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Identification of potential key molecular and signaling pathways for psoriasis based on weighted gene co-expression network analysis and miRNA-mRNA regulatory network

Key molecular and pathways of psoriasis

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Abstract

BACKGROUND

Psoriasis is a chronic inflammatory skin disease, the pathogenesis of which is more complicated and often requires long-term treatment. Especially, moderate to severe psoriasis usually requires systemic treatment. Psoriasis is also associated with many diseases such as cardiometabolic diseases, malignant tumors, infections and mood disorders. Psoriasis can appear at any age, and lead to a substantial burden for individuals and society. So far, psoriasis is still a treatable but incurable disease. Previous studies have found that microRNAs (miRNAs) play an important regulatory role in the progression of various diseases. Currently, miRNAs studies in psoriasis and dermatology are relatively new. Therefore, the identification of key miRNAs in psoriasis is helpful to elucidate the molecular mechanism of psoriasis.

AIM

To identify key molecular markers and signaling pathways to provide potential basis for the treatment and management of psoriasis.

METHODS

The miRNA and mRNA data were obtained from the Gene Expression Omnibus (GEO) database. Then, differentially expressed mRNAs (DEmRNAs) and differentially expressed miRNAs (DEmiRNAs) were screened out by limma R package. Subsequently, DEmRNAs were analyzed for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) functional enrichment. The “WGCNA” R package was used to analyze the co-expression network of all miRNAs. In addition, we constructed miRNA-mRNA regulatory networks based on identified hub miRNAs. Finally, *in vitro* validation was performed. All experimental procedures were approved by the ethics committee of Chinese PLA General Hospital (S2021-012-01).

RESULTS

A total of 639 DEmRNAs and 84 DE miRNAs were identified. DEmRNAs screening criteria were $\text{adj.P.Val} < 0.01$ and $|\log\text{FoldChange}| (|\log\text{FC}|) > 1$. DE miRNAs screening criteria were $\text{adj.P.Val} < 0.01$ and $|\log\text{FC}| > 1.5$. KEGG functional analysis demonstrated that DEmRNAs were significantly enriched in immune-related biological functions, for example, toll-like receptor signaling pathway, cytokine-cytokine receptor interaction and chemokine signaling pathway. In weighted gene co-expression network analysis (WGCNA), turquoise module was the hub module. Moreover, 10 hub miRNAs were identified. Among these 10 hub mRNAs, only 8 hub mRNAs predicted the corresponding target mRNAs. 97 negatively regulated miRNA-mRNA pairs were involved in the miRNA-mRNA regulatory network, for example, hsa-miR-21-5p-CLDN8, hsa-miR-30a-3p-IL1B and hsa-miR-181a-5p/hsa-miR-30c-2-3p-CXCL9. RT-PCR results showed that IL1B and CXCL9 were up-regulated and CLDN8 was down-regulated in psoriasis with statistically significant differences.

CONCLUSION

The identification of potential key molecular markers and signaling pathways provides potential research directions for further understanding the molecular mechanisms of psoriasis. This may also provide new research ideas for the prevention and treatment of psoriasis in the future.

Key Words: psoriasis; miRNAs; weighted gene co-expression network analysis; functional enrichment; miRNA-mRNA regulatory network

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Core Tip: Psoriasis is a common chronic, recurrent, immune-regulatory skin and joint disease. Although psoriasis is widespread and has significant negative impact on

patients' life quality, it has not yet been fully diagnosed and treated. Moreover, it is also associated with many other diseases. So far, psoriasis is still a treatable but incurable disease. We use WGCNA to identify key modules and miRNAs related to psoriasis, and explore potential key pathways related to psoriasis through the targeting relationship of miRNA-mRNA. This provides new research ideas for the prevention and treatment of psoriasis in the future.

