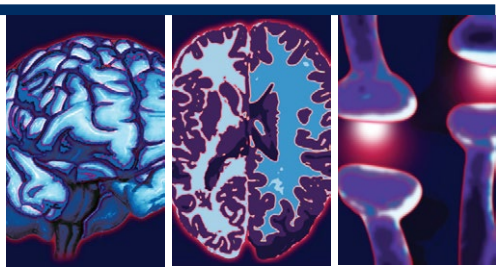

Increased Levels of miR-30e, miR-132, miR-185, and miR-212 at Baseline and Increased Brain-derived Neurotrophic Factor Protein and mRNA Levels after Treatment in Patients with Major Depressive Disorder

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Abstract

Background:

Brain-derived neurotrophic factor (BDNF) is involved in the pathophysiology of major depressive disorder (MDD). In recent years, epigenetic modifications of BDNF in patients of MDD had been investigated, and the role of microRNAs (miRNAs) had not been widely addressed.

Methods:

From December 2015 to November 2016, patients with MDD and healthy controls were recruited from a medical center in Taiwan. Their serum BDNF protein, BDNF mRNA, and ten miRNAs related to BDNF (miR-16, miR-30e, miR-34c-5p, miR-128, miR-132, miR-134, miR-182, miR-183, miR-185, miR-212) levels were measured and analysed. Some patients required hospitalization, and those markers were measured again after four-week antidepressant treatment.

Results:

Seventy-four subjects were recruited, including thirty-four patients with MDD and forty healthy controls. Using t-test, patients with MDD had higher levels of miR-30e, miR-132, miR-185, and miR-212 than healthy controls ($p = 0.006$, 0.000, 0.024, and 0.008, respectively). Using ANCOVA adjusted for sex and age, no statistical significance was found, however. Nineteen patients received one-month antidepressant treatments. Using Wilcoxon signed rank test, BDNF protein and mRNA levels increased significantly after treatment ($p = 0.016$ and 0.033, respectively). Their miRNA levels showed no significant change after the treatment.

Conclusion:

Serum miR-30e, miR-132, miR-185, and miR-212 levels were significantly increased in patients with MDD at baseline compared to healthy controls, suggesting miRNAs could be used as potential diagnostic biomarkers. BDNF protein and mRNA levels increased significantly after antidepressant treatment. Antidepressant treatment did not change miRNA levels significantly in our study, probably due to shorter treatment period or small sample size.

Keywords:

Antidepressant, BDNF, Major depression, MicroRNA, MRNA

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Introduction

Major depressive disorder (MDD) is a debilitating brain disorder that profoundly affects mood, thought, and behaviors. Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal survival and neuroplasticity in the brain [1]. Binding of BDNF with the transmembrane tropomyosin-related kinase B (TrkB) activates the signaling pathway [2]. BDNF could also cross blood-brain barrier [3]. Lower BDNF levels had been observed in patients with MDD [4,5], and antidepressant treatment could increase BDNF levels [6]. Decreased peripheral BDNF mRNA levels could also be reversed by antidepressant treatment [7].

Epigenetic abnormalities of DNA methylations, histone modifications, and noncoding RNAs of MDD were targets of interest in the recent years [8,9]. The most well-studied noncoding RNAs are microRNA (miRNA), which are 21-23 nucleotides long and can inhibit the translation of messenger RNA (mRNA), regulating protein expression via post-transcriptional gene silencing. Several miRNAs could control the expression of BDNF in the prefrontal cortex [10]. Several miRNAs could directly repress BDNF protein expression by binding to BDNF 3' untranslated region [11]. Differentially expressed levels of miRNAs in CSF and serum of patients with MDD were investigated [12], as well as change of miRNA levels after antidepressant treatment [13]. Despite increasing number of miRNA studies, the exact mechanisms of miRNA in MDD remained unclear, partly due to the fact that each miRNA could have numerous target sites and influence multiple proteins at the same time.

Therefore, in this study, we aimed to investigate the serum levels of miRNAs related to BDNF pathway, which were miR-16, miR-30e, miR-34, miR-128, miR-132, miR-134, miR-182, miR-183, miR-185 and miR-212, in addition to serum BDNF protein and mRNA levels, in patients of MDD and healthy controls. Patients with MDD who were hospitalized would be assessed again after four weeks of antidepressant treatment to evaluate the relationship of those biomarkers with treatment.

