system, resulting in nerve injury and inflammation (Chennaoui et al., 2015). The nuclear factor kappa-B (NF-κB) plays a regulatory role in inflammation by inducing the release of cytokines that regulate immune responses, such as IL-1, IL-6, IL-8, and TNF-α (Hoesel & Schmid, 2013). Due to its crucial role in neural development, synaptic transmission, synaptic plasticity, and other functions, NF-κB has become a significant subject of research in the neurobiology of depression in recent years (Caviedes et al., 2017). Studies have shown that SD induces the activation of NF-κB in different brain regions (Basheer et al., 2001). The bidirectional interaction between BDNF and the NF-κB pathway exhibits an opposite trend. Therefore, we hypothesize that miR-155-5p may impact the cognitive function of SD mice through the BDNF/NF-κB pathway.

In this study, an SD mice model was established to investigate the impact of SD on cognitive function in mice. The research delved into the mechanism of SD-induced cognitive impairment. Moreover, the study examined the influence of miR-155-5p on cognitive function in SD mice through the BDNF/NF-κB pathway, aiming to lay a foundation for potential treatments for SD.

METHODS

Cell culture and treatment

The mouse hippocampal neuron cell line HT22 (20190512, KALANG, China) was cultured in the DMEM medium (D5796, Sigma, USA) with fetal bovine serum (FBS, 10%) (10099141, Gibco, USA) and double antibiotics (Penicillin/Streptomycin, 1%) (SV30010, Beyotime, China). Cells were taken for trypsin digestion, passage, and transfection experiments after the culture *in vitro* at 37°C and 5% CO_2. Lipofectamine 2000 transfection reagent (11668-019, Invitrogen, USA) was employed for transfection. Cell experiments were grouped as follows: mimics NC: transfection with NC mimics; miR-155-5p mimics: transfection with miR-155-5p mimics; inhibitor NC: transfection with NC inhibitor; miR-155-5p inhibitor: transfection with miR-155-5p inhibitor. Negative control (NC) represented non-loaded vector. All the mimics and inhibitor were provided by Changsha Abiowell Biotechnology Co., Ltd.

Experimental animals and groups

A total of 45 adult male C57BL/6 mice (weight: 20~25 g) were bought from Hunan SJA Laboratory Animal Co., Ltd. The experiment was conducted after 1 week of adaptive feeding. Experiment 1: Normal: without any treatment; SD: sleep deprivation; Negative control (NC): SD after injection of inhibitor NC (Artificially synthesized empty RNA oligos); anti-miR-155-5p: SD after injection of miR-155-5p inhibitor (Artificially synthesized miR-155-5p oligos). Experiment 2: overexpression (oe)-NC: SD after injection of oe-NC; oe-BDNF: SD after injection of oe-BDNF. Experiment 3: NC: SD after injection of inhibitor NC; anti-miR-155-5p: SD after injection of miR-155-5p inhibitor; anti-miR-155-5p+si-BDNF: SD after injection of miR-155-5p inhibitor and si-BDNF. Five mice were tested in each group. All the mimics and inhibitors were provided by Changsha Abiowell Biotechnology Co., Ltd. The mice in the SD, NC, anti-miR-155-5p, oe-NC, oe-BDNF, and anti-miR-155-5p+si-BDNF groups all suffered from SD for 21 consecutive days (20 h/d) while the mice in the Normal group could sleep freely. On day 14 after SD, mice were fixed and injected with

pentobarbital (50 mg/kg body weight). Subsequently, si-NC, si-miR-155-5p, si-BDNF, oe-NC, and oe-BDNF (2 μL, 0.4 μL/min) were injected bilaterally into the hippocampus of corresponding mice respectively (Shao, 2021; Wu et al., 2019). Next, the cognitive function of mice was tested. This work was approved by the Animal Ethical and Welfare Committee of Central South University Xiangya School of Medicine Affiliated Haikou Hospital (2022-121).

Construction of SD model

The SD model was created using the flower pot technique as described (Zhang et al., 2017). The experiment took place in a water tank measuring 50×40×20 cm, fitted with multiple cylindrical platforms (5 cm in height, 3 cm in diameter, positioned 1 cm above the horizontal) where mice had access to food and water. These platforms were spaced 4 cm apart to allow for unrestricted movement. Prior to constructing the SD model, the mice were acclimated to the testing environment for 3 days. During the experiment, the mice exhibited decreased muscle tone, prompting them to awaken upon contact with water if they entered rapid eye movement (REM) sleep. The room temperature was maintained at 22-25°C, and a 40 W fluorescent lamp provided illumination throughout the experiment. The mice were kept awake for 20 hours each day and allowed to sleep for only 4 hours (from 8 a.m. to 12 p.m.) over a period of 21 days.

Preparation of hippocampal tissue

After the experiment, the mice were anesthetized by intraperitoneal injection of 0.1% pentobarbital solution with a 1 mL syringe. The mice were carefully positioned on their backs, with their limbs and teeth secured to an anatomical board using rubber bands. Using tissue scissors, a horizontal incision was made along the skin in the midsection, gradually dissecting through the layers to expose the abdominal cavity. The diaphragm was then cut to open the chest, followed by cutting both sides of the ribs to lift the sternum and expose the pericardium to access the heart. The right auricle was then precisely cut with scissors, and the heart was immobilized for further procedures. Cold saline solution was swiftly injected three times into the heart using a 10 mL syringe to drain the blood from the mouse's body. Subsequently, the skull was carefully separated from the occipital bone's foramen magnum, unveiling the brain tissue. The skull was delicately peeled back to fully expose the intact brain, which was then extracted from the skull base. The isolated brain tissue was transferred to a petri dish and immersed in a PBS solution of appropriate osmotic pressure concentration. Using a clean surgical blade, the brain was halved, and the hippocampus was carefully extracted with the aid of microscopic tweezers.

Morris water maze test

The Morris water maze test was conducted in accordance with the procedures outlined in the literature (Tabuchi et al., 2007). The localization navigation test was used to evaluate the learning and memory capabilities of the mice. The training spanned 5 days, with 4 sessions per day (test interval: 20-30 min apart). Mice were placed in a quadrant (excluding the target platform quadrant) to measure their escape latency. Upon successfully reaching the platform, mice were allowed to rest on it for 20 seconds. If a mouse failed to reach the platform within 120 seconds, it was manually placed on the platform for 20 seconds and the escape latency was recorded as 120 seconds. During the test, the average swimming speed (cm/s) and escape latency (s) were analyzed. On day 6, the target platform was removed to assess spatial memory. Mice were placed in a quadrant and the time spent in each quadrant was recorded within a 120-second timeframe.

Bioinformatic prediction

The binding sites of miR-155-5p and BDNF was predicted on the Starbase website (https://rnasysu.com/encori/index.php).

