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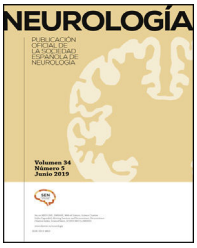

ORIGINAL ARTICLE

The miRNA neuroinflammatory biomarkers in COVID-19 patients with different severity of illness

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KEYWORDS
miRNAs;
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Abstract
Introduction: The expression of specific miRNAs and their mRNA targets are changed in infectious disease. The aim of this study was to analyze the expression of pro-neuroinflammatory miRNAs, anti-neuroinflammatory miRNAs, and their mRNA targets in the serum of COVID-19 patients with different grades.
Methods: COVID-19 patients with different grades were enrolled in this study and the expression of pro-neuroinflammatory miRNAs, anti-neuroinflammatory miRNAs, and their target mRNAs was analyzed by q-PCR.
Results: The relative expression of anti- neuroinflammatory miRNAs (*mir-21*, *mir-124*, and *mir-146a*) was decreased and the relative expression of their target mRNAs (*IL-12p53*, *Stat3*, and *TRAF6*) was increased. Also, the relative expression of pro-neuroinflammatory miRNAs (*mir-326*, *mir-155*, and *mir-27b*) was increased and the relative expression of their target mRNA (*PPARS*, *SOCS1*, and *CEBPA*) was decreased in COVID-19 patients with increase of disease grade. A negative significant correlation was seen between *mir-21* and *IL-12p53* mRNA, *mir-124* and *Stat3* mRNA, *mir-146a* and *TRAF6* mRNA, *mir-27b* and *PPARS* mRNA, *mir-155* and *SOCS1* mRNA, and between *mir-326* and *CEBPA* mRNA in COVID-19 patients ($P < 0.05$).
Conclusions: This study showed that the relative expression of anti- neuroinflammatory miRNAs was decreased and the relative expression of their targeted mRNAs was increased in COVID-19 patients from asymptomatic to critical illness. Also, this study showed that the relative expression of pro-neuroinflammatory miRNAs was increased and the relative expression of their targeted mRNA was decreased in COVID-19 patients from asymptomatic to critical illness.

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PALABRAS CLAVE
miARN;
COVID-19;
Pro-neuroinflamatorio;
Anti-neuroinflamatorio

Los biomarcadores neuroinflamatorios miARN en pacientes con COVID-19 con diferente gravedad de la enfermedad

Resumen
Introducción: La expresión de miARN específicos y sus dianas de ARNm se modifican en las enfermedades infecciosas. El objetivo de este estudio fue analizar la expresión de miARN pro-neuroinflamatorios, miARN anti-neuroinflamatorios y sus ARNm dianas en el suero de pacientes con COVID-19 de diferentes grados.
Métodos: Se incluyeron en este estudio pacientes con COVID-19 de diferentes grados y se analizó la expresión de miARN pro-neuroinflamatorios, miARN anti-neuroinflamatorios y sus ARNm diana mediante q-PCR.
Resultados: La expresión relativa de miARN anti-neuroinflamatorios (mir-21, mir-124 y mir-146a) disminuyó y la expresión relativa de sus ARNm diana (IL-12p53, Stat3 y TRAF6) aumentó. Además, la expresión relativa de miARN pro-neuroinflamatorios (mir-326, mir-155 y mir-27b) aumentó y la expresión relativa de su ARNm diana (PPARS, SOCS1 y CEBPA) disminuyó en pacientes con COVID-19 con aumento del grado de enfermedad. Se observó una correlación negativa significativa entre ARNm de mir-21 e IL-12p53, ARNm de mir-124 y Stat3, ARNm de mir-146a y TRAF6, ARNm de mir-27b y PPARS, ARNm de mir-155 y SOCS1, y entre mir-326 y ARNm de CEBPA en pacientes con COVID-19 ($p < 0,05$).
Conclusiones: Este estudio mostró que la expresión relativa de miARN anti-neuroinflamatorios disminuyó y la expresión relativa de sus ARNm diana se incrementó en pacientes con COVID-19 de enfermedad asintomática a crítica. Además, este estudio mostró que la expresión relativa de miARN pro-neuroinflamatorios aumentó y la expresión relativa de su ARNm diana disminuyó en pacientes con COVID-19 de enfermedad asintomática a crítica.

Introduction

Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), known as COVID-19, is a new infectious disease first seen in late December 2019 in Wuhan, China, and similar outbreaks occurred in the hospital in neighboring countries. Major clinical symptoms include fever, dry cough, diarrhea, muscle aches, pneumonia, and in severe cases death.[1,2] COVID-19 also is associated with neurological manifestations such as encephalopathy and encephalomyelitis, ischemic stroke and intracerebral hemorrhage, anosmia, neuromuscular diseases, and neuroinflammation diseases.[3]

Since COVID-19 is a new disease, complete information on its etiology, cellular mechanisms, and possible risk factors is not available. COVID-19 may be similar to recent acute respiratory syndromes, such as SARS and MERS.[4] Theoretically, after the SARS-CoV-2 enters the human body, different types of immune cells are stimulated. These cells trigger the proper immune response by producing different cytokines, chemokines, antibodies, etc. SARS-CoV-2 can infect the CNS following the entry of the virus into the nose or the eye. The viral particles are transmitted to the olfactory bulb and then to the brainstem, and then all parts of the brain.[5] In addition to the direct attack of nerve cells, the SARS-CoV-2 can systematically cross the BBB through the blood vessels and reach the CNS. The main feature of systemic infection in COVID-19 is the massive increase in pro-inflammatory factors in the blood, which is described as a ''cytokine stor''.[6] This leads to BBB permeability and transmission of SARS-CoV-2 and peripheral immune cells. Once the coronavirus enters the CNS, it is the turn of the astrocytes and microglia to fight it. The immune response of astrocytes and microglia is regulated by different microRNAs (miRNAs). Previous studies showed inflammatory processes in CNS are guided by pro-neuroinflammatory miRNAs (such as *mir-155*, *mir-27b*, *mir-326*) and anti- neuroinflammatory miRNAs (such as *mir-146a*, *mir-124*, and *mir-21*).[7,8]

This study aimed to analyze the expression of pro-neuroinflammatory miRNAs, anti-neuroinflammatory miRNAs, and their mRNA targets in the serum of COVID-19 patients with different grades.

