Effective Biomarkers and Therapeutic Targets of Nerve-Immunity Interaction in The Treatment of Depression: A Integrated Analysis of miRNA-mRNA Regulatory Networks

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Abstract

Background: Major depressive disorder (MDD) is an emotional disorder that has a negative effect on patients' studies and daily lives. A great number of studies have found that miRNAs play an important role in the development of MDD and that they can be used as a biomarker for the diagnosis and treatment of MDD. However, there have been few investigations on nerve-immunity interaction therapy for MDD patients' brains.

Methods: We attempted to evaluate MDD in the gene expression matrix database and miRNAs in plasma samples from healthy controls using bioinformatics methods. Four plasma miRNAs (DE-miRNAs) samples were found from MDD patients. Funrich planned the transcription factors and target genes of miRNAs, and the enrichment of TF and GO was examined. The intersecting mRNAs were discovered by comparing the various expressions of the projected target genes and 5 mRNAs (DE-mRNAs) samples. In the end, 34 DE-miRNAs, 386 DE-mRNAs, and 17 intersecting mRNAs were detected. Intersecting core genes were then investigated using GO and KEGG enrichment analysis to find the intersecting mRNA. Identify particular candidate genes and pathways in neurology and immunology that may be associated with MDD for further investigation.

Results: We discovered 17 important HUB genes by the advance of a miRNA-mRNA network, and 5 HUB DE-MRNAs were derived following CytoNCA topology.

Conclusion: Our findings from a comprehensive bioinformatics analysis of miRNAs and mRNAs in MDD show that DE-miRNAs like miR-338-3P and miR-206 may be excellent biomarkers and potential therapeutic targets for the treatment of MDD via nerve-immunity interaction.

1 Introduction

Major depressive disorder (MDD) is a crippling mental illness defined by depression and emotional disturbance induced by a variety of psychological variables^[1]. The high prevalence rate, recurrence rate, and disability rate is all tough issues to address the prevention and treatment of MDD^[2]. At the moment, at least 20% of the world's populations is affected by MDD, and the WHO projects that the disease burden of MDD will surpass that of heart disease by 2030^[3-4]. The occurrence of MDD not only decreases patients' quality of life, but also places a significant cost on the family and society^[5]. Monoamine transmitter, immune-inflammation, stress, neuroscientist, and other popular ideas of MDD are now under investigation^[6]. Scholars have paid increasing emphasis in recent years to research on the pathophysiology of immunity-inflammation and MDD^[7-8]. Depending on several research, the activation of the peripheral immune system is linked to MDD^[9]. However, the pathobiology behind MDD inflammation is not well known, and more study is needed to uncover precise diagnostic biomarkers. The interplay of chronic moderate stress, neuroinflammation, and immune response alterations has been demonstrated to have a role in the pathogenesis of MDD^[10]. More and more research has revealed that the neurological system is inseparable from the immune system.

Currently, chemical medications are utilized mostly in the clinical treatment of MMD in Western medicine, whereas Chinese material media, acupuncture, and moxibustion are frequently used in traditional Chinese medicine (TCM)^[11]. Traditional antidepressants, on the other hand, can only relieve part of the symptoms of MMD patients, and 20% to 30% of MMD patients do not react to antidepressant medications. Neuroinflammation is seen as one of the causes of treatment ineffectiveness^[12]. After an injury, neuroinflammation is the result of an imbalance in the production and release of pro-inflammatory and anti-inflammatory cytokines from central or peripheral sources^[13]. The most noticeable aspect of neuroinflammation is microglia activation. Microglia activation results in the production of nutritional and anti-inflammatory substances under physiological settings. In pathological conditions, such as chronic stress or infection, microglia become over-estimated, resulting in not only higher amounts of inflammatory chemicals in the brain, but also neuronal damage and death^[14]. Therefore, identifying particular diagnostic biomarkers from the perspective of nerve-immunity interaction in order to effectively manage neuroinflammation and restore the function of neurotropism and neurotransmitters is critical to basic or clinical MMD research.

MicroRNAs (MiRNAs) are a type of endogenous intracellular tiny yet non-coding RNA. It can regulate biological processes such as cell proliferation, division, apoptosis, and metabolism as a regulator of many biological processes by encouraging the degradation of mRNAs and blocking their translation, so that its targeted mRNAs play a negative feedback regulation role^[15]. MiRNAs have been examined as a potential therapeutic target for a variety of central nervous system illnesses since they are involved in practically all fundamental cellular activities. It is well established that changes in miRNA expression are linked to the pathophysiology of several neurodegenerative illnesses and have significant therapeutic potential in the treatment of mood disorders, including clinical MDD^{[16-17a][i][18a]}. Detecting changes in the level of pathogenic miRNAs in the brain will be a very good technique to identify patients with early MMD. Bioinformatics is an important instrument for advancing biological understanding and therapeutic development^[19]. Therefore, the purpose of this study is to analyze and synthesize the evidence of immunity or of nerve-related miRNA in MDD plasma using bioinformatics, which is important for future fundamental research and clinical application of nerve-immunity interaction therapy of MDD. Figure 1 depicts a summary of this strategy.

