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Altered microRNA Expression in Peripheral Blood Mononuclear Cells from Young Patients with Schizophrenia

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Abstract Schizophrenia (SZ) is a debilitating psychotic disorder of unknown etiology, and the diagnosis is essentially based on clinical symptoms. So it is urgent to find an objective and feasible clinical diagnostic index for SZ. MicroRNA array was performed in peripheral blood mononuclear cells (PBMCs) obtained from young SZ patients and gender-, age-, and ethnicity-matched healthy controls. Then, real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to verify the top 10 microRNAs (miRNAs) with the highest fold change values in 55 SZ patients and 28 healthy controls, and 9 miRNAs demonstrate significant differences in expression levels ($P<0.01$). Receiver operating characteristic (ROC) curve analysis showed that the combining area under the ROC curve (AUC) of the nine miRNAs was 0.973 (95 % confidence interval (CI): 0.945–1.000). miRNA target gene prediction and functional annotation analysis showed that there were 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 miRNAs may be involved in mechanism of SZ. We conclude that altered expression of miRNAs in PMBCs might be involved in young SZ pathogenesis and may serve as noninvasive biomarker for SZ diagnosis.

Hui-min Fan, Xin-yang Sun, and Wei Niu agreed to share the first authorship position together.

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Introduction

Schizophrenia (SZ) is a severe, chronic, and disabling mental disorder that affects about 1 % of the world's population throughout life (Saha et al. 2005). Until today, the diagnosis of SZ is mainly based on clinical symptoms varying greatly between individuals. Moreover, there are considerable symptomatic overlap between some mental disorders (Haller et al. 2014), which may lead to misdiagnosis or different diagnosis between doctors. So it is urgent to find an objective and feasible clinical diagnostic index for SZ.

microRNAs (miRNAs) are endogenous small noncoding RNAs that negatively regulate gene expression by translational repression or mRNA cleavage at the post-transcriptional level. Accumulating evidence indicates that miRNAs are involved in a wide variety of biological processes, including development, differentiation, proliferation, apoptosis, invasion, and metastasis (Bartel 2009; Zhuo et al. 2013; Moreno-Moya et al. 2014), and play an important role in brain development and function (Bian and Sun 2011; Iyengar et al. 2014; Smalheiser 2014). There is also growing evidence showing that miRNA expression profiles are altered in psychiatric disorders such as SZ, bipolar disorder, autism spectrum disorders (Miller and Wahlestedt 2010; Xu et al. 2010; Song et al. 2014), suggesting that miRNAs may contribute to the etiology and pathogenesis of psychiatric disorders. Since 2008, many studies have reported that specific miRNA expression can be detected in peripheral blood in many diseases. From then on, circulating miRNAs have become a hot issue of current research. Because of its characteristics of high stability, noninvasiveness, easy accessibility, high measurement accuracy, and cost-effectiveness (Ajit 2012), circulating miRNAs may serve as promising biomarkers of many diseases. Although the research on circulating miRNAs as biomarkers is still at a groping stage, hundreds of papers have already been published on the subject in various diseases such as cancer and cardiovascular diseases (Wittmann and Jäck 2010; Ajit 2012; Xu et al. 2012). However, there is a very limited number of study on the miRNA expression profile in peripheral blood of psychiatry diseases. In terms of schizophrenia, the only study we could found was conducted by Lai et al., who identified a seven-miRNA signature (hsa-miR-34a, miR-449a, miR-564, miR-548d, miR-572, and miR-652 upregulated; miR-432 downregulated) in blood mononuclear leukocytes which could distinguish SZ patients from normal controls (Lai et al. 2011).

Given that the onset of schizophrenia generally occurs between the late teens and the mid-30s (American Psychiatric Association 2013) and that the expression of miRNA is influenced by age (Noren Hooten et al. 2010; Pena-Chilet et al. 2014), we speculate that young SZ patients would be a good sample to identify altered miRNAs as biomarker for diagnosis. The present study screened the differently expressed miRNAs in peripheral blood mononuclear cells (PBMCs) from young SZ patients and healthy controls using miRNA microarray, and then the candidate miRNAs were validated in a larger sample of cases and controls using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Finally, target genes were predicted by bioinformatics tools and were subjected to gene ontology (GO) analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis.

