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Original Article

Circulating miR-134 is a potential biomarker for diagnosis and monitoring of major depressive disorder

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Abstract: Aim: to investigate the diagnostic value of circulating miR-134 in major depressive disorder (MDD). Plasma miR-134 levels were determined in patients with MDD, bipolar disorder (BD), and schizophrenia (SCZ), as well as in healthy controls using quantitative real-time PCR. The diagnostic value of circulating miR-134 was assessed using receiver-operator characteristic (ROC) curve analysis. A chronic unpredictable mild stress (CUMS) rat model was established to evaluate the miR-134 expression pattern in serum and diseased brain tissues. Plasma miR-134 levels in MDD patients were significantly lower than those in healthy controls and patients with BD or SCZ. In addition, plasma miR-134 levels could be used to discriminate MDD patients from healthy controls (areas under the curve (AUC) = 0.901), healthy controls + patients with BD or SCZ (AUC = 0.864), patients with SCZ (AUC = 0.856), patients with SCZ or BD (AUC = 0.828), and patients with BD (AUC = 0.799), respectively. In CUMS rats, the miR-134 levels in plasma ($P < 0.001$), prefrontal cortex ($P = 0.031$), and hippocampus ($P = 0.006$) were consistently reduced compared with those in control group, with the exception of those in olfactory bulb ($P = 0.565$). Circulating miR-134 a potential biomarker for MDD diagnosis and monitoring.

Keywords: Major depressive disorder, biomarker, miR-134, chronic unpredictable mild stress

Introduction

Major depressive disorder (MDD) is one of the most prevalent neuropsychiatric disorders worldwide, resulting in heavy social and economic burdens [1]. The heritability of MDD is approximately 40%. However, the causal factors contributing to the other 60% remain unclear [2]. Currently, diagnosis and treatment monitoring of MDD primarily rely on depression-rating scales, such as Hamilton Rating Scale for Depression (HAM-D), self-report Beck Depression Inventory (BDI), and Beck-Rafaelsen Manic Scale (BRMS). Compared with objective laboratory-based testing, these rating scales subjectively assess patient symptoms, mental status, and clinical behaviors, thus increasing the risks of underdiagnosis, misdiagnosis, and delayed treatment adjustments [3]. Therefore, it is of great importance to identify potential biomarkers for early and accurate diagnosis and timely treatment adjustments for MDD.

MicroRNAs (miRNAs) are small, non-coding RNA molecules (19-25 nucleotides in length) that posttranscriptionally regulate gene expression by suppressing target mRNAs [4-6]. miRNAs have been found to regulate neural plasticity and higher brain functioning [7]. Accumulating evidence has demonstrated that miRNAs play important roles in neuropsychiatric diseases, including schizophrenia, autism, Parkinson's disease, Huntington's disease, Tourette's syndrome, Fragile X syndrome, DiGeorge syndrome, Down syndrome, and Alzheimer's disease [8]. Circulating miRNAs in the blood stream have been implicated in various diseases, such as cancers, mental disorders, and cardiovascular diseases [9-11], being increasingly recognized as promising biomarkers [12]. Recently, altered miRNA expression in post-mortem brain tissues and blood samples has been linked to MDD [13, 14]. However, the role of miRNAs in MDD development remains largely unknown.

Table 1. The demographic and clinical features were recorded for MDD patients (baseline and 8-week follow-up) and healthy volunteers in the 'MiR-134 expression pattern analysis' set

	HC (n = 35)	MDD (n = 35)		*P*-value	
		Baseline	8-week follow-up	HC vs. MDD (Baseline)	MDD (Baseline) vs. MDD (8-week follow-up)
Sex (male/female)‡	13/22	10/25		0.445	
Average age (years)†	34.4 ± 10.5	36.9 ± 12.7		0.378	
BMI	23.3 ± 3	22.9 ± 3.2	22.5 ± 3	0.563	0.606
HAM-D score†	0.9 ± 1	23.4 ± 3.7	12.9 ± 6.4	< 0.001	< 0.001
BDI score†	0.4 ± 0.6	19.6 ± 6	12.1 ± 8	< 0.001	< 0.001

‡Two-sided χ^2 test; †Independent-Sample Student's *t*-test. HC: healthy controls; MDD: major depressive disorder; BMI: body mass index; HAM-D: Hamilton Depression Rating Scale; BDI: self-reported Beck Depression Inventory.

