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ORIGINAL ARTICLE

Differential exosomal microRNA profile in the serum of a patient with depression

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Abstract
Background and objectives: Accumulating evidence suggests that exosomal miRNAs play important roles in disease pathogenesis; however, their role in the pathogenesis of depression remains largely unknown. We explored the miRNA profile of circulating exosomes in a patient with depression and compared it with a healthy volunteer.
Methods: Serum exosomes were isolated from a patient and volunteer, and exosomal miRNAs were analyzed by miRNA-sequencing. MiRTarbase and gene ontology (GO) analysis were used to predict the downstream signaling pathways of the depression-specific miRNAs.
Results: miRNA-sequencing analysis identified 12 up-regulated miRNAs and 20 down-regulated miRNAs in the depressed patient. MiRTarbase and GO analysis revealed that the up-regulated miRNAs regulated the expression of genes involved in histone/chromatin modification and the neurotrophin signaling pathway. The down-regulated miRNAs regulated genes were associated with apoptosis, hypoxia, cell growth, and immune response.
Conclusions: Depression-specific miRNAs may modulate neurotrophin signaling, chromatin/histone modification, immune system function, inflammation, depression, apoptosis, hypoxia, and cell growth. Identifying the networks that may be regulated by these depression-specific miRNAs and their target genes may provide a novel, high-throughput screening platform for the early detection of depression as well as determining its severity and response to treatment.

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Introduction

Cells of the central nervous system (CNS) release exosomes that could have an active role in CNS function, development, and disease initiation or progression, such as Alzheimer's disease, Parkinson's disease, prion diseases, multiple sclerosis, brain tumors, and schizophrenia. In all of these diseases, exosomes mediate the spread of mutated or misfolded proteins, which serve as templates for the formation of disease-producing oligomers.[1] However, the role of exosomes in the pathogenesis or physiological function of patients with depression is largely unknown.

In addition to proteins, lipids, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) can also be delivered via circulating exosomes as intercellular messengers.[2,3] Moreover, exosomal miRNAs play important roles in disease pathogenesis, especially in cancers.[4,5] In addition, Banigan et al.[6] reported the differential exosomal microRNA profiles in the prefrontal cortices of patients with schizophrenia and bipolar disorder compared to healthy controls.[6] These studies suggest that exosomal miRNAs have a role in the pathogenesis of psychiatric disorders.

The structure of exosomes permits their incorporation into the cell membrane and allows them to easily cross the blood-brain barrier (BBB). Recently, Yang et al.[7] demonstrated that exosomes isolated from astrocytoma, glioblastoma, endothelial, and neuroectodermal brain tumor cells can across the BBB for drug delivery in zebrafish. Exosomes with lower miR-193b levels have also been observed in the cerebral spinal fluid (CSF) of dementia of Alzheimer-type (DAT) patients.[8] Raymond et al.[9] also demonstrated that exosomes expressing HIV negative factor can break down the BBB and regulate neuroimmune pathogenesis.

The neuroimmune system represents a dense network of biochemical signals, including neurotransmitters, neuropeptides, neurotrophin, neurohormones, cytokines, chemokines, and growth factors synthesized by neurons, glial cells, and immune cells to maintain systemic homeostasis. Endogenous and/or exogenous, noxious stimuli in any tissue are detected by sensor cells to inform the brain; likewise, signals originating in the CNS are transmitted to peripheral immune effectors, which react to central stimuli. This multidirectional information flow makes it possible for the CNS to respond to peripheral damage and for alterations in brain function to be reflected in peripheral immune changes.[10,11] Studies have demonstrated the association between depression and altered immune response, such as inflammation.[12–15] In this study, we explored the microRNA profile of circulating exosomes in a patient with depression and compared it with a healthy control patient. Given the interplay between the neuroimmune system, inflammation, miRNAs, and exosomes,[16] we hypothesized that a differential exosomal microRNA profile may play a role in modifying the pathogenesis of depression. Identifying the networks that may be regulated by these candidate miRNAs and their target genes may provide a novel, high-throughput screening platform for the early detection of depression as well as determining its severity and response to treatment.