^[i] Lauren Allen, Yogesh Dwivedi. MicroRNA mediators of early life stress vulnerability to depression and suicidal behavior^[J]. *Molecular Psychiatry*, 2020, 25(2):

2 Materials And Methods

1.1 Data processing of DE-miRNAs

We select data-sets from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>). Strategy for searching ('depression' [MeSH] and miRNA [All Fields] and normal) AND ('Homo sapiens' [Organism] AND 'Non-coding RNA profiling by array' [Filter]). The following was the inclusion criteria: Plasma miRNA levels in MMD patients and healthy persons. Finally, four sets of MMD miRNA expression

profile data were compiled. The data-set GSE5 8105 is based on the GPL18743 platform; the data-set GSE81152 is based on the GPL21814 platform; the data-set GSE152267 is based on the GPL21572-124634 platform; and the data-set GSE182194 is based on the GPL24741 platform, and it contains 88 MMD samples and 42 normal samples. The four miRNA chip data sets mentioned above are merged by perl software, and the VENN package of the four miRNA chip data sets are formed (Figure 2) in the R4.1.0 VENN package (<https://cran.r-project.org/web/packages/venn/>). The R4.1.0 Sva and Limma are used to multi-chip data-set for data rectification (batchNormalize). Genes acquired by the Limma software were determined to be significantly differentially expressed miRNAs using the corrected miRNA chip data-set and $\log_2$ (fold change) > 2 or $\log_2$ (fold change) < -2 as screening thresholds (DE-miRNAs). In R4.1.0, the pheatmap package (<https://cran.r-project.org/web/packages/pheatmap/>) is used to construct DEGS heat maps and volcanoes.

1.2 Identification of TF and targeted genes of DE-miRNAs

The FunRich3.1.3 software is used to annotate DEMs' GO function, which includes annotations of pathways and transporters (TF), as well as biological processes (BP), cellular components (CC), molecular functions (MF), etc^[20]. FunRich was used to classify miRNAs with statistical significance, up-regulation, and down-regulation in this work, and the first 10 transporters and target genes of DE-miRNAs were found.

1.3 Data processing of DE-mRNAs

Choosing a GEO data-set from the database. ('depression' [MeSH] and mRNA [All Fields] and normal) AND ('Homo sapiens' [Organism] AND 'Expression profiling by array' [Filter]). The following were the inclusion criteria: MMD sufferers' or healthy people's plasma mRNA Finally, five sets of MMD mRNA expression profile data were acquired. The data-set GSE19738 is based on the GPL6848-9572 platform, while the data-sets GSE32280, GSE44593, GSE53987, and GSE98793 are based on the GPL570-55999 platform, and include 80 MMD samples and 175 normal samples. Perl software is used to integrate the aforementioned five mRNA chip data-sets, and VENN diagrams of the five mRNA chip data-sets are created using the R4.1.0 VENN package (Figure 3). The Sva and Limma of R4.1.0 were then employed exclusively for multi-chip data-set data rectification (batchNormalize). Genes acquired by the Limma package were determined to be significantly differentially expressed mRNA using the corrected mRNA data-set, with $P < 0.05$ and $\log_2(\text{fold change}) > 2$ or $\log_2(\text{fold change}) < 2$ as screening conditions (DE-mRNAs). The pheatmap package in R software is used to construct DEGS heat maps and volcanoes.

1.4 Intersection genes of target mRNAs and DE-mRNAs

Given the negative regulation relationship between miRNA and target genes, perl software intersects the target genes up-regulated DE-miRNAs and down-regulated DE-mRNAs, and the target genes down-regulated DE-miRNAs are intersected with up-regulated DE-mRNAs, and the intersection genes are defined as core genes. The target genes of DE-miRNAs predicted by FunRich were then compared to DE-miRNAs

forecast by GEO. The crossing genes were represented by a Venn diagram, and overlapping mRNAs were discovered.

1.5 Construction of miRNA-mRNA network and Protein-protein interaction network

Cytoscape3.7.2 is utilized to build the miRNA-mRNA network of mRNAs^[21]. The String online tool (<https://string-db.org/>)^[22] is used to create the PPI network of aberrant mRNA. With the protein type "Homo sapiens" and the highest level of confidence (0.150). The PPI network model is then created by Cytoscape3.7.2.

1.6 Go and KEGG Enrichment analysis of mirNA-mrna regulatory network.

Following the acquisition of the core target, the ClusterProfiler, Colorspace, stringi, ggplot2, DOSE, enrichplot, and org. The R4.1.0 Hs.eg.db was utilized to examine the enrichment of GO and KEGG of the core target. These seven packages can be obtained from bioconductor (<http://www.bioconductor.org/>). GO (<http://geneontology.org/>) enrichment primarily examines the target's biological process, cell composition, and the molecular function, whereas KEGG (<https://www.kegg.jp/>) enrichment examines the target's potential biological pathways and activities.

