The European Journal of Psychiatry 37 (2023) 15—23

THE EUROPEAN JOURNAL OF PSYCHIATRY

www.elsevier.es/ejpsy

ORIGINAL ARTICLE

Relationship between the expression level of miRNA-4485 and the severity of depressive symptoms in major depressive disorder patients

Hong-tao Song[a,b], Xin-yang Sun[c], Wei Niu[d], Qiao-li Zhang[e], Li-yi Zhang[f,*], Ai-fang Zhong[g]

[a] *School of Mental Health, Bengbu Medical College, Bengbu, Anhui 233030, People's Republic of China*
[b] *Department of Mental Health, Bengbu First People's Hospital, Bengbu, Anhui 233030, People's Republic of China*
[c] *Shanghai United Family Xintiandi Clinic, Shanghai 200051, People's Republic of China*
[d] *Department of Rehabilitation, No. 904 Hospital of Joint Logistics Unit, Changzhou, Jiangsu 213003, People's Republic of China*
[e] *Third Affiliated Hospital of Soochow University, Changzhou First People's Hospital, Changzhou, Jiangsu 213003, People's Republic of China*
[f] *Prevention and Treatment Center for Psychological Diseases, No. 904 Hospital of Joint Logistics Unit, Peace Road 55, Changzhou, Jiangsu 213003, People's Republic of China*
[g] *Department of Laboratory, No. 904 Hospital of Joint Logistics Unit, Changzhou, Jiangsu 213003, People's Republic of China*

Received 27 August 2021; accepted 22 September 2022
Available online 9 October 2022

KEYWORDS
Major depression disorder;
Depressive symptoms;
miRNA-4485;
Biomarker;
Antidepressant treatment

Abstract
Background and objectives: Despite the growing pieces of evidence on the relationship between the altered expression level of miRNAs and major depressive disorder (MDD), few studies have focused on the relationship between the altered expression of miRNAs and the severity of depressive symptoms. This study aimed to investigate the relationship between the expression level of miRNA-4485 and the severity of depressive symptoms in major depressive disorder (MDD) patients.
Methods: Eighty MDD patients without antidepressants and 45 healthy controls were placed and tested for the expression level of miRNA-4485 using quantitative RT–PCR. At the same time, the Hamilton Depression Scale (HAMD) was used to assess depression symptoms for MDD patients. Twenty-nine out of 80 MDD patients were selected for miRNA expression level testing and symptomatology assessments before and after three weeks of treatment.
Results: The expression level of miRNA-4485 in the MDD group was significantly overexpressed compared to that in healthy controls ($P < 0.05$), and the expression level of miRNA-4485 in the higher HAMD group was also much higher than that in the lower HAMD group and healthy controls ($P < 0.05$). The expression level of miRNA-4485 in MDD patients was negatively correlated with HAMD total score, anxiety/somatization, and bodyweight factor score ($P < 0.05$), accounting for 9.4%, 12.4% and 5.7%, respectively. MiRNA-4485 significantly predicted MDD and the severity of depressive symptoms ($P < 0.05$). Compared with that before treatment, the expression level of

* Corresponding author.
E-mail address: zly102@126.com (L.-y. Zhang).

https://doi.org/10.1016/j.ejpsy.2022.09.005

miRNA-4485 was significantly downregulated after treatment, while the patient's depressive symptoms were improved ($p < 0.05$). The improvement in depressive symptoms was positively correlated with the downregulation of miRNA-4485, which could significantly predict the effects of antidepressant treatment on MDD ($P < 0.05$).

Conclusion: MiRNA-4485, which is significantly related to depressive symptoms and its improvement, could be a valuable biomarker or drug target to predict MDD, the severity of depressive symptoms and the effects of antidepressant treatment on MDD.

Introduction

The World Health Organization reports that the global incidence of major depressive disorder (MDD) is approximately 5% to 19%, with a total of approximately 300 million people with depression.[1,2] MDD not only brings great pain to the individual and family but also places a considerable burden on society.[3] Presently, the etiology of MDD is unknown, and there are no biological markers for the diagnosis and treatment of depression. Although the etiology of MDD is not clear, many studies have consistently shown that genetic risk factors play an integral role in the pathogenesis of MDD. Compared with those for schizophrenia (70%-80%), the heritability estimates (∼40%) of MDD are lower.[4,5] This indicates that other factors may also play a role in MDD, such as the external environment, being under pressure and experiencing adverse life events. Recently, it has been suggested that epigenetics plays an important role in the pathogenesis of MDD, which may be driven by genetic and environmental factors.[6,7] MiRNAs, as prominent members of epigenetics, have strong developmental sequence specificity and tissue specificity.[8,9] The expression of some miRNAs changes with cell proliferation, regional differentiation and the establishment of conduction pathways during the formation of the cortex.[10,11] Therefore, miRNAs play an essential role in the development and functional regulation of the brain. Some studies have shown that miRNAs are not only involved in the regulation of a variety of neural functions but also related to synaptic plasticity.[12,13] Large-scale miRNA microarray analysis of brain tissue indicated that compared with normal subjects, 17% of miRNAs were significantly downregulated in the prefrontal cortex in the depressive suicide population.[14] A study reported that miRNA expression was altered in the prefrontal cortex of patients with bipolar depression.[15] Among them, miRNA-504, miRNA-145, miRNA-145*, miRNA-22*, miRNA-133B, miRNA-154* and miRNA-889 expression was upregulated, while miRNA-104-3p, miRNA-29a, miRNA-32, miRNA-454*, miRNA-874 and miRNA-520c-3p expression was obviously downregulated.[15] Changes in miRNA expression levels participate in the occurrence and development of MDD by affecting the expression of many neural-related genes in the brain. A study on 314 MDD patients and 252 normal controls reported that different DGCR8rs3757 alleles were associated with a high tendency of depression suicide and a high response to depression treatment, while AGO1rs636832 was related to a decrease in the high suicide risk of MDD and a reduction in the MDD treatment response.[16] Thus, miRNAs were associated with depressive symptoms, and some of them were involved in the pathogenesis of MDD through corresponding targets.