Dual-luciferase reporter assay

The dual luciferase reporter plasmids psiCHECK-2-musBDNF-3U and psiCHECK-2-musBDNF-3U-Mut were all purchased from Changsha Abiowell Biotechnology Co., Ltd. Four groups of 293A cells (HG-NC071, HonorGene, China) were cultured and set into musBDNF-3U + mimics NC, musBDNF-3U + miR-155-5p mimics, musBDNF-3U-Mut + mimics NC, and musBDNF-3U-Mut + miR-155-5p mimics groups. Cells were transfected with plasmids and miRNAs, respectively. Detection was performed according to the Dual-Luciferase Reporter Assay Kit (E1910, Promega, USA). After washing with PBS, 100 μL of PLB lysate was added to the cell solution. The cell lysate was collected after gently shaking the culture plate for 15 min at room temperature. Then, 20 μL of the above solu-

tion was added, followed by 100 µL of LARII solution. The mixture was loaded into a luminescence detector to measure the initial luminescence (firefly luciferase activity). Subsequently, 100 µL of Stop&Glo detection solution was added to halt the first luminescence and initiate the second luminescence. Following mixing, the mixture was analyzed in the luminescence detector (GloMax 20/20, Promega) to determine the second luminescence value (Renilla luciferase activity).

Detection of IL-1β, IL-6, and TNF-α levels by enzyme-linked immunosorbent assay (ELISA)

Mouse hippocampal tissue was made into homogenate. The homogenate was performed at 5000 g for 5 min at 4°C. Then, the supernatant was taken for the experiment. Before detection, the protein concentration was measured through Bicinchoninic Acid (BCA) method (Kielkopf et al., 2020). Each well was added with 100 µL of standard or sample solution followed by incubation at 37°C for 2 h. Then, 100 µL of biotin-labeled antibody was added, incubating at 37°C for another 1 h. Next, 100 µL of horseradish peroxidase-labeled avidin (avidin-HRP) was added to incubate at 37°C for 40 min. The substrate solution started the catalytic reaction to develop color at 37°C for 20 min, and then the reaction was terminated with 100 µL of termination solution. The protein concentration was found on the standard curve according to the OD value. IL-1β (KE10003, Proteintech, USA), IL-6 (KE10007, Proteintech, USA), TNF-α (KE10002, Proteintech, USA) levels in the supernatant samples were detected by the ELISA reader (MB-530, HEALES, China).

Pathological changes of hippocampal neurons were observed by hematoxylin-eosin (HE) staining

The dissected hippocampus tissue underwent gradual dehydration in a 4% paraformaldehyde solution and was subsequently embedded in a paraffin block. The paraffin-embedded block was securely placed on a paraffin slicer, with the slicer's barrier adjusted to ensure a parallel section surface with a set slice thickness of 5 µm for continuous slicing. After cutting, the slices were gently transferred into water for unfolding, then separated with a slide and allowed to air dry naturally. The slices were dewaxed to water by gradient ethanol (95-100%) with 5 min in each stage. After being soaked with distilled water for 5 min, they were stained with hematoxylin for 5 min. Then, slices were returned to blue with PBS after washing with distilled water. Eosin staining for 3-5 min was followed by gradient ethanol (95-100%) dehydration for 5 min in each stage. Next, slides were placed in xylene for 10 min followed by sealing with neutral gum. Hippocampal neurons were observed with the microscope (BA210T, Motic, China). The 3 visual fields were randomly selected and photographed (magnification: 100× and 400×) in the hippocampal CA1 region. The rationale for selecting CA1 region was based on the studies on long-term enhancement of synaptic plasticity in the hippocampal CA1 region shown to be closely related to spatial learning and memory. The long-term potentiation (LTP) in the CA1 region is reduced in diseases characterized by cognitive impairments or accompanied by cognitive impairments (Xu et al., 2021).

Detection of apoptosis of hippocampal neurons by TUNEL

After the hippocampal tissue sections were dewaxed, dehydrated, and rinsed, they were performed according to the TUNEL Cell Apoptosis Detection Kit (40306ES50, Yeasen, China). Each slice was added with 100 µL of proteinase K solution, reacting at 37°C for 20 min followed by washing with PBS. The equilibrium solution and TDT enzyme buffer were prepared as the instructions described. Each slice was dripped with 100 µL of equilibrium solution and incubated for 10-30 min. After equilibration, the water was absorbed with absorbent paper and 50 µL of TDT enzyme buffer was added, incubating at 37°C for 1 h. After absorbing water with absorbent paper, DAPI working solution was added dropwise followed by incubation at 37°C for 10 min in the dark. Slices were blocked after washing with PBS. Three visual fields were randomly selected and photographed (magnification: 200×) by the fluorescence microscope (BA410T, Motic, China) in the hippocampal CA1 region. Green fluorescence was the positive signal while blue fluorescence was the nuclear staining signal. The apoptosis index = TUNEL positive cells/total number of cells.

Detection of miR-155-5p and BDNF mRNA expression levels by quantitative real-time polymerase chain reaction (RT-qPCR)

HT22 cells and hippocampal tissue were lysed to extract RNA by Trizol total RNA Extraction Kit (15596026, Thermo, USA). miR-155-5p and U6 (internal reference) were reverse transcribed using the miRNA Reverse Transcription Kit (CW2141, CWBIO, China), and BDNF and β-actin (internal reference) were reverse transcribed using the mRNA Reverse Transcription Kit

(CW2569, CWBIO, China) to obtain the corresponding cDNA for the amplification reaction.
miR-155-5p: FP: TTAATGCTAATTGTGATAGGGGT,
RP: GCTGTCAACGATACGCTACGTAA;
U6: FP: CTCGCTTCGGCAGCACA,
RP: AACGCTTCACGAATTTGCGT;
BDNF: FP: AGCAGAGTCCATTCAGCACC,
RP: TCTGACTCTCTCTCCAGCCC;
β-actin: FP: ACATCCGTAAAGACCTCTATGCC,
RP: TACTCCTGCTTGCTGATCCAC.
Primers were purchased from Beijing Qingke Biotechnology Co., Ltd. The ultra SYBR (CW2601, CWBIO) mixture was applied to execute in a qRT-PCR instrument (PIKOREAL96, Thermo, USA). The gene levels of miR-155-5p and BDNF were measured by the $2^{-\Delta\Delta Ct}$ approach.

