

Original Article

miR-132 targets PTEN to regulate cognitive impairment and neural plasticity in mice with depression through PI3K/AKT signaling pathway

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Abstract: Objective: To investigate the mechanism of miR-132 targeting on PTEN and acting on cognitive impairment and neuroplasticity in mice with depression through PI3K/AKT signaling pathway. Methods: The targeting relationship between miR-132 and PTEN was validated by dual-luciferase reporter assay. Ninety mice were included, among which 15 were in a Normal group, and the remaining ones were constructed as depression models and divided into 5 groups: Model group, NC group, miR-132 mimics group, si-PTEN group, and miR-132 mimics + si-PTEN group. The mRNA and protein levels of PTEN, Bcl-2, Bax and Caspase-3 etc. were measured. Results: Dual-luciferase reporter assay demonstrated that miR-132 targeted negative regulation of PTEN. Compared with the Normal group, the Model group showed reduced expression of miR-132 and Bcl-2 mRNA, reduced phosphorylation of PI3K and AKT protein, reduced level of GAP-43, but elevated mRNA and protein expressions of PTEN, Bax and Caspase-3; while the miR-132 mimics and si-PTEN groups showed the opposite results when comparing with the Model group. Compared with the Normal group, the other groups showed significantly longer escape latency of the hidden platform, fewer times of crossing the platform within 2 min, shorter time of tail suspension, and higher neuronal apoptosis rate (all $P < 0.05$), while the miR-132 mimics and si-PTEN groups showed the opposite results when comparing with the Model group. Additionally, the increase and decrease were more significant in miR-132 mimics + si-PTEN group. Conclusion: The cognitive impairment and neuroplasticity in mice with depression can be improved by miR-132 inhibiting PTEN through PI3K/AKT signaling pathway.

Keywords: miR-132, PTEN, depression mice, PI3K/AKT signaling pathway, cognitive impairment, neuroplasticity

Introduction

Depression is a common mood disorder featured by high prevalence, high mortality, long duration, and easy recurrence [1]. The specific etiology and pathogenesis of depression are still not clear yet, but may be related to neuronal apoptosis, inflammatory reaction, and alteration of intestinal flora [2]. In recent years, it has been found that changes in hippocampal neuroplasticity are closely associated with the pathogenesis of depression [3]. The hippocampus is a brain structure contributing to learning and memory. Patients with depression develop pathological changes such as a decrease in the size of hippocampus or in the number of neurons [4, 5]. Cognitive dysfunction as one of the core symptoms of depression, is characterized

by decreased ability to think and concentrate, as well as difficulty in making decisions [6].

MicroRNAs play an important role in the development, differentiation, proliferation and apoptosis of neurons, the synaptic plasticity, the ability of cognition, learning and memory, as well as the occurrence of depression [7]. The miRNAs are a class of endogenous non-coding RNAs of about 20-25 nucleotides in size that bind to target mRNAs, and regulate gene expression [8]. The miR-132 is a highly conserved miRNA located on chromosome 17p13.3, mainly in hippocampus, and it is transcribed at the gene gap of human chromosome 17 via transcription factor cAMP-binding protein [9]. The miRNA-132 plays an important role in nervous system diseases and participates in

neuronal differentiation and apoptosis [10]. Latest research has found that miR-132 has a targeted relationship with the phosphatase and tensin homolog deleted on the chromosome 10 (PTEN) gene [11].

PTEN, as the first-found tumor suppressor gene with phosphatase activity, is located on chromosome 10q23, about 218 kb in length, consists of 9 exons and 8 introns, and encodes protein of 403 amino acids [12]. PTEN gene may be associated with depression and depressive symptoms [13]. The PI3K/AKT signaling pathway is one of the most important signaling pathways *in vivo*. Antidepressants are closely related to PI3K/AKT signaling, which can promote the treatment efficacy of antidepressants [14, 15]. That is, both PTEN and PI3K/AKT signaling pathway are closely related to depression.

Therefore, we explored the role of miR-132 targeting PTEN gene via PI3K/AKT signaling pathway in depression mice, and its effect on cognitive impairment and neuroplasticity, so as to provide a scientific basis for new drug target in depression treatment.

Materials and methods

Dual-luciferase reporter assay

The target gene analysis of miR-132 was performed using a biological prediction site, and the dual-luciferase reporter assay was used to verify whether PTEN is a direct target gene of miR-132. The 3'UTR segment of the PTEN gene was cloned and amplified, and the PCR product was cloned into the multiple cloning site downstream of the luciferase reporter gene using pmiRGLO (e1330, Promega, USA), named Wt-PTEN. Then, the specific binding site of miR-132 and PTEN was predicted by bioinformatics website for mutagenesis and construction of Mut-PTEN vector, which was then cloned to pmiRGLO (e2241, Promega, USA) as controls. The miR-132 mimic and the miR-132 mimic NC were co-transfected into 293T cells with luciferase reporter vector, respectively, and the luciferase activity in each group was detected.

Animals

Ninety male C57BL/6J mice, weighing 18-22 g, were purchased from the Experimental Animal Center of Guangzhou University of Chinese

Medicine. All mice were fed normal diet and housed under specific pathogen-free conditions. All experimental procedures were in accordance with the International Laboratory Animal Ethics and complied with national regulations. The experiment protocol was approved by the Ethics Committee of The Third Affiliated Hospital of Guangzhou Medical University.

