

## PDGFRB as a potential therapeutic target of ankylosing spondylitis: validation following bioinformatics analysis

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**Abstract:** Ankylosing spondylitis (AS) is a chronic, progressive, and inflammatory disease that mainly affects the central axis joint. Although this disease has already been well documented and studied, its pathogenesis is still not well understood. This study aimed to screen and identify key candidate genes involved in the progression of AS. For this purpose, expression profiles of GSE39340 and GSE41038 were downloaded from the Gene Expression Omnibus and displayed in the form of volcano plots and heatmaps. Differentially expressed genes (DEGs) were identified by the Limma package in R and functional enrichment analyses were performed. Moreover, STRING and Cytoscape were utilized to construct protein-protein interaction (PPI) networks and screen significant modules. Immunohistochemistry (IHC) in tissue chips of AS and normal human synovial tissues was performed to confirm the major proteins associated with its development. Western blotting (WB) and alizarin red staining were applied to validate the expression level of platelet-derived growth factor receptor beta (PDGFRB) and function during osteogenesis differentiation of fibroblasts in AS. A total of 256 DEGs were screened, including 191 up-regulated genes and 65 down-regulated genes. The enriched functions of these identified genes mainly included adherens junction, focal adhesion, and cell-substrate adherens junction. The pathways most highly associated with the progression of AS were TGF- $\beta$  signaling pathway, the Hippo signaling pathway, and the AGE-RAGE signaling pathway. In addition, IHC showed that mitogen-activated protein kinase 1 (MAPK1), C-X-C motif chemokine receptor 4 (CXCR4), and PDGFRB were highly expressed in AS. PDGFRB was found upregulated during osteogenesis of fibroblasts and stimulates osteogenesis in AS. These findings may improve our understanding of the molecular mechanisms controlling AS. Pharmacological targeting of PDGFRB may initiate a possible suppression of bone formation in AS.

**Key words:** Ankylosing spondylitis; Bioinformatics; Differentially expressed gene; Protein-protein interaction network; Functional analysis.

### Introduction

Ankylosing spondylitis (AS) is a type of chronic rheumatic disease that mainly affects the spine and sacroiliac joints (the sacroiliac joint is the joint between the sacrum and the pelvis). Spondylitis means inflammation of the spine, and ankylosing spondylitis means that the bones tend to stick together. AS causes the vertebrae in the spine to heal (1-3).

In ankylosing spondylitis, the ligaments around the spine become inflamed at the junction of the vertebrae. In other words, the source of ankylosing spondylitis is inflammation of these ligaments. This inflammation stimulates the bone-forming cells and bone is made inside the ligaments, and after a while, the ligaments turn into bone. Gradually, these bones become larger, and because the ligaments themselves are connected to the adjacent vertebrae from all around, the bones created inside the ligaments connect like a bridge between adjacent vertebrae and connect the vertebrae. After a while, the vertebrae of the spine are connected and the movement between them disappears. The same thing happens with the sacroiliac joints, causing the sacroiliac joints to fuse. The sacroiliac joint is located between the sacrum and the ilium and is located behind the pel-

vic ring (1-3). AS is characterized by inflammation and progressive structural damage. Inflammatory lesions often spread from the sacroiliac joint to the central axis and the peripheral joint. Fibrous or bony stiffness often occurs in the late stages of AS, usually causing severe deformities and disability. The global prevalence of AS has been estimated to be between 0.1% and 1.4% (4, 5). In 1973, Sehlosstein et al. and Brewerton et al. reported a significant association between AS and HLA-B27 (6, 7). This finding was considered a milestone in the study of AS. In recent years, the application of genome-wide association studies has led to the discovery of many AS-related genes, which has improved our understanding of its pathogenesis (8). Based on these findings, various hypotheses regarding the pathogenesis of AS have been developed, such as the arthritis antigen peptide hypothesis, protein misfolding hypothesis, and cell surface HLA-B27 homodimer hypothesis (8, 9). However, our understanding of the molecular mechanisms leading to AS development remains unclear. Identifying the complex mechanisms controlling the link between genetic polymorphisms and disease manifestations remains a challenging task. Therefore, the screening and identification of AS candidate genes are particularly important to improve our understanding of its molecular mecha-

nisms to find therapeutic targets.

Microarray technology and bioinformatics are two new and closely related technologies in the field of life science research. Bioinformatics has promoted the research and application of microarray technology, while false-positive rates in independent microarray analyses make it difficult to obtain reliable results (10-15).

