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Long Non-Coding RNA: Potential Diagnostic and Therapeutic Biomarker for Major Depressive Disorder

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Background: The criteria for diagnosing depression are based on behavioral observation and self-reporting of symptoms by the patients or guardians without any biological validation of the disease. This study aimed to identify long non-coding RNAs (lncRNAs) in peripheral blood mononuclear cells (PBMCs) as robust and predictive biomarkers for diagnosis and therapy response in major depressive disorder (MDD).

Material/Methods: We used human lncRNA 3.0 microarray profiling (which covers 30,586 human lncRNAs), using PBMCs from five MDD patients and five controls. Differentially expressed lncRNAs in the PBMCs of MDD patients were identified, of which 10 candidate lncRNAs were selected for real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis in a larger cohort of 138 MDD patients and 63 healthy controls. Then among the 138 MDD patients who received standard antidepressant treatment, 30 were randomly selected for lncRNAs expression retesting and symptomatology assessments after three-weeks and six-weeks of antidepressant treatment.

Results: Six lncRNAs (TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) were significantly downregulated in MDD patients compared to control patients, and the area under the receiver operator curve (ROC) of these six lncRNAs cases, combined, was 0.719 (95% confidence interval (CI): 0.617–0.821). There was no difference in the expression of these six lncRNAs based on gender ($p>0.05$) or age ($p>0.05$).

Conclusions: These results suggest that the combined expression of six lncRNAs in PBMCs may serve as a potential biomarker for diagnosis and therapy response of MDD in the clinical setting.

MeSH Keywords: Biological Markers • Depressive Disorder, Major • RNA, Long Noncoding

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Background

Major depressive disorder (MDD) is a debilitating mental disorder characterized by a depressed mood, anhedonia, and feelings of worthlessness and guilt [1]. More than 350 million people in the world suffer from MDD. By the year 2020, unipolar depressive disorder is predicted to become the second leading cause of disease burden worldwide [2]. MDD results in a significant burden to society because of its high incidence, early onset, chronic course, and its impairment of social function and cognitive ability, and unnatural death [3]. The ratio of attempted suicide to suicide completely in MDD patients is much higher than in the general population [4]. If it was possible to identify patients in advance of their diagnosis [5] during the months (or perhaps years) before meeting diagnostic criteria, there could be a substantial reduction in suffering and healthcare-related costs. Hence, there is an urgent need to investigate potential biomarkers for MDD.

To address this need, substantial efforts are being made. In addition to examining metabolic dysregulation (e.g., insulin resistance) [6] and changes in brain structure and function [7], there is a need to identify biomarkers in peripheral tissue that are easily accessible (e.g., blood). These biomarkers include growth factors and/or pro-inflammatory cytokines and endocrine factors (e.g., hypothalamic-pituitary-adrenal (HPA), and sex steroids) [8–11]. Although considerable progress has been made [12,13], the predictive factors of these biomarkers for diagnostic purpose typically have shown high false-positive rates [14]. Recent research has established that environmental events and behavioral experience can induce epigenetic changes at specific gene *loci*, and that these changes can help shape neuronal plasticity, function, and behavior, hence contributing to the pathogenesis of depression [15]. Therefore, the identification of biomarkers for MDD has gradually shifted to epigenetic mechanisms.

