# Identification of MicroRNA-mRNA Regulatory Networks in Periodontitis by Bioinformatics Analysis

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# Abstract

**Background**: Periodontitis is a complex infectious disease with various causes and contributing factors. In recent years, microRNAs (miRNAs) have been commonly accepted as having key regulatory functions in periodontal disease. The aim of this study was to identify miRNAs and hub genes involved in periodontal disease pathogenesis using a miRNA-mRNA interaction network.

**Methods:** The GSE54710 miRNA microarray dataset and the gene expression microarray dataset GSE16134 were downloaded from the Gene Expression Omnibus database. The differentially expressed miRNAs (DEMis) and mRNAs (DEMs) were screened using $P < 0.05$ and $|\log FC| \geq 1$. Potential upstream transcription factors and downstream target genes of candidate DEMis were predicted using the FunRich and miRNet programs, respectively. Subsequently, DEMs were uploaded to the STRING database, a protein-protein interaction (PPI) network was established, and the cytoHubba plugin was used to screen out key hub mRNAs. The key genes in the miRNA-mRNA regulatory network were extracted by intersecting the target genes of candidate DEMis and DEMs. Cytoscape software was used to visualise the interaction between miRNAs and mRNAs and to predict the hub genes. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were used to analyse the key genes in the regulatory network.

**Results:** Ten DEMis and 161 DEMs were filtered out, from which we constructed a miRNA-mRNA network consisting of six miRNAs and 32 mRNAs. KEGG pathway analysis showed that mRNAs in the regulatory network were mainly involved in the IL-17 signalling pathway. Hsa-miR-203/CXCL8, hsa-miR-203/BTG2, and hsa-miR-203/DNAJB9 were identified as four potential regulatory pathways for periodontitis.

**Conclusion:** In this study, a potential miRNA–mRNA regulatory network was first constructed and four regulatory pathways were identified for periodontitis to help clarify the aetiology of the disease and provide potential therapeutic targets.


# Background

Periodontitis is a widespread, infectious, human chronic inflammatory disease characterised by gum bleeding, attachment loss, bone resorption, and eventual tooth loss. The amount of tissue destruction is generally commensurate with dental plaque levels, host defence, and related risk factors [1]. Periodontal disease is highly prevalent worldwide, with serious periodontitis affecting 10.8% of people worldwide [2]. The prevalence of periodontitis increases gradually with age [3], particularly in adults over 50 years of age [4]. Usually, periodontal disease is a silent and subclinical disease; however, it can negatively affect eating habits, facial aesthetics, and speaking [5]. However, the aetiology of periodontitis remains unclear; therefore, investigating the pathogenesis of periodontitis is extremely important for treatment and prevention of the disease.

MicroRNAs (miRNAs) are endogenous, non-coding, small RNAs containing approximately 22 nucleotides [6]. MiRNAs regulate target genes at transcriptional or translational levels based on the sequence

complementarity between miRNA and the 3'untranslated region of target genes [7]. In recent years, miRNAs have gained increased attention from researchers for periodontal disease studies. For example, miR-146a is highly expressed in the serum of patients with chronic periodontitis and its expression is directly proportional to disease severity [8]. Zhou et al. suggested that miR-21 downregulates Porphyromonas gingivalis lipopolysaccharide (LPS) -induced inflammation, and that miR-21 plays a protective role in periodontitis progression [9]. Akkouch et al. suggested that local treatment with miR-200c was effective for alveolar bone resorption in a rat model of periodontitis [10]. However, few studies have been conducted to clarify the role of the miRNA-mRNA regulatory network in periodontal disease.

The aim of the present study was to identify key genes and miRNAs involved in periodontitis and to construct miRNA-mRNA regulatory networks using bioinformatics methods. Compared with traditional biomarker research, this study focuses on the function of a single molecule and its relationship with the whole. Our study may be helpful in exploring the underlying molecular mechanisms of periodontitis.

## Methods

# Data download and screening strategy

We used “periodontitis” as the keyword for our search in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). We obtained one miRNA microarray dataset (GSE54710) containing 200 gingival tissue samples from 86 patients with periodontitis and one mRNA microarray dataset (GSE16134) containing 310 gingival tissue samples from 120 patients with periodontitis. The former dataset was based on GPL-15159, and the latter was based on GPL570-55599. All RNA information for the selected samples was downloaded for further analysis.

The original expression matrix was normalised and processed using the R (4.0.4) software. The “limma” package was used to screen differentially expressed miRNAs (DEMis) and mRNAs (DEMs) in periodontitis cases and healthy controls. The P-values for the gene expression was calculated using the t-test, and Benjamini and Hochberg's method was used to calculate the adjusted P-value to reduce the false positive rate. The DEMis and DEMs were screened for those with $P < 0.05$ and $|\log FC| \geq 1$, and these were visualised using volcano maps by the plot in R.

# Prediction of potential transcription factors and GO enrichment analysis

To predict the transcription factors of the included DEMis, they were uploaded to FunRich (3.1.3), which is used mainly for functional enrichment and interaction network analysis for genes and proteins [11]; it identifies the top 10 predicted transcription factors. Gene Ontology (GO) enrichment analysis included three categories: biological process (BP), cellular component (CC), and molecular function (MF), which were also applied to FunRich for prediction of potential transcription factors [12].

# Protein-protein interaction network construction and analysis

To gain insight into the interactions of the proteins, a protein-protein interaction (PPI) network was constructed. The DEMs were uploaded to the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/), a database covering 9,643,763 proteins from 2,031 organisms. Results with a score of > 0.4 were imported into Cytoscape v.3.8.2 [13, 14]. The CytoHubba plugin was then used to identify the ten key hub mRNAs according to degree, and the Molecular Complex Detection (MCODE) plugin was used to filter out significant modules with a degree cut-off of 2, node score cut-off of 0.2, k-Core of 2, and maximum depth of 100. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of genes in the first gene cluster was performed using the “enrichplot” and “ggplot2” packages in R [15].

# Construction of the miRNA-mRNA regulatory network

A combined analysis of DEMs and target genes of DEMis was conducted by drawing Venn diagrams. Overlapping genes were considered key genes in the regulatory network. Subsequently, the miRNA-mRNA network was constructed by loading all the DEMi-DEM pairs into the Cytoscape software, which was used to visualise all the pairs at once. The hub nodes were simultaneously screened using the CytoHubba plugin.

# GO and KEGG enrichment analyses for mRNAs in the network

GO and KEGG enrichment analyses for the mRNAs were performed using the “enrichplot” and “ggplot2” packages in R. Statistical significance was set at $P < 0.05$.

# Expression analysis of hub genes by the GSE10334 dataset

The GSE10334 dataset, downloaded from the GEO database, was used to analyse the expression of hub genes. The dataset was based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array), in which 183 and 64 diseased and healthy samples were selected, respectively. Student’s *t*-test was used to identify the differentially expressed genes (DEGs) in periodontitis samples and healthy controls. Only those DEGs with $P < 0.05$ were considered.

