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Silence of MiR-9 protects depression mice through Notch signaling pathway

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Abstract. – OBJECTIVE: To investigate the potential influence of micro ribonucleic acid (miR)-9 on depressive behaviors of depression mice.

MATERIALS AND METHODS: Depression model in mice was established via chronic unpredictable mild stress. A total of 36 C57BL/6 mice were randomly divided into control group (control mice, n=12), model group (depression mice administrated with control lentivirus, n=12) and miR-9 low-expression group (depression mice administrated with LV-miR-9-shRNA lentivirus, n=12). After 6 weeks, the depressive behaviors of mice were evaluated by behavioral experiments. Mice were then sacrificed for harvesting the hippocampus. Relative level of miR-9 in mouse hippocampus was determined via quantitative polymerase chain reaction (qPCR). Moreover, the number of newborn neurons in the hippocampus in each group was detected via immunofluorescence assay. Expression levels of doublecortin (DCX), post-synaptic density protein 95 (PSD95) and Notch signaling pathway in the hippocampus in each group were detected via Western blotting.

RESULTS: In the open field test, there were no significant differences in the crossing score and rearing score among control group, model group and miR-9 low-expression group (p>0.05). The immobility time of mice in tail suspension test and forced swimming test increased significantly in model group compared with that in control group (*p*<0.01), while it was significantly shorter in miR-9 low-expression group than that in model group (*p*<0.01). The expression of miR-9 in mouse hippocampus in model group was significantly higher than that in control group and miR-9 low-expression group (*p*<0.01). In miR-9 low-expression group, the number of newborn neurons in the hippocampus was significantly larger than that in control group and model group (*p*<0.01). Protein levels of both DCX and PSD95 were significantly higher in control group than those in model group (*p*<0.01). Besides, protein levels of Notch intracellular domain (NICD), Hes1 and Jag1 in the hippocampus in model group were remarkably declined compared with those in control group (*p*<0.01), which were higher in miR-9 low-expression group.

CONCLUSIONS: MiR-9 is upregulated in the hippocampus of depression mice. Silence of miR-9 in the hippocampus can effectively activate the Notch signaling pathway, promote the neuronal regeneration in the hippocampus and improve the depressive symptoms of mice.

Key Words:
miR-9, Notch signaling pathway, Nerve regeneration.

Introduction

Depression is a common central nervous system disease that can cause neurasthenia, which is mainly characterized by the long-term persistent depressed mood and retardation of thinking, accompanied by suicidal tendency[1,2]. The morbidity of depression is on the rise these years. As suggested by the World Health Organization, depression is one of the most important problems seriously affecting human mental health, posing a heavy burden on patients, families and society[3]. At present, the first-line antidepressants have disadvantages as slow effect, serious adverse reactions and low effectiveness[4]. Merino et al[5] followed up 1,829 adult depression patients who used to take antidepressants in the past 5 years, and found that 60% had emotional anesthesia and up to 39% had suicidal tendency. Therefore, it is significant to further study the pathogenesis of depression, which helps to search for new therapeutic targets for depression, develop novel antidepressants and overcome various limitations of existing antidepressants. In recent years, micro ribonucleic acids (miRNAs) are found to be extremely important for the regulation of vital ac-

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tivities. They regulate the gene transcription and affect the expressions of downstream target proteins through binding to target genes, thereby exerting biological effects[6]. Wan et al[7] analyzed the human genes via bioinformatics and found that more than one third of the genes are regulated by miRNAs and miRNA expression has tissue and time specificity. MiR-9, a brain-derived miRNA, is closely associated with the neuronal growth and development. Its expression level is downregulated during neuronal regeneration, but upregulates during neuronal differentiation[8]. Notch signal is highly conserved during the transduction. Notch receptor binds to its ligand (Jag1) to release the Notch intracellular domain (NICD) from the inner side of the cell membrane, thereby inducing the expression of the downstream target Hes1 and participating in the hippocampal neuronal regeneration and differentiation[9]. However, the effect of miR-9 on the Notch signaling pathway is less studied. The potential associations of the occurrence and development of depression with miR-9 level in the hippocampus remain unclear. In the present study, depression model in mice was established *via* chronic unpredictable mild stress, so as to evaluate the effect of miR-9 on depressive behaviors of mice and study whether the Notch signaling pathway is involved in the regulation.

Materials and Methods

Animals and Grouping

A total of 36 male C57BL/6 mice weighing 20-22 g were purchased from Guangdong Medical Laboratory Animal Center. Mice were adaptively fed in a specific pathogen-free environment for 7 d under the temperature of (22±1)°C, humidity of (45±2)% and regular circadian rhythm and had free access to food and water. They were randomly divided into control group, model group and miR-9 low-expression group. Mice in control group were healthy without any treatment, whereas mice in the other two groups were the depression ones. In addition, mice in model group and miR-9 low-expression group were administrated with control lentivirus and LV-miR-9-shRNA lentivirus, respectively. This study was approved by the Ethics Committee of Jining Psychiatric Hospital, and the animal experimental operations were performed in strict accordance with the Guide for the Feeding and Use of Laboratory Animals issued by the National Institutes of Health (NIH, Bethesda, MD, USA).