Materials and methods

Materials

All primers were provided from Bioneer, South Korea. MirPremier microRNA isolation kit was sourced from Sigma-Aldrich, USA. Mir-X miRNA First-Strand Synthesis kit and cDNA matermix were purchased from Takara bio inc, USA. SYBR® Green Real-Time Master Mix was from Invitrogen, UK.

Bioinformatics

In this study, to determine the miRNAs associated with the COVID-19, we used online bioinformatics Softwares.[9] In the first step, mirTarP (https://mcube.nju.edu.cn/jwang/mirTar/docs/mirTar/) was used to the list of appropriate miRNAs.[10,11] In the second step, to reduce

Table 1 The characteristics of study groups.

	Study group 1	Study group 2	Study group 3	Study group 4	Study group 5	Control
Number (*n*)	21	20	20	21	21	20
Age distribution ± SD	50 ± 10	50 ± 10	50 ± 10	50 ± 10	50 ± 12	50 ± 12
Sex percentage ± SD	Female (52% ± 2%) Male (48% ± 1%)	Female (51% ± 2%) Male (49% ± 2%)	Female (50% ± 3%) Male (50% ± 1%)	Female (53% ± 1%) Male (47% ± 2%)	Female (52% ± 1%) Male (48% ± 2%)	Female (50% ± 3%) Male (50% ± 1%)
Severity of illness[a]	*Grade 5 Critical illness:* Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.	*Grade 4 Severe illness:* Individuals who have SpO_2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mm Hg, respiratory frequency > 30 breaths/min, or lung infiltrates > 50%.	*Grade 3 Moderate illness:* Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have saturation of oxygen (SpO_2) ≥94% on room air at sea level.	*Grade 2 Mild illness:* Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.	*Grade 1 Asymptomatic:* Individuals who test positive for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test or an antigen test) but who have no symptoms that are consistent with COVID-19.	*Healthy people*
Comorbidities	No	No	No	No	No	No
Inflammatory autoimmune diseases	No	No	No	No	No	No
Drug treatment	No	No	No	No	No	No

[a] The severity of COVID-19 was categorized according to NIH guidelines, https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum.

the number of selected miRNAs, we selected some limited pro-neuroinflammatory and anti-neuroinflammatory miRNAs that were previously reported in other studies. In the third step, the miRDB online database (http://mirdb.org/) was used to find the target of selected miRNAs.[12] Target genes of the differentially regulated miRNAs were predicted using the mirPath tool (version 3.0).[13] KEGG molecular pathways were also retrieved using the same tool.[14] Pathways and processes regulated with *P* values lower than 0.05 were considered significant.

Study groups

Table 1 shows the full characteristics of 6 study groups enrolled in this study. The licensing committee that approved the experiments, including any relevant details was Zahedan University of Medical Sciences, Zahedan, Iran. All experiments were under the guidelines of the National Institute of Health, and the ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran. (Ethical code: IR.ZAUMS.REC.1399.317). Also, informed consent was obtained from all participants. Five ml of whole blood was collected from each person and their serum was separated by centrifugation at 3000 rpm/min for 10 min at 4 °C. In this study, only COVID-19 patients with English variant of SARS-COV-2 (Lineage B.1.1.7; GISAID accession number: EPI-ISL-2227268) were included.

Small RNA isolation, first-strand cDNA synthesis, and quantification of miRNAs and mRNAs by qPCR

Here, Small RNA was isolated from blood samples using mir-Premier microRNA isolation kit. Briefly, 1000 μL of the lysis buffer was added to 100 μL of serum samples, vortexed for 2 min, and incubated at 55 °C for 5 min. The samples were then centrifuged for 5 min at 14,000 × *g* to remove cellular debris, genomic DNA, and large RNA. The lysate supernatant was filtered through the filtration column and binding column. After binding, the column was first washed with 700 μL of 100% ethanol and centrifuged at 14,000 × *g* for 30 s and again the flow-through was discarded. The second wash was done by adding 500 μl of binding solution into the column and centrifuged at maximum speed (14,000 × *g*) for 1 min. Subsequently, 500 ml of the ethanol-diluted wash

solution 2 was added to the column for a third wash. After centrifugation at maximum speed (14,000 × *g*) for 30 s, the flow-through was discarded. Next, the column was dried by centrifuging at maximum speed (14,000 × *g*) for 1 min. The column-tube assembly was carefully removed from the centrifuge to avoid splashing of the residual flow-through liquid to the dried column. Small RNA was eluted from the column using 50 ml elution solution and by centrifugation at 16,000 × *g* and the process was repeated to improve small RNA yield. The purity of the RNA samples was analyzed by NanoDrop ND-1000 UV-VIS spectrophotometer. The A260 nm/A280 nm ratio of all samples was between 1.8 and 2.1. The quantity of RNA samples was analyzed by agarose gel electrophoretic separation. For first-strand cDNA synthesis, small RNAs were polyadenylated and reverse transcribed using the Mir-X miRNA First-Strand Synthesis kit. Briefly, 5 μl mRQ buffer (2×), 5 μg RNA and 1.25 μl mRQ enzyme was mixed in a reaction volume of 10 μl and incubated in a thermocycler for 1 h at 37 °C, then terminate at 85 °C for 5 min to inactivate the enzymes. After reverse transcription, the cDNA was diluted. For quantification of miRNA by qPCR, Mir-X miRNA qPCR SYBR Kit was used. Briefly, 10 μl PCR reaction mixture was prepared to comprise of 1× SYBR advantage premix, 0.2 mM of both forward and reverse primers, and 50 ng of the first-strand cDNA. qPCR reactions were incubated in a 96 well plate at 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 20 s. Amplification cycles were followed by a melting curve analysis ranging from 56 to 95 °C. Finally, the threshold cycle (Ct) values were recorded. For mRNA, total RNA was extracted using an RNA extraction kit. Then, the cDNA was synthesized in the presence of the superscript enzyme and hexamers. For real-time PCR, 2 μL of cDNA, 2 μL of forward primer, and 2 μL of reverse primer of each gene were added to 10 μL of SYBR® Green Real-Time Master Mix. In this study, the relative expression of *mir-155*, *mir-27b*, *mir-326*, *mir-124*, *mir-146a*, *mir-21*, *IL-12p53*, *Stat3*, *TRAF6, PPARS*, *SOCS1*, and *CEBPA* was analyzed. The expression of microRNA and mRNA was normalized to *RNU 48* and *GAPDH*, respectively.

Table 2 The human gene targets of pro-neuroinflammatory miRNAs and anti- neuroinflammatory miRNAs, obtained from miRDB online database.