## Results

# DEMi screening in periodontitis

GSE54710 is an miRNA expression profile dataset that was downloaded from the NCBI GEO database. It contains 41 healthy gingival tissue samples and 159 tissue samples of periodontitis. Ten DEMis were obtained after the two groups of samples were screened based on the following criteria: $P < 0.05$, and $|\log FC| \geq 1$. Four upregulated miRNAs and six downregulated miRNAs were included these DEMis were

subsequently assessed. All ten DEMis are presented in Table 1. A volcano map was drawn to show the distribution of differential miRNA expression between periodontitis and healthy controls. (Fig. 1)

Table 1
Key DEMis accessed from GSE54710

| miRNA_ID | Log FC | P Value | Adjusted P value |
|---|---|---|---|
| hsa-miR-3917 | 1.085309938 | < 0.001 | < 0.001 |
| hsa-miR-1246 | -1.390929316 | < 0.001 | < 0.001 |
| hsa-miR-486-5p | 1.30391312 | < 0.001 | < 0.001 |
| hsa-miR-1260 | -1.04054174 | < 0.001 | < 0.001 |
| hsa-miR-483-5p | 1.043113657 | < 0.001 | < 0.001 |
| hsa-miR-671-5p | 1.193440192 | < 0.001 | < 0.001 |
| hsa-miR-451 | 1.281609232 | < 0.001 | < 0.001 |
| hsa-miR-223 | 1.238010714 | < 0.001 | < 0.001 |
| hsa-miR-203 | -1.061388711 | < 0.001 | < 0.001 |
| hcmv-miR-UL70-3p | 1.123636686 | < 0.001 | < 0.001 |

# Transcription Factor Enrichment and GO Enrichment Analyses

The DEMis were uploaded to the FunRich software (3.1.3), and we filtered out the following top 10 transcription factors significantly associated with miRNAs: RORA, DBX2, HOXD8, CDX2, EGR1, HOXA9, HMX1, HOXB7, HOXB9, and POU2F1 (Fig. 2). The regulatory relationship of these transcription factors with DEMis was indicated when the enrichment of the targets of the transcription factors was examined.

The significantly enriched BPs were signal transduction, cell communication, regulation of gene expression, epigenetic processes, and cell-cell adhesion (Fig. 3a). Further, the cytoplasm, perinuclear region, Golgi membrane, and activin responsive factor complex accounted for the majority of the enriched CCs (Fig. 3b). Functions in protein serine/threonine kinase activity, DNA binding, cell adhesion molecule activity, and receptor signalling complex scaffold activity were the most significantly enriched MFs (Fig. 3(c)).

# DEM screening in periodontitis

We intended to identify the DEMs in periodontal and healthy samples using the NCBI GEO database. The GSE16134 dataset is an mRNA expression profile dataset containing 41 healthy gingival tissue samples and 159 tissue samples of periodontitis. Those DEMs with $P < 0.05$ and $|\log FC| \geq 1$ were identified.

Consequently, 129 upregulated and 32 downregulated DEMs were identified. The top 20 upregulated and downregulated genes are listed in Table 2.

Table 2
Top 20 downregulated and upregulated differentially expressed mRNAs.

| Gene_ID | logFC | P Value | Adjusted P value | Gene_ID | logFC | P Value | Adjusted P value |
|---|---|---|---|---|---|---|---|
| Top 20 downregulated mRNAs | | | | Top 20 upregulated mRNAs | | | |
| DSC1 | -2.212 | < 0.001 | < 0.001 | MZB1 | 2.492 | < 0.001 | < 0.001 |
| FLG2 | -1.744 | < 0.001 | < 0.001 | TNFRSF17 | 2.461 | < 0.001 | < 0.001 |
| KRT2 | -1.704 | < 0.001 | < 0.001 | IGLL5 | 2.437 | < 0.001 | < 0.001 |
| LOR | -1.371 | < 0.001 | < 0.001 | IGK | 2.312 | < 0.001 | < 0.001 |
| BPIFC | -1.324 | < 0.001 | < 0.001 | SPAG4 | 2.204 | < 0.001 | < 0.001 |
| ELOVL4 | -1.305 | < 0.001 | < 0.001 | IGHM | 2.194 | < 0.001 | < 0.001 |
| CLDN20 | -1.299 | < 0.001 | < 0.001 | LOC100293211 | 2.156 | < 0.001 | < 0.001 |
| CALML5 | -1.298 | < 0.001 | < 0.001 | FAM46C | 2.088 | < 0.001 | < 0.001 |
| FLG | -1.297 | < 0.001 | < 0.001 | CXCL6 | 2.034 | < 0.001 | < 0.001 |
| NEFL | -1.265 | < 0.001 | < 0.001 | CD79A | 2.033 | < 0.001 | < 0.001 |
| AADAC | -1.260 | < 0.001 | < 0.001 | CXCL1 | 1.897 | < 0.001 | < 0.001 |
| SLC27A6 | -1.253 | < 0.001 | < 0.001 | IGLV1-44 | 1.896 | < 0.001 | < 0.001 |
| RORA | -1.248 | < 0.001 | < 0.001 | CD27 | 1.869 | < 0.001 | < 0.001 |
| ATP6V1C2 | -1.191 | < 0.001 | < 0.001 | SLAMF7 | 1.864 | < 0.001 | < 0.001 |
| ABCA12 | -1.164 | < 0.001 | < 0.001 | CXCR4 | 1.809 | < 0.001 | < 0.001 |
| LCE2B | -1.151 | < 0.001 | < 0.001 | LAX1 | 1.744 | < 0.001 | < 0.001 |
| NPR3 | -1.139 | < 0.001 | < 0.001 | CHST2 | 1.738 | < 0.001 | < 0.001 |

| Gene_ID | logFC | P Value | Adjusted P value | Gene_ID | logFC | P Value | Adjusted P value |
|---|---|---|---|---|---|---|---|
| MAMDC2 | -1.137 | < 0.001 | < 0.001 | LOC101929272 | 1.712 | < 0.001 | < 0.001 |
| SLC16A9 | -1.111 | < 0.001 | < 0.001 | CYTIP | 1.700 | < 0.001 | < 0.001 |
| EPCAM | -1.100 | < 0.001 | < 0.001 | CPNE5 | 1.683 | < 0.001 | < 0.001 |

# PPI Network Construction and Analysis of Modules

Subsequently, we mapped the DEMs identified based on the screening criteria into the STRING database and constructed a PPI network of these genes, with 117 nodes and 490 edges. Hub proteins are those proteins in a network that are highly connected, and are the master keys of regulation. To explore the hub genes in the PPI network, the node pairs were input into the Cytoscape software and analysed using the CytoHubba plugin. The top 10 hub genes were *CXCR4, CXCL8, CXCL1, IL1B, MMP3, MMP7, CTGF, MMP1, MMP13*, and *CXCL12* (Fig. 4a). MCODE was used to process the data downloaded from STRING to further mine gene clusters, and eight cluster modules were obtained. The first gene cluster with the highest scores contained five hub genes (Fig. 4b). KEGG enrichment analysis revealed that the genes in this gene cluster were mainly involved in viral protein interactions with cytokines and cytokine receptors, cytokine-cytokine receptor interactions, and chemokine signalling pathways (Table 3). These pathways play an important role in the inflammatory response.