1.7 Identification of potential Hub mRNAs and hub miRNAs

Based on the PPI network obtained above, according to the topological characteristics of the network, three most important parameters were selected by CytoNCA plug-in to screen the Hub mRNAs: Degree Centrality (DC)^[23], Closeness Centrality (CC)^[24], and Betweenness Centrality (BC)^[25]. DC relates to the number of other nodes associated with a node in the network. The higher the degree centrality, the greater the importance of the node. BC estimates the number of shortest paths through a node. The more the number of shortest paths through a node, the higher its intermediary centrality. CC calculates the sum of the distances from one point to all other points. The smaller the sum, the shorter the path from this point to all other points, which means that the point is closer to all other points. The levels of these three parameters represented the topological importance of the nodes in the network, they reflected the role and influence of the corresponding nodes in the whole network and importance of the nodes was positively correlated with the output value of the network. According to relevant literature reports, the target showing two-fold the median value was selected for DC^[26], and the target with the median value for BC and CC^[27] was selected to obtain more accurate core targets. Then, according to the miRNA-mRNA interaction network obtained above, the hub miRNAs corresponding to hub mRNAs is obtained.

3 Results

The purpose of this study is to investigate, evaluate, and summarize evidence of nerve-immunity-related miRNA in the plasma of MMD patients in order to determine useful biomarkers, new therapeutic targets, and prognosis evaluation methodologies in MDD nerve-immunity interaction therapy. We predict miRNA of TFs, route Hub miRNAs and mRNAs, and build a miRNA-mRNA network and a PPI network.

3.1 Identification of DE-miRNAs and DE-mRNAs.

The combined data set of 42 healthy people and 88 MMD patients revealed 34 DE-miRNAs (20 up-regulated and 14 down-regulated). Following the screening with $P < 0.05$ and $\log_2(\text{fold change}) \geq 2$. To visualize the DE-miRNAs, a volcano map and a heat map are constructed (Figure 4). The DE-mRNAs of 80 MMD patients and 175 healthy controls were analysed. A total of 386 DE-mRNAs were identified after screening with $P < 0.05$ and $\log_2(\text{fold change}) \geq 2$ (181 up-regulated and 205 down-regulated). To visualize the DE-mRNAs, the volcano map and heat map are utilized (Figure 5). The top 20 miRNAs and mRNAs exhibit differential expression (Table.1A,1B).

Table 1A. The table shows the information of Top 20 differentially expressed miRNAs.

id	logFC	adj.P.Val	Regulated
hsa-miR-574-5p	-110.9542898	1.49E-36	down
hsa-miR-940	39.47752153	2.16E-06	up
hsa-miR-486-5p	-38.71374032	1.32E-14	down
hsa-miR-1225-5p	-37.92807878	4.51E-19	down
hsa-miR-1207-3p	18.78576063	3.88E-06	up
hsa-miR-1207-5p	-17.826831	1.07E-09	down
hsa-miR-137	13.40197958	0.012279941	up
hsa-miR-320c	-12.34701801	0.007034566	down
hsa-miR-568	12.06373429	0.030023	up
hsa-miR-595	-10.80147681	2.64E-17	down
hsa-miR-1825	10.66823191	0.005454226	up
hsa-miR-1290	9.531297239	0.016235855	up
hsa-miR-450b-3p	8.988693552	0.002201271	up
hsa-miR-621	7.682599653	0.035136805	up
hsa-miR-1246	-6.396313788	0.000500662	down
hsa-miR-630	-6.024802257	8.95E-09	down
hsa-miR-875-3p	4.4232763	0.012241154	up
hsa-miR-325	4.178569237	1.23E-05	up
hsa-miR-127-3p	4.163199662	0.015960629	up
hsa-miR-483-5p	-4.110638734	0.00430359	down

Table 1B. The table shows the information of Top 20 differentially expressed mRNAs.

id	logFC	adj.P.Val	Regulated
P2RY8	-3.420464032	5.27E-11	down
C16orf54	-3.414050815	5.07E-10	down
PPBP	-3.288699839	2.10E-10	down
PLP1	3.192684606	4.51E-07	up
PLAC8	-3.1541482	3.41E-11	down
IL2RG	-3.145809707	7.92E-12	down
S100A9	-3.127308745	1.67E-09	down
CXCR2	-3.112538855	1.08E-08	down
MPEG1	-3.112269482	1.12E-11	down
CSF2RB	-3.102043325	7.06E-12	down
CMTM2	-3.069217774	7.29E-12	down
CSTA	-3.061488684	2.90E-11	down
C5AR1	-3.014644308	1.18E-09	down
NCF2	-3.012015175	1.70E-08	down
SELL	-2.992395615	1.10E-10	down
S100A8	-2.96753421	3.42E-08	down
IL7R	-2.949694197	4.84E-11	down
EOMES	-2.941185679	4.82E-11	down
PLBD1	-2.936378649	7.59E-12	down
FCGR3B	-2.910908361	2.51E-09	down