In our previous study, we found that 26 miRNAs (21 upregulated miRNAs and 5 downregulated miRNAs) were significantly differentially expressed between MDD patients and healthy controls by microarray analysis.[17] With the purpose of further validation in a larger sample, 9 upregulated miRNAs (miRNA-26b, miRNA-29b, miRNA-146b, miRNA-1244, miRNA-4485, miRNA-1972, miRNA-4498, miRNA-4743, and miRNA-874) and 1 downregulated miRNA (miRNA-338) chosen from the 26 differentially expressed miRNAs were compared in 81 MDD patients and 46 healthy controls. In the results, the expression levels of 5 miRNAs (miRNA-1972, miRNA-26b, miRNA-4485, miRNA-4498, and miRNA-4743) were significantly different, suggesting that these 5 abnormal miRNAs closely associated with MDD could be used as biological markers to diagnose MDD. Although much evidence indicates that miRNAs play a vital role in the occurrence and development of MDD, there has been little research analyzing the relationship between the abnormal expression of miRNAs and the depressive symptoms of MDD patients.

Thus, we hypothesize that abnormal miRNAs are associated with depressive symptoms and that regulation of miRNAs is correlated with the improvement of depressive symptoms, and some of them can be used as biomarkers or drug targets to judge the severity and effects of antidepressant treatment of MDD. Based on our previous research, miRNA-4485 was selected for further study to analyze the relationship between miRNA-4485 and depressive symptoms and its antidepressant response to provide a basis for further elucidating the role of miRNA in the development and recovery of MDD in this study.

Materials and methods

Participants

Eighty MDD patients aged 18 to 68 years who met the diagnostic criteria of depression in the Diagnostic and Statistical Manual 4th edition (DSM-IV) were enrolled as the experimental subjects. All patients were first-episode patients treated without antidepressants or antipsychotics or relapsed within at least 3 months without antidepressants or antipsychotics. The patients had no history of other mental diseases, physical or nervous system diseases such as brain trauma, mental retardation, alcoholism or drug abuse. In addition, patients who received electroconvulsive therapy for less than six months or were given a blood transfusion in one month were excluded. The subjects of this study provided informed

consent. This research was approved by the local ethics review committee.

Hamilton depression scale

The Hamilton Depression Scale (HAMD) is a commonly used scale for MDD that is used to measure the severity of depressive symptoms. This research used the 24-item version of HAMD. It includes seven dimensions, including anxiety/somatization, body weight, cognitive disorder, day and night change, retardation, sleep disorder and hopelessness. According to the division boundary of Davis JM, if the score exceeds 35, it may be severe depression; if the score exceeds 20, it may be mild or moderate depression. If the score is less than 8, the patient will not have depressive symptoms. In this research, the Chinese version of this scale, which has been naturalized and verified for its reliability and validity, was used.[18]

Blood collection and RNA extraction

Samples of whole blood (5 ml) were collected from each object by an EDTA anticoagulant tube and processed within 3 h at 7 a.m. the next day after enrollment. Peripheral blood mononuclear cells (PBMCs) separated from the blood by using Ficoll density centrifugation were transferred into a fresh RNase/DNase-free 2 ml microcentrifuge tube and stored at -80 °C until use. According to the manufacturer's protocol, total RNA extracted from the PBMCs by TRIzol reagent (Invitrogen®, USA) was quantified by a NanoDrop ND-2100 (Thermo Scientific). RNA integrity was assessed using an Agilent 2100 (Agilent Technologies). To ensure a robust analysis for the following procedures, samples with an RNA integrity number (RIN) less than 8 were excluded.[17]

Real-time quantitative reverse-transcription PCR (qRT–PCR)

According to previous studies, miRNA-4485 was chosen for further validation with real-time quantitative reverse transcription polymerase chain reaction (qRT–PCR). Blood samples from 80 MDD patients were utilized to validate the findings from miRNA profiling. Total RNA was isolated from the PBMCs using TRIzol reagent (Invitrogen®, USA) for quantitative detection of miRNA. Complementary DNA was synthesized using the Reverse Transcription TaqMan MiRNA Reverse Transcription Kit and miRNA-specific stem–loop primers in accordance with the manufacturer's instructions. Each RT reaction consisted of 5μL of total RNA, 0.15 μL dNTPs with dTTP (100 mM), 1.00 μL Multiscribe RT enzyme (50 U/μL), 1.5 μL 10 $\times$ RT Buffer, RNase Inhibitor 0.19 μL (20 U/μL), Nuclease-free water 4.16 μL, 3 μL TaqMan MicroRNA Assays, in a total volume of 15 μL. Reactions were performed under the following conditions: 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C, and held at 4 °C. Real-time PCR was accomplished by Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems, Inc., USA), with a 10 μL PCR mixture that included 2 μL of the cDNA, 5 μL of 2 $\times$ TaqMan Universal Master Mix II (Applied Biosystems, CA.), 0.5 μL of TaqMan MicroRNA Assays (Applied Biosystems, CA), and 2.5 μL of double distilled water. PCRs in a 384-well plate were run at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Each sample was tested in triplicate. The miRNA-specific stem–loop primers were provided by TaqMan MicroRNA Assays (Applied Biosystems, CA.) on the basis of the microRNA sequences obtained from the miRBase database. SDS 2.4 software was used to collect the data. Using RNU48 as a reference for normalization, the expression levels of miRNA-4485 were calculated by the fold change (FC) using the $2^{-\Delta\Delta CT}$ method.[17,19]

Medication intervention

Using systematic random sampling, 29 subjects were selected from 80 MDD patients for clinical intervention observation. Antidepressant treatment was applied, including sertraline hydrochloride with a daily dosage range from 100 to 200 mg. MiRNA-4485 was detected, and the HAMD scale was evaluated before and after three weeks of antidepressant treatments.