Initially, many scholars studied the differential expression of microRNAs (miRNAs) in patients with depression. miRNA-26b, miRNA-1972, miRNA-4743, miRNA-4485, and miRNA-4498 were considered to be potential biomarkers for MDD [16]. Subsequently, long non-coding RNAs (lncRNAs), defined as non-protein-coding RNAs that are greater than 200 nt in length, were found to serve very important functions in the epigenetic regulation of the human genome, regulating DNA methylation, histone modification, and chromatin remodeling through epigenetic, transcriptional, and post-transcriptional regulation, resulting in gene silencing or activation. Moreover, lncRNAs are widely involved in various biological processes of the central nervous system, such as hippocampal development, oligodendrocyte myelination, and brain aging [17]. LncRNAs have been associated with many human diseases, such as cancer [18], spinocerebellar ataxia [19], and cardiovascular disease [20]. However, there has been little research on lncRNAs that are differentially expressed in patients with depression; only one study showed that there were eleven differentially expressed lncRNAs in patients with MDD compared to normal controls. In that study, which examined the co-expression network of lncRNAs and mRNAs, there were significantly related expression patterns and, in particular, three lncRNAs that were located in chr10: 874695–874794, chr10: 75873456–75873642, and chr3: 47048304–47048512 that may be important factors in regulating the expression of mRNAs [21]. However, the sample size was small: the study included only ten MDD patients and ten healthy controls, without validation in a larger cohort using qRT-PCR, without predicting treatment responders, and with no classification by gender or age, hence the study design had apparent deficiencies. Besides, an ideal biomarker should not only reflect a patient's odds of developing an illness and the severity of the illness, but also the response to therapeutic intervention. In our study, we used lncRNA microarray technology to evaluate differences in the lncRNA expression profiles of MDD patients, then chose 10 candidate lncRNAs to validate the expression patterns through RT-PCR, and compared their expression level before and after treatment, including comparison with healthy controls, to confirm the diagnostic and therapeutic value of lncRNA biomarkers for MDD.

Material and Methods

Subjects

A total of 138 patients who met the criteria for MDD as described in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) were enrolled from the Changzhou Maternity and Child Health Care Hospital and No. 102 Hospital of the Chinese People's Liberation Army from May 2014 to February 2015. Diagnoses were independently made by two attending psychiatry doctors using the Chinese version of the Modified Structured Clinical Interview for DSM-IV, patient version (SCID-I/P) [22]. The severity of MDD was assessed using the 24-item Hamilton Depression Rating Scale ($HAMD_{24}$) [23]. The inter-rater reliability was 0.87. The inclusion criteria were as follows: 1) patients were either first-time visitors or enrolled previously for any clinical treatment; 2) patients had not taken any antidepressants for at least three months before enrollment in the study, 3) the age of the patients ranged between 18 and 60 years [24]; 4) patients had no previous history of organic disease (such as heart disease, diabetes, or Parkinson's disease), 5) female patients were not currently pregnant; 6) patients had no severe negative life events within the last six months before their diagnosis; and 7) patients had no other psychiatric disorders. The 63 healthy controls who were recruited from the community nearby did not have any family history of mental disorders (e.g.,

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schizophrenia, MMD, bipolar disorder), did not have any history of severe traumatic events within the last month of being enrolled in the study, and were diagnosed as not having mental disease according to the SCID-I/P. Patients and healthy controls were matched in age, ethnicity, and gender at a ratio of two to one. The study was approved by the Ethical Committee for Medicine of Changzhou Maternity and Child Health Care Hospital and No. 102 Hospital of Chinese People's Liberation Army, China. All participants and their legal guardians gave written informed consent.

Sample preparation and RNA extraction

Whole blood samples from 138 MDD patients and 63 healthy controls were collected using EDTA anticoagulant tubes and centrifuged (400 g for 40 minutes) within 2 hours. Blood samples of 30 MDD patients among the 138 were randomly collected again after three-weeks and six-weeks of antidepressant treatment. PBMCs were separated from the blood using density gradient centrifugation and stored at –80°C until used. Total RNA was extracted from the PBMCs using TRIzol (Invitrogen, Carlsbad, CA, USA) and purified by QIAGEN RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. The RNA was quantified using the NanoDrop ND-2000 (Thermo Scientific, Delaware, ME, USA), treated with RNase-free-DNase (Promega, Madison, WI, USA) and reverse transcribed with Invitrogen (Superscript III, USA). The integration of RNA was assessed using the Agilent Bioanalyzer 2100 (Agilent Technologies, USA).