Table 2. Antidepressant medication was recorded for MDD patients (baseline and 8-week follow-up) in the 'MiR-134 Expression Pattern Analysis' set

Drug Use (% of MDD patients)	Baseline (n = 35)	8-week follow-up (n = 35)
Heterocyclics/TCAs	0.0	0.0
SSRIs	40.0	74.3
SNRIs	5.7	8.6
MAOIs	0.0	0.0
Atypical antidepressants	28.6	65.7
Hypnotics	25.7	28.6
No drug	42.9	5.7

MDD: major depressive disorder; TCAs: tricyclic antidepressants; SSRIs: selective serotonin reuptake inhibitors; SNRIs: serotonin and norepinephrine reuptake inhibitors; MAOIs: monoamine oxidase inhibitors.

miR-134 is one of the tissue-specific miRNAs found in mouse brain [15]. A recent study suggests that miR-134 impairs synaptic plasticity in epilepsy through inhibiting LIM domain kinase 1 and cAMP-responsive element binding protein (CREB) [16]. Impaired synaptic plasticity is closely associated with various neurobiological disorders, including MDD [17-20]. Therefore, we hypothesize that miR-134 is related to development and progression of MDD.

In the present study, we compared the plasma expression pattern of miR-134 between MDD patients and healthy controls as well as in large cohorts of patients with MDD, schizophrenia (SCZ), or bipolar disorder (BD) [21]. The optimal cut-off plasma level of miR-134 was determined and tested for distinguishing MDD from healthy controls and other mental disorders with overlap symptoms. A chronic unpredictable mild stress (CUMS) rat model was established to explore whether the alteration in circulating miR-134 levels was consistent with that in local brain tissues. Our results identified circulating miR-134 as a potential biomarker for MDD diagnosis and therapeutic response monitoring.

Material and methods

Patients

This study was approved by The Ethics Committee of Chongqing Medical University (Chongqing, China). From August 2009 to November 2011, 100 patients with MDD and 50 patients with BD were recruited from the First Affiliated Hospital of Chongqing Medical University. 50 patients with SCZ were recruited from the Chongqing Municipal Psychiatric Hospital. A total of 100 healthy volunteers aged 16-80 years were recruited as healthy controls.

35 MDD patients and 35 healthy volunteers were randomly selected as a 'MiR-134 Expression Pattern Analysis' set (**Table 1**) to determine miR-134 levels in peripheral blood. Clinical features, including body mass index (BMI), HAM-D (17-item version) [22] score, and BDI (13-item version) [23] score, were evaluated. For MDD patients, medical information, including antidepressant medication, was recorded at baseline and at 8-week follow-up, respectively (**Table 2**).

An enlarged 'MiR-134 Expression Pattern Analysis' set (**Table 3**) including 100 healthy controls, 100 MDD, 50 BD, and 50 SCZ patients was used to further validate the differential miR-134 expression among these groups.

Table 3. The demographic and clinical features were recorded for MDD, BD and SCZ patients (baseline) and healthy volunteers in the enlarged 'MiR-134 Expression Pattern Analysis' set

	HC (n = 100)	MDD (n = 100)	BD (n = 50)	SCZ (n = 50)
Sex (male/female)	43/57	37/63	24/26	14/36
Average age (years)	32.4 ± 11.8	35.9 ± 11.5	29.8 ± 13.5	42.6 ± 11.2
BMI	23.1 ± 2.9	22.6 ± 3.2	22.3 ± 3.3	22.8 ± 3
HAM-D score	0.7 ± 0.9	22.9 ± 3.8	13.6 ± 9.5	
BRMS score			6.2 ± 8.1	
PANSS score				59 ± 14.5

HC: healthy controls; MDD: major depressive disorder; BD: bipolar disorder; SCZ: schizophrenia; BMI: body mass index; HAM-D: Hamilton Depression Rating Scale; BRMS: Bech-Rafaelsen Mania Scale; PANSS: Positive and Negative Syndrome Scale.

All diagnoses were performed according to the criteria in the 'Diagnostic and Statistical Manual of Mental Disorders' by at least two psychiatric experts. Inclusion criteria for all 3 types of mental illnesses were as follows: ① aged 16-80 years; ② meeting the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders; ③ first-episode patients who did not receive any treatment including psychotherapy. Additional inclusion criteria for MDD was HAM-D score ≥ 17. Exclusion criteria for all 3 types of mental illnesses were as follows: ① History of or current mental illnesses other than MDD, BD, or SCZ; ② Traumatic brain injury; ③ Severe or chronic physical diseases, including hypertension, diabetes, chronic bronchitis, pulmonary heart disease, active tuberculosis, asthma, coronary heart disease, cardiomyopathy, hepatitis, cirrhosis, pancreatitis, hyperthyroidism, hypothyroidism, diabetes, blood system diseases, immune system diseases, and tumors; ④ Abnormal blood test results; ⑤ A history of drug or substance abuse in the prior year. A written informed consent was obtained from each participant in this study.

Blood sample collection

Peripheral blood samples were collected at the first visit. For the MDD patients in the 'MiR-134 Expression Pattern Analysis' set, blood samples were also collected at 8-week follow-up. All the blood samples were collected in EDTA anticoagulant tubes in the morning (8:00 a.m.-12:00 p.m.), followed by centrifugation at 3000 g for 15 minutes to spin down the blood cells. The plasma was then transferred to a 1.5 ml RNase-free centrifuge tube and stored at -80°C until use.