Establishment of mouse model of depression

After a 3-day acclimation period, 75 mice were housed separately and exposed to an environment of unpredictable chronic mild stress. The stressors applied within the 5-week period included 24-hour food deprivation, 24-hour water shortage, 24-hour ramped cage (45°), 2-hour shaking, 5-minute cold swimming (4°C), 2-hour body restraint, 24-hour wet bedding, and night lighting. The stressors above were performed 1-2 times a day in a random order for 5 consecutive weeks. After successful modeling, the depression mice were randomly divided into 5 groups: Model group (depression mice), NC group (depression mice transfected with miR-132 mimics NC sequence), miR-132 mimics group (depression mice transfected with miR-132 mimics sequence), si-PTEN group (depression mice transfected with si-PTEN plasmid), and miR-132 mimics + si-PTEN group (depression mice transfected with the miR-132 mimics + si-PTEN plasmid), with 15 mice in each group. Lentiviral vector containing miR-132 mimics, si-PTEN or negative control plasmid (Shanghai Jima, China) was injected into the tail vein (the amount of virus injected was 2×10^7 IU) once every 2 days, continuously for 5 times. Three weeks after successful modeling, all mice were euthanized (by cervical dislocation after intraperitoneal injection of 30 mg/kg of 0.3% pentobarbital sodium) following behavioral tests to collect the hippocampal tissue samples. Five samples in each group were randomly selected for TUNEL experiment, and the rest were used for qRT-PCR and Western blot.

Morris water maze

Three weeks after successful modeling, the water maze test was performed on 10 random mice in each group using the SLY-WMS Morris Water Maze Analysis System (Shuolinyuan Technology, China). (1) Place navigation: The mice were put into the pool from 4 different directions with their head towards the pool wall, and the time required for a mouse to reach the

hidden platform was escape latency (EL), which reflected the learning ability of mice. If the mice failed to locate the platform within 60 s, they would be guided to the platform and allowed to stay on it for 15 seconds (EL recorded as 60 s). The mean EL values of 4 times was recorded as its final score for that day. The test was carried out from day 1 to day 4. (2) A probe trial was performed with the absence of a platform. The mouse was put into the pool with their head towards the pool wall, and the number of times that the mouse crossed the location of the platform within 2min was recorded.

Tail suspension test (TST)

Depressed mice are often in a state of immobility. In this study, TST was used to evaluate the level of depression and the motor ability of each group after the intervention of miR-132 and PTEN. TST was first performed after the successful modeling and again 3 weeks after. The 10 mice in each group subjected to the Morris water maze test were then subjected to tail suspension test. The mouse was made to grasp at a horizontal glass rod using its front legs and was hanging 45 cm above the desk-top. The time (sec) from the mouse was lifted to it fell down was recorded.

Inclined plane test

Due to the state of immobility in mice with depression, this study used inclined plane test to evaluate the level of depression and the motor ability of each group after the intervention of miR-132 and PTEN. The inclined plane test was also carried out 3 weeks after successful modeling. Ten mice in each group subjected to TST were then subjected to an inclined plane test. The head of mouse was placed upside down on a 45° inclined plane, and the time (sec) a mouse needed to turn its head upward at an angle of over 135° was recorded.

TUNEL assay for detecting apoptosis

After the behavioral experiments, 5 mice were randomly selected from each group, and the left hippocampal CA1 tissue was harvested and prepared as paraffin sections in thickness of 5 μm. The procedures were according to the instructions of TUNEL Apoptosis Detection Kit (YT137, Beijing Biolab, China). First, paraffin sections were dewaxed with xylene, hydrated in

gradient alcohol, washed with distilled water, immersed in 3% H₂O₂ for 10 min, washed again 3 times with distilled water, 3 min for each time, and digested with proteinase K (20 pg/mL) for 7 min. Second, each sample was washed 3 times with TBS, 1 min for each time, added with 20 μL of buffer, placed in a wet box at 37°C for 2 h, and washed again 3 times with TBS, 3 min for each time. Third, 50 μL of TBS diluted streptavidin-biotin complex was added in the sample area, and treated for 1 h at 37°C, and then the samples were washed 4 times with TBS, 5 min for each time. Fourth, the sections were stained with dimethylaniline for 10 min, and washed with distilled water for 5 min. Fifth, the samples were stained with hematoxylin for 1 min, and then rinsed with TBST and distilled water. Last, the sections were sealed with a neutral gum. Thereafter, 5 slices were selected in each group, and 5 random fields of each slice were selected for statistics (×400). All images were analyzed with the use of Image-pro+6.0 software. Apoptosis rate = number of apoptotic cells/total number of cells × 100%.

qRT-PCR

After the behavioral experiments, the left hippocampus tissues of 5 mice in each group were harvested to prepare a homogenate. Total RNA was extracted from the homogenate of cerebral cortex using Trizol (10296028, Thermo Fisher, USA), which was also used to determine the purity and concentration of the extracted RNA. Then, cDNA was synthesized using an RT-reverse transcription kit (11939823001, Merck, USA). The 10 μL qRT-PCR system included: 0.5 μL of forward primer, 0.5 μL of reverse primer, 1 μL of cDNA template (Shanghai Renjie, China), 3 μL of ddH₂O (Shanghai Fortuneibo-tech, China), and 5 μL of (2×) SYBR® Premix Ex Taq™ II (Dalian TaKaRa, China). The reaction procedures were conducted under the following conditions: pre-denaturation at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s and 60°C for 30 s, and another 72°C for 5 min. U6 gene was the internal reference of miR-132. GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) was internal reference of Bcl2, Bax and Caspase3. The primers are shown in **Table 1**, and were synthesized by Sangon Biotech, China. The expression of the product was calculated as follows: $\Delta\Delta Ct = \Delta Ct_{\text{remaining group}} - \Delta Ct_{\text{control group}}$, $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{GAPDH}}$. Ct refers to the amplification cycles when the

Table 1. Primer sequence

Name	Sequence
miR-132	F: 5'-TGCGGCTGGTGAGTCCG-3' R: 5'-GCGGAAGGCAAGGAGG-3'
PTEN	F: 5'-TCGATCACACAGCACTGACG-3' R: 5'-GAGGCAGTTCCGTATGGAGG-3'
GAP-43	F: 5'-CGAAGATGCACAGTTCGGAA-3' R: 5'-CACCTCCTCTCAATCTCTCC-3'
caspase-3	F: 5'-TGAGCTGATAGAGTAGCGCG-3' R: 5'-TCCATCCAGCATGCAATCGC-3'
Bcl-2	F: 5'-TATAAGCTGTGCGAGAGGGG-3' R: 5'-TGACGCTCTCCACACACATG-3'
Bax	F: 5'-TGCCAGCAAAGTGGTCTCA-3' R: 5'-GCACTCCCGCCACAAAGATG-3'
U6	F: 5'-CGCTTCTGCCACACAGG-3' R: 5'-ACACTCGAGATTACGTG-3'
GAPDH	F: 5'-AGGTCGGGTGTGAACGGATTTG-3' R: 5'-GGGGTCTGTGATGGCAACA-3'

Note: GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

fluorescence intensity reaches the threshold after qPCR.