Target score	miRNA Name	Gene Symbol	Gene description
98	miR-155	SOCS1	Suppressor Of Cytokine Signaling 1
99	miR-155	ZNF629	Zinc finger protein 629
99	miR-155	CREBRF	CREB3 regulatory factor
99	miR-155	DENND1B	DENN domain containing 1B
98	miR-155	PTPN21	Protein tyrosine phosphatase, non-receptor type 21
98	miR-27b	PPARs	Peroxisome Proliferator Activated Receptor Gamma
97	miR-27b	AFF4	AF4/FMR2 family member 4
97	miR-27b	GXYLT1	Glucoside xylosyltransferase 1
97	miR-27b	ARFGEF1	ADP ribosylation factor guanine nucleotide exchange factor 1
96	miR-27b	GCC2	GRIP and coiled-coil domain containing 2
99	miR-326	CEBPA	CCAAT Enhancer Binding Protein Alpha
99	miR-326	ETS1	ETS proto-oncogene 1, transcription factor
99	miR-326	CEP85	Centrosomal protein 85
98	miR-326	FGF11	Fibroblast growth factor 11
98	miR-326	GPD2	Glycerol-3-phosphate dehydrogenase 2
98	miR-124	Stat3	Signal Transducer And Activator Of Transcription 3
98	miR-124	OSBPL3	Oxysterol binding protein like 3
98	miR-124	SLC50A1	Solute carrier family 50 member 1
98	miR-124	ITGB1	Integrin subunit beta 1
98	miR-124	SIX4	SIX homeobox 4
100	miR-146a	TRAF6	TNF Receptor Associated Factor 6
100	miR-146a	FOXC1	Forkhead box C1
100	miR-146a	CPLX2	Complexin 2
100	miR-146a	STXBP6	Syntaxin binding protein 6
100	miR-146a	ZFX	Zinc finger protein X-linked
99	miR-21	IL-12p53	Interleukin 12 p53 protein
99	miR-21	STK38L	Serine/threonine kinase 38 like
99	miR-21	PCDH19	Protocadherin 19
99	miR-21	LAMP1	Lysosomal associated membrane protein 1
99	miR-21	GRIA2	Glutamate ionotropic receptor AMPA type subunit 2

Table 3 Important pathways of pro-neuroinflammatory miRNAs and anti- neuroinflammatory miRNAs, extracted from KEGG molecular pathway.

KEGG pathway	*P*-value	Genes	miRNAs
Adherens junction	0.0001	30	6
Endometrial cancer	0.0001	24	6
Small cell lung cancer	0.0001	36	6
Regulation of actin cytoskeleton	0.0001	67	56
Bladder cancer	0.0002	21	4
PI3K-Akt signaling pathway	0.0002	105	5
Shigellosis	0.0002	26	4
Thyroid hormone signaling pathway	0.0003	44	4
Lysine degradation	0.001	15	3
Non-small cell lung cancer	0.001	23	4
mRNA surveillance pathway	0.001	34	4
mTOR signaling pathway	0.002	25	4
Oocyte meiosis	0.003	38	4
Prolactin signaling pathway	0.003	28	4
Melanoma	0.003	25	5
Ubiquitin mediated proteolysis	0.004	48	5
Fatty acid metabolism	0.004	11	3
Fatty acid elongation	0.004	6	3
Arrhythmogenic right ventricular	0.006	16	3
Estrogen signaling pathway	0.006	31	5
Signaling pathways regulating of stem cells	0.007	42	5
Gap junction	0.008	27	5
Sphingolipid signaling pathway	0.009	38	5
Amoebiasis	0.01	30	4
Pantothenate and CoA biosynthesis	0.01	6	3
MAPK signaling pathway	0.01	72	5
Insulin signaling pathway	0.02	45	5
Wnt signaling pathway	0.02	41	4
Axon guidance	0.02	37	4
Pathogenic Escherichia coli infection	0.02	21	5
AMPK signaling pathway	0.02	41	5
Hepatitis C	0.03	20	5
Vibrio cholerae infection	0.03	23	4
Epithelial cell signaling in Helicobacter pylori infection	0.04	54	4
Platelet activation	0.04	39	5
Steroid biosynthesis	0.04	5	3
Salmonella infection	0.04	27	5

Statistical analysis

All data were reported as the mean ± standard deviation. To find significant differences between groups, a one-way ANOVA method was applied. A *P*-value of less than 0.05 was considered statistically significant. Also, Spearman's correlation coefficient was used to correlate the expression of miRNAs and their mRNA targets.

Results

Bioinformatics analysis

Five-top human mRNA targets for pro-neuroinflammatory miRNAs (*mir-155*, *mir-27b*, and *mir-326*) and anti- neuroinflammatory miRNAs (*mir-124*, *mir-146a*, and *mir-21*) are shown in Table 2. It should be noted that each miRNA has many targets, but here we have listed only 5 important mRNA targets with the highest target score. Theoretically, all of them can be affected by pro-neuroinflammatory and anti-neuroinflammatory miRNAs.

Based on KEGG database (Table 3), we found that both pro-neuroinflammatory miRNAs and anti-neuroinflammatory miRNAs are significantly enriched in important cellular pathways, such as PI3K-Akt signaling pathway, mRNA surveillance pathway, mTOR signaling pathway, MAPK signaling pathway, Wnt signaling pathway, and AMPK signaling pathway.

The expression of anti-neuroinflammatory miRNAs and their mRNA targets

We found that the relative expression of anti-neuroinflammatory miRNAs, including *mir-21*, *mir-124*,

