

# Bioinformatics study on different gene expression profiles of fibroblasts and vascular endothelial cells in keloids

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## Abstract

Keloid is a benign fibroproliferative skin tumor. The respective functions of fibroblasts and vascular endothelial cells in keloid have not been fully studied. The purpose of this study is to identify the respective roles and key genes of fibroblasts and vascular endothelial cells in keloids, which can be used as new targets for diagnosis or treatment.

The microarray datasets of keloid fibroblasts and vascular endothelial cells were obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were screened out. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used for functional enrichment analysis. The search tool for retrieval of interacting genes and Cytoscape were used to construct protein-protein interaction (PPI) networks and analyze gene modules. The hub genes were screened out, and the relevant interaction networks and biological process analysis were carried out.

In fibroblasts, the DEGs were significantly enriched in collagen fibril organization, extracellular matrix organization and ECM-receptor interaction. The PPI network was constructed, and the most significant module was selected, which is mainly enriched in ECM-receptor interaction. In vascular endothelial cells, the DEGs were significantly enriched in cytokine activity, growth factor activity and transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling pathway. Module analysis was mainly enriched in TGF- $\beta$  signaling pathway. Hub genes were screened out separately.

In summary, the DEGs and hub genes discovered in this study may help us understand the molecular mechanisms of keloid, and provide potential targets for diagnosis and treatment.

**Abbreviations:** DAVID = database for annotation, visualization and integrated discovery, DEGs = differentially expressed genes, GEO = Gene Expression Omnibus, GO = gene ontology, KEGG = kyoto encyclopedia of genes and genomes, MCC = maximal clique centrality algorithm, PDGF = platelet-derived growth factor, PPI = protein-protein interaction, TGF- $\beta$  = transforming growth factor- $\beta$ .

**Keywords:** bioinformatics, differentially expressed genes, fibroblasts, hub genes, keloid, protein-protein interactions, vascular endothelial cells

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All data were downloaded from public databases. No patients were involved in our study and there was no written informed consent.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## 1. Introduction

Keloid is a benign fibroproliferative dermal tumor, characterized by excessive deposition of extracellular matrix, especially collagen, originated from skin trauma or spontaneous, and often grows beyond the normal range of the original wound, and often continues to grow, and rarely subsides spontaneously.<sup>[1,2]</sup> At present, the exact etiology of keloid disease is still unclear, but some possible factors have been identified. Skin trauma was once considered a necessary and important irritation event, but recent studies have shown that keloid scars can also occur spontaneously.<sup>[3]</sup> The genetic component is recognized in keloid diseases. People with darker skin have a higher risk of developing keloids. Individuals in Africa and Asia are more likely to develop keloids. Caucasians are less likely to develop. There are no reported cases of albinism.<sup>[4]</sup>

During normal and abnormal wound healing, fibroblasts are the main source of extracellular matrix and collagen. These effects are mainly driven by various cytokines, such as transforming growth factor- $\beta$  (TGF- $\beta$ ), platelet-derived growth factor (PDGF), fibroblast growth factor  $\beta$  and insulin-like growth factor I.<sup>[5]</sup> Fibroblasts isolated from keloid tissues are more sensitive to TGF- $\beta$ 1, PDGF and insulin-like growth factor I in

vitro than normal skin fibroblasts, leading to high expression of collagen and extracellular matrix-associated genes.<sup>[6–8]</sup>

Several studies have shown that in the process of keloid formation, the abnormal functions of vascular endothelial cells and their interactions with immune cells and fibrotic cells may affect the deposition of extracellular matrix and the progression of inflammation.<sup>[9,10]</sup> Disordered angiogenesis, low capillary density, microvascular occlusion, flat lumen and other pathological changes are likely to lead to hypoxia in keloid tissues, which in turn leads to abnormal metabolism of extracellular matrix.<sup>[11,12]</sup>

Surgical resection is currently the main method for the treatment of keloids, but when used as the only treatment, most patients will relapse, and usually lead to stronger collagen accumulation and larger lesion formation. In most cases, the combination of surgery and several adjuvant treatments, such as laser ablation, radiotherapy, compression therapy and corticosteroid injection therapy, can make the lesions get better treatment, but the degree of recurrence varies.<sup>[13–19]</sup> Few targeted therapies have been developed and have not yet been used in clinical treatments, so it is very important to understand the pathological and molecular mechanisms of keloids.<sup>[20,21]</sup> New diagnostic or therapeutic targets are urgently needed to be explored.

The rapid development of genechip and next-generation sequencing technologies has enabled us to understand diseases at the molecular level. In this study, the microarray datasets of keloid fibroblasts and vascular endothelial cells were obtained from the GEO database, and the differentially expressed genes (DEGs) were screened out. GO and KEGG were used for functional enrichment analysis. String and Cytoscape were used to construct the PPI networks and analyze gene modules. The hub genes were screened out, and the relevant interaction networks and biological process analysis were carried out. In conclusion, in keloid fibroblasts, a total of 375 DEGs were identified; in keloid vascular endothelial cells, a total of 239 DEGs were identified, and 10 hub genes were selected from the 2 types of cells respectively, which may be the diagnostic or therapeutic targets for keloid.