3.2 Predicting TF and target genes of miRNAs.

SP1, SP4, KLF7EGR1, HNF4A, POU2F1, GABPA, ETS1, CTCF, and RREB1 are the top ten TF in DE-miRNAs (Figure 6-A). SP1, SP4, KLF7, EGR1, HNF4A, CTCF, POU2F1, NFYA, GABPA, and RREB1 were the top ten TFs of up-regulated DE-miRNAs (Figure 6-B). SP1, SP4, KLF7, EGR1, HNF4A, POU2F1, GABPA, ETS1, MEF2A, and NFIC are the top ten TFs of down-regulated DE-miRNAs. (Figure 6-C) successfully predicted the target mRNA for 578 up-regulated and 635 down-regulated DE-miRNAs. See (Appendix Table 1) for TF analysis and miRNA target genes (Appendix Table 2).

3.3 Functional annotation of DE-miRNAs

FunRich program discovered 59 biological processes (BP), 267 cellular composition (CC), and 116 molecular functions (MF) using GO enrichment analysis of DE-mRNAs (Figure 7). It is essentially connected to Molecular function, Transcription activity, and DNA-RNA-Protein activity in the MF, Molecular function uncertain, Transcription factor activity, Transcription regulator activity, DNA-RNA binding, Protein serine/threonine kinase activity, and so on. It is primarily connected to signal and cell communication in the BP, such as signal transduction, cell communication, biological process unknown, and regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolism. It is mainly associated with Cytoplasm, Nucleus, Plasma membrane, Exosomes, and Lysosomes in the CC (Appendix Table 3-5).

3.4 Identifying the intersection mRNAs.

The 34 DE-miRNAs (20 up-regulated and 14 down-regulated) and 578 up-regulated and 635 down-regulated target mRNAs effectively predicted were compared to the 386 DE-mRNAs (181 up-regulated and 205 down-regulated) acquired from GEO data screening. The VENN map (Figure 8) was constructed, which included 17 overlapping DE-mRNAs. Crossover genes are represented in (Table 2).

Table 2. The table shows the information of intersection genes.

miRNA	mRNA	Target	mirnaLogFC	mrnaLogFC
hsa-miR-206	GJA1	target	-3.251413682	2.682957112
hsa-miR-206	MAL2	target	-3.251413682	2.253644367
hsa-miR-206	PCDH17	target	-3.251413682	2.035934044
hsa-miR-206	SNAP25	target	-3.251413682	2.533742062
hsa-miR-206	SOX9	target	-3.251413682	2.070698257
hsa-miR-320c	GNAI1	target	-12.34701801	2.155037624
hsa-miR-320c	LMO3	target	-12.34701801	2.604451601
hsa-miR-320c	TUSC3	target	-12.34701801	2.034421167
hsa-miR-338-3p	CACNA2D1	target	-2.295135247	2.092690723
hsa-miR-338-3p	CHL1	target	-2.295135247	2.234787943
hsa-miR-338-3p	KCND2	target	-2.295135247	2.141220267
hsa-miR-338-3p	MYT1L	target	-2.295135247	2.069355937
hsa-miR-338-3p	NOVA1	target	-2.295135247	2.202273914
hsa-miR-296-5p	RAB37	target	2.171635035	-2.470323316
hsa-miR-137	BAZ1A	target	13.40197958	-2.100731
hsa-miR-137	RNASE6	target	13.40197958	-2.659559722
hsa-miR-137	SASH3	target	13.40197958	-2.661132519
hsa-miR-127-3p	RAB37	target	4.163199662	-2.470323316

3.5 Construction of miRNA-mRNA network and PPI network

Because miRNA and mRNA have a negative regulatory interaction, the previously collected intersecting DE-mRNAs were employed in Cytoscape3.7.2 to construct a miRNA-mRNA regulatory network. The network contains 6 miRNA and 17 mRNA (Figure 9). Table 1 indicates the comparable expression of miRNA-mRNA in MMD.

3.6 Construction of PPI network and Screening of hub mRNAs and miRNAs.

To obtain the PPI network, the common differentially expressed genes from the two data sets are introduced in the String database, and the unconnected targets are deleted. Cytoscape3.7.2 BisoGenet revealed that the network had 369 nodes and 2485 edges. The CytoNCA plug-in is then used to further evaluate the network's core targets, depending on the features of the network architecture. Depending on the DC, the target with twice the median value is chosen, whereas BC and CC to choose the target of the

median value. Following screening, five hub mRNAs and their corresponding hub miRNAs (Appendix Table 6-7) are defined, and the node transmission information and transmission efficiency are chosen as the "primary target" (Figure 10) for further investigation. It can be pointed out that the core genes include KCND2, MYT1L, GJA1, CHL1, and SNAP25 (Table 3).

Table 3. Information on 5 core targets.