Western blot (WB)

After the behavioral experiments, the left hippocampus tissues of 5 mice in each group were harvested to prepare homogenate, which was lysed on ice and extracted for total protein. The samples were centrifuged at 13,000 rpm for 2 min, then the supernatant was taken and detected for protein concentration with the use of BCA kit (P0012S, Shanghai Beyotime, China). After that, 10 µg of supernatant was mixed with 4 µL of 5 × SDS loading buffer and then bathed in boil water for 10 min. The loading quantity of sample was 2 µL. The samples were transferred to a PVDF membrane after SDS-PAGE gel electrophoresis, then blocked with 5% skim milk at room temperature for 2 h and incubated with primary antibodies at room temperature for 2 h. Primary antibodies were rabbit polyclonal antibodies against PTEN (ab31392, 1/1,000, Abcam, USA), PI3K (ab86714, 1:10,000, Abcam, USA), p-PI3K (p110 delta/p85 alpha, ab125633 1:10,000, Abcam, USA), AKT (ab8805, 1:10,000, Abcam, USA), p-AKT (phospho T308, ab38449, 1:10,000, Abcam, USA), Bax (ab32503, 1:1,000, Abcam, USA), Bcl2 (ab32124, 1:1,000, Abcam, UK), Cleaved-Caspase-3 (ab2302, 1/1,000, Abcam, USA), GAP-43 (ab232772, 1/1,000, Abcam, USA),

and GAPDH (ab181602, 1/10,000, Abcam, USA). The samples were then washed 3 times with TBST, 5 min for each time, incubated with secondary antibody rabbit anti-goat IgG (ab6721, 1/2,000, Abcam, USA) for 2 h at room temperature, and washed again 3 times with TBST, 5 min for each time. Thereafter, the PVDF membrane was placed in a gel imager (Bio-Rad, USA) with developer spotted, and photographed with the use of image analysis system (The ChemiDoc MP, BIO-RAD, USA). Then, the gray value of protein bands was analyzed with the use of the Quantity One software (BIO-RAD, USA). The relative content of phosphorylated protein = phosphorylated protein/total protein, and the internal reference of the rest proteins was GAPDH. The relative protein content = gray value of protein/gray value of internal reference.

Statistical analysis

SPSS 21.0 (IBM SPSS Statistics, Chicago, IL, USA) was used for statistical analysis. The measurement data were expressed as mean ± sd and compared with the use of one-way ANOVA and the Bonferroni post-hoc test. The difference was statistically significant at P<0.05.

Results

Different expressions of miR-132, PTEN and PI3K/AKT signaling pathway between normal and depression mice

After successful modeling, the mRNA expressions of miR-132 and PTEN were detected by qRT-PCR, and the protein expressions of PTEN, as well as PI3K, p-PI3K, AKT and p-AKT in PI3K/AKT signaling pathway were detected by WB (**Figure 1**). Compared with the Normal group, the Model group had significantly reduced miR-132, elevated mRNA and protein levels of PTEN, and reduced phosphorylation levels of PI3K and AKT proteins in hippocampus (all P<0.05). It can be seen that the PI3K/AKT signaling pathway was inhibited in mice with depression.

Different expressions of apoptosis factors Bcl-2, Bax and Caspase-3, and growth-associated protein GAP-43 between normal and depression mice

After successful modeling, the mRNA and protein expressions of apoptosis factors Bcl-2, Bax and Caspase-3 and growth-associated protein