Methods

Study participants

The inclusion criteria for the patient with depression were as follows[17]: (1) 20–64 years of age, (2) a recent diagnosis of depression, (3) no history of taking any drugs, and (4) a total score of 13–34 on the Montgomery–Asberg Depression Scale (MADRS-S) indicative of mild-to-moderate depression. In addition, the patient had to meet one of the following four items to be included in the study. (1) The patient had >8 weeks of symptoms described in the MADRS-S at the time of hospital admission. (2) Under special conditions, the patient had a MRDS-S score of 13–34 at the time of admission and again 8 weeks after admission without treatment over the 8-week period. (3) The patient had <8 weeks of symptoms of depression described in the MADRS-S at the time of admission, which were treated. (4) The patient had <8 weeks of symptoms of depression described in the MADRS-S at the time of admission with aggravating symptoms during the follow-up, which were treated. Before the treatment, the patient was again evaluated using the MADRS-S. The patient received treatment only if the results of first evaluation were maintained. If the score was >34, the disease was considered severe, and treatment was not applied. Participants (both the control participant and the patient with depression) were excluded from the study if they had severe personality disorder, risk of suicide, pregnancy, thyroid disease, use of current psychotherapy of any kind, and participation in any other psychiatric intervention study.[17]

The patient completed MADRS-S questionnaires at baseline and after 8 weeks of follow-up (0–6, normal/without symptoms; 7–19, mild depression; 20–34, moderate depression; and >34, severe depression). The patient received antidepressants and tranquilizers, if necessary. Blood samples were collected at the same time as the assessment of self-rated symptoms before treatment. The healthy control participant was age-matched with the patient with depression; they had (1) no history of depression, (2) a MADRS-S score of 0–4 for >8 weeks, and (3) none of the conditions described in the exclusion criteria.

This study was approved by the Institutional Review Board of the First Hospital of Harbin City. Both participants provided informed consent prior to enrollment.

Sample collection

Serum samples were collected in the patient with depression prior to administration of any treatment, if needed as well as in the healthy control participant. At least 2 mL of venous blood was collected from the participants in collection tubes without anticoagulant. After centrifugation at 3000 rpm for 10 min at room temperature, the serum

samples (4–6 mL/sample ideally) were stored in RNase antifreeze tubes at −80 °C.

Exosome isolation

Exosomes were isolated using the Total Exosome Isolation kit (cat# 4478360; Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. In brief, the serum samples were centrifuged at 2000 × *g* for 30 min. After the supernatants containing the clarified serum were transferred to a new tube, 0.2 volume of the Total Exosome Isolation reagent was added. The solution was vigorously mixed and incubated at 2–8 °C for 30 min. After centrifugation at 10,000 × *g* for 10 min at room temperature, the pellets containing the exosomes were isolated, resuspended in PBS, and stored at −20 °C until further use.

Exosome RNA extraction

Exosome RNA, including miRNA, was isolated using an ExoRNeasy Serum/Plasma Midi Kit (cat# 77044, Qiagen, Germantown, MD, USA) following the manufacturer's instructions. Briefly, cold, sterile RNase-free PBS was used to dilute the serum samples to final volume 1200 μL. After centrifugation for 10 min at 3000 × *g* at 4 °C, the supernatant was collected and filtered using a 0.8 μm Millipore Millex-AA filter syringe (cat. no. SLAA033SB). miRNA was isolated from 1 mL of sample using an exoRNeasy Serum/Plasma Midi Kit. The yield and quality of the isolated RNA were analyzed using an Agilent 2100 Bioanalyzer (Santa Clara, CA, USA).