INTRODUCTION

Psoriasis is a common chronic, recurrent, immune-regulatory skin and joint disease. It has a variety of clinical skin manifestations, but the most common clinical manifestations are erythematous, scaling papules and plaques^[1]. Previous studies have found that the incidence of psoriasis varies with age and geographic area^[2]. Mechanical stress, air pollutants, sun exposure, infection, lifestyle, obesity, dyslipidemia and mental stress are associated with the progression of psoriasis^[3]. The pathogenesis of psoriasis is more complicated and often requires long-term treatment. ¹ Mild to moderate psoriasis can be treated topically with a combination of glucocorticoids, vitamin D analogues, and phototherapy. Moderate to severe psoriasis usually requires systemic treatment^[4]. Psoriasis is also associated with many diseases, for example, cardiometabolic diseases, malignant tumors, infections and mood disorders^[5]. So far, psoriasis is still a treatable but incurable disease. Therefore, identification of key genes and signaling pathways are of great significance for understanding the molecular mechanism of psoriasis, and provides potential basis for the treatment and management of psoriasis.

⁸ MicroRNAs (miRNAs) are a class of small non-coding single-stranded RNAs that regulate gene expression^[6]. MiRNAs play a vital role in the progression of diseases, for example, cancer, infectious diseases, and immune diseases^[7]. MiRNAs can regulate cell proliferation, keratinocyte differentiation, apoptosis and atypical immune activation in psoriasis^[8]. Previous studies have found that miR-187 can inhibit the proliferation of keratinocytes by targeting B7 homolog 3 protein (B7-H3). In addition, in the mouse model of psoriasis, overexpression of miR-187 can reduce acanthosis and reduce the

severity of the disease^[9]. MiR-183-3p can inhibit the proliferation and migration of keratinocytes in psoriasis by inhibiting growth factor receptor binding 2-associated binding protein 1 (GAB1)^[10]. MiR-320b negatively regulates keratinocyte proliferation in psoriasis by targeting AKT serine/threonine kinase 3 (AKT3)^[11]. The low expression of miR-31 in dermal mesenchymal stem cells (DMSCs) in patients with psoriasis results in the increased expression of some of its target genes, which in turn promotes the activation of T lymphocyte by inhibiting the proliferation of DMSCs, thereby contributing to the pathogenesis of psoriasis^[12]. Previous studies have found that miR-146 regulates inflammatory responses in keratinocytes and skin fibroblasts, which may affect the pathogenesis of psoriasis^[13]. Furthermore, in human keratinocytes, the expression of miR-146a is induced by pro-inflammatory cytokines tumor necrosis factor- α (TNF- α), interleukin-1 β (IL- β) and interleukin-17a (IL-17a), and the expression of miR-146b is induced by interferon γ (IFN- γ) and interleukin-22 (IL-22). MiR-203 promotes keratinocyte proliferation by targeting liver X receptor- α (LXR- α) and peroxisome proliferator-activated receptor- γ (PPAR- γ) in psoriasis^[14]. MiR-125 regulates keratinocytes proliferation by regulating targeted genes^[15, 16]. MiR-197 overexpression inhibits keratinocytes proliferation induced by IL-22 and keratinocytes migration^[17]. In addition, miR-99 also plays an important role in regulating the abnormal proliferation and differentiation of keratinocytes in psoriasis^[18]. These studies demonstrate that miRNAs play a vital role in the pathogenesis of psoriasis. Currently, miRNAs studies in psoriasis and dermatology are relatively new. Therefore, the identification of key miRNAs in psoriasis is helpful to elucidate the molecular mechanism of psoriasis.

Weighted gene co-expression network analysis (WGCNA) can be used for finding clusters (modules) of highly correlated genes^[19]. WGCNA has been used to identify key genes for many diseases, including cancer^[20], cardiovascular disease^[21] and immunological diseases^[22]. In addition, WGCNA has also been used to identify key mRNAs and long noncoding RNAs (lncRNAs) in psoriasis. So far, we have not found studies that use WGCNA to identify key miRNAs in psoriasis. Therefore, we use

WGCNA to identify key modules and miRNAs related to psoriasis, and explore potential key pathways related to psoriasis through the targeting relationship of miRNA-mRNA.

MATERIALS AND METHODS

Data sources and processing

The miRNA and mRNA data were obtained from the Gene Expression Omnibus (GEO) database^[23]. The keyword we searched in the GEO database was “psoriasis”. Then, cell line or animal level studies and single-sample studies were excluded. Five datasets GSE13355, GSE66511, GSE145054, GSE142582 and GSE129373 were involved in this study (Table 1). All data were derived from skin samples. GSE13355 and GSE66511 were mRNA data from GPL570 and GPL16288 platform, respectively. GSE145054, GSE142582 and GSE129373 were miRNA data from GPL19117, GPL20301 and GPL11154, respectively. The gene expression matrix files in GSE13355 and GSE145054 were downloaded. The GPL annotation file was used to annotate the expression matrix. The probe ID was converted to the gene symbol. Multiple probes correspond to the same gene, take the average value. The probe that corresponds to multiple genes was removed. The original data of gene expression profile in GSE66511, GSE142582 and GSE129373 were downloaded, and performed by logarithm processing. Then, the combat function in “sva” R package was used to remove batch effects.