## 2. Methods

### 2.1. Microarray data

GEO (<http://www.ncbi.nlm.nih.gov/geo>) database is a public functional genomics data repository.<sup>[22]</sup> The latest gene expression datasets of fibroblasts (GSE145725) and vascular endothelial cells (GSE121618) in keloids were downloaded from GEO.<sup>[23,24]</sup> The GSE145725 dataset contains 19 samples, of which 9 are keloid fibroblasts and 10 are normal dermal fibroblasts. The GSE121618 dataset contains 11 samples, of which 5 are keloid endothelial cells and 6 are normal tissue endothelial cells.

### 2.2. Identification of differentially expressed genes

The DEGs in the samples were identified by the limma package (version 3.46.0) of R (R x 64 4.0.4; <https://www.r-project.org>). The Benjamini & Hochberg False Discovery Rate correction method was used to correct the *P* values. Fold change >2 and adj. *P*-value <.05 were considered to be statistically significant. In addition, hierarchical clustering analysis was performed on

DEGs of fibroblasts and endothelial cells, and the heatmap package of R was used to visualize them, and the heatmaps were plotted.

### 2.3. Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (version 6.8) (<https://david.ncifcrf.gov>) provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes.<sup>[25]</sup> GO annotation is a statement about the function of a particular gene. These statements comprise a “snapshot” of biological knowledge.<sup>[26]</sup> KEGG is a database resource for understanding high-level functions of the biological system from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies.<sup>[27]</sup> These tools were used to analyze the biological functions and pathways of the DEGs. *P* < .05 was considered statistically significant.

### 2.4. Protein-protein interaction network construction and module analysis

The search tool for retrieval of interacting genes (version 11.0) (<https://www.string-db.org>) is a database of known and predicted protein-protein interactions, which was used in this study to construct the PPI network of the DEGs.<sup>[28]</sup> All settings were default, and the minimum interaction scores of 0.4 was considered statistically significant. Cytoscape (version 3.8.0) is an open-source software platform, used to visualize molecular interaction networks.<sup>[29]</sup> In this study, Cytoscape was used to draw the PPI networks, and its plug-in MCODE was used to filter the most significant modules. The selection criteria were as follows: degree cut-off=2, node score cut-off=0.2, K-core=2, maximum depth=100, MCODE score>5. Then, GO and KEGG enrichment analysis on the genes in the most important modules are performed.

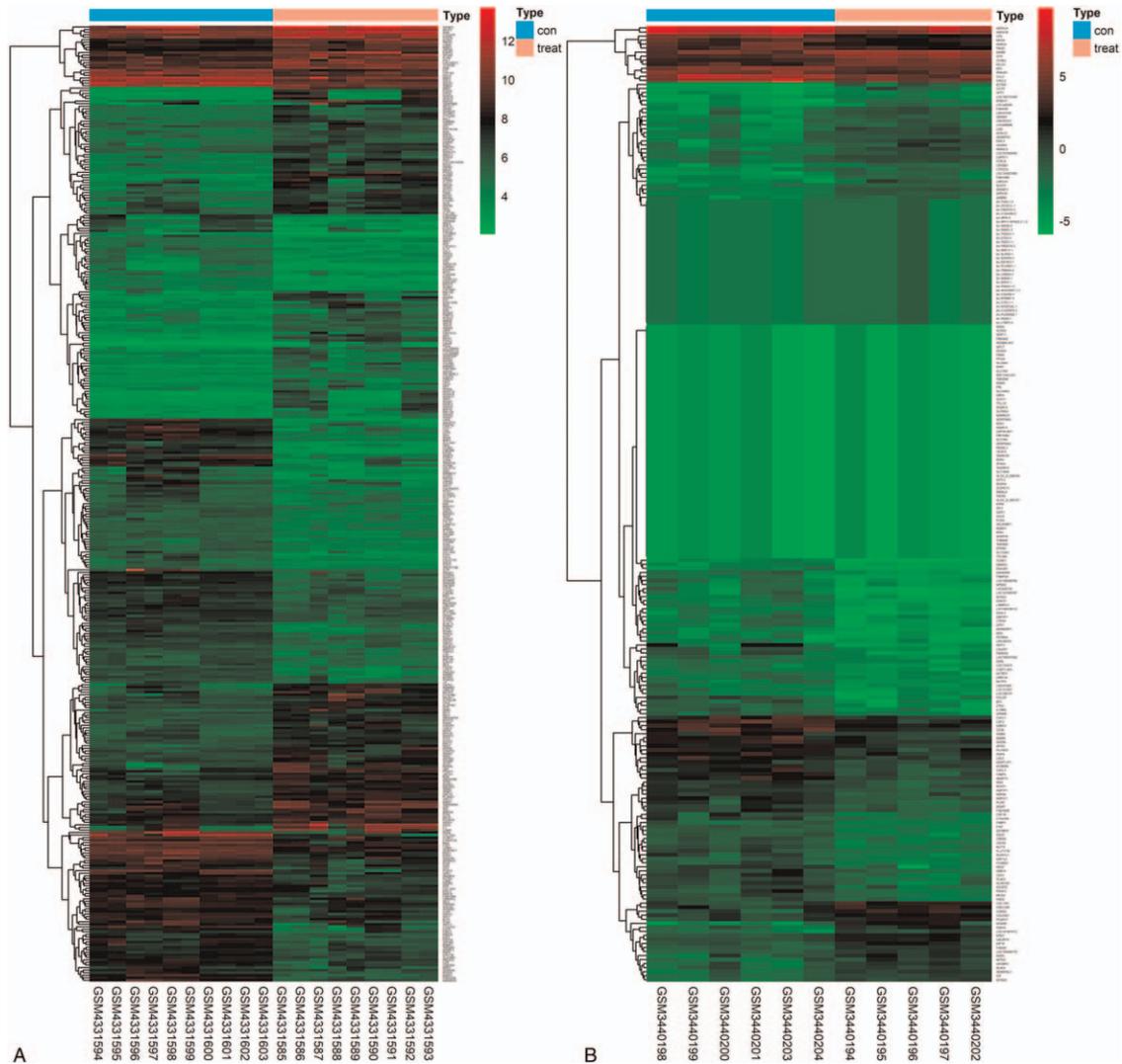
### 2.5. Selection and analysis of the hub gene

