Detection of Circulating miRNA Levels in Schizophrenia

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Objective: Diagnosis of schizophrenia is currently dependent on symptom-based criteria and lacks objective indicators. In this study, the authors investigated whether circulating miRNA can serve as a diagnostic biomarker for schizophrenia.

Methods: Global plasma miRNAs were profiled in a test cohort of 164 schizophrenia patients and 187 control subjects, using Solexa sequencing, TaqMan Low Density Array, and quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays. The captured miRNAs were then validated by qRT-PCR assays in an independent cohort of 400 schizophrenia patients, 213 control subjects, and 162 patients with nonschizophrenia psychiatric disorders; the 400 schizophrenia patients underwent a 12-month follow up study of regular treatment with an atypical antipsychotic (risperidone and aripiprazole).

Results: The global plasma miRNA screening revealed eight miRNAs that were up-regulated in schizophrenia, as revealed by both assay platforms. The qRT-PCR analysis showed the up-regulation of miR-130b and miR-193a-3p in schizophrenia but not in nonschizophrenia disorders.

Conclusions: The up-regulation of miR-130b and miR-193a-3p is a state-independent biomarker for schizophrenia, and these two miRNAs could be used to develop a diagnostic tool for schizophrenia.

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Schizophrenia is one of the most common severe mental disorders, with a lifetime risk of 1% in the population worldwide (1). Over the years, the diagnosis of schizophrenia has remained symptom based, relying mainly on self-reports from patients, mental state examination, and clinical interviews, and lacking objective laboratory tests (2). Such a diagnostic strategy can sometimes lead to misdiagnosis and has been criticized widely (3). To remedy this embarrassing state of affairs, a set of biomarkers has been proposed based on physical and biological tests (4).

MicroRNAs (miRNAs) are a class of small noncoding RNAs of 19–23 nucleotides in length that can inactivate a target mRNA sequence through binding to its 3′-UTR region (5). Increasing evidence shows that miRNAs take part in many biological processes, including cell proliferation, differentiation, migration, and apoptosis (6). It has been suggested that 70% of known miRNAs are expressed in the CNS, some of which are brain specific or brain-region specific (7), implying that these miRNAs may play a vital role in the development of diseases in the CNS (8). Altered miRNA levels have been found in CSF and postmortem brain of schizophrenia patients (9, 10), but no ideal miRNAs have been identified in the circulation that might be used as biomarkers for clinical applications. As plasma and serum samples can be easily collected, it is important to detect circulating miRNAs of interest as diagnostic biomarkers, as opposed to potential biomarkers in other tissues. Circulating miRNAs, first identified in 2008 (11), have been found to be highly stable under extreme conditions, such as ribonuclease digestion and extreme pH and temperature (12, 13). While levels of circulating miRNAs appear to be relatively low in healthy individuals, they may be increased in pathological conditions (14). Numerous studies have reported that circulating miRNA levels are highly associated with various diseases in humans, such as diabetes, cancer, and immunological diseases (13, 15), but there has been no systematic research on circulating miRNAs in psychiatric diseases.

In this study, we designed a multistage case-control study with follow-up plan to investigate the plasma miRNA profile as noninvasive biomarkers for schizophrenia. We globally screened plasma miRNAs initially with both Solexa sequencing and TaqMan Low Density Array (TLDA) chips, followed by a stem-loop quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay. (For a flowchart of the project strategy, see Figure S1 in the data supplement that accompanies the online edition of this article.)

METHOD

Participants

A total of 726 patients with mental disorders were recruited in this study, of whom 564 were diagnosed as having

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TABLE 1. Demographic and Clinical Characteristics of Participants in a Study of Circulating miRNA Levels