Construction of Mice with Low Expression of miR-9

Mice in miR-9 low-expression group were subjected to intra-hippocampal injection of LV-miR-9-shRNA lentivirus purchased from Shanghai Genechem (Shanghai, China). Briefly, mice were anesthetized and fixed on the stereotaxic apparatus, and the skull skin was cut to expose the anterior fontanel. With the anterior fontanel as the original point, the parameters of the stereotaxic apparatus were adjusted as follows: AP: +2.15 mm, ML: ±0.28 mm, and DV: -1.16 mm, which located to the hippocampus of mice[10]. Next, the skull was drilled, and 2 μL of lentivirus was injected into the hippocampus using a micro syringe pump at a rate of 0.2 μL/min. Finally, the wound was sutured and penicillin was applied to prevent infection. After resuscitation, the mice were placed back to the cage.

Establishment of Depression Model in Mice

Depression model in mice was established *via* chronic unpredictable mild stress. In brief, stress factors included deprivation of food for 24 h, swimming at 4°C for 3 min, tail clamping for 2 min, deprivation of water for 24 h, foot shock for 10 s, cage wetting for 24 h, cage tilt at 45°C for 24 h, whole body binding for 2 h, feeding in separate cages for 24 h, and swimming at 56°C for 3 min. A single different stress factor was given every day during modeling for consecutive 6 weeks. Sucrose preference test (SPT), forced swimming test (FST) and tail suspension test (TST) were conducted to verify the success of model establishment.

Evaluation of Depressive Behaviors of Mice via Behavioral Experiments

Depressive behaviors of mice were evaluated *via* behavioral experiments.

Open field test: a square open box (60 cm × 60 cm × 20 cm) was divided into 36 equal-sized grids. After the odor in the open box was removed with alcohol, the mice were placed in the center of each quadrant, and the movement of mice in the open box within 5 min was recorded and evaluated by the crossing score (the number of grids crossed) and rearing score (the times of forelimb upright).

TST: The end of tail was fixed with tape, and the mice were hung on the suspension device with the head downwards at about 10 cm above the

ground. The immobility time (no movement of the limbs and body) of mice was recorded within 6 min to evaluate the movement of mice.

FST: The mice were placed in a transparent plastic barrel (diameter: 13 cm, height: 24 cm) filled with thermostatic water. The movement of mice in each group was recorded within 6 min, and the immobility time (no movement of the limbs and body) in the last 4 min was also recorded.

Quantitative Polymerase Chain Reaction (qPCR)

After behavioral experiments, the mice were immediately executed, and the hippocampus was isolated and incubated in TRIzol (Invitrogen, Carlsbad, CA, USA) at a volume ratio of 1:9. Tissue homogenization was performed using an ultrasonic homogenizer, followed by standing at room temperature for 10 min and centrifugation at 4°C and 12000 rpm for 10 min. Next, the supernatant was transferred into a new centrifuge tube to extract the total RNA according to the RNA extraction method. The optical density (OD) value was measured using an ultraviolet spectrophotometer (BioTek, Biotek Winooski, VE, USA), and the quality of RNA was evaluated using the agarose gel. The RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using the kit (TaKaRa, Otsu, Shiga, Japan) under the following reaction conditions: 37°C for 15 min and 85°C for 5 s. The qPCR system was prepared: 2 μL 2 × SYBR Primer Ex Taq II, 2 μL cDNA, 10 μM Primer R, 10 μM Primer F, 0.4 μL 50× ROX Reference Dye II, and 6 μL ddH_2O. The qPCR conditions were as follows: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 45 s and extension at 72°C for 1 min, for a total of 35 cycles, and extension at 72°C for 5 min finally. The primer sequences were shown in Table I. The primers were synthesized by Invitrogen (Carlsbad, CA, USA), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference. The relative expression level of miR-9 was calculated by $2^{-\Delta\Delta Ct}$ based on the Ct-value of amplification.

Immunofluorescence Detection of the Number of Newborn Neurons

After behavioral experiments, the mice were immediately sacrificed for collecting the brain. The whole brain was dehydrated with sucrose water in an equal gradient, fixed with 4% paraformaldehyde, sliced into 40 μm-thick brain slices using the freezing microtome and placed into a 24-well plate containing 0.1 M phosphate-buffered saline (PBS). After PBS was discarded, 500 μL freshly-prepared 0.3% Triton was added into each well, followed by transparent at room temperature for 15 min. The slices were sealed with 500 μL 10% goat serum at room temperature for 2 h and incubated with 300 μL primary antibody (diluted in 5% goat serum) in a refrigerator at 4°C overnight. After the primary antibody was discarded, the slices were incubated with 300 μL secondary antibody (diluted in 5% goat serum) in the refrigerator at 4°C for 2 h. The brain slices were placed on the glass slide, added with 0.1 M PBS, sealed with an appropriate amount of anti-quenching mounting medium and covered with the cover glass. Images were captured under a confocal fluorescence microscope (Nikon, Tokyo, Japan). Finally, the number of newborn neurons in the hippocampus was calculated.

Detection of Protein Expression Levels via Western Blotting

Mouse hippocampus was isolated and added with radioimmunoprecipitation assay (RIPA) lysis buffer (CST, Danvers, MA, USA) at a mass/volume ratio of 100 mg/1 mL. Lysis was subjected to homogenization using the ultrasonic homogenizer, followed by standing for 10 min and centrifugation at 4°C and 12000 rpm for 10 min. After that, the supernatant was taken to detect the total protein concentration in the hippocampus using the diaminobenzidine (DAB) protein quantification kit (Solarbio, Beijing, China). The hippocampus in

Table I. Primer sequences.