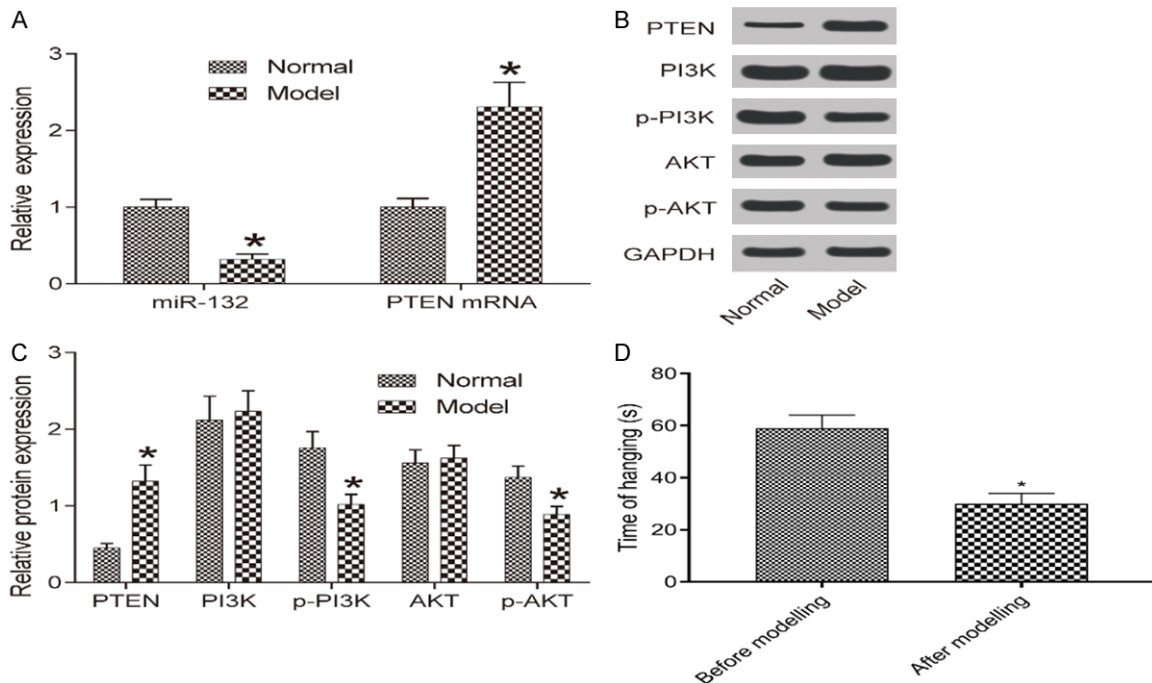


Figure 1. Different expressions of miR-132, PTEN and PI3K/AKT signaling pathways between normal and depression mice. A. Expressions of miR-132 and PTEN mRNA in hippocampus of mice (by qRT-PCR, n=10); B. Protein bands of PTEN as well as PI3K, p-PI3K, AKT, p-AKT in PI3K/AKT signaling pathways in hippocampus of mice (by Western blot); C. Protein expressions of PTEN, PI3K, p-PI3K, AKT, p-AKT (by Western blot, n=10). D. Tail suspension time before and after modelling (n=75). Compared with the Normal group, *P<0.05.

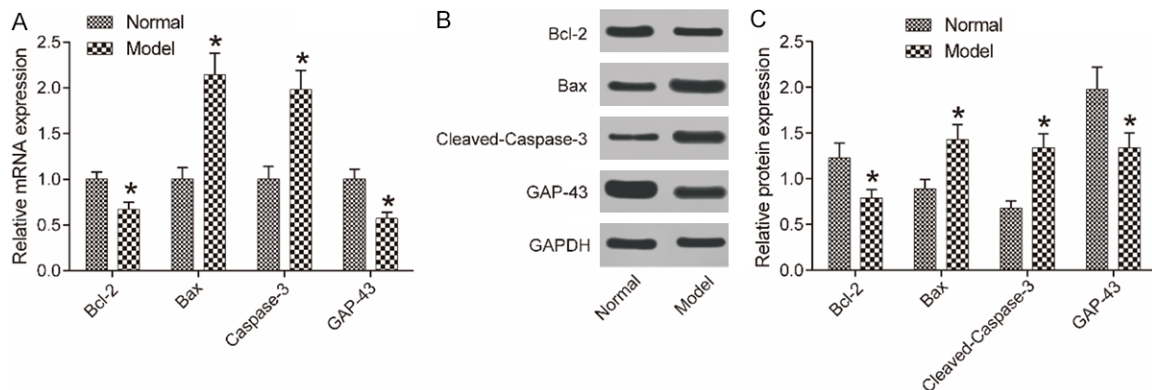


Figure 2. Expressions of apoptosis factors Bcl-2, Bax and Caspase-3, and growth-associated protein GAP-43 mRNA and protein in normal and depression mice between normal and depression mice. A. mRNA expressions of Bcl-2, Bax, Caspase-3 and GAP-43 in mouse hippocampus (by qRT-PCR, n=10); B. Protein bands of Bcl-2, Bax, cleaved-Caspase-3 and GAP-43 in mouse hippocampus; C. Protein levels of Bcl-2, Bax, cleaved-Caspase-3 and GAP-43 (n=10). Compared with the Normal group, *P<0.05.

GAP-43 in mouse hippocampus were detected by qRT-PCR and WB (Figure 2). Compared with the Normal group, the Model group had significantly elevated mRNA levels of Bax and Caspase-3, elevated protein levels of Bax and cleaved-Caspase-3, reduced levels of Bcl-2 mRNA and growth-associated protein GAP-43 (all P<0.05).

Negative regulation of PTEN targeted by miR-132

The biological prediction site TargetScan predicted that miR-132 and PTEN had specific binding sites. The dual-luciferase reporter assay showed that the luciferase activity in the Wt-PTEN subgroup of miR-132 mimic group

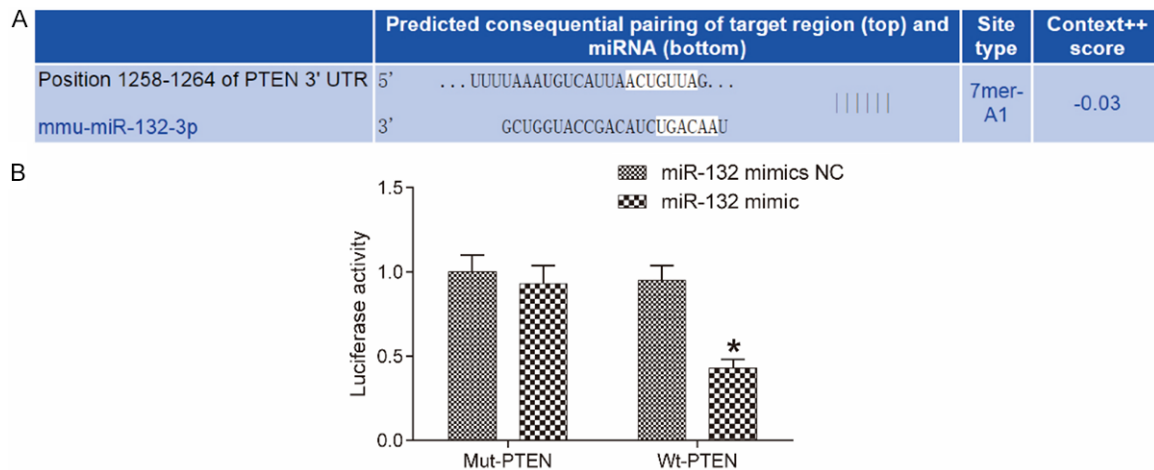


Figure 3. Negative regulation of PTEN by miR-132. A. The sequence of the 3'-UTR segment of miR-132 binding to PTEN; B. Luciferase activity (by dual luciferase assay, n=3). Compared with the miR-132 mimics NC group, *P<0.05.

was significantly lower than that in the miR-132 mimics NC group (P<0.05). However, the luciferase activity in the Mut-PTEN subgroup did not change significantly (P>0.05). It can be seen that miR-132 could target the negative regulation of the PTEN gene. See **Figure 3**.