Methods

■ Subjects and Study Design

From November 2015 to November 2016, inpatients and outpatients diagnosed with MDD were recruited at the Chang Gung Memorial Hospital. MDD was diagnosed by a psychiatrist according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) [14]. Only patients aged between 20 and 65 years old were included. Patients with systemic diseases, such as cardiovascular diseases, liver diseases and thyroid diseases, heavy smokers, or patients with alcohol dependence were excluded. The severity of depression was assessed by the 17-item Halmiton Depression Rating Scale (Ham-D) [15]. Inpatients would receive a second evaluation after four weeks of antidepressant treatment in addition to baseline assessment. The choice of antidepressants depended on what the clinicians considered best for the patients. Healthy controls were recruited and assessed by semi-structured interviews to rule out psychiatric disorder according to DSM-IV criteria. Written informed consent was provided by all participants after the content and context of the study was fully explained. The institutional review board (IRB) of Chang Gung Memorial Hospital approved the study design (IRB 104-7392C).

■ Laboratory Data

Venous blood of 15 ml was drawn from each participant in the morning following a 6-hour fast at baseline. Inpatients would have a second blood draw after 4-week antidepressant treatment. Serum BDNF protein levels were measured using a commercially available ELISA kit of the sandwich type (BDNF Emax Immunoassay System; Promega; USA), which measures mature BDNF. Each system contained anti-BDNF mAb, Block&Sample 5X buffer, BDNF standard, antihuman BDNF pAb, anti-IgY HRP, TMB solution, peroxidase substrate, and protocol. All samples were assayed or duplicated by the same senior laboratory assistant.

BDNF mRNA and miRNA levels were determined by polymerase chain reaction (PCR). Total RNA was isolated with mirVana miRNA Isolation kit (Ambion, Life technologies) and concentration was determined by photometric measurements. A High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used to synthesize cDNA from 200 ng of RNA, following manufacturer's recommendations. qRT-PCR amplification mixtures contained 20 ng template cDNA, 2X Power SYBR Green Master mix (10 µL) (Applied Biosystems) and 400–600 nM forward and reverse primers in a final volume of 20 µL. TaqMan® MicroRNA

Assays (Applied Biosystems) were used to assess miR-16, miR-30e, miR-34, miR-128, miR-132, miR-134, miR-182, miR-183, miR-185 and miR-212 expression levels and included two steps: reverse transcription and real-time PCR. The total RNA (2.5 ng/reaction) from samples was reverse-transcribed with specific looped RT primers. Fifteen μL reactions, in turn, were performed using reagents from the High-Capacity cDNA Archive Kit (Applied Biosystems) and 1.9 U RNase inhibitor (Applied Biosystems) and were and incubated for 30 minutes at 16°C, 30 minutes at 42°C, and 5 minutes at 85°C. As for the real-time PCR step, 4.5 μL 1:5 diluted cDNA samples were used as templates in 10 μL reactions containing primers and probes for miR-16, miR-30e, miR-34, miR-128, miR-132, miR-134, miR-182, miR-183, miR-185 and miR-212, according to manufacturer instructions. All reactions were run in duplicate on an ABI7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95°C, for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and 60°C, for 1 minute. Total RNA input was normalized based on the Ct values obtained for RNU6B, which is a nucleolar RNA used as an endogenous control in this type of analysis. Relative expressions levels of RNAs were calculated by 2^-ΔΔCt.

Statistical Analysis

All results are represented as mean ± standard deviation. The levels of BDNF protein, BDNF mRNA, and miRNAs between patients with MDD and healthy controls were analysed with independent *t*-test and analysis of co-variants (ANCOVA) adjusted for age and sex. The levels of BDNF protein, BDNF mRNA, and miRNAs of patients before and after 4-week antidepressant treatment were analyzed with Wilcoxon signed rank test. Pearson correlation was used to assess the relationship with the associated parameters. Data analysis was performed using SPSS 19 (Chicago, IL, U.S.A.). p values of <0.05 were considered statistically significant.