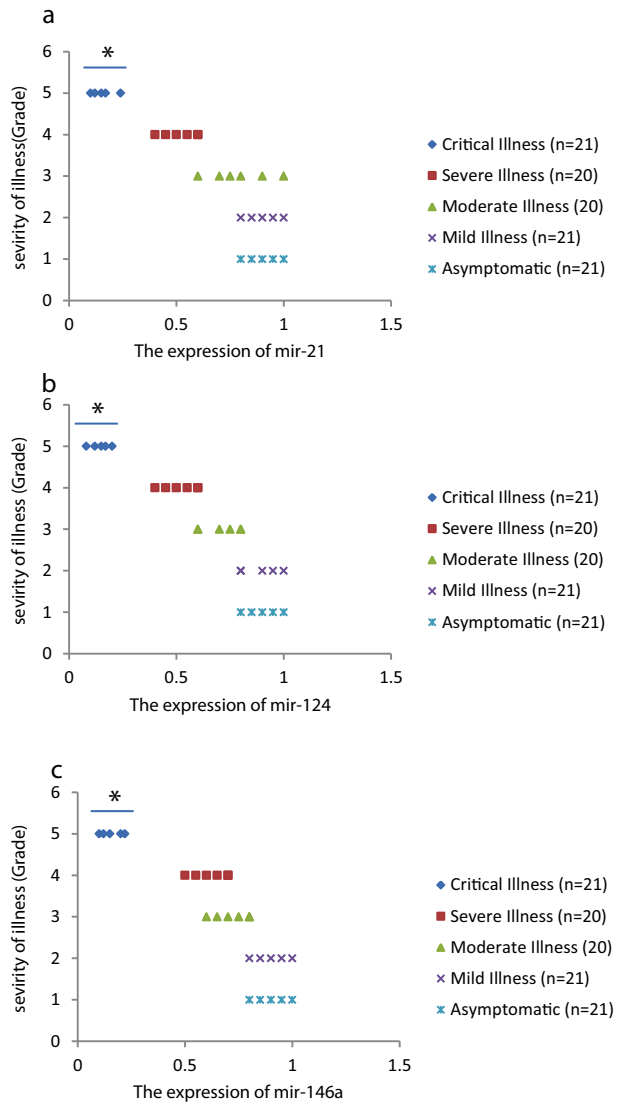

Figure 1 The relative expression of *mir-21* (a), *mir-124* (b), and *mir-146a* (c) in COVID-19 patients with different grades. *$P < 0.05$ compared with Mild Illness and Asymptomatic by one-way ANOVA.

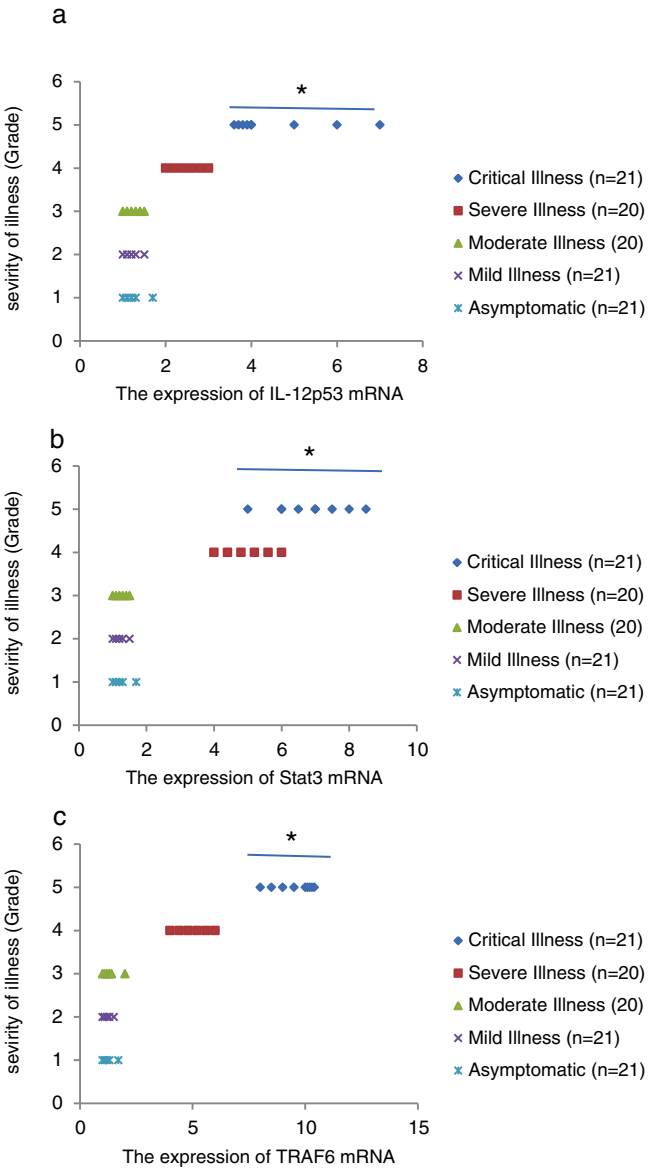

Figure 2 The relative expression of *IL-12p53* (a), *Stat3* (b), and *TRAF6* (c) mRNAs in COVID-19 patients with different grades. *$P < 0.05$ compared with Mild Illness and Asymptomatic by one-way ANOVA.

and *mir-146a*, was significantly decreased with increase of COVID-19 grade ($P < 0.05$) (Fig. 1(a–c)). Interestingly, the relative expression of human mRNA targets, including *IL-12p53*, *Stat3*, and *TRAF6*, of anti-neuroinflammatory miRNAs was significantly increased with increase of COVID-19 grade ($P < 0.05$) (Fig. 2(a–c)). A negative significant correlation was seen between the expression of (*mir-21* and *IL-12p53* mRNA), (*mir-124* and *Stat3* mRNA), and (*mir-146a* and *TRAF6* mRNA) in COVID-19 patients at all grades ($P < 0.05$) (Fig. 3(a–c)).

The expression of pro-neuroinflammatory miRNAs and their mRNA targets

The relative expression of pro-neuroinflammatory miRNAs, including *mir-326*, *mir-155*, and *mir-27b*, was significantly increased with increase of COVID-19 grade ($P < 0.05$) (Fig. 4(a–c)). Interestingly, the relative expression of human mRNA targets, including *PPARS*, *SOCS1*, and *CEBPA*, of pro-neuroinflammatory miRNAs was significantly decreased with increase of COVID-19 grade ($P < 0.05$) (Fig. 5(a–c)). A negative significant correlation was also seen between the expression of (*mir-27b* and *PPARS* mRNA), (*mir-155* and *SOCS1* mRNA), and (*mir-326* and *CEBPA* mRNA) in COVID-19 patients at all grades ($P < 0.05$) (Fig. 6(a–c)).

