Identification and Interaction Analysis of Key Genes and MicroRNAs in Systemic Sclerosis by Bioinformatics Approaches*

Yan-hong SUN[1, 2†], Meng XIE[1†], Shi-di WU[1], Jing ZHANG[3], Chang-zheng HUANG[1#]
[1]*Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*
[2]*Department of Dermatology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China*
[3]*Department of Dermatology, General Hospital of the Central Theater Command of the People's Liberation Army, Wuhan 430070, China*

Summary: Systemic sclerosis (SSc) is a highly heterogeneous autoimmune disease with a high mortality rate. However, the cellular and molecular mechanisms of SSc remain unclear. Here, we identified the key hub genes and microRNAs (miRNAs) that modulate the occurrence and development of SSc. We downloaded the microarray dataset GSE95065 from the Gene Expression Omnibus (GEO) database and then analyzed the data by using GEO2R. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used for functional pathway enrichment analyses of differentially expressed genes (DEGs), and Cytoscape software was used to generate the protein-protein interaction (PPI) network. In addition, OmicsNet was used to predict the miRNAs for the hub genes of SSc. As a result, 783 DEGs were identified, of which 770 genes (142 up-regulated genes and 628 down-regulated genes) were matched to the genes in SSc skin samples. Gene Ontology (GO) analyses by DAVID indicated that the up-regulated genes were mainly involved in immune response, and the down-regulated genes were greatly enriched in glycinergic synaptic transmission. In the PPI network, 22 nodes were selected as key genes, including several members of the chemokine family. Furthermore, after uploading these key genes to the OmicsNet tool, we found that hsa-miR-26b-5p might target CXCL9 and CXCL13. Moreover, we demonstrated that the hsa-miR-26b-5p inhibitor might inhibit fibrosis in TGF-β-activated fibroblasts, which would be a promising target for SSc therapy.
Key words: systemic sclerosis; bioinformatics analysis; CXCL9; CXCL13; hsa-miR-26b-5p

Systemic sclerosis (SSc) is a highly heterogeneous autoimmune disease, involving clinical findings of extensive fibrosis, vascular changes and immune abnormalities[1]. SSc most commonly affects the skin, causing an increase in collagen and extracellular matrix deposition in the skin. In addition, it also involves internal organs, such as the heart, lungs, kidneys, and digestive tract, leading to serious complications such as pulmonary fibrosis and pulmonary hypertension. More than half of those diagnosed with SSc eventually die as a direct result of internal organ damage, which imposes a heavy physical and psychological burden on patients[2]. Based on in-depth study of the pathogenesis of SSc, scholars believe that the increase in extracellular matrix production is caused by the excessive activation of fibroblasts caused by the interaction of endothelial cells, lymphocytes, macrophages and other cells. However, the cause of SSc and the underlying mechanism remain unclear[3]. There is no specific treatment, including a targeted treatment for progressive fibrosis. Therefore, exploring the mechanisms associated with the pathogenesis of SSc is very important for improving the treatment of the disease.

The bioinformatics analysis of microarray data, as a high-throughput technology, has been extensively used as an efficient tool in oncological research to detect genetic alterations. Such analysis has also played an important role in identifying valuable biomarkers in SSc. In recent years, many researches on differentially expressed genes (DEGs) and their molecular functions in SSc have been conducted[4]. Moreover, many recent reports have shown that serum microRNAs (miRNAs), such as miR-21 and miR-92a, might be promising biomarkers for the diagnosis, prognosis and

Yan-hong SUN, E-mail: sunyh1128@foxmail.com; Meng XIE, E-mail: 2547693245@qq.com
†These authors contributed equally to this work.
#Corresponding author, E-mail: hcz0501@126.com
*This project was supported by National Natural Science Foundation of China (No. 81472886).

treatment of SSc[5, 6]. However, the interaction between DEGs and miRNAs and the molecular mechanism by which the dysregulated expression of miRNA affects the pathogenesis of SSc remain unclear[7]. Here, we further investigated the interactions between DEGs and miRNAs in SSc through microarray technology and bioinformatics analysis, aiming to explore their potential mechanisms of pathogenesis in SSc.

1 MATERIALS AND METHODS

1.1 Microarray Data

We downloaded a gene expression profile (GSE95065) including 31 samples (18 SSc samples and 15 control samples) from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). All SSc tissue samples were collected from SSc patients, while the normal skin tissue samples from healthy volunteers served as controls.

1.2 Identification of DEGs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) is an open-source tool that can be used to identify DEGs by comparing gene expression data from GEO series[8]. The volcano map was performed by the RStudio. An adjusted P value of <0.05 and a log fold change (FC) value of ≥1 were considered to be the appropriate criteria for the selection of DEGs. The heat map of DEGs (top 50 genes based on the difference in expression) was performed by using Network Analyst (http://www.networkanalyst.ca/)[9].