Detection of BDNF, p65, p-p65 expression levels by Western blot

HT22 cells and hippocampal tissue were lysed to extract total proteins. The protein concentration was measured by the BCA method. The protein was boiled with the loading buffer (4:1, V) for subsequent experiments. Each well was loaded with 10 μL of the above mixture. After protein separation by electrophoresis, it was transferred to the membrane and blocked in 5% skimmed milk powder for 1.5 h. The percentage of the gel used for resolving BDNF, p-p65/p65 and β-actin proteins was 15%, 10%, and 10~12%, respectively. The monoclonal antibodies of BDNF (1 μg/mL, ab203573, abcam, USA), p-p65 (1:1000, ab76302, Abcam, UK), p65 (1:1000, 10745-1-AP, Proteintech, USA), and β-actin (internal reference, 1:5000, 66009-1-Ig, Proteintech, USA) were added respectively and incubated overnight at 4°C. After incubation, membranes were washed with PBST 3 times. HRP goat anti-mouse IgG (1:5000, SA00001-1, Proteintech, USA) and HRP goat anti-rabbit IgG (1:6000, SA00001-2, Proteintech, USA) were added and incubated for 1.5 h. After incubation, membranes were all washed with PBST 3 times. ECL reagent (ABW0005, Abiowell, China) was added. Photos were taken by the chemiluminescence imaging system (ChemiScope6100, Clinx, China). The gray values of the bands were obtained using Image J to visualize the expression levels of the target proteins.

Data analysis

GraphPad Prism 9 was used for the data analysis. All experimental data were presented as mean ± standard deviation (SD). Prior to statistical analysis, normality and homogeneity of variance were assessed using the Shapiro-Wilk test and F-test, respectively. The data demonstrated normal distribution and homogeneity of variance. Statistical comparisons between two groups were conducted using an unpaired Student's t-test. Differences among multiple groups were evaluated using one-way ANOVA, followed by Tukey's *post hoc* test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The effect of miR-155-5p on cognitive function in SD mice

The classic Morris water maze test was carried out to evaluate the effect of SD on the learning and memory ability of mice. The level of miR-155-5p related to sleep regulation was quantitatively detected by RT-qPCR. miR-155-5p was significantly up-regulated in the hippocampus of mice in the SD group compared to normal group (Fig. 1A). However, miR-155-5p level was significantly decreased under the condition of si-miR-155-5p injection in SD mice (Fig. 1A). Along with the extension of training time, the escape latency was gradually shortened, and the swimming velocity was gradually accelerated in four groups (Fig. 1B-D). The difference was statistically significant on the 5th day (Fig. 1B-D). In the SD group, the escape latency was significantly prolonged, the swimming velocity was slower, and the time spent in the target platform quadrant was shorter than that in normal group, indicating the successful construction of the SD model (Fig. 1B-D). However, SD mice after injection of si-miR-155-5p significantly shortened the escape latency, accelerated the swimming velocity, and significantly prolonged the time spent in the target platform quadrant (Fig. 1B-D). The results above revealed that SD significantly impacted the cognitive function of mice, likely through the modulation of miR-155-5p expression levels. Furthermore, the detrimental effects of SD on the mice's learning and memory abilities were mitigated by downregulating miR-155-5p expression levels.

The effect of miR-155-5p on hippocampal tissue damage and inflammation in SD mice

To investigate the mechanism by which miR-155-5p impacts the cognitive function of SD mice, we conducted HE staining to observe pathological changes in the hippocampus CA1 region, as depicted in Fig. 2A. Normal group neuronal cells have regular morphology, orderly arrangement, and intact structure. In the SD

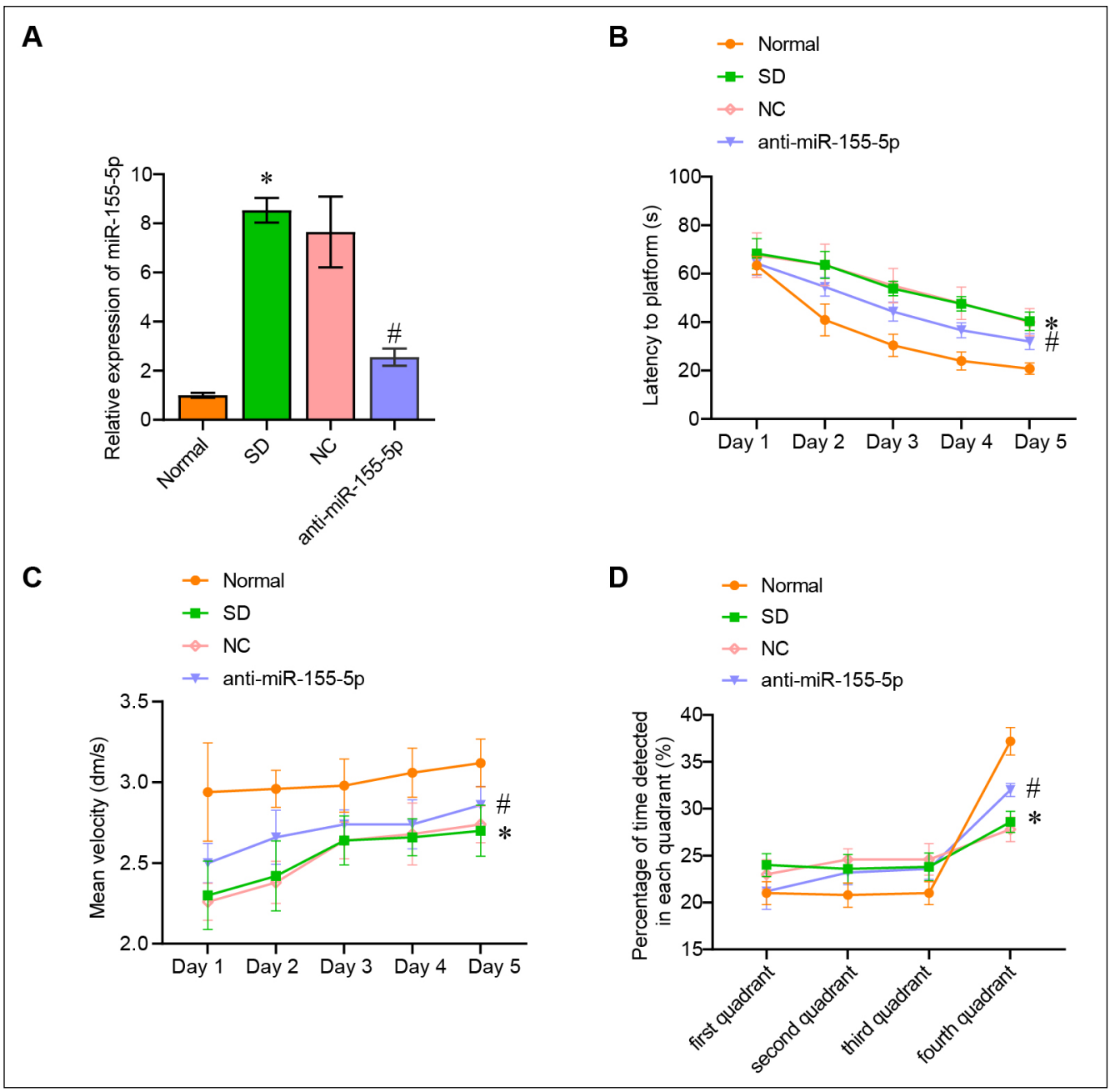

Fig. 1. The effect of miR-155-5p on cognitive function of SD mice. (A) RT-qPCR detection of miR-155-5p expression level in the hippocampus; (B) Escape latency (s); (C) Mean swimming velocity (dm/s); (D) Time spent in each platform quadrant (%). * P<0.05 *vs.* Normal; # P<0.05 *vs.* NC.

