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RESEARCH ARTICLE

Bioinformatics Analysis of Altered lncRNAs in Peripheral Blood Molecular Cells from Major Depressive Disorder (MDD) Patients

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Abstract

Objectives: Based on the prior studies, altered lncRNAs in peripheral blood Molecular Cells (PBMC) from depression patients were chosen to perform informatics analysis for lncRNA target gene prediction and functional annotation.

Methods: Microarray was first used to screen dys regulated lncRNAs in the PBMCs of MDD patients, of which 10 lncRNAs were selected for quantitative real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) study, as well as bioinformatics analysis.

Results: According to microarray results, the top 10 lncRNAs with highest expression changes were chosen for further validation with qRT-PCR, 9 lncRNAs demonstrate significant down-regulation in expression levels (TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045, NONHSAT142707) ($P < 0.05$). lncRNA target gene prediction and functional annotation analysis showed a significant enrichments in several gene ontology (GO) biological process and Kyoto encyclopedia of genes and genomes (KEGG) pathways associated with nervous system and brain functions, suggesting that the differentially expressed lncRNAs may be involved in mechanism of MDD. Cytoscape network chart indicated that TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707 also provide clues for the association of lncRNAs with MDD.

Conclusions: Altered expression of lncRNAs (TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707) might be involved in MDD pathogenesis and may serve as noninvasive biomarker for MDD diagnosis.

Keywords

lncRNA, Depression, Target gene, Bioinformatics

Introduction

Major Depression Disorder, a mood disorder with high suicide risk [1], affects approximately 300 million people worldwide [2]. It has been suggested MDD was co-effected by genetics, personalities, cognitive, poor life event, biochemical disease, somatopathy and so on [3-5], of which, nearly 50% of the risk for depression is contributed to genetic factors [6-8].

Long non-coding RNA (lncRNA) was a molecular

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with a length of over 200 nt, widely existing in animals, plants, yeasts, prokaryotes and viruses. A numerous study recently indicated lncRNA's function in genetics due to its conservative secondary structures and shear forms. A numerous study recently indicated lncRNA's function in genetics due to its conservative secondary structures and shear forms [9].

Because of the inconsistent between sequence and function of lncRNA, it is very hard to confirm their function. But fortunately, a large number of studies have indicated the association of lncRNA with mental disease [10-14]. In this study, through long non-coding RNA (lncRNA) expression profiling and reverse transcription-polymerase chain reaction, 2115 down-regulated and 534 up-regulated lncRNAs were screened. 10 lncRNAs (TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, ENST00000414201, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707) showed a marked differences ($P < 0.01$ or $P < 0.05$).

Based on the prior studies, altered lncRNAs in peripheral blood Molecular Cells (PBMC) from Depression patients were chosen to perform informatics analysis for lncRNA target gene prediction and functional annotation.

Materials and Methods

Subjects

138 MDD patients who met the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V) were enrolled from No.102 Hospital of the Chinese People's Liberation Army from May 2014 to February 2015. Diagnoses were made independently by three attending psychiatric doctors using the Chinese version of the modified Structured Clinical Interview for DSM-V. All patients were presenting for their first visit without any prior clinical treatment. They had not taken antipsychotic, antidepressant, anxiolytic drugs for at least three months before study enrollment, had no previous history of organic disease (such as heart disease, diabetes, and Parkinson's disease) and no other psychiatric disorders.

The 63 healthy controls were recruited from No.102 Hospital of the Chinese People's Liberation Army from May 2014 to February 2015, without any family history of major psychiatric disorders (including schizophrenia, bipolar disorder and anxiety disorders) and without any history of severe traumatic events in the past month.

Prior to study entry, all risks, benefits and potential adverse events associated with participation in the study were explained to the participants and their legal guardians, who provided written informed consent according to a protocol approved by the Ethical Committee for Medicine of No.102 Hospital of Chinese People's Liberation Army, PR, China.

Experimental methods

RNA extraction: Whole blood (5 ml) was collected in EDTA anticoagulant tube from each subject and processed within 1 hour. PBMCs were isolated from the blood through density gradient centrifugation and stored at -80 °C until use. Total RNAs were extracted from the PBMCs with Trizol (Invitrogen, Carlsbad, CA, USA) and the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, quantified by Nano Drop ND-2000 (Thermo Scientific, Delaware, ME, USA), DNase treated (Turbo DNase, Life Technologies) and reverse transcribed (Superscript III; Invitrogen). The integration of RNA was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies).