Statistical analysis

All data were processed by SPSS v26.0 and GraphPad Prism 5. Real-time quantitative PCR data were collected by SDS 2.4 software. The Mann–Whitney U test, Kruskal–Wallis H test, independent sample t test and chi-square test were used to test the differences in miRNA-4485 and demographic variables between the higher and lower HAMD group, MDD group and healthy controls. Spearman's correlation test was carried out to test the relationship between the miRNA-4485 expression level and depressive symptoms. Regression analysis was performed to determine the accountability of miRNA-4485 for depressive symptoms. Finally, an ROC curve was established to test the predictability of miRNA-4485 for depressive disorder, the severity of depressive symptoms and the effects of antidepressant treatment for MDD. All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Comparisons of demographic variables

There were no differences in age, sex, residence, educational level or marital status between the MDD group and healthy controls ($P > 0.05$, Table 1). The HAMD total score of MDD in this study ranged from 20 to 49. According to the criteria for severe depression, participants whose HAMD total score was lower than 35 were allocated to the lower HAMD group ($n = 45$, HAMD total score: 22.91 $\pm$ 2.30), while those whose HAMD total score was higher than or equal to 35 were allocated to the higher HAMD group ($n = 35$, HAMD total score: 36.94 $\pm$ 2.57). As shown in Table 1, there were no differences in age, sex, residence, educational level or marital status between the higher and lower HAMD groups ($P > 0.05$, Table 1).

miRNA-4485 expression in the MDD group, healthy controls, and higher and lower HAMD groups

As shown in Fig. 1, the Mann–Whitney U test results revealed that the expression level of miRNA-4485 in the MDD group

Table 1 Comparisons of demographic variables.

Group	Age	Gender		Residence		Educational level		Marital status	
		Girls	Boys	Urban	Rural	Junior high and below	Senior high and above	Married	Unmarried and divorce
MDD group	33.710 ± 15.244 (15–68)	47	33	47	33	40	40	46	34
Healthy controls	32.600 ± 14.739 (15–65)	22	23	20	25	23	22	26	19
t/x^2	-0.396	1.132		2.370		0.014		0.001	
P	0.693	0.287		0.124		0.905		0.976	
Lower HAMD group	34.340 ± 15.094 (15–67)	27	18	30	15	20	25	24	21
Higher HAMD group	33.220 ± 15.512 (15–67)	20	15	17	18	20	15	22	13
t/x^2	0.324	0.066		2.660		1.270		0.731	
P	0.747		0.797	0.103		0.260		0.393	

(FC = 1.667) was significantly overexpressed compared to that in the healthy control group (FC = 1.000) (Z = -2.268, P = 0.023, Fig. 1 A). The expression level of miRNA-4485 was significantly different between the higher HAMD group, lower HAMD group and healthy controls by Kruskal–Wallis H Test (H = 10.589, p = 0.005). Furthermore, post hoc test showed that the expression level of miRNA-4485 in the higher HAMD group(FC = 2.223) was significantly overexpressed as compared to the lower HAMD group(FC = 1.234, H = -19.051, p = 0.020) and healthy controls(FC = 1.000,H = -26.029, p = 0.001,Fig. 1 B), while there was no significant difference between the lower HAMD group and healthy controls(H = -6.978, P = 0.361,Fig. 1 B).

Correlation between abnormal expression of miRNAs-4485 and depressive symptoms

The results of Spearman correlation analysis of the expression of miRNA-4485 and depression symptoms in MDD patients demonstrated that the total score of HAMD was negatively correlated with the expression level of miRNA-4485 (r = -0.398, P = 0.007). The factor score of anxiety/somatization was negatively correlated with the expression level of miRNA-4485 (r = -0.356, P = 0.001). The factor score of body weight was negatively correlated with the expression level of miRNA-4485 (r = -0.371, P = 0.015), while there was no significant correlation between other factors of HAMD and miRNA-4485 ($P > 0.05$).

Stepwise regression analysis of the effects of miRNA-4485 expression on depressive symptoms

Taking the expression level of miRNA-4485, age, sex, residence, educational level and marital status as independent variables and the HAMD total score, anxiety/somatization and body weight as dependent variables, stepwise regression analysis was carried out. As Table 2 indicates, miRNA-4485 was entered into the regression function with HAMD total score and anxiety/somatization as the independent variable, accounting for 9.4%, 12.4% and 5.7%, respectively.

Prediction of depressive disorder and its severity by miRNA-4485

The expression level of miRNA-4485 was taken as the independent variable, and the group of higher and lower HAMD (0 represents the lower HAMD group and 1 represents the higher HAMD group) was taken as the dichotomous dependent variable. Stepwise logistic regression analysis was performed. The results revealed that miRNA-4485 was entered into the regression function, accounting for 7.5% of the severity of depression symptoms. The odds ratio determined by the higher HAMD group against the lower HAMD group was 0.829.