1.3 Gene Ontology and KEGG Analyses

Gene Ontology (GO) analysis is a widely used bioinformatics analysis method that constructs ontologies of gene function of individual genomic products. There are three key GO categories: molecular function (MF), biological process (BP) and cellular component (CC), which describe the attributes of genes and gene products[10, 11]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource that can annotate genome sequences or high-throughput data[12]. GO and KEGG analyses can be performed on the Database for Annotation, Visualization and Integrated Discovery (DAVID) website (https://david.ncifcrf.gov/), which is an online functional annotation tool that aims to provide a functional interpretation of the genes input into the system[13]. The DAVID was used to perform GO and KEGG analyses of the DEGs identified in GSE95065. P<0.05 was considered a significant difference.

1.4 Protein-protein Interaction (PPI) Network Analysis of DEGs

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://string-db.org/) is online software that aims to assess and integrate the interactions between proteins[14]. The DEGs were mapped to the STRING database to construct a PPI network. The cut-off criterion for the PPI network was a combined score of >0.9. Cytoscape, an open-source software tool, was applied to construct a visual network of interactions between proteins, genes and so on[15]. The DEGs were visualized with Cytoscape to obtain the hub genes, and the cut-off criterion was a node degree of >10. Then, we used Molecular Complex Detection (MCODE) to generate hub gene modules.

1.5 Prediction of MiRNAs and Transcription Factors

The miRNAs and transcription factors for the hub genes were predicted through OmicsNet (http://www.omicsnet.ca/), a visual network analytics system that aims to create and explore relationships among genes, proteins, miRNAs, transcription factors, and others in three-dimensional (3D) form[16].

1.6 Cell Culture

Adult foreskin was taken and digested with 2 mg/mL of collagenase Ⅰ for 4 h at 37°C to prepare a single-cell suspension. Then, the cells were collected by centrifugation and resuspended in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) containing 10% heat-inactivated fetal bovine serum (FBS) (Gibco). The cells were seeded in cell culture dishes and maintained at 37°C with 5% CO_2. After incubation for 72 h, adherent dermal fibroblasts were obtained, and 3–10 passages of cells were used for experiments.

1.7 MiRNA Transfection and Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)

After seeding in 12-well plates for 24 h, skin fibroblasts were transfected with 100 nmol/L hsa-miR-26b-5p inhibitor or negative control by using Lipofectamine 2000 reagent (Invitrogen, USA) for 24 h. Then, the transfection medium was removed, and the cells were recovered overnight in complete DMEM. Before stimulation, the cells were incubated in serum-reduced DMEM (1% FBS) for 24 h and then stimulated with or without 10 ng/mL TGF-β (R&D Systems, USA) for another 24 h. Next, fibroblasts were collected to analyze the expression of fibrosis-related gene mRNAs and collagen synthesis-related gene mRNAs by RT-qPCR. The primer sequences are shown in table 1. These experiments were performed at least three times. The data were input into GraphPad Prism 7 for analysis, and the results were shown as the mean±standard deviations (SD). Student's t-test was used to determine differences between the two groups, and P<0.05 was considered statistically significant.

1.8 *In Vitro* Alpha-Smooth Muscle Actin (α-SMA) Detection

Skin fibroblasts were transfected with 100 nmol/L hsa-miR-26b-5p inhibitor or negative control and incubated with 10 ng/mL TGF-β as previously mentioned. Then, the cells were fixed with 4% paraformaldehyde for 30 min and incubated with

Table 1 Primer sequences for RT-qPCR

Name	Forward	Reverse
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
α-SMA	AGCCACTGTGCCTAGCCTGAG	CACTTGAGACCTGGAGCTTGACAC
FAP	AGCAGTGGTCGGAATGTTCAAGTG	ATGTCTCGCCTCCTCTGTCTTCTG
Col1A2	CTCCATGGTGAGTTTGGTCTC	CTTCCAATAGGACCAGTAGGAC
Col4A1	TGAGTTTTATTTCGACTTGCGG	TCCTTTCAATCCTACAGAACCC
CXCL9	AAGACCTTAAACAATTTGCCCC	TGCTGAATCTGGGTTTAGACAT
CXCL13	CAAGGTGTTCTGGAGGTCTATT	TGAATTCGATCAATGAAGCGTC

anti-α-SMA antibody (1:400, Abcam, UK) overnight at 4°C. After washing with phosphate-buffered saline (PBS), the cells were incubated with Cy3-conjugated goat anti-rabbit IgG secondary antibody (1:800, Boster, China) for one h. Next, 4',6-diamidino-2-phenylindole (DAPI) incubation was applied for 5 min for nuclear staining. Images of α-SMA expression fluorescence in fibroblasts were captured under a confocal microscope.

2 RESULTS

2.1 Identification of DEGs in SSc

After the analyses of GSE95065, 783 DEGs were identified, 770 of which matched unique genes in SSc skin tissue samples as compared with normal skin samples. In addition, we identified 142 up-regulated genes and 628 down-regulated genes (fig. 1A). The expression of the top 50 dysregulated DEGs is shown by the heat map in fig. 1B.