LncRNA microarray expression profiling: RNA samples were used for lncRNA microarray profiling. LncRNA expression was measured by Human lncRNA 3.0 array (Arraystar, Santa Clara, CA, USA). The sample labeling, microarray hybridization and washing were performed based on the manufacturer's standard protocols. Afterwards, the labeled RNAs were hybridized onto the microarray. Having washed and stained the slides, the arrays were scanned by the Agilent Scanner G2505C (Agilent). The scanned images were analyzed using Feature Extraction software (version 10.7.1.1, Agilent Technologies) and Genespring software (version 12.5; Agilent Technologies).

Real-time quantitative reverse-transcription PCR (qRT-PCR): According to microarray results, the top 10 lncRNAs with the highest differentially expression were chosen for further validation with qRT-PCR. Blood samples from 69 GAD patients and 41 controls were used to validate the candidate lncRNAs. Total RNAs were isolated from the PBMCs using Trizol reagent (Invitrogen®, USA) for quantitative detection of lncRNA. Complementary RNA was synthesized using the Reverse Transcription TaqMan RNA Reverse Transcription Kit (Applied Biosystems, inc., USA) according to the manufacturer's instructions. Real-time PCR was performed using Applied Biosystems 7900 HT Real-Time PCR System (Applied Biosystems, Inc., USA). Data were collected using the SDS 2.3 software (Applied Biosystems, Inc.) and Data Assist v3.0 software. After normalized to β-Actin, the expression levels of lncRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.

Data acquisition: Feature Extraction (version 10.7.1.1, Agilent Technologies) was applied to act on original image for extracting original data, then Genespring.

Results

Qrt-PCR result showed that TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707 were identified as significantly different from the control group ($P < 0.01$

Table 1: Comparison of lncRNA expression levels between MDD group and control group (ΔCt, $\overline{X}$ ± s).

lncRNA	GAD (n = 138)	Control group (n = 63)	Fold change	Up-down	*P*-value
TCONS_L2_00001212	6.91 ± 2.11	5.78 ± 2.36	5.686522	Down	0.0067
NONHSAT102891	7.12 ± 2.21	6.15 ± 2.41	3.558301	Down	0.0242
TCONS_00019174	6.95 ± 2.46	5.77 ± 2.78	3.775364	Down	0.0153
ENST00000566208	6.36 ± 2.51	5.34 ± 2.70	3.697919	Down	0.0373
ENST00000414201	6.22 ± 2.60	5.26 ± 2.67	5.115068	Down	0.0559
NONHSAG045500	8.24 ± 2.52	7.23 ± 2.57	3.514152	Down	0.0376
ENST00000591189	4.84 ± 1.51	4.15 ± 1.81	4.610542	Down	0.0243
ENST00000517573	7.50 ± 2.69	6.34 ± 2.69	3.242618	Down	0.0237
NONHSAT034045	7.01 ± 2.71	5.98 ± 2.76	3.405084	Down	0.0457
NONHSAT142707	9.14 ± 2.57	7.97 ± 2.76	5.130027	Down	0.0193

Table 2: Enrichment items of lncRNA target genes in GO.

GO number	Item	*P*-value	Gene name
GO: 0010467	gene expression	3.76e-05	splicing factor 3a, subunit 1, 120 kDa
GO: 0007596	blood coagulation	7.1e-04	CAP, adenylate cyclase-associated protein 1 (yeast)
GO: 0044267	cellular protein metabolic process	3.35e-06	defender against cell death 1
GO: 0016032	viral reproduction	9.45e-04	nucleoporin 50 kDa
GO: 0030168	platelet activation	3.21e-05	CAP, adenylate cyclase-associated protein 1 (yeast)
GO: 0006412	translation	1.46e-04	ribosomal protein L 10-like
GO: 0044419	interspecies interaction between organisms	9.53e-03	acyl-CoA thioesterase 8
GO: 0007411	axon guidance	7.55e-03	myosin, light chain 12B, regulatory
GO: 0031018	endocrine pancreas development	3.18e-09	glucokinase (hexokinase 4)
GO: 0006414	translational elongation	1.29e-09	eukaryotic translation elongation factor 1 alpha 1
GO: 0019058	viral infectious cycle	8.39e-10	ribosomal protein L 3-like
GO: 0006415	translational termination	1.83e-09	ribosomal protein L 3-like
GO: 0019083	viral transcription	7.42e-10	ribosomal protein L 3-like
GO: 0048011	nerve growth factor receptor signaling pathway	2.29e-02	BCL2-like 11 (apoptosis facilitator)

Table 3: Enrichment items of lncRNA target genes in KEGG.