or NC group, neuronal cells show obvious shrinkage, irregular morphology in some neurons, reduced and deformed cell bodies, disappearance of Nissl bodies in the cytoplasm, appearing dark red. In addition, the cytoplasmic vacuolization of hippocampal tissue cells in the SD group mice indicates excessive brain tissue damage. Vacuolization damage also appeared in the NC group mice, but the extent of damage varied, possibly due to some differences in the surgical modeling process. After injecting si-miR-155-5p, the structural damage of neuronal cells in SD mice was improved with relatively intact cell structure and orderly arrangement compared to the SD mice. The dispersion of the upper pyramidal neurons in the CA1 region suggests that anti-miR-155-5p has played a certain role in improving hippocampal tissue damage, but it has not been completely improved. Furthermore, downstream inflammation markers were assessed by ELISA, and their levels were shown in Fig. 2B. The levels of IL-1β, IL-6, and TNF-α were increased in the SD mice. In addition, the

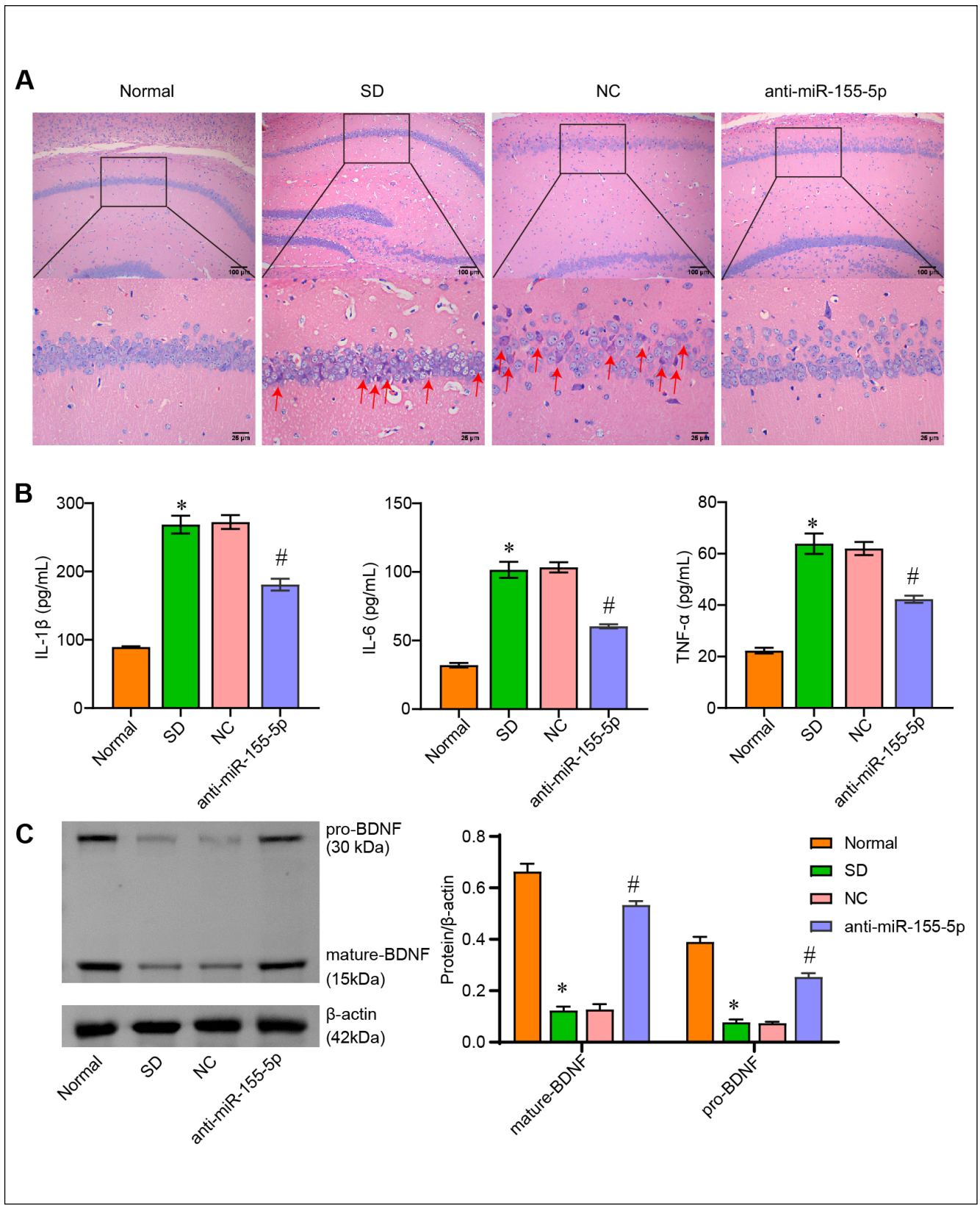

Fig. 2. The effect of miR-155-5p inhibition on hippocampal tissue damage and inflammation in SD mice. (A) The pathological changes of hippocampal neurons were detected by HE staining (upper figures: 100×, lower figures: 400×). Damaged neuronal cells were marked with red arrows; (B) Detection of IL-1β, IL-6, and TNF-α levels by ELISA; (C) Western blot results of mature and pro-BDNF expression level. * P<0.05 *vs.* Normal; # P<0.05 *vs.* NC.

inflammation in the hippocampus was alleviated after si-miR-155-5p injection (Fig. 2B). Western blot was applied to evaluate the effect of miR-155-5p on BDNF expression levels in the hippocampus of SD mice (Fig. 2C). SD significantly decreased the mature and pro-BDNF expression levels in the hippocampus compared with the Normal group (Fig. 2C). While, the expression of the mature and pro-BDNF protein was significantly increased after the injection of si-miR-155-5p (Fig. 2C). The above results indicated that SD led to hippocampal tissue damage and inflammation in mice, both of which were suppressed by the silencing of miR-155-5p.

