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

Chin-Chuen Lin¹, Chien-Te Lee², Ming-Hsiang Sun¹, Tiao-Lai Huang¹†

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-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
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-30c, 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
miRNAs were summarized in levels of BDNF protein, BDNF mRNA, and controls. Their demographic data and serum thirty-four patients with MDD and forty healthy Seventy-four subjects were recruited, including Results values of <0.05 were p (Chicago, IL, U.S.A.). Data analysis was performed using SPSS 19 the relationship with the associated parameters. Pearson correlation was used to assess treatment were analyzed with Wilcoxon signed of patients before and after 4-week antidepressant levels of BDNF protein, BDNF mRNA, and miRNAs between patients with MDD and healthy controls were analysed with ANCOVA adjusted for age and sex. The levels of BDNF protein, BDNF mRNA, and miRNAs were calculated by 2^\triangle\triangle Ct.

**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 mRNA 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, 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-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-128, 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-134, 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 = 0.335, p = 0.004; r = 0.253, p = 0.030, respectively). Using ANCOVA adjusted for sex and age, no statistical significance was found, however.


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 s178077483 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>
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<th>Table 1: Patients with MDD and healthy controls.</th>
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BDNF: brain-derived neurotrophic factor; BMI: body mass index; Ham-D: 17-item Haldimton Depression Rating Scale; MDD: major depressive disorder *: p < 0.05
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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 antidepressant 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 miRNA 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.

Acknowledgement

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