Table 3
Top 5 KEGG pathway enriched in module 1

| ID | Description | Count |
|---|---|---|
| hsa04061 | Viral protein interaction with cytokine and cytokine receptor | 5 |
| hsa04060 | Cytokine-cytokine receptor interaction | 6 |
| hsa04062 | Chemokine signaling pathway | 5 |
| hsa05323 | Rheumatoid arthritis | 4 |
| hsa04064 | NF-kappa B signaling pathway | 4 |

# Prediction of target genes of DEMis and reconstruction of the miRNA-mRNA network

miRNet 2.0, which is an online tool based on three bioinformatic algorithms (miRTarbase v8.0, TarBase v8.0, miRecords), was utilised to predict the potential target genes of aberrant miRNAs. A total of 5632 target genes were identified. The target genes predicted by the miRNA molecules and DEMs in the mRNA

expression profile datasets were analysed for intersection to screen out reliable target genes; 32 overlaps were identified (Fig. 5). Subsequently, an miRNA-mRNA regulatory network of 32 mRNAs and 6 miRNAs was constructed to further demonstrate the interaction between DEMis and DEMs (Fig. 6). Understanding of this interaction would help clarify the role of miRNAs in periodontitis. The following ten nodes with the highest degrees were identified using the cytoHubba plugin in Cytoscape: hsa-miR-203, hsa-miR-671-5p, hsa-miR-223, hsa-miR-486-5p, hsa-miR-1246, *ENTPD1, CXCL8, BTG2, SEL1L3*, and *DNAJB9*. (Fig. 7). These nodes were screened for a negative regulatory relationship between the miRNA and mRNA. In a negative regulatory relationship, upregulated target genes for DEMis are downregulated, or downregulated target genes for DEMis are upregulated. Consequently, hsa-miR-203/CXCL8, hsa-miR-203/BTG2, hsa-miR-203/SEL1L3, and hsa-miR-203/DNAJB9 were identified as four potential regulatory pathways in periodontitis.

# Functional and pathway enrichment analysis of mRNAs in the regulatory network

To further elucidate the function of mRNAs in the regulatory network, we used the “enrichplot” package in R to perform GO and KEGG analyses. GO analysis of the mRNAs in the regulatory network showed that the following BP terms were most significantly enriched in response to molecules of bacterial origin: cellular response to molecules of bacterial origin, cellular response to biotic stimulus, response to lipopolysaccharide, and humoral immune response. The most enriched MF terms included cytokine activity, cytokine receptor binding, *CXCR*-chemokine receptor binding, G-protein alpha-subunit binding, and misfolded protein binding. No CC term was observed to be significantly enriched (Fig. 8a). The relationships between the mRNAs and enriched pathways are shown in Fig. 8b. KEGG pathway analysis showed that mRNAs in the regulatory network were mainly involved in the IL-17 signalling pathway, coronavirus disease-2019, rheumatoid arthritis, legionellosis, and pertussis (Fig. 9a). The relationships between the mRNAs and the enriched KEGG pathways are shown in Fig. 9b.

# Validation of hub gene expression

Based on the miRNA-mRNA-hub gene network, the levels of expression of four hub genes (*CXCL8*, *BTG2*, *DNAJB9*, and *SEL1L3*) were identified using the GSE10334 dataset. The expression of *CXCL8, BTG2*, and *DNAJB9* was observed to be consistently decreased in the periodontal samples (Fig. 10). Therefore, hsa-miR-203/CXCL8, hsa-miR-203/BTG2, and hsa-miR-203/DNAJB9 were identified as potential regulatory pathways in chronic obstructive pulmonary disease.

# Discussion

Periodontitis is a complex infectious disease with various causes and contributing factors [16]. An increasing number of studies are now being conducted on the diagnosis and treatment of periodontitis. However, due to the limited understanding of the pathogenesis of periodontitis, the prognosis of patients with periodontitis remains poor. MicroRNAs are a family of endogenous, non-coding, small RNA molecules that play important roles in regulating gene expression at the post-transcriptional level [17].

Recently, microarray technology has been used to reveal thousands of gene changes in the development of various diseases.

The expression of miRNA can be modulated by transcription factors [18, 19], and thus, we identified those that could potentially regulate candidate DEMis in periodontitis. Egr-1, a zinc finger transcription factor [20], was predicted to account for the highest percentage of downregulated DEMis. Trabandt et al. showed that Egr-1 plays a crucial role in cellular proliferation and collagenase expression in in vivo gingival and periodontal tissue destruction [21]. Shi et al. found that curcumin promotes osteogenic differentiation of human periodontal ligament stem cells by inducing *EGR1* expression [22].

We reconstructed an miRNA-mRNA regulatory network comprising 32 mRNAs and 6 miRNAs. We also calculated the degree, closeness, and betweenness of the genes in the network. The top 10 nodes included hsa-miR-203, hsa-miR-671-5p, hsa-miR-223, hsa-miR-486-5p, hsa-miR-1246, *ENTPD1, CXCL8, BTG2, SEL1L3*, and *DNAJB9*. Among the ten hub genes, hsa-miR-203 showed a significant inverse correlation with *CXCL8, BTG2, SEL1L3*, and *DNAJB9* expression, which are believed to play critical roles in the development and pathological mechanisms of periodontitis.

MicroRNA-203 was first identified in the pathogenesis of psoriasis and is involved in various physiological and pathological processes [23]. Zhang et al. demonstrated that miR-203 overexpression suppresses *TRAF6*-induced IL-β, IL-6, and TNF-α activation in human renal mesangial cells and proximal tubular cell line cells [24]. A recent study showed that miR-203 protects against microglia-mediated brain injury by targeting the protein MyD88 to modulate the inflammatory response [25]. Furthermore, Wang et al. showed that miR-203 inhibits inflammation to alleviate myocardial ischemia-reperfusion injury [26].

*CXCL8, BTG2*, and *DNAJB9* were selected as key genes in the miRNA-mRNA network. *CXCL8*, a potent neutrophil chemoattractant and activator, plays a crucial role in the progression of chronic periodontitis [27]. It causes the destruction of periodontium via accumulation and degranulation of neutrophils [28, 29]. Moreover, *CXCL8* significantly upregulates elastase release by neutrophils, which contributes to the progression of human chronic periodontitis [30]. *BTG2*, also known as pheochromocytoma cell 3 or tetradecanoyl phorbol acetate-inducible sequence, belongs to the antiproliferative (APRO) gene family [31]. Previous studies have reported that *BTG2* expression is positively correlated with development, particularly with terminal differentiation [32, 33]. Normally, *DNAJB9* is localised to the endoplasmic reticulum and protects cells from stress-mediated apoptosis [34]. Nasr et al. identified *DNAJB9* as a specific immunohistochemical marker for fibrillary glomerulonephritis [35], and its role in the diagnosis and treatment of periodontitis needs to be further elucidated.