GO number	Item	*P*-value	Gene name
03010	ribosome	2.24e-09	ribosomal protein L 10-like
04670	leukocyte transendothelial migration	4.91e-03	myosin, light chain 12B, regulatory
05414	dilated cardiomyopathy	1.29e-02	Calcium channel, voltage-dependent, gamma subunit 3
04146	peroxisome	1.43e-02	acyl-CoA thioesterase 8
04970	salivary secretion	3.11e-02	Adenylate cyclase 9
04612	antigen processing and presentation	1.43e-02	cAMP responsive element binding protein 1
05110		1.63e-02	Sec61 beta subunit
05340	primary immunodeficiency	2.75e-02	Interleukin 2 receptor, gamma

or $P < 0.05$, Table 1). Qrt-PCR result showed that TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707 were identified as significantly different from the control group ($P < 0.01$ or $P < 0.05$, Table 1).

lncRNA target gene prediction and functional annotation analysis showed a significant enrichments in several gene ontology (GO) associated with nervous system and brain functions, suggesting that the differentially expressed lncRNAs may be involved in mechanism of MDD (Table 2).

lncRNA target gene prediction and functional annotation analysis showed a significant enrichments in Kyoto encyclopedia of genes and genomes (KEGG) pathways associated with nervous system and brain functions, suggesting that the differentially expressed lncRNAs may be involved in mechanism of MDD. Cytoscape network chart indicated that TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707 also provide clues for the association of lncRNAs with MDD (Table 3).

Discussion

Given the evidence that estimated 90% of non-coding proteins in human genome have important role in genetics, not just "translational noise", it seems reasonable that lncRNA may play an important role in regulation of transcription and post-transcription [15]. Research also demonstrated that lncRNA acts as the molecular scaffold, making sense for the transmit of biological signals, interaction of molecules, and the specificities and dynamics of signal regulation [16]. Until now, MDD is a mental illness with the functional changes of neurotransmitter, and more and more evidences have revealed the association of differentially expressed

lncRNAs with neurodevelopment, neurodegeneration and neuroimmunological disorders [17-19].

Given the consistent evidences, it makes sense to further explore the biological function on the nine down-regulated lncRNAs.

LncRNA is found to serve as a very important epigenetic regulation factor in human genome, regulating DNA methylation, histone modification and chromatin remodeling through epigenetic, transcriptional regulation and post-transcription regulation, making the gene silencing or activation. In this study, at first, we re-tested the nine up-regulated differentially expressed lncRNAs through gene ontology (GO), indicated that the lncRNAs expression in MDD had a enrichment in translation, DNA dependency transcriptional regulation, axon elongation, dendritic morphology, cell differentiation, long-term memory, RNA transfer, neurons development, and so on. This was similar to the existing results. A study demonstrated that MDD was significantly enriched in central system, including axon elongation, dendritic morphology, cell differentiation, long-term memory [20].

Simultaneously, we also perform Kyoto encyclopedia of genes and genomes (KEGG) pathways, and the result illustrated that there is a enrichment in central nervous system. Among which, the low-regulation of Wnt signaling pathway may cause hippocampal neuron denaturation [21], Ca^{2+}/calmodulin-dependent protein kinase IV and MAPK signaling pathway are closely associated with the regulation processes of depression behavior [22-25]; biological analysis related to molecular pathway of depression revealed the enrichment in VEGF signaling pathway, GnRH signaling pathway and LTP pathway [26]. In addition to this, TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707 were seen to be posited in the centre of network, thus it is considered that these nine lncRNAs could be biomarkers of MDD.

In conclusion, we speculate that TCONS_L2_00001212, NONHSAT102891, TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000591189, ENST00000517573, NONHSAT034045 and NONHSAT142707 could be potential indicators for the diagnosis of MDD. The clinical significance of this study was given advice for the early forecast and diagnosis of MDD.

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