Next-generation sequencing analysis

RNA fragments of 17–30 nucleotides or 15–35 nucleotides in size were isolated by gel purification. Adapters were ligated to both ends of the isolated RNA segments, which were then reverse transcribed into cDNA as previously described.[18] Expansion was done by PCR to establish a sequence library, which was subjected to Illumina HiSeq high-throughput sequencing using the SE50 strategy.[18] Genomic notes in the miRBase database (Release 21) or the miRNA sequence in the database was compared to those in the genome to obtain information about the location of known mature and precursor miRNAs in the genome. Clean reads in the genome were matched to the location of miRNA[19] to identify known miRNAs, and their expression, sequence, and structure were further analyzed. miRNA target genes were predicted from the sequence information using miRanda (3.3a) analysis as previously described.[20] The target genes of differentially expressed miRNA were subjected to gene ontology (GO) analyses as previously described.[21,22] Gender-specific and sex-hormone regulating miRNAs were excluded from the analysis.

Statistical analysis

For continuous variables, data were expressed as mean ± s.d. Differences between groups were estimated using Student's *t*-tests. All statistical analyses were performed using JMP 5 software (JMP, Cary, NC, USA), and the findings were considered significant when the *P*-value was <0.05.[17]

Table 1 Up-regulated candidate miRNAs in secreted exosomes in a patient with depression as compared to a healthy control subject.

miRNA	Fold-change	Adjusted *P*-value
hsa-miR-1255a	31.5672	0.000555
hsa-miR-3161	17.2185	0.016613
hsa-miR-99a-3p	14.3487	0.031591
hsa-miR-205-5p	5.02206	0.004325
hsa-miR-26a-1-3p	5.02206	0.046379
hsa-miR-139-5p	4.2472	8.23E−11
hsa-miR-7849-3p	4.01764	0.009700
hsa-miR-195-5p	3.18861	0.006295
hsa-miR-125b-2-3p	3.15672	0.046047
hsa-miR-664a-3p	2.63060	0.012340
hsa-let-7c-5p	2.09323	4.30E−07
hsa-miR-197-3p	2.06263	0.000316

Adjusted *P*-values were determined using Student's *t*-test.

Results

Identification of candidate miRNAs in a patient with depression using next-generation sequencing analysis

This study included one patient with depression and one healthy control. Using next-generation sequencing, miRNAs analyzed were considered candidate miRNAs if they were up- or down-regulated by ≥2-fold and the adjusted *P*-value was <0.05. As shown in Table 1, there were 12 up-regulated miRNAs: hsa-miR-1255a, hsa-miR-3161, hsa-miR-99a-3p, hsa-miR-205-5p, hsa-miR-26a-1-3p, hsa-miR-139-5p, hsa-miR-7849-3p, hsa-miR-195-5p, hsa-miR-125b-2-3p, hsa-miR-664a-3p, hsa-let-7c-5p, and hsa-miR-197-3p. There were also 20 down-regulated miRNAs identified by next-generation sequencing analysis (Table 2).

GO analysis of the up- and down-regulated exosomal miRNAs isolated from a patient with depression

We first used the miRNA database, miRTarbase, and GO Enrichment analysis to predict the possible downstream targets of the candidate miRNAs. As shown in Table 3, GO analysis of the up-regulated candidate miRNAs revealed that some have roles in chromatin/histone modification and posttranscriptional regulation of gene expression as well as neurotrophin signaling pathway. Overlap analysis revealed that the number of predicted downstream target genes of the candidate miRNAs identified versus the total number of genes in the GO gene expression network was as high as 30% (for the TGF-β signaling pathway).

As shown in Table 4, GO analysis of the down-regulated genes showed that some of the miRNAs have roles in apoptotic signaling, oxidation, hypoxia, cell cycle, and cellular growth. Of note, there was a 34% overlap in the cellular

Table 2 Down-regulated candidate miRNAs in secreted exosomes in a patient with depression as compared to a healthy control subject.

