miR-98-5p plays a critical role in depression and antidepressant effect of ketamine

Chaoli Huang1,2,5, Yuanyuan Wang1,5, Zifeng Wu1, Jiali Xu1, Ling Zhou1, Di Wang1, Ling Yang3, Bin Zhu4, Guiquan Chen2, Cunming Liu1,5, and Chun Yang1,5

© The Author(s) 2021

INTRODUCTION

Mounting studies demonstrate that ketamine exerts a robust antidepressant effect, especially for refractory depression [1]. The antidepressant effect of ketamine is characterized by a rapid onset of action within hours and a long-lasting effect up to 2 weeks after a single dose [2, 3], attracting increasing scientific attentions and concerns. However, despite its clinical efficacy, the detailed mechanisms of ketamine exerting facilitating effects for depression are still obscure. Recent studies propose that in addition to its ability to inhibit N-methyl-D-aspartate (NMDA) receptors, the antidepressant effect of ketamine might involve epigenetic mechanisms, such as microRNA (miRNA) regulation [4].

miRNAs are a subset of endogenous small noncoding RNA molecules that serve as post-transcriptional regulators. It is well elucidated that miRNAs are associated with the pathophysiology and therapeutic mechanisms of depression [5]. Several miRNAs have been reported to be involved in pathogenesis and response to treatment of depression, and could act as potential targets for novel antidepressant treatment [6–9]. Very recently, the antidepressant effect of ketamine is closely related to the regulation of various miRNAs in the brain [10, 11].

In the present study, we adopted microarray analysis to assess miRNA expressions in the prefrontal cortex (PFC) of resilient and susceptible phenotypes in chronic social defeat stress (CSDS) mice, and 3 miRNAs were selected as potential candidates in the regulation of depression. We demonstrated that interference of these miRNAs attenuated depression-like behaviors in CSDS-susceptible mice. More importantly, we found that an antidepressant dose of ketamine led to an increased expression of miR-98-5p, and inhibition of miR-98-5p diminished the antidepressant effect of ketamine in CSDS mouse model.

MATERIALS AND METHODS

Animals

Male adult C57BL/6 mice aged eight weeks (body weight 20–25 g) and male adult ICR mice aged 13–15 weeks (body weight 40–50 g) were purchased from GemPharmatech (Nanjing, China). Animals were maintained in a specific pathogen-free (SPF) level of animal room in the facility of the Model Animal Research Center (MARC) of Nanjing University. The mice were kept under constant humidity and temperature (25 ± 1 °C) and 12-h light/dark cycles (lights on 7:00 AM–19:00 PM) with ad libitum food and water. The protocol was approved by the Nanjing University Institutional Animal Care and Use Committee.

Chronic social defeat stress paradigm

The procedure of CSDS was performed as previously reported [3, 12–14]. In the CSDS model, 8-week-old C57BL/6 male mice were exposed to a different CD-1 mouse for consecutive 10 days (10 min/day). C57BL/6/J mouse was defeated by a larger CD-1 mouse. After the social defeat session, the defeated mouse was subjected to continuous psychological stress from CD-1 mouse through a clear perforated divider that allows visual, olfactory, and auditory contact in a shared home cage for the remaining 24 h. The C57BL/6 mice were rotated daily, while the CD-1 mice were kept in the same cage during the 10-day defeat procedure. In order to select the susceptible and resilient mice, social-interaction test (SIT) was performed on the next day after the last attack of defeat stress. The SIT was conducted in an interaction-test box (42 × 42 cm) with an empty perforated plastic box (10 × 4.5 cm) located at one end. The 8-cm-wide area surrounding the box was defined as the “interaction zone”. The test...
The establishment of SDS mouse model.  

**Fig. 1** The schedule of CSDS model and behavioral tests.  

**A** The schedule of CSDS model and behavioral tests.  

**B** and **C** The time spent in the interaction zone during the SIT. SIT was performed at day 11 to segregate the defeated mice into resilient and susceptible subgroups.  