The targeted inhibition of miR-155-5p on BDNF

The Starbase website was employed to predict the binding site between miR-155-5p and BDNF. miR-155-5p regulated BDNF by binding the 3'UTR of BDNF to degrade it or hinder translation (Fig. 3A). The dual-luciferase reporter assay was applied to further verify the binding of miR-155-5p to BDNF. The results are shown in Fig. 3B. In the wild-type group, the luciferase activity ratio was decreased after the insertion of miR-155-5p sequence, so miR-155-5p had a targeted regulatory effect on BDNF expression level. RT-qPCR was employed

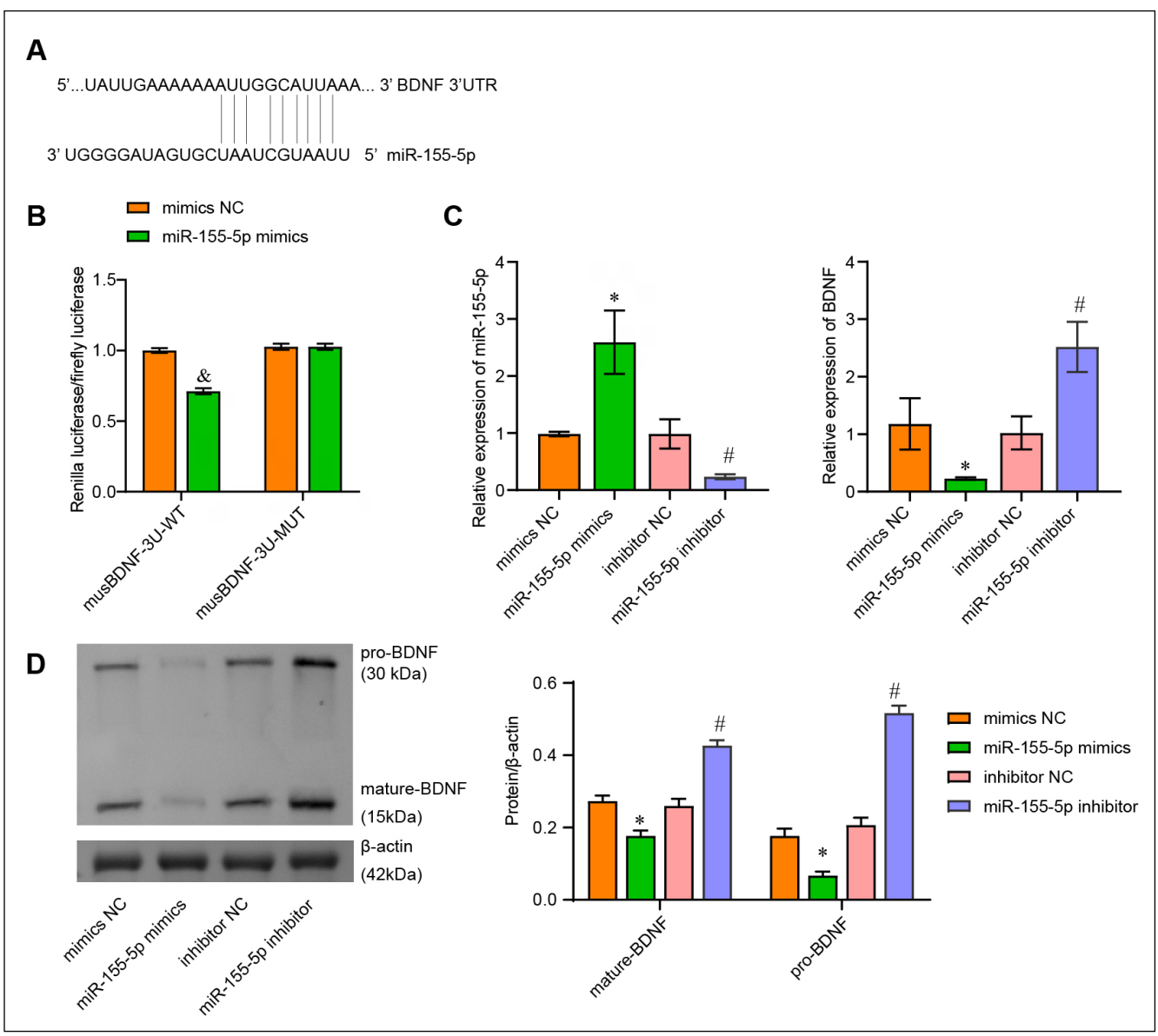

Fig. 3. The targeted inhibition of miR-155-5p on BDNF. (A) Starbase website (https://starbase.sysu.edu.cn) analysis of the binding sites between miR-155-5p and BDNF; (B) Dual-luciferase reporter assay; (C) RT-qPCR detection of miR-155-5p and BDNF mRNA expression levels; (D) Western blot analysis of mature and pro-BDNF expression level. & P<0.05 *vs.* the musBDNF-3U-WT+mimics NC group; * P<0.05 *vs.* mimics NC; # P<0.05 *vs.* inhibitor NC.

to quantify the miR-155-5p and BDNF mRNA expression levels. The results were shown in Fig. 3C. In the miR-155-5p mimics group, miR-155-5p was significantly up-regulated, while BDNF mRNA expression was significantly lower than that in mimics NC group. After silencing of miR-155-5p, the BDNF mRNA expression level in hippocampal neurons significantly increased, while that of miR-155-5p significantly decreased compared to inhibitor NC group (Fig. 3C). Next, the BDNF expression level in hippocampal neuronal cells was analyzed by western blot. The results were shown in Fig. 3D. The mature and pro-BDNF protein expression levels in hippocampal neurons with miR-155-5p mimics transfection was significantly lower, while that in hippocampal neurons silenced with miR-155-5p was significantly higher. These findings provided evidence that miR-155-5p bound to BDNF, leading to the inhibition of BDNF mRNA and protein expression levels in SD mice.