miRNA	Fold-change	Adjusted *P*-value
hsa-miR-499a-5p	0.03878	5.51E−08
hsa-miR-4732-3p	0.05519	0.002701
hsa-miR-222-5p	0.06522	0.007088
hsa-miR-1291	0.06522	0.007088
hsa-miR-668-3p	0.07972	0.018124
hsa-miR-425-3p	0.13665	0.000904
hsa-miR-6511a-3p	0.14349	0.026290
hsa-miR-145-3p	0.20498	0.019027
hsa-miR-200a-3p	0.26904	0.026290
hsa-miR-143-3p	0.27107	3.09E−166
hsa-miR-196b-5p	0.28185	2.69E−05
hsa-miR-99a-5p	0.34668	6.01E−10
hsa-miR-144-3p	0.37808	9.26E−18
hsa-miR-584-5p	0.39186	1.90E−25
hsa-miR-210-3p	0.40043	0.004850
hsa-miR-183-5p	0.40854	1.08E−07
hsa-miR-107	0.45366	6.70E−28
hsa-miR-130b-5p	0.46058	0.000592
hsa-miR-589-5p	0.46677	0.000598
hsa-miR-1910-5p	0.47829	0.000605

Adjusted *P*-values were determined using Student's *t*-test.

response to oxygen levels and a 28% overlap in the intrinsic apoptotic signaling pathway.

Putative target genes of the differentially expressed exosomal miRNAs in a patient with depression

Combining the data obtained by the next-generation sequencing analysis, miRTarbase target prediction, and GO Enrichment Analysis, we identified the possible genes targeted by the differentially expressed candidate miRNAs in a patient with depression. As shown in Table 5, there were eight up-regulated target genes with roles in depression, neurotrophin signaling, chromatin/histone modification, immune system function, and inflammation. There were also 12 down-regulated target genes involved in depression, apoptosis, hypoxia, immune system function, and cell growth (Table 5).

Discussion

Because the role of exosomal miRNA in the pathogenesis or physiological function of patients with depression is largely unknown, the present study compared exosomal miRNA levels from a patient with depression with that obtained from a healthy control participant. We found 12 candidate up-regulated miRNAs and 20 candidate down-regulated miRNAs. GO analysis revealed that some of the up-regulated candidate miRNAs regulated the expression of genes involved in histone/chromatin modification and neurotrophin signaling pathway. Those down-regulated miRNAs regulated genes involved in apoptosis, hypoxia, and cell growth. Analysis of potential gene targets of the dysregulated exosomal miRNAs in the depressed patient identified genes involved in neurotrophin signaling, chromatin/histone modification, immune system function, inflammation, depression, apoptosis, hypoxia, and cell growth.

The dysregulated exosomal miRNA expression observed in the present study is consistent with Belzeaux et al.[23] who found changes in several miRNAs (has-miR-107, miR-133a, miR-148a, miR-200c, miR-381, miR-425-3p, miR-494, miR-517b, miR-579, miR-589, miR-636, miR-652, miR-941, and miR-1243) in peripheral blood mononuclear cells of patients with major depressive disorder (MDD) compared with healthy controls. Of them, two miRNAs showed stable overexpression in MDD patients after an 8-week follow-up (miR-941 and miR-589).[23]

Studies have shown that neuroinflammation has a crucial role in the pathogenesis of depression, and exosomes may regulate some critical pathways associated with depression.[16,24] For example, interferon gamma (IFNγ) may

Table 3 GO analysis of the up-regulated candidate miRNAs in secreted exosomes in a patient with depression as compared to a healthy control subject.

Term	Overlap	*P*-value	Adjusted *P*-value
Gene expression (GO:0010467)[a]	145/672	8.15E−14	3.82E−10
Posttranscriptional regulation of gene expression (GO:0010608)[a]	104/438	2.80E−12	6.55E−09
Regulation of translation (GO:0006417)[a]	62/264	1.07E−07	0.000144
Neurotrophin signaling pathway (GO:0038179)[b]	62/278	4.98E−07	0.000212
Chromatin modification (GO:0016568)[a]	92/475	2.85E−07	0.000212
Fc receptor signaling pathway (GO:0038093)	66/352	3.30E−05	0.004182
Neurotrophin TRK receptor signaling pathway (GO:0048011)[b]	61/274	6.47E−07	0.000233
Histone modification (GO:0016570)[a]	64/293	6.10E−07	0.000233
Fibroblast growth factor receptor signaling pathway (GO:0008543)	44/163	3.21E−07	0.000212
Transforming growth factor beta receptor signaling pathway (GO:0007179)	40/134	1.23E−07	0.000144