**D**–**F** The number of squares crossed during LMT, immobility time during FST, and sucrose-preference ratio during SPT of CSDS-resilient and -susceptible mice. LMT, FST, and SPT were performed to analyze the depression-like behaviors of CSDS-resilient and -susceptible mice at day 12 and day 13. Data are shown as mean ± SEM (n = 8). **P** < 0.01, ***P** < 0.001, N.S. not significant.
GenePix Pro 6.0 software (Axon Instruments, Foster City, CA, USA) for grid alignment and data extraction. miRNAs, whose intensities are more than 50 in all samples, were used to calculate a normalization factor. Expressed data were normalized by median normalization. After normalization, the miRNAs that were significantly differentially expressed were identified through Volcano Plot filtering. Finally, the data were subjected to hierarchical clustering and depicted in a heat-map format using Gene-Spring GX software v7.3 (Agilent Technologies, California, United States).

Statistical analysis
The data were expressed as the mean ± standard error of the mean (SEM). Analyses were performed with GraphPad Prism 8.0 (GraphPad Software Inc, La Jolla, CA, USA). Data in this study were analyzed by one-way, two-way analysis of variance (ANOVA) followed by Tukey post hoc analysis or unpaired t-test. P < 0.05 was considered significant.

RESULTS
Expression of miRNAs in PFC and hippocampus of CSDS-resilient and -susceptible mice
Multiple lines of evidence suggest that miRNAs are associated with the pathogenesis of depression [16]. To examine the effect of CSDS on miRNA expressions in PFC and hippocampus, we first established CSDS mouse model (Fig. 1a–c). After 10 days of CSDS, we carried out behavioral tests and observed that mice in the susceptible group had significantly increased immobility time in the FST, and significantly lowered sucrose-preference ratio in the SPT (Fig. 1d–f). Therefore, the depression model in mice has been successfully established. Subsequently, we collected PFC samples three days after CSDS and conducted miRNA microarray analysis. We identified 30 sets of miRNAs that expressed differentially in the CSDS-susceptible group compared with the resilient group (Fig. 2a), including 10 upregulated miRNAs and 20 downregulated miRNAs (Fig. 2b). In particular, 4 miRNAs (miR-23a-5p, miR-193a-5p, miR-98-5p, and miR-3968) with stable expressions and significant differences between the two groups were selected for further validation according to the P value and the FC value.

We next performed qPCR to validate the expressions of the 4 miRNAs in the hippocampus and found that three of them (miR-23a-5p, miR-98-5p, and miR-3968) show significant expression differences (Fig. 2c). These data indicate that miR-23a-5p, miR-98-5p, and miR-3968 may be involved in the pathogenies of depression.

Effects of miR-23a-5p/-98-5p/-3968 interferences on the depression-like behaviors of CSDS-susceptible mice
To further explore the roles of miR-23a-5p, miR-98-5p, and miR-3968 in the CSDS-induced depression-like behavior, we evaluated the effects of interfering these 3 miRNAs in CSDS-susceptible mice separately. Since miR-23a-5p is significantly upregulated in
CSDS-susceptible mice while miR-98-5p and miR-3968 are downregulated, we treated the susceptible mice with antagonist for miR-23a-5p or agonist for miR-98-5p or miR-3968 to interfere with these miRNAs, respectively, and then conducted behavioral tests to examine their depression-like behaviors (Fig. 3a). qPCR was conducted first to verify the efficiency of the miRNA interference (Fig. s1a). The results showed that expressions of miR-23a-5p in PFC and hippocampus were significantly downregulated by the antagonist and expressions of miR-98-5p and miR-3968 were significantly upregulated by the respective agonists (Fig. s1b–d), suggesting that interfering the miRNAs with antagonist or agonist is efficient. Subsequently, the behavioral tests demonstrated that all three groups with miRNA interference had significantly less immobility time in the FST and higher ratio of sucrose preference in the SPT than those in the control group (Fig. 3c, d). Therefore, interference of miR-23a-5p/-98-5p/-3968 could alleviate the depression-like behaviors of CSDS-susceptible mice.

Effects of ketamine on the expression of miR-23a-5p/-98-5p/-3968 in CSDS-susceptible mice
Recent studies have linked miRNAs to the antidepressant effect of ketamine [9, 10]. Therefore, we investigated whether the expressions of miR-23a-5p, miR-98-5p, and miR-3968 in PFC and hippocampus respond to ketamine treatment. We first treated CSDS-susceptible mice with an antidepressant dose of ketamine after CSDS, then performed behavioral tests to validate the antidepressant effect of ketamine, and adopted qPCR to analyze the expressions of the 3 miRNAs (Fig. 4a). The behavioral tests illustrated that ketamine markedly decreased the immobility time of the FST and increased sucrose-preference ratio (Fig. 4b–d), confirming the remarkable antidepressant effect of ketamine. qPCR results showed that in both PFC and hippocampus, the levels of miR-98-5p were increased by ketamine treatment, but the levels of miR-23a-5p and miR-3968 were not significantly affected (Fig. 4e–g). These results imply that miR-98-5p is a ketamine-sensitive miRNA,