2.2 GO and KEGG Enrichment Analyses

To further explore the function and mechanism of the 770 identified DEGs, the DAVID was used to perform GO and KEGG analyses. As shown in fig. 2A and 2B, the GO term enrichment analysis indicated that for the BP category, the up-regulated genes were mainly involved in immune response, while the down-regulated genes were involved in glycinergic synaptic transmission. In addition, MF analysis indicated that the up-regulated genes were mainly involved in heparin binding, while the down-regulated genes were mainly enriched in integrin binding. Moreover, for CC, the up-regulated genes were significantly associated with the extracellular region, whereas the down-regulated genes were related to the plasma membrane. In addition, as shown in fig. 2C, the KEGG pathway analysis revealed that the up-regulated genes took part

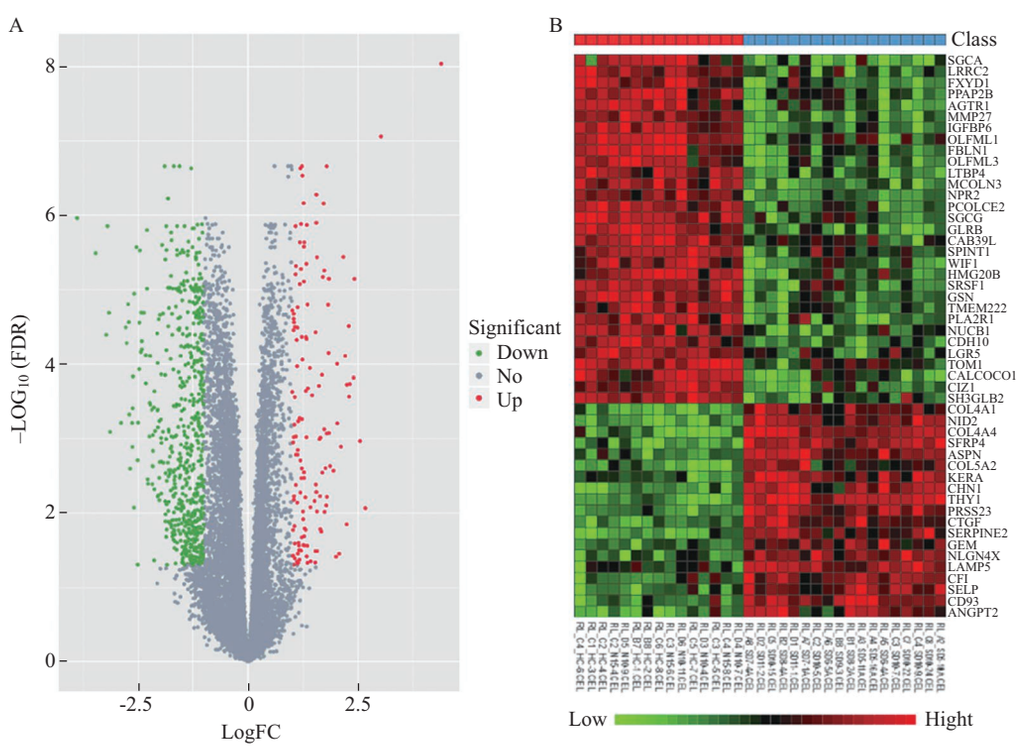

Fig. 1 Differential expression of genes between the SSc group and the control group

A: Volcano plots showing DEGs between the SSc and normal samples; B: the heatmap of DEGs (top 50 genes in terms of difference in gene expression). The 15 GEO samples (GSM) in the left columns were taken from the healthy skin tissues of healthy volunteers, while the 18 GSM samples in the right columns were collected from the lesioned skin of patients with SSc.