Behavioral tests

In order to confirm that the depression models were successfully established, we conducted behavioral tests in Normal group and modeled groups. On the 35th day of modeling, Morris water maze test, tail suspension test and inclined plane test were performed in each group (**Figure 4**). In the place navigation test, the model groups showed significantly longer EL, fewer times of crossing the platform within 2 min, shorter time of hanging, and longer time in inclined plane test than the Normal group (all P<0.05). To better understand the mechanism of miR-132 and PTEN on cognitive impairment and neuroplasticity in mice with depression, we transfected miR-132 mimics and si-PTEN in depression mice. The miR-132 mimics group and si-PTEN group had significantly shorter EL, more times of crossing the platform within 2 min, longer hanging time, and shorter time in inclined plane test than the Model group (all P<0.05). Furthermore, the tendencies were even more significant in the miR-132 mimics + si-PTEN group. It can be seen that overexpression of miR-132 or silence of PTEN can improve the memory ability of mice with depression.

Neuronal apoptosis in hippocampal CA1 detected by TUNEL assay

The apoptosis of neurons in CA1 was detected by TUNEL staining (**Figure 5**). Compared with the Normal group, the neuronal apoptosis rates in the CA1 region were elevated significantly in other groups (all P<0.05). Compared with the Model group, the apoptosis rates were significantly reduced in the miR-132 mimics group, si-PTEN group, and miR-132 mimics + si-PTEN group (all P<0.05). Compared with the miR-132 mimics group and the si-PTEN group, the apoptosis rate in the miR-132 mimics + si-PTEN group was significantly reduced (both P<0.05). It can be seen that overexpression of miR-132 or silence of PTEN can reduce the neuronal apoptosis in CA1 region of depression mice.

Different expressions of miR-132, PTEN and PI3K/AKT signaling pathway between each group

To better understand the mechanism of miR-132 and PTEN on cognitive impairment and neuroplasticity in mice with depression, we detected miR-132 and PTEN mRNA by qRT-PCR, and detected protein expressions of PTEN as well as PI3K, p-PI3K, AKT, and p-AKT in PI3K/AKT signaling pathway by WB after we transfected miR-132 mimics and si-PTEN in depression mice (**Figure 6**). Compared with the Model group, the miR-132 mimics group had elevated level of miR-132 in hippocampus; the miR-132 mimics group, si-PTEN group and miR-