Fig. 3 Effects of miR-23a-5p/-98-5p/-3968 inhibition on depression-like behaviors of CSDS-susceptible mice. a The schedule of miRNA antagonist or agonist administration and behavioral tests after CSDS. b–d The number of squares crossed during LMT, immobility time during FST, and sucrose-preference ratio during SPT. LMT, FST, and SPT were performed to analyze the depression-like behaviors of CSDS-susceptible mice after administration with antagonist to miR-23a-5p or agonist to miR-98-5p or miR-3968. Data are shown as mean ± SEM (n = 8). ***P < 0.001, N.S. not significant.
raising the possibility that upregulation of this miRNA may contribute to the antidepressant effect of ketamine.

**Effects of miR-98-5p inhibition on the antidepressant effect of ketamine**

To determine whether the antidepressant effect of ketamine requires upregulation of miR-98-5p, we tested the effects of miR-98-5p on the antidepressant actions of ketamine. We first treated CSDS-susceptible mice with miR-98-5p antagonist and then treated them with ketamine, and finally conducted behavioral tests to examine their depression-like behaviors (Fig. 5a). Although ketamine significantly decreased immobility time in the FST and increased sucrose-preference ratio in the SPT, it had no obvious effect on the mice treated with miR-98-5p antagonist (Fig. 5b–d). These results suggest that the antidepressant effect of ketamine is dependent upon the upregulation of miR-98-5p.

**DISCUSSION**

miRNAs regulate multiple gene expressions, significantly affect cellular functions, and are implicated in many psychiatric diseases [17]. Growing evidence indicated that miRNAs might play a key role in the pathogenesis of depression [16]. Clinical studies have identified altered expressions of many miRNAs in the peripheral tissues of patients with major depression or bipolar disorder [18, 19], and also have detected aberrant levels of several miRNAs in the brain of patients with major depressive symptoms who died by suicide [18]. Subsequent animal studies replicated miRNAs' role in depression by interfering some miRNAs to reverse the depression-like behaviors in animal models of depression [20, 21]. To further explore which miRNAs are potentially involved in depression, we took use of CSDS mouse model and profiled the miRNA expressions in the PFC of CSDS-resilient and -susceptible mice by utilizing a microarray technique. Four miRNAs with the most distinctive difference between the two conditions were selected for further validation.
groups were noted and three of them were selected after validating their expressions in the hippocampus, including miR-23a-5p, miR-98-5p, and miR-3968. We interfered their expression in CSDS-susceptible mice by the respective agonist or antagonists and found that reversing the altered expression of any of these miRNAs could drastically reduce the social stress-induced depression-like behaviors. Thus, we implicated 3 miRNAs in the progression of depression. Even though our following studies excluded two of these miRNAs to be critically involved in the mechanism of ketamine's antidepressant effect, they may still serve as therapeutic targets in depression and worth further investigating.

In recent years, ketamine has become a promising candidate to produce a rapid and sustained antidepressant effect in treatment-resistant patients. Although the antidepressant mechanism of ketamine has not been fully understood, several studies have associated it with regulation of miRNAs [9, 10]. Thus, the 3 miRNAs selected from the expression profile may be critically involved in the mechanism of ketamine's antidepressant effect, they may still serve as therapeutic targets in depression and worth further investigating.

In summary, we implicate miR-23a-5p, miR-98-5p, and miR-3968 in the pathogenesis of depression. Moreover, we uncover a novel role of miR-98-5p in ketamine's antidepressant effect.
However, the precise mechanisms about how ketamine regulates miR-98-5p expression and how miR-98-5p affects the progression of depression still require future exploration.