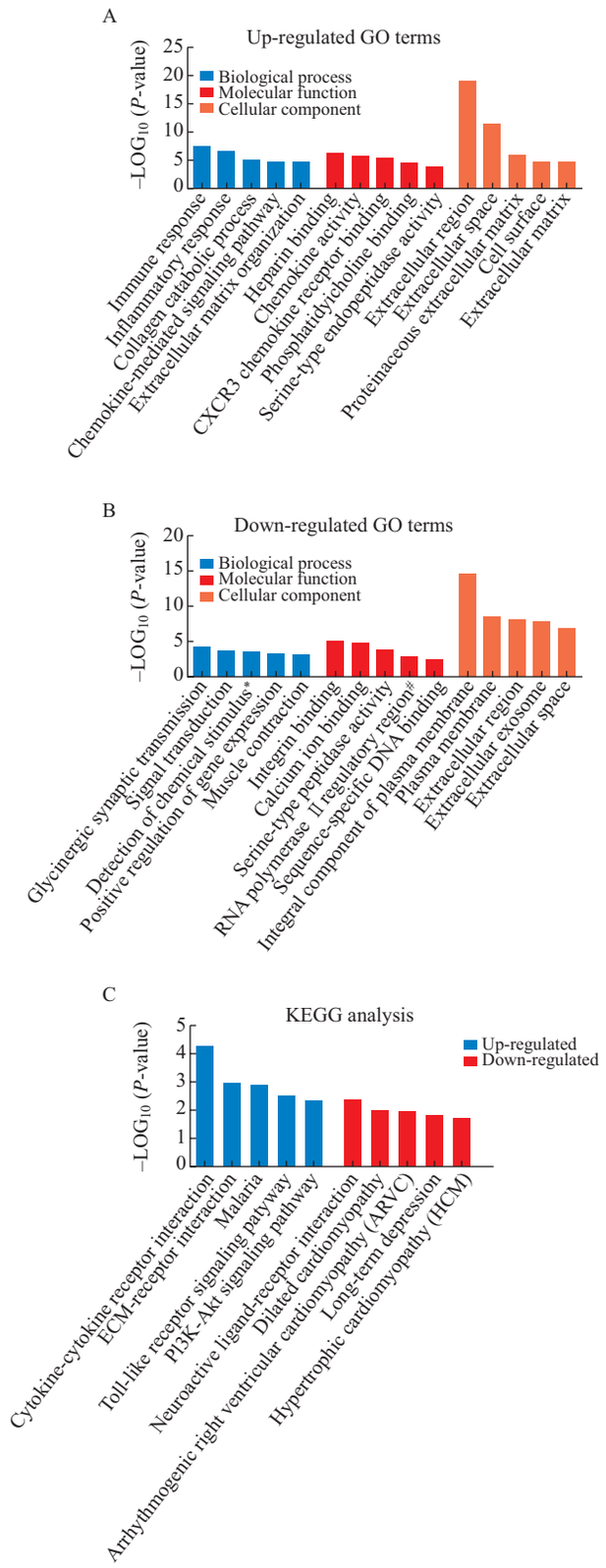

Fig. 2 Functional and pathway enrichment analyses
A: functional enrichment analysis of up-regulated DEGs in SSc; B: functional enrichment analysis of down-regulated DEGs in SSc; C: KEGG pathway enrichment analysis of DEGs in SSc. *The full name of this GO term is detection of chemical stimulus involved in sensory perception of bitter taste. #The full name of this GO term is RNA polymerase Ⅱ regulatory region sequence-specific DNA binding.

in the cytokine-cytokine receptor interaction pathway, extracellular matrix-receptor interaction and the PI3K-Akt signaling pathway, whereas the down-regulated genes mainly participated in the neuroactive ligand-receptor interaction.

2.3 PPI Network Analysis of DEGs

The 770 identified DEGs were uploaded to the STRING database to construct a PPI network (combined score >0.9), and a total of 309 nodes and 819 edges were generated (fig. 3A). The 22 highest-scoring nodes, including CXC motif chemokine ligand family members (CXCL9, CXCL10, CXCL11, and CXCL13) and CC motif chemokine ligand family members (CCL19, CCL21, and CCL25), were defined as key genes (fig. 3B). Furthermore, MCODE generated a significant module including these 22 key nodes and 231 edges.

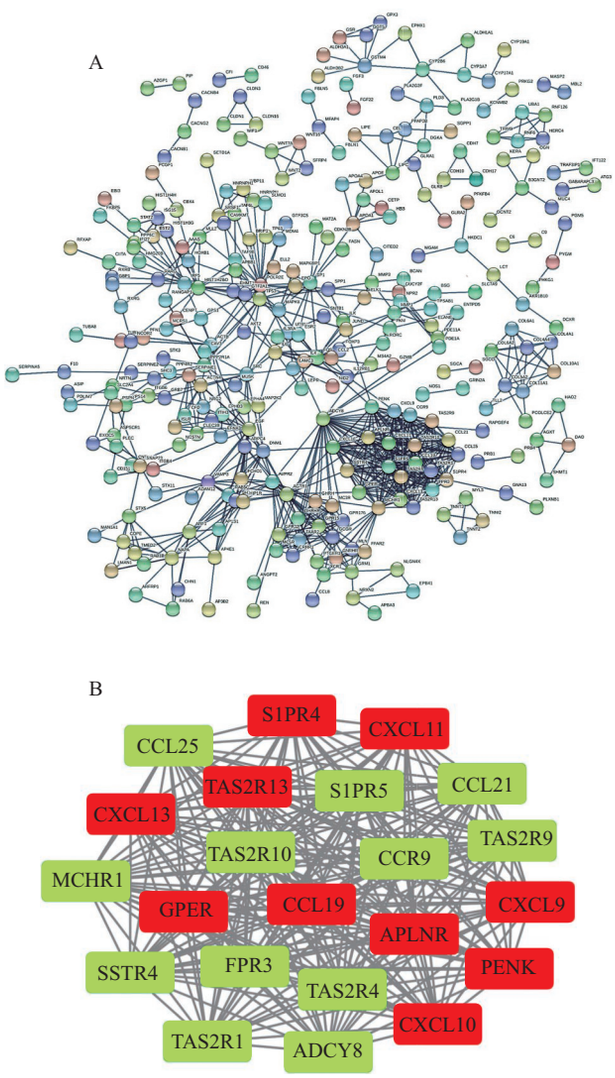

Fig. 3 PPI network and a significant module
A: The PPI network of DEGs is shown by using the STRING database. B: The significant module selected from the PPI network consists of 22 nodes and 231 edges. Red genes are considered up-regulated, while green genes are considered down-regulated.

2.4 Prediction of miRNAs and Transcription Factors

All of the hub genes were uploaded to OmicsNet, a web-based tool, to predict the miRNAs and transcription factors. As shown in fig. 4, the results, output by the powerful 3D visualization system, indicated that hsa-miR-26b-5p might target CXCL9 and CXCL13 and that hsa-miR-335-5p might target CXCL9, CCL19 and CCL25. In addition, transcription factors might also modulate the expression of several key genes, such as early growth response 1 (EGR1) and IKAROS family zinc finger 1 (IKZF1).