Characteristic	Test Cohort				Validation Cohort			
	Control Subjects (N=187)		Schizophrenia Patients (N=164)		Control Subjects (N=213)		Schizophrenia Patients (N=400)	
	N	%	N	%	N	%	N	%
Male	88	47.1	81	49.4	105	49.3	189	47.3
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	28.7	9.5	29.2	9.8	26.2	5.6	25.0	7.5
Education (years)	15.0	3.3	11.0	2.9	16.1	4.0	13.3	3.8
Positive and Negative Syndrome Scale								
Positive subscale score			17.8	6.1			22.9	5.4
Negative subscale score			17.6	6.8			20.9	7.4
General psychopathology score			38.5	9.7			41.5	8.8
Total score			79.6	20.7			85.2	5.3

schizophrenia according to DSM-IV criteria and 162 as having nonschizophrenia disorders (Table 1), including major depression, anxiety, bipolar disorder, and other atypical psychiatric diseases (see Table S1 in the data supplement). All patients were diagnosed by at least two consultant psychiatrists using a structured interview and a strict assessment process. A total of 400 healthy subjects, matched on age, gender, smoking history, and education level, were recruited as control subjects. All participants were of Chinese Han origin and met all of the study's inclusion criteria (see Table S2 in the data supplement).

Participants were divided into a test cohort and a validation cohort. The test cohort comprised 164 patients with schizophrenia, who were recruited from Shanxi Province; the validation cohort comprised 400 patients with schizophrenia, who were recruited from multiple mental health centers in Beijing, Shanghai, Sichuan, and Hunan Provinces. The 162 patients with nonschizophrenia disorders were recruited from Shanxi Province. The control subjects were recruited from local communities around each institution and showed no evidence of psychotic symptoms. All participants gave written informed consent to take part in the study, as approved by local ethics committees and in conformity with the requirements of the Declaration of Helsinki.

The schizophrenia patients in the validation cohort underwent a follow-up study under regular treatment with atypical antipsychotic drugs—risperidone and aripiprazole. The dosage for risperidone started at 1 mg/day and was gradually increased to 2–6 mg/day over 2–3 weeks, and that for aripiprazole started at 5 mg/day and was increased to 14–20 mg/day. Benzodiazepines were used temporarily in small dosages, if necessary, in patients with insomnia or anxiety, but combinations of other antipsychotics, antidepressants, or mood stabilizers were not allowed. A total of 107 patients reached the study endpoint, which was 12 months of treatment (Table 1). The definition of remission used in our study was that proposed by the Remission in Schizophrenia Working Group (16), in which both symptom and duration criteria must be met: participants had to maintain scores ≤3 on eight core items on the Positive and Negative Syndrome Scale (PANSS)—delusions (P1), concept disorganization (P2), hallucinatory behavior (P3), unusual thought content (G9), mannerisms/posturing (G5), blunted affect (N1), passive/apathetic social withdrawal (N4), and lack of spontaneity and flow of conversation (N6)—for at least 6 months.

Blood Sample Collection and RNA Extraction

Whole blood was obtained between 9 a.m. and 11 a.m. through venipuncture of a forearm vein and treated with EDTA. The blood sample was separated into plasma and cellular fractions by centrifugation (800 g, 10 minutes, 4°C) within 2 hours after collection; the plasma fraction was then centrifuged again (12,000 g, 10 minutes, 4°C) to remove cell debris. The plasma samples were then aliquoted, stored at −80°C, and transported by dry ice.

An aliquot of 100 μL of plasma was diluted with 300 μL of DEPC-treated water, and then diluted further with a sequencing mix of 200 μL of phenol-water and 200 μL of chloroform. The sample stood at room temperature for several minutes before being centrifuged at 12,000 rpm for 20 minutes, after which the upper aqueous layer was collected. A 1/10-volume of 3-M sodium acetate and a twofold volume of isopropyl alcohol were added and mixed, and the total RNA was precipitated after incubation at −20°C for 1 hour. Subsequently, the total RNA pellet was collected by centrifugation at 12,000 rpm for 20 minutes, washed with 75% ethanol by centrifugation at 7,500 rpm for 10 minutes, and dried for 15 minutes at room temperature. Finally, the total RNA was dissolved in 20 μL of ribonuclease-free water and stored at −80°C.