KEGG pathway analysis showed that mRNAs in the regulatory network were mainly enriched in the IL-17 signalling pathway. In previous studies, the activation of the IL-17 signalling pathway has been shown to be associated with periodontitis. A study performed by Satoru showed that IL-17A may promote the progression of periodontitis through pro-inflammatory cytokine production [36]. Moreover, IL-17 is essential for the maintenance of bone mass as it orchestrates osteoclast differentiation and activation [37]. Inana revealed that IL-17 inhibited both the proliferation and migration of periodontal ligament

mesenchymal stem cells and decreased their osteogenic differentiation by activating ERK1/2 and JNK mitogen-activated protein kinases [38]. Therefore, inhibition of the IL-17 signalling pathway may be a therapeutic strategy for periodontitis.

Although we constructed the potential miRNA–mRNA regulatory network by integrating multiple microarray datasets for the first time, our study is limited by the fact that predictions were made based on public databases; further in vivo and in vitro studies are required to validate our findings.

# Conclusions

In this study, we constructed an miRNA-mRNA network for periodontal pathogenesis and identified hsa-miR-203/CXCL8, hsa-miR-203/BTG2, and hsa-miR-203/DNAJB9 as pathways that could be involved in the pathogenesis of periodontitis, providing potential therapeutic targets.

# Abbreviations

BP, biological process

CC, cellular component

DEG, differentially expressed genes

DEMis, differentially expressed miRNAs

DEMS, differentially expressed mRNAs

GEO, Gene Expression Omnibus

GO, Gene Ontology

KEGG, Kyoto Encyclopaedia of Genes and Genomes

LPS, lipopolysaccharides

MCODE, Molecular Complex Detection

MF, molecular function

MiRNA, microRNA

NCBI, National Center for Biotechnology Information

PPI, protein-protein interaction

STRING, Search Tool for the Retrieval of Interacting Genes

## Declarations

# Ethics approval and consent to participate

Not applicable

# Consent for publication

Not applicable.

# Availability of data and materials

The datasets (GSE54710, GSE16134 and GSE10334) generated and analyzed during the current study are available in GEO Datasets repository. https://www.ncbi.nlm.nih.gov/gds.

# Competing interests

The authors declare that they have no competing interests

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# Authors' contributions

DZ conceived of the procedure for the research; XG and JH analysed study data; XG wrote the manuscript; ZZ and ZW revised the draft. All authors read and approved the final manuscript.

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# Figures

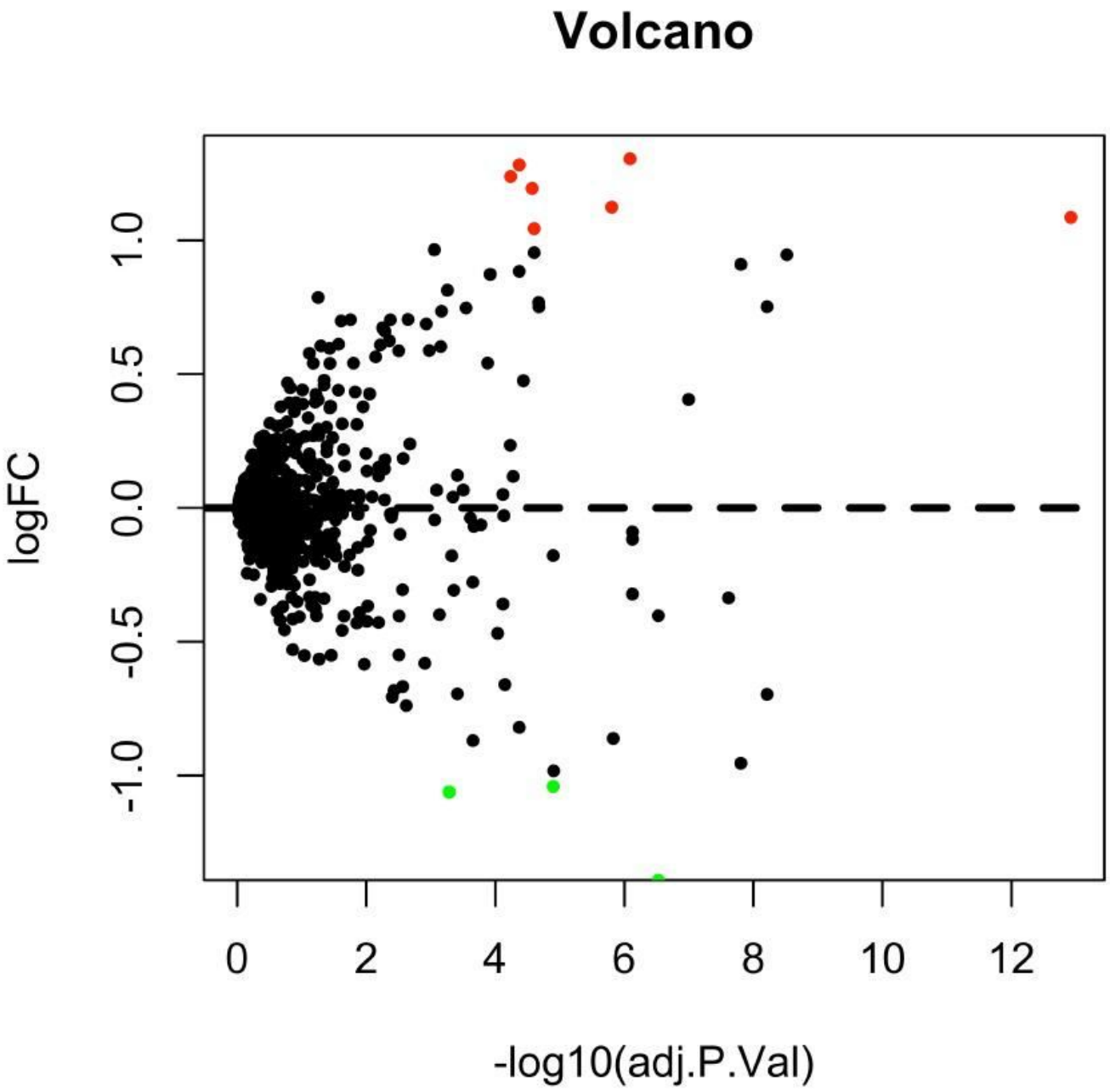
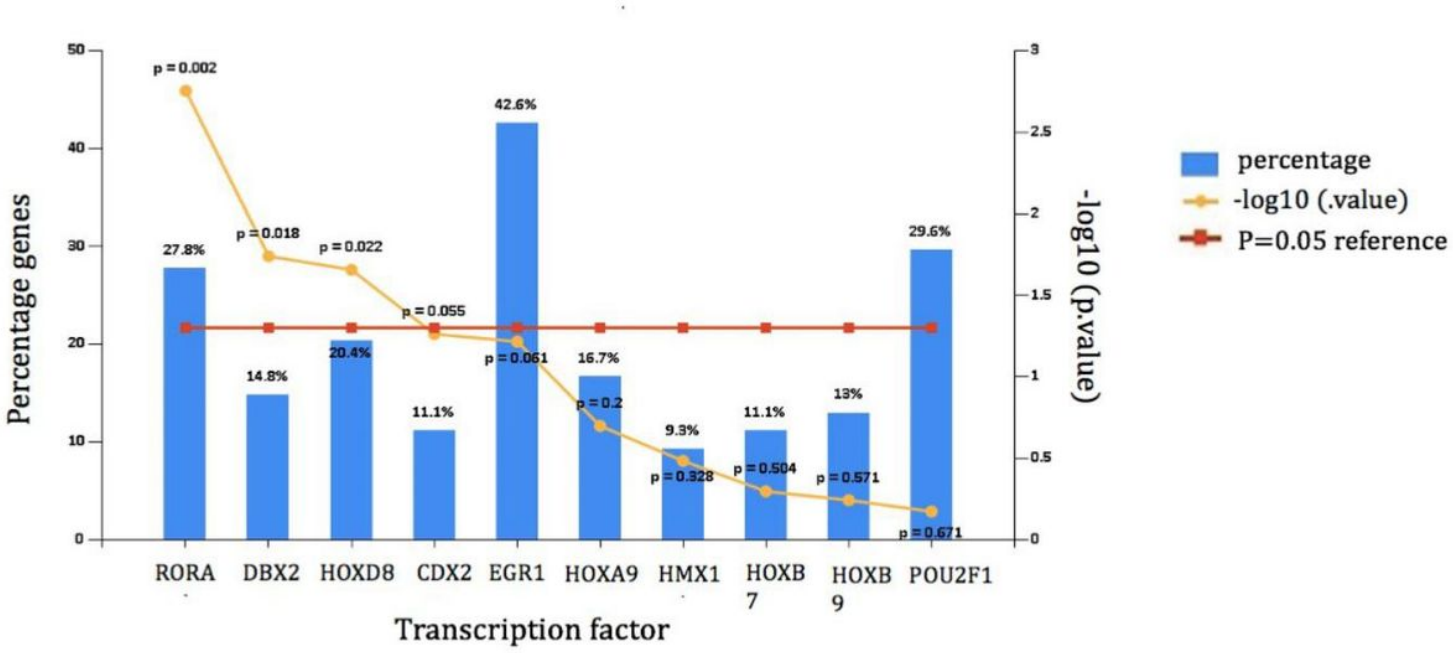