Results

Seventy-four subjects were recruited, including thirty-four patients with MDD and forty healthy controls. Their demographic data and serum levels of BDNF protein, BDNF mRNA, and miRNAs were summarized in **Table 1**. Using independent *t*-test, patients with MDD had higher levels of miR-30e, miR-132, miR-185, and miR-212 than healthy controls (p = 0.006, 0.000, 0.024, and 0.008, respectively). Using ANCOVA adjusted for sex and age, no statistical significance was found, however.

Nineteen patients received one-month antidepressant treatments, and presented with more severe depressive symptoms as shown by higher Ham-D scores. Their clinical data and serum levels of BDNF protein, BDNF mRNA, and miRNAs were summarized in **Table 2**. Eighteen out of the nineteen patients were females. Twelve patients were prescribed of selective serotonin reuptake inhibitors (fluoxetine: 4; paroxetine: 6; escitalopram: 2); Six were prescribed of serotonin–norepinephrine reuptake inhibitor (venlafaxine: 4; duloxetine: 2); and one was prescribed of valproic acid. Using Wilcoxon signed rank test, BDNF protein and mRNA levels increased significantly after treatment (p = 0.016 and 0.033, respectively). Their miRNA levels showed no significant change after the treatment.

In terms of correlation, BDNF protein levels correlated significantly with Ham-D score, miR-34, and miR-134 levels (r = -0.513, p = 0.002; r = 0.335, p = 0.004; r = 0.253, p = 0.030, respectively). BDNF mRNA levels did not correlate with BDNF protein levels, miRNA levels, or Ham-D score. miR-30e level correlated significantly with miR-16, miR-132, miR-134, miR-182, miR-185, and miR-212 levels (r = 0.370, p = 0.001; r = 0.498, p = 0.000; r = 0.261, p = 0.024; r = 0.307, p = 0.008; r = 0.502, p = 0.000; r = 0.321, p = 0.005, respectively). miR-132 level correlated significantly with miR-16, miR-30e, miR-34, miR-128, miR-134, miR-182, miR-185, and miR-212 levels (r = 0.574, p = 0.000; r = 0.498, p = 0.000; r = 0.468, p = 0.000; r = 0.251, p = 0.031; r = 0.467, p = 0.000; r = 0.511, p = 0.000; r = 0.583, p = 0.000; r = 0.680, p = 0.000, respectively). miR-185 level correlated significantly with miR-16, miR-30e, miR-34, miR-128, miR-132, miR-134, miR-182, and miR-212 levels (r = 0.739, p = 0.000; r = 0.502, p = 0.000; r = 0.244, p = 0.036; r = 0.295, p = 0.011; r = 0.583, p = 0.000; r = 0.355, p = 0.002; r = 0.511, p = 0.000; r = 0.645, p = 0.000, respectively). miR-212 level correlated significantly with Ham-D score, miR-16, miR-30e, miR-34, miR-128, miR-132, miR-134, miR-182, miR-183, and miR-185 levels (r = -0.500, p = 0.003; r = 0.573, p = 0.000; r = 0.321, p = 0.005; r = 0.506, p = 0.000; r = 0.383, p = 0.001; r = 0.680, p = 0.000; r = 0.317, p =

0.006; r = 0.453, p = 0.000; r = 0.397, p = 0.000; r = 0.645, p = 0.000, respectively). Those results are summarized in Table 3.

Discussion

The first major finding of the study was that serum miR-30e, miR-132, miR-185, and miR-212 levels were increased in patients with MDD at baseline compared to healthy controls. Among the ten miRNAs investigated, those four miRNAs also highly correlated with the other miRNAs (as miR-30e correlated with six other miRNAs; miR-132 and miR-185 correlated with eight other miRNAs; miR-212 correlated with nine other miRNAs), providing evidences that those miRNAs could be associated within a network, possibly BDNF pathway. Several studies investigated miRNAs and BDNF simultaneously in the past. Maussion, *et al.* reported that in the postmortem tissue of suicide completers, a significant correlation was found between miR-185 and TrkB-T1, a BDNF receptor lacking the tyrosine kinase domain [16]. Li, *et al.* showed that patients with depression had lower BDNF levels and higher serum miR-132 and miR-182 levels, which decreased BDNF protein levels in human neuronal cells [17]. Yi, *et al.* demonstrated that oleanolic acid would increase miR-132 level, which activated BDNF pathway [18]. Su, *et al.* found increased miR-132 level and decreased BDNF level in the peripheral blood of patients with MDD and a similar pattern in the hippocampi in animal models of depression [19]. Liu, *et al.* also reported increased miR-132 levels in patients with MDD, and miR-132 levels significantly correlated with visual memory [20]. Xu, *et al.* found significant association between the polymorphism ss178077483 in the miR-30e precursor and MDD [21]. Our findings on miR-30e, miR-132, and miR-185 were in line with those earlier studies.