SUID	Gene symbol	Protein name	Betweenness	Closeness	Degree
77	KCND2	Potassium voltage-gated channel subfamily D member 2	0.666666667	1	4
83	CHL1	Neural cell adhesion molecule L1-like protein	0.666666667	1	4
73	SNAP25	Synaptosomal-associated protein 25	0.666666667	1	4
92	MYT1L	Myelin transcription factor 1-like protein	0	0.8	3
75	GJA1	Gap junction alpha-1 protein	0	0.8	3

3.7 Go and KEGG Enrichment analysis of miRNA-mRNA regulatory network.

470 biological process (BP), 83 cellular composition (CC) and 84 molecular function (MF) (Figure 11) were identified by GO enrichment analysis of common DEMs by R4.1.0 software. In terms of molecular function, mainly including channel activity (GO:0015267), passive transmembrane transporter activity (GO:0022803), immune receptor activity (GO:0140375), nucleoside binding (GO:0001882). In the biological process, mainly including neutrophil activation (GO:0042119), neutrophil activation involved in immune response (GO:0002283), neutrophil degranulation (GO:0043312), neutrophil mediated immunity (GO:0002446). In the cellular composition, mainly including presynapse (GO:0098793), external side of plasma membrane (GO:0009897), secretory granule membrane (GO:0030667), synaptic membrane (GO:0097060) (Appendix Table 8-10). We selected the first 20 feature-rich processes to draw clusters and circles, as showed in (Figure 9). In addition, we identified the main signal pathways involved in the occurrence and development of MMD in KEGG enrichment analysis, and screened out the first 20 signal pathways related to MMD and significantly enriched. Including Cytokine-cytokine receptor interaction (hsa04060), Chemokine signaling pathway (hsa04062), Phagosome (hsa04145), Tuberculosis (hsa05152), Viral protein interaction with cytokine and cytokine receptor (hsa04061), Neutrophil extracellular trap formation (hsa04613) (Appendix Table 11). We select the first 20 principal signal pathways to draw cluster diagrams and circles, such as figure (Figure 12).

4 Discussion

The incidence of MMD is increasing year by year as a result of the pressures of job and life. Patients with MMD not only suffer from the condition, but they also make an unbelievable load on their family and

society as a whole^[28]. Modern medicine examines the pathogenic basis of MMD to be a disrupted metabolic process of the neuro-endocrine-immune network consists of the neurological system, hypothalamus-pituitary-adrenal axis (HPA axis), and immune system^[29][a30a]. Depending on studies, nerve-immunity network malfunction causes excessive secretion of inflammatory components and induces glucocorticoid secretion, which mediates the onset of MMD^[31]. The increased expression of pro-inflammatory factors such as TNF-, IL-6, and IL-1 in the serum, cerebrospinal fluid, and hippocampus of MMD patients and animal models forms a negative feedback regulation of the anti-inflammatory factor IL-10, resulting in neuronal inflammation and neuronal damage in the central nervous system^[32][a33a].

At present, scholars place great importance on nerve-immunity interactive therapy MDD. Commonly used clinical treatments such as escitalopram, fluoxetine, acupuncture and Chinese materia medica are related to nerve-immunity interactive therapy. It has been found that common antidepressants such as escitalopram and fluoxetine can enhance central function by enhancing central serotonergic nerve activity. It can also effectively regulate the levels of serum brain-derived neurotrophic factors and inflammatory factors in patients^[34][a35a]. Acupuncture can increase the content of serum 5-HT, up-regulate the content of anti-inflammatory cytokine IL-10, and reduce the content of proinflammatory cytokine IL-6 and TNF- α ^[36][a37a]. Chinese materia medica such as Rhizoma Cyperi, Rhizoma Coptidis, Cinnamomum and Radix Paeoniae Alba can improve the growth and development of neurons by mediating neurotrophic factors and other signal pathways, resist the neuronal damage caused by MMD, and regulate the signal transduction pathways such as NLR, ErbB and chemokines to regulate inflammatory response and immune response, resulting in antidepressant effects^[38][a39a[ii]][a40a[ii]] [a41a]. It has been reported that miRNAs plays a major role in the occurrence and development of MMD, but the interaction between miRNAs and mRNA is not clear. Therefore, it is of profound significance to study MMD at the molecular level and to find the mechanism of nerve-immunity interactive therapy of MDD, so as to provide reference ideas for future basic research and clinical application.

We discovered 17 important Hub genes by the development of a miRNA-mRNA network, and after CytoNCA topology, we discovered 5 Hub DE-MRNAs: KCND2, MYT1L, GJA1, CHL1, and SNAP25, all of which are up-regulated genes. The comparable miRNAs are hsa-miR-206 and hsa-miR-338-3p, which are both down-regulated in MMD. Although five pairs of regulatory relationships between miRNA and mRNA have not previously been reported, this study validates hsa-miR-206 with GJA1, SNAP25, hsa-miR-338-3p with KCND2, MYT1L through the miRDB database (<http://mirdb.org/>), and hsa-miR-338-3p with CHL1 through the TargetScan database (http://www.targetscan.org/vert_71/), and predicts the possibility of interaction between five pairs of miRNA-mRNA. It provides a theoretical foundation for additional experimental validation of the coupling of these miRNA and mRNA.