LncRNA microarray expression profiling

RNA samples from 5 MDD patients (male, 23 years, HAMD 28; male, 31 years, HAMD 32; male, 33 years, HAMD 35; female, 52 years, HAMD 29; female, 45 years, HAMD 35) in the 138 MDD group and 5 RNA samples from controls (male, 20 years, HAMD10; male, 33 years, HAMD 9; female, 63 years, HAMD 8; male, 35 years, HAMD 8; female, 43 years, HAMD 11) in the 63 healthy control group were randomly selected for Human lncRNA 3.0 array (Agilent, USA) analysis which covers 30,586 human lncRNAs. Sample labeling, microarray hybridization, and washing were performed based on the manufacturer's standard protocols (Agilent, Inc.). Subsequently, the labeled RNAs were hybridized onto the microarray. After washing and staining the slides, the arrays were scanned using the Agilent Scanner G2505C (Agilent Technologies). The scanned images were analyzed using the Feature Extraction software (version10.7.1.1, Agilent Technologies) and GeneSpring software (version 12.5; Agilent Technologies) [25].

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

According to microarray expression profiling and informed by the literature, candidate lncRNAs were selected using qRT-PCR. Blood samples from 138 MDD patients, 63 healthy controls, and 30 MDD patients after three-week and six-week antidepressant treatment were used to verify the findings from lncRNA microarray expression profiling. Total RNAs were extracted from the PBMCs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) for quantitative detection of lncRNAs. Complementary DNA (cDNA) was synthesized using the TaqMan RNA Reverse Transcription Kit (ABI, USA) according to the manufacturer's protocol. Each RT reaction included 10 uL of total RNA, 3.0 uL TaqMan MicroRNA Assay, 4.16 uL nuclease free water, 0.19 uL RNase inhibitor, 1 uL Multiscribe Reverse Transcriptase, 0.15 uL dNTP, 1.5 uL 10xRT Buffer, in a total volume of 15 uL. Reactions were implemented using the following conditions: 30 minutes at 16°C, 30 minutes at 42°C, 5 minutes at 85°C, and 10 minutes at 4°C. Each sample was processed in duplicate for analysis; real-time PCR was performed using the Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems, Inc., USA). The SDS 2.3 software (Applied Biosystems, Inc.) and DataAssist v3.0 software were used to collect the data. After normalization to β-Actin, the expression levels of lncRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.

Gene ontology (GO) and pathway analysis

GO and pathway analyses were employed to determine the biological pathways or GO terms that were associated with the coding genes located closest to the lncRNAs. Pathway analysis is a functional analysis for mapping genes to the KEGG pathway. Fisher's exact test is a statistical significance test used in the analysis of contingency tables to select a significant pathway. A lower *p* value denotes a more significant GO term and pathway; a *p* value <0.05 and FDR <0.01 indicated statistically significant results.

Medical intervention

Thirty MDD patients in the 138 MDD group were randomly selected for a subsequent antidepressant study. Among the 30 patients, ten were treated with citalopram (initial dosage of 20 mg, average dosage of 30 mg, and dosage range of 20–40 mg) combined with mirtazapine (initial dosage of 7.5 mg, average dosage of 15 mg, and dosage range of 7.5–22.5 mg); eight with sertraline (initial dosage of 50 mg, average dosage of 100 mg, and dosage range of 50–150 mg) combined with mirtazapine (initial dosage of 7.5 mg, average dosage of 15 mg, and dosage range of 7.5–22.5 mg); twelve with fluvoxamine (initial dosage of 50 mg, average dosage of 100 mg, and dosage range of 50–150 mg) combined mirtazapine (initial dosage of 7.5 mg, average dosage of 15 mg, and dosage range

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Table 1. Clinical characteristics of MDD patients and healthy controls.

	MDD (n=138)	Controls (n=63)	Comparison	
			Statistics	P-value
Age (SD)	36.41 (16.6)	39.58 (13.4)	t=0.354	0.478
Sex(Female/Male)	84/54	33/30	χ^2=108.32	0.254
HAMD score, mean (SD)	27.6 (6.94)	8.2 (2.5)	t=12.3	<0.0001
Ethnic	Han	Han		

MDD – major depressive disorder; HAMD – Hamilton depression scale.

of 7.5–22.5 mg). The severity of symptoms and their changes were assessed by attending psychiatrists using the $HAMD_{24}$. Patients were assessed by HAMD at baseline and reassessed at three-weeks and six-weeks after antidepressant treatment. Using the qRT-PCR, we measured the expression levels of candidate lncRNAs in these 30 MDD patients after three-weeks and six-weeks of treatment. MDD patients who had ≥50% reduction in the $HAMD_{24}$ total scores from baseline met criteria of responder, those who had ≥75% reduction in the HAMD total scores from baseline met the criteria of remission [26].