The hub genes were selected using the cytoHubba plug-in, and the top 10 genes were selected as hub genes using the Maximal Clique Centrality algorithm (MCC) method, and the interaction networks of the hub genes was analyzed as well. Subsequently, the biological process of hub genes was analyzed and visualized using Biological Networks Gene Ontology tool plug-in.

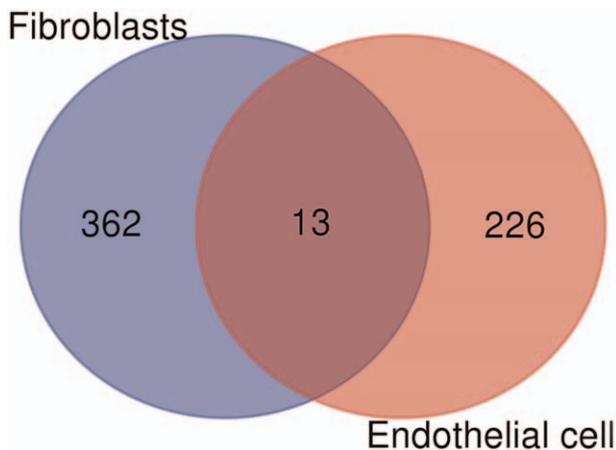
## 3. Results

### 3.1. Identification of differentially expressed genes

After normalizing the data in the microarray, a total of 375 DEGs were identified in the fibroblast samples, of which 175 were up-regulated and 200 were down-regulated (Fig. 1A); a total of 239 DEGs were identified in the vascular endothelial cell samples, of which 89 were up-regulated and 150 were down-regulated (Fig. 1B). In these 2 types of cell, only 13 genes were differentially expressed at the same time, as shown in the Venn diagram (Fig. 2). Most genes were differentially expressed in only one type of cell, which indicates that fibroblasts and vascular endothelial cells may play different roles in the occurrence and development of keloids.



**Figure 1.** Heatmap of the DEGs in fibroblasts (A) and vascular endothelial cells (B). Expression up-regulation is marked as red, and expression down-regulation is marked as green. The "treat" groups are keloid tissues, and the "con" groups are normal skin tissues. DEGs = differentially expressed genes.