Differential expression analysis

After the above pretreatment, differentially expressed mRNAs (DEmRNAs) and differentially expressed miRNAs (DEmiRNAs) were screened out by limma R package^[24]. DEmRNAs screening criteria were $\text{adj.P.Val} < 0.01$ and $|\log\text{FoldChange}| (|\log\text{FC}|) > 1$. DEmiRNAs screening criteria were $\text{adj.P.Val} < 0.01$ and $|\log\text{FC}| > 1.5$.

Functional enrichment analysis

To investigate the possible involvement of DEmRNAs in the biological processes related to psoriasis, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) functional enrichment analyses. David database was used

for the enrichment analysis of DEmRNAs^[25], and the screening criterion was $P < 0.05$. GO enrichment analysis, which is comprised of biological processes (BP), cellular components (CC) and molecular functions (MF). The “GOplot” R package was used to visualize the enrichment results.

Construction of weighted gene co-expression network

The “WGCNA” R package was used to analyze the co-expression network of all miRNAs. In order to detect outliers, the “hclust” function was used to cluster the sample data. Subsequently, in order to construct a scale-free topology, the “pickSoftThreshold” function was used to select an appropriate soft threshold power regulator (height 0.90, β value 2). Calculate the adjacency matrix based on the kernel value. The adjacency matrix was transformed into topological overlap matrix (TOM) and corresponding dissimilarity matrix (1-TOM). Genes with similar expression patterns were gathered together, and modules were divided according to the function of “cutreeDynamic” with default parameters. Since the modules identified by the dynamic tree cutting algorithm may be similar, they are combined with a truncation of 0.25 height^[26].

Hub modules and miRNAs

To identify the important modules associated with psoriasis, the module eigengene (ME) of each module was calculated using the “moduleEigengenes” function. Then, the correlation between ME and psoriasis was analyzed using Pearson method. Subsequently, the module with the highest correlation with psoriasis was identified as the hub module. According to module connectivity (MM) and clinical trait relationship (GS) of each gene in the hub module, the candidate hub miRNAs were screened out^[27]. The screening criteria were $MM > 0.8$ and $GS > 0.5$. Finally, the intersection of candidate hub miRNAs and DEmiRNAs were selected as real hub miRNAs.

MiRNA-mRNA network

To explore the correlation between miRNA and mRNA, we constructed a miRNA-mRNA regulatory network. Target mRNAs of miRNAs were predicted using miRDB (<http://mirdb.org>) database. MiRNA-mRNA pairs involved in common DEmRNA and

negatively regulated were selected to construct the network. Cytoscape (www.cytoscape.org/) was used to visualize the miRNA-mRNA regulatory network.

In vitro validation

We collect skin tissue samples from healthy control individuals and patients with psoriasis. Basic information (including age, sex, *etc.*) of patients and healthy controls were recorded in detail during sample collection (Table 2). The specific inclusion criteria of patients with psoriasis: (1) patients had at least one well-circumscribed erythematous and squamous lesion, which had been confirmed by at least two dermatologists; (2) patient's pathological tissues were confirmed by clinical histopathology; (3) patients had no systemic anti-psoriatic treatment 2 wk before the skin biopsy; (4) patients had no topical anti-psoriatic treatment 1 wk before the skin biopsy. Patients with psoriasis who were treated with immune-related drugs before sampling and whose clinical information was incomplete were excluded. The individuals in the normal control group were gender and age matched with the psoriasis group, and without history of psoriasis or other autoimmune diseases.