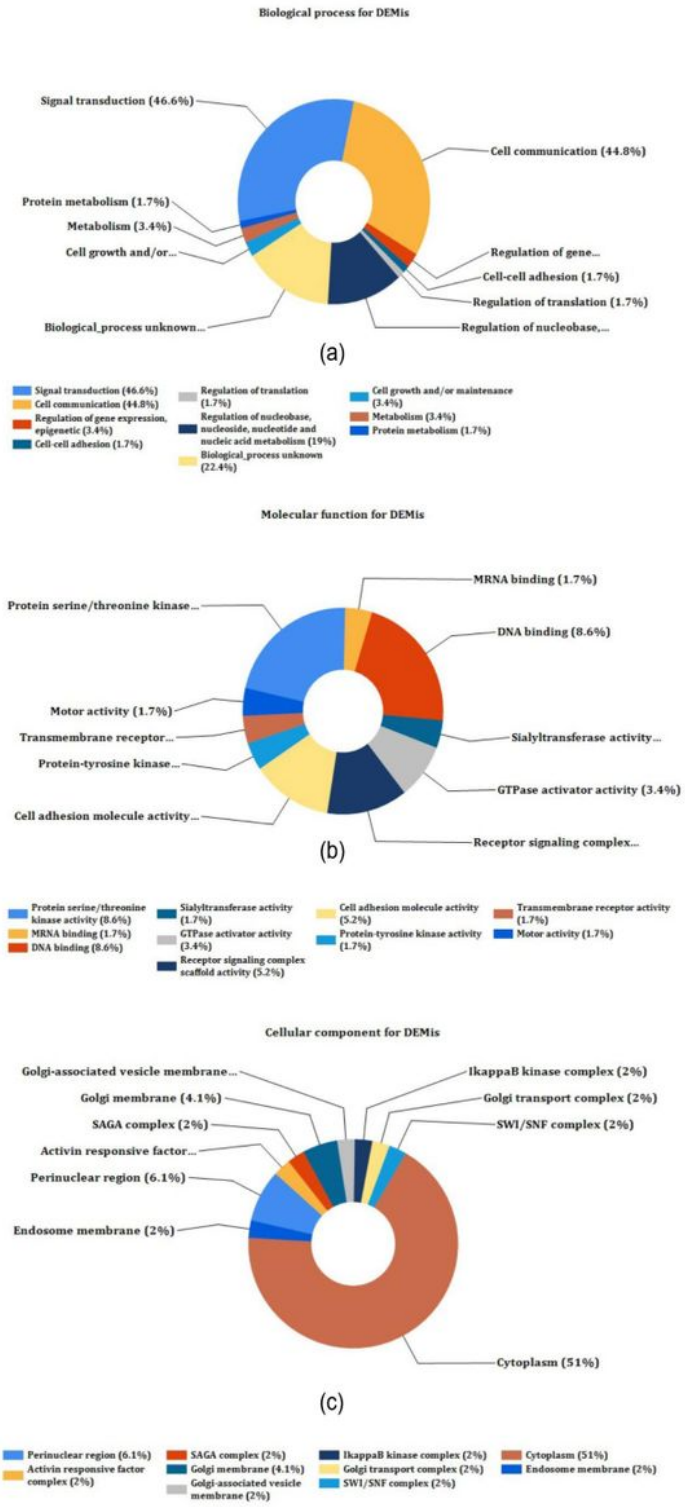

**Figure 1**

Volcano map of DEMis. Red spots represent upregulated genes; green spots represent downregulated genes.