The overlap value indicates the number of predicted downstream target genes of the candidate miRNAs identified versus the total number of genes in the GO gene expression network.
P-values were determined using Student's *t*-test; adjusted *P*-values were determined using Student's *t*-test.
[a] Chromatin/histone modification and posttranscriptional regulation of gene expression.
[b] Neurotrophin signaling pathway.

Table 4 GO analysis of the down-regulated candidate miRNAs in secreted exosomes in a patient with depression as compared to a healthy control subject.

Term	Overlap	*P*-value	Adjusted *P*-value
Apoptotic signaling pathway (GO:0097190)[a]	82/327	8.50E−10	4.06E−06
Positive regulation of cell cycle (GO:0045787)[c]	65/278	4.19E−07	0.000200
Response to oxygen levels (GO:0070482)[b]	62/259	3.89E−07	0.000200
Response to hypoxia (GO:0001666)[b]	59/241	3.94E−07	0.000200
Response to decreased oxygen levels (GO:0036293)[b]	59/245	6.27E−07	0.000262
Cell cycle phase transition (GO:0044770)[c]	66/280	2.66E−07	0.000200
Cellular response to oxygen levels (GO:0071453)[b]	37/109	9.69E−08	0.000200
Mitotic cell cycle phase transition (GO:0044772)[c]	65/277	3.76E−07	0.000200
Intrinsic apoptotic signaling pathway (GO:0097193)[a]	48/170	1.51E−07	0.000200
Growth (GO:0040007)[c]	72/329	8.67E−07	0.000296

The overlap value indicates the number of predicted downstream target genes of the candidate miRNAs identified versus the total number of genes in the GO gene expression network.
P-values were determined using Student's *t*-test; adjusted *P*-values were determined using Student's *t*-test.
[a] Apoptotic signaling.
[b] Oxidation and hypoxia.
[c] Cell cycle and cellular growth.

Table 5 Putative target genes of the differentially expressed miRNAs in secreted exosomes in a patient with depression as compared to a healthy control subject.

Genes	Number of miRNAs targeted	miRNA expression	Reference(s)	Function(s)
LCOR	4 (hsa-miR-1255a, 139-5p, 7849-3p, 205-5p)	Up	[38]	Chromatin/Histone modification
IGF1R	3 (hsa-miR-139-5p, 125b-2-3p, let-7c-5p)	Up	[15]	Depression, neurotrophin
VEGFA	2 (hsa-miR-195-5p, 205-5p)	Up	[15]	Neurotrophin
FASN	2 (hsa-miR-195-5p, 197-3p)	Up	[15,25]	Antidepressant effects of immune and inflammation related processes
APP	1 (hsa-miR-195-5p)	Up	[15,25]	Innate immune response, immune system process
GSK3A	1 (hsa-let-7c-5p)	Up	[11,26]	Depression
GSK3B	1 (hsa-miR-195-5p)	Up	[11,26]	Depression
ICAM1	1 (hsa-miR-1255a)	Up	[57]	Depression, inflammation
GATA6	5 (hsa-miR-425-3p, 6511a-3p, 200a-3p, 196b-5p, 183-5p)	Down	[28]	Innate immune response
MAPK1	4 (hsa-miR-668-3p, 143-3p, 196b-5p, 584-5p)	Down	[14,15]	Immune response, innate immune response, immune system process
CELF1	4 (hsa-miR-499a-5p, 6511a-3p, 200a-3p, 183-5p)	Down	[57]	Cell growth
SGPL1	4 (hsa-miR-222-5p, 143-3p, 144-3p, 4732-3p)	Down	[47]	Apoptosis
TGFBR3	4 (hsa-miR-196b-5p, 144-3p, 107, 130b-5p)	Down	[27]	Depression
NOTCH2	3 (hsa-miR-183-5p, 107, 130b-5)	Down	[15,51]	Hypoxia, immune system process, immune system development
VAV3	3 (hsa-miR-425-3p, 6511a-3p, 130b-5p)	Down	[15,49]	Apoptosis, hypoxia, innate immune response
BCL2	2 (hsa-miR-143-3p, 196b-5p)	Down	[15]	Apoptosis, innate immune response, immune system process and development
PMAIP1	2 (hsa-miR-499a-5p, 589-5p)	Down	[15,48]	Apoptosis, immune system process
XIAP	1 (hsa-miR-143-3p)	Down	[43]	Apoptosis, depression
PCNA	1 (hsa-miR-222-5p)	Down	[43]	Apoptosis, depression
BNIP3	1 (hsa-miR-210-3p)	Down	[50]	Apoptosis, depression