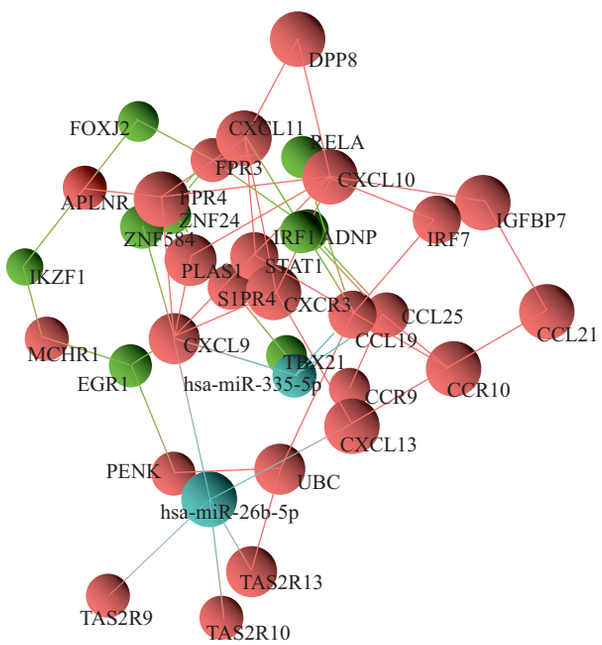

Fig. 4 MiRNAs and transcription factors potentially regulating the significant module
Red nodes are regarded as key genes of the module, blue nodes are considered miRNAs, and green nodes are represented as transcription factors.

2.5 Effects of Hsa-miR-26b-5p on Fibrosis-related Gene Expression in Fibroblasts

Based on previous studies, we hypothesized that hsa-miR-26b-5p might have an effect on fibroblast fibrosis in SSc. To test our hypothesis, we transferred the hsa-miR-26b-5p inhibitor into human fibroblasts and then tested fibrosis-related gene expression by RT-qPCR and α-SMA expression by immunofluorescence after TGF-β stimulation. As a result, the decreased hsa-miR-26b-5p expression led to the decreased expression of α-SMA mRNA and fibroblast activation protein (FAP) mRNA, two known biomarkers of fibrosis, compared to the miR-NC group after TGF-β stimulation (fig. 5A and 5B). Accordingly, the collagen-type Ⅰ alpha 2 (Col1A2) and collagen-type Ⅳ alpha 1 (Col4A1) mRNA expression of hsa-miR-26b-5p inhibitor group was also significantly lower in TGF-β-activated fibroblasts than that of the miR-NC group (fig. 5C and 5D). In addition, transfection with the hsa-miR-26b-5p inhibitor reduced the mRNA expression of CXCL9 and CXCL13 (fig. 5E and 5F). Moreover, after TGF-β stimulation, the fluorescence intensity (FI) of α-SMA in the hsa-miR-26b-5p inhibitor group was lower than that in the miR-NC group (fig. 5G and 5H).

3 DISCUSSION

Although researchers have been investigating the mechanism of SSc over the past few decades and many experiments have been conducted, our understanding of the mechanism of SSc is still limited. Therefore, exploring the mechanisms of SSc progression to improve the therapy of the disease is necessary. Fortunately, with the development of microarray technology, the general genetic alterations of SSc are easier to find, which may greatly help improve the treatment of the disease.

In this study, 770 DEGs in SSc were identified, including 142 up-regulated genes and 628 down-regulated genes. The up-regulated genes were significantly enriched in immune response, while the down-regulated genes were involved in glycinergic synaptic transmission. Moreover, we identified several high-degree genes, including CXCL9 and CXCL13, by using the OmicsNet tool to create a PPI network and found that hsa-miR-26b-5p might target both genes.

CXCL9, a member of the CXC family, plays a crucial role in the chemotaxis of immune cells and is related to the pathogenesis of various diseases[17]. In the past few years, much experimental evidence has revealed that CXCL9 is closely related to the diagnosis, treatment and prognosis of cancer[18]. Various studies have found an increase in serum CXCL9 levels associated with many skin diseases, such as psoriasis[19], sarcoidosis[20], cutaneous lupus erythematosus[21], and other diseases. Moreover, a recent study showed that CXCL9 was a promising biomarker of disease activity in limited cutaneous scleroderma (morphea)[22]. Several studies also reported that CXCL9 was significantly elevated in SSc skin and serum compared to the controls[23–26], which was consistent with the results of our bioinformatics analysis.

CXCL13, the major B-cell chemoattractant, is also a member of the CXC family and induces chemotaxis of lymphocytes through its receptor CXCR5[27, 28]. As previously reported, CXCL13 is involved in multiple human autoimmune diseases, such as rheumatoid arthritis[29], systemic lupus erythematosus[30] and Sjögren's syndrome[31]. Recently, levels of CXCL13 were proven to increase in the serum of SSc patients, and CXCL13 likely leads to tissue fibrosis, vascular anomalies and immune abnormalities in SSc[32, 33]. In addition, CXCL13 may be a valuable biomarker for the detection and treatment of cancers[34–36].