ROC curves were also established using the expression level of miRNA-4485 as the testing variable and the higher and lower HAMD groups, MDD group and healthy controls as state variables. As shown in Fig. 2, the results revealed that the expression level of miRNA-4485 could significantly predict MDD (AUC = 0.623, p = 0.023, 95% CI 0.519-0.726), especially severe MDD (AUC = 0.714, p = 0.001, 95% CI 0.603 −0.826). The expression level of miRNA-4485 could also

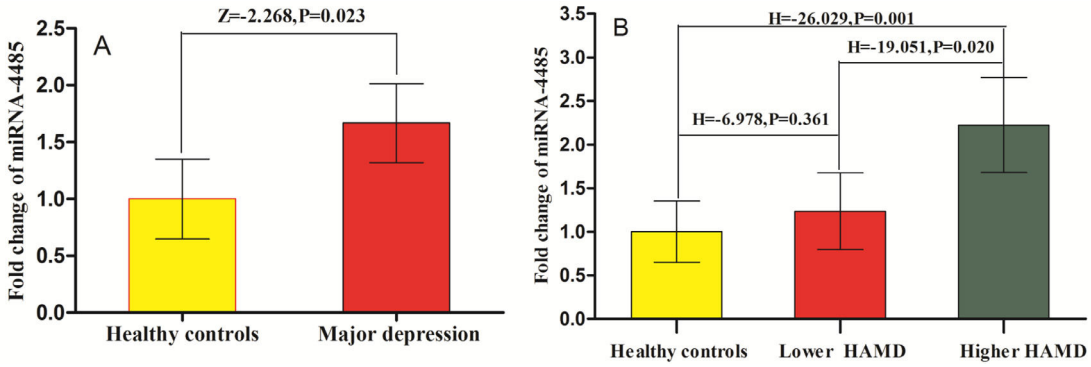

Fig. 1 The comparison of miRNA-4485 between the MMD group, Healthy controls, Lower and Higher HAMD groups.

Table 2 Stepwise regression analysis of the effects of miRNA-4485 expression upon depressive symptoms.

Dependent variables	Regression model	B	SE	β	ΔR^2	t value	*P* value
HAMD total score	constant	23.619	0.788	—	—	29.980	<0.001
	miRNA-4485	-0.486	0.160	-0.325	0.094	3.033	0.003
Anxiety/somatization	constant	4.868	0.393	—	—	12.372	<0.001
	miRNA-4485	-0.279	0.080	-0.367	0.124	3.489	0.001
Body weight	constant	0.833	0.100	—	—	8.330	<0.001
	miRNA-4485	-0.049	0.020	-0.262	0.057	-2.402	0.019

significantly predict the severity of depressive symptoms (AUC = 0.646, $p = 0.025$, 95% CI 0.526—0.766).

Correlation between the downregulation of miRNA-4485 expression and symptom improvement

After three weeks of antidepressant treatments, the miRNA-4485 expression level significantly decreased (FC = 0.384, $Z = -2.930$, $P = 0.003$, Fig. 3 A), while the total HAMD score was significantly lower than that before treatment ($t = 7.364$, $P < 0.001$, Fig. 3 B). Symptom improvements were measured by the differences in HAMD total scores before and after treatment. The downregulation of miRNA-4485 expression was measured by the difference in miRNA-4485 expression levels before and after treatment. The results of Spearman correlation analysis demonstrated that the downregulation of miRNA-4485 was positively correlated with the improvement of depressive symptoms ($r = 0.451$, $p = 0.014$).

MiRNA-4485 downregulates the accountability of symptom improvement and predictability of treatment effects

To explore miRNA-4485 as a predictive marker for the effects of antidepressant treatment on MDD symptoms, 29 MDD patients were divided into higher and lower treatment effect subgroups by HAMD score reduction rate (Spre-Spost/Spre, where Spre represents the premedication score and Spost represents the postmedication score). The patients whose

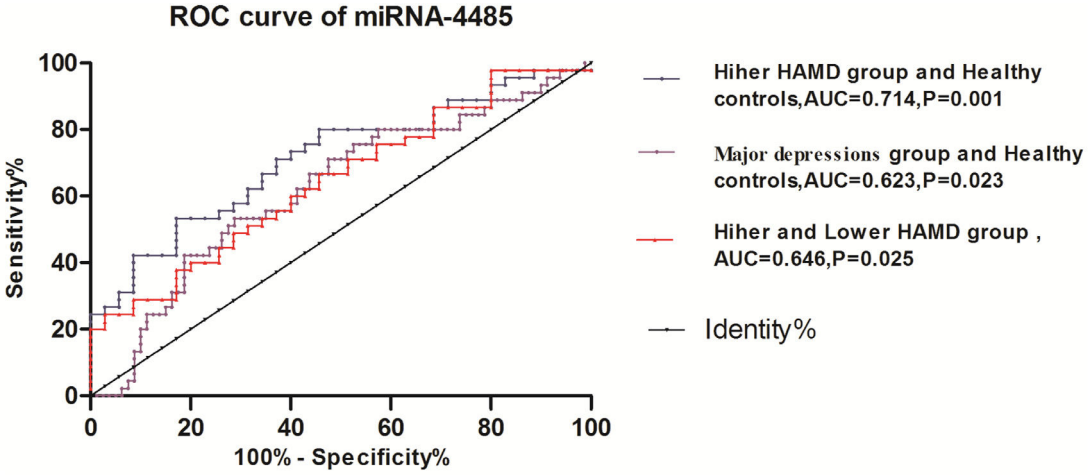

Fig. 2 Prediction of depressive disorder and its severity by miRNA-4485, AUC=area under the curve, miRNA=microRNA.