Materials and Methods

Patients

A total of 55 SZ patients between 18 and 30 years old who met the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition for schizophrenia, were enrolled from No.102 Hospital of the People's Liberation Army from December 2012 to May 2013. Patients were either first onset or drug naive from any antidepressant for at least 3 months before enrollment. Patients with severe medical diseases, other psychiatric disorders, structural brain disorders, mental retardation, mood incongruent psychotic symptoms, and primary substance abuse were excluded. Patients who had brain injury causing traumatic amnesia longer than 24 h and who received blood transfusion within 1 month or electroconvulsive therapy within 6 months were also excluded from the study.

Twenty-eight healthy controls without any family history of major psychiatric disorders (SZ, bipolar disorder, and major depressive disorder) were recruited. All healthy controls were without any history of blood transfusion or severe traumatic event within 1 month. Patients and healthy controls were matched in gender, age, and ethnicity on a ratio of 2:1. The study was approved by the local ethics committee. Written informed consent was obtained from all subjects.

Blood Collection and RNA Extraction

Whole blood (5 ml) was collected in EDTA anticoagulant tube from each subject and processed within 1 h. PBMCs were isolated through density gradient centrifugation and stored at −80 °C until use. Total RNAs were extracted from the PBMCs

with the mirVana™miRNA Isolation Kit (Ambion, LOT:1406120 AM1561) according to the manufacturer's protocol. To ensure a robust analysis for the following procedures, samples with an RNA integrity number (RIN) inferior to 8 were excluded.

miRNA Microarray Expression Profiling

RNA samples from three SZ patients (male, 20 years; male, 21 years; female, 19 years) and three controls (male, 20 years; male, 21 years; female, 19 years) were used for miRNA microarray profiling. miRNA expression was measured by Affymetrix miRNA 3.0 array (Affymetrix, Santa Clara, CA, USA). The sample labeling, microarray hybridization, and washing were performed based on the manufacturer's standard protocols. Briefly, total RNA were tailed with poly A and then labeled with Biotin. Afterward, the labeled RNAs were hybridized onto the microarray. Having washed and stained the slides, the arrays were scanned by the Affymetrix Scanner 3000 (Affymetrix). The scanned images were analyzed using Expression Console software (version 1.3.1, Affymetrix).

Real-Time Quantitative Reverse Transcription PCR

According to microarray results, the top 10 miRNAs with the highest expression changes were chosen for further validation with qRT-PCR. Blood samples from 55 SZ patients and 28 controls were used to validate the candidate miRNAs. Total RNAs were isolated from the PBMCs using Trizol reagent (Invitrogen®, USA) for quantitative detection of miRNA. Complementary DNA was synthesized using the Reverse Transcription TaqMan MicroRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied Biosystems, Inc., USA, P/N: 4366596) according to the manufacturer's instructions. Real-time PCR was performed using Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems, Inc., USA). The 5× RT primers (miRNA-specific stem-loop primers) and 20× miRNA-specific PCR primer/probe mix were supplied by the TaqMan MicroRNA Assays (Applied Biosystems, Inc.). Data were collected using the SDS 2.3 software (Applied Biosystems, Inc.). After normalized to RNU48, the expression levels of miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008).

miRNA Target Prediction and Pathway Analysis

The predicted target genes of the differentially expressed miRNAs were obtained using two open access databases (miRDB, http://mirdb.org/miRDB/index.html; and DIANA-

Table 1 Demographic data of patients with SZ and healthy controls

	Cases (n=55)		Controls (n=28)		P value[a]
Variable	Number	Percent	Number	Percent	
Female	23	(41.82)	13	(46.43)	0.689
Male	32	(58.18)	15	(53.57)	
Urban	26	(47.27)	15	(53.57)	0.587
Rural	29	(52.73)	13	(46.43)	
Age (SD)	33.28	14.96)	33.35	(15.43)	0.647

SD standard deviation

[a] *t* test was used for quantitative variables and chi-square test for categorical variables

Table 2 Differentially expressed microRNAs identified by microarray analysis in PBMCs from SZ patients versus controls