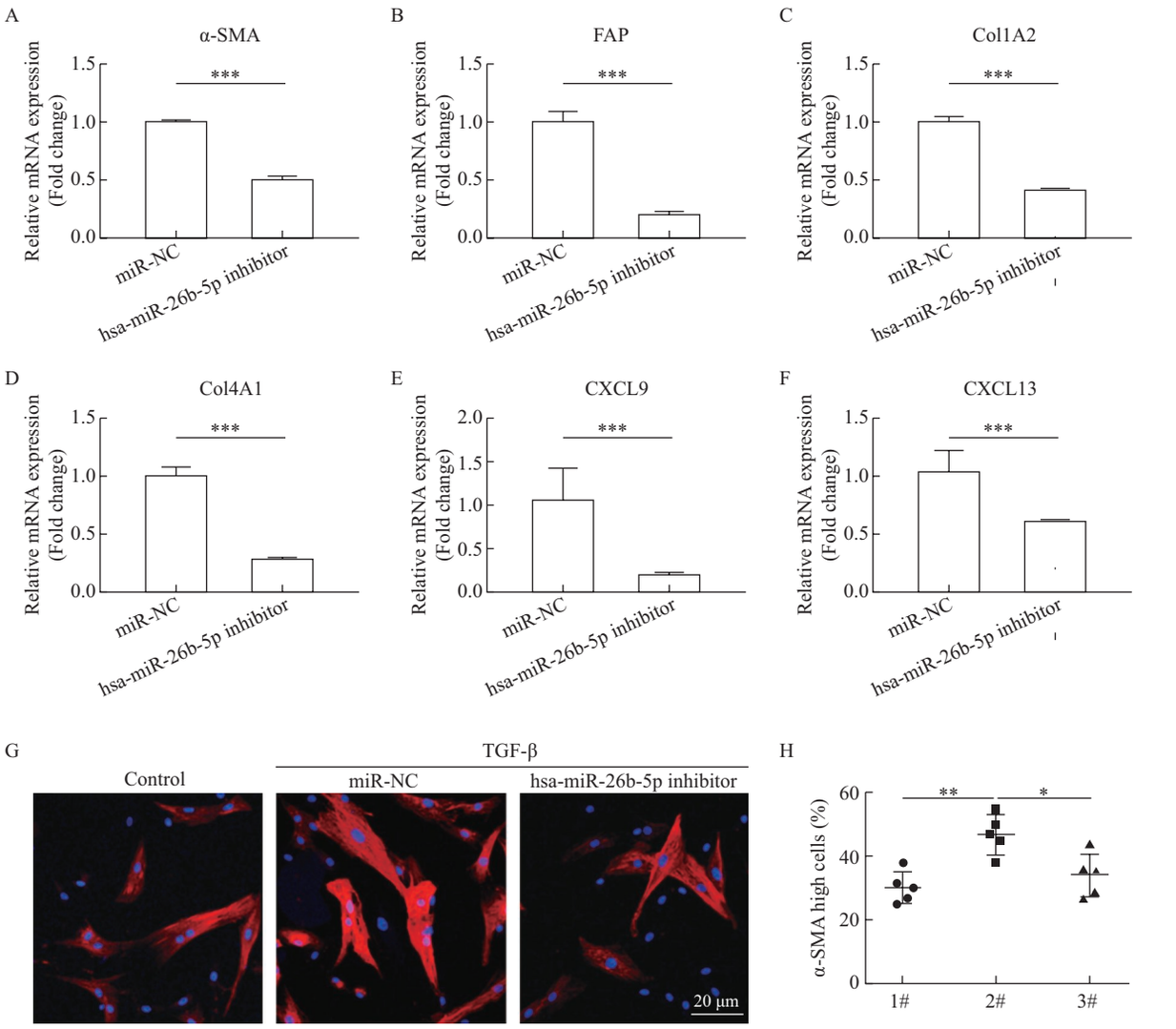

Fig. 5 Hsa-miR-26b-5p inhibitor regulated fibroblast fibrosis

A–F: The relative mRNA expression of α-SMA (A), FAP (B), Col1A2 (C), Col4A1 (D), CXCL9 (E) and CXCL13 (F) in fibroblasts with a low expression of hsa-miR-26b-5p after TGF-β stimulation for 24 h. G–H: Images of α-SMA (red) expression and proportion of highly expressed α-SMA cells. 1#, 2# and 3#: the control group, the miR-NC group and the hsa-miR-26b-5p inhibitor group after TGF-β stimulation, respectively. All experiments were performed in triplicate. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.0001$

According to our work, CXCL13 was also found to be a high-expression hub gene. However, the molecular mechanism of CXCL9 and CXCL13 and the interaction between them have rarely been explored in SSc.