MiRNAs are tiny non-coding RNAs that directly regulate more than 30% of the genes in cells by inhibiting the degradation or translation of target mRNAs^[42][a43a]. It is linked to nearly every fundamental biological function, including MMD and anxiety disorders^[44][a45a]. It is understood that changes in miRNA expression are linked to the pathophysiology of numerous neurodegenerative disorders^[46]. MiR-

206, a member of the muscle-specific miR-1 family, was first considered to govern skeletal muscle embryonic development^[47]. MiR-338-3p was first thought to be a cancer-related miRNA, but it was later found to be involved in neuronal regulation, including the Parkinson's disease pathway^[48]. Neurological aspect: Brain-derived neurotrophic factor (BDNF) participates in neuroplasticity and protection throughout brain development, as well as playing an important role in emotion-related brain activities^[49][a50a]. MiR-206 has the capacity to suppress the production of BDNF following transcription, which is vital in the regulation and participation in MMD^[51]. It was also discovered that using miR-206 antagonists could attenuate stress-induced aggressive behavior while increasing BDNF expression in SI mice. Up-regulation of miR-206 has been demonstrated to impair neuron function and result in poor adaptive behavior^[52]. MiR-338 can increase myelination in the nervous system, and over-expression of miR-338 can boost oligodendrocyte differentiation and inhibit myelin negative regulators Sox6 and Hes5, hence encouraging myelin formation^[53][a54a]. MiR-338-5p has been found to safeguard the cognitive function of transgenic APP/PS1 mice by lowering neuronal death^[55]. Other research has shown that silencing miR-338-5p can result in neuronal polarity loss and a considerable decrease in the number of neurons. Thus, activating miR-338 can be, to some extent, influences the emotional function of the brain^[56]. Immune aspects: MiR-206 is also linked to the TNF signaling pathway^[57]. The activated TNF pathway mediated by miR-206 can, in turn, drive NF- κ B to enter the nucleus and boost the creation and release of inflammatory mediators such as TNF- α , IL-8, IL-6, and others to be involved in inflammatory and immunological processes^[58]. TNF inflammatory cytokines, which are plentiful in tumor microenvironments, can promote tumor growth, disrupt cell proliferation and death, and impair the innate immune response to cancer cells^[59]. MiR-338-3p is also related to the TNF signaling pathway. TNF- α may cause inflammation by activating the NF- κ B/MAPK signaling pathway and lowering the expression of miR-338-3p^[60]. Overexpression of miR-338-3p has been shown to block the ERK/p38MAPK signaling pathway and reduce the expression of pro-inflammatory genes VCAM-1 and ICAM-1RNA^[61].

KCND2 is a Potassium Voltage-Gated Channel Subfamily D protein coding gene that can encode a type A potassium channel protein that is important during the repolarization stage of an action potential^[62]. By controlling potassium transport across the excitatory membrane in the brain, voltage-gated potassium channels regulate dendritic A-type current I (SA) in brain neurons^[63]. By controlling potassium transport across the excitatory membrane in the brain, voltage-gated potassium channels regulate dendritic A-type current I (SA) in brain neurons^[64]. Myt1L (myelin transcription factor 1 like) is a fellow of the Myt/neural zinc finger (NZF) family that encodes a neural transcription factor with six zinc fingers. Its expression has so far only been detected in neural tissue^[65]. This gene is important in order to neural differentiation, and mutations in it have been linked to autism spectrum disorder and an autosomal dominant form of cognitive dysfunction^[66]. Mutations in Myt1L in chromosomal band 2p25.3 have been linked to intellectual disability, while Myt1L repetition has been linked to schizophrenia and MMD^[67]. GJA-1 (Gap junction alpha-1 protein) expression had proven to be down regulated in MDD patients, as was the Cx43 mRNA that encoded it^[68]. CHL1 (Cell Adhesion Molecule L1 Like) is a cell adhesion molecule that belongs to the L1 family. This gene's deletion and repetition have been associated with a variety of neurological

illnesses including autism, Parkinson's disease, and mental impairment^[69]. The study revealed that the level of serum CHL1 in patients with MMD declined dramatically and was inversely proportional to the severity of the disease, implying that CHL1 may be involved in the pathogenesis of MMD and immune cell dysfunction^[70]. SNAP-25 has been associated with the intensity of depressive symptoms in patients with MMD as judged by the Beck MMD Rating Scale in numerous studies^{[71-][a72a[iii]][a73a]}. It can be seen that both the hub miRNA and the mRNA generated by establishing a miRNA-mRNA network plays a role in the occurrence and progression of MMD via nerve-immunity interaction.