microRNA	Fold change	P value	Style	Mirbase no.
hsa-miR-1228	2.298578	0.022124	Up	MIMAT0005582
hsa-miR-1246	4.513534	0.002745	Up	MIMAT0005898
hsa-miR-1273d	11.57571	0.002882	Up	MIMAT0015090
hsa-miR-1303	4.630197	0.038842	Up	MIMAT0005891
hsa-miR-1908	2.563516	0.023536	Up	MIMAT0007881
hsa-miR-1910	3.567002	0.021187	Up	MIMAT0007884
hsa-miR-21	4.740135	0.004294	Up	MIMAT0004494
hsa-miR-3064-5p	7.426971	0.012482	Up	MIMAT0019864
hsa-miR-3131	5.398245	0.0034	Up	MIMAT0014996
hsa-miR-3156-5p	2.949549	0.023226	Up	MIMAT0015030
hsa-miR-3188_st	2.517869	0.012923	Up	MIMAT0015070
hsa-miR-3617	2.598172	0.045114	Up	MIMAT0017997
hsa-miR-3687	6.095347	0.049142	Up	MIMAT0018115
hsa-miR-3916	3.60074	0.00155	Up	MIMAT0018190
hsa-miR-3937	3.387137	0.018038	Up	MIMAT0018352
hsa-miR-4271	3.49536	0.008433	Up	MIMAT0016901
hsa-miR-4428	4.275544	0.031099	Up	MIMAT0018943
hsa-miR-4436b-5p	3.036439	0.018361	Up	MIMAT0019940
hsa-miR-4467	3.554171	0.00776	Up	MIMAT0018994
hsa-miR-4486	2.262619	0.011133	Up	MIMAT0019020
hsa-miR-4488	2.259933	0.036646	Up	MIMAT0019022
hsa-miR-4492	4.855994	0.038624	Up	MIMAT0019027
hsa-miR-4506	2.894774	0.006503	Up	MIMAT0019042
hsa-miR-4508	4.061554	0.027825	Up	MIMAT0019045
hsa-miR-4646-5p	2.126904	0.047362	Up	MIMAT0019707
hsa-miR-4701-3p	2.950271	0.039956	Down	MIMAT0019799
hsa-miR-4708-5p	2.599784	0.042219	Up	MIMAT0019809
hsa-miR-4725-3p	4.710764	0.011814	Up	MIMAT0019844
hsa-miR-4753-5p	2.093268	0.001562	Up	MIMAT0019890
hsa-miR-5096	4.022421	0.021726	Up	MIMAT0020603
hsa-miR-885-3p	3.059504	0.028828	Up	MIMAT0004948
hsa-miR-885-5p	3.372775	0.035697	Up	MIMAT0004947
hsa-miR-92b	2.615707	0.033428	Up	MIMAT0004792

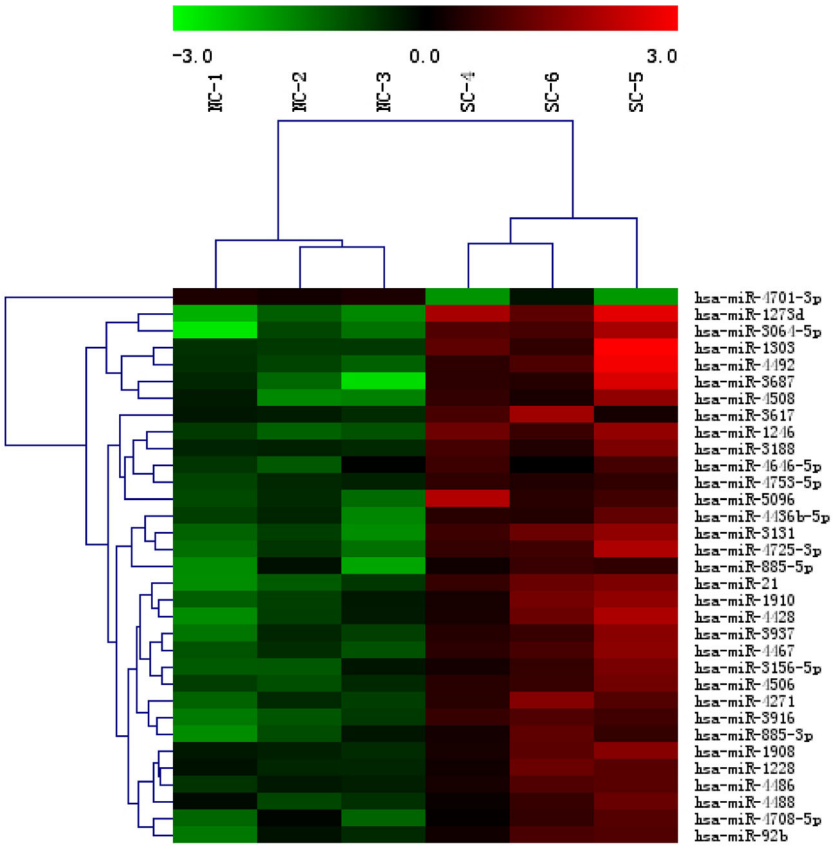

Fig. 1 Heat map showing 33 differentially expressed miRNAs in PBMCs from SZ patients (n=3) and controls (n=3). *Rows* represent miRNA species, and *columns* represent individual blood sample. The relative miRNA expression is depicted according to the color scale. *Red* indicates upregulation; *green* indicates downregulation. The *numbers* with SZ denote schizophrenia; *numbers* with NC denote normal controls (Color figure online)

microT v5.0, http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index). The genes co-identified by both programs were considered as potential target genes of a given miRNA. We next performed GO and KEGG pathway analyses using the public database FunNet (http://www.funnet.ws/) to explore the functional annotation of candidate target genes.