Solexa Sequencing and TLDA Assay

For Solexa sequencing and TLDA assay, a 5-μL aliquot of each total RNA sample from 150 schizophrenia patients and 150 control subjects in the test cohort was taken for pooling, and the pooled samples were then re-extracted with the mirVana miRNA Isolation Kit (Applied Biosystems, Foster City, Calif.). Solexa sequencing analysis was performed on the Illumina Solexa Sequencer (Illumina, San Diego) as described

Patients With Nonschizophrenia Disorders (N=162)		Remitted Patients, Baseline Values (N=79)		Remitted Patients, 1-Year Values (N=79)		Unremitted Patients, Baseline Values (N=28)		Unremitted Patients, 1-Year Values (N=28)	
N	%								
73	45.1								
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
36.0	14.8								
11.8	3.1								
		22.3	5.2	7.3	1.1	22.1	6.3	10.7	5.2
		21.5	6.8	9.5	2.8	21.5	7.3	16.7	6.2
		41.4	7.4	18.4	2.1	39.5	7.4	23.6	6.3
		85.1	5.9	35.2	4.4	83.1	15.9	51.0	14.7

previously (12), and the clean readouts were compared with the miRBase database (http://microrna.sanger.ac.uk, release 12.0). The total RNAs were reverse-transcribed into cDNAs with the TaqMan miRNA Reverse Transcription Kit (Applied Biosystems) for the TLDA Chip (Applied Biosystems, version 3.0) screening. The TLDA chip was processed on the 7900HT PCR system (Applied Biosystems) to profile the miRNA expression pattern. The results were analyzed with the RQ Manager software program (Applied Biosystems) and calculated by the comparative threshold (Ct) method ($2^{\triangle\triangle}$Ct).

Quantitative Real-Time PCR Analysis

TaqMan miRNA Assay (Applied Biosystems) was used to quantify mature miRNAs in plasma samples in accordance with the manufacturer's instructions. Briefly, 2 μL of total RNA was reverse-transcribed to cDNA using M-MLV reverse transcriptase (Promega, Madison, Wisc.) and stem-loop RT primers (Applied Biosystems). Real-time PCR was performed using TaqMan miRNA probes (Applied Biosystems) on the CFX-96 system (Bio-Rad, Hercules, Calif.). All reactions, including the no-template controls, were run in triplicate. After real-time PCR amplification, the Ct values were determined using the fixed threshold settings. Each miRNA was reverse transcribed and amplified separately.

Quantification of qRT-PCR Results

To calibrate plasma levels of the target miRNAs identified in the test cohort, an external standard curve was generated with synthetic miRNAs for every qRT-PCR assay in the validation cohort based on the method described by Schmittgen et al. (17). For example, synthetic miR-16 oligonucleotides were used as the external standard to calibrate plasma levels of all miRNAs of interest, with concentrations ranging from 10 to 10^7 fmol/L (see Figure S2A in the online data supplement). We also repeated the experiments in the test cohort using synthetic miR-130b and miR-193a-3p as the external standards (see Figures S2B and S2C). The concentrations of all miRNAs tested in individual plasma samples fell along the standard curves.

To assess the accuracy of the calibration of plasma miRNAs with the miR-16 standard curve, 10-pM synthetic miR-130b and miR-193a-3p oligonucleotides were put in phosphate-buffered saline containing 5% BSA; the recovery rates were calculated according to the resulting data from the exact detection process as described above. In brief, cDNA samples were diluted successively in a twofold series, and the levels of miR-130b and miR-193a-3p were then detected by qRT-PCR assay, calibrated by their corresponding standard curves; their recovery rates were then worked out, and the correlation in recovery rates between synthetic miR-16 and miR-130b and between synthetic miR-16 and miR-193a-3p was tested (see Tables S3 and S4 in the data supplement).

Quality Control and Data Analysis

To assess the reproducibility of qRT-PCR assay, plasma samples from 10 healthy subjects and schizophrenia patients were pooled and then aliquoted into five portions (>100 μL each). Total RNA was extracted and the levels of miR-130b and miR-193a-3p were measured every other day by qRT-PCR analysis. The coefficients of variation (CV), representing the interassay deviation (i.e., the reproducibility of the qRT-PCR assay), were calculated.

Because the range of plasma miRNA measurements was quite wide among individual samples, the Mann-Whitney U test and percentile ranking were used to compare the differences in plasma miRNA levels between the patient group and the control group. The paired t test was also used to compare the plasma miRNA levels between baseline and individual tests during 1 year of treatment in the follow-up group. A p value <0.01 (two-tailed) was considered statistically significant.