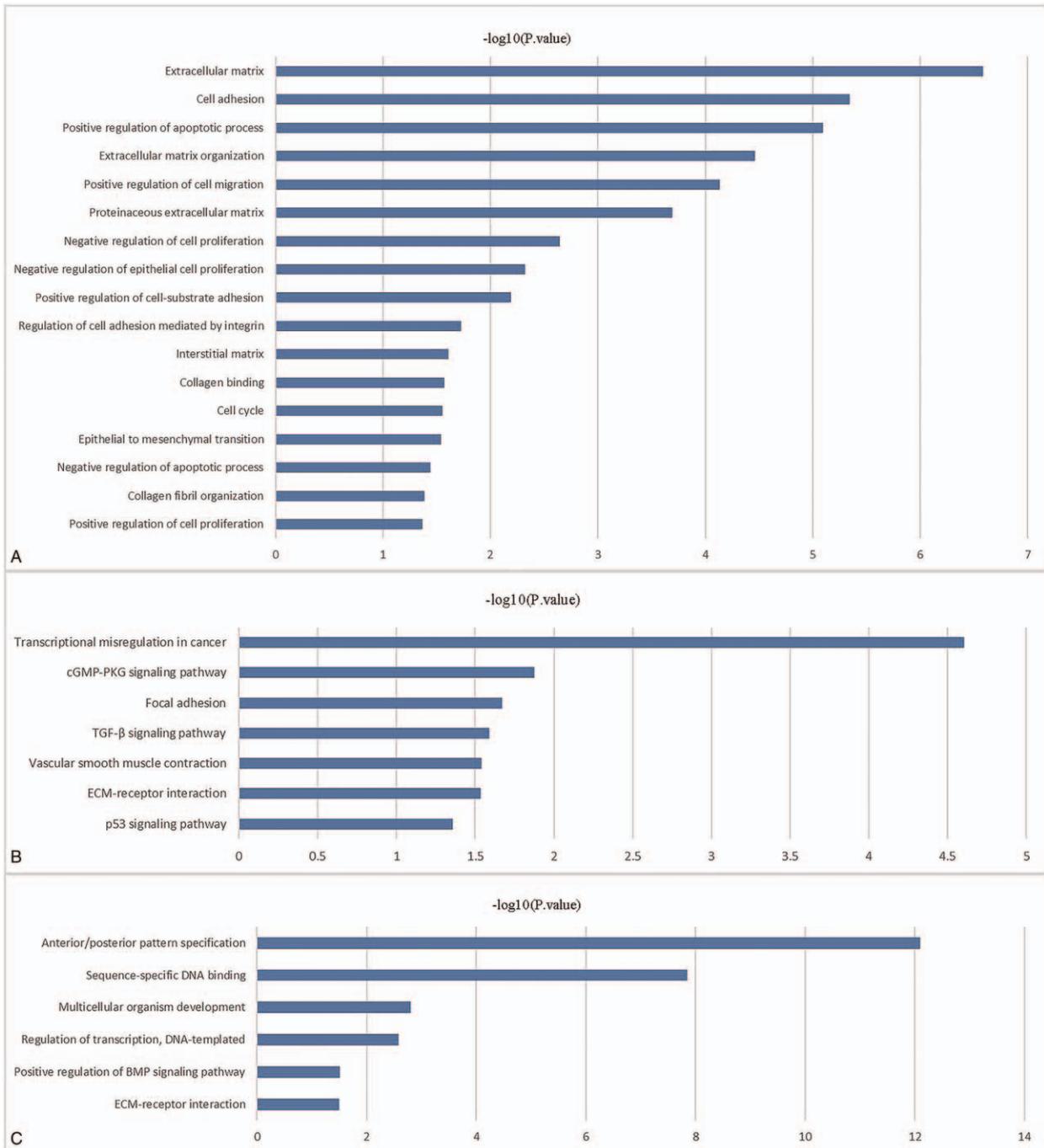


**Figure 2.** Venn diagram of the genes that are differentially expressed in two types of cell at the same time.

### 3.2. Functional enrichment analysis of the differentially expressed genes

To further explore the biological functions of the DEGs, GO and KEGG enrichment analysis was performed using DAVID. In fibroblasts, GO enrichment analysis results showed that these DEGs are significantly enriched in collagen fibril organization, extracellular matrix organization, cell adhesion and regulation of cell adhesion mediated by integrin. KEGG enrichment analysis results show that these DEGs are significantly enriched in ECM-receptor interaction, transcriptional misregulation in cancer, focal adhesion and TGF-β signaling pathway (Fig. 3A–B).

In vascular endothelial cells, GO enrichment analysis results showed that these DEGs are significantly enriched in cytokine activity, receptor ligand activity, signaling receptor activator activity and cytokine receptor binding. KEGG enrichment analysis results show that these DEGs are significantly enriched in TGF-β signaling pathway, cytokine-cytokine receptor interaction, PPAR signaling pathway and IL-17 signaling pathway (Fig. 4A–B).



**Figure 3.** GO (A) and KEGG (B) enrichment analysis of the DEGs in fibroblasts. GO and KEGG enrichment analysis of the DEGs in the most significant module (C) in fibroblasts. DEGs = differentially expressed genes. GO = Gene Ontology. KEGG = Kyoto Encyclopedia of Genes and Genomes.