Our finding that miR-212 was upregulated in patients with MDD did not correlate with earlier studies. In a postmortem study, miR-132 and miR-212 were initially identified as differentially expressed in bipolar disorder [22]. In patients with postpartum psychosis, miR-212 was found to be decreased in monocytes [23]. The role of miR-212 in MDD will need further investigations to validate.

The second finding of the study was that BDNF protein and mRNA levels significantly increased after four weeks of antidepressant treatment. Our findings of BDNF protein and mRNA levels in response to antidepressant treatment were in line with earlier studies [4-7,17]. In our sample, eighteen out of nineteen patients of this sample were females, suggesting that gender might also play a role in BDNF levels in MDD. At baseline, female patients with MDD had significantly lower serum BDNF protein levels than controls, but that phenomenon was not observed in male patients [4]. While BDNF levels increased significantly after antidepressant treatment, only

Table 1: Patients with MDD and healthy controls.

	Patients with MDD	Healthy Controls	
Size	n = 34	n = 40	
Sex	males = 6 females = 28	males = 7 females = 33	
Age (years)	45.4 ± 12.9	38.6 ± 7.9	
BMI (kg/m^2)	23.7 ± 5.1	22.0 ± 3.2	
Ham-D scores	17.3 ± 12.1		
BDNF protein levels (ng/mL)	8.7 ± 2.1	8.5 ± 1.8	
BDNF mRNA	1.8 ± 3.3	2.2 ± 2.9	
miR-16	5.3 ± 4.7	3.2 ± 6.0	
miR-30e*	5.0 ± 4.0	2.5 ± 3.7	p = 0.006
miR-34	5.0 ± 6.2	3.1 ± 6.2	
miR-128	2.5 ± 3.2	2.3 ± 3.2	
miR-132*	8.3 ± 6.4	2.5 ± 3.3	p = 0.000
miR-134	9.5 ± 18.2	4.3 ± 8.5	
miR-182	9.7 ± 10.6	7.9 ± 17.1	
miR-183	2.7 ± 2.8	4.7 ± 12.9	
miR-185*	5.8 ± 5.0	3.0 ± 5.5	p = 0.024
miR-212*	4.0 ± 3.1	2.1 ± 2.8	p = 0.008

BDNF: brain-derived neurotrophic factor; BMI: body mass index; Ham-D: 17-item Halmiton Depression Rating Scale; MDD: major depressive disorder
*: $p < 0.05$

Table 2: Before and After Antidepressant Treatment.

	Baseline	After	
Size	n = 19		
Sex	males = 1 females = 18		
Age (years)	42.4 ± 14.5		
BMI (kg/m^2)	23.8 ± 5.6	23.8 ± 5.3	
Ham-D score	25.7 ± 6.3	10.7 ± 6.7	
BDNF protein levels (ng/mL)*	8.0 ± 2.0	9.1 ± 1.7	$p = 0.016$
BDNF mRNA*	2.5 ± 4.9	9.1 ± 11.6	$p = 0.033$
miR-16	2.4 ± 1.8	3.7 ± 4.8	
miR-30e	1.6 ± 1.6	1.5 ± 1.6	
miR-34	1.6 ±1.8	2.0 ± 2.1	
miR-128	2.6 ±4.3	2.0 ± 1.9	
miR-132	1.6 ± 1.5	1.3 ± 0.9	
miR-134	2.7 ± 6.4	2.1 ± 2.1	
miR-182	1.5 ± 1.7	1.3 ± 1.0	
miR-183	1.5 ± 1.7	1.5 ± 1.0	
miR-185	1.2 ± 0.7	1.4 ± 1.2	
miR-212	1.2 ± 0.8	1.8 ± 2.1	

BDNF: brain-derived neurotrophic factor; BMI: body mass index; Ham-D: 17-item Halmiton Depression Rating Scale; MDD: major depressive disorder
*: $p < 0.05$

Table 3: Correlations between variables.