Statistical Analysis

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) for Windows 22.0, DataAssist 3.0, and Graphpad Prism 5.01. Demographic variables were compared between patients and controls with chi-square test for qualitative variables and t test for quantitative variables. Expression levels of miRNAs were compared using the Mann–Whitney U test. miRNA data are presented as fold change relative to the control group (control = 1). Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were analyzed to assess specificity and sensitivity of single miRNA and their combination using multiple logistic regression analysis. All statistical tests were two-tailed and P values of <0.05 were considered to indicate significant differences.

Results

Clinical Characteristics of the Patients

The mean age (mean±SD) of patients and health controls was 21.7±4.09 and 22.1±4.00 years, respectively. All the subjects were of Han nationality, and there were no significant differences in age, sex, or residential locations between SZ patients and healthy controls (Table 1).

Microarray Expression Analysis

There were 33 miRNAs significantly differentially expressed in three SZ patients compared with three controls (fold change ≧ 2; P<0.05); all but one (hsa-miR-4701-3p) of which were upregulated (Table 2). Heat map was generated to visualize the results of hierarchical clustering, where a general distinction between samples of SZ and control were clearly observed (Fig. 1).

Real-Time qRT-PCR Validation

To validate the results of the microarray assay, 10 miRNAs (miR-1273d, miR-1303, miR-21, miR-3064-5p, miR-3131, miR-3687, miR-3916, miR-4428, miR-4725-3p, and miR-5096, all of which were upregulated) with

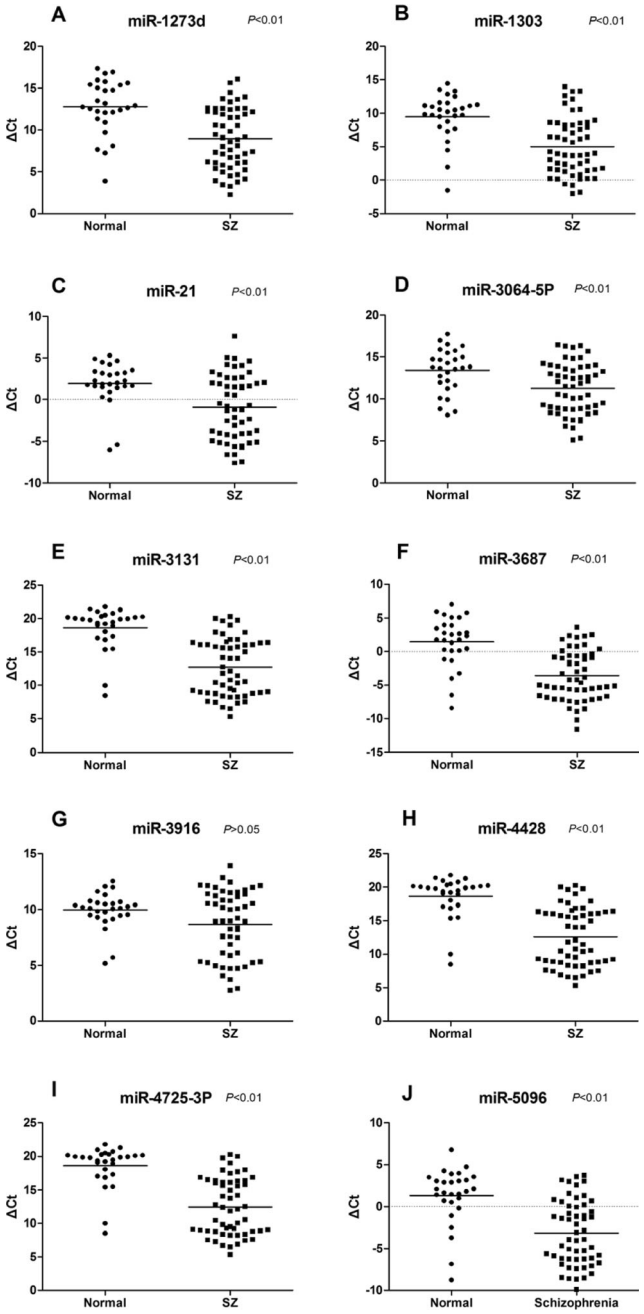

Fig. 2 Validation of microRNA expression by qRT–PCR analysis in PBMCs from SZ (n=55) and normal controls (n=28). The *line* represents the median value. The plots were constructed using GraphPad Prism 5 software and statistical difference was analyzed by Mann–Whitney U test