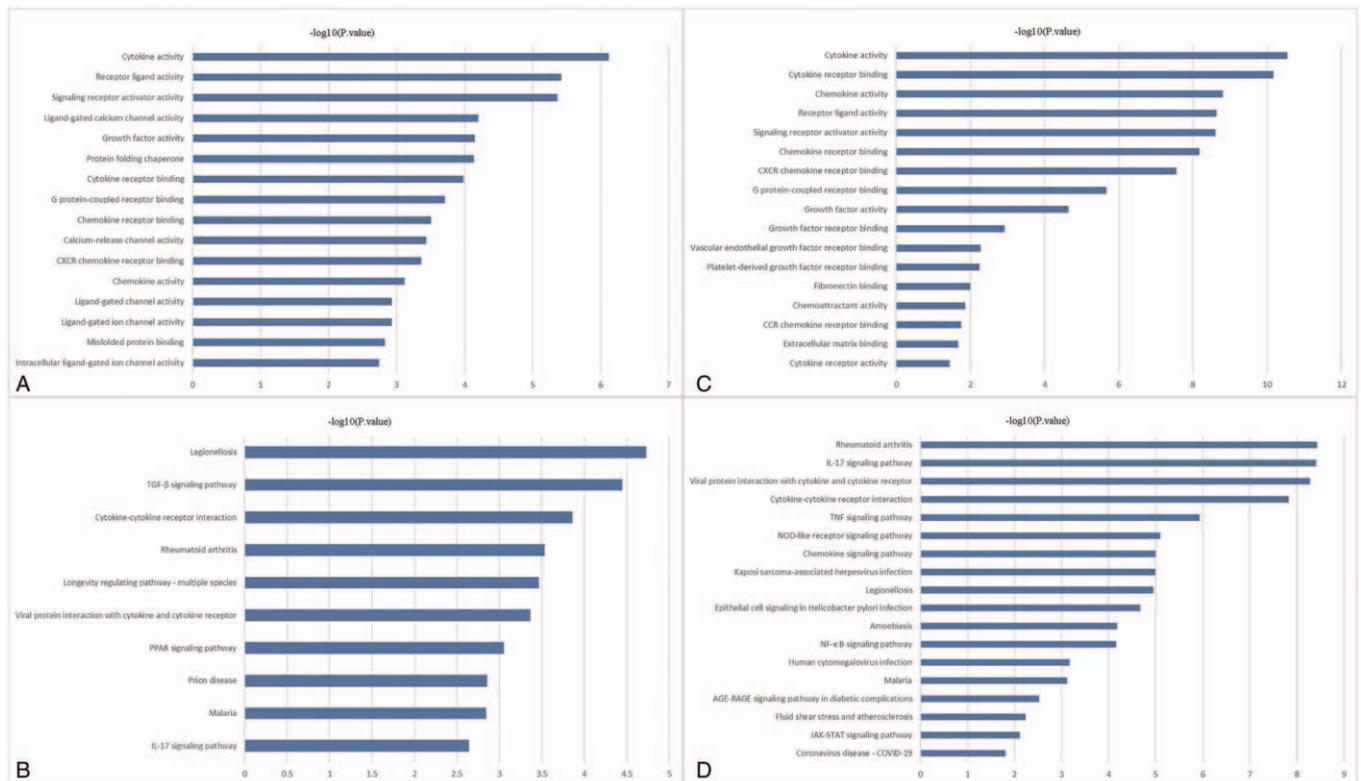
### 3.3. Protein-protein interaction network construction and module analysis

The PPI networks of the DEGs in fibroblasts and vascular endothelial cells were constructed separately (Fig. 5 A and C), and the most significant modules were selected (Fig. 5 B and D). Functional enrichment analysis of the genes in the modules was performed using DAVID. In fibroblasts, the results showed that the genes are mainly enriched in regulation of transcription, regulation of BMP signaling pathway and ECM-receptor

interaction (Fig. 3C); in vascular endothelial cells, the results showed that the genes are mainly enriched in vascular endothelial growth factor receptor binding, fibronectin binding and cytokine activity (Fig. 4C–D).

### 3.4. Hub gene selection and analysis

The MCC method of cytoHubba was used to select hub genes. In fibroblasts and vascular endothelial cells, according to the ranking, the top 10 genes in each are regarded as hub genes. In



**Figure 4.** GO (A) and KEGG (B) enrichment analysis of the DEGs in vascular endothelial cells. GO (C) and KEGG (D) enrichment analysis of the DEGs in the most significant module in vascular endothelial cells. DEGs = differentially expressed genes. GO = Gene Ontology. KEGG = Kyoto Encyclopedia of Genes and Genomes.