Gene		Sequences of PCR primers
miR-9	Sense	5'-AAGCTAGATAACCGAAAGTAG-3'
	Antisense	5'-GAGTGCCACAGAGCCGTAACTT-3'
GAPDH	Sense	5'-AGGTCGGTGTGAAACGGATTTG-3'
	Antisense	5'-TGTAGACCATGTAGTTGAGGTCA-3'

each group was prepared into protein samples in an equal concentration, and boiled for 10 min to inactivate the protein. Protein sample was separated *via* sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) using the wet method and sealed with the freshly-prepared 5% skim milk powder for 2 h. The target band was cut according to the molecular weight of the target protein, and incubated with the monoclonal primary antibodies (1:1000) against doublecortin (DCX) (CST, Danvers, MA, USA), post-synaptic density protein 95 (PSD95) (CST, Danvers, MA, USA), NICD (CST, Danvers, MA, USA), Hes1 (Abcam, Cambridge, MA, USA), Jag1 (Abcam, Cambridge, MA, USA), and GAPDH (CST, Danvers, MA, USA) at 4°C overnight. After the protein band was washed with Tris-buffered saline and Tween-20 (TBST), it was incubated with the secondary antibody (Wuhan Boster Biological Technology Co., Ltd., Wuhan, China) at room temperature for 2 h, and washed with TBST for 3 times (5 min/time). Next, electrochemiluminescence (ECL) solution (Thermo Fisher Scientific, Waltham, MA, USA) was prepared for image development in a dark room. Finally, the protein in each group was quantitatively analyzed using ImageJ software, and the expression level of protein in the hippocampus was calculated.

Statistical Analysis

Experimental data were expressed as ($\bar{x} \pm s$) and analyzed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA). Comparison between groups was done using one-way analysis of variance (ANOVA) followed by Post-Hoc Test LSD (Least Significant Difference). Bonferroni's method was adopted for the pairwise comparison in the case of homogeneity of variance, while Games-Howell test was adopted in the case of heterogeneity of variance. $p<0.05$ suggested that the difference was statistically significant.

Results

Autonomic Movement of Mice in Each Group Evaluated by Open Field Test

The autonomic movement of mice in each group was evaluated by open field test. As shown in Figure 1, there were no significant differences in the crossing score and rearing score among control group, model group and miR-9 low-expression group ($p>0.05$).

Depressive Behaviors of Mice Evaluated by Behavioral Experiments

Depressive behaviors of mice in each group were evaluated by TST and FST. As shown in Figure 2, the immobility time of mice in TST and FST was significantly longer in model group than that in control group ($p<0.01$), while it was significantly shorter in miR-9 low-expression group than that in model group ($p<0.01$).

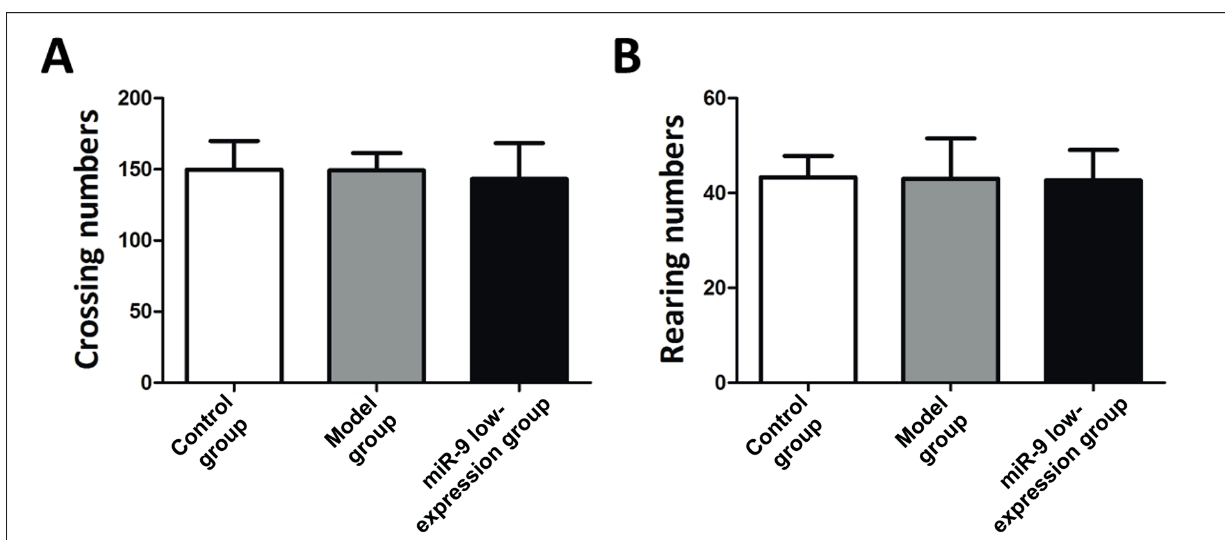

Figure 1. Autonomic movement of mice in each group evaluated by open field test. ***A,*** Crossing score, ***B,*** Rearing score.