RESULTS

Global Profiling of Plasma miRNAs in Schizophrenia

Solexa sequencing and TLDA assay identified 255 and 247 miRNAs, respectively, in pooled plasma RNA samples from

TABLE 2. Comparison of Plasma miRNA Levels in Control Subjects and Schizophrenia Patients in the Test Cohort and the Validation Cohort

Group and miRNA	Mean Rank		p
Test cohort	Control	Schizophrenia	
miR-122	70.14	64.34	0.389
miR-130a	63.20	56.47	0.298
miR-130b	97.62	265.38	<0.001
miR-193a-3p	95.02	268.33	<0.001
miR-193b	92.17	93.84	0.830
miR-502-3p	75.07	73.92	0.871
miR-652	74.44	67.28	0.300
miR-886-5p	54.03	55.92	0.755
Validation cohort	Control	Schizophrenia	
miR-130b	107.00	413.50	<0.001
miR-193a-3p	107.17	413.41	<0.001
Nonschizophrenia disorders	Control	Nonschizophrenia disorders	
miR-130b	278.84	288.07	0.542
miR-193a-3p	274.84	297.95	0.127

control subjects, and 215 and 235 miRNAs, respectively, in pooled plasma RNA samples from patients. The Solexa data revealed that miRNAs were a major fraction of small RNAs (<30 bp) in plasma (see Figure S3 in the data supplement) and that patients with schizophrenia had 126 plasma miRNAs with increased levels of >twofold changes; TLDA chips confirmed 41 miRNAs with altered levels of ~10-fold changes.

We selected miRNAs of interest for qRT-PCR validation by the following criteria: 1) having at least 50 copies with >twofold changes in plasma levels as shown by Solexa sequencing, 2) Ct value below 30 with >10-fold changes in plasma levels as shown by TLDA, and 3) having the same direction of altered expression shown by both Solexa

TABLE 3. Percentile Rank Analysis of Plasma miR-130b and miR-193a-3p Levels in Control Subjects and Schizophrenia Patients in the Test Cohort and the Validation Cohort and in Participants With Nonschizophrenia Disorders

miRNA, Groups, and Percentile	Cutoff (fmol)[a]	Control Group[b]	Case Group[b]	χ^2	Odds Ratio	95% CI	p
miR-130b							
Test cohort, control versus schizophrenia group							
Median	43.07	93/187	164/164	112.60	∞		<0.001
75th percentile	115.05	45/187	164/164	209.15	∞		<0.001
95th percentile	524.51	9/187	132/164	208.19	81.58	44.87–148.34	<0.001
Validation cohort, control versus schizophrenia group							
Median	43.07	157/213	400/400	115.74	∞		<0.001
75th percentile	115.05	125/213	400/400	192.96	∞		<0.001
95th percentile	524.51	25/213	400/400	509.23	∞		<0.001
Control versus nonschizophrenia disorders group							
Median	43.07	250/400	105/162	0.27	1.11	0.75–1.65	0.630
75th percentile	115.05	170/400	64/162	0.43	0.88	0.60–1.29	0.571
95th percentile	524.51	34/400	28/162	9.06	2.25	1.33–3.82	0.004
miR-193a-3p							
Test cohort, control versus schizophrenia group							
Median	152.76	93/187	164/164	110.96	∞		<0.001
75th percentile	202.98	46/187	164/164	206.69	∞		<0.001
95th percentile	273.87	8/187	156/164	289.67	436.31	60.73–878.61	<0.001
Validation cohort, control versus schizophrenia group							
Median	152.76	106/213	400/400	243.43	∞		<0.001
75th percentile	202.98	62/213	400/400	376.25	∞		<0.001
95th percentile	273.87	33/213	380/400	399.70	103.64	65.76–163.35	<0.001
Control versus nonschizophrenia disorders group							
Median	152.76	199/400	84/162	0.20	1.09	0.75–1.59	0.710
75th percentile	202.98	108/400	51/162	1.14	1.24	0.84–1.84	0.302
95th percentile	273.87	41/400	33/162	10.33	2.24	1.37–3.66	0.002

[a] The cutoff points were set based on the miRNA expression levels in control subjects in the test cohort.
[b] In the sample counts, the first number lists subjects with higher miRNA expression than cutoffs and the second lists subjects.

sequencing and TLDA assay. A total of eight miRNAs (miR-122, miR-130a, miR-130b, miR-193a-3p, miR-193b, miR-502-3p, miR-652, and miR-886-5p) were identified by both assay platforms and then validated by qRT-PCR analysis (see Figure S4 in the data supplement).