According to screening criteria, 7 normal and 7 patient skin tissue samples were obtained. Firstly, total RNA of the samples were extracted using TRIzol. Then, fastKing cDNA first strand synthesis kit and miRNA first strand cDNA synthesis kit (Tailing Reaction) were used for mRNA and miRNA reverse transcription, respectively. Subsequently, SuperReal PreMix Plus (SYBR Green) and miRNA quantitative PCR kit (SYBR Green Method) were used to perform real-time polymerase chain reaction (RT-PCR) validation of mRNA and miRNA, respectively. GAPDH, ACTB and hsa-U6 were internal reference. ABI7300 fluorescence quantitative PCR instrument was used for detection. Finally, the $2^{-\Delta\Delta C_t}$ method was used for relative quantitative analysis of the data^[28]. This study complied with the Declaration of Helsinki. The informed consent was obtained from the participants. All experimental procedures were approved by the ethics committee of Chinese PLA General Hospital (S2021-012-01).

Statistical analysis

All statistical analyses were performed using R software. Limma R package was used to screen for DEmRNAs and DEmiRNAs. Pearson's method was used to analyze the correlation between ME and psoriasis. RT-PCR validation data were statistically analyzed by one-way anova. $P < 0.05$ was statistically significant.

RESULTS

Analysis of DEmRNAs and DEmiRNAs

After pretreatment of the raw data, 15,780 mRNAs and 837 miRNAs were screened out (Figure 1). Compared with the control group, 639 DEmRNAs were identified in the psoriasis group (adj.P.Val < 0.01 and $|\log FC| > 1$). Among them, 497 were up-regulated and 142 were down-regulated. Compared with the control group, 84 DEmiRNAs were identified in the psoriasis group (adj.P.Val < 0.01 and $|\log FC| > 1.5$). Among them, 70 were up-regulated and 14 were down-regulated. Heat maps showed that there were significant differences of mRNA (Figure 2A) and miRNA (Figure 2B) expression between psoriasis group and control group. The volcano maps of DEmRNAs and DEmiRNAs were shown in Figure 2C-D.

Analysis of biological functional

Functional analysis of 639 DEmRNAs was performed using David database ($P < 0.05$). GO enrichment results showed that most of the DEmRNAs were distributed in inflammatory response (GO:BP), immune response (GO:BP), cytosol (GO:CC), extracellular exosome (GO:CC), protein homodimerization activity (GO:MF) and other biological processes (Figure 3A-C). KEGG enrichment results demonstrated that DEmRNAs were significantly enriched in metabolic pathways, influenza A, chemokine signaling pathway and cytokine-cytokine receptor interaction (Figure 3D and Table 3).

WGCNA

WGCNA was performed on 837 miRNAs. The samples were clustered to remove abnormal samples (GSM3711960 and GSM3711959) (Figure 4A, B). After calculation, when the soft threshold β was 2, it was approximately a scale-free topology (Figure 4C). After determining the soft threshold, the cluster tree graph was constructed.

Subsequently, set the minimum number of genes for modules to 20, and then the modules with dissimilarity <25% merged using the dynamic tree cutting method. Finally, 6 modules were determined (Figure 4D).

Uncovering of hub modules and miRNAs

The correlation between modules and psoriasis was analyzed by Pearson's method. The results showed that turquoise module had the highest correlation with psoriasis ($r = 0.96$) (Figure 5A). Therefore, turquoise module was considered hub module. Subsequently, 21 miRNAs were screened out from the turquoise module as candidate hub miRNAs (MM >0.8 and GS >0.5) (Figure 5B). Intersection of DEmiRNAs and candidate hub miRNAs was obtained (Figure 5C). 10 intersecting miRNAs were identified as real hub miRNA. Among them, 5 hub miRNAs were up-regulated (hsa-miR-21-3p, hsa-miR-21-5p, hsa-miR-31-5p, hsa-miR-18a-5p and hsa-miR-33b-3p) and 5 hub miRNAs were down-regulated (hsa-miR-181a-2-3p, hsa-miR-181a-5p, hsa-miR-6510-3p, hsa-miR-30a-3p and hsa-miR-30c-2-3p).