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1) Ham-D score												
(2) BDNF protein	**-0.513** **0.002***											
(3) BDNF mRNA	0.073 0.682	-0.011 0.924										
(4) miR-16	**-0.438** **0.010***	0.211 0.071	0.027 0.819									
(5) miR-30e	-0.142 0.424	-0.008 0.946	-0.066 0.576	**0.370** **0.001***								
(6) miR-34	-0.269 0.124	**0.335** **0.004***	-0.114 0.333	**0.314** **0.006***	0.174 0.139							
(7) miR-128	0.000 1.000	0.221 0.058	0.079 0.502	**0.232** **0.047***	0.076 0.520	0.204 0.082						
(8) miR-132	-0.280 0.109	0.204 0.081	-0.060 0.611	**0.574** **0.000***	**0.498** **0.000***	**0.468** **0.000***	**0.251** **0.031***					
(9) miR-134	-0.145 0.413	**0.253** **0.030***	-0.070 0.555	**0.385** **0.001***	**0.261** **0.024***	**0.285** **0.014***	0.190 0.105	**0.467** **0.000***				
(10) miR-182	-0.287 0.100	0.179 0.127	-0.112 0.344	**0.753** **0.000***	**0.307** **0.008***	**0.322** **0.005***	0.096 0.414	**0.511** **0.000***	**0.391** **0.001***			
(11) miR-183	-0.120 0.498	0.006 0.961	-0.102 0.385	**0.240** **0.040***	0.098 0.408	0.202 0.084	**0.261** **0.025***	0.153 0.194	0.226 0.053	**0.491** **0.000***		
(12) miR-185	0.099 0.401	0.157 0.181	0.153 0.192	**0.739** **0.000***	**0.502** **0.000***	**0.244** **0.036***	**0.295** **0.011***	**0.583** **0.000***	**0.355** **0.002***	**0.511** **0.000***	0.202 0.084	
(13) miR-212	0.227 0.052	0.191 0.103	0091 0.442	**0.573** **0.000***	**0.321** **0.005***	**0.506** **0.000***	**0.383** **0.001***	**0.680** **0.000***	**0.317** **0.006***	**0.453** **0.000***	**0.397** **0.000***	**0.645** **0.000***

In each cell Pearson's r and *p* value were shown in first and second row, respectively.
BDNF: brain-derived neurotrophic factor; Ham-D: 17-item Halmiton Depression Rating Scale
*: $p < 0.05$

female responders had significantly increased changes in serum BDNF protein levels [4].

We found no significant change in miRNA levels after four weeks of antidepressant treatment, however. Earlier studies also reported many miRNA changes associated with antidepressant treatment. Oved, *et al.* reported that miR-30b, miR-132, and miR-212 levels differed significantly between paroxetine-sensitive and non-sensitive cell lines [24]. In the dentate gyrus

and whole blood of rats, miR-212 levels increased after electroconvulsive stimulation [25]. In mice model of depression, duloxetine treatment upregulated miR-132 level in hippocampus [26]. Bocchio-Chiavetto, *et al.* found that levels of both miR-30b and miR-132, along with other 26 miRNAs, were statistically increased after 12 weeks of escitalopram treatment in ten patients with MDD [27]. Though we did not find statistical differences after four weeks of antidepressant treatment, we did find miR-30e and miR-132 levels to be statistically increased at baseline. Other miRNAs not investigated in this study had also been associated with antiderpessant treatment. In raphe nuclei of mice, fluoxetine treatment increases miR-16 levels, and miR-16 decreased serotonin transporter expression [28]. In hippocampus of mice, fluoxetine treatment also decreased miR-16 level, and human CSF analysis revealed miR-16 as a regulator between fluoxetine treatment and BDNF [29]. miR-1202 level was significantly increased in remitters after eight weeks of antidepressant treatment, and change in miR-1202 level correlated negatively with change in depression severity [30]. miR-335 levels were decreased in 18 patients with MDD as compared to healthy controls, and the miR-334 levels were increased after citalopram treatment, though the exact treatment period was not reported [31]. Lower baseline miR-1202 levels were found in responders as compared to non-responders, and miR-1202 levels increased after eight weeks of antidepressant treatment [32]. We did not find the investigated miRNAs to be statistically different after four weeks of antidepressant treatment. The shorter treatment period might be the cause of this discrepancy.