TABLE 4. Expression Levels of miR-130b and miR-193a-3p in Plasma (fmol/L) at Baseline and After 1 Year of Antipsychotic Treatment in Schizophrenia Patients With and Without Remission at 1 Year

	miRNA Concentration (fmol/L)					
	Baseline		1 Year			
Stage and miRNA	Mean	SD	Mean	SD	Proportion of 1 Year to Baseline	p
Remitted (N=79)						
miR-130b	10,570	12,300	4,636	5,380	0.44	<0.0001
miR-193a-3p	3,395	4,641	1,041	2,281	0.31	<0.0001
Unremitted (N=28)						
miR-130b	15,800	7,097	12,840	4,559	0.81	0.051
miR-193a-3p	543.7	319.3	371.3	399.2	0.68	0.072

Evaluation of miRNA Expression by Quantitative Real-Time PCR Analysis

The qRT-PCR validation showed that of these eight miRNAs identified, only miR-130b and miR-193a-3p showed significant differences (Table 2). Percentile rank analysis showed that patients with schizophrenia had higher levels of plasma miR-130b than control subjects, including the median (χ^2=112.60, p<0.001; odds ratio=∞), the 75th percentile (χ^2=209.15, p<0.001; odds ratio=∞), and the 95th percentile (χ^2=208.19, p<0.001; odds ratio=81.58, 95% CI=44.87–148.34). As shown in Table 3, the level of plasma miR-193a-3p was also higher in the patient group than the control group, including the median (χ^2=110.96, p<0.001; odds ratio=∞), the 75th percentile (χ^2=206.96, p<0.001; odds ratio=∞), and the 95th percentile (χ^2=289.67, p<0.001; odds ratio=436.31, 95% CI=60.73–878.61). These two miRNAs were further evaluated in the validation cohort, and the pattern of altered miRNAs was consistent with that shown in the test cohort (Tables 2 and 3). Further analysis of plasma miR-130b and miR-193a-3p levels was performed in 162 patients with nonschizophrenia disorders but failed to show significant differences. Based on the calculation of odds ratios, these two miRNAs have a lesser effect on nonschizophrenia disorders than on schizophrenia (Tables 2 and 3), suggesting that the altered levels of plasma miR-130b and miR-193a-3p may be specific for schizophrenia.

To clarify whether altered levels of these two plasma miRNAs were state-dependent, a 1-year follow-up study was carried out on patients treated with antipsychotics. Among 107 schizophrenia patients who completed the 1-year follow-up, 79 achieved the remission criteria. The levels of plasma miR-130b and miR-193a-3p declined significantly in patients who remitted but not in those without remission (Table 4). We also compared the baseline levels of these two miRNAs between patients who remitted and those who did not, and found that the magnitude of miRNA level change was greater in those who remitted (miR-130b, mean percentage of baseline for remitted patients, 0.35 [SD=0.33], mean percentage for unremitted patients, 1 [SD=0.64], p<0.001; miR-193a-3p, mean percentage of baseline for remitted patients, 0.29 [SD=0.25], mean percentage for unremitted patients, 0.82 [SD=0.93], p<0.001). These results demonstrate that the altered levels of plasma miR-130b and miR-193a-3p were state dependent in schizophrenia.

TABLE 5. The Reproducibility of the Quantitative Reverse Transcription Polymerase Chain Reaction Assay[a]

	Time Point (Day)							
miRNA	1	2	3	4	5	Mean	SD	CV (%)
miR-130b (Ct)	25.41	24.64	24.89	26.10	24.37	25.08	0.61	2
miR-193a-3p (Ct)	29.05	28.99	29.39	30.32	31.08	29.77	0.91	3

[a] Ct=comparative threshold; CV=coefficient of variation.

Accuracy Assessment of the RNA Extraction and qRT-PCR

As shown in Table 5, the miR-130b assay had a CV of 2.44% and the miR-193a-3p assay had a CV of 3.05%. The correlation (r) in recovery rates between synthetic miR-16 and miR-130b was 0.752 (p=0.032) and that between synthetic miR-16 and miR-193a-3p was 0.953 (p=0.0003). These results suggest that the RNA extraction and qRT-PCR assay were reproducible and reliable.