The effect of BDNF on hippocampal tissue damage repair in SD mice

To assess the reparative effect of BDNF on hippocampal tissue damage induced by SD, we utilized western blotting to evaluate the expression level of BDNF in mice from each experimental group. Compared with the oe-NC group, the mature and pro-BDNF protein expression levels in the hippocampus of SD mice was increased significantly after injection of oe-BDNF (Fig. 4A). The corresponding HE staining results were shown in Fig. 4B. After injecting oe-NC, the CA1 neurons in the hippocampus of SD mice showed severe damage, with a small number, irregular arrangement, and uneven morphology. The cell nuclei were severely condensed and the cell bodies shrunk and deformed. However, after the oe-BDNF treatment, the CA1 neurons in the hippocampus of SD mice were repaired, with an increased number, orderly arrangement, and uniform morphology. The results of apoptosis was detected by TUNEL were shown in Fig. 4C. The TUNEL positive rate of hippocampal CA1 neurons was notably high in the oe-NC group, suggesting a substantial presence of apoptotic cells. Following oe-BDNF treatment, the TUNEL positive rate of hippocampal CA1 neurons in the SD mice decreased significantly, indicating a marked alleviation in the severity of apoptosis. As shown in Fig. 4D, compared with the oe-NC group, the p65 phosphorylation level was significantly decreased in the hippocampus of SD mice after oe-BDNF treatment. To identify the effect of BDNF on the release of downstream inflammatory factors caused by SD, ELISA was carried out to detect IL-1β, IL-6, and TNF-α levels in the hippocampus of mice in each group. The results were shown in Fig. 4E. IL-1β, IL-6, and TNF-α levels in the SD mice after oe-BDNF treatment were significantly decreased. These results indicated that oe-BDNF inhibited neuronal apoptosis, reduced p65 phosphorylation level and related inflammatory factors level, which promoted the repair of hippocampal tissue damage caused by SD.

The effect of silencing miR-155-5p on the activation of the BDNF/NF-κB pathway in SD mice

The impact of miR-155-5p on the BDNF/NF-κB signaling pathway was investigated through the silencing of miR-155-5p and BDNF. Expression levels of genes and proteins associated with the pathway were assessed. Compared with the mice injected with si-NC, the BDNF mRNA expression level in the mice injected with anti-miR-155-5p was higher. However, it was decreased in the hippocampus of mice injected with anti-miR-155-5p and si-BDNF at the same time (Fig. 5A). Western blot results were shown in Fig. 5B-C. Compared with the mice injected with si-NC, the mature and pro-BDNF protein expression levels in mice injected with anti-miR-155-5p was significantly increased, but the p65 phosphorylation level was significantly decreased. The mature and pro-BDNF protein expression levels in the hippocampus of mice injected with anti-miR-155-5p and si-BDNF was significantly decreased and the p65 phosphorylation level was significantly increased. The level of inflammatory factors IL-1β, IL-6, and TNF-α in the hippocampus of mice in each group was detected by ELISA. The results were shown in Fig. 5D. IL-1β, IL-6, and TNF-α levels in the hippocampus of mice injected with anti-miR-155-5p were all decreased compared with mice injected with si-NC. The IL-1β, IL-6, and TNF-α levels in mice injected with anti-miR-155-5p and si-BDNF simultaneously were increased compared to the anti-miR-155-5p group. The above results indicated that silencing miR-155-5p inhibited the NF-κB pathway through BDNF to improve hippocampal inflammation and neuronal damage in SD mice.

DISCUSSION

Adequate sleep not only promotes brain development but also enhances body immunity. However, SD has significant detrimental effects on cognitive function, impacting memory, learning, and emotional well-being (Abel et al., 2013). Previous studies have revealed the association of several miRNAs with sleep regulation (Goodwin et al., 2018), including miR-155-5p, which has been linked to

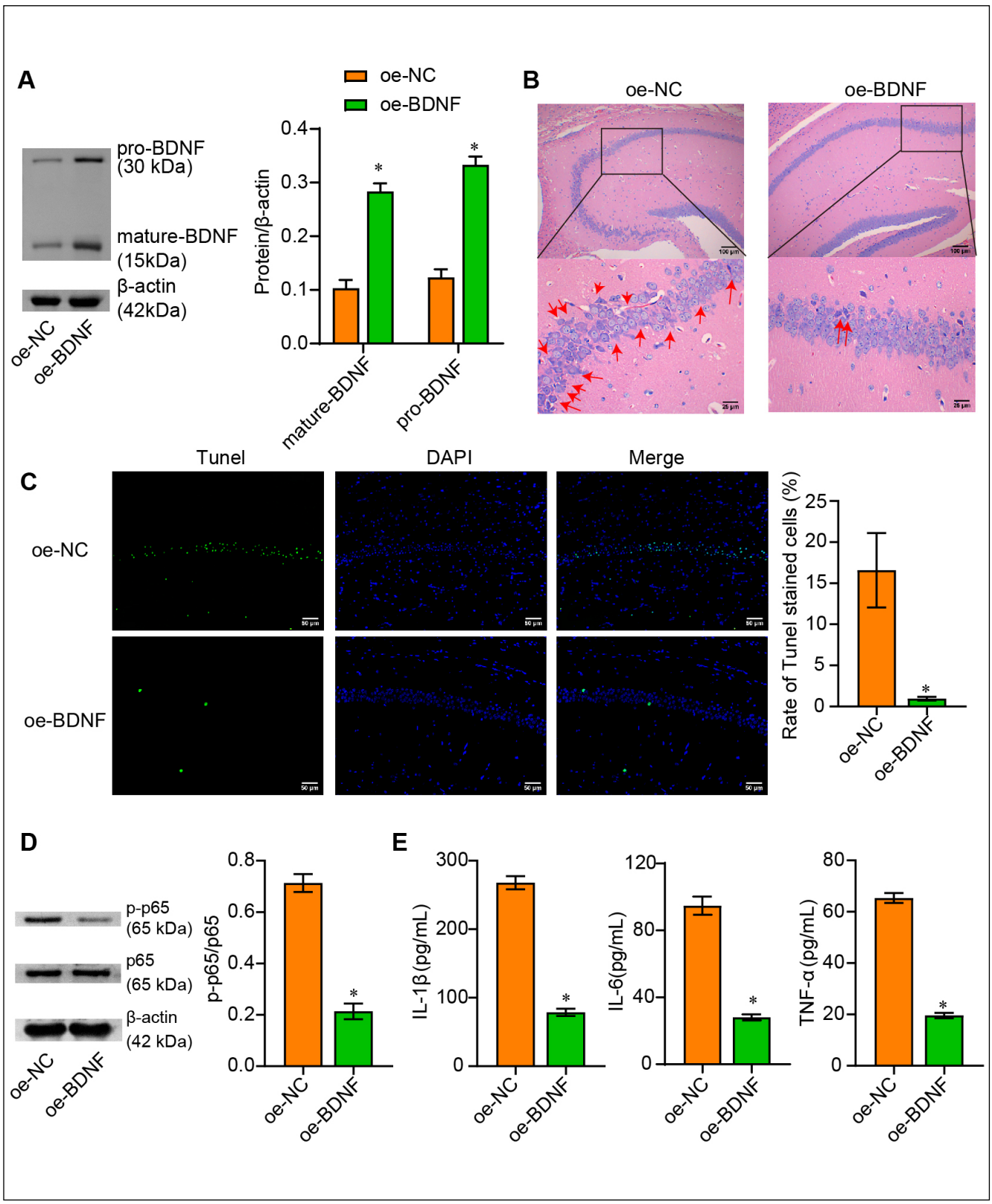

Fig. 4. The effect of BDNF on hippocampal tissue damage repair in SD mice. (A) Western blot analysis of mature and pro-BDNF expression level; (B) The pathological changes of hippocampal neurons were detected by HE staining (upper figures: 100×, lower figures: 400×). Damaged neuronal cells were marked with red arrows; (C) The apoptosis rate in the hippocampus was detected by TUNEL (magnification: 200×); (D) Western blot analysis of p65, p-p65 expression levels; (E) Detection of IL-1β, IL-6, and TNF-α levels by ELISA. * P<0.05 *vs.* oe-NC.