Discussion

This study showed that the relative expression of anti-neuroinflammatory miRNAs (*mir-21*, *mir-124*, and *mir-146a*)

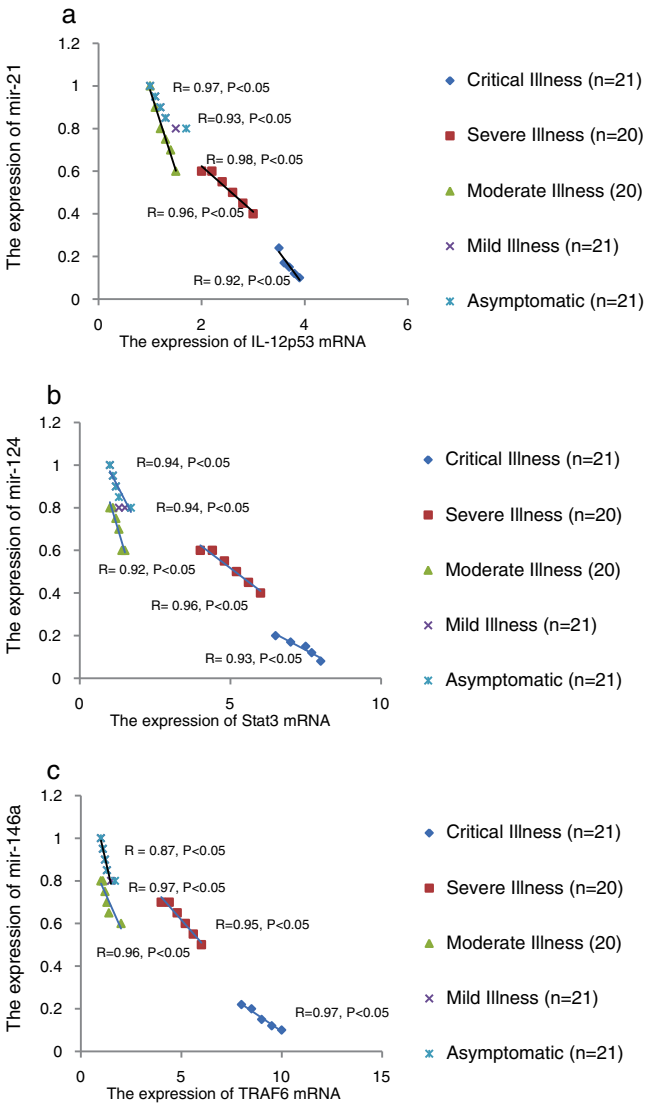

Figure 3 The correlation between the relative expression of *mir-21* and *IL-12p53* mRNA (a), *mir-124* and *Stat3* mRNA (b), and *mir-146a* and *TRAF6* mRNA (c) in COVID-19 patients with different grades. Spearman's correlation coefficient was used to correlate these parameters.

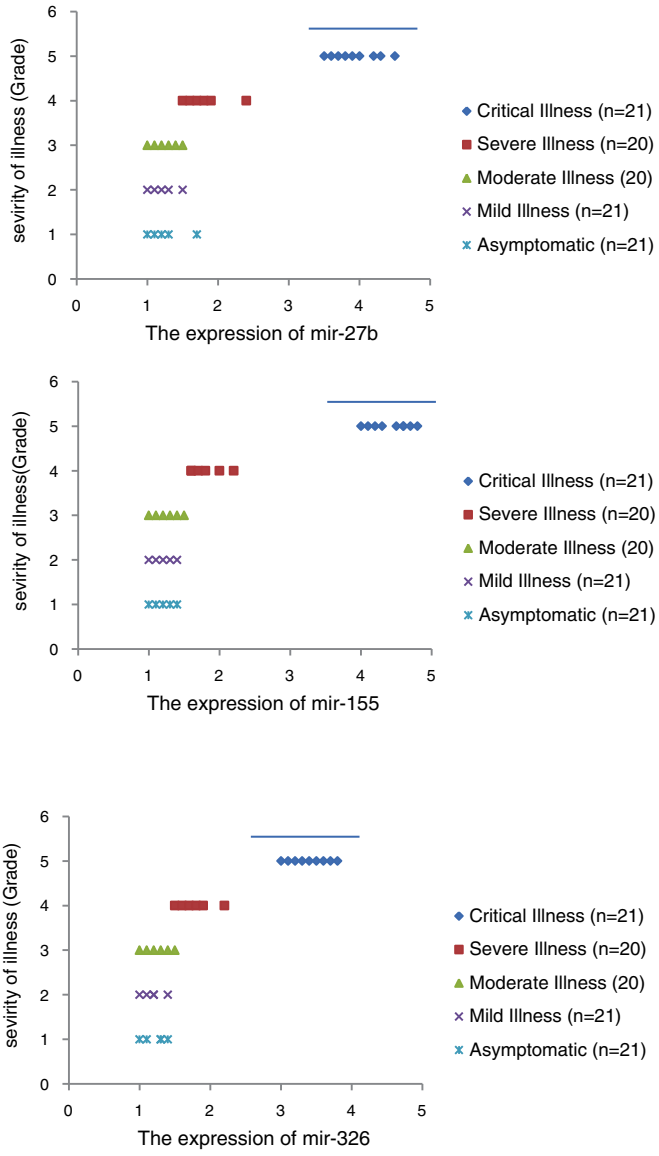

Figure 4 The relative expression of *mir-27b* (a), *mir-155* (b), and *mir-326* (c) in COVID-19 patients with different grades. **P* < 0.05 compared with Mild Illness and Asymptomatic by one-way ANOVA.

was decreased and the relative expression of their target mRNAs (*IL-12p53*, *Stat3*, and *TRAF6*) was increased in COVID-19 patients with increase of disease grade from asymptomatic to critical illness. Also, this study showed that the relative expression of pro-neuroinflammatory miRNAs (*mir-326*, *mir-155*, and *mir-27b*) was increased and the relative expression of their target mRNA (*PPARS*, *SOCS1*, and *CEBPA*) was decreased in COVID-19 patients with increase of disease grade. A negative significant correlation was seen between each miRNA and its target mRNA. Based on bioinformatics analysis, some important pathways are affected by these pro-neuroinflammatory and anti-neuroinflammatory miRNAs, including PI3K-Akt, mRNA surveillance, mTOR, MAPK, Wnt, and AMPK signaling pathways. What we have found is that in patients with high severity of illness, the expression of pro-inflammatory miRNAs is increased, and conversely, the expression of anti-inflammatory miRNAs is decreased. Of course, it is clear that this situation follows a cytokine storm. Unfortunately, we have to say that this special condition not only causes serious damage to the brain but also causes damage to several organs and leads to multiple organ failure. We think that when immune cells are highly stimulated, cytokines and miRNAs can travel through the bloodstream to the whole body. This phenomenon has been mentioned by some researchers.[15,16]