Statistical analysis

Chi-square test and the independent *t*-test were used to compare the demographic variables between MDD patients and controls. The Mann-Whitney U test was used to test the differences in the expression levels of lncRNAs between the patient group and the control group, and the Wilcoxon rank sum test was used to test the differences between different genders and ages in the MDD and control groups. Volcano Plot filtering was used to identify differentially expressed lncRNAs between the two groups (fold change ≥2.0, $p<0.05$). The repeated measures design analysis of variance was used to test the differences of lncRNA expression at three times. The specificity and sensitivity of single lncRNAs and their combination were assessed by receiver operating characteristic (ROC) curves. All statistical analyses were conducted using SPSS version 20.0 software (Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). A $p<0.05$ (two-tailed) was considered with statistical significance.

Results

Clinical characteristics of the patients

According to chi-square test and *t*-test, there were no significant differences between the MDD group and control group in age or sex distribution, with the exception of HAMD scores. The demographic data of the patients and controls are shown in Table 1.

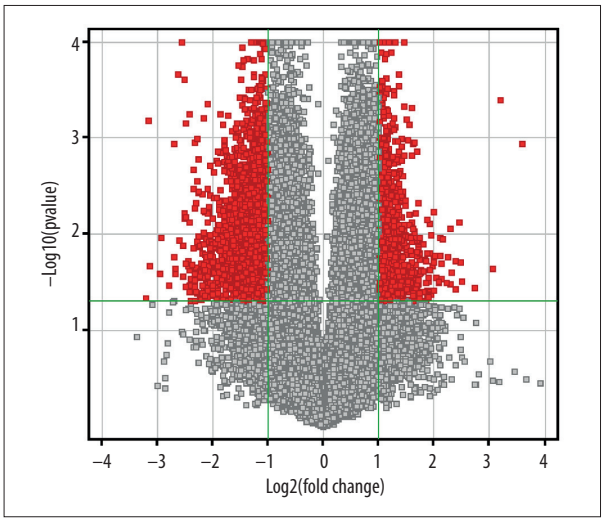

Figure 1. Volcano plot of lncRNAs. Volcano plot is used for visualizing differential expression between two groups. The vertical lines correspond to 2.0-fold up and down, respectively, and the horizontal line represents a *p*-value of 0.05. The red point in the plot represents the differentially expressed lncRNAs with statistical significance.

LncRNA microarray expression profiling

Differentially expressed lncRNAs between MDD patients and controls were identified through volcano plot filtering ($p<0.05$, fold change ≥2) (Figure 1). Using ten blood samples (five MDD patients and five controls) in microarray profiling, a total of 2,649 differentially expressed lncRNAs, which included 534 upregulated and 2,115 downregulated lncRNAs, were identified between the two groups. Ten candidate lncRNAs were selected to verify in a larger cohort (138 patients and 63 controls) using the qRT-PCR, shown in Table 2. These ten lncRNAs were selected using the following the criteria: lncRNAs with higher fold change in microarray results; lncRNAs that had been reported in lncRNA databases, such as lncrnadb, lncRNABase, lncRNA Disease and UCSC_kg; lncRNAs that could be detected in every sample.

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Table 2. The ten candidate lncRNAs in MDD patients and controls.