MiRNAs are a class of 18–25-nt-long noncoding RNAs that exist in many organisms. MiRNAs regulate gene expression at the posttranscriptional level by complementary binding to the 3' noncoding region (3'-UTR) of their target mRNA molecule[37]. They are involved in a series of important steps in all life processes, including early development, cell proliferation, differentiation and apoptosis, and substance metabolism, and play an important role in the pathogenesis of many diseases. In recent years, increasing evidence has revealed that abnormal miRNA expression may play a significant role in the pathogenesis of SSc[38]. In this study, we uploaded the hub genes to OmicsNet and found that the two hub genes CXCL9 and CXCL13 might be targeted by hsa-miR-26b-5p in SSc. Hsa-miR-26b-5p has been reported to participate in the pathogenesis of several diseases, such as multiple myeloma[39], intrahepatic cholangiocarcinoma[40] and diabetic cardiomyopathy[41]. Our study found that transfection with hsa-miR-26b-5p inhibitor in TGF-β-activated fibroblasts could modulate fibrosis-related gene expression. Moreover, the reduced CXCL9 and CXCL13 mRNA expression suggested that hsa-miR-26b-5p down-regulation might inhibit the fibrosis process by targeting CXCL9 and CXCL13. However, a recent study reported that hsa-miR-26b levels were significantly decreased in the serum of SSc patients as compared with normal

controls, suggesting that a down-regulation in hsa-miR-26b might lead to the promotion of fibrosis[42]. The above mentioned discrepancy in the function of hsa-miR-26b in SSc might be due to racial differences since the lack of appropriate demonstration of variability in the clinical characteristics of participants. In addition, RT-qPCR results showed that CXCL9 and CXCL13 mRNA expression was down-regulated after hsa-miR-26b-5p inhibitor transfection, which was contrary to our expected results. We supposed that hsa-miR-26b-5p inhibitor might induce high expression of negative regulators in NF-κB or JAK/STAT pathway[43], leading to the inhibition of CXCL9 and CXCL13 expression. We only observed the changes in fibrosis related-gene expression induced by hsa-miR-26b-5p inhibitor transfection, but the mechanism by which the hsa-miR-26b-5p regulates the fibrosis process of scleroderma fibroblasts is still not very clear, which is worth further exploration.

In summary, through bioinformatics approaches, our study identified 770 unique DEGs and several key genes in SSc compared to normal controls. We also predicted that hsa-miR-26b-5p could regulate fibroblast fibrosis, which might be related to targeting CXCL9 and CXCL13. This finding might provide new insight into the treatment of SSc.

Conflict of Interest Statement

The authors declare no competing interests.

REFERENCES

1 Barnes J, Mayes MD. Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. Curr Opin Rheumatol, 2012,24(2):165-170

2 Denton CP. Advances in pathogenesis and treatment of systemic sclerosis. Clin Med (Lond), 2016,16(1):55-60

3 Denton CP. Systemic sclerosis: from pathogenesis to targeted therapy. Clin Exp Rheumatol, 2015,33(4 Suppl 92):S3-S7

4 Zuo X, Zhang L, Luo H, *et al*. Systematic approach to understanding the pathogenesis of systemic sclerosis. Clin Genet, 2017,92(4):365-371

5 Sing T, Jinnin M, Yamane K, *et al*. microRNA-92a expression in the sera and dermal fibroblasts increases in patients with scleroderma. Rheumatology (Oxford), 2012,51(9):1550-1556

6 Zhu H, Luo H, Li Y, *et al*. MicroRNA-21 in scleroderma fibrosis and its function in TGF-beta-regulated fibrosis-related genes expression. J Clin Immunol, 2013,33(6):1100-1109

7 Miao CG, Xiong YY, Yu H, *et al*. Critical roles of microRNAs in the pathogenesis of systemic sclerosis: New advances, challenges and potential directions. Int Immunopharmacol, 2015,28(1):626-633

8 Clough E, Barrett T. The Gene Expression Omnibus Database. Methods Mol Biol, 2016,1418:93-110

9 Xia J, Gill EE, Hancock RE. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat Protoc, 2015,10(6):823-844

10 Gene Ontology C. The Gene Ontology (GO) project in 2006. Nucleic Acids Res, 2006,34(Database issue):D322-326

11 Lewis SE. The Vision and Challenges of the Gene Ontology. Methods Mol Biol, 2017,1446:291-302

12 Kanehisa M, Sato Y, Kawashima M, *et al*. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res, 2016,44(D1):D457-462

13 Huang DW, Sherman BT, Tan Q, *et al*. DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Res, 2007,35(Web Server issue):W169-175

14 Szklarczyk D, Franceschini A, Wyder S, *et al*. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res, 2015,43(Database issue):D447-452

15 Su G, Morris JH, Demchak B, *et al*. Biological network exploration with Cytoscape 3. Curr Protoc Bioinformatics, 2014,47:8.13.1-24

16 Zhou G, Xia J. OmicsNet: a web-based tool for creation and visual analysis of biological networks in 3D space. Nucleic Acids Res, 2018,46(W1):W514-W522

17 Elia G, Guglielmi G. CXCL9 chemokine in ulcerative colitis. Clin Ter, 2018,169(5):e235-e241

18 Ding Q, Lu P, Xia Y, *et al*. CXCL9: evidence and contradictions for its role in tumor progression. Cancer Med, 2016,5(11):3246-3259

19 Duarte GV, Boeira V, Correia T, *et al*. Osteopontin, CCL5 and CXCL9 are independently associated with psoriasis, regardless of the presence of obesity. Cytokine, 2015,74(2):287-292

20 Su R, Nguyen ML, Agarwal MR, *et al*. Interferon-inducible chemokines reflect severity and progression in sarcoidosis. Respir Res, 2013,14:121