sleep disorders. The BDNF plays a crucial role in memory and sleep regulation and was found to be down-regulated in the hippocampus of SD rats (Looti Bashiyan et al., 2021). However, the specific mechanism through which miR-155-5p affects cognitive function in SD mice *via* BDNF remains unclear. SD often triggers an inflammatory response and activates the NF-κB inflammatory pathway (Irwin et al., 2008). The morris water maze test demonstrated the impaired learning and memory abilities in mice subjected to SD, with the mechanism linked to increased expression of miR-155-5p in hippocampal tissues. In relevant studies, elevated levels of miR-155-5p have been observed in the hippocampus of rats with Alzheimer's disease (Liu et al., 2019). Nevertheless, treat-

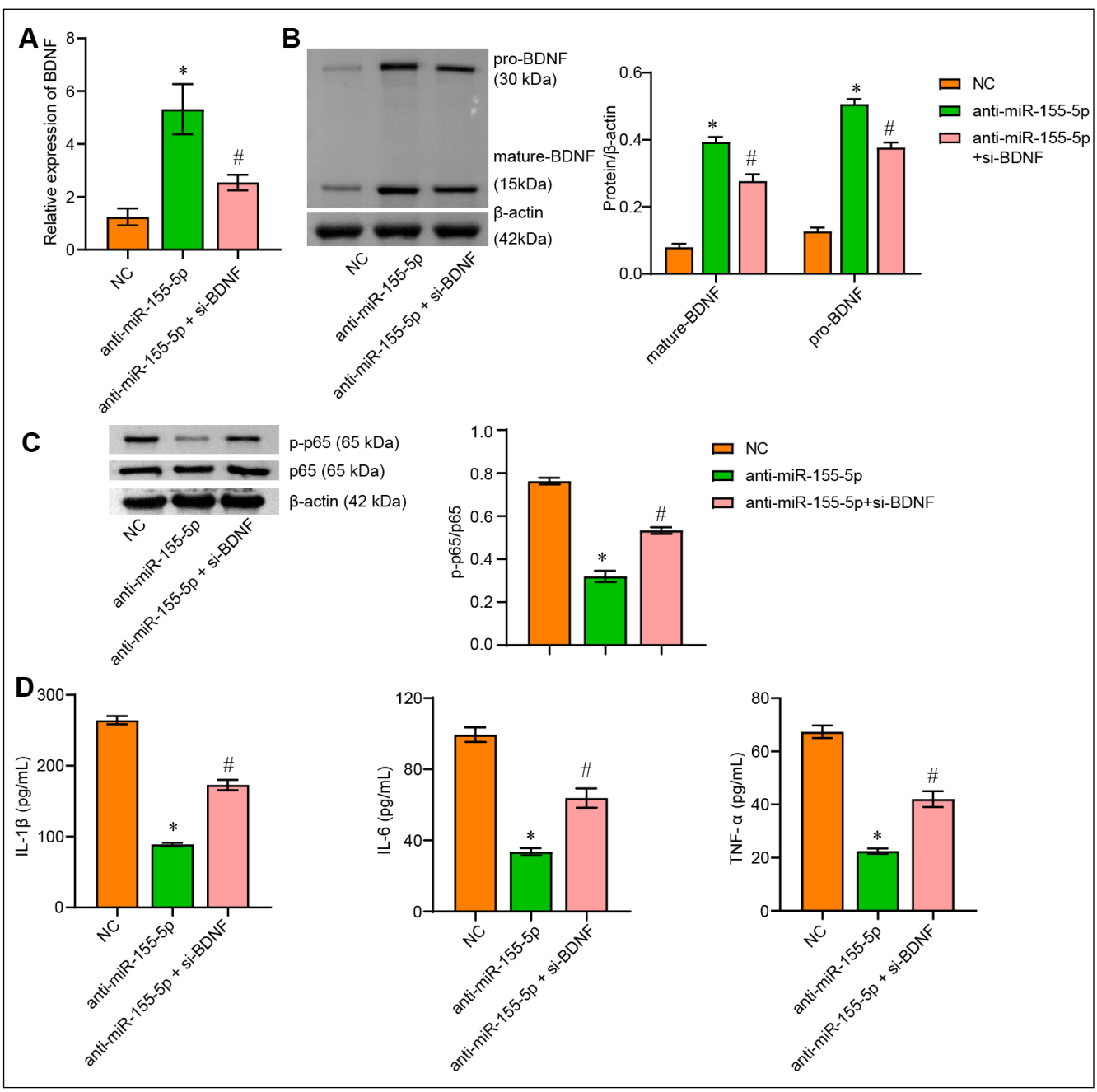

Fig. 5. The effect of silencing miR-155-5p on the activation of the BDNF/NF-κB pathway in SD mice. (A) RT-qPCR detection of BDNF mRNA expression level; (B) Western blot analysis of mature and pro-BDNF expression level; (C) Western blot analysis of p65, p-p65 expression levels; (D) Detection of IL-1β, IL-6, and TNF-α levels by ELISA. * P<0.05 *vs.* NC. # P<0.05 *vs.* anti-miR-155-5p.

ment with anti-miR-155-5p significantly restored the learning ability of SD mice, suggesting an effective regulation of memory impairment by miR-155-5p.

The HE staining results revealed a reduction in the number of neurons after SD, characterized by prominent pyknosis, irregular morphology, and tangled shape. This pathological observation suggests that SD has negative effects on nervous system development and neuronal differentiation. SD induced an inflammatory response and the release of inflammatory factors during oxidative stress (Everson et al., 2005). The ELISA results of this study showed that the IL-1β, IL-6, and TNF-α levels were significantly increased in SD mice. It has been reported that a high level of IL-1β inhibited neuronal excitability and synaptic function in the hippocampus tissue, thus leading to cognitive dysfunction (Samatra et al., 2018). The elevated level of IL-6 was closely associated with cognitive dysfunction and caused tissue damage in brain (Kim et al., 2017). TNF-α played a key role in sleep/wake regulation, which also increased after SD or sleep fragmentation (Kaushal et al., 2012). Studies have shown that the BDNF, as a neurotrophic factor, has an important protective effect on the hippocampus (Rahmani et al., 2020). This work showed that SD reduced the mature and pro-BDNF protein expression levels in the hippocampus tissue, which was consistent with the literature (Alzoubi et al., 2013). Previous studies also have illustrated that miR-155-5p could directly regulate the BDNF expression (Varendi et al., 2014). The results of cell experiments in this work showed that miR-155-5p could bind to the 3'-UTR of BDNF to inhibit its mRNA and protein expression.