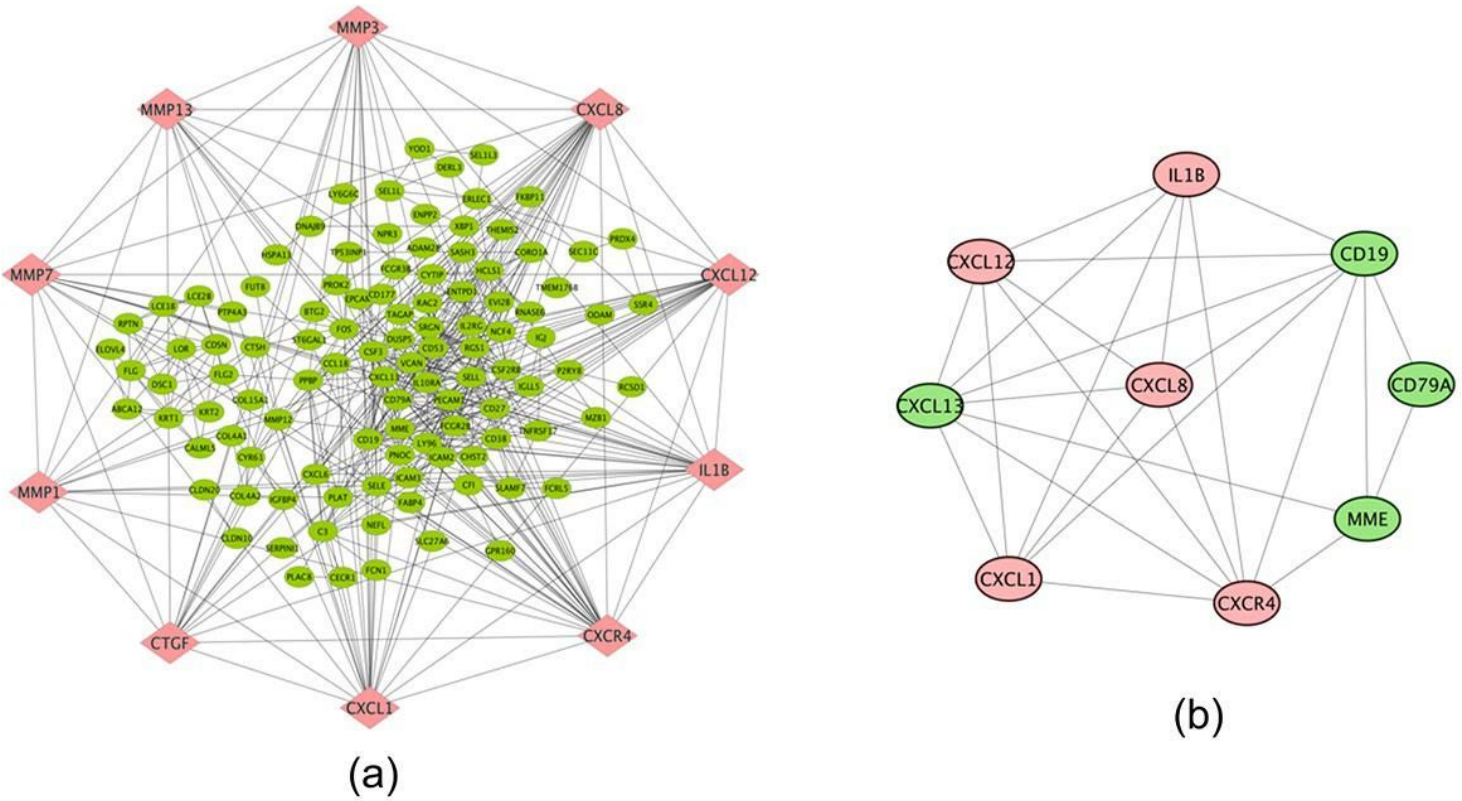

## Figure 2

Potential transcription factors of DEMis predicted by FunRich



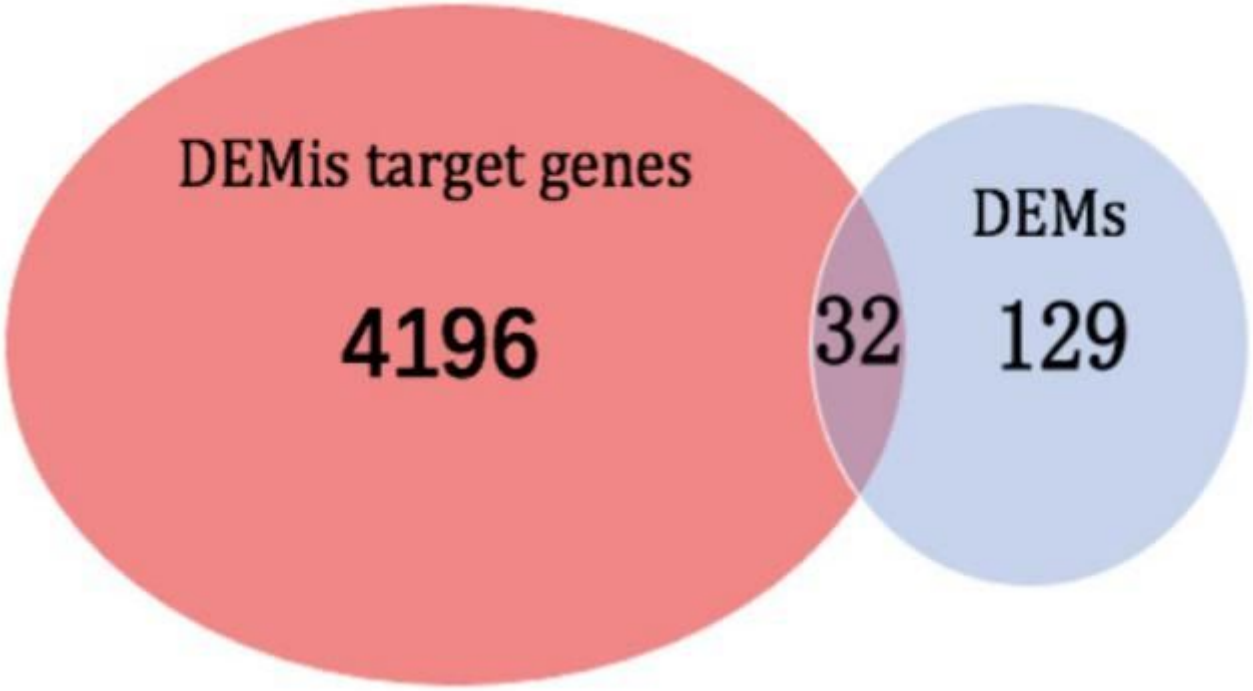

## Figure 3

The transcription factor enrichment for DEMis. Genes/mRNAs involved in biological process terms for DEMis(a); Genes/mRNAs involved in molecular function terms for DEMis (b); Genes/mRNAs involved in cellular component terms for DEMis (c).