the highest fold change values were examined in a larger sample (55 cases and 28 controls) using qRT-PCR method. Using RNU48 as normalization control, all the 10 miRNAs were upregulated in SZ patients compared with normal controls, showing the same tendency with the microarray results, and 9 of them demonstrate significant difference (P<0.01) except miR-3916 (Fig. 2).

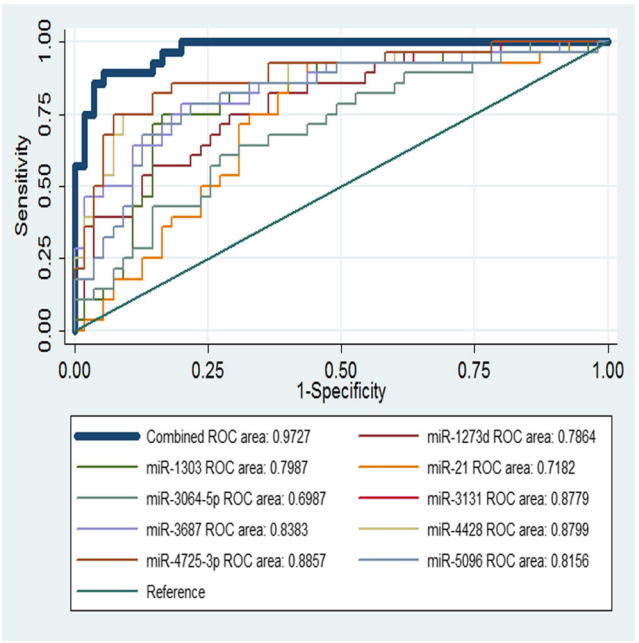

Fig. 3 ROC curve of the nine differentially expressed miRNAs and their combined ROC curve

To evaluate the potential of the 10 miRNAs as biomarkers of SZ, we performed ROC analysis of data from the qRT-PCR results. ROC curve analysis showed that the AUC of nine miRNAs (miR-1273d, miR-1303, miR-21, miR-3064-5p, miR-3131, miR-3687, miR-4428, miR-4725-3p, miR-5096) could distinguish SZ cases from normal controls with an AUC ranging from 0.699 to 0.886 (all P<0.01), while the AUC of miR-3916 did not reach statistical significance (P= 0.128). By using logistic regression approach, we examined

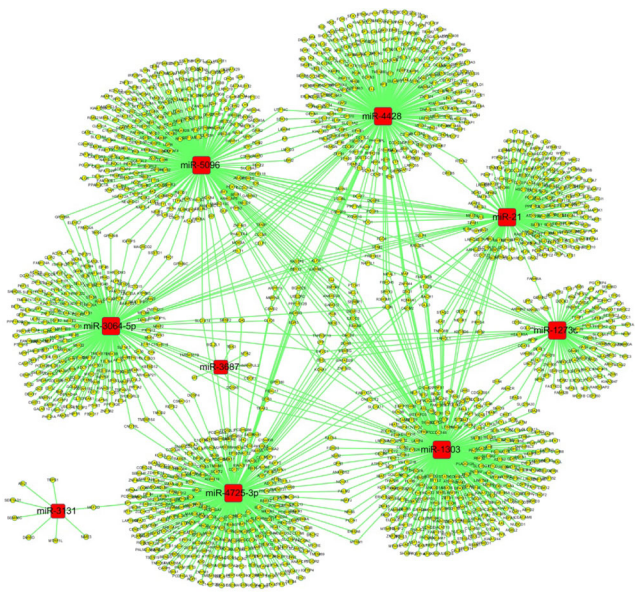

Fig. 4 MicroRNA–gene network. The *yellow circle* represents gene (mRNA); *red square* represents microRNA. The relationship between microRNA and gene is represented by one *green line* (Color figure online)

Table 3 The enriched GO biological process terms associated with the brain development and functions