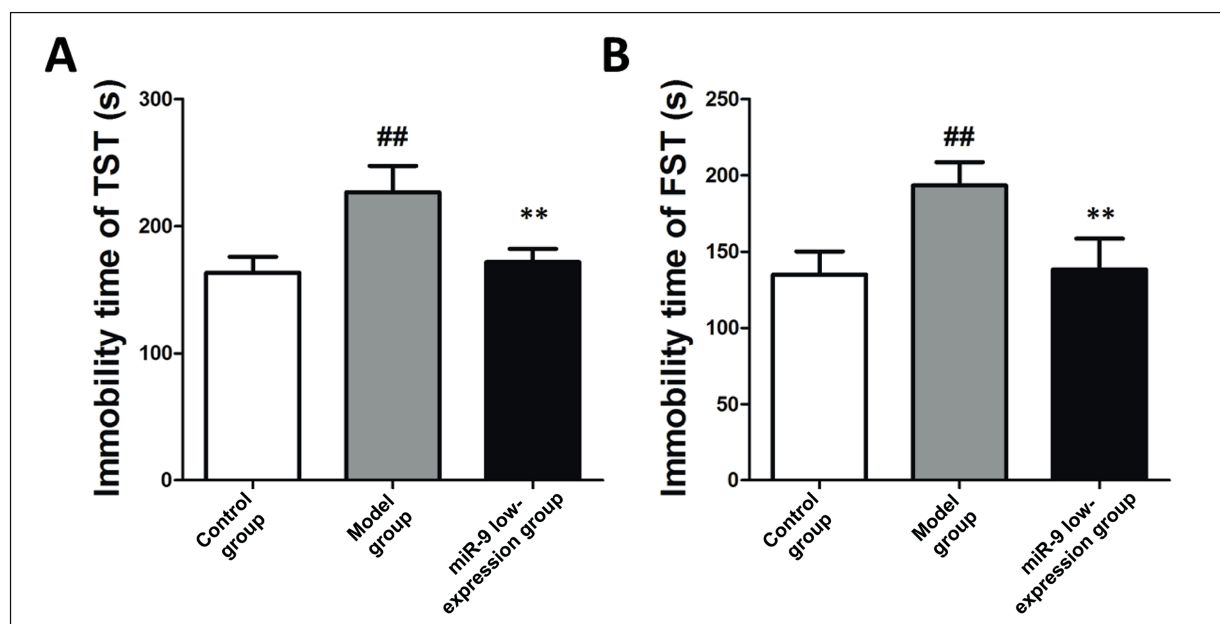

Figure 2. Depressive behaviors of mice evaluated by behavioral experiments. ***A,*** TST, ***B,*** FST. The immobility time of mice in TST and FST is increased significantly in model group compared with that in control group, while it is significantly shorter in miR-9 low-expression group than that in model group. ##p<0.01 *vs.* control group, **p<0.01 *vs.* model group.

Expression Level of miR-9 in Hippocampus in Each Group Detected via qPCR

Expression level of miR-9 in the hippocampus in each group was detected *via* qPCR. As shown in Figure 3, miR-9 in the hippocampus was significantly upregulated in model group (p<0.01), while it was significantly declined in miR-9 low-expression group after intervention with lentivirus (p<0.01).

Number of Newborn Neurons in Hippocampus in Each Group Detected Via Immunofluorescence

The number of newborn neurons in the hippocampus in each group was detected *via* immunofluorescence. The results revealed that the number of newborn neurons in the hippocampus significantly decreased in model group compared with that in control group (p<0.01), while it significantly increased in miR-9 low-expression group after intervention with lentivirus (p<0.01) (Figure 4).

Expression Levels of Nerve Regeneration-Related Proteins Detected via Western Blotting

After the behavioral experiments, mice were sacrificed to harvest hippocampus. Western blotting results showed that the expression levels of DCX and PSD95 in the hippocampus remarkably declined in model group compared with those in control group (p<0.01), while they significantly increased in miR-9 low-expression group (p<0.01) (Figure 5).

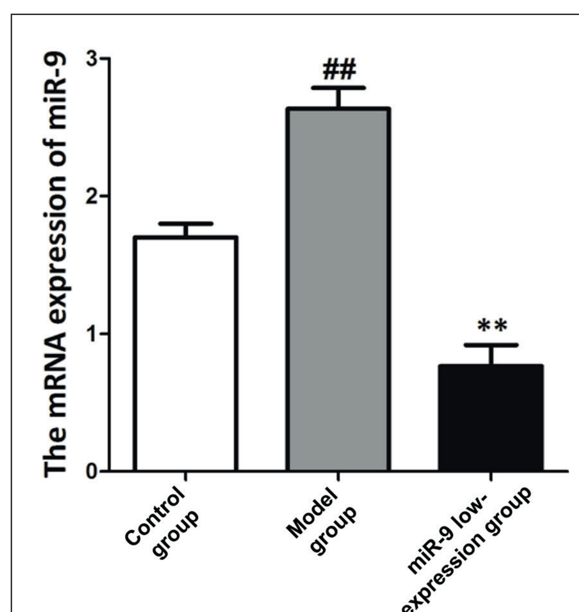

Figure 3. Expression level of miR-9 in hippocampus. The expression of miR-9 in the hippocampus is significantly higher in model group than that in control group, while it is significantly lower in miR-9 low-expression group than that in model group. ##p<0.01 *vs.* control group, **p<0.01 *vs.* model group.