CUMS model

Sixteen healthy adult male Sprague-Dawley rats weighing approximately 155 g were purchased from Chongqing Medical University Laboratory Animal Center (Chongqing, China) and were housed under standard laboratory conditions (12-h light/dark cycle, lights on from 7:00 a.m.-7:00 p.m.; temperature = 22 ± 1°C; relative humidity = 52 ± 2%) with free access to standard rodent chow and water. After a week of accommodation, all rats were trained to get accustomed to 1% sucrose solution (w/v) for 5 weeks (week 2-6). Two bottles of 1% sucrose solution or 1 bottle of 1% sucrose solution plus 1 bottle of water were randomly placed in each cage. The rats were randomly divided into CUMS and control groups (n = 8/group). During week 7-10, rats in the CUMS group were housed individually and subjected to stressors, including cage tilting (45°), 24-h light, daytime stroboscopic, horizontal vibration, water deprivation, watering with an empty bottle, wet bedding, and cohabitating. Rats in the control group were housed under the standard condition with free access to food and water without any stress [24, 25]. The experimental design diagram was shown in Figure S1.

Sucrose preference test (SPT)

SPT was used to assess whether rats in the CUMS group developed stress-induced anhedonia [26]. Briefly, rats were deprived of food and water for 24 h prior to SPT. Two bottles of pre-weighed liquid (1% sucrose solution and water in each) were placed in the cage at random positions. 24 h later, the two bottles were re-weighed. Sucrose preference (%) was calculated as Sucrose Consumption (g)/[Sucrose Consumption (g) + Water Consumption (g)] × 100% [27].

Open field test (OFT)

OFT was used to measure spatial exploration in rats [25]. The test was performed between 8:00 a.m.-12:00 p.m. All rats were placed in a

Table 4. Primer sequences used for RT-qPCR

Gene	RT primer sequences (5'---3')	PCR primer sequences (5'---3')
U6	CGCTTCACGAATTTGCGTGTCAT	F: GCTTCGGCAGCACATATACTAAAAT R: CGCTTCACGAATTTGCGTGTCAT
hsa-miR-134	GTCGTATCCAGTGCGTGTCGTGGAGTCG	F: GGGTGTGACTGGTTGACC
rno-mir-134	GCAATTGCACTGGATACGACCCCCTC	R: CAGTGCGTGTCGTGGAGT

soundproof room 1 hour prior to the test. During the test, a single rat was placed at the center of a black square cage (100 cm × 100 cm × 40 cm) divided into multiple squares (25 × 25 cm^2). Following 30 s of adaptation, the number of squares that the rat crossed with all four paws and rear frequency (a posture with hind limbs on the floor) were recorded for 5 minutes using a Sony DCR-SR45E camera (Japan) located 190-200 cm above the arena.

Animal specimens

Whole blood of anesthetized rats was collected by cardiac puncture using a heparin-laced syringe, followed by immediate centrifugation at 3000 g for 15 minutes to obtain plasma samples. The plasma was stored at -80°C until use. The rats were then euthanized by using sharp thread scissors to cut off the head quickly. The prefrontal cortex (PFC), hippocampus (HIP), and olfactory bulb (OB) tissues were collected and stored in liquid nitrogen until use.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from plasma or brain tissue using Trizol reagent (Invitrogen life technologies, USA) following the manufacturer's instruction. RNA concentration was determined using NanoDrop ND-1000 (Thermo Company, USA). Reverse transcription was performed using specific miRNA stem-loop primers and a Taqman microRNA reverse-transcription kit (Shanghai Sangon Biological Engineering Co., LTD) following the manufacturer's instructions. The amplification was performed using a TaKaRa PCR Thermal Cycler (Bao Biological Engineering Co. LTD). The primer sequences are shown in **Table 4**. Small RNA U6 was used as an internal control. Relative quantification of miRNA was conducted using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analyses were carried out using SPSS 19.0 software (IBM, Armonk, NY, USA). Differences between two groups were assessed using XXX or One-way ANOVA. Statistical significance was evaluated using Student's *t*-test, paired t-test, Wilcoxon Mann-Whitney test, or χ^2 test. The potential of plasma miR-134 level for distinguishing MDD patients from healthy controls and patients with other mental disorders was evaluated using receiver-operator characteristic (ROC) curves. $P < 0.05$ was considered significant.

Results

Plasma miR-134 levels are downregulated in MDD patients and restored following antidepressant therapy

To explore a possible relationship between circulating miR-134 and MDD, we performed qRT-PCR to measure the plasma miR-134 levels in the 'MiR-134 Expression Pattern Analysis' set. The demographic and clinical characteristics of the patients are summarized in **Table 1**. As shown in **Figure 1A**, plasma miR-134 levels in untreated MDD patients (presented as MDD baseline) were significantly decreased compared with those in healthy controls, suggesting that plasma miR-134 level might be a potential diagnostic marker for MDD.