Mir-155 is a central pro-inflammatory mediator in CNS by NF-κB dependent TLR signaling. It is synthesized inside macrophages and microglia.[17—19] *mir-155* targets anti-inflammatory regulators such as *SOCS1*,[17,19] *SHIP1*,[20] *C/EBP-β*[21] and *IL13Rα1*.[22] *mir-155* inhibits the suppression of anti-inflammatory signaling and induces neuroinflammation. When *mir-155* is expressed, it stimulates the

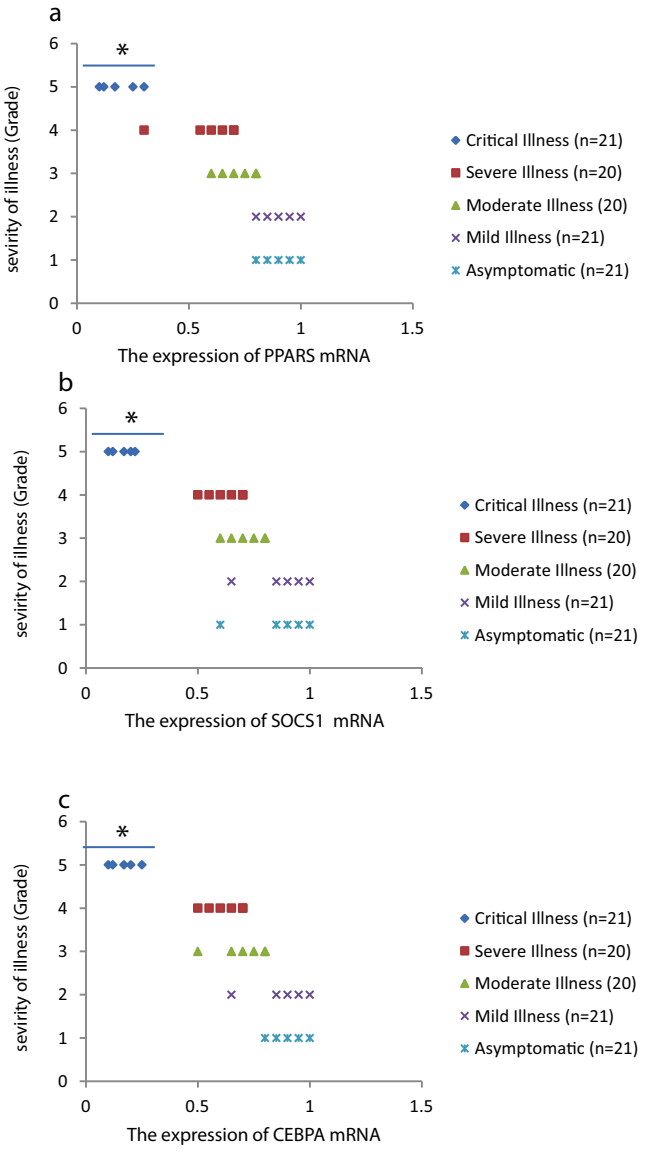

Figure 5 The relative expression of *PPARS* (a), *SOCS1* (b), and *CEBPA* (c) mRNAs in COVID-19 patients with different grades. *$P < 0.05$ compared with Mild Illness and Asymptomatic by one-way ANOVA.

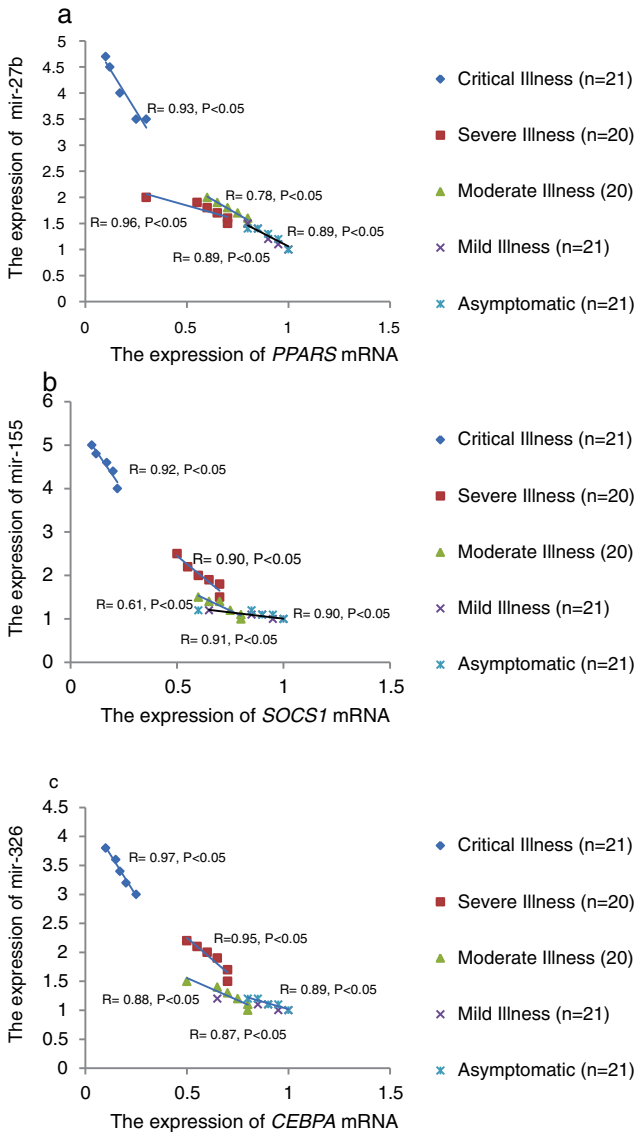

Figure 6 The correlation between the relative expression of *mir-27b* and *PPARS* mRNAs (a), *mir-155* and *SOCS1* mRNAs (b), and *mir-326* and *CEBPA* mRNA (c) in COVID-19 patients with different grades. Spearman's correlation coefficient was used to correlate these parameters.