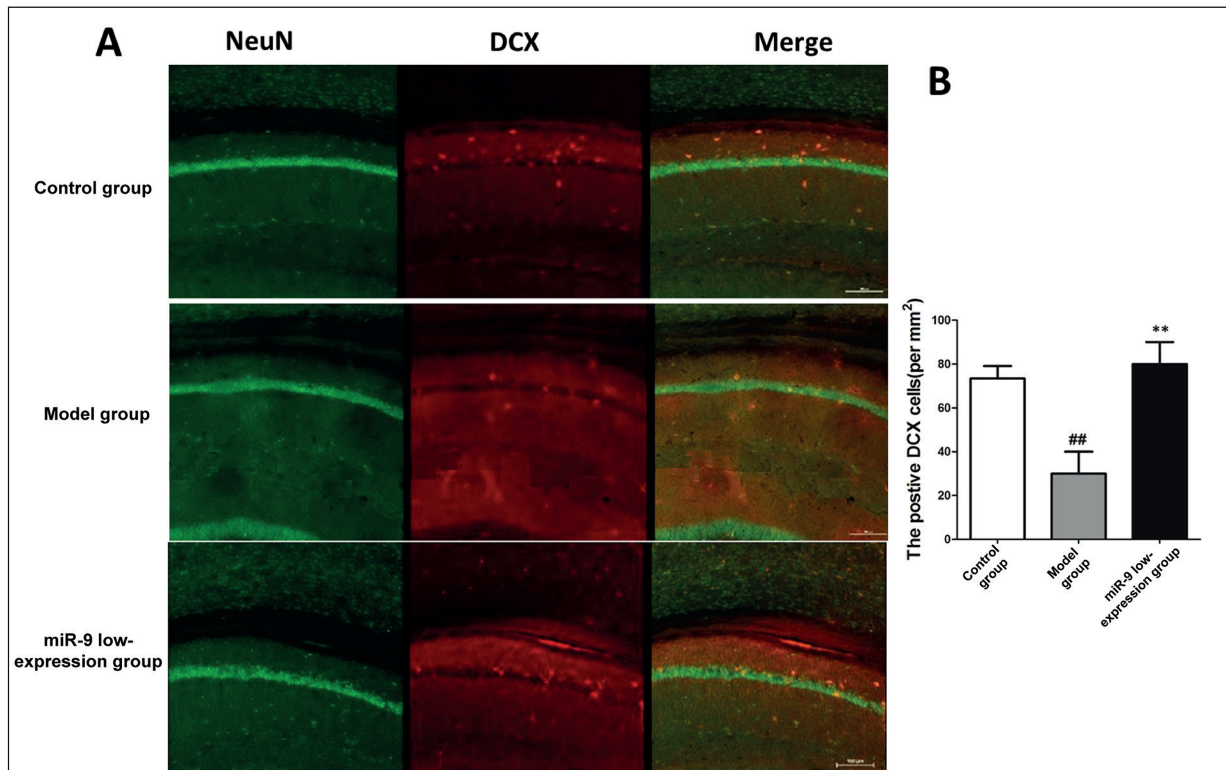

Figure 4. Number of newborn neurons in hippocampus in each group detected *via* immunofluorescence. ***A,*** Immunofluorescence results, ***B,*** statistical graph. The number of newborn neurons in the hippocampus is significantly smaller in model group than that in control group and miR-9 low-expression group. $^{\#\#}p<0.01$ *vs.* control group, $^{**}p<0.01$ *vs.* model group, scale bar=50 μm.

Expression Levels of Notch Signaling Pathway-Related Proteins Detected via Western Blotting

Expression levels of Notch signaling pathway-related proteins in each group were examined *via* Western blotting. As shown in Figure 6, protein levels of NICD, Hes1 and Jag1 in the hippocampus were remarkably declined in model group compared with those in control group ($p<0.01$), while they were remarkably upregulated in miR-9 low-expression group ($p<0.01$).

Discussion

The pathogenic factors and pathological mechanism of depression, as a refractory complex neuropsychiatric disease, have not been clarified yet. At present, the antidepressants developed based on the monoamine neurotransmitter hypothesis improve the depressive symptoms of depression patients mainly through increasing levels of neurotransmitters, such as 5-hydroxytryptamine, dopamine and norepinephrine in the synaptic cleft. However, most of them have a low effective rate. It is believed that depression is not only associated with the monoamine neurotransmitters in the synaptic cleft[11]. Currently, the pathogenesis of depression is studied in the human trial mainly through the imaging and molecular biological results after autopsy. Since it is difficult to obtain clinical specimens, the depression model is established in mice for clinical research[12]. In the present study, depression model in mice was established *via* chronic unpredictable mild stress, which completely simulated the whole process of human depression. Depressive symptoms are similar to those of depression patients after long-term chronic unpredictable mild stress, such as anhedonia, decline in sociability and behavioral despair. Finally, the success of depression model establishment was evaluated *via* behavioral experiments. Kitzlerova et al[13] found that the depression model established *via* chronic unpredictable mild stress is more stable and can evaluate the effect of antidepressants more effectively than that established *via* intraperitoneal injection of