Seqname	Fold change	Regulation	P-value	chr
TCONS_l2_00001212	5.686522	Down	0.001366	chr1: 47347875-47351132
NONHSAT102891	3.558301	Down	0.003411	chr5: 95768987-95770845
TCONS_00019174	3.775364	Dowd	0.012859	chr11: 116367616-116371347
ENST00000566208	3.697919	Down	0.023714	chr16: 8348494-8349774
ENST00000414201	5.115068	Down	0.004037	chr13: 61491176-61494141
NONHSAG045500	3.514152	Down	0.03892	chr6: 170426388-170430482
ENST00000591189	4.610542	Up	0.047744	chr6: 143825121-143827284
ENST00000517573	3.242618	Down	0.039281	chr8: 20831308-20832540
NONHSAT034045	3.405084	Down	0.010672	chr13: 55976660-55981315
NONHSAT142707	5.130027	Down	0.016325	chr16: 56716393-56718108

"Up" – represents the expression of lncRNAs in patients were higher than in the normal control, but the number of fold changes were smaller than the normal control; "Down" – represents the opposite.

Table 3. Comparison of $^{\Delta}$CT between MDD patients and healthy controls ($\bar{\chi}\pm s$).

Seqname	MDD (n=138)	NC (n=63)	*P* value
TCONS_L2_00001212	6.91±2.11	5.78±2.36	**0.0067**
NONHSAT102891	7.12±2.21	6.15±2.41	**0.0242**
TCONS_00019174	6.95±2.46	5.77±2.78	**0.0153**
ENST00000566208	6.36±2.51	5.34±2.70	**0.0373**
ENST00000414201	6.22±2.60	5.26±2.67	0.0559
NONHSAG045500	8.24±2.52	7.23±2.57	**0.0376**
ENST00000591189	4.84±1.51	4.15±1.81	**0.0243**
ENST00000517573	7.50±2.69	6.34±2.69	**0.0237**
NONHSAT034045	7.01±2.71	5.98±2.76	**0.0457**
NONHSAT142707	9.14±2.57	7.97±2.76	**0.0193**

MDD – major depressive disorder; NC – normal control; **bold** – significant difference.

Validation of microarray profiling data using qRT-PCR

The ten lncRNAs were verified in 138 MDD patients and 63 controls using qRT-PCR. As shown in Table 3, the $^{\Delta}$CT values for all lncRNAs were bigger in MDD patients than in healthy controls, indicating that their actual expression trend was lower in MDD patients. There was no significant difference in ENST00000414201 between the two groups. Moreover, the expression pattern of ENST00000591189 was opposite to what was observed in the microarray. Therefore, only TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707 were considered to be significantly different between the two groups.

Comparison of lncRNAs expression before and after antidepressant treatment in MDD patients

Among the 138 MDD patients who received formal antidepressant treatment, 30 of them (sex: male 10, female 20; ages: 40.3±6.7 years; HAMD 29.6±6.47) were randomly selected to retest lncRNAs expression at three-weeks and six-weeks after treatment. In all, 24 achieved the criteria of remission and 6 achieved criteria of response after six-weeks of antidepressant therapy. The eight lncRNAs expressions were subsequently analyzed at three time points. Through repeated measures analysis of variance, all of the lncRNAs except NONHSAT102891 were significantly upregulated ($p<0.05$), and only six lncRNAs

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Table 4. Comparison of lncRNAs $^{\Delta}$CT value before and after antidepressant treatment ($\bar{\chi}\pm s$).

Seqname	MDD (n=30)	3w (n=30)	6w (n=30)	P value	NC (n=63)	P value
TCONS_L2_00001212	6.89±2.01	5.89±1.08	3.04±5.51	**0.002**	5.78±2.36	**<0.0001**
NONHSAT102891	7.26±2.19	7.21±3.14	6.02±2.57	0.085	6.15±2.41	**0.018**
TCONS_00019174	7.34±2.58	7.08±4.80	4.40±4.15	**0.006**	5.77±2.78	0.360
ENST00000566208	6.74±2.73	7.07±4.29	4.56±3.52	**0.011**	5.34±2.70	0.397
NONHSAG045500	8.62±2.53	8.67±5.16	5.96±4.13	**0.009**	7.23±2.57	0.894
ENST00000517573	7.75±2.80	7.76±5.15	4.99±4.34	**0.013**	6.34±2.69	0.349
NONHSAT034045	7.37±2.85	7.78±5.13	5.02±4.11	**0.011**	5.98±2.76	0.503
NONHSAT142707	9.47±2.46	8.74±5.45	6.17±4.48	**0.014**	7.97±2.76	0.257
HAMD	27.60±6.94	15.23±7.10	10.90±4.30	**<0.001**	8.2±2.5	0.084

The first P value represents the results of variance analysis for lncRNAs expression in 30 MDD patients. The second P value represents the contrast analysis result of lncRNAs expression between the 30 MDD patients after six-week treatment and the controls.
MDD – major depressive disorder; NC – normal control; HAMD – Hamilton depression scale; **bold** – significant difference.

(TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) expression levels had no difference between MDD and healthy controls ($p>0.05$) (Table 4). Notably, the HAMD score was significantly reduced after six-weeks of treatment ($p<0.05$).

The diagnostic value of the six lncRNAs that were significantly differentially expressed were evaluated by ROC curve analysis. The results showed that the AUC of TCONS_00019174 was 0.646 (95% CI: 0.545–0.748), ENST00000566208 was 0.596 (95% CI: 0.493–0.698), NONHSAG045500 was 0.601 (95% CI: 0.499–0.703), ENST00000517573 was 0.621 (95% CI: 0.521–0.720), NONHSAT034045 was 0.603 (95% CI: 0.502–0.703), and NONHSAT142707 was 0.627 (95% CI: 0.524–0.729). The predictive value of these six lncRNAs combined was also examined, the AUC was 0.719, with 82.4% sensitivity and 72.0% specificity (Figure 2).

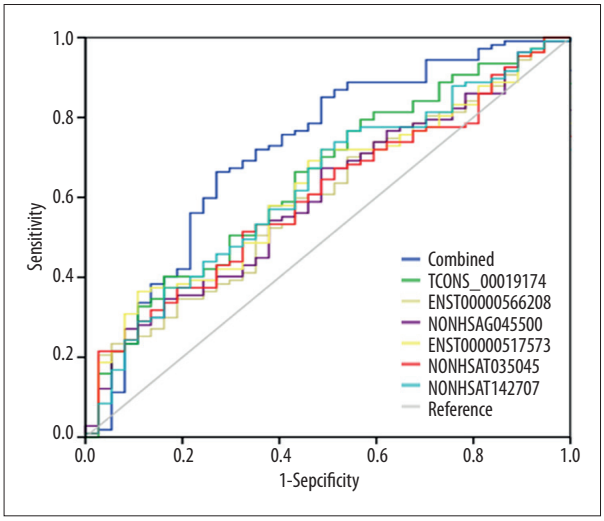

Figure 2. The receiver operating characteristics curve (ROC) of the eight differentially expressed lncRNAs and combined ROC of the six lncRNAs.

Comparison of lncRNA expression across gender and age

According to the WHO guidelines for age segmentation [27], 138 MDD patients were divided into two age groups: youths (18–44 years, n=89) and the middle-aged (>44 years, n=49). The independent sample *t*-test revealed that there were no differencex in the expression levels of the six lncRNAs among the two age groups (t=1.200–1.811, $p>0.05$), and between males (n=84) and females (n=54) (t=–1.724–0.391, $p>0.05$).

GO and pathway analyses

Gene ontology (*hrrp://www.geneontology.org*) covers three domains: biological processes, cellular components, and molecular functions. Using the gene ontology database, the significance level of GO terms for the differentially expressed genes was analyzed.

In our existing data, the coding genes adjacent to the down-regulated lncRNAs mainly involved the following functions: (1) translational elongation, (2) protein transport, (3) establishment of protein localization, (4) protein complex biogenesis, (5) protein complex assembly, (6) translation, (7) macromolecular complex assembly, (8) macromolecular complex subunit organization, (9) proteasomal ubiquitin-dependent protein catabolic processes, and (10) proteasomal protein catabolic processes (Figure 3A).