BDNF participated in the process of learning and memory, acting as a central regulator of hippocampal neuronal plasticity (von Bohlen & Halbach, 2018). The results of this study determinted that overexpression of BDNF repaired the damage of hippocampal neuronal cells with the increase of cell number and the decrease of cell apoptosis. Previous studies have found that long-term SD could trigger autophagy and apoptosis of hippocampal neurons, thereby reducing learning and memory abilities (Cao et al., 2019). In contrast, activation of the BDNF/TrkB signaling pathway can reduce apoptosis of hippocampal neurons (Ding et al., 2021). The results of this work showed that oe-BDNF reduced p-p65/p65 protein level and inhibited the release of IL-1β, IL-6, and TNF-α to alleviate the damage and inflammation of hippocampal tissue in SD mice. The research on the BDNF/NF-κB signaling pathway primarily centers on the neurobiology of depression and anxiety (Caviedes et al., 2017; Olugbemide et al., 2021). This study explored the impact of silencing miR-155-5p on the activation of the BDNF/NF-κB pathway in SD mice. The results proved that silencing miR-155-5p promoted the high expression of BDNF mRNA and protein, decreased the p-p65/p65 protein level and the IL-1β, IL-6, and TNF-α levels in SD mice. Relevant studies also have shown that the knockdown of miR-155 can reduce neuroinflammation and is conducive to the improvement and repair of nervous system-related diseases (Gaudet et al., 2016). All these proved that silencing of miR-155-5p could improve the learning and memory ability in SD mice through BDNF.

miR-155-5p has been found to inhibit cognitive impairment in APP/PS1 mice, promote neuronal regeneration, and reduce Aβ deposition (Wang et al., 2022). It has also been identified as a key regulatory factor in autism (Rastegari et al., 2023). Furthermore, miR-155-5p may serve as a potential target for treating cognitive impairment induced by 1,2-diethylbenzene during risperidone therapy (Nguyen et al., 2023). These findings indicate that miR-155-5p could be a promising target for managing mental disorders. Moreover, recent studies have shown that exosomes carrying either overexpressed or silenced miR-155 can modulate melanoma angiogenesis (Zhou et al., 2018). Delivery of exosomal-miR-155-5p in immunocompetent mice has been demonstrated to inhibit ovarian cancer progression, reduce macrophage infiltration, and enhance the function of CD8+ T cells (Li et al., 2022). Additionally, miR-155-5p coated with positively charged polyethyleneimine-polysaccharide nanoparticles can effectively enter endothelial cells and sustain the release of miR-155-5p for exerting biological functions (Antunes et al., 2019). These investigations highlight the potential of exosome or nanoparticle delivery of silenced miR-155-5p in improving inflammation and memory impairment in SD mice. This opens up new possibilities for clinical applications and advancements in gene therapy for SD-related diseases, warranting further exploration.

CONCLUSION

In the current study, the silence of miR-155-5p led to the inhibition of the NF-κB pathway activation *via* BDNF, thus ameliorating cognitive impairment in SD mice. These findings offer novel insights into the mechanistic understanding of SD and may pave the way for potential clinical interventions.

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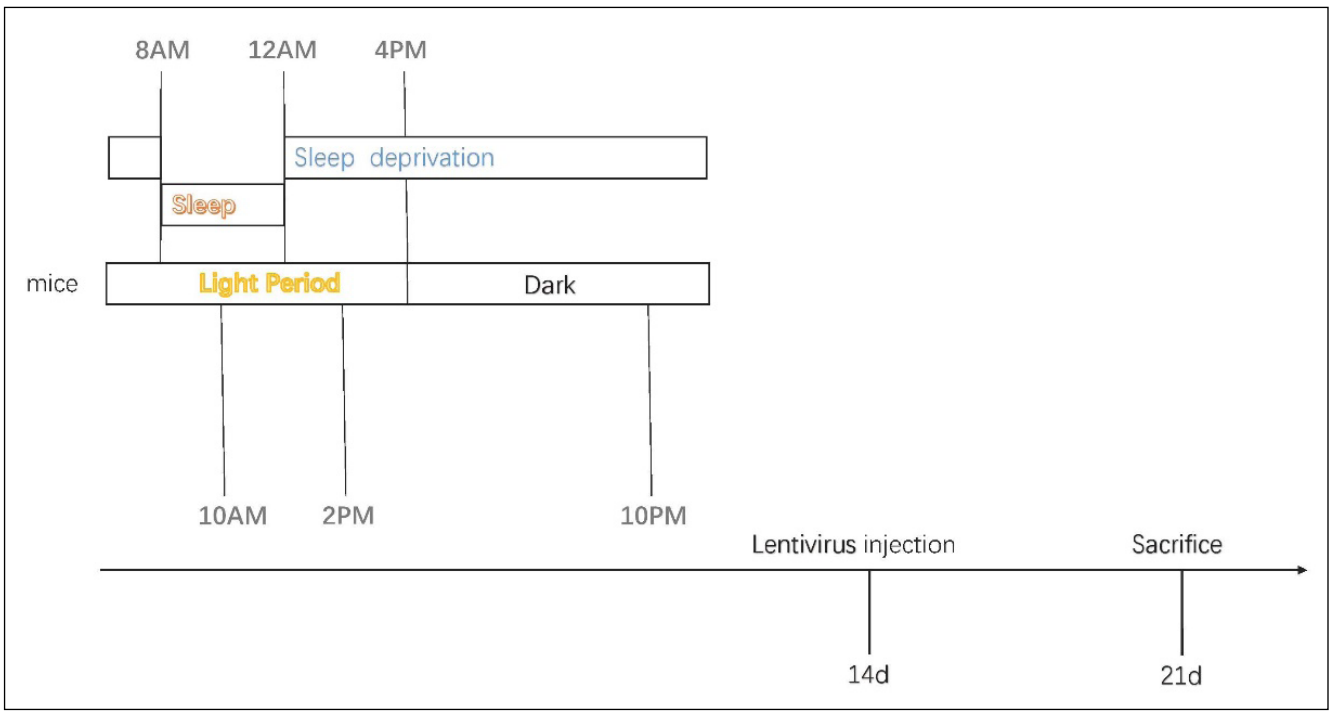
SUPPLEMENTARY MATERIALS



Supplementary Fig. 1. The experimental design for SD modelling.