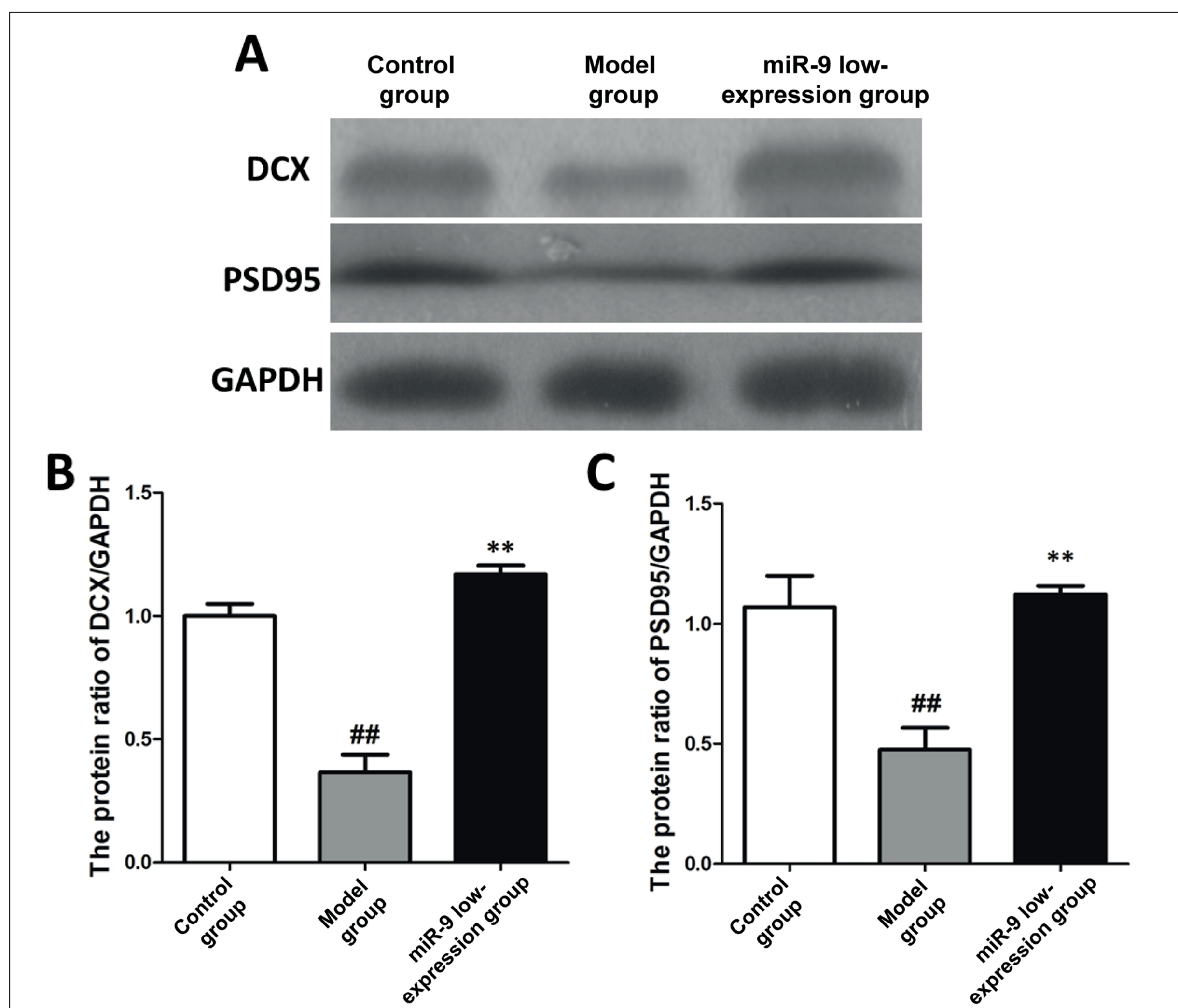

Figure 5. Expression levels of nerve regeneration-related proteins detected *via* Western blotting, ***A,*** Protein band, ***B,*** expression level of DCX, ***C,*** expression level of PSD95. The expression levels of PSD95 and DCX in the hippocampus in model group are significantly lower than those in control group and miR-9 low-expression group. $^{\#\#}p<0.01$ *vs.* control group, $^{**}p<0.01$ *vs.* model group.

LPS and CD-1. Both TST and FST are classic behavioral despair tests that can effectively evaluate the depressive behaviors of subjects[14]. In this experiment, the immobility time of mice in TST and FST increased in model group after stress for 6 weeks, strongly suggesting that the mouse model of depression was established successfully via stress. Xue et al[15] found that the expression of miR-9, a brain-derived miRNA, is differentially expressed in brain tissues, and it is closely associated with the occurrence and development of central nervous system diseases, such as Parkinson's disease and Alzheimer's disease. Besides, Junn et al[16] studied the substantia nigra of 63 patients with Parkinson's disease and found that the expression level of miR-9 significantly increased compared with that in normal people. They proposed that high expression of miR-9 may induce neuronal apoptosis and inhibit neuronal regeneration, and the expression level of synapse-associated protein PSD95 also significantly declines. In the present study, it was found that the expression level of miR-9 in the hippocampus of depression mice was significantly higher than that in control group, and the number of newborn neurons in the hippocampus of depression mice significantly decreased. Moreover, miR-9 knockdown improved the depressive behaviors of depression mice, increased the number of newborn neurons