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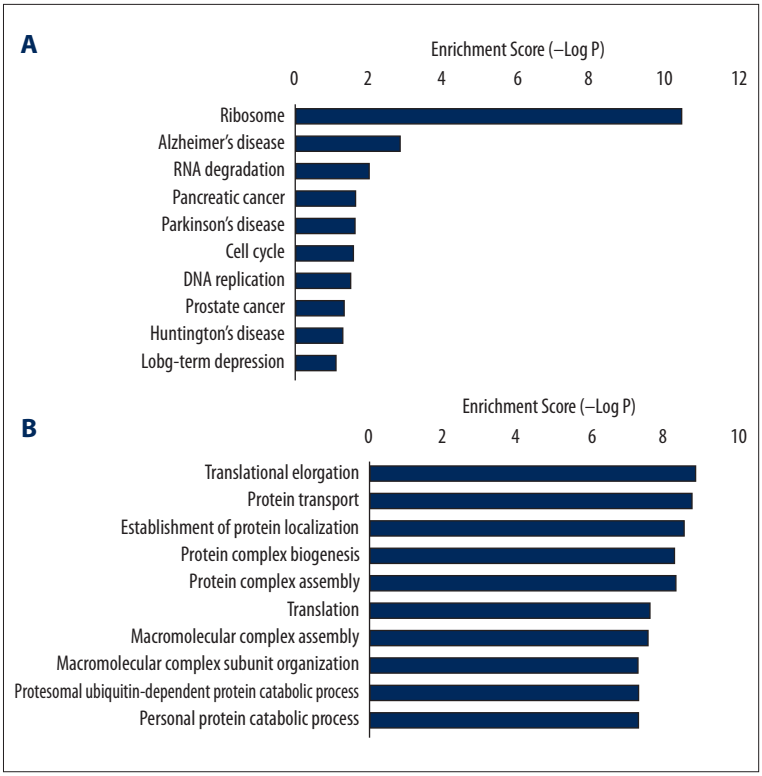

Figure 3. GO and pathway analysis. The top 10 GO terms for the function of the coding genes associated with downregulated lncRNAs are listed. The top 10 pathways for the functions of the coding genes associated with lncRNAs are listed.

Pathway analysis is a functional analysis that maps genes to the KEGG pathway. Based on the Kyoto Encyclopedia of Genes and Genomes database (*http:www.genome.jp/kegg/*), the significance levels of differentially expressed gene pathways were analyzed. In our existing data, the functions of the coding genes adjacent to the downregulated lncRNAs mainly involved the following pathways: (1) ribosome activities; (2) Alzheimer's disease; (3) RNA degradation; (4) pancreatic cancer; (5) Parkinson's disease; (6) cell cycle; (7) DNA replication; (8) prostate cancer; (9) Huntington's disease; and (10) long-term depression (Figure 3B).

Discussion

At present, there were many studies about epigenetic biomarkers for MDD, such as DNA methylation and miRNA. Numata [28] et al. showed that significant diagnostic differences in DNA methylation were observed at 363 CpG sites in the discovery set for MDD patients, and maternal depression was associated with DNA methylation alterations in maternal T lymphocytes, neonatal cord blood T lymphocytes, and adult offspring hippocampi [29]. In addition, miRNAs (miR-16 [30,31], miR-221-3p, miR-34a-5p, let-7d-3p, and miR-451a [32], miRNA-26b, miRNA-1972, miRNA-4743, miRNA-4498, and miRNA-4485 [16]) were found to involve the development of MDD, and the changes of miRNA expression were correlated with the improvement of symptoms [33–35]. These findings suggest research attention to should include another non-coding RNA, lncRNA.

To the best of our knowledge, no previous studies have used a large sample size to investigate the expression profiles of lncRNAs in MDD, the changes of lncRNA expression following treatment, or the expression of lncRNAs between sexes and among different age groups. In this study, first we examined the differential expression of lncRNAs in MDD patients using microarray analysis. A total of 2,649 lncRNAs, consisting of 534 upregulated and 2,115 downregulated lncRNAs, were found to be differentially expressed. Then, ten of these lncRNAs were chosen to validate this finding using qRT-PCR. The result confirmed that eight lncRNAs (TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) were differentially expressed in PBMCs of MDD patients. An ideal biomarker has not only clinical diagnostic value (diagnostic biomarkers) but also can be used to identify and/or predict those who respond or do not respond to treatment (treatment biomarkers). So, the biomarker may either be present at baseline and predict response to treatment or, alternatively, may temporarily change in a way that it predicts the ultimate response. Previous studies on miRNAs [16,34] and lncRNAs [21] as MDD biomarkers only examined the expression level according to disease status, without comparing expression level after treatment. Although some studies compared the differences of miRNAs before and after treatment, they did not analyze the difference in miRNA expression after treatment between patients and normal controls [33,35]. At last, in this study, the expression of eight lncRNAs at three different time

