Journal of Affective Disorders 238 (2018) 609–614

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

CSF miR-16 expression and its association with miR-16 and serotonin transporter in the raphe of a rat model of depression

Shao Qiong-Yan[a], You Fei[b], Zhang Yong-Hua[a], Hu Lin-Lin[c], Liu Wen-Juan[c], Liu Yi[c], Li Jing[c], Wang Sheng-Dong[c], Song Ming-Fen[c,a,d,*]

[a] Internal Medicine of Traditional Chinese Medicine, The 4nd Clinical Medical College, Zhejiang Chinese Medical University, Zhejiang, China
[b] Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Zhejiang, China
[c] Molecular Biology Laboratory, Hangzhou Seventh People's Hospital, Zhejiang, China
[d] Mental Health Center, School of Medicine, Zhejiang University, Zhejiang, China

ARTICLE INFO

Keywords:
Depression
Chronic unpredictable mild stress
miR-16
Serotonin transporter

ABSTRACT

Background: Depression is a common mental disorder with unknown mechanism. Emerging evidence shows that miRNAs play a critical role in the process of depression. Here we reported the cerebrospinal fluid (CSF) miR-16 expression and its association with miR-16 and serotonin transporter (SERT) in the raphe of a rat model of depression.

Methods: 20 rats were randomized to the control or CUMS (chronic unpredictable mild stress) group. The rats in the CUMS group underwent CUMS for 21 days, while those in the control group received no treatment. After anesthetization, CSF was collected for the measurement of miR-16. Then raphes from all rats were separated for determination of miR-16 and SERT protein.

Results: The expression levels of miR-16 in CSF and raphe of the CUMS group were significantly lower than those of the control group ($P = 0.007$ and 0.031). However, SERT protein in raphe of the CUMS group was obviously increased as compared that of the control group ($P = 0.005$). There was a positive correlation between CSF miR-16 and raphe miR-16 ($r = 0.95$, $P = 0.000$). Meanwhile, negative correlations between miR-16 and SERT protein in raphe ($r = -0.70$ $P = 0.02$), between CSF miR-16 and raphe SERT protein ($r = -0.86$, $P = 0.002$) were observed in the CUMS group.

Limitations: We have not explored the reason why CSF miR-16 was decreased in the rat model of depression and only tested the association of miR-16 between CSF and raphe.

Conclusions: CSF miR-16 was involved in the pathogenesis of depression via reflecting raphe miR-16 level, and thus affecting raphe SERT expression.

1. Introduction

Depression, as a common mental disorder, will be the second contribution to the global burden and leading cause of disease worldwide just after heart disease by 2020 (Chapman and Perry, 2008). Although increasing researches on depression have been performed worldwide, the cellular and molecular mechanism is still unclear.

MicroRNAs (miRNAs) are a kind of non-coding, small-molecule and single-stranded RNAs with a length of 20–25 nucleotides (Ambros, 2004; Bartel, 2004). They can post-transcriptionally regulate gene expression and play important roles in almost all the biological processes (Breving and Esquelakerscher, 2010; Li and Rana, 2014). Although the long history of miRNA research (first reported in 1993 Lee et al., 1993), their power of regulation function of gene-expression has not been taken widely until recent years. It is believed that miRNAs regulate neurogenesis and synaptic plasticity, as well as neurotransmitter homeostasis, and thus participate in the pathophysiology of neuropsychiatric disorders and the corresponding biological changes (Cristino et al., 2014; Maffioletti et al., 2014; Saharan et al., 2013).

Recent studies have shown that miRNA-16 (miR-16) may be involved in the occurrence and progression of depression via targeting serotonin transporter (SERT) gene and regulating its translational level, thereby affecting the neurotransmitter function of 5-HT in brain tissue (Baudry et al., 2010; Moya et al., 2013). As we know, SERT is the pharmacological target of selective serotonin reuptake inhibitor (SSRI) antidepressants (Coleman et al., 2016). Baudry et al. (2010) reported that miR-16 contributed to the therapeutic action of SSRI antidepressants in serotonergic raphe of two mouse models of depression.

* Corresponding author at: Hangzhou Seventh People's Hospital, No. 305, Tianmushan Road, Hangzhou, Zhejiang 310013, China.
E-mail address: songmingfen2005@163.com (M.-F. Song).

https://doi.org/10.1016/j.jad.2018.06.034
Received 2 April 2018; Received in revised form 15 May 2018; Accepted 13 June 2018
Available online 19 June 2018
0165-0327/

Our previous study demonstrated that CSF miR-16, raphe miR-16 and raphe SERT in a rat model of depression were abnormal (Song et al., 2015). However, it is unknown how CSF miR-16 is involved in the pathophysiological process of depression. In this study, we aimed to identify whether CSF miR-16 level would reflect raphe miR-16 and SERT expression. Thus, we used a rat model of depression induced by chronic unpredictable mild stress (CUMS) to clarify the correlation between CSF miR-16 and miR-16, SERT expression in raphe.

2. Materials and methods

2.1. Ethics statement

Twenty 8-week Sprague Dawley rats were purchased from Zhejiang Academy of Medical Sciences and housed at (22 ± 1) °C with food and water ad libitum and a reversed 12:12 h light cycle. All animal procedures were performed in accordance with China National Guidelines for Animal Care and were approved by the Animal Care and Use Committee in Hangzhou Seventh People's Hospital.