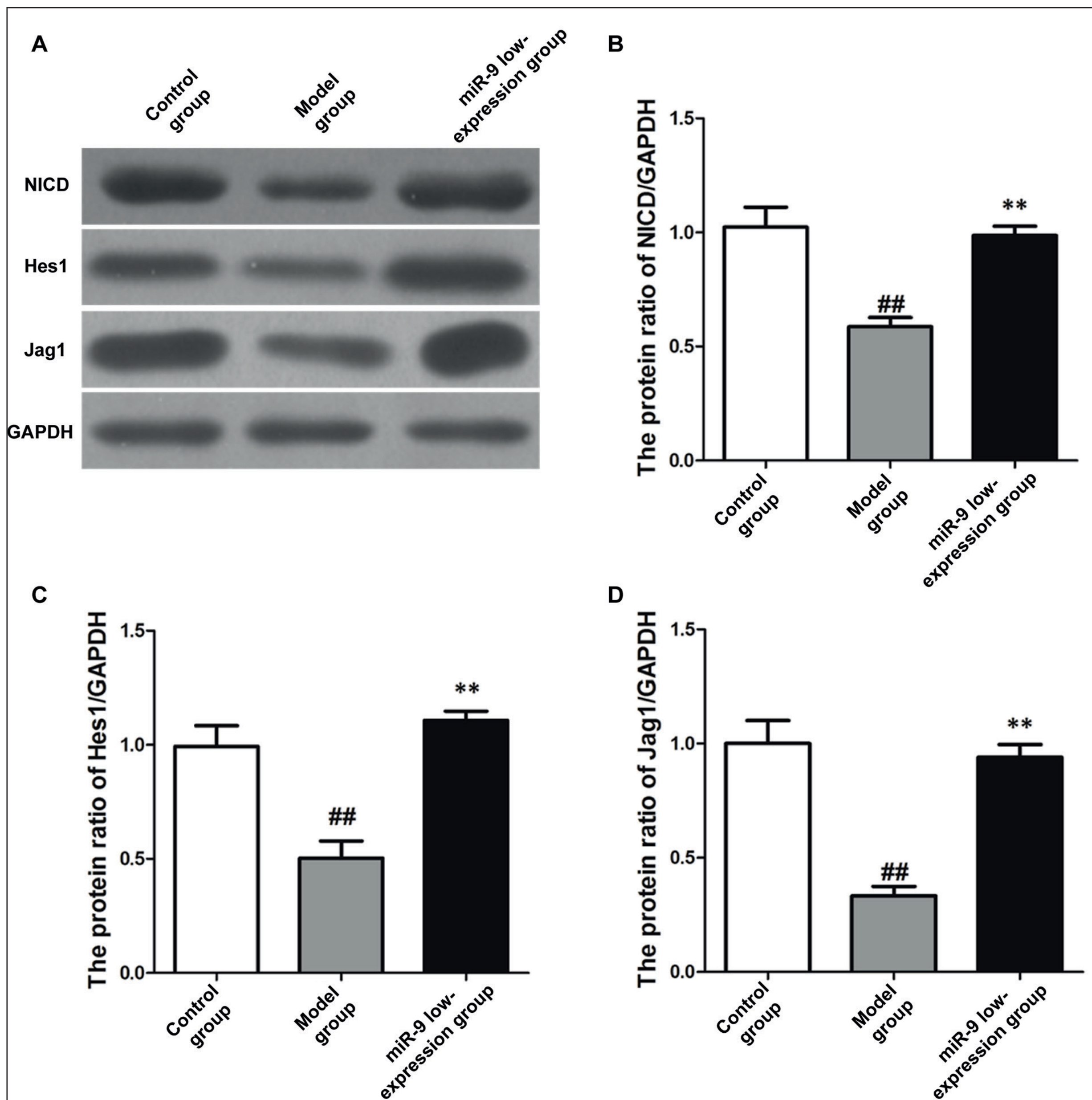

Figure 6. Expression levels of Notch signaling pathway-related proteins detected *via* Western blotting, ***A,*** Protein band, ***B,*** statistical graph of NICD, ***C,*** statistical graph of Hes1, ***D,*** statistical graph of Jag1. The expression levels of NICD, Hes1 and Jag1 in the hippocampus in model group are significantly lower than those in control group and miR-9 low-expression group. $^{\#\#}p<0.01$ *vs.* control group, $^{**}p<0.01$ *vs.* model group.

in the hippocampus and upregulated PSD95, indicating that the expression level of miR-9 in the hippocampus was closely related to the depressive behaviors of mice. The Notch signaling pathway is closely related to the body growth and development, showing potential functions in self-renewal, proliferation, differentiation and apoptosis of neural stem cells[17,18]. Zeng et al[19] found that in the early stage of embryonic development, the Notch signaling pathway is activated to release NICD, thereby promoting cell development and differentiation. Notch signaling pathway acts on the downstream target protein Hes1, thus promoting the differentiation of neural stem cells into astrocytes and participating in the pathophysiological process of nervous system diseases, such as Alzheimer's disease and epilepsy[20]. In the present study, the

Notch signaling pathway was inhibited in the hippocampus, and the expressions of NICD, Hes1 and Jag1 were significantly downregulated in depression mice. At the same time, the typical depressive symptoms developed in depression mice. Silence of miR-9 in the hippocampus could effectively activate the Notch signaling pathway in the hippocampus, upregulate expressions of NICD, Hes1 and Jag1, and improve the depressive symptoms. Coincidentally, this study found that the number of newborn neurons in the hippocampus of depression mice obviously increased after the Notch signaling pathway was activated, suggesting that the activation of Notch signaling pathway regulated the nerve regeneration in the hippocampus of mice.