prevent spreading depression, which was mediated by induction of exosome release by microglia.[24] Thus, we hypothesized that a differential exosomal miRNA profile, particularly in those miRNAs that regulate neuroimmune system function and inflammation, may play a role in modifying the pathogenesis of depression. In the present study, miRNAs targeting genes involved in depression control as well as inflammation and immune system function, including *FASN*, *APP*, *GSK3A/B*, *ICAM1*, *MAPK1*, and *TGFBR3*, were both up- and down-regulated. Given the strong link between depression and depression control with inflammation and immune system function,[11,15] this finding was not surprising. Specifically, two miRNAs that potentially target *GSK3A/B* were up-regulated in the present study. Immune responses mediated by GSK signaling may play a role in depression control.[25,26] Furthermore, four miRNAs that potentially target *TGFBR3* were down-regulated. Genetic variants in *TGFBR3*, a member of the human activin system, also predict antidepressant treatment response in depression.[27] In addition, five miRNAs that potentially target *GATA6* and four that potentially target *MAPK1* were down-regulated in the present study. Both *GATA6*[28] and *MAPK1*[14] have been implicated in mediating immune response in depression control.[28] Thus, studies have described the potential clinical benefit of determining miRNAs, including hsa-miR-26a, hsa-miR-107, hsa-miR-130b, hsa-miR-183, hsa-miR-200c, hsa-miR-425-3p, hsa-miR-589, and hsa-miR-664, in blood and peripheral tissues to monitor response to treatment.[29]

Roles for synaptic plasticity and neurotrophin factors, such as IGF1, BDNF, p75NTR, VEGFR, neurotrophin-3, and the Trk pathway, in the depression mechanism have been reported. For example, genetic association analysis of polymorphisms in 10 genes contributing to IGF-I signaling revealed associations with susceptibility for depression and treatment response.[30] The present study identified miRNAs, including miR-125, miR-195, miR-222, miR-139, and miR-183, that regulate synaptic plasticity and neurotrophin signaling were also dysregulated in the patient with depression. Similarly, bioinformatic analysis indicated that the predicted targets of miR-195 and miR-329 had roles in neuronal apoptosis and synaptic plasticity.[31] MiR-125b, which targets *NR2A* and negatively regulates synaptic plasticity,[32] was significantly elevated in synaptosome supernatants after depolarization, suggesting that that secreted miRNAs might be functionally active after being taken up by the synaptic fraction via the endocytic pathway.[33] Furthermore, treatment with a selective serotonin reuptake inhibitor decreased the levels of miR-222, which targets *ITGB3* that is necessary for serotonin transporter (SERT) activity.[34] Finally, miR-183 is known to contribute brain-derived neurotrophic factor action in the brain,[35–37] and miR-183 may play a role in the pathogenesis of psychiatric disorders and in the mechanism of action of antipsychotic drugs and mood stabilizers.[35]