2.2. Treatment of rats

All rats were randomly assigned into the control group (10 rats) and the CUMS group (10 rats). Rats in the control group received no treatment while those in the CUMS group were given 7 kinds of different chronic unpredictable stresses with a duration of 21 days followed by a previous report (Bai et al., 2012). Briefly, rats were exposed to water deprivation for 24 h, food deprivation for 24 h, swimming in cold water (4 °C) for 5 min, exposure to an elevated open platform (10 cm × 10 cm, 160 cm in height) for 2 h, restraint stress for 2 h or electric foot shock for 20 s (800 mA, 1 s duration, average 1 shock/10 s). Every day, a type of stimulation was randomly selected for performing one time of stimulation. On average, each of the rats received each kind of stimulation for 3 times. The reliability of the model was evaluated by the experiment of sucrose consumption test and open field test.

2.3. Assessment of depressive behaviors

The depression-like behaviors were evaluated by sucrose consumption test and open field test as described by Bai et al. (2012).

In open field test, the arena was made of an open rectangular plastic box (100 cm × 100 cm × 40 cm) with 25 squares (20 cm × 20 cm) painted on the floor. Rats were placed individually at the center of the field and were allowed to explore any areas freely. The rat activity was recorded for 5 min by an overhanging camera that was linked to a computer. Ethovision XT (Noldus, The Netherlands) was used to analyze the behaviors. The number of a rat crossing grid lines with all four paws was calculated as its horizontal score and the number of rearing activities was considered as its vertical score. The arena was cleaned with 70% alcohol between trials to make sure a rat's behaviors were not affected by the imprint of previous rats.

In sucrose consumption test, the whole test took 3 days. On day 1, each rat was housed individually and given two bottles of sucrose solution (1%, w/v). On day 2, one bottle of sucrose solution was replaced with water. On day 3, rats were deprived of water and food for 23 h, and then given two pre-weighed bottles of solution: 100 ml of sucrose solution (1%, w/v) and 100 ml of water. One hr later, the consumed volume in both bottles was recorded. The sucrose preference rate was calculated using the following formula: Sucrose preference rate = sucrose solution consumption/(water consumption + sucrose solution consumption) × 100%.

2.4. Collection of CSF and raphe

The harvest of CSF and raphe was performed as our previous study described (Song et al., 2015). Briefly, a longitudinal incision (about 2 cm) was cut in the dorsal skin for blunt separation of muscles and exposure of foramen magnum under isoflurane anesthesia. About 0.1 ml of CSF samples were immediately drawn through foramen magnum for miR-16 determination. For the obtainment of raphe, brains were immediately removed from rats after CSF obtainment, then brain stem tissues from the anterior margin of quadrigeminal bodies to the posterior margin of cerebella were dissected. Finally, raphe tissues were obtained by vertical incision at 0.45 mm lateral to the midline and prepared as homogenate for each group.

2.5. Western blot test for SERT protein

SERT protein in raphe was quantified via Western blot method. Briefly, the membrane protein of raphe tissues was extracted with Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit (Thermo Fisher Scientific Inc., MA, USA) and quantified via the Bradford method. The next steps of Western blot were followed as previously described (Huff et al., 2013). The anti-SERT (1:250 dilution), anti-β-actin (1:250 dilution), and HRP-conjugated second antibodies (1:5000 dilution) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The protein bands were analyzed using ChemiDocTM XRS + with Image LabTM Software (Bio-rad, California, USA) and corrected for the β-actin signal.

2.6. qRT-PCR test for miR-16 in CSF and raphe

Quantitative RT-PCR was used to detect miR-16 in CSF and raphe. Briefly, total miRNAs in CSF or raphe were extracted using miRcute miRNA Isolation Kit (TIANGEN BIOTECH Co., Ltd., Beijing, China). The concentration and purity were measured by nanodrop (Thermos scientific company, USA). 2 μg RNA was taken for the obtainment of cDNA by reverse transcription reaction. In the step of real-time quantitative PCR amplification, miRcute miRNA qPCR Detection Kit (SYBR Green) (TIANGEN BIOTECH Co., Ltd., Beijing, China) was used for miR-16 expression determination through ABI StepOne Plus Real Time PCR System. The forward primer sequence of miR-16 was 5′-TAGCAGCAC GTAAATTGGCG-3′. U6 was used as the internal reference gene and its forward primer sequence was 5′-ACGCAAATTCGTGAAGCGTTCCAT-3′. The reverse primers of miR-16 and U6 were universal and provided in the kit by manufacture but shown no sequence.

Samples were amplified for an initial denaturation at 94 °C for 2 min, followed by 40 cycles each consisting denaturation at 94 °C for 20 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s. Each sample was assayed triplicate. The data were obtained by standard curve method and normalized by U6.

2.7. Statistical analyses

All statistical analyses were conducted using SPSS19.0 software package (SPSS Inc, America). Student *t*-test was used for the comparisons of CSF miR-16, raphe miR-16, and raphe SERT protein between groups. The correlation analyses were assayed by Pearson correlation test. $P < 0.05$ was considered statistically significance.

3. Results

3.1. Estimation of depression-like behavioral indicators

Before treatment, the sucrose consumption rate, the horizontal score and vertical score of open field test in the CUMS group had no significantly difference as compared with those in the control group ($P > 0.05$). After received CUMS, these three indicators were all significantly lower than those in the control group ($P < 0.05$ or $P < 0.01$). See Table 1.