ID	Term	List hits	List total	Population hits	Population total	*P* value
GO:0007268	Synaptic transmission	40	1129	312	14200	1.78E-03
GO:0007411	Axon guidance	34	1129	301	14200	2.37E-02
GO:0048011	Nerve growth factor receptor signaling pathway	32	1129	214	14200	3.81E-04
GO:0007605	Sensory perception of sound	13	1129	90	14200	2.53E-02
GO:0007219	Notch signaling pathway	9	1129	53	14200	2.27E-02
GO:0030900	Forebrain development	9	1129	51	14200	1.80E-02
GO:0007220	Notch receptor processing	4	1129	16	14200	3.34E-02
GO:0048169	Regulation of long-term neuronal synaptic plasticity	4	1129	17	14200	4.10E-02
GO:0001841	Neural tube formation	3	1129	9	14200	2.93E-02
GO:0021954	Central nervous system neuron development	3	1129	10	14200	3.94E-02
GO:0021952	Central nervous system projection neuron axonogenesis	2	1129	4	14200	3.40E-02
GO:0007270	Neuron-neuron synaptic transmission	2	1129	4	14200	3.40E-02
GO:2000171	Negative regulation of dendrite development	2	1129	3	14200	1.79E-02
GO:0010626	Negative regulation of Schwann cell proliferation	2	1129	4	14200	3.40E-02

List hits is the number of genes annotated by the considered GO biological process category or annotation cluster within the analyzed list of target genes. List total is the number of genes within the analyzed list of target genes having at least one GO biological process annotation. Population hits is the number of genes, available on the entire microarray, annotated by the considered GO biological process. Population total is the number of genes available on the entire microarray and having at least one GO Biological Process annotation. *P* value is the significance *P* value of the gene enrichment of the considered GO biological process category or annotation cluster, calculated with a unilateral Fisher exact test

the predictive power of the combined ROC of the nine miRNAs. The AUC of the combined ROC curve was 0.973 (95 % confidence interval (CI) 0.945–1.000) with 89.29 % sensitivity and 94.55 % specificity (Fig. 3).

miRNA Target Analysis

Using miRDB and DIANA-microT v5.0, a total number of 1457 genes of the nine differentially expressed miRNAs were identified. Then, the microRNA–gene network was established to outline the interactions of miRNAs and the targets (Fig. 4). GO biological process analysis indicate that the target genes have participated in a wide variety of physiological and pathophysiological processes, such as regulation of transcription, signal transduction, cell adhesion, ion transport, blood coagulation, synaptic transmission, axon guidance, small GTPase mediated signal transduction, interspecies interaction between organisms, and nerve growth factor receptor signaling pathway. Among which, biological processes related to brain development and function are listed in Table 3, which may be involved in the pathophysiology of SZ. Likewise, KEGG pathway analysis showed a significant enrichment in several important pathways related to neuronal brain function, such as neurotrophin signaling pathway, Jak-STAT signaling pathway, Wnt signaling pathway, Axon guidance, ErbB signaling pathway, chemokine signaling pathway, TGF-beta signaling pathway, and Notch signaling pathway (Table 4).

Discussion

Peripheral blood is an ideal tissue for basic and clinical research for its easy obtainment. Generally, peripheral blood samples can be divided into two categories, PBMCs and plasma/serum, which were widely applied in blood-based miRNA research. Our previous study had demonstrated that aberrant microRNA expression could be detected in both plasma and PBMCs and that the expression level of miR-30e is more significant in plasma than in PBMCs (Sun et al. 2014). But, in the present study, we chose to use PBMCs for the microarray and PCR experiment for several reasons. First, there is more and more evidence indicating close relationship between inflammation and SZ (Nikkila et al. 2001; Miller et al. 2011; Fineberg and Ellman 2013; Miller et al. 2013), and most of the blood-based miRNA research studies having relation to inflammation were conducted in PBMCs (Lai et al. 2011; Yao et al. 2011; Ma et al. 2014; Munshi et al. 2014). Second, several studies have indicated that transcriptional alterations in PBMCs may reflect the molecular and cellular changes in the brain (Fisar and Raboch 2008; Cattaneo et al. 2010). Furthermore, evidence suggests that PBMCs may share a common miRNA expression pattern with the brain (Liang et al. 2007).