Conclusions

We showed that the expression level of miR-9 in the hippocampus of depression mice significantly increased. Silence of miR-9 in the hippocampus can effectively activate the Notch signaling pathway, promote the neuronal regeneration in the hippocampus and improve the depressive symptoms of mice.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Ma K, Zhang H, Baloch Z. Pathogenetic and therapeutic applications of tumor necrosis factor-alpha (TNF-alpha) in major depressive disorder: a systematic review. Int J Mol Sci 2016; 17: 733.
2) Liu J, Xu ZQ, Yi X, Wang YJ, Zhou HD. A study on related factors of hemodynamic depression in carotid artery stenting. Eur Rev Med Pharmacol Sci 2018; 22: 5255-5263.
3) Parikh SV, Quilty LC, Ravitz P, Rosenbluth M, Pavlova B, Grigoriadis S, Velyvis V, Kennedy SH, Lam RW, MacQueen GM, Milev RV, Ravindran AV, Uher R. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: section 2. Psychological treatments. Can J Psychiatry 2016; 61: 524-539.
4) Oluboka OJ, Katzman MA, Habert J, McIntosh D, MacQueen GM, Milev RV, McIntyre RS, Blier P. functional recovery in major depressive disorder: providing early optimal treatment for the individual patient. Int J Neuropsychopharmacol 2018; 21: 128-144.
5) Merino H, Senra C, Ferreiro F. Are worry and rumination specific pathways linking neuroticism and symptoms of anxiety and depression in patients with generalized anxiety disorder, major depressive disorder and mixed anxiety-depressive disorder? PLoS One 2016; 11: e156169.
6) Alural B, Genc S, Haggarty SJ. Diagnostic and therapeutic potential of microRNAs in neuropsychiatric disorders: past, present, and future. Prog Neuropsychopharmacol Biol Psychiatry 2017; 73: 87-103.
7) Wan Y, Liu Y, Wang X, Wu J, Liu K, Zhou J, Liu L, Zhang C. Identification of differential microRNAs in cerebrospinal fluid and serum of patients with major depressive disorder. PLoS One 2015; 10: e121975.
8) Shi Z, Piccus Z, Zhang X, Yang H, Jarrell H, Ding Y, Teng Z, Tchernichovski O, Li X. miR-9 regulates basal ganglia-dependent developmental vocal learning and adult vocal performance in songbirds. eLife 2018; 7: e29087.
9) Salazar JL, Yamamoto S. Integration of drosophila and human genetics to understand notch signaling related diseases. Adv Exp Med Biol 2018; 1066: 141-185.
10) Jo SJ, Yim HW, Jeong H, Song HR, Ju SY, Kim JL, Jun TY. Prevalence of depressive disorder of outpatients visiting two primary care settings. J Prev Med Public Health 2015; 48: 257-263.
11) D'Ostilio K, Garraux G. The network model of depression as a basis for new therapeutic strategies for treating major depressive disorder in Parkinson's disease. Front Hum Neurosci 2016; 10: 161.
12) Wilkinson PO, Croudace TJ, Goodyer IM. Rumination, anxiety, depressive symptoms and subsequent depression in adolescents at risk for psychopathology: a longitudinal cohort study. BMC Psychiatry 2013; 13: 250.
13) Kitzlerova E, Fisar Z, Lelkova P, Jirak R, Zverova M, Hroudova J, Manukyan A, Martasek P, Raboch J. Interactions among polymorphisms of susceptibility loci for Alzheimer's disease or depressive disorder. Med Sci Monit 2018; 24: 2599-2619.
14) Pedrelli P, Borsari B, Lipson SK, Heinze JE, Eisenberg D. Gender differences in the relationships among major depressive disorder, heavy alcohol use, and mental health treatment engagement among college students. J Stud Alcohol Drugs 2016; 77: 620-628.
15) Xue Q, Yu C, Wang Y, Liu L, Zhang K, Fang C, Liu F, Bian G, Song B, Yang A, Ju G, Wang J. miR-9 and miR-124 synergistically affect regulation of dendritic branching via the AKT/GSK3beta pathway by targeting Rap2a. Sci Rep 2016; 6: 26781.
16) Junn E, Mouradian MM. MicroRNAs in neurodegenerative diseases and their therapeutic potential. Pharmacol Ther 2012; 133: 142-150.
17) Steinbuck MP, Winandy S. A review of notch processing with new insights into ligand-indepen-

dent notch signaling in T-cells. Front Immunol 2018; 9: 1230.

18) Kannan R, Cox E, Wang L, Kuzina I, Gu Q, Giniger E. Tyrosine phosphorylation and proteolytic cleavage of Notch are required for non-canonical Notch/Abl signaling in Drosophila axon guidance. Development 2018; 145: dev151548.

19) Zeng D, He S, Yu S, Li G, Ma C, Wen Y, Shen Y, Yu Y, Li H. Analysis of the association of MIR124-1 and its target gene RGS4 polymorphisms with major depressive disorder and antidepressant response. Neuropsychiatr Dis Treat 2018; 14: 715-723.

20) Tian H, Li X, Tang Q, Zhang W, Li Q, Sun X, Zhao R, Ma C, Liu H, Gao Y, Han F. Yi-nao-jie-yu Prescription exerts a positive effect on neurogenesis by regulating notch signals in the hippocampus of post-stroke depression rats. Front Psychiatry 2018; 9: 483.