MiRNA-mRNA regulatory network

Target mRNAs of 10 hub miRNAs were predicted using miRDB database, but only 8 hub miRNAs (4 up-regulated and 4 down-regulated) were predicted to the corresponding target mRNAs. 11 target mRNAs of up-regulated hub miRNAs (hsa-miR-21-3p, hsa-miR-21-5p, hsa-miR-18a-5p and hsa-miR-33b-3p) were matched with DEmRNAs, and 72 target mRNAs of down-regulated hub miRNAs (hsa-miR-181a-2-3p, hsa-miR-181a-5p, hsa-miR-30a-3p and hsa-miR-30c-2-3p) were matched with DEmRNAs. 97 negatively regulated miRNA-mRNAs were involved in the miRNA-mRNA regulatory network (Figure 6), for example, hsa-miR-21-3p/hsa-miR-18a-5p-F3, hsa-miR-21-5p-CLDN8, hsa-miR-33b-3p-PLCB4, hsa-miR-30a-3p-IL1B, hsa-miR-181a-5p-CCL8, hsa-miR-181a-5p/hsa-miR-30c-2-3p-CXCL9 and hsa-miR-30c-2-3p-KYNU.

Functional enrichment of target DEmRNAs

Functional analysis of 83 target DEmRNAs was performed using David database ($P < 0.05$). GO enrichment results demonstrated that most of the target DEmRNAs were distributed in signal transduction (GO:BP), immune response (GO:BP), integral

component of membrane (GO:CC), plasma membrane (GO:CC), ATP binding (GO:MF) and other biological processes (Figure 7A-C). KEGG enrichment results demonstrated that the target DEmRNAs were significantly enriched in influenza A, hepatitis C, measles and chemokine signaling pathway (Figure 7D and Table 4).

RT-PCR validation

IL1B, CXCL9, CLDN8, CCL8, hsa-miR-181a-5p, hsa-miR-30a-3p and hsa-miR-21-5p related to psoriasis were selected from hub miRNAs and target DEmRNAs for *in vitro* validation. Primers for each mRNAs and miRNAs were shown in Table 5. IL1B, CXCL9 and CCL8 were up-regulated and CLDN8 and hsa-miR-181a-5p were down-regulated in psoriasis tissues (Figure 8). Among them, IL1B, CXCL9 and CLDN8 showed significant difference in expression levels. In addition, we found that expression trends of hsa-miR-30a-3p and hsa-miR-21-5p were contrary to the results of bioinformatics analysis. The reason for the inconsistency between RT-PCR and bioinformatics analysis results may be the small sample size. Further research is needed.

DISCUSSION

Although psoriasis is widespread and has significant negative impact on patients' life quality, it has not yet been fully diagnosed and treated. In the research, we used the WGCNA method to identify 10 hub miRNAs that may be related to the pathogenesis of psoriasis. Then, target mRNAs of hub miRNAs were predicted by using miRDB database. Only 8 hub miRNAs were predicted to the corresponding target mRNAs. Subsequently, to understand the key biological functions involved in DEmRNAs and target DEmRNAs, we performed GO and KEGG functional analysis. Results demonstrated that they were significantly enriched in immune-related biological functions, for example, immune response, cell chemotaxis and chemokine signaling pathway.

Hsa-miR-21-5p is abnormally expressed in psoriasis, but the specific molecular mechanism remains unclear^[29]. In this study, results demonstrated that hsa-miR-21-5p expression was up-regulated and negatively regulated with claudin 8 (CLDN8). CLDN8

is down-regulated in psoriasis and has an important molecular regulatory role^[30]. CLDN8 has also been identified as a key downstream component of the interleukin (IL) 9 and IL23 inflammatory cascade^[31, 32]. In addition, KEGG functional enrichment analysis showed that CLDN8 was enriched in hepatitis C. Arnon D Cohen *et al* found that psoriasis is associated with hepatitis C^[33]. Cathelicidin, toll like receptor 9 (TLR9), IFN- γ and TNF- α are inflammatory cytokines. Hepatitis C may increase susceptibility to psoriasis by up-regulating these inflammatory factors^[34, 35]. Thus, we hypothesized that hsa-miR-21-5p may play a vital regulatory role in hepatitis C through regulating CLDN8 and thus affect the pathogenesis of psoriasis.