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points (before treatment, three-weeks after treatment, and six-weeks after treatment) were analyzed. The results indicated that, after six-weeks of treatment, the expression levels of seven downregulated lncRNAs were significantly upregulated, but only six lncRNAs (TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) had no significant difference compared to normal controls. After six-weeks of standard antidepressant treatment, 24 patients achieved the criteria of remission, and six achieved the criteria of response, the total HAMD score for patients was not significantly different from controls. Then, the diagnostic value of the six lncRNAs was assessed using ROC curve. The AUC of the six lncRNAs ranged from 0.596 to 0.646, and the combined AUC was 0.719, with a sensitivity of 82.4% and specificity of 72.0%. Therefore, if the six lncRNAs were used as a whole, their diagnostic and therapeutic value for MDD would improve further. We also conducted preliminary GO and pathway analyses of the biological functions of the differentially regulated lncRNAs. In our existing data, the main biological processes involving the differentially expressed lncRNAs included translational elongation, protein transport, establishment of protein localization, protein complex biogenesis, and protein complex assembly. Pathway analysis showed that the genes associated with the differentially expressed lncRNAs involved central nervous system diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease, long-term depression) as well as several cancers (pancreatic cancer, prostate cancer). Further study is needed on the mechanisms of lncRNAs involvement in biological processes of the central nervous system, especially hippocampal development, brain aging, and GABA neurons, which have been associated with the development of depression.

The incidence rate of many diseases has sex and age bias, for example, the morbidity of ischemic stroke, cardiovascular disease, schizophrenia, and depression are higher in women than in men, and higher in the elderly than in adults or adolescents. Recently, the role of age and sex on circulating ncRNAs was explored in peripheral blood using microarray technology, indicated that there were age-associated (has-miR-126-3p and has-miR-21-5p) and sex-related (has-miR-145-5p, has-miR0451a, miR-26a and miR-26b) miRNA levels in the blood [24,36–38]. In addition, a lncRNA named LINC-RBE (rat brain-expressed transcript) [39] was found to be expressed in an age-dependent manner, with significantly higher levels of LINC-RBE in the adult brain (16 week) compared to both immature (4 week) and old (70 week) rats. Thus, the expression of LINC-RBE increased from the immature stage to adulthood and declined further in old age. Therefore, in this study, we further compared the expression of six lncRNAs between different sexes and across ages, the results showed that lncRNAs expression in males was higher than in females, lower in youths than in middle-aged individuals, but the differences did not reach the significant level. Consequently, we hypothesis that in the clinical setting, we can use different reference ranges of these six lncRNAs as an index to assess the disease status for different sex and age groups of MDD patients.

Conclusions

Non-coding RNAs in cellular blood represent a promising source for the development of diagnostic and prognostic tools, as the method of sample collection is minimally invasive, lncRNAs exhibit high stability, and these lncRNAs can be easily quantified using standard techniques. These six lncRNAs (TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) may be used as stable biomarkers for MDD in those of Han Chinese ethnic origin, regardless of gender or age.

Limitation

The samples from this study were all collected in mainland China, so the samples are solely of Han Chinese nationality. The 63 healthy controls in this study, didn't reach a ratio of one to one for patients and controls. In compliance with the Ethical Committee for Medicine, we only compared the difference of lncRNA expression before and after treatment in 30 MDD patients who had dosage and combined antidepressant treatment, without including a placebo group, so we are not sure whether there was a time effect in the alteration of lncRNA

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