To further investigate whether circulating miR-134 plays a prognostic role in MDD, we measured plasma miR-134 levels in MDD patients receiving antidepressant therapy (**Table 2**). The mean HAM-D and BDI scores at 8-week follow-up were both significantly decreased compared with those at baseline (all $P < 0.001$, **Table 1**), suggesting that the clinical symptoms of these patients were noticeably improved after antidepressant therapy. We then classified these patients into response (n = 24), partial response (n = 6), and nonresponse (n = 5) groups based on their symptom improvement. As shown in **Figure 1A**, the plasma miR-134 levels in all three groups were significantly lower than those in healthy controls ($P < 0.05$),

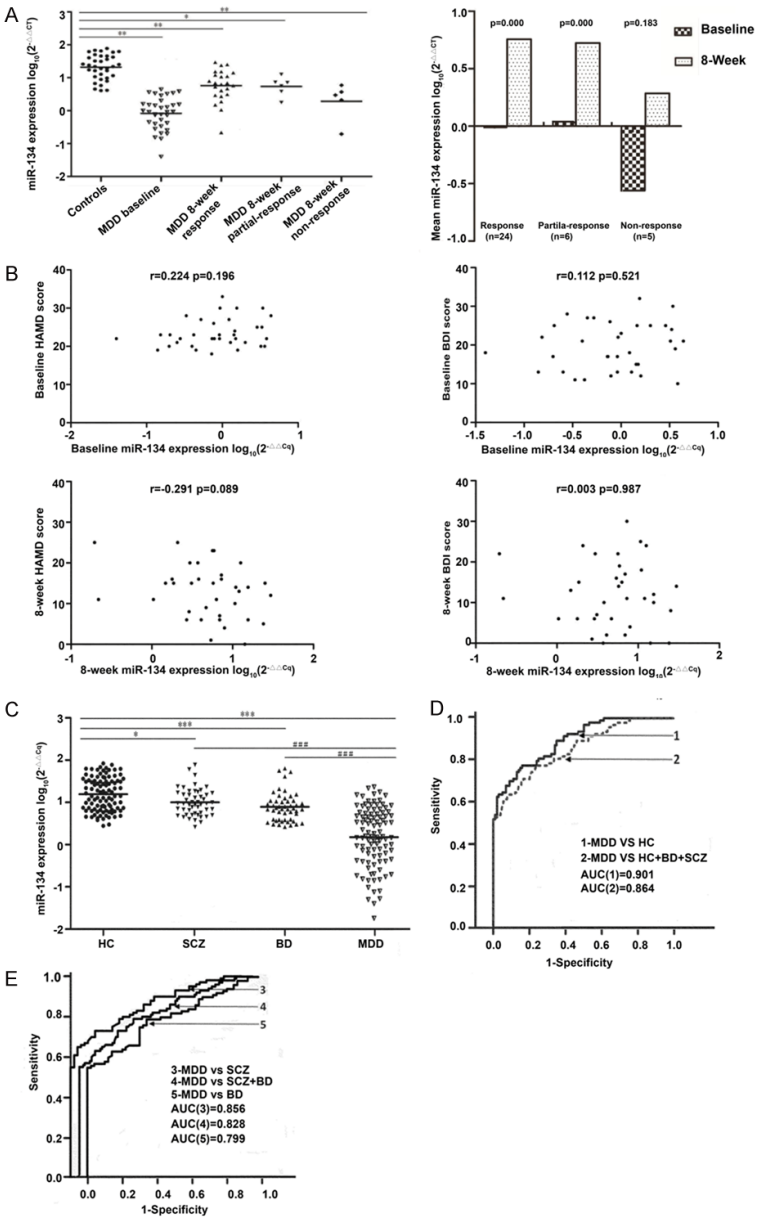

Figure 1. Diagnostic potential of circulating miR-134 in major depressive disorder (MDD). A. Left panel: 35 MDD patients receiving different antidepressants were classified into response (n = 24), partial response (n = 6), and nonresponse (n = 5) groups based on their symptom improvement after 8 weeks of treatment. Response, partial response, and non-response were defined according to the depression-like symptoms and HAM-D score. The patients were divided into three groups: 1) treatment-ineffective group (no clinical symptoms improved); 2) some effective group (some clinical symptoms improved, HAM-D score decreased by more than 25% and less than 50%); 3) significant group (clinical symptoms improved significantly, HAM-D score decreased by more than 50% or less than 15% respectively). The plasma miR-134 levels in these patients were determined (qRT-PCR) at baseline and 8 weeks after antidepressant therapy, respectively, using quantitative real-time PCR. The plasma miR-134 levels in 35 healthy controls (HC) were also determined. Data are expressed as mean ± standard deviation (SD). MDD baseline vs. Controls, $P < 0.001$; MDD 8-week response vs. Controls, $P < 0.001$; MDD 8-week partial-response vs. Controls, $P < 0.05$; MDD 8-week non-response vs. Controls, $P = 0.138$. $*P < 0.05$, $**P < 0.001$. Right panel: The plasma miR-134 levels of response (n = 24), partial-response (n = 6), and non-response (n = 5) groups at baseline and 8 weeks after antidepressant therapy. B. Correlations between the plasma miR-134 levels and HAM-D/BDI scores at baseline and 8 weeks post antidepressant therapy, respectively. C. The plasma miR-134 levels in HC (n = 100) and patients with MDD (n = 100), schizophrenia (SCZ; n = 50), and bipolar disorder (BD; n = 50). Data are expressed as mean ± SD. $***P < 0.001$ vs. HC; $###P < 0.001$, vs. MDD. D and E. Receiver-operator characteristic curve analysis for diagnostic potential of plasma miR-134 in distinguishing MDD from HC (1, n = 100), BD + SCZ + HC (2, n = 200), SCZ (3, n = 50), SCZ + BD (4, n = 100), and BD (5, n = 50), respectively. MDD, major depressive disorder; SCZ, schizophrenia; BD, bipolar disorder; HC, healthy controls; HAM-D, Hamilton Rating Scale for Depression; BDI, self-reported Beck Depression Inventory.