In this study, microarray was first used to screen dysregulated miRNAs in the PBMCs of young SZ patients. As shown in Table 2, a total of 33 miRNAs were identified to be differentially expressed, in which 32 miRNAs were upregulated and only 1 miRNA (hsa-miR-4701-3p) was downregulated. Next,

we chose the top 10 miRNAs with the highest fold change values (miR-1273d, miR-1303, miR-21, miR-3064-5p, miR-3131, miR-3687, miR-3916, miR-4428, miR-4725-3p, miR-5096; all of which were up-regulated) for qRT-PCR validation and 9 of them (except miR-3916) reached statistical significance in SZ patients compared with normal controls. To further explore the diagnostic potential of these nine miRNAs as biomarkers for SZ diagnosis, receiver operating characteristic (ROC) curves were constructed on the basis of the nine miRNA expression levels between two groups. When the expression levels of these nine miRNAs were subjected to combined analysis by multiple logistic regression, the ROC curve reflected a higher ability to differentiate patients with SZ from healthy controls (AUC value 0.973, 95 % CI 0.945–1.000), demonstrating that the diagnostic accuracy of the nine-miRNA signature is an effective biomarker for SZ diagnosis.

Among the nine differentially expressed miRNAs, miR-21 was the most frequently reported one. The function of miR-21 is complex and has elicited considerable interest in diverse fields including embryonic development (Ramachandra et al. 2008), tumorigenesis (Wang et al. 2014), fibrosis (Zhang et al. 2013), and immune reaction (Smigielska-Czepiel et al. 2013). Until now, the majority of the studies of miRNA-21 were related to various human cancers such as breast cancer (Mar-

Table 4 KEGG pathway annotations of the differentially expressed miRNA predicted targets

ID	Term	List hits	List total	Population hits	Population total	*P* value
5200	Pathways in cancer	38	426	327	5981	1.62E-03
4062	Chemokine signaling pathway	29	426	189	5981	5.82E-05
4510	Focal adhesion	23	426	200	5981	1.46E-02
4144	Endocytosis	22	426	203	5981	3.07E-02
4722	Neurotrophin signaling pathway	19	426	127	5981	1.49E-03
4630	Jak-STAT signaling pathway	18	426	155	5981	2.63E-02
4310	Wnt signaling pathway	18	426	151	5981	2.07E-02
4110	Cell cycle	18	426	128	5981	3.94E-03
4910	Insulin signaling pathway	18	426	138	5981	8.68E-03
4120	Ubiquitin mediated proteolysis	18	426	139	5981	9.33E-03
5162	Measles	17	426	134	5981	1.38E-02
5145	Toxoplasmosis	17	426	133	5981	1.28E-02
4360	Axon guidance	16	426	130	5981	2.16E-02
5215	Prostate cancer	16	426	89	5981	4.61E-04
5160	Hepatitis C	16	426	135	5981	2.96E-02
5220	Chronic myeloid leukemia	15	426	73	5981	1.51E-04
5221	Acute myeloid leukemia	15	426	58	5981	8.31E-06
4270	Vascular smooth muscle contraction	14	426	116	5981	3.52E-02
4114	Oocyte meiosis	14	426	114	5981	3.09E-02
4916	Melanogenesis	14	426	101	5981	1.17E-02
4012	ErbB signaling pathway	12	426	87	5981	1.96E-02
5213	Endometrial cancer	12	426	52	5981	2.19E-04
4350	TGF-beta signaling pathway	12	426	85	5981	1.65E-02
4971	Gastric acid secretion	11	426	74	5981	1.48E-02
4520	Adherens junction	11	426	73	5981	1.35E-02
5412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	11	426	74	5981	1.48E-02
5211	Renal cell carcinoma	10	426	70	5981	2.54E-02
5214	Glioma	10	426	65	5981	1.57E-02
5223	Non-small cell lung cancer	10	426	54	5981	4.23E-03
4961	Endocrine and other factor-regulated calcium reabsorption	8	426	49	5981	2.11E-02
0565	Ether lipid metabolism	7	426	36	5981	1.21E-02
4330	Notch signaling pathway	7	426	47	5981	4.68E-02
5216	Thyroid cancer	7	426	29	5981	3.49E-03
0120	Primary bile acid biosynthesis	5	426	16	5981	4.05E-03

List hits is the number of genes annotated by the considered GO biological process category or annotation cluster within the analyzed list of target genes. List total is the number of genes within the analyzed list of target genes having at least one GO biological process annotation. Population hits is the number of genes, available on the entire microarray, annotated by the considered GO biological process. Population total is the number of genes available on the entire microarray and having at least one GO Biological Process annotation. *P* value is the significance *P* value of the gene enrichment of the considered GO biological process category or annotation cluster, calculated with a unilateral Fisher exact test