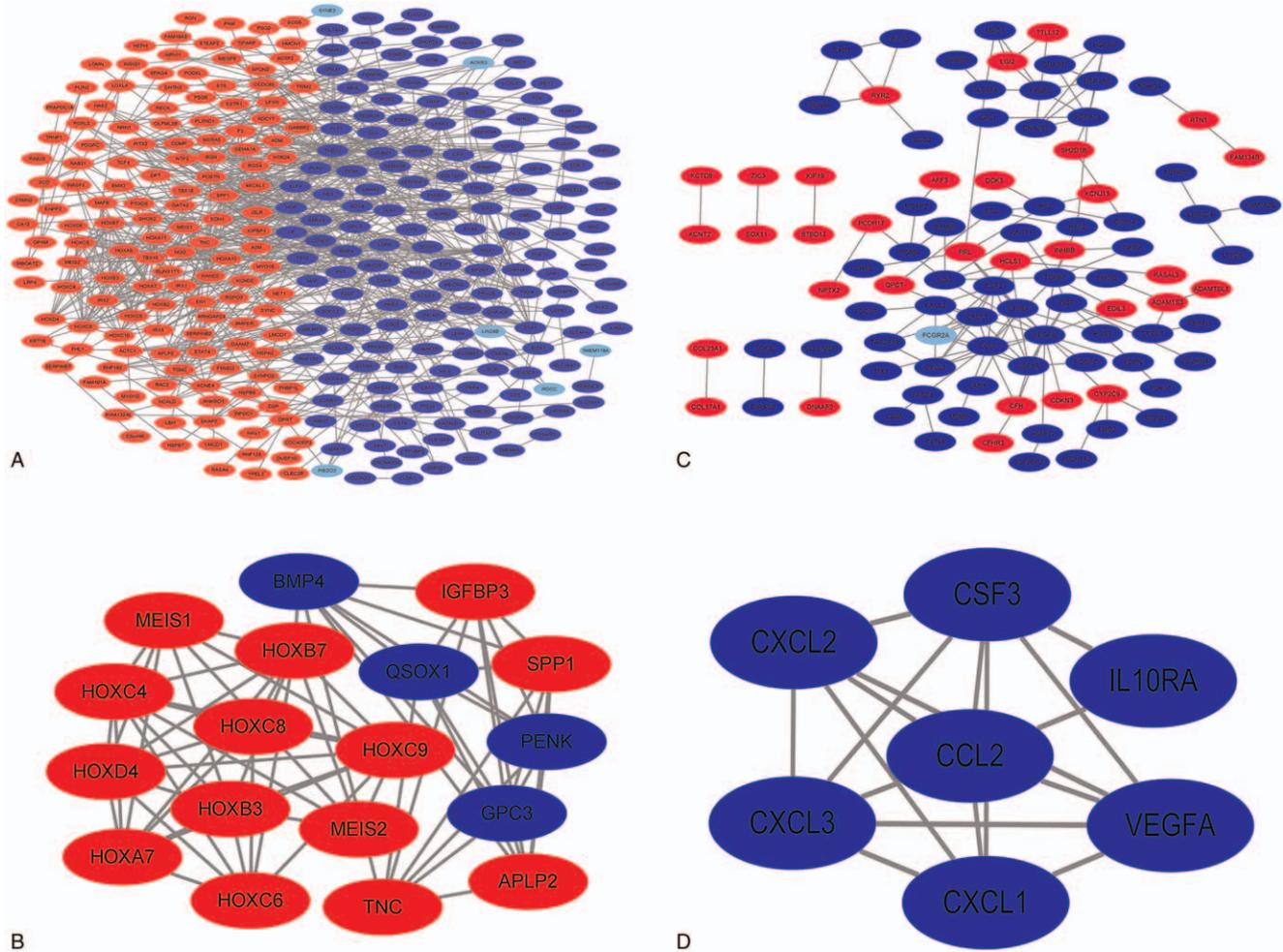
fibroblasts, the hub genes are as follows: HOXA5, HOXC9, HOXB7, HOXC4, HOXA7, HOXD4, HOXB3, HOXC6, MEIS1, and BMP4; In vascular endothelial cells, the hub genes are as follows: CCL2, CSF3, CXCL1, VEGFA, CXCL2, CXCL3, CSF1R, FCGR2A, IL10RA, and HSPA1A. The interaction network and biological processes of the hub genes were further analyzed and visualized to understand the molecular mechanism of keloids (Fig. 6).

#### 4. Discussion

Keloid is a common disease in plastic surgery, which brings serious cosmetic and psychological burden to patients. The etiology of keloids can be roughly divided into genetic factors and environmental factors. In terms of genetic factors, keloids are related to single nucleotide polymorphisms.<sup>[30,31]</sup> Elastic tension and mechanical stress on the skin, adolescence, pregnancy, and endothelial dysfunction are environmental factors for the etiology of keloids.<sup>[32–34]</sup> Fibroblasts, the main source of collagen, play a vital role in keloid lesions. Various cytokines, such as TGF- $\beta$ , EGF, PDGF, VEGF, and IGF-1, are crucial in the progression of keloids.<sup>[35]</sup> Recent studies have shown that abnormal vascular endothelial cell function and hypertension are found to be associated with the progression of keloids.<sup>[36,37]</sup> The treatment methods of keloid are currently limited. The main method is surgery, combined with a variety of auxiliary treatment methods, but they all have different recurrence rates. The molecular mechanisms of keloids are not very clear. Several studies have shown that FOXF1 overexpression significantly inhibits cell proliferation, invasion, and extracellular matrix deposition in

keloid fibroblasts.<sup>[38]</sup> PENK induced by oxidative stress can induce the translocation and phosphorylation of p38 MAPK in keloid, and p38 MAPK is considered to be a regulator of inflammatory molecules and inhibitors and MMPs.<sup>[39]</sup> COMP promotes the formation of keloids by accelerating collagen deposition, and COMP gene knockout reduces the amount of collagen.<sup>[40]</sup> In decades of research, although many characteristics of keloid have been described, our understanding of the promoting factors of the disease is still incomplete. Therefore, in order to further clarify the molecular mechanism of the occurrence and development of keloids, we explored the gene expression and function of fibroblasts and vascular endothelial cells in keloids.

In this study, the microarray datasets of fibroblasts and vascular endothelial cells in keloids were analyzed, and their respective DEGs were obtained. GO and KEGG enrichment analyses were performed. In fibroblasts, these DEGs are significantly enriched in collagen fibril organization, extracellular matrix organization, collagen binding and TGF- $\beta$  signaling pathway. Previous studies reported that these biological functions of fibroblasts play an important role in the progression of keloids. These research conclusions are consistent with our results.<sup>[41–45]</sup> Little is known about the role of vascular endothelial cells and their interaction with fibroblasts in keloids. Vascular endothelial cells can secrete a variety of cytokines. We speculate that vascular endothelial cells may play a role in the development of keloids by regulating other types of cells. In vascular endothelial cells, these DEGs are mainly enriched in cytokine activity, receptor ligand activity, cytokine receptor binding and TGF- $\beta$  signaling pathway. The results show that our speculation may be correct.