but significantly higher than MDD baseline ($P < 0.001$), with the exception of nonresponse group ($P = 0.183$). These results demonstrated that antidepressant therapy could partially restore the circulating miR-134 levels while improving MDD symptoms, suggesting that elevated plasma miR-134 levels may be associated with good prognosis in MDD. However, we did not observe significant correlation between plasma levels of miR-134 and HAM-D/BDI scores either at baseline or at 8 weeks' follow-up in these patients (**Figure 1B**).

The plasma level of miR-134 is a reliable indicator in distinguishing MDD from healthy controls and other mental disorders

To further investigate whether circulating miR-134 has diagnostic value for different types of mental disorders, we mea-

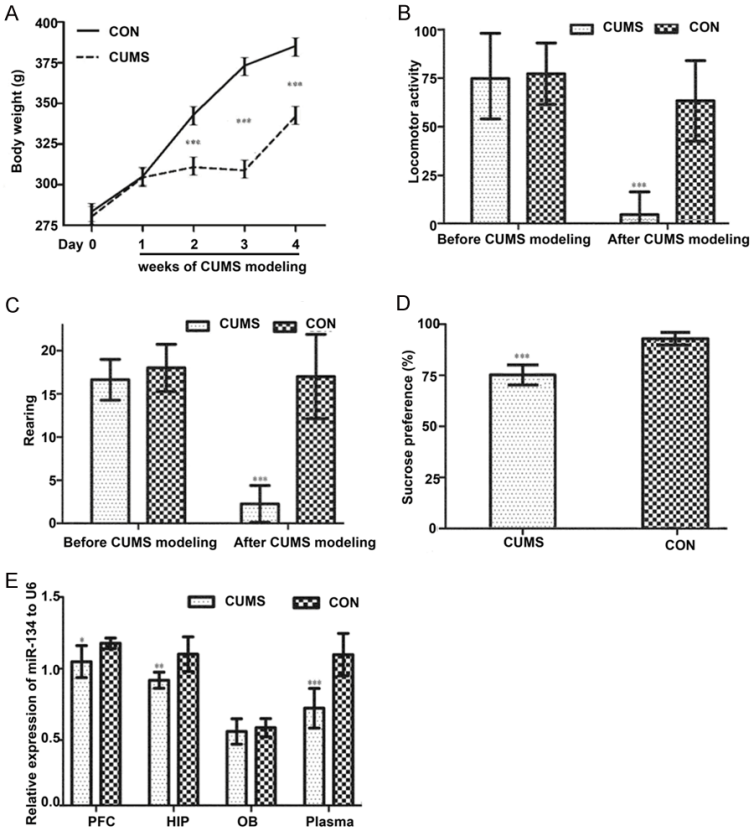

Figure 2. Quality of the depressed rat model and comparison of miR-134 expression between the plasma and brain tissue of rats. Sixteen healthy adult male Sprague-Dawley rats (average weight: 155 g) were trained to get accustomed to 1% sucrose solution (w/v) for 5 weeks and then randomly divided into chronic unpredictable mild stress (CUMS) and control groups (n = 8/group). Rats in CUMS group were housed individually in separate cages and subjected to stressors for 4 weeks to induce CUMS. The quality of CUMS model was assessed by comparing body weights (A), the numbers of locomotion with all four paws (B), the numbers of rears with hind limbs (C), and sucrose preference (D) between CUMS and control rats at baseline and 4 weeks after CUMS exposure, respectively. Sucrose Preference (%) = Sucrose Consumption (g)/[Sucrose Consumption (g) + Water Consumption (g)] × 100%. (E) miR-134 levels in the rat plasma or different brain regions (prefrontal cortex, hippocampus, and olfactory bulb) were detected at 4 weeks after CUMS modeling. Data are presented as mean ± SD. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ vs. CON group; n = 8. PFC, prefrontal cortex; HIP, hippocampus; OB, olfactory bulb; CUMS, chronic unpredictable mild stress; CON, control.

sured plasma miR-134 levels in the enlarged 'MiR-134 Expression Pattern Analysis' set. The demographic and clinical characteristics of these patients are summarized in **Table 3**. As shown in **Figure 1C**, the plasma miR-134 levels were significantly decreased in all three types of mental disorders compared with those in healthy controls. Intriguingly, the plasma levels of miR-134 in MDD patients were lower than those of SCZ or BD patients, suggesting that circulating miR-134 may be used to distinguish MDD from SCZ and BD.