Aguilar et al. 2013), lung cancer(Tang et al. 2013), colorectal cancer (Kanaan et al. 2012), and gastric cancer(Li et al. 2012). However, several recent studies have found that miRNA-21 played an important role in many physiological and pathological processes of the central nervous system. In a rat stroke model, Buller et al. (2010) found that miR-21 levels had been markly improved in neurons of the ischemic boundary zone, and overexpression of miR-21 in neurons significantly reduced Faslg protein levels which is known to contribute to ischemic injury of neurons (Barone et al. 1997). In another study, miR-21 was found exerting the function of reducing neuronal apoptosis through activating the PTEN-Akt signaling pathway (Han et al. 2014). Montalban et al. (2014) reported that miR-21 could enhance neurotrophin signaling and control neuronal differentiation induced by Ngf. In the same study, miR-21 was able to preserve the neurite network and to support viability of the neurons in a situation mimicking neurodegeneration.

In several studies, miR-1303 was reported to be concerned with cancers such as breast cancer, gastric cancer, colorectal cancer, and hepatocellular carcinoma. So far, the other seven miRNAs differentially expressed in our study have scarcely been reported before. All these nine miRNAs were found to be related with SZ for the first time. This may provide new clues for SZ research.

The microRNA–gene network showed that there were 138 genes targeted by at least two miRNAs in which 1 gene (*EIF2C1*) was targeted by four miRNAs and 11 genes (*CLIC6*, *DCAF7*, *DGKB*, *DSEL*, *ESRRG*, *GLDN*, *KCNA1*, *LPP*, *PCGF5*, *RAB22A*, *ZNF445*) were targeted by three miRNAs, suggesting that these miRNAs may have potential roles in the pathogenesis of SZ. Indeed, *CLIC6*, a member of the intracellular chloride channel family, was proven to be involved in dopamine D(2)-like receptor complex (Griffon et al. 2003). *Kcna1*, a gene involved in voltage-gated ion channels, was supposed to play a role in mediating antipsychotic drug effects (Duncan et al. 2008).

In order to gain insight into the function of miRNAs, GO term and KEGG pathway annotation were applied to their target gene pool. GO enrichment analysis showed that biological processes regulated by differentially expressed miRNAs including diverse terms, and some important terms, such as synaptic transmission, nerve growth factor receptor signaling pathway, axon guidance, Notch signaling pathway, and regulation of long-term neuronal synaptic plasticity, had direct relationship with central nervous system (CNS) and brain functions. These biological processes may contribute to the etiopathogenesis of SZ based on recent data from many studies (Kalkman 2009; Chen et al. 2011; Earls et al. 2012; Masana et al. 2012; Ikeda et al. 2013). KEGG pathway analysis revealed a close linkage between signaling pathways identified and SZ, as shown in Table 4. For example, neurotrophin 3 levels in serum were found significantly decreased in SZ patients compared to controls, suggesting that the neurotrophin 3 signaling system may play a role in the pathophysiology of SZ (Vargas et al. 2008). JAK-STAT signaling has been proven to play a role in treatment of schizophrenia with olanzapine (Singh et al. 2007). Wnt signaling is a highly conserved pathway that plays a prominent role in the CNS, and an increasing number of evidence suggest a close connection between Wnt signaling and the pathogenesis and therapeutics of schizophrenia (Singh 2013; Peng et al. 2014). NTNG1 is a well-known axon guidance factor, whose allelic variation was proven to be a very important contributor to the risk for schizophrenia (Ohtsuki et al. 2008; Wilcox and Quadri 2014). Besides, ErbB signaling pathway, Chemokine signaling pathway, TGF-beta signaling pathway, and Notch signaling pathway are also found to be implicated in the pathologic mechanisms of SZ (Chertkow et al. 2007; Ohtsuki et al. 2008; Kerns et al. 2010; Frydecka et al. 2013).

There are several limitations of this study that should be mentioned. First, our sample size was relatively small; therefore, further validation in larger cohorts is needed to better evaluate the sensitivity and specificity of the nine-miRNA signature as biomarkers. Second, the predicted genes and their regulation function should be validated by future functional experimental research.

In conclusion, we have identified nine miRNAs (miR-1273d, miR-1303, miR-21, miR-3064-5p, miR-3131, miR-3687, miR-4428, miR-4725-3p, miR-5096) that are upregulated in PBMC of young SZ patients and may serve as useful noninvasive biomarkers for SZ diagnosis. The bioinformatics analysis suggested that these miRNAs may be involved in the pathogenesis of SZ.

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