To examine the molecular mechanism of MDD, we investigated the key targets using GO and KEGG enrichment. The core targets added value in immunological and neural aspects in BP, CC, and MF, according to GO and KEGG enrichment analysis. Presynapse, synaptic membrane, neuronal cell body, synaptic connections between neurons, and voltage-gated sodium channel complex were among the 83 CC that were found to be enriched. There were 84 enriched elements in the MF, most of which were immune receptor activity, neurotransmitter receptor activity, chemokine binding, etc. There were 470 enriched elements in the BP, most of which were connected with neutrophil, T, and B cell activation, activation of surface receptors and signal pathways of immune response, and modulation of chemical synaptic transmission, among other things. In addition, 39 MMD-related pathways were determined and evaluated, including immune and neuroregulation pathways.

Among the signaling pathways investigated in this work. The signal pathways listed below being associated with immunological and neurological control. Specifically, the B and T cell receptor signal pathways, the chemokine signal route, the JAK-STAT signal pathway, Th1 and Th2 cell differentiation, and Th17 cell differentiation. Th17, Th1, and Th2 CD4+T lymphocyte subsets of the human immune system serve essential roles in promoting inflammation and immunosuppression, respectively, and contribute in immune system homeostasis while supporting B cell activation and having a role in humoral immunity^{[74-][a75a]}. ■ TTh17 cell differentiation: Th17 plays a role in the pathophysiology of MDD by increasing the generation of autoantibodies and the proclivity for autoimmunity^[76]. Furthermore, Th17 and Treg are connected in differentiation and function, and they are frequently in a state of dynamic equilibrium. If the proportion of them is no longer part balanced, it will result in aberrant immunological responses such as inflammatory, tumor, and autoimmune reactions.^[77] Th17 had proven to be elevated in the brains of MMD model mice, but ROR γ t defective mice demonstrated resistance to learning helplessness. As a result, an increase in Th17 can cause MMD, whereas suppressing Th17 production or function can lessen the risk of MMD in mice^[78]. ■ Th1 and Th2 cell differentiation: Both Th1 and Th2 cells can release cytokines, which can boost their own proliferation while inhibiting each other in order to maintain a relative equilibrium. When the equilibrium is disrupted, also known as "Th1/Th2 drift," it can be expected to result in the emergence and development of a variety of disorders associated with immunological escape^{[79-][a80a]}. ■ T and B signaling pathways: In MMD patients, immunological function was compromised, T cell apoptosis increased, neutrophils rose, and total lymphocytes decreased. T cell function degradation could be due to inflammatory substances such as tumor necrosis factor alpha destroying T cell function. B cells mediate humoral immunity via antibodies;

as the number of B cells falls, so does the rate of transformation^[81]. **Chemokine signaling pathway:** Chemokines' major purpose is to control leukocyte migration (homing) to their proper regions during inflammation and homeostasis. Some regulate the chemotaxis of immune cells during the immune surveillance process, and others recruit neutrophils to the site of infection or tissue injury for anti-infection, which serves a guiding role in the cells of the innate immune system and the adaptive immune system. Furthermore, chemokines play a vital function in advance^[82-]a83a]. **JAK/STAT signal pathway:** Not only gets this one of the most essential transmembrane signal transduction pathways, but it is also the most critical signal pathway triggered by inflammatory cytokines^[84]. Furthermore, JAK and STAT are key components of many signaling pathways that regulate cell growth, differentiation, survival, and pathogen resistance, including those involving the IL-6 (gp130) receptor family, which aids in the regulation of B cell differentiation, plasma cell production, and acute phase response^[85-]a86a]. **Neural aspect:** The JAK/STAT signaling pathway is widely implicated in the proliferation and differentiation of NSC during central nervous system development, and it plays a key role in regulating hippocampal nerve remodeling^[87]. A member of its family, the JAK2/STAT3 signal pathway, is involved in neuronal death and nervous reorganization in ischemic encephalopathy^[88]. Numerous studies have revealed that TNF- α causes JAK/STAT signal pathway activation via the protein-gp130 on the cell membrane's surface, and so contributes to the control of central nervous system degenerative alterations^[89-]a90a]. In addition, the JAK/STAT signaling pathway can influence neuron reorganization via inflammatory cytokines, brain-derived neurotrophic factors, neurotransmitters, etc^[91-]a92a[iv]]a93a]. As a consequence, medications and other treatment interventions can suppress neuronal death and promote nerve reorganization by altering the JAK/STAT signal pathway's associated molecules.

Because of the blood-brain barrier, the central nervous system is commonly considered to be an immunological zone, according to conventional wisdom. However, as more study is undertaken, this concept is gradually shifting^[94-]a95a]. Chemokines, which include Th17, Th1, and Th2, are key chemicals in the immunological and neurological systems. They can coordinate this migration of T and B cells to inflammatory areas, promote T cell activity and help B cell activation, and play a function in regulating immunity-inflammation in the immune system^[96]. Following psychological stress, activated T cells can get into the central nervous system in a variety of ways, reduce inflammation, and support neuronal integrity via cellular immunity, so modifying depressed behavior^[97-]a98a]. Furthermore, the JAK/STAT signaling system influences brain remodeling by activating inflammatory cytokines and altering the metabolism of the neuro-endocrine-immune network.