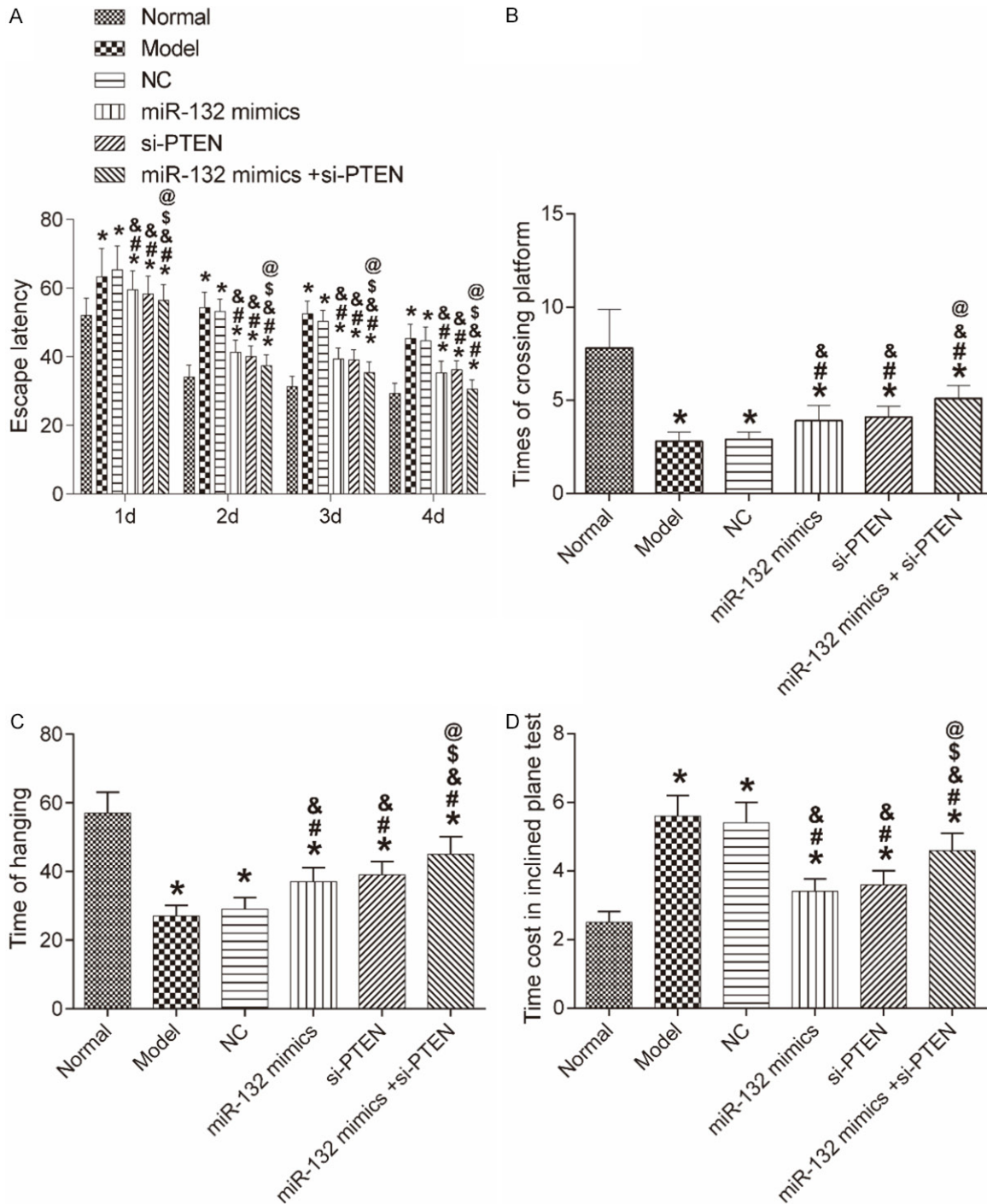


Figure 4. Data of Morris water maze test, TST and inclined plane test. A. Escape latency in Morris water maze test (n=15); B. Times of crossing platform (n=15); C. Time of hanging (n=15); D. Time cost in inclined plane test (n=15). Compared with the Normal group, *P<0.05; compared with the Model group, #P<0.05; compared with the NC group, &P<0.05; compared with the miR-132 mimics group, \$P<0.05; compared with the si-PTEN group, @P<0.05. TST: Tail suspension test.

132 mimics + si-PTEN group all had reduced mRNA and protein levels of PTEN, as well as elevated protein phosphorylation levels of PI3K

and AKT (all P<0.05). Compared with the si-PTEN group and miR-132 mimics group, the miR-132 mimics + si-PTEN group had signifi-

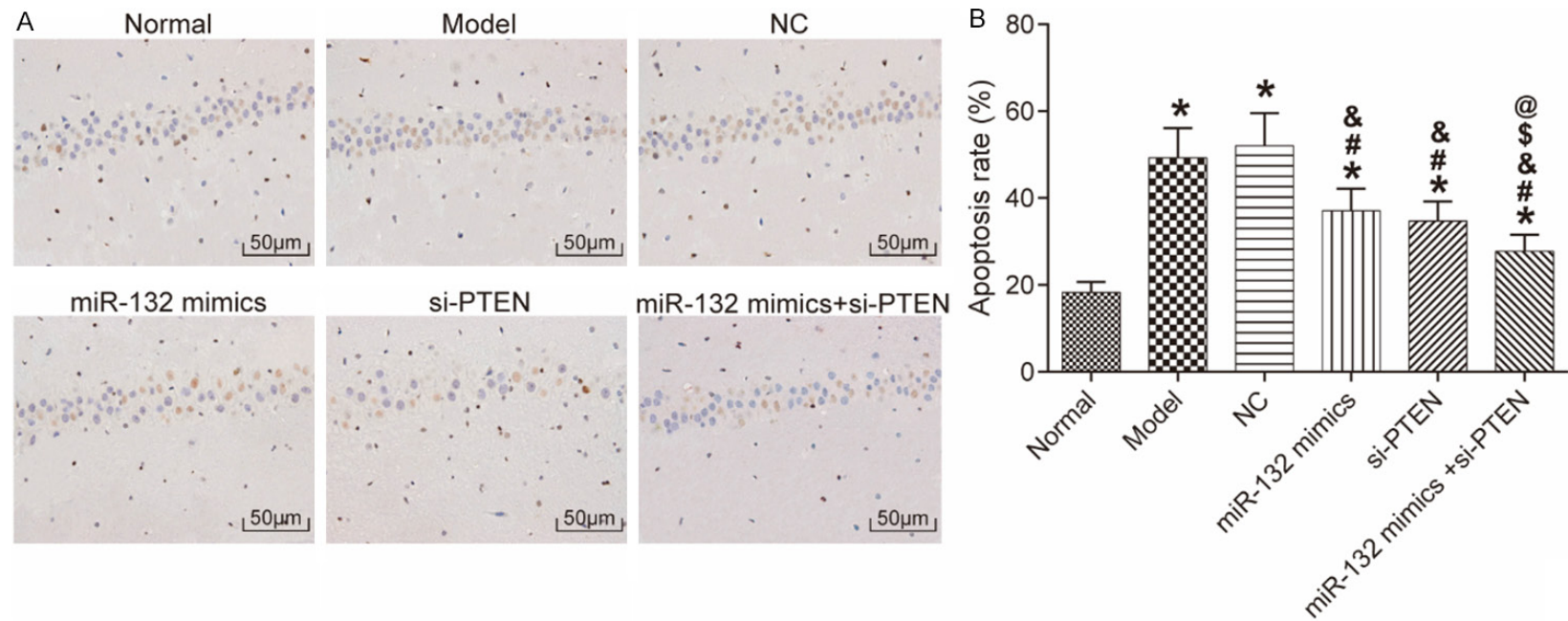


Figure 5. Neuronal apoptosis in hippocampal CA1 region (TUNEL staining, $\times 400$). A. Neuronal apoptosis in hippocampal CA1 region (TUNEL staining, $\times 400$); B. Neuronal apoptosis rate in the CA1 region of each group of mice ($n=5$). Compared with the Normal group, * $P<0.05$; compared with the Model group, # $P<0.05$; compared with the NC group, & $P<0.05$; compared with the miR-132 mimics group, \$ $P<0.05$; compared with the si-PTEN group, @ $P<0.05$.