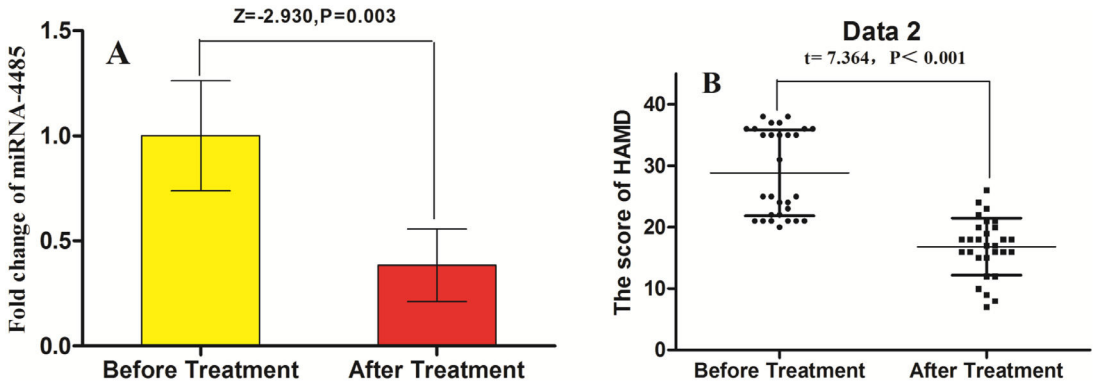

Fig. 3 Comparison of miRNA-4485 expression levels and depressive symptoms before and after treatments.

HAMD score reduction rate was equal to or greater than 50% were categorized into the higher treatment effect group, while the patients whose HAMD score reduction rate was less than 50% were categorized into the lower treatment effect group. As shown in Fig. 4, the miRNA-4485 downregulation of the higher treatment effect group was much higher than that of the lower treatment effect group (Fig. 4 A), which could significantly predict the effects of antidepressant treatment on MDD (Fig. 4 B).

Discussion

Studies have shown that miRNAs, as prominent epigenetic regulators, participate in a series of important pathophysiological processes, including the pathogenesis of depression.[6] In this research, we found that the expression level of miRNA-4485 in the MDD group was significantly overexpressed compared with that in healthy controls. MiRNA-4485 could be a valuable biomarker to predict MDD. Deregulation of miRNAs could affect various cellular and molecular targets involved in depression pathogenesis.[20] Among various types of biomarkers, miRNAs have emerged as powerful tools to diagnose patients with depression.[21] Based on a study of 1088 MDD patients and 1102 healthy controls, Xu found a statistically significant positive association between miRNA-30ess178077483 and MDD.[22] These findings suggested that miRNA polymorphisms might play a significant role in MDD susceptibility and implied that miRNAs might be involved in the etiology of MDD.

The altered expression of miRNAs in plasma and brain tissue might be one of the mechanisms of MDD. According to Fan, miRNA-1972, miRNA-26b, miRNA-4743, miRNA-4498, and miRNA-4485 in plasma were differentially expressed between MDD patients and healthy controls and could be used as biomarkers to diagnose MDD.[17] Nevertheless, few studies have explored the relationship between the differentially expressed miRNAs and depressive symptoms. In this study, the expression level of miRNA-4485 in the higher HAMD group was significantly overexpressed compared with that in the lower HAMD group. In comparison, there was no significant difference between the lower HAMD group and healthy controls. The total HAMD score and the anxiety/somatization and body weight factor scores were negatively correlated with the expression level of miRNA-4485. Consequently, miRNA-4485 is significantly related to depressive symptoms and could be a useful biomarker to predict depressive disorder and the severity of depressive symptoms. A high proportion of MDD patients present the symptoms of abnormal timing of sleep and wakefulness. Interestingly, these manifestations of abnormal circadian function return to normality with antidepressant or mood stabilizer treatment and patient recovery.[23] According to research on 359 MDD patients and 341 healthy controls, Ester found that miRNA-182 rs76481776 was related to late insomnia in MDD patients. MDD patients carrying the T allele

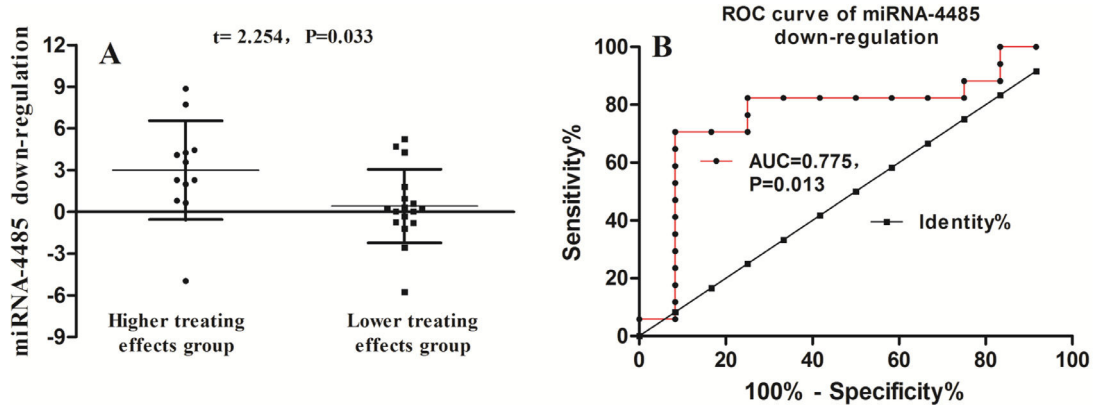

Fig. 4 MiRNA-4485 downregulates the accountability of symptom improvement and predictability of treatment effects.