Hsa-miR-30a-3p can affect the migration and proliferation of cancer cells by targeting related genes^[36, 37]. Hsa-miR-30a-3p is also contacted with platelet apoptosis and adhesion in immune thrombocytopenia^[38]. However, we have not found any report about hsa-miR-30a-3p in psoriasis. In this study, we found that hsa-miR-30a-3p was up-regulated and negatively regulated with multiple DEmRNAs in psoriasis. It may play a vital regulatory role in psoriasis by targeting these DEmRNAs. We found the pro-inflammatory factor interleukin 1 beta (IL1B) in target DEmRNAs of hsa-miR-30a-3p. IL1B has been found to play an important role in autoimmune or autoinflammatory conditions^[39]. IL1B is abundant in the tissue fluid of psoriasis and participates in the disease progression through dual effects^[40]. First, it induces insulin resistance through p38 mitogen-activated protein kinase (p38MAPK), preventing insulin-dependent differentiation of keratinocytes, while IL1B promotes keratinocyte proliferation, both hallmarks of psoriasis. Through KEGG functional enrichment analysis, we also found that IL1B participates in multiple signal pathways. For example, measles, toll-like receptor signaling pathway and cytokine-cytokine receptor interaction. Measles can relieve psoriasis through immunosuppressive effect^[41]. Toll-like receptor signaling pathway play an important role in psoriasis by affecting keratinocyte production^[42]. The cytokine-cytokine receptor interaction is related to the occurrence and progression of psoriasis through combined transcriptomic analysis^[43], which is consistent with our analysis. Thus, we hypothesized that hsa-miR-30a-3p may play a vital molecular role in

the progression of psoriasis by targeting DEmRNAs to regulate multiple biological signaling pathways.

Hsa-miR-181a-5p is involved in the catabolic pathway of chondrocytes and oxidative stress in osteoarthritis^[44]. Hsa-miR-181a-5p can also regulate the pathogenesis of sepsis-related inflammation through CRNDE/hsa-miR-181a-5p/TLR4 pathway^[45]. In a case-control study, hsa-miR-181a-5p was significantly down-regulated in psoriasis^[29]. The miRNA-mRNA regulatory network result demonstrated that hsa-miR-181a-5p was negatively regulated with multiple DEmRNAs. Among these DEmRNAs, we found inflammatory mediators C-X-C motif chemokine ligand 9 (CXCL9) and C-C motif chemokine ligand 8 (CCL8). CXCL9 is an important chemokine involved in T cell recruitment and is up-regulated in the plasma of patients with psoriasis^[46, 47]. Increased CXCL9 expression can aggravate the progression of psoriasis^[48]. CXCL9 and hsa-miR-30c-2-3p were also negatively regulated in the miRNA-mRNA regulatory network. Oncology studies have shown that hsa-miR-30c-2-3p play a vital role in tumor pathogenesis by regulating the proliferation, apoptosis, migration and invasion of cancer cells^[49, 50]. So far, we have not found any studies on hsa-miR-30c-2-3p in psoriasis. This article may be the first to report that hsa-miR-30c-2-3p plays a role in the pathogenesis of psoriasis. As a chemokine, CCL8 is involved in immune regulation and inflammatory processes in a variety of diseases^[51-53]. Although no relevant studies on CCL8 have been found in psoriasis, the expression of CCL8 is up-regulated in atopic dermatitis^[54]. In addition, CXCL9 and CCL8 were found to be enriched in chemokine signaling pathway and cytokine-cytokine receptor interaction by functional analysis. Therefore, this further suggests that hsa-miR-181a-5p and hsa-miR-30c-2-3p may play a regulatory role in psoriasis by targeting DEmRNAs to mediate multiple biological signaling pathways.

Current research results highlighted that silencing hsa-miR-181a-2-3p could enhance cadmium-induced inflammatory response and activation of inflammasome^[55]. In the research, we found that hsa-miR-181a-2-3p was negatively regulated with multiple DEmRNAs. Among these DEmRNAs, OAS3 was found to be involved in hepatitis C

and Measles in KEGG functional enrichment. Some researchers have found that the OAS3 is concerned with the occurrence and progression of psoriasis through transcriptomic analysis, which is consistent with our analysis^[56, 57]. Thus, we hypothesized that hsa-miR-181a-2-3p may play a regulatory role in the progression of psoriasis by targeting OAS3 to mediate hepatitis C and Measles. This provides potential molecular research directions for further research on the pathogenesis of psoriasis.