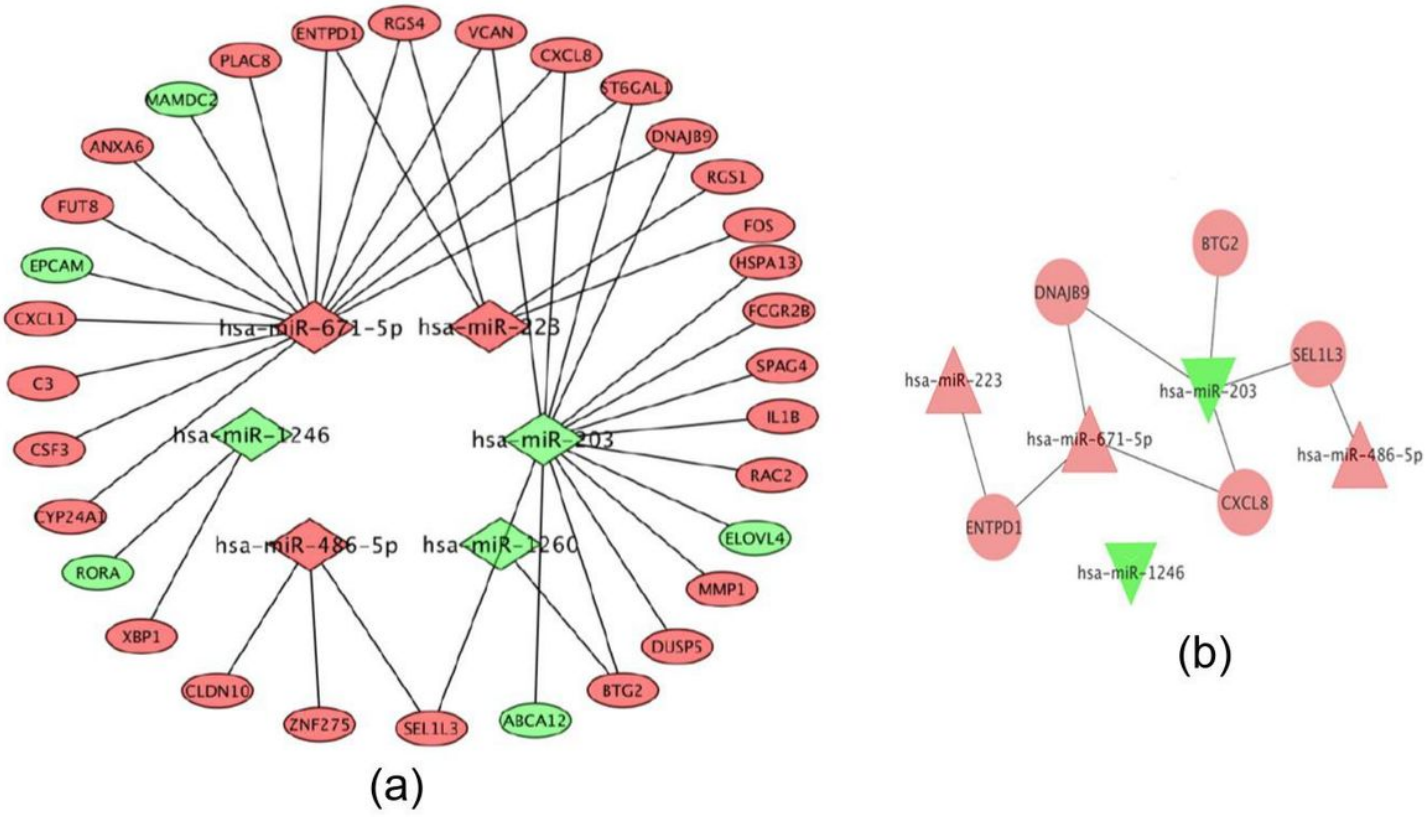

## Figure 4

The PPI networks of DEMs. The red colors represent the 10 highest degree genes and the circles with green represent the remaining genes. (a) The top one module from the PPI network. (b)



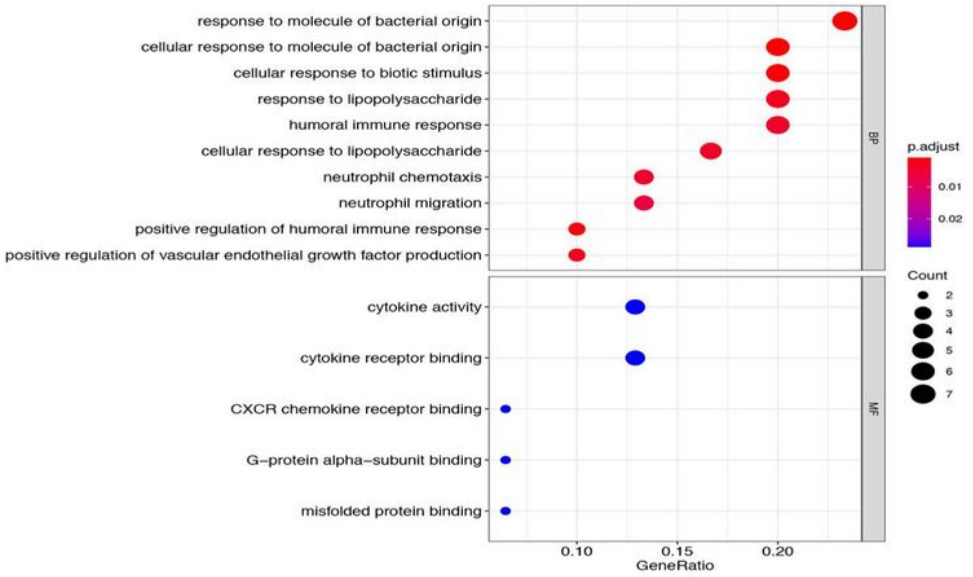
## Figure 5

A Venn-diagram between DEMis target genes and DEMs

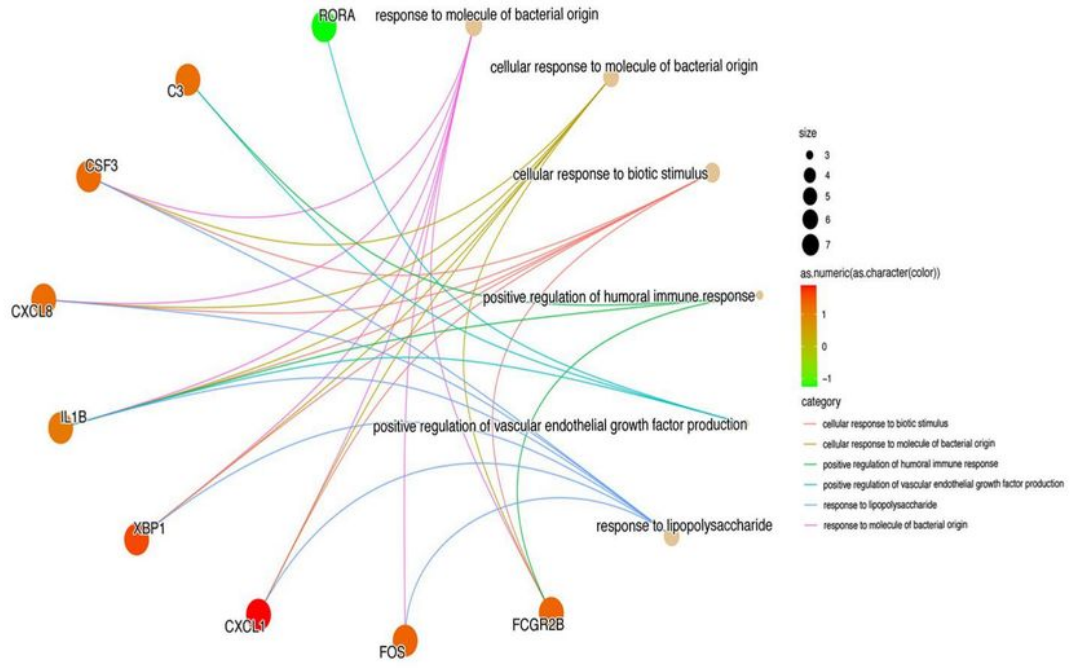



## Figure 6

The miRNA-mRNA network and the top 10 nodes in the regulatory network. The upregulated genes were exhibited by the red color, while the blue color exhibited the downregulated genes.