had a higher risk of presenting late insomnia.[24] Suicidal behavior, a significant cause of death and morbidity worldwide, is a severe and frequent symptom of MDD.[25—27] Compared with nonpsychiatric controls who died of other causes, global miRNA showed considerably decreased expression in the prefrontal cortex of depressed suicide completers. MiRNA-185 was reported to regulate TrkB-T1 linked to suicidal behavior upon truncation.[28] The changes in miRNA-4485 before and after antidepressant treatments in patients with MDD were also reported to be related to suicidal ideation.[29]

Further study showed that downregulation of miRNA-4485 was positively correlated with the improvement of depressive symptoms and could significantly predict the effects of antidepressant treatment on MDD. Serotonin (5-HT) receptor, corticosteroid receptor (GR) and brain-derived neurotrophic factor (BDNF) are considered to be closely related to the pathogenesis of MDD.[30] A large number of studies have shown that miRNA-96, miRNA-196, miRNA-195, miRNA-15A and miRNA-15b can affect the expression of 5-HT receptors, and miRNA-18a and miRNA-124a can affect the development of hippocampal neurons by affecting the expression of glucocorticoid receptor (GR).[31,32] MiRNA-30a and miRNA-195 influence the expression of brain-derived neurotrophic factor (BDNF).[33] Selective serotonin reuptake inhibitors (SSRIs), as first-line agents for antidepressive therapy, could improve synaptic 5-HT by affecting the targets of the serotonin transporter (SERT). Baudry found that SERT was the target of miRNA-16 in a rat model of depression.[34] After long-term treatment with fluoxetine, the expression of miRNA-16 increased in the raphe nucleus. The antidepressant effect of fluoxetine was similar to that of directly injecting miRNA-16 into the raphe nucleus. Further research found that miRNA-16 in human tissues can also inhibit the expression of SERT.[35,36] Fluoxetine promoted the transformation of pre/pri-miRNA-16 to miRNA-16 by activating glycogen synthase kinase-3 (GSK-3), and the expression of SERT was reduced with the upregulation of miRNA-16 in the raphe nucleus. Thus, the inhibition of SERT by upregulation of miRNA-16 induced by fluoxetine reduced the reuptake of synaptic 5-HT, which may be the antidepressive mechanism of fluoxetine. In summary, miRNAs could affect the expression of the 5-HT receptor and improve synaptic 5-HT by affecting the targets of the serotonin transporter (SERT).

Based on our previous research, we found that the downregulation of miRNA-1972, miRNA-4485, miRNA-4498 and miRNA-4743 was positively correlated with the improvement of retardation symptoms after antidepressant treatments.[37] The downregulation of miRNA-26b was negatively correlated with the improvement of day and night change symptoms. Our previous research indicated that miRNA-4743, miRNA-4498, miRNA-4485, miRNA-1972 and miRNA-26b might be therapeutic targets for MDD treatment but did not deeply analyze the relationship between the expression level of miRNAs and the severity of depressive symptoms. In this study, we found that the miRNA-4485 expression level significantly decreased, while the total HAMD score was significantly lower than that before treatment. The downregulation of miRNA-4485 was positively correlated with the improvement of depressive symptoms, which could significantly predict the effects of antidepressant treatments on MDD. Many reports have found that miRNAs may be targets of antidepressants in the treatment of depression. Lope's research showed that miRNA-146a-5p, miRNA-146b-5p, miRNA-425-3p and miRNA-24-3p are markers of the antidepressant response by regulating mitogen-activated protein kinase (MAPK)/Wnt-system genes.[38] In addition, miRNA-1202 could predict the antidepressant response by regulating the expression of the metabotropic glutamate receptor 4 (GRM4) gene.[39] Granulocyte colony-stimulating factor (G-CSF), as a promoter of neuronal plasticity, may play a biological role in depression-like behaviors.[40] Compared with healthy controls, the serum level of G-CSF in adolescents with MDD was much higher. After four weeks of treatment with fluoxetine, the expression level of G-CSF in serum significantly decreased.[41] G-CSF may be the target of antidepressants and may be associated with depressive symptoms. G-CSF regulates the phenotypic changes of NK cells with 40% downregulation of NKp46.[42] Compared with healthy controls, the expression of NK cells in patients with depression is abnormal.[43] The levels of NKp46-positive NK cells in patients with depressive symptoms were significantly lower than those in the control group.[44] Interestingly, hsa-miRNA-4485-5p was reported to regulate NKp46 expression by linc-EPHA6-1.[45] We assume that NKp46 may be the key target gene of hsa-miRNA-4485-5p and G-CSF. Hsa-miRNA-4485-5p and G-CSF may play an important role in the pathogenesis of depression by regulating NKp46. Our findings provide additional evidence that the aberrant expression of miRNAs may play an important role in the development and recovery of depressive symptoms.

In conclusion, miRNA-4485 had a significant association with depressive symptoms. The expression of miRNA-4485 was significantly different in the higher and lower HAMD groups, MDD group and healthy controls. Abnormal expression of miRNA-4485 might be closely connected with the severity of depressive symptoms. The expression of miRNA-4485 and depressive symptoms changed during antidepressant treatment. The downregulation of miRNA-4485 was positively correlated with the improvement of depressive symptoms. MiRNA-4485 could be a useful biomarker or drug target to predict depressive disorder, the severity of depressive symptoms and the effects of antidepressant treatment on MDD.