miR-132 targets PTEN to regulate cognitive impairment and neural plasticity

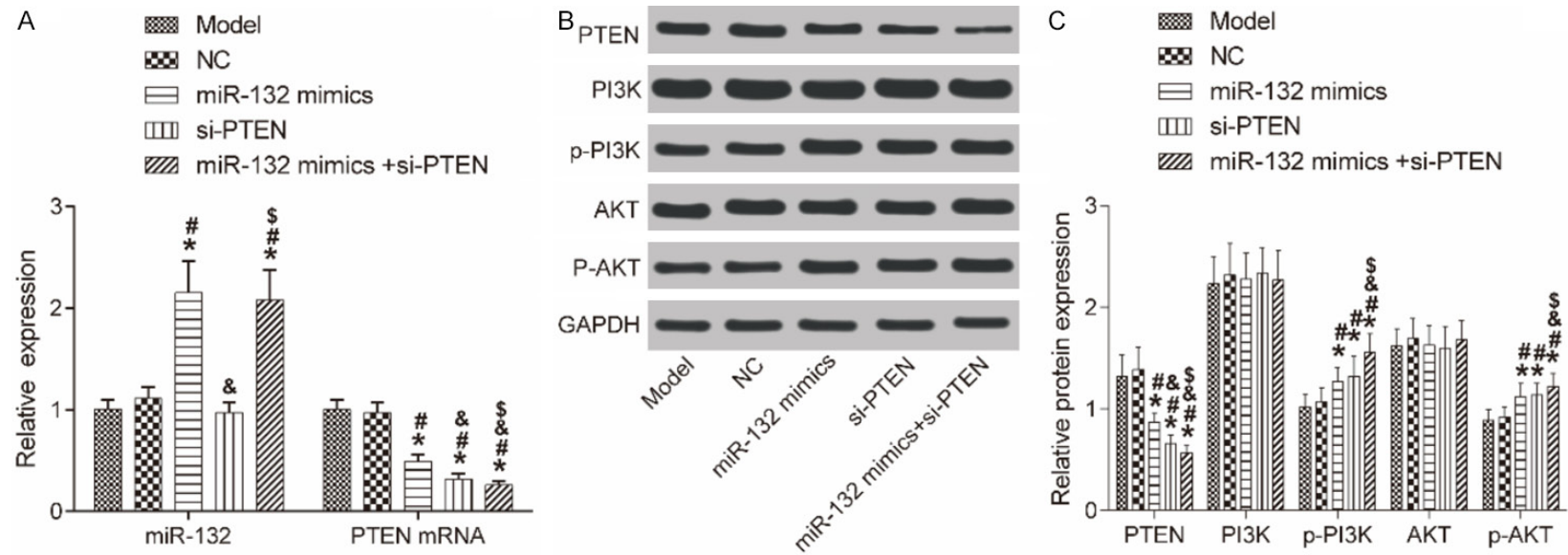


Figure 6. Different expressions of miR-132, PTEN and PI3K/AKT signaling pathways in each group. A. Expressions of miR-132 and PTEN mRNA in hippocampus of mice (by qRT-PCR, n=10); B. Protein bands of PTEN as well as PI3K, p-PI3K, AKT, p-AKT in PI3K/AKT signaling pathways in hippocampus of mice (Western blot); C: Protein expressions of PTEN, PI3K, p-PI3K, AKT, and p-AKT s (by Western blot, n=10). Compared with the Model group, *P<0.05; compared with the NC group, #P<0.05; compared with the miR-132 mimics group, &P<0.05; compared with the si-PTEN group, \$P<0.05.

cantly reduced mRNA and protein levels of PTEN, as well as elevated protein phosphorylation levels of the PI3K and AKT (all $P < 0.05$). It can be seen that overexpression of miR-132 can inhibit the expression of the target gene PTEN, thereby activating the PI3K/AKT signal.

Different expressions of apoptosis factors Bcl-2, Bax and Caspase-3, and growth-associated protein GAP-43 between each group

The mRNA and protein expressions of apoptosis factors Bcl-2, Bax and Caspase-3, and growth-associated protein GAP-43 in mouse hippocampus were detected by qRT-PCR and WB (**Figure 7**). Compared with the Model group, the miR-132 mimics group, si-PTEN group and miR-132 mimics + si-PTEN group had significantly reduced mRNA levels of Bax and Caspase-3, reduced protein levels of Bax and cleaved-Caspase-3, and elevated mRNA and protein levels of Bcl-2 and GAP-43 (all $P < 0.05$). Compared with miR-132 mimics group and si-PTEN group, the miR-132 mimics + si-PTEN group showed similar results (all $P < 0.05$). It can be seen that overexpression of miR-132 or silence of PTEN can inhibit apoptosis and neuronal damage in hippocampus of mice with depression. Furthermore, the combination of the two can synergistically inhibit the apoptosis and neuronal damage.

Discussion

Depression poses a major threat to people's physical and mental health, also brings serious social and family burden. However, the current treatments for depression generally have disadvantages of slow effect and unfavorable side effects. Therefore, it is imperative to reveal the pathogenesis of depression, so as to explore new targets for drug treatment and find new and more effective treatments for depression.

Cognitive impairment is a major symptom of depression. There are increasing evidences indicating that changes in hippocampal neuroplasticity are closely related to the pathogenesis of depression [16]. Lots of studies have found that miR-132 may be involved in antidepressant mechanisms. For example, miR-132 expression was up-regulated in depression mice after 3 weeks of antidepressant treatment, and miR-132 inhibitors can inhibit the proliferation of hippocampal neurons and the

production of post-synaptic proteins [17]. The level of miR-132 in hippocampus was significantly increased after long-term duloxetine treatment for depression mice [18]. In our experiment, we also found that the expression of miR-132 was significantly decreased in the Model group as compared with the Normal group; besides, overexpression of miR-132 could improve the memory of mice with depression and reduce the neuronal apoptosis in CA1. It is speculated that antidepressants could elevate the expression of miR-132, so as to regulate the downstream target genes and related proteins thereby improving cognitive impairment and neuroplasticity. A number of previous studies have confirmed that miR-132 is closely related to cognitive impairment and synaptic plasticity [19, 20]. Elevated level of miR-132 was associated with memory dysfunction in patients with depression [21]. Overexpression of miR-132 can increase the number of synaptic branches and dendritic spines, while silence of miR-132 can impact the development of neuronal dendrites [22].