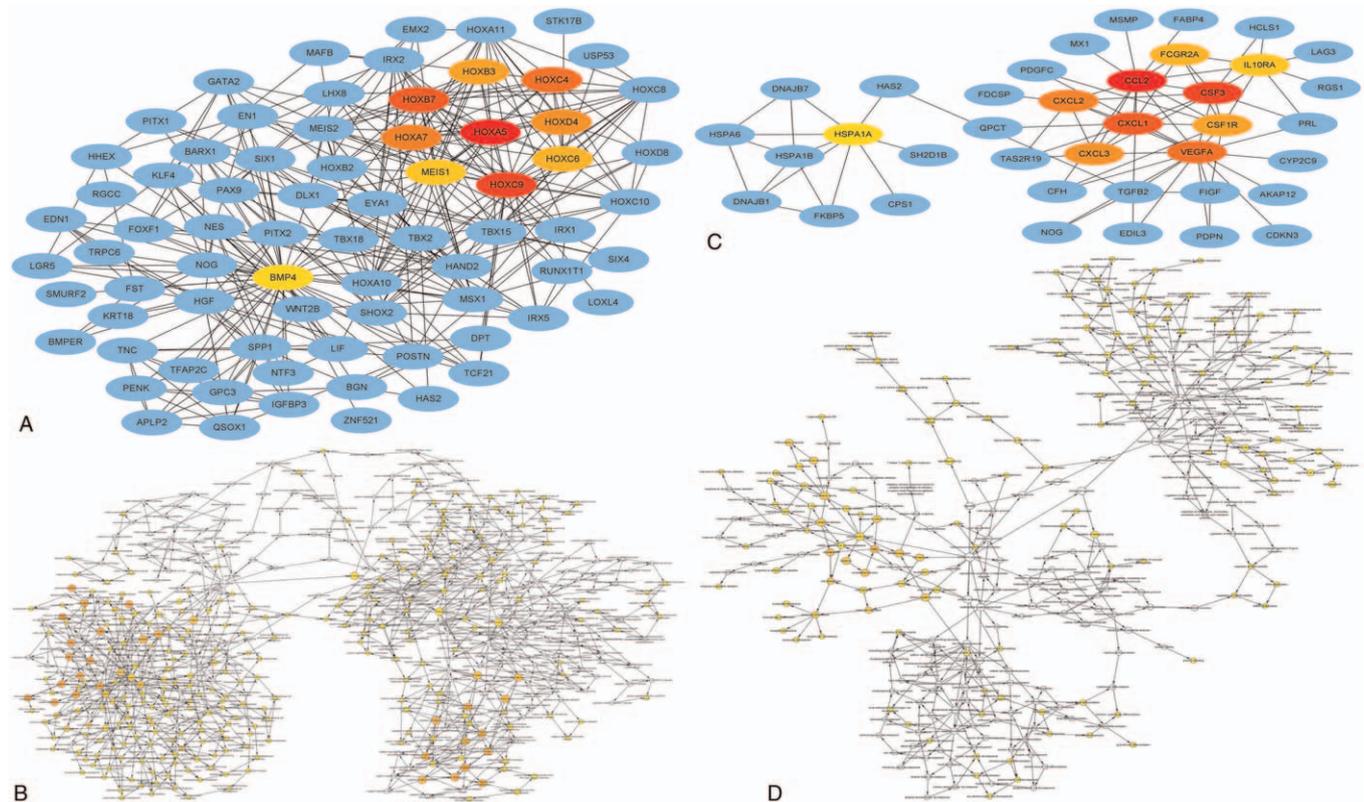


**Figure 5.** The PPI networks and the most significant modules of the DEGs in fibroblasts and vascular endothelial cells. (A) The PPI network of the DEGs in fibroblasts. (B) The most significant module in the PPI network of the DEGs in fibroblasts. (C) The PPI network of the DEGs in vascular endothelial cells. (D) The most significant module in the PPI network of the DEGs in vascular endothelial cells. Expression up-regulation is marked as red, and expression down-regulation is marked as blue. DEGs = differentially expressed genes. PPI = protein-protein interaction.

After PPI networks construction and module analyses were carried out, 10 hub genes were screened out from the networks. In fibroblasts, the 10 hub genes are as follows: HOXA5, HOXC9, HOXB7, HOXC4, HOXA7, HOXD4, HOXB3, HOXC6, MEIS1, and BMP4, of which only BMP4 is down-regulated, and the rest are all up-regulated. Hox genes encode homeodomain transcription factors controlling morphogenesis and have definite functions in development and evolution. HOXA5 plays a role in extracellular matrix deposition and controlling the proliferation, differentiation and apoptosis of epidermal cells.<sup>[46]</sup> The TGF- $\beta$  signaling pathway is closely related to the pathogenesis of keloids. BMP4 encodes a secreted ligand of the TGF- $\beta$  superfamily of proteins. Ligands of this family bind various TGF- $\beta$  receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression.<sup>[47]</sup> In addition, many genes, in DEGs, have been studied and proved to be related to the occurrence and progression of keloids, such as: POSTN, SERPINB2, SIX1, CCND2, and HOXC10.<sup>[48,49]</sup> For example, studies have shown that hypoxia initiates hyperplasia of keloid fibroblasts and increases POSTN expression; POSTN is involved in the

pathogenesis of keloids, which indicates that POSTN may be a novel therapeutic target for keloids.<sup>[50]</sup>