DISCUSSION

Abnormal miRNA expression has widely been reported in numerous human diseases. Postmortem examinations have revealed an alteration of several miRNAs in brain tissue from patients with schizophrenia (18–20). However, such examinations cannot be applied to living patients, and the specificity and accuracy of abnormal miRNAs detected in the postmortem brain have yet to be fully investigated. In the peripheral circulatory system, Gardiner et al. (21) found that several miRNAs were down-regulated in peripheral blood mononuclear cells of schizophrenia patients, indicating a significant relationship between miRNA expression and schizophrenia (21). Recently, abnormal miRNA levels in the circulation were found in some human diseases, suggesting the potential for them to serve as a novel biomarker in clinical diagnosis and prognosis of these diseases (22, 23). Abnormalities of circulating miRNAs have not been confirmed in psychiatric diseases, although Shi et al. (24) tested the levels of 25 plasma miRNAs in 115 patients with schizophrenia and

40 control subjects and found that nine plasma miRNAs showed abnormal levels in schizophrenia; no firm conclusions could be drawn, however, because of the study's small sample size (24).

To profile circulating miRNA levels, we used both Solexa sequencing and TLDA to detect miRNAs in plasma. The Solexa sequencing is a next-generation sequencing technology with the ability to read short fragments in a high-throughput pattern (25). Because next-generation sequencing is not fully matured and can be influenced by sequencing errors, we used TLDA to filter out the false signal. To obtain a large amount of total RNA for genome-wide miRNA profiling (20 μg for Solexa sequencing and 2 μg for TLDA chips), we pooled the samples of low total RNA extraction yields (about 500 ng per 100 μL plasma), although this strategy's analysis accuracy has limitations. The pooled samples could mask variance and obscure heterogeneity, and some miRNAs of small effect may be missed. In addition, the pooled samples may generate a miRNA cumulative effect, leading to a false positive signal in the genome-wide screening stage. This may be the reason why the other six candidate miRNAs failed to show a significant change in the test cohort of schizophrenia. To avoid generating a false positive outcome, however, we performed qRT-PCR validation individually in several independent sample groups to confirm an initial finding. Based on the assessment of the interassay deviations (Table 5), the reproducibility of the miRNA assay used was satisfactory, suggesting that our findings should be reliable. Moreover, there is no endogenous reference miRNA available for normalization of qRT-PCR data; this is the reason why we applied the miR-16 external standard curve to calibrate circulating levels of eight candidate miRNAs, as proposed in a previous study (26). The recovery rate of miR-130b and miR-193a-3p, which was calculated through the miR-16 calibration, was highly correlated with that through the miR-130b and miR-193a-3p calibrations (see Tables S3 and S4 in the data supplement), suggesting that the results from the miR-16 external standard curve are reliable. However, the lower recovery rate of miR-16 calculation may affect the sensitivity of miRNA assay, which means that some useful signals may be missed. This is a limitation of this study.

Analysis of global miRNA profiling in a large sample identified eight miRNAs that showed differential profiling in plasma from patients with schizophrenia, in which plasma miR-130b and miR-193a-3p levels were up-regulated in schizophrenia in both study cohorts. This work suggests that these miRNAs are novel biomarkers that can be used to develop a laboratory-based test for diagnosis of schizophrenia. In this study, we also found that the increased levels of miR-130b and miR-193a-3p in plasma could be suppressed in remitted patients after 1 year of treatment with antipsychotic drugs (aripiprazole and risperidone), suggesting that these two miRNAs can also be used as biomarkers for prognosis of schizophrenia patients on medication.