MDD and miRNAs had been studied in cells, animals, postmortem tissues, and various body fluids (such as CSF, serum, or specific types of blood cells) of clinical patients [33]. Some studies showed that miRNA alterations are limited to certain regions. For example, in the mouse model of depression, several miRNA levels changed significantly after induction of depression in the frontal cortex, but the changes were not observed in hippocampus [34]. Others showed concordant changes across different tissues of different species. For example, miR-132 and BDNF levels had similar patterns in the hippocampi of mouse model of depression and peripheral blood of patients with MDD [19]. Given that different miRNAs could have different distributions in the body, and each has multiple targets, the study on miRNAs is a challenge where every piece of evidence counts.

There are several limitations to the study. The sample size was small, so a bigger sample size would be needed to verify our findings. The four-week treatment period was shorter compared to most of the earlier studies, which lasted eight to twelve weeks? Lastly, we did not limit the choice of antidepressants, which might be a confounding factor.

Conclusion

We found that serum miR-30e, miR-132, miR-185, and miR-212 levels were increased in patients with MDD at baseline compared to healthy controls, suggesting miRNA could be used as a potential diagnostic biomarker. BDNF protein and mRNA levels increased significantly after antidepressant treatment. Antidepressant treatment did not change miRNA levels significantly in our study, probably due to shorter treatment period or small sample size. Further investigation with a larger sample size would be needed to confirm the roles of miRNAs in MDD.

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References

1. Maisonpierre PC, Belluscio L, Friedman B, *et al.* NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. *Neuron* 5(4), 501-509 (1990).
2. Nagappan G, Lu B. Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. *Trends. Neurosci* 28(9), 464-471 (2005).
3. Pan W, Banks WA, Fasold MB, *et al.* Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology* 37(12), 1553-1561 (1998).
4. Huang TL, Lee CT, Liu YL. Serum brain-derived neurotrophic factor levels in patients with major depression: effects of antidepressants. *J. Psychiatr. Res* 42(7), 521-525 (2008).
5. Molendijk ML, Bus BA, Spinhoven P, *et al.* Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. *Mol. Psychiatry* 16(11), 1088-1095 (2011).
6. Molendijk ML, Spinhoven P, Polak M, *et al.*

Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). *Mol. Psychiatry* 19(7), 791-800 (2014).