transcription factor p53, and it targets the c-Maf transcription factor, which induces differentiation and inflammatory responses.[23] *Mir-146a* is an anti-inflammatory regulator in nerve cells, microglia, and astrocytes. It activates by NF-κB dependent TLR signaling.[24,25] The *Mir-146a* targets MyD88 signaling complex, including IRAK1 and *TRAF6*, and acts as an NF-κB signaling regulator. In addition, *Mir-146a* targets other pro-inflammatory mediators including *STAT*-1,[26,27] *IRF-5*[27] and *CFH*.[28,29] The polarization of macrophages and microglia are also altered by *mir-146a*.[30] *mir-124* is also an anti-inflammatory miRNA and has a major role in neuronal differentiation[31] and is highly expressed in microglia under normal conditions, but is not expressed in peripheral macrophages.[32] Expression of *mir-124* in microglia leads to anti-inflammatory effects[33] by M2 phenotype.[34] It is clear that *mir-124* has anti-inflammatory activity by reducing inflammatory mediators and limiting microglia to activity. The role of *mir-21* is very prominent in different types of CNS cells such as microglia[35] and astrocytes,[36] neurons,[37] and oligodendrocytes.[38] *Mir-21* is an anti-inflammatory regulator activated by TLR signaling. This induces the expression of the anti-inflammatory cytokine such as IL-10.[39] In addition, *mir-21* decreases TNF-α secretion in macrophages and microglia.[40] *mir-27b* targets an anti-inflammatory transcriptional activator, *PPAR-γ*; in human macrophages, this interaction blocks the induction of an anti-inflammatory phenotype. Inhibiting *mir-27b* also limits its inflammatory signaling. It leads to produce inflammatory cytokines including IL-6 and TNF-α.[41] *mir-326* is another pro-

inflammatory miRNAs and can affect on differentiation of IL-17-producing Th17 cells. It was found that silencing *mir-326* reduced EAE pathology.[42] miRNAs have a cumulative effect on neuronal signaling and act together in inflammatory or anti-inflammatory pathways. For example, both *mir-146a* and *mir-21* target different components of the TLR/MyD88/NF-κB and JAK-STAT pathways.[26,28] In contrast, *mir-155*, *mir-27b*, and *mir-326* activate the JAK-STAT pathway by targeting *SOCS1* and *SHIP1*.[19] It is interesting to note that miRNAs are also present in extracellular exosomes and can participate in intercellular communication.[43] For example, *mir-124*, *mir-21*, and let-7 are found in exosomes and stimulate and regulate adjacent cells such as microglia and contribute to inflammatory signaling.[44]

One of main limitations of this study was to find and to collect COVID-19 patients with no comorbidities, no inflammatory autoimmune diseases, and no drug treatments. Theoretically, these factors can affect the expression of mRNAs and miRNAs. Second limitation was that we did not include COVID-19 patients caused by different variants of SARS-COV-2. Here, only COVID-19 patients with English variant (Lineage B.1.1.7) were included. We think that the expression of mRNAs and miRNAs may also be affected by virus variants. The third limitation was that we evaluated only 6 neuroinflammatory miRNAs in COVID-19 patients and it is suggested that other neuroinflammatory miRNAs could be studied in future studies.

Conclusions

This study showed that the relative expression of anti-neuroinflammatory miRNAs (*mir-21*, *mir-124*, and *mir-146a*) was decreased and the relative expression of their mRNAs (*IL-12p53*, *Stat3*, and *TRAF6*) was increased in COVID-19 patients from asymptomatic to critical illness. Also, this study showed that the relative expression of pro-neuroinflammatory miRNAs (*mir-326*, *mir-155*, and *mir-27b*) was increased and the relative expression of their mRNA (*PPARS*, *SOCS1*, and *CEBPA*) was decreased in COVID-19 patients from asymptomatic to critical illness. A negative significant correlation was seen between *mir-21* and *IL-12p53* mRNA, *mir-124* and *Stat3*, between *mir-146a* and *TRAF6*, between *mir-27b* and *PPARS*, between *mir-155* and *SOCS1*, and between *mir-326* and *CEBPA* mRNA in COVID-19 patients ($P < 0.05$).

Authors' contributions

(I) Conception and design: R.K. and A.J., (II) Administrative support: R.K. and A.J., (III) Provision of study materials or patients: R.K., (IV) Collection and assembly of data: A.J., (V) Data analysis and interpretation: R.K. and A.J., (VI) Manuscript writing: All authors, (VII) Final approval of manuscript: All authors.

Ethics approval and consent to participate

''The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.'' All experiments were under the guidelines of the National Institute of Health, the provisions of the Declaration of Helsinki,and the ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran. (Ethical code: IR.ZAUMS.REC.1399.317).

Consent for publication

Not.

Availability of data and material

Not.

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Conflict of interest

There is no conflict of interest.

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References

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020:102433.
2. Zu ZY, Jiang MD, Xu PP, Chen W, Ni QQ, Lu GM, et al. Coronavirus disease 2019 (COVID-19): a perspective from China. Radiology. 2020;200490.
3. Berlit P, Bösel J, Gahn G, Isenmann S, Meuth SG, Nolte CH, et al. ''Neurological manifestations of COVID-19''-guideline of the German society of neurology. Neurol Res Pract. 2020;2: 1—14.
4. Peeri NC, Shrestha N, Rahman MS, Zaki R, Tan Z, Bibi S, et al. The SARS MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned? Int J Epidemiol. 2020.
5. Li K, Wohlford-Lenane C, Perlman S, Zhao J, Jewell AK, Reznikov LR, et al. Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human dipeptidyl peptidase 4. J Infect Dis. 2016;213: 712—22.

6. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 2020;53:25—32.
7. Gaudet AD, Fonken LK, Watkins LR, Nelson RJ, Popovich PG. MicroRNAs: roles in regulating neuroinflammation. Neuroscientist. 2018;24:221—45.
8. Slota JA, Booth SA. MicroRNAs in neuroinflammation: implications in disease pathogenesis, biomarker discovery and therapeutic applications. Non-coding RNA. 2019;5:35.
9. Ekimler S, Sahin K. Computational methods for microRNA target prediction. Genes. 2014;5:671—83.
10. Shu X, Zang X, Liu X, Yang J, Wang J. Predicting microRNA mediated gene regulation between human and viruses. Cells. 2018;7:100.
11. Sardar R, Satish D, Gupta D. Identification of novel SARS-CoV-2 drug targets by host microRNAs and transcription factors co-regulatory interaction network analysis. Front Genet. 2020;11:1105.
12. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res. 2015;43:D146—52.
13. Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, et al. DIANA-miRPath v3.0 deciphering microRNA function with experimental support. Nucleic Acids Res. 2015;43:W460—6.
14. Kanehisa M, Goto S, Kawashima S, Nakaya A. The KEGG databases at GenomeNet. Nucleic Acids Res. 2002;30:42—6.
15. Latimer G, Corriveau C, DeBiasi RL, Jantausch B, Delaney M, Jacquot C, et al. Cardiac dysfunction and thrombocytopenia-associated multiple organ failure inflammation phenotype in a severe paediatric case of COVID-19. Lancet Child Adolescent Health. 2020;4:552—4.
16. Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, et al. Comorbidities and multi-organ injuries in the treatment of COVID-19. Lancet. 2020;395:e52.
17. Wang P, Hou J, Lin L, Wang C, Liu X, Li D, et al. Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1. J Immunol. 2010;185:6226—33.
18. Bala S, Marcos M, Kodys K, Csak T, Catalano D, Mandrekar P, et al. Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor α (TNFα) production via increased mRNA half-life in alcoholic liver disease. J Biol Chem. 2011;286:1436—44.
19. Cardoso AL, Guedes JR, Pereira de Almeida L, Pedroso de Lima MC. miR-155 modulates microglia-mediated immune response by down-regulating SOCS-1 and promoting cytokine and nitric oxide production. Immunology. 2012;135:73—88.
20. O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci USA. 2009;106:7113—8.
21. Worm J, Stenvang J, Petri A, Frederiksen KS, Obad S, Elmen J, et al. Silencing of microRNA-155 in mice during acute inflammatory response leads to derepression of c/ebp Beta and down-regulation of G-CSF. Nucleic Acids Res. 2009;37:5784—92.
22. Martinez-Nunez RT, Louafi F, Sanchez-Elsner T. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor α1 (IL13Rα1). J Biol Chem. 2011;286:1786—94.
23. Su W, Hopkins S, Nesser NK, Sopher B, Silvestroni A, Ammanuel S, et al. The p53 transcription factor modulates microglia behavior through microRNA-dependent regulation of c-Maf. J Immunol. 2014;192:358—66.
24. Taganov KD, Boldin MP, Chang K-J, Baltimore D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA. 2006;103:12481—6.
25. Cui JG, Li YY, Zhao Y, Bhattacharjee S, Lukiw WJ. Differential regulation of interleukin-1 receptor-associated kinase-1 (IRAK-1) and IRAK-2 by microRNA-146a and NF-κB in stressed human astroglial cells and in Alzheimer disease. J Biol Chem. 2010;285:38951—60.
26. He X, Tang R, Sun Y, Wang Y-G, Zhen K-Y, Zhang D-M., et al. MicroR-146 blocks the activation of M1 macrophage by targeting signal transducer and activator of transcription 1 in hepatic schistosomiasis. EBioMedicine. 2016;13:339—47.
27. Tang Y, Luo X, Cui H, Ni X, Yuan M, Guo Y, et al. MicroRNA-146a contributes to abnormal activation of the type I interferon pathway in human lupus by targeting the key signaling proteins. Arthritis Rheumat. 2009;60:1065—75.
28. Li YY, Cui JG, Dua P, Pogue AI, Bhattacharjee S, Lukiw WJ. Differential expression of miRNA-146a-regulated inflammatory genes in human primary neural, astroglial and microglial cells. Neurosci Lett. 2011;499:109—13.
29. Lukiw WJ, Zhao Y, Cui JG. An NF-κB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. J Biol Chem. 2008;283:31315—22.
30. Huang C, Liu X-j, Xie J, Ma T-t, Meng X-m, Li J. MiR-146a modulates macrophage polarization by inhibiting Notch1 pathway in RAW264 7 macrophages. Int Immunopharmacol. 2016;32:46—54.
31. Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell. 2007;27:435—48.
32. Ponomarev ED, Veremeyko T, Barteneva N, Krichevsky AM, Weiner HL. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU. 1 pathway. Nat Med. 2011;17:64—70.
33. Ma C, Li Y, Li M, Deng G, Wu X, Zeng J, et al. microRNA-124 negatively regulates TLR signaling in alveolar macrophages in response to mycobacterial infection. Mol Immunol. 2014;62:150—8.
34. Veremeyko T, Siddiqui S, Sotnikov I, Yung A, Ponomarev ED. IL-4/IL-13-dependent and independent expression of miR-124 and its contribution to M2 phenotype of monocytic cells in normal conditions and during allergic inflammation. PLoS ONE. 2013;8:e81774.
35. Zhang L, Dong LY, Li YJ, Hong Z, Wei WS. miR-21 represses FasL in microglia and protects against microglia-mediated neuronal cell death following hypoxia/ischemia. Glia. 2012;60:1888—95.
36. Li H-J, Pan Y-B, Sun Z-L, Sun Y-Y, Yang X-T, Feng D-F. Inhibition of miR-21 ameliorates excessive astrocyte activation and promotes axon regeneration following optic nerve crush. Neuropharmacology. 2018;137:33—49.
37. Han Z, Chen F, Ge X, Tan J, Lei P, Zhang J. miR-21 alleviated apoptosis of cortical neurons through promoting PTEN-Akt signaling pathway in vitro after experimental traumatic brain injury. Brain Res. 2014;1582:12—20.
38. Miguel-Hidalgo JJ, Hall KO, Bonner H, Roller AM, Syed M, Park CJ, et al. MicroRNA-21: expression in oligodendrocytes and correlation with low myelin mRNAs in depression and alcoholism. Prog Neuropsychopharmacol Biol Psychiatry. 2017;79:503—14.
39. Sheedy FJ, Palsson-McDermott E, Hennessy EJ, Martin C, O'leary JJ, Ruan Q, et al. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol. 2010;11:141—7.
40. Barnett RE, Conklin DJ, Ryan L, Keskey RC, Ramjee V, Sepulveda EA, et al. Anti-inflammatory effects of miR-21 in the macrophage response to peritonitis. J Leukoc Biol. 2016;99:361—71.
41. Jennewein C, von Knethen A, Schmid T, Brüne B. MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor γ (PPARγ) mRNA destabilization. J Biol Chem. 2010;285:11846—53.

42. Du C, Liu C, Kang J, Zhao G, Ye Z, Huang S, et al. MicroRNA miR-326 regulates T H-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol. 2009;10:1252.
43. Bayraktar R, Van Roosbroeck K, Calin G.A. Cell-to-cell communication: microRNAs as hormones. Mol Oncol. 2017;11:1673—86.
44. Pinto S, Cunha C, Barbosa M, Vaz AR, Brites D. Exosomes from NSC-34 cells transfected with hSOD1-G93A are enriched in miR-124 and drive alterations in microglia phenotype. Front Neurosci. 2017;11:273.