Although the origin of circulating miRNAs remains unclear, it has been reported that they may be derived from three different pathways: passive leakage from broken cells, active secretion via microvesicles, and active secretion through an RNA-binding protein-dependent pathway (27). Numerous reports have suggested that active secretion was the major source of circulating miRNAs (28). Several studies reported that both miR-130b and miR-193a-3p were up-regulated in pathologic lymphocytes, in which miR-130b was able to be packed into microvesicles and taken up by recipient cells (29–31). Most recently, the Schizophrenia Working Group of the Psychiatric Genomics Consortium confirmed 108 schizophrenia-associated loci and found that these loci were significantly enriched in B-lymphocyte lineages (32). It is possible that up-regulated plasma miRNAs in schizophrenia comes from these abnormally activated lymphocytes. Functional studies of these miRNAs would be helpful for a better understanding of their contribution to the pathophysiology of schizophrenia. The validated downstream target genes for miR-130b include PDGFRA, RUNX3, ITGB1, PPARG, FMR1, and STAT3, and for miR-193a-3p they include ErbB4, S6K2, and MCL1 (33–39). These genes can be classified into three groups: schizophrenia susceptibility genes (PDGFRA, PPARG, ErbB4), neurodevelopment-related genes (RUNX3, ITGB1, FMR1, STAT3), and neuroprotective genes (S6K2 and MCL1). The down-regulation of these genes by miRNAs may disturb neuronal function, leading in turn to dysfunction of the neural circuits. Interestingly, both the upstream regions of miR-130b and miR-193a-3p genes contain a CpG island, which can be regulated by DNA methylation and histone modification (40, 41). These two miRNAs may contribute to the pathogenesis of schizophrenia via epigenetic pathways, although additional studies are needed to clarify the precise mechanism by which miR-130b and miR-193a-3p may play a crucial role in developing schizophrenia.

In conclusion, plasma miR-130b and miR-193a-3p may be useful biomarkers for the development of a diagnostic tool for clinical application. Abnormal levels of these two plasma miRNAs also provide a clue to the natural history of the illness.