REFERENCES

1. Yang C, Yang J, Luo A, Hashimoto K. Molecular and cellular mechanisms underlying the antidepressant effects of ketamine enantiomers and its meta-
2. Corrigger A, Pickering G. Ketamine and depression: a narrative review. Drug Des Devel Ther. 2019;13:3051–67.
3. Yang C, Shirayama Y, Zhang JC, Ren Q, Yao W, Ma M, et al. R-ketamine: a rapid-
4. Potter DE, Choudhury M. Ketamine: repurposing and redefining a multifaceted drug. Drug Disc Today. 2014;19:1848–54.
5. O’Connor RM, Grehnem S, Dinan TG, Cryan JF. microRNAs as novel antidepressant targets: converging effects of ketamine and electroconvulsive shock therapy in the rat hippocampus. Int J Neuropsychopharmacol. 2013;16:1885–92.
6. Issler O, Haramati S, Paul ED, Maeno H, Navon I, Zwang R, et al. MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact ser-
7. Baudry A, Mouillet-Richard S, Schneider B, Launay JM, Kellermann O. miR-16 targets the serotonin transporter: a new facet for adaptive responses to anti-
8. Lopez JP, Fiori LM, Cruceanu C, Lin R, Labonte B, Cates HM, et al. MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. Nat Commun. 2017;8:15497.
9. Wan YO, Feng JG, Li M, Wang MZ, Li L, Liu X, et al. Prefrontal cortex miR-29b-3p plays a key role in the antidepressant-like effect of ketamine in rats. Exp Mol Med. 2018;50:1–4.
10. Yang X, Yang Q, Wang X, Luo C, Wan Y, Li J, et al. MicroRNA expression profile and functional analysis reveal that miR-206 is a critical novel gene for the expression of BDNF induced by ketamine. Neurochemical Med. 2014;16:594–605.
11. Mao J, Li T, Fan D, Zhou H, Feng J, Liu L, et al. Abnormal expression of mco-
12. Yang C, Qu Y, Abe M, Nozawa D, Chaki S, Hashimoto K. (R)-Ketamine shows
13. Yang C, Cu Y, Abe M, Nozawa D, Chaki S, Hashimoto K. (R)-Ketamine shows greater potency and longer lasting antidepressant effects than its metabolite (2R,6R)-hydroxynorketamine. Biol Psychiatry. 2017;82:e3–4.
14. Yang C, Qu Y, Fujita Y, Ren Q, Ma M, Dong C, et al. Possible role of the gut microbe-brain axis in the antidepressant effects of (R)-ketamine in a social defeat model. Transl Psychiatry. 2017;7:1294.
15. Yang C, Ren Q, Qu Y, Zhang JC, Ma M, Dong C, et al. Mechanistic target of rapamycin-independent antidepressant effects of (R)-ketamine in a social defeat model. Biol Psychiatry. 2018;83:18–28.
16. Zhang K, Yang C, Yang C, Chang L, Sakamoto A, Suzuki T, Fujita Y, et al. Essential role of microglial transforming growth factor-beta1 in antidepressant actions of (R)-ketamine and the novel antidepressant TGF-beta1. Transl Psychiatry. 2020;10:32.
17. Tavakolizadeh J, Roshanaei K, Salmaninejad A, Yari R, Nahand JS, Sarkarzi HK, et al. MicroRNAs and exosomes in depression: potential diagnostic biomarkers. J Cell Biochem. 2018;119:3783–97.
18. Gruzdev SK, Yakovlev AA, Drushkova TA, Guekht AB, Gulyaeva NV. The missing link: how exosomes and miRNAs can help in bridging psychiatry and molecular biology in the context of depression, bipolar disorder and schizophrenia. Cell Mol Neurobiol. 2019;39:729–50.
19. Penner-Goeke S, Binder EB. Epigenetics and depression. Dialogues Clin Neurosci. 2019;21:397–405.
20. Maffioletti E, Cattaneo A, Rosso G, Maina G, Maj C, Gennarelli M, et al. Peripheral whole blood microRNA alterations in major depression and bipolar disorder. J Affect Disord. 2016;200:250–8.
21. Wang SS, Mu RH, Li CF, Dong SQ, Geng D, Liu Q, et al. microRNA-124 targets glucoorticoid receptor and is involved in depression-like behaviors. Prog Neuropsychopharmacol Biol Psychiatry. 2017;79:417–25.
22. Deng ZF, Zheng HL, Chen JG, Luo Y, Xu XF, Zhao G, et al. miR-214-3p targets beta-
23. Chen H, Vandorpe DH, Xie Y, Alper SL, Zeidel ML, Yu W. Disruption of Cav1.2-mediated signaling is a pathway for ketamine-induced pathology. Nat Commun. 2020;11:4328.