The present study identified four up-regulated miRNAs (miR-1255a, miR-139-5p, miR-7849-3p, and miR-205-5p) that may target *LCOR*. LCOR interacts with histone deacetylase 3 (HDAC3) and HDAC6, both of which are key modulators of chromatin remodeling.[38] In addition to chromatin remodeling, the polyamine system has been reported to play a role in predisposition to suicidal behavior.[39,40] Our analysis identified that miR-139-5p was up-regulated in the patient with depression, which is consistent with previous studies showing that miR-139-5p was inversely correlated with the polyamine gene, *SAT1*.[40] Thus, miRNAs may regulate polyamine gene expression in the suicidal brain via post-transcriptional activity.[41,42]

Studies have suggested that neurons are more vulnerable to degenerative signals in patients with MDD.[43] In addition, chronic stress, hypoxia, and cell death have been linked in depression,[44–46] and some mood stabilizers may prevent apoptosis in the CNS.[46] Some of the down-regulated miRNAs identified in the present study may also target genes involved in apoptosis, including *XIAP*, *PCNA*, *BNIP3*, *BCL-2*, *VAV3*, *SGPL1*, and *PMAIP1*,[15,43,47–50] as well as hypoxia, such as *NOTCH2* and *VAV3*.[15,49,51] Further studies will confirm whether these genes have a role in the pathology of depression or may be useful as markers of disease severity or treatment effects.

Changes in the exosomal miRNA profile may have therapeutic implications as a potential delivery strategy given that naturally occurring exosomes are capable of incorporation into cell membranes and crossing the BBB,[7,52] and miRNAs, including miR-132, function as intercellular signals, mediating neurovascular communication and regulating vascular integrity of the brain.[53] In addition to small molecules, intravenously injected exosomes have the potential to deliver miRNAs specifically to neurons, microglia, and oligodendrocytes in the brain, resulting in knockdown of specific genes.[52] Indeed, exosomes isolated from patients' cells may be engineered to express particular cell surface receptors recognized by specific cell types and loaded with therapeutic miRNAs, which may be used for personalized autologous therapies.[16] In addition, mixing miRNAs and exosomes as nanoparticles has potential for delivery of therapeutic siRNAs across the BBB.[54] Thus, further studies will evaluate the therapeutic potential of delivering the five down-regulated miRNAs that potentially target GATA6 and four that potentially target MAPK1 identified in the present study in exosomes for modulation of the neuroimmune response and depression control. We also identified that miR-139-5p was up-regulated, which may target LCOR and the polyamine gene, SAT1. Thus, we will also examine the impact of exosomal delivery of a miR-139-5p inhibitor in a model of depression.

In addition to being a potential new therapeutic delivery system, serum exosomes as well as exosomal miRNAs may represent accessible prognostic and diagnostic biomarkers for depression. Moreover, the resistance of exosomal miRNAs to RNase-dependent degradation permits their detection in circulating plasma and serum, which is necessary for broad clinical diagnostic applications.[55] Given the presence of cell-type specific markers of the surface of exosomes, neural-derived exosomes may be isolated from serum samples using a neural-specific surface marker for diagnosis of psychiatric conditions.[52] Exosomes may also contain disease biomarkers other than miRNAs, which may be of diagnostic importance.[56] Thus, further studies are necessary to fully explore the diagnostic potential of exosomes and exosomal miRNA for psychiatric disorders, including depression.

The present study is limited in that the exosomal miRNA of only one patient with depression was compared with only one healthy control. Furthermore, we only used one miRNA database, miRTarbase, for target gene prediction without

validation. Thus, further studies will examine the levels of target genes in patients with depression.

In conclusion, exosomal miRNAs with the potential to regulate neurotrophin signaling, chromatin/histone modification, immune system function, inflammation, depression, apoptosis, hypoxia, and cell growth are differentially expressed in depression. Further studies will evaluate whether the dysregulation of any specific miRNA or combination of miRNAs has a role in the pathology of depression or whether they may represent potential markers of the disease or treatment response.

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Conflict of interest

None.

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