21 Wenzel J, Tuting T. Identification of type I interferon-associated inflammation in the pathogenesis of cutaneous lupus erythematosus opens up options for novel therapeutic approaches. Exp Dermatol, 2007,16(5):454-463

22 O'Brien JC, Rainwater YB, Malviya N, *et al*. Transcriptional and Cytokine Profiles Identify CXCL9 as a Biomarker of Disease Activity in Morphea. J Invest Dermatol, 2017,137(8):1663-1670

23 Rabquer BJ, Tsou PS, Hou Y, *et al*. Dysregulated expression of MIG/CXCL9, IP-10/CXCL10 and CXCL16 and their receptors in systemic sclerosis. Arthritis Res Ther, 2011,13(1):R18

24 Hasegawa M, Fujimoto M, Matsushita T, *et al*. Serum chemokine and cytokine levels as indicators of disease activity in patients with systemic sclerosis. Clin Rheumatol, 2011,30(2):231-237

25 Hasegawa M, Asano Y, Endo H, *et al*. Serum chemokine levels as prognostic markers in patients with early systemic sclerosis: a multicenter, prospective, observational study. Mod Rheumatol, 2013,23(6):1076-1084

26 Kuo PT, Zeng Z, Salim N, *et al*. The Role of CXCR3 and Its Chemokine Ligands in Skin Disease and Cancer. Front Med (Lausanne), 2018,5:271

27 Fazilleau N, Mark L, McHeyzer-Williams LJ, *et al*.

Follicular helper T cells: lineage and location. Immunity, 2009,30(3):324-335
28 Lim HW, Hillsamer P, Kim CH. Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell–driven B cell responses. J Clin Invest, 2004,114(11):1640-1649
29 Shi K, Hayashida K, Kaneko M, *et al*. Lymphoid Chemokine B Cell-Attracting Chemokine-1 (CXCL13) Is Expressed in Germinal Center of Ectopic Lymphoid Follicles within the Synovium of Chronic Arthritis Patients. J Immunol, 2001,166(1):650-655
30 Ishikawa S, Sato T, Abe M, *et al*. Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by C11b+CD11c+ dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. J Exp Med, 2001,193(12):1393-1402
31 Kramer JM, Klimatcheva E, Rothstein TL. CXCL13 is elevated in Sjogren's syndrome in mice and humans and is implicated in disease pathogenesis. J Leukoc Biol, 2013,94(5):1079-1089
32 Wutte N, Kovacs G, Berghold A, *et al*. CXCL13 and B-cell activating factor as putative biomarkers in systemic sclerosis. Br J Dermatol, 2013,169(3):723-725
33 Taniguchi T, Miyagawa T, Toyama S, *et al*. CXCL13 produced by macrophages due to Fli1 deficiency may contribute to the development of tissue fibrosis, vasculopathy and immune activation in systemic sclerosis. Exp Dermatol, 2018,27(9):1030-1037
34 Ammirante M, Shalapour S, Kang Y, *et al*. Tissue injury and hypoxia promote malignant progression of prostate cancer by inducing CXCL13 expression in tumor myofibroblasts. Proc Natl Acad Sci USA, 2014,111(41):14776-14781
35 Wei Y, Lin C, Li H, *et al*. CXCL13 expression is prognostic and predictive for postoperative adjuvant chemotherapy benefit in patients with gastric cancer. Cancer Immunol Immunother, 2018,67(2):261-269
36 Zhu Z, Zhang X, Guo H, *et al*. CXCL13-CXCR5 axis promotes the growth and invasion of colon cancer cells via PI3K/AKT pathway. Mol Cell Biochem, 2015,400(1-2):287-295
37 Cai Y, Yu X, Hu S, *et al*. A brief review on the mechanisms of miRNA regulation. Genomics, Proteomics Bioinformatics, 2009,7(4):147-154
38 Miao CG, Xiong YY, Yu H, *et al*. Critical roles of microRNAs in the pathogenesis of systemic sclerosis: New advances, challenges and potential directions. Int Immunopharmacol, 2015,28(1):626-633
39 Jia CM, Tian YY, Quan LN, *et al*. miR-26b-5p suppresses proliferation and promotes apoptosis in multiple myeloma cells by targeting JAG1. Pathol Res Pract, 2018,214(9):1388-1394
40 Fan F, Lu J, Yu W, *et al*. MicroRNA-26b-5p regulates cell proliferation, invasion and metastasis in human intrahepatic cholangiocarcinoma by targeting S100A7. Oncol Lett, 2018,15(1):386-392
41 Tang CM, Zhang M, Huang L, *et al*. CircRNA_000203 enhances the expression of fibrosis-associated genes by derepressing targets of miR-26b-5p, Col1a2 and CTGF, in cardiac fibroblasts. Sci Rep, 2017,7:40342
42 Chouri E, Servaas NH, Bekker CPJ, *et al*. Serum microRNA screening and functional studies reveal miR-483-5p as a potential driver of fibrosis in systemic sclerosis. J Autoimmun, 2018,89:162-170
43 Richmond A. Nf-kappa B, chemokine gene transcription and tumour growth. Nat Rev Immunol, 2002,2(9):664-674

(Received Mar. 8, 2019; revised June 4, 2019)