7. Cattaneo A, Bocchio-Chiavetto L, Zanardini R, *et al.* Reduced peripheral brain-derived neurotrophic factor mRNA levels are normalized by antidepressant treatment. *Int. J. Neuropsychopharmacol* 13(1), 103-108 (2010).
8. Menke A, Binder EB. Epigenetic alterations in depression and antidepressant treatment. *Dialogues. Clin. Neurosci* 16(3), 395-404 (2014).
9. Saavedra K, Molina-Márquez AM, Saavedra N, *et al.* Epigenetic Modifications of Major Depressive Disorder. *Int. J. Mol. Sci* 17(8) E1279 (2016).
10. Darcq E, Warnault V1, Phamluong K, *et al.* MicroRNA-30a-5p in the prefrontal cortex controls the transition from moderate to excessive alcohol consumption. *Mol. Psychiatry* 20(10), 1219-1231 (2015).
11. Varendi K, Kumar A, Härma MA, *et al.* miR-1, miR-10b, miR-155, and miR-191 are novel regulators of BDNF. *Cell. Mol. Life. Sci* 71(22), 4443-4456 (2014).
12. Wan Y, Liu Y, Wang X, *et al.* Identification of Differential MicroRNAs in Cerebrospinal Fluid and Serum of Patients with Major Depressive Disorder. *PLos. One* 10(3), e0121975 (2015).
13. Bocchio-Chiavetto L, Maffioletti E, Bettinsoli P, *et al.* Blood microRNA changes in depressed patients during antidepressant treatment. *Eur. Neuropsychopharmacol* 23(7), 602-611 (2013).
14. American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (Text Revision). Washington DC: American Psychiatric Publishing, Inc (2000).
15. Hamilton M. Development of a rating scale for primary depressive illness. *Br. J. Soc. Clin. Psychol* 6(4), 278-296 (1967).
16. Maussion G, Yang J, Yerko V, *et al.* Regulation of a truncated form of tropomyosin-related kinase B (TrkB) by Hsa-miR-185* in frontal cortex of suicide completers. *PLoS. One* 7(6), e39301 (2012).
17. Li YJ, Xu M, Gao Z, *et al.* Alterations of serum levels of BDNF-related miRNAs in patients with depression. *PLoS. One* 8(5), e63648 (2013).
18. Yi LT, Li J, Liu BB, *et al.* BDNF-ERK-CREB signalling mediates the role of miR-132 in the regulation of the effects of oleanolic acid in male mice. *J. Psychiatry. Neurosci* 39(5), 348-359 (2014).
19. Su M, Hong J, Zhao Y, *et al.* MeCP2 controls hippocampal brain-derived neurotrophic factor expression via homeostatic interactions with microRNA132 in rats with depression. *Mol. Med. Rep* 12(4), 5399-5406 (2015).
20. Liu Y, Yang X, Zhao L, *et al.* Increased miR-132 level is associated with visual memory dysfunction in patients with depression. *Neuropsychiatr. Dis. Treat* 12(1), 2905-2911 (2016).
21. 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* 127(1-3), 332-336 (2010).
22. Azevedo JA, Carter BS, Meng F, *et al.* The microRNA network is altered in anterior cingulate cortex of patients with unipolar and bipolar depression. *J. Psychiatr. Res* 82(1), 58-67 (2016).
23. Weigelt K, Bergink V, Burgerhout KM, *et al.* Down-regulation of inflammation-protective microRNAs 146a and 212 in monocytes of patients with postpartum psychosis. *Brain. Behav. Immun* 29(1), 147-155 (2013).
24. Oved K, Morag A, Pasmanik-Chor M, *et al.* Genome-wide miRNA expression profiling of human lymphoblastoid cell lines identifies tentative SSRI antidepressant response biomarkers. *Pharmacogenomics* 13(10), 1129-1139 (2012).
25. Ryan KM, O'Donovan SM, McLoughlin DM. Electroconvulsive stimulation alters levels of BDNF-associated microRNAs. *Neurosci. Lett* 549(1), 125-129 (2013).
26. Pan B, Liu Y. Effects of duloxetine on microRNA expression profile in frontal lobe and hippocampus in a mouse model of depression. *Int. J. Clin. Exp. Pathol* 8(11), 15454-15461 (2015).
27. Bocchio-Chiavetto L, Maffioletti E, Bettinsoli P, *et al.* Blood microRNA changes in depressed patients during antidepressant treatment. *Eur. Neuropsychopharmacol* 23(7), 602-611 (2013).
28. Baudry A, Mouillet-Richard S, Schneider B, *et al.* miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. *Science* 329(5998), 1537-1541 (2010).
29. Launay JM, Mouillet-Richard S, Baudry A, *et al.* Raphe-mediated signals control the hippocampal response to SRI antidepressants via miR-16. *Transl. Psychiatry* 1(11), e56 (2011).
30. 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* 20(7), 764-768 (2014).
31. Li J, Meng H, Cao W, *et al.* MiR-335 is involved in major depression disorder and antidepressant treatment through targeting GRM4. *Neurosci. Lett* 606(1), 167-172 (2015).
32. Fiori LM, Lopez JP, Richard-Devantoy S, *et al.* Investigation of miR-1202, miR-135a, and miR-16 in Major Depressive Disorder and Antidepressant Response. *Int. J. Neuropsychopharmacol* (2017).
33. Dwivedi Y. Emerging role of microRNAs in major depressive disorder: diagnosis and therapeutic implications. *Dialogues. Clin. Neurosci* 16(1), 43-61 (2014).
34. Rinaldi A, Vincenti S, De Vito F, *et al.* Stress induces region specific alterations in microRNAs expression in mice. *Behav. Brain. Res* 208(1), 265-269 (2010).