In vascular endothelial cells, CCL2, CSF3, CXCL1, VEGFA, CXCL2, CXCL3, CSF1R, FCGR2A, IL10RA, and HSPA1A were identified as the hub genes, and they are all down-regulated genes. CCL2 displays chemotactic activity for monocytes and basophils.<sup>[51]</sup> Evidence suggests that CCL2 has a strong oncogenic role in cancer by influencing the recruitment and activation of monocytes / macrophages, inducing angiogenesis and promoting metastasis.<sup>[52]</sup> CSF3 encodes a member of the IL-6 superfamily of cytokines. The encoded cytokine controls the production, differentiation, and function of granulocytes.<sup>[53]</sup> IL-6 and TGF- $\beta$  have been shown to play a role in skin development and maintenance.<sup>[54-56]</sup> Studies show that IL-6 has the function to modulate the expression of TGF- $\beta$  and TGF- $\beta$ 2 in the skin, which may provide a mechanism for defining the role of IL-6 in skin maintenance and a new association of IL-6 with TGF- $\beta$  in pathologies associated with fibrosis.<sup>[57]</sup> CXCL1, CXCL2, CXCL3 encode a member of the CXC subfamily of chemokines. The encoded protein is a secreted growth factor that signals through the G-protein coupled receptor, CXC receptor 2. This



**Figure 6.** The interaction network and biological processes of the hub genes. (A) The interaction network of the hub genes in fibroblasts. (B) The biological processes of the hub genes in fibroblasts. (C) The interaction network of the hub genes in vascular endothelial cells. (D) The biological processes of the hub genes in vascular endothelial cells. In A and C, the deeper the red, the higher the MCC score and the higher the ranking. In B and D, the color depth of nodes refers to the adj. *P* value of ontologies, and the size of nodes refers to the numbers of genes that are involved in the ontologies. Adj. *P* < .05 was considered statistically significant. MCC = maximal clique centrality algorithm.

protein plays a role in inflammation and as a chemoattractant for neutrophils.<sup>[58]</sup> Studies have reported on CXCL1 expression in epithelial cells and that secretion of CXCL1 from these epithelial cells induces angiogenesis.<sup>[59]</sup> VEGFA is a member of the PDGF/VEGF growth factor family. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis.<sup>[60]</sup> The protein encoded by CSF1R is the receptor for colony stimulating factor 1, a cytokine which controls the production, differentiation, and function of macrophages.<sup>[61]</sup> FCGR2A encodes one member of a family of immunoglobulin fold change receptor genes found on the surface of many immune response cells.<sup>[62]</sup> Proteomics analysis showed that FCGR2A mediates angiogenesis in a rodent model of vascular disease.<sup>[63]</sup> The protein encoded by IL10RA is a receptor for interleukin 10. It has been shown to mediate the immunosuppressive signal of interleukin 10, and thus inhibits the synthesis of proinflammatory cytokines.<sup>[64]</sup> HSPA1A encodes a 70kDa heat shock protein which is a member of the heat shock protein 70 family.<sup>[65]</sup> Previous studies showed that HSPA1A binds to and is internalised by human endothelial cell populations.<sup>[66]</sup>

The above studies have shown that most of these hub genes promote angiogenesis, or at least are related to angiogenesis. In keloid vascular endothelial cells, the expression of these genes is down-regulated, so we speculate that angiogenesis in keloids is reduced. In fact, there are studies that have proved the reduction

of blood vessels in keloids.<sup>[67]</sup> The reduction of blood vessels leads to the formation of a local hypoxic microenvironment in keloids. Studies have shown that hypoxia can lead to increased collagen deposition.<sup>[68]</sup> Therefore, the down-regulation of the expression of these hub genes in keloid vascular endothelial cells directly promotes collagen deposition. In addition, studies have shown that vascular endothelial cells in keloids can also secrete cytokines to regulate fibroblasts, promote myofibroblast differentiation, and indirectly promote collagen deposition.<sup>[69]</sup> Therefore, we conclude that fibroblasts in keloids are the main source of extracellular matrix and play a major role in disease progression. Keloid vascular endothelial cells play an important role in the occurrence and development of diseases by directly promoting collagen deposition and indirectly regulating fibroblasts.

## 5. Conclusion

In summary, we have studied the gene profiles of keloid fibroblasts and vascular endothelial cells. The two types of keloid-derived cells show different gene expressions and different functional categories. We have identified the DEGs and hub genes respectively. These genes, especially hub genes, can be used as targets for the diagnosis, treatment and prognosis of keloids. However, further molecular biology experiments are needed to verify our results.

## Author contributions

**Data curation:** Weihan Zheng, Guojian Lin.

**Formal analysis:** Guojian Lin.

**Methodology:** Weihan Zheng, Guojian Lin.

**Writing – original draft:** Weihan Zheng.

**Writing – review & editing:** Zhizhou Wang.

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