Limitations

This study found a close relationship between the severity of depressive symptoms and the expression level of miRNA-4485, and the downregulation of miRNA-4485 was positively correlated with the improvement of depressive symptoms. Nevertheless, we did not analyze their internal mechanism. According to our findings, although miRNA-4485 could predict the severity of depressive symptoms and the effects of antidepressant treatment, using miRNA-4485 as a biomarker or drug target to judge the severity and the effects of antidepressant treatment on MDD in the clinic needs further research.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Declaration of Competing Interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical considerations

This study was conducted anonymously. Informed consent was obtained from the researcher before the study, and the researcher was allowed to withdraw. All participants in the study provided a written informed consent form. The study was approved by the local institutional review committee.

References

1. Andrade L, Caraveo-Anduaga JJ, Berglund P, et al. The epidemiology of major depressive episodes: results from the international consortium of psychiatric epidemiology (ICPE) Surveys. Int J Methods Psychiatr Res. 2003;12(1):3—21. https://doi.org/10.1002/mpr.138.
2. Vos T, Flaxman AD, Naghavi M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990—2010: a systematic analysis for the global burden of disease study 2010. Lancet. 2012;380(9859):2163—96. https://doi.org/10.1016/S0140-6736(12)61729-2.
3. Andersen I, Thielen K, Bech P, Nygaard E, Diderichsen F. Increasing prevalence of depression from 2000 to 2006. Scand J Public Health. 2011;39(8):857—63. https://doi.org/10.1177/1403494811424611.
4. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 2003;60(12):1187—92. https://doi.org/10.1001/archpsyc.60.12.1187.
5. McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. Arch Gen Psychiatry. 2003;60(5):497—502. https://doi.org/10.1001/archpsyc.60.5.497.
6. Miao C, Chang J. The important roles of microRNAs in depression: new research progress and future prospects. J Mol Med. 2021;99(5):619—36. https://doi.org/10.1007/s00109-021-02052-8. (Berl).
7. Penner-Goeke S, Binder EB. Epigenetics and depression. Dialogues Clin Neurosci. 2019;21(4):397—405. https://doi.org/10.31887/DCNS.2019.21.4/ebinder.
8. Reinhart BJ, Slack FJ, Basson M, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000;403(6772):901—6. https://doi.org/10.1038/35002607.
9. Wheeler G, Ntounia-Fousara S, Granda B, Rathjen T, Dalmay T. Identification of new central nervous system specific mouse microRNAs. FEBS Lett. 2006;580(9):2195—200. https://doi.org/10.1016/j.febslet.2006.03.019.
10. Miska EA, Alvarez-Saavedra E, Townsend M, et al. Microarray analysis of microRNA expression in the developing mammalian brain. Genome Biol. 2004;5(9):R68. https://doi.org/10.1186/gb-2004-5-9-r68.
11. Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol. 2004;5(3):R13. https://doi.org/10.1186/gb-2004-5-3-r13.
12. Cheng HY, Papp JW, Varlamova O, et al. microRNA modulation of circadian-clock period and entrainment. Neuron. 2007;54(5):813—29. https://doi.org/10.1016/j.neuron.2007. 05.017.
13. Schratt GM, Tuebing F, Nigh EA, et al. A brain-specific microRNA regulates dendritic spine development. Nature. 2006;439(7074):283—9. https://doi.org/10.1038/nature04367.
14. Smalheiser NR, Lugli G, Rizavi HS, Torvik VI, Turecki G, Dwivedi Y. MicroRNA expression is down-regulated and reorganized in prefrontal cortex of depressed suicide subjects. PLoS One. 2012;7(3):e33201. https://doi.org/10.1371/journal.pone.0033201.
15. Kim AH, Reimers M, Maher B, et al. MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. Schizophr Res. 2010;124(1-3):183—91. https://doi.org/10.1016/j.schres.2010.07.002.
16. He Y, Zhou Y, Xi Q, et al. Genetic variations in microRNA processing genes are associated with susceptibility in depression. DNA Cell Biol. 2012;31(9):1499—506. https://doi.org/10.1089/dna.2012.1660.
17. Fan HM, Sun XY, Guo W, et al. Differential expression of microRNA in peripheral blood mononuclear cells as specific biomarker for major depressive disorder patients. J Psychiatr Res. 2014;59:45—52. https://doi.org/10.1016/j.jpsychires.2014.08.007.
18. Hamilton M. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol. 1967;6(4):278—96. https://doi.org/10.1111/j.2044-8260.1967.tb00530.x.
19. Smalheiser NR, Lugli G, Zhang H, Rizavi H, Cook EH, Dwivedi Y. Expression of microRNAs and other small RNAs in prefrontal cortex in schizophrenia, bipolar disorder and depressed subjects. PLoS One. 2014;9(1):e86469. https://doi.org/10.1371/journal.pone.0086469.
20. Torres-Berrío A, Lopez JP, Bagot RC, et al. DCC Confers susceptibility to depression-like behaviors in humans and mice and is regulated by miR-218. Biol Psychiatry. 2017;81(4):306—15. https://doi.org/10.1016/j.biopsych.2016.08.017.
21. Tavakolizadeh J, Roshanaei K, Salmaninejad A, et al. MicroRNAs and exosomes in depression: potential diagnostic biomarkers. J Cell Biochem. 2018;119(5):3783—97. https://doi.org/10.1002/jcb.26599.
22. Xu Y, Liu H, Li F, et al. A polymorphism in the microRNA-30e precursor associated with major depressive disorder risk and P300 waveform. J Affect Disord. 2010;127(1-3):332—6. https://doi.org/10.1016/j.jad.2010.05.019.
23. McClung CA. Circadian genes, rhythms and the biology of mood disorders. Pharmacol Ther. 2007;114(2):222—32. https://doi.org/10.1016/j.pharmthera.2007.02.003.
24. Saus E, Soria V, Escaramís G, et al. Genetic variants and abnormal processing of pre-miR-182, a circadian clock modulator, in major depression patients with late insomnia. Hum Mol Genet. 2010;19(20):4017—25. https://doi.org/10.1093/hmg/ddq316.
25. Innamorati M, Pompili M, Gonda X, et al. Psychometric properties of the Gotland scale for depression in Italian psychiatric inpatients and its utility in the prediction of suicide risk. J Affect Disord. 2011;132(1-2):99—103. https://doi.org/10.1016/j.jad.2011. 02.003.
26. Pompili M, Rihmer Z, Akiskal H, et al. Temperaments mediate suicide risk and psychopathology among patients with bipolar disorders. Compr Psychiatry. 2012;53(3):280—5. https://doi.org/10.1016/j.comppsych.2011.04.004.
27. Serafini G, Pompili M, Innamorati M, et al. The role of microRNAs in synaptic plasticity, major affective disorders and suicidal behavior. Neurosci Res. 2012;73(3):179—90. https://doi.org/10.1016/j.neures.2012.04.001.
28. Serafini G, Pompili M, Hansen KF, et al. The involvement of microRNAs in major depression, suicidal behavior, and related disorders: a focus on miR-185 and miR-491-3p. Cell Mol