Depression is associated with changes in hippocampal structure and function, and inhibition of hippocampal apoptosis may be an important drug target for the treatment of depression, as well as the regulation of mood and memory [23]. PTEN is a tumor suppressor gene with phosphatase activity, involved in cell proliferation, apoptosis and migration as well as regulation of cell cycle [24]. Silencing PTEN can increase cell proliferation and decrease apoptosis [25]. PTEN can negatively regulate the PI3K/Akt signaling pathway. Specifically, the encoded product of PTEN is a PIP3 phosphatase. PIP3 activates AKT by activating phosphorylation sites on AKT, then activated AKT protein relocates to the cytoplasm or nucleus to promote the phosphorylation of a variety of downstream substrates, thereby negatively regulating the PI3K/AKT signaling pathway, ultimately inhibiting cell proliferation and promoting apoptosis [26-28]. Our study found that miR-132 can negatively regulate PTEN, and overexpression of miR-132 can inhibit the expression of target gene PTEN, thereby activating PI3K/AKT signaling. The relationship between miRs and PTEN has been confirmed in many experiments. For example, studies have found that PTEN is a direct target of miR-21, and overexpression of miR-21 can inhibit the expression of PTEN, while inhibition of miR-21

miR-132 targets PTEN to regulate cognitive impairment and neural plasticity

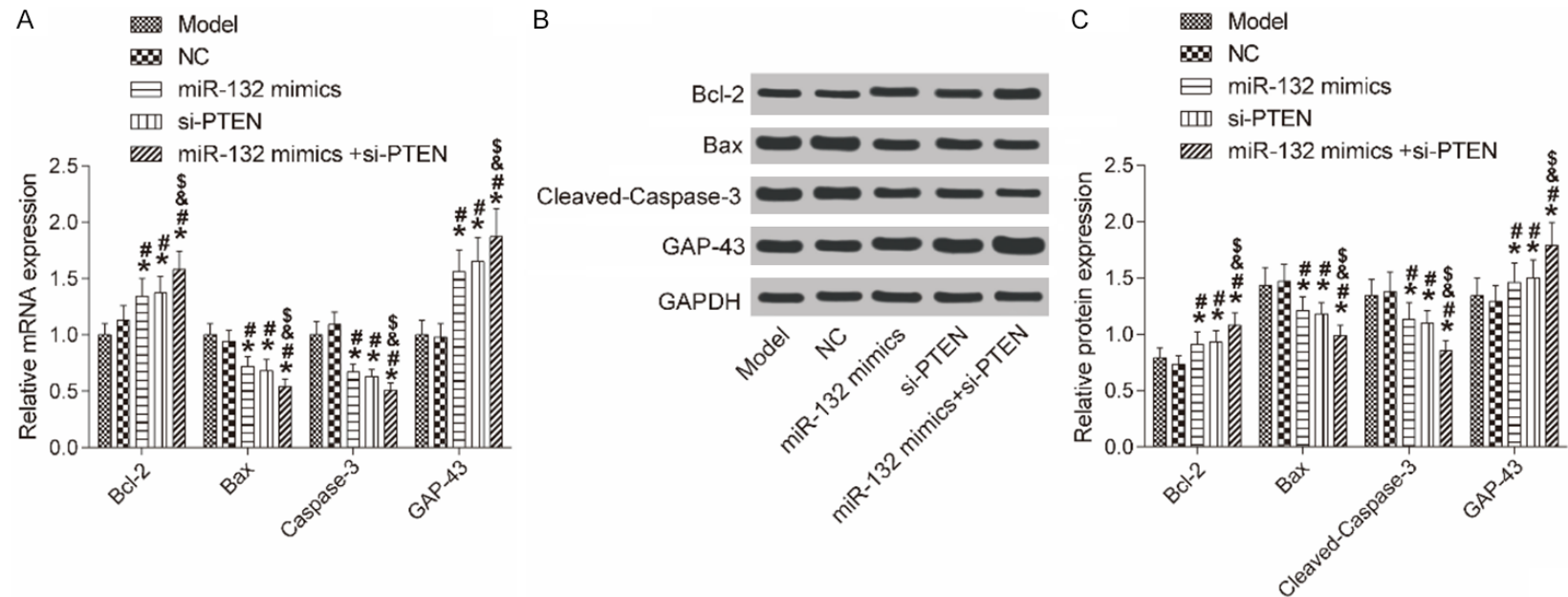


Figure 7. Expressions of apoptosis factors Bcl-2, Bax and Caspase-3, and growth-associated protein GAP-43 mRNA and protein in different groups. A. mRNA expressions of Bcl-2, Bax, Caspase-3 and GAP-43 in mouse hippocampus (by qRT-PCR, n=10); B. Protein bands of Bcl-2, Bax, cleaved-Caspase-3 and GAP-43 in mouse hippocampus; C. Protein levels of Bcl-2, Bax, cleaved-Caspase-3 and GAP-43. Compared with the Model group, *P<0.05; compared with the NC group, #P<0.05; compared with the miR-132 mimics group, &P<0.05; compared with the si-PTEN group, \$P<0.05.

can promote the expression of PTEN, which are consistent with our findings [29, 30].

In summary, miR-132 can negatively regulate PTEN gene, and the overexpression of miR-132 or the silence of PTEN can improve the memory ability of mice with depression and reduce the apoptosis of neurons in CA1, which may be related to the activation of the PI3K/AKT signaling pathway. However, this experiment only used animals as models, therefore, the mechanism in humans and the side effects need to be further studied.

Disclosure of conflict of interest

None.

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