The expression of hsa-miR-21-3p in psoriasis was significantly increased^[58]. Moreover, hsa-miR-21-3p¹³ plays a pro-inflammatory role in keratinocytes, and high expression in the skin is concerned with psoriasis^[59]. So far, we have not found relevant reports about hsa-miR-18a-5p in psoriasis. However, hsa-miR-18a-5p promotes the proliferation and migration of pulmonary smooth muscle cells by targeting notch receptor 2 (NOTCH2)^[60]. Oncology studies have shown that hsa-miR-18a-5p promotes melanoma cell proliferation by targeting EPH receptor A7 (EPHA7) signaling^[61]. Hsa-miR-18a-5p can affect keratinocytes apoptosis by targeting B-cell lymphoma/Leukemia-2-like protein 10 (BCL2L10) in patients with toxic epidermal necrolysis (TEN), and is related to the skin erythema or erosion area of drug eruptions^[62]. The miRNA-mRNA regulatory network regulatory network result demonstrates that hsa-miR-21-3p and hsa-miR-18a-5p jointly negatively regulate coagulation factor III, tissue factor (F3). This finding provides new research ideas for the pathogenesis of psoriasis in the future.

Results of previous studies demonstrate that down-regulation of interleukin 1 receptor associated kinase 3 (IRAK3) by hsa-miR-33b-3p can alleviate the inflammation and apoptosis induced by IL1B in CHON-001 cells^[63]. As a key miRNA of major depression disorder and kawasaki disease, hsa-miR-33b-3p play an important role in their pathogenesis^[64, 65]. Hsa-miR-33b-3p has also been reported in cancer.^[66] In the miRNA-mRNA regulatory network, phospholipase C beta 4 (PLCB4) is the only negatively regulated target DEMRNA of hsa-miR-33b-3p. Neutrophils are an important part of the innate immune system and an early line of defense against microbial invasion. PLCB4 can regulate the number of neutrophils.^[67] In addition, KEGG functional analysis result demonstrated that PLCB4 was enriched in chemokine signaling pathway. Thus, we

hypothesized that hsa-miR-33b-3p may play a vital molecular role in psoriasis by targeting PLCB4 to regulate chemokine signaling pathway.

This study has some limitations. First, sample size of *in vitro* validation was small, leading to a certain degree of error between RT-PCR validation results and bioinformatics analysis results. Further studies with a larger sample size are needed. Secondly, the specific regulatory mechanism of the identified genes and signaling pathways in psoriasis remain unclear, so further research is needed.

CONCLUSION

In conclusion, identification of potential key molecular markers and signaling pathways provides potential molecular research directions for further understanding the pathological mechanisms of psoriasis. This may also provide new research ideas for the prevention and treatment of psoriasis in the future.

ARTICLE HIGHLIGHTS

Research background

This study provide new research ideas for the prevention and treatment of psoriasis in the future.

Research motivation

The identification of potential key molecular markers and signaling pathways provides potential research directions for further understanding the molecular mechanisms of psoriasis.

Research objectives

We identified a large number of DEMRNAs and screened out possible signaling pathways related to psoriasis, for example, toll-like receptor signaling pathway, cytokine-cytokine receptor interaction and chemokine signaling pathway. 10 hub miRNAs were identified by WGCNA. 8 hub mRNAs predicted the corresponding

target mRNAs. 97 negatively regulated miRNA-mRNA pairs were involved in the miRNA-mRNA regulatory network, for example, hsa-miR-21-5p-CLDN8, hsa-miR-30a-3p-IL1B and hsa-miR-181a-5p/hsa-miR-30c-2-3p-CXCL9.

Research methods

Differentially expressed mRNAs (DEmRNAs) and differentially expressed miRNAs (DEmiRNAs) were screened out by limma R package. DEmRNAs were analyzed for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) functional enrichment. The “WGCNA” R package was used to analyze the co-expression network of all miRNAs. We constructed miRNA-mRNA regulatory networks based on identified hub miRNAs.

Research results

Identification of key miRNAs in psoriasis is helpful to elucidate the molecular mechanism of psoriasis

Research conclusions

Although psoriasis is widespread and has significant negative impact on patients' life quality, it has not yet been fully diagnosed and treated.

Research perspectives

Previous studies have found that microRNAs (miRNAs) play an important regulatory role in the progression of various diseases. Currently, miRNAs studies in psoriasis and dermatology are relatively new

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