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REFERENCES

1. Mayilyan KR, Weinberger DR, Sim RB: The complement system in schizophrenia. Drug News Perspect 2008; 21:200–210
2. Kane JM, Correll CU: Pharmacologic treatment of schizophrenia. Dialogues Clin Neurosci 2010; 12:345–357
3. Wakefield JC: Misdiagnosing normality: psychiatry's failure to address the problem of false positive diagnoses of mental disorder in a changing professional environment. J Ment Health 2010; 19:337–351
4. Pillai A, Buckley PF: Reliable biomarkers and predictors of schizophrenia and its treatment. Psychiatr Clin North Am 2012; 35:645–659
5. Farh KK, Grimson A, Jan C, et al: The widespread impact of mammalian microRNAs on mRNA repression and evolution. Science 2005; 310:1817–1821
6. Bartel DP: MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215–233
7. Cao X, Yeo G, Muotri AR, et al: Noncoding RNAs in the mammalian central nervous system. Annu Rev Neurosci 2006; 29:77–103
8. Hsu R, Schofield CM, Dela Cruz CG, et al: Loss of microRNAs in pyramidal neurons leads to specific changes in inhibitory synaptic transmission in the prefrontal cortex. Mol Cell Neurosci 2012; 50:283–292
9. Gallego JA, Gordon ML, Claycomb K, et al: In vivo microRNA detection and quantitation in cerebrospinal fluid. J Mol Neurosci 2012; 47:243–248
10. Moreau MP, Bruse SE, David-Rus R, et al: Altered microRNA expression profiles in postmortem brain samples from individuals with schizophrenia and bipolar disorder. Biol Psychiatry 2011; 69:188–193
11. Gilad S, Meiri E, Yogev Y, et al: Serum microRNAs are promising novel biomarkers. PLoS ONE 2008; 3:e3148
12. Chen X, Ba Y, Ma L, et al: Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008; 18:997–1006
13. Mitchell PS, Parkin RK, Kroh EM, et al: Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008; 105:10513–10518
14. Hull ML, Nisenblat V: Tissue and circulating microRNA influence reproductive function in endometrial disease. Reprod Biomed Online 2013; 27:515–529
15. Zampetaki A, Kiechl S, Drozdov I, et al: Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010; 107:810–817
16. Andreasen NC, Carpenter WT Jr, Kane JM, et al: Remission in schizophrenia: proposed criteria and rationale for consensus. Am J Psychiatry 2005; 162:441–449
17. Schmittgen TD, Lee EJ, Jiang J, et al: Real-time PCR quantification of precursor and mature microRNA. Methods 2008; 44:31–38
18. Perkins DO, Jeffries CD, Jarskog LF, et al: microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol 2007; 8:R27
19. Santarelli DM, Beveridge NJ, Tooney PA, et al: Upregulation of dicer and microRNA expression in the dorsolateral prefrontal cortex Brodmann area 46 in schizophrenia. Biol Psychiatry 2011; 69:180–187
20. Miller BH, Zeier Z, Xi L, et al: MicroRNA-132 dysregulation in schizophrenia has implications for both neurodevelopment and adult brain function. Proc Natl Acad Sci USA 2012; 109:3125–3130
21. Gardiner E, Beveridge NJ, Wu JQ, et al: Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells. Mol Psychiatry 2012; 17:827–840
22. Sheinerman KS, Umansky SR: Circulating cell-free microRNA as biomarkers for screening, diagnosis, and monitoring of neurodegenerative diseases and other neurologic pathologies. Front Cell Neurosci 2013; 7:150
23. Zheng H, Liu JY, Song FJ, et al: Advances in circulating microRNAs as diagnostic and prognostic markers for ovarian cancer. Cancer Biol Med 2013; 10:123–130
24. Shi W, Du J, Qi Y, et al: Aberrant expression of serum miRNAs in schizophrenia. J Psychiatr Res 2012; 46:198–204
25. Morozova O, Marra MA: Applications of next-generation sequencing technologies in functional genomics. Genomics 2008; 92:255–264
26. Liu R, Zhang C, Hu Z, et al: A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. Eur J Cancer 2011; 47:784–791
27. Zen K, Zhang CY: Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev 2012; 32:326–348
28. Liang H, Gong F, Zhang S, et al: The origin, function, and diagnostic potential of extracellular microRNAs in human body fluids. Wiley Interdiscip Rev RNA 2014; 5:285–300
29. Ma S, Tang KH, Chan YP, et al: miR-130b Promotes CD133(+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. Cell Stem Cell 2010; 7:694–707
30. Lindberg RL, Hoffmann F, Mehling M, et al: Altered expression of miR-17-5p in CD4+ lymphocytes of relapsing-remitting multiple sclerosis patients. Eur J Immunol 2010; 40:888–898
31. Pan S, Yang X, Jia Y, et al: Microvesicle-shuttled miR-130b reduces fat deposition in recipient primary cultured porcine adipocytes by inhibiting PPAR-g expression. J Cell Physiol 2014; 229:631–639
32. Schizophrenia Working Group of the Psychiatric Genomics Consortium: Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014; 511:421–427
33. Bobbs AS, Saarela AV, Yatskievych TA, et al: Fibroblast growth factor (FGF) signaling during gastrulation negatively modulates the abundance of microRNAs that regulate proteins required for cell migration and embryo patterning. J Biol Chem 2012; 287:38505–38514
34. Lai KW, Koh KX, Loh M, et al: MicroRNA-130b regulates the tumour suppressor RUNX3 in gastric cancer. Eur J Cancer 2010; 46: 1456–1463
35. Zhao Y, Miao G, Li Y, et al: MicroRNA- 130b suppresses migration and invasion of colorectal cancer cells through downregulation of integrin β1 [corrected]. PLoS ONE 2014; 9:e87938
36. Wang YC, Li Y, Wang XY, et al: Circulating miR-130b mediates metabolic crosstalk between fat and muscle in overweight/obesity. Diabetologia 2013; 56:2275–2285
37. Yu T, Li J, Yan M, et al: MicroRNA-193a-3p and -5p suppress the metastasis of human non-small-cell lung cancer by downregulating the ERBB4/PIK3R3/mTOR/S6K2 signaling pathway. Oncogene 2015; 34:413–423
38. Nakano H, Yamada Y, Miyazawa T, et al: Gain-of-function microRNA screens identify miR-193a regulating proliferation and apoptosis in epithelial ovarian cancer cells. Int J Oncol 2013; 42:1875–1882
39. Gong X, Zhang K, Wang Y, et al: MicroRNA-130b targets Fmr1 and regulates embryonic neural progenitor cell proliferation and differentiation. Biochem Biophys Res Commun 2013; 439:493–500
40. Yang C, Cai J, Wang Q, et al: Epigenetic silencing of miR-130b in ovarian cancer promotes the development of multidrug resistance by targeting colony-stimulating factor 1. Gynecol Oncol 2012; 124:325–334
41. Ma K, He Y, Zhang H, et al: DNA methylation-regulated miR-193a-3p dictates resistance of hepatocellular carcinoma to 5-fluorouracil via repression of SRSF2 expression. J Biol Chem 2012; 287:5639–5649