Depending on the Integrated Analysis of miRNA-mRNA Regulatory Networks, miR-338-3p and miR-206 can coordinate the regulation of the neuro-endocrine-immune network by regulating chemokines such as Th17, Th1 and Th2, immune cells such as T/B and the JAK/STAT signaling pathway, among other things. Realize bi-directional regulation of the HPA axis, which can normalize nerve conduction, minimize immuno-inflammatory responses, and restore normal neural, endocrine, and immunological function, and so play a therapeutic role in MMD. To research useful biomarkers and treatment targets from the perspective of MMD and the host neuro-endocrine-immune network in order to regulate the level of

immune inflammatory cytokines in the microenvironment and balance neuro-endocrine-immune homeostasis at the molecular level. It is critical to improve the critical mechanism of neuro-endocrine-immune imbalance in MMD patients.

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Conclusions

To summarize, the occurrence and progression of MMD are a function of multi-target, multi-pathway, multi-signal pathway, and multi-mechanism interactions, and the regulation process is synergistic and bi-directional. MiRNA, which differs from miR-206 and miR-338-3p, may activate or inhibit the B cell receptor signal pathway, T cell receptor signal pathway, and chemokine signal pathway by governing the expression of KCND2, Myt1L, GJA-1, CHL1, and SNAP-25. JAK-STAT and other signal pathways regulate immune-inflammatory response, nerve remodeling, and other functions, as well as mediating the occurrence and progression of MMD. Although this study presents some theoretical foundations and research ideas for nerve-immunity interaction, it has several drawbacks. Improvement suggestions: ¶ Because the current data is derived from the GEO database, it is impossible to assess the dependability and quality of the statistical data. In the future, we will be added to the number of data sources and decrease the data offset. ¶ Conduct additional scientific and clinical research to see if acupuncture, TCM, and other treatments can improve the neurological and immunological function of MMD patients by modulating the level of miRNA in the brain.

Abbreviations

MDD	Major depressive disorder	GEO	Gene Expression Omnibus GEO,
GO	Gene Ontology	MiRNAs	MicroRNAs MiRNAs)
TCM	traditional Chinese medicine	BP	Biological processes
MF	molecular functions	CC	cellular components
KEGG	Kyoto Encyclopedia of Genes and Genomes	DEGs	differentially expressed genes

Declarations

Data availability

1. All the data can be obtained from the open source website we provide, and the conclusion can be drawn through the analysis of the relevant software.
2. The [miRNA] data used to support the findings of this study have been deposited in the [GEO] repository (<https://www.ncbi.nlm.nih.gov/geo/>).
3. The [mRNA] data used to support the findings of this study have been deposited in the [GEO] repository (<https://www.ncbi.nlm.nih.gov/geo/>).
4. The [Integrated Analysis of miRNA-mRNA Regulatory Networks] data used to support the findings of this study are included within the supplementary information file(s).

Ethics approval and consent to participation

This manuscript is not a clinical trial, hence the ethics approval and consent to participation is not applicable.

Consent for publication

All authors have read and approved this manuscript to be considered for publication.

Competing interests

The authors declare no competing financial interests.

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Author Contributions

Zixuan Wu drafted and revised the manuscript. Xuyan Huang and Minjie Cai are in charge of data collection. Peidong Huang conceived and designed this article, in charge of syntax modification and revise of the manuscript. Zunhui Guan revised the manuscript. All the authors have read and agreed to the final version manuscript.

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Figures

Figure 1

Framework based on an integration strategy of integrating analysis of miRNA-mRNA Regulatory Networks.

Figure 2

Venn diagram of miRNA of the merger GSE58105,GSE81152,GSE152267 and GSE182194.

Figure 3

Venn diagram of mRNA of the merger GSE19738, GSE32280, GSE44593, GSE53987 and GSE98793.

Figure 4

Differential genes volcano map and heat map are jointly analyzed by 4 miRNA chips. miRNA from normal group and MMD group.

Figure 5

Differential genes volcano map and heat map are jointly analyzed by 5 mRNA chips. mRNA from normal group and MMD group.

Figure 6

(A) Top 10 TF of DE-miRNAs. (B) Top 10 TF of up-regulated DE-miRNAs. (C) Top 10 TF of down-regulated DE-miRNAs..

Figure 7

GO enrichment analysis of DE-miRNAs.

Figure 8

Venn diagram of overlapping DE-mRNAs.

Figure 9

The miRNA-mRNA networks of all dysregulated mRNAs. Red stands for up-regulation, green for down-regulation, triangle for miRNA, and prototype for mRNA.

Figure 10

The process of topological screening for the PPI network.

Figure 11

The GO enrichment analysis of core nodes. Including cellular components, molecular functions, biological processes

Figure 12

The KEGG enrichment analysis of core nodes.

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