We next sought to assess the effectiveness of the plasma miR-134 level in distinguishing MDD from healthy controls and other mental disorders using ROC analysis. As shown in **Figure 1D**, when MDD was compared with healthy control and healthy control + SCZ + BD, respectively, at the given cut-off value of 5.99 (relative quantity of miR-134 normalized to U6), the area under the curve (AUC) was 0.901 (95% CI, 0.861 to 0.941, $P < 0.001$) and 0.864; sensitivity was 79% and specificity was 84%. These data suggest that circulating miR-134 may serve as a reliable biomarker for distinguishing MDD from healthy controls or healthy controls + SCZ + BD. In addition, this cut-off value was also effectively used for distinguishing MDD from SCZ (AUC = 0.856), BD (AUC = 0.799), or SCZ + BD (AUC = 0.828) (**Figure 1E**).

miR-134 expression was consistently suppressed in plasma and different brain regions of CUMS rats

To evaluate whether alteration in plasma miR-134 is consistent with that in local brain tissue, we established a CUMS rat model to examine miR-134 expression pattern in plasma and different brain regions of CUMS rats. The results showed that CUMS rats exhibited significantly decreased body weights, locomotion activity, rear frequency, and relative sucrose intake compared with control ones after 4 weeks of CUMS exposure (all $P < 0.001$; **Figure 2A-D**), suggesting that the rat depression model was successfully established. As shown in **Figure 2E**, miR-134 expression was significantly reduced in plasma ($P < 0.001$), PFC ($P < 0.05$), and HIP ($P < 0.01$) of CUMS rats compared with that in control ones, with the exception of miR-134 expression in OB ($P = 0.565$), indicating that miR-134 is con-

stantly downregulated in plasma and local brain tissues in response to CUMS.

Discussion

MDD is a common chronic mood illness that leads to psychosocial impairment, poor quality of life [8], and significant disability [28], morbidity, and mortality [29-31]. Dysregulation of miRNA is associated with a variety of diseases, including MDD [32]. Due to the presence and/or release of miRNAs in the bloodstream, circulating miRNAs are considered potential biomarkers in MDD pathogenesis and therapeutic response monitoring [33, 34]. In this study, we found that the baseline plasma levels of miR-134 in MDD patients were significantly lower than those in normal controls. After 8 weeks of antidepressant therapy, plasma miR-134 was partially restored in both response and partial response groups, but not in the nonresponse group. These findings suggest that miR-134 could be a biomarker for diagnosis and therapy response monitoring in MDD. However, we did not observe any relevance between plasma levels of miR-134 and HAM-D/BDI scores at baseline or follow-up possibly due to small sample size or the subjectivity of the HAM-D/BDI scores.

MDD, BD, and SCZ are three major mental diseases with overlapping symptoms and genetic alterations [21]. Our results showed that the plasma miR-134 level was commonly decreased in these three disorders compared with normal controls. Importantly, the plasma levels of miR-134 in MDD patients were lower than those in BD or SCZ patients. The results of ROC analysis suggest that circulating miR-134 may be a useful biomarker for diagnosis of MDD and BD or SCZ.

Blood-based biomarkers have been widely used as diagnostic/prognostic tools because the "Sentinel Principle" suggests that blood may provide information on the health or disease of any particular tissue by a change in gene expression pattern [35]. In order to examine whether miR-134 in peripheral blood has similar expression pattern to that in local brain tissue, we detected the expression of miR-134 in both plasma and emotion-related brain regions of CUMS rats. The results demonstrated the decreased miR-134 expression in the PFC and HIP from CUMS rats compared with that in the control ones, which is consistent with the data observed in plasma. Therefore, miR-134 alteration in the plasma may dynamically reflect the pathologic changes in brain, serving as a reliable biomarker for MDD diagnosis and progression.

Impaired synaptic plasticity is a primary contributing factor for MDD [36]. CREB and brain-derived neurotrophic factor (BDNF) play critical roles in modulating synapse formation and synaptic plasticity [37]. Gao et al have reported that Sirtuin 1 (Sirt1) can suppress miR-134 expression a in central nervous system-derived clonal catecholaminergic cell line. Abnormal miR-134 expression resulting from Sirt1 deficiency may cause posttranscriptional downregulation of CREB and BDNF expression, thereby impairing synaptic plasticity [38, 39]. In this study, we observed downregulation of miR-134 in MDD patients and a CUMS rat model. We speculate that downregulation of miR-134 in MDD patients and rats may be a self-protective mechanism to restore BDNF and CREB expression, which counteracts the adverse effects of decreased BDNF and CREB on synaptic plasticity. However, we did not determine BDNF and CREB expression in this study. The underlying mechanism of miR-134 downregulation in MDD requires further investigation.