Neurobiol. 2014;34(1):17—30. https://doi.org/10.1007/s10571-013-9997-5.
29. Kong LM, Yao GF, He MJ, Zhu XL, Zhang LY. Effects of antidepressants on auicide in depressive patients and its association with expression level of miRNA in peripheral blood mononuclear cells. J Prev Med Chin PLA. 2019;37(07):9—11. https://doi.org/10.13704/j.cnki.jyyx.2019.07.005.
30. Horowitz MA, Zunszain PA. Neuroimmune and neuroendocrine abnormalities in depression: two sides of the same coin. Ann N Y Acad Sci. 2015;1351:68—79. https://doi.org/10.1111/nyas.12781.
31. Dwivedi Y. Evidence demonstrating role of microRNAs in the etiopathology of major depression. J Chem Neuroanat. 2011;42 (2):142—56. https://doi.org/10.1016/j.jchemneu. 2011.04.002.
32. Mouillet-Richard S, Baudry A, Launay JM, Kellermann O. MicroRNAs and depression. Neurobiol Dis. 2012;46(2):272—8. https://doi.org/10.1016/j.nbd.2011.12.035.
33. Mellios N, Huang HS, Grigorenko A, Rogaev E, Akbarian S. A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. Hum Mol Genet. 2008;17(19):3030—42. https://doi.org/10.1093/hmg/ddn201.
34. Baudry A, Mouillet-Richard S, Schneider B, Launay JM, Kellermann O. miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. Science. 2010;329 (5998):1537—41. https://doi.org/10.1126/science.1193692.
35. Moya PR, Wendland JR, Salemme J, Fried RL, Murphy DL. miR-15a and miR-16 regulate serotonin transporter expression in human placental and rat brain raphe cells. Int J Neuropsychopharmacol. 2013;16(3):621—9. https://doi.org/10.1017/S1461145712000454.
36. Issler O, Haramati S, Paul ED, et al. MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. Neuron. 2014;83(2):344—60. https://doi.org/10.1016/j. neuron.2014.05.042.
37. Zhang QL, Lu J, Sun XY, et al. A preliminary analysis of association between plasma microRNA expression alteration and symptomatology improvement in major depressive disorder (MDD) patients before and after antidepressant treatment. Eur. J. Psychiat. 2014;28(4):252—64. https://doi.org/10.4321/S0213-61632014000400006.
38. Lopez JP, Fiori LM, Cruceanu C, 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. https://doi.org/10.1038/ncomms15497.
39. Lopez JP, Lim R, Cruceanu C, et al. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nat Med. 2014;20(7):764—8. https://doi.org/10.1038/nm.3582.
40. Li H, Linjuan-Li WY. G-CSF improves CUMS-induced depressive behaviors through downregulating Ras/ERK/MAPK signaling pathway. Biochem Biophys Res Commun. 2016;479(4):827—32. https://doi.org/10.1016/j.bbrc.2016.09.123.
41. Becerril-Villanueva E, Pérez-Sánchez G, Alvarez-Herrera S, et al. Alterations in the levels of growth factors in adolescents with major depressive disorder: a longitudinal study during the treatment with fluoxetine. Mediat Inflamm. 2019;2019:9130868. https://doi.org/10.1155/2019/9130868.
42. Schlahsa L, Jaimes Y, Blasczyk R, Figueiredo C. Granulocyte-colony-stimulatory factor: a strong inhibitor of natural killer cell function. Transfusion. 2011;51(2):293—305. https://doi.org/10.1111/j.1537-2995.2010.02820. x.
43. Goyal S, Srivastava K, Kodange C, Bhat PS. Immunological changes in depression. Ind Psychiatry J. 2017;26(2):201—6. https://doi.org/10.4103/ipj.ipj_22_18.
44. Kopecký J, Slováček L, Slováčková B, et al. Effect of Depressive mood on NK cells in patients with pancreatic tumor—pilot study. J Adv Med Med Res. 2016;14(11):1—8. https://doi.org/10.9734/BJMMR/2016/25200.
45. Li S, Zhu A, Ren K, Li S, Chen L. IFNβ-induced exosomal linc-EPHA6-1 promotes cytotoxicity of NK cells by acting as a ceRNA for hsa-miR-4485-5p to up-regulate NKp46 expression. Life Sci. 2020;257:118064. https://doi.org/10.1016/j.lfs.2020.118064.