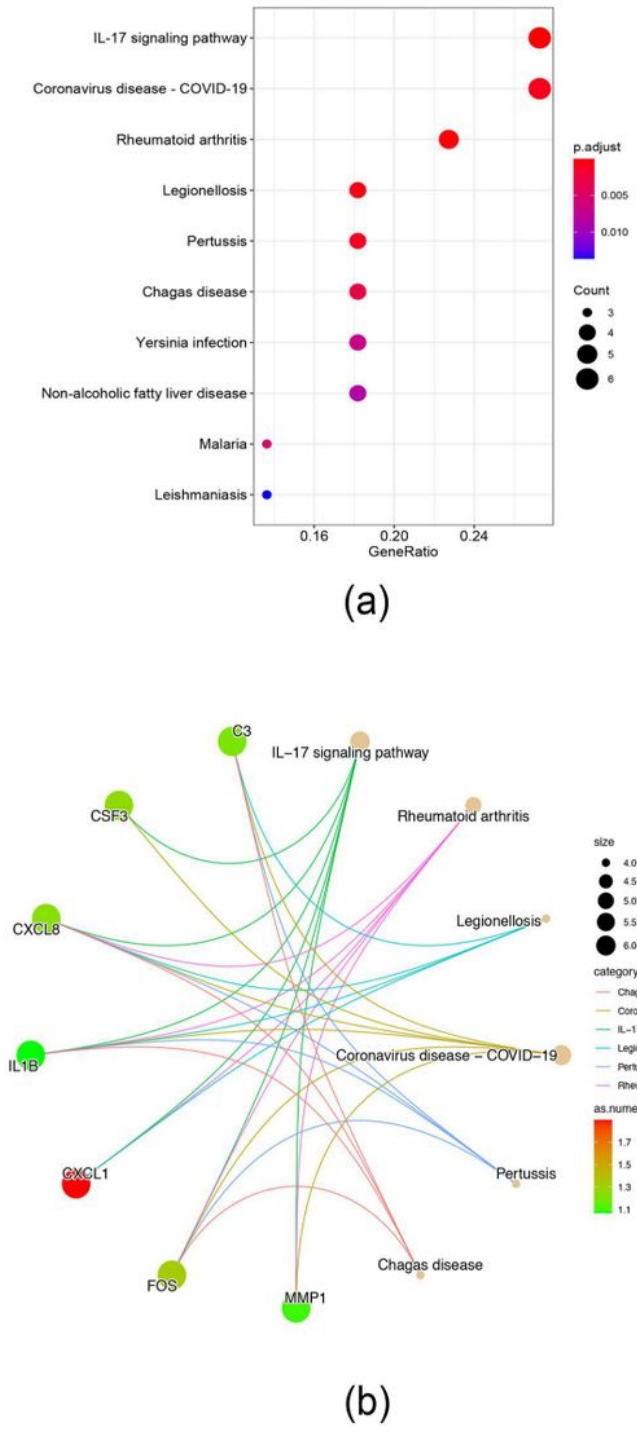

(a)




(b)

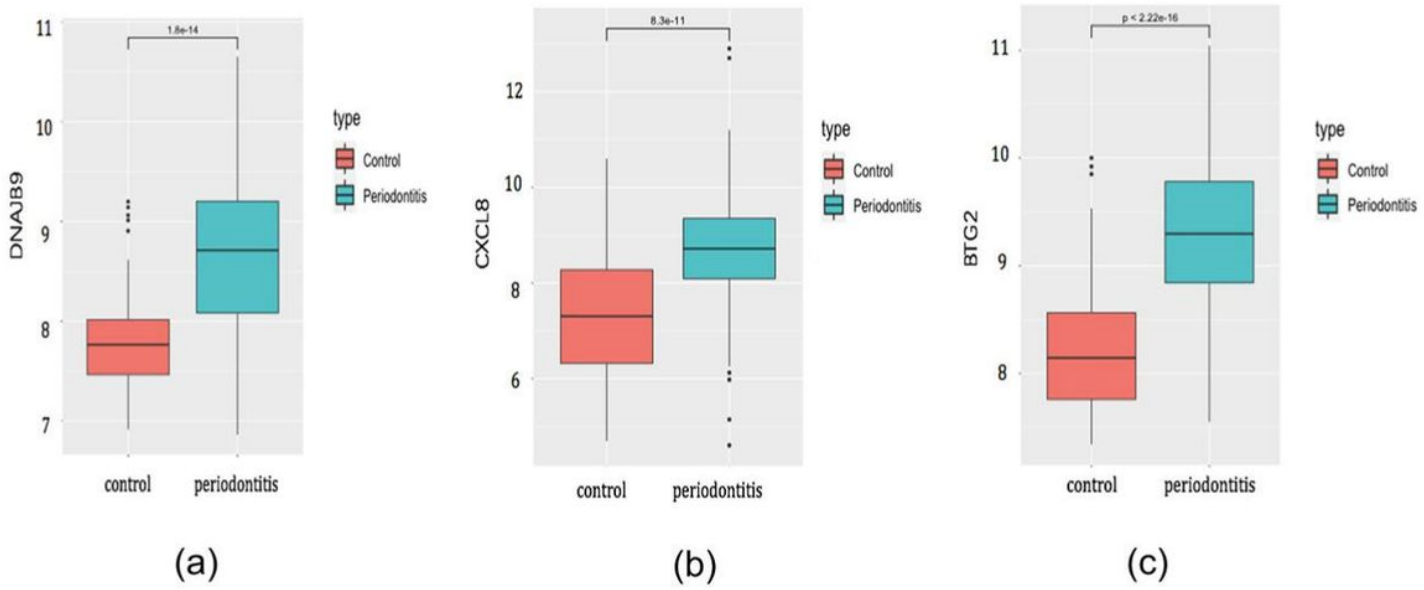
## Figure 7

GO analysis of the mRNAs in the regulatory network (a). The relationship between enriched mRNAs in GO analysis (b).




## Figure 8

The KEGG pathway enrichment analysis of mRNAs in the network (a). The relationship between enriched mRNAs in KEGG pathways (b).




## Figure 9

The mRNA expression of the key genes was determined from the GSE10334 dataset.