In sum, in the present study, we identified plasma miR-134 as a potential biomarker for MDD, which provides a more objective and minimally invasive approach for MDD diagnosis and monitoring.

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Disclosure of conflict of interest

None.

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References

[1] Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basanez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabe E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fevre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gosselin R, Grainger R, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Ma J, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA, 3rd, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, De Leon FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh PH, Zaidi AK, Zheng ZJ, Zonies D, Lopez AD, Murray CJ, AlMazroa MA and Memish ZA. 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: 2163-2196.

[2] Liu Z, Li X, Sun N, Xu Y, Meng Y, Yang C, Wang Y and Zhang K. Microarray profiling and co-expression network analysis of circulating lncRNAs and mRNAs associated with major depressive disorder. PLoS One 2014; 9: e93388.

[3] Bech P. Rating scales in depression: limitations and pitfalls. Dialogues Clin Neurosci 2006; 8: 207-215.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Carthew RW and Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell 2009; 136: 642-655.

[6] Flynt AS and Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 2008; 9: 831-842.

[7] Follert P, Cremer H and Beclin C. MicroRNAs in brain development and function: a matter of

flexibility and stability. Front Mol Neurosci 2014; 7: 5.
[8] Dwivedi Y. Emerging role of microRNAs in major depressive disorder: diagnosis and therapeutic implications. Dialogues Clin Neurosci 2014; 16: 43-61.
[9] Esquela-Kerscher A and Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.
[10] Wang Z, Zhang C, Huang J, Yuan C, Hong W, Chen J, Yu S, Xu L, Gao K and Fang Y. MiRNA-206 and BDNF genes interacted in bipolar I disorder. J Affect Disord 2014; 162: 116-119.
[11] Choi E, Cha MJ and Hwang KC. Roles of Calcium regulating microrna in cardiac ischemia-reperfusion injury. Cells 2014; 3: 899-913.
[12] Kroh EM, Parkin RK, Mitchell PS and Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods 2010; 50: 298-301.
[13] Smalheiser NR, Lugli G, Rizavi HS, Torvik VI, Turecki G and Dwivedi Y. MicroRNA expression is down-regulated and reorganized in prefrontal cortex of depressed suicide subjects. PLoS One 2012; 7: e33201.
[14] Fan HM, Sun XY, Guo W, Zhong AF, Niu W, Zhao L, Dai YH, Guo ZM, Zhang LY and Lu J. Differential expression of microRNA in peripheral blood mononuclear cells as specific biomarker for major depressive disorder patients. J Psychiatr Res 2014; 59: 45-52.
[15] Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W and Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002; 12: 735-739.
[16] Ye Y, Xu H, Su X and He X. Role of MicroRNA in governing synaptic plasticity. Neural Plast 2016; 2016: 4959523.
[17] Holtmaat A and Svoboda K. Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci 2009; 10: 647-658.
[18] Kessels HW and Malinow R. Synaptic AMPA receptor plasticity and behavior. Neuron 2009; 61: 340-350.
[19] Yoshihara Y, De Roo M and Muller D. Dendritic spine formation and stabilization. Curr Opin Neurobiol 2009; 19: 146-153.
[20] Duman RS, Aghajanian GK, Sanacora G and Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med 2016; 22: 238-249.
[21] Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, Gordon-Smith K, Fraser C, Forty L, Russell E, Hamshere ML, Moskvina V, Nikolov I, Farmer A, McGuffin P, Wellcome Trust Case Control C, Holmans PA, Owen MJ, O'Donovan MC and Craddock N. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry 2010; 15: 1016-1022.
[22] Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23: 56-62.
[23] Beck AT and Beamesderfer A. Assessment of depression: the depression inventory. Mod Probl Pharmacopsychiatry 1974; 7: 151-169.
[24] Mu J, Xie P, Yang ZS, Yang DL, Lv FJ, Luo TY and Li Y. Neurogenesis and major depression: implications from proteomic analyses of hippocampal proteins in a rat depression model. Neurosci Lett 2007; 416: 252-256.
[25] Yang D, Li Q, Fang L, Cheng K, Zhang R, Zheng P, Zhan Q, Qi Z, Zhong S and Xie P. Reduced neurogenesis and pre-synaptic dysfunction in the olfactory bulb of a rat model of depression. Neuroscience 2011; 192: 609-618.
[26] Katz RJ. Animal model of depression: pharmacological sensitivity of a hedonic deficit. Pharmacol Biochem Behav 1982; 16: 965-968.
[27] Willner P, Towell A, Sampson D, Sophokleous S and Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 1987; 93: 358-364.
[28] Broadhead WE, Blazer DG, George LK and Tse CK. Depression, disability days, and days lost from work in a prospective epidemiologic survey. JAMA 1990; 264: 2524-2528.
[29] Pompili M, Innamorati M, Rihmer Z, Gonda X, Serafini G, Akiskal H, Amore M, Niolu C, Sher L, Tatarelli R, Perugi G and Girardi P. Cyclothymic-depressive-anxious temperament pattern is related to suicide risk in 346 patients with major mood disorders. J Affect Disord 2012; 136: 405-411.
[30] Serafini G, Pompili M, Innamorati M, Fusar-Poli P, Akiskal HS, Rihmer Z, Lester D, Romano A, de Oliveira IR, Strusi L, Ferracuti S, Girardi P and Tatarelli R. Affective temperamental profiles are associated with white matter hyperintensity and suicidal risk in patients with mood disorders. J Affect Disord 2011; 129: 47-55.
[31] Innamorati M, Pompili M, Gonda X, Amore M, Serafini G, Niolu C, Lester D, Rutz W, Rihmer Z and Girardi P. 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: 99-103.
[32] Rinaldi A, Vincenti S, De Vito F, Bozzoni I, Oliverio A, Presutti C, Fragapane P and Mele A. Stress induces region specific alterations in microRNAs expression in mice. Behav Brain Res 2010; 208: 265-269.
[33] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W,

Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J and Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008; 18: 997-1006.

[34] Dwivedi Y. microRNAs as biomarker in depression pathogenesis. Ann Psychiatry Ment Health 2013; 1: 1003.

[35] Liew CC, Ma J, Tang HC, Zheng R and Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med 2006; 147: 126-132.

[36] Castren E and Hen R. Neuronal plasticity and antidepressant actions. Trends Neurosci 2013; 36: 259-267.

[37] Flavell SW and Greenberg ME. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu Rev Neurosci 2008; 31: 563-590.

[38] Gao J, Wang WY, Mao YW, Graff J, Guan JS, Pan L, Mak G, Kim D, Su SC and Tsai LH. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 2010; 466: 1105-1109.

[39] Libert S, Pointer K, Bell EL, Das A, Cohen DE, Asara JM, Kapur K, Bergmann S, Preisig M, Otowa T, Kendler KS, Chen X, Hettema JM, van den Oord EJ, Rubio JP and Guarente L. SIRT1 activates MAO-A in the brain to mediate anxiety and exploratory drive. Cell 2011; 147: 1459-1472.

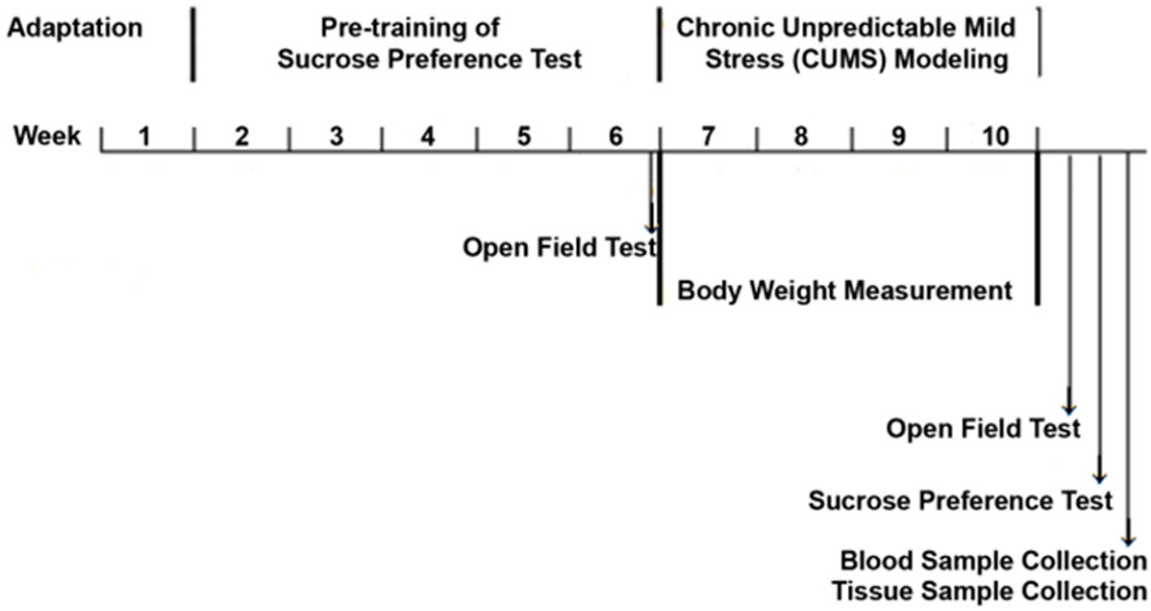

Figure S1. Experimental design diagram of the *in vivo* study.