This study was conducted to screen and identify key candidate genes involved in the progression of AS based on the technique of bioinformatics analysis. Two mRNA microarray datasets from the Gene Expression Omnibus (GEO) were downloaded and analyzed to obtain differentially expressed genes (DEGs) between AS tissues and osteoarthritis (OA) tissues. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed. Furthermore, significant modules in protein-protein interaction (PPI) networks were screened out. Immunohistochemistry (IHC), lentivirus infection, western blotting (WB) and alizarin red staining were used to validate the expression and function of key genes. Among them, platelet-derived growth factor receptor beta (PDGFRB) was found upregulated during osteogenesis of fibroblasts and stimulates osteogenesis in AS.

## Materials and Methods

### Microarray data

Two original microarray datasets were downloaded for the current study: GSE39340, which is based on the GPL10558 Illumina HumanHT-12 V4.0 expression beadchip and contains five AS tissue samples and seven OA samples, and GSE41038, which is based on the GPL6883 Illumina HumanRef-8 v3.0 expression beadchip and contains two AS tissue samples and three OA samples (16).

### Identification of DEGs

The DEGs between AS and OA synovial biopsy samples were screened and identified using the Limma package in R (V 3.0.1). When a gene had multiple probes, the average value of the results was given. Unnamed genes were excluded. Volcano plots and heatmaps of the selected genes from the two datasets were constructed using the gplots package in R. Values of  $|\log_{2}FC(\text{fold change})| > 1$  and  $P < 0.05$  were considered statistically significant.

### KEGG and GO enrichment analyses of DEGs

DAVID was used to analyze gene function. GO and pathway analysis were carried out and visualized using the clusterProfiler package in R and KEGG PATHWAY. Furthermore, Cytoscape was used for the visualization of functional enrichment analysis. The BiNGO plugin of Cytoscape was used to classify the functions of the enrichment analysis and present them as a network diagram; the Cluego plugin was then used to more clearly show the relationship between the pathways and the enrichment of genes within those pathways. A value of  $P < 0.05$  was considered statistically significant.

### PPI network construction and module analysis

The STRING online database was employed to identify the proteins encoded by the major identified DEGs

to provide an improved understanding of AS pathogenesis (17). A combined score of  $> 0.4$  was considered statistically significant. The MCODE plugin of Cytoscape was used to cluster large gene or protein networks to build functional modules (18). PPI networks were drawn using Cytoscape and the most significant modules in the PPI networks were identified using MCODE. The selection criteria were as follows: degree cut-off = 2, node score cut-off = 0.2, Max depth = 100, and k-score = 2.

### Selection of hub genes

Cytohubba provides a simple interface to analyze networks with many kinds of scoring methods (19). Using this software, we screened the top thirty genes as hub genes, ranked with the maximal clique centrality (MCC) scoring method.

### Validation of the hub genes by Immunohistochemical staining

Immunohistochemical staining of tissue chips was performed to identify the major proteins encoded by DEGs in AS and normal human knee synovial tissues. In this study, knee synovial tissues were obtained from AS patients who met the Modified New York criteria for AS and the patients whose tibial plateau was fractured without other diseases. They ever had undergone total hip replacement at the Orthopaedic Department of Changhai Hospital in Shanghai between June 2017 and June 2019. All patients provided written informed consent. This study was approved by the Shanghai Changhai Hospital Ethics Committee. The knee synovial tissues were fixed in 4% paraformaldehyde and made into tissue chips. The primary antibodies were MAPK1 (Servicebio, 1:100), CXCR4 (Proteintech, 1:100), CASP3 (Servicebio, 1:100), and PDGFRB (Bioss, 1:200). The slides were incubated in a secondary antibody (Servicebio, 1:200) at 37°C.

### Evaluation of the function of the hub gene PDGFRB ex vivo using Western Blotting and lentivirus infection

Fibroblasts were isolated from the knee synovial tissues of AS patients. To induce osteogenic differentiation, cells were equally dispersed in a 24-well plate cultured in the osteogenic induction medium ( $\alpha$ -MEM complemented with 10% FBS, 10 mM  $\beta$ -glycerophosphate, 100 nM dexamethasone, and 50 mg/ml ascorbic acid) for 21 days. Alizarin red staining was applied to test osteogenic differentiation ability and 10% cetylpyridinium chloride for quantification.

The total protein of fibroblast cells was extracted. Thirty micrograms of total protein were loaded into SDS-PAGE gel. The protein was transferred onto a nitrocellulose membrane. The blot was blocked for 1 h and incubated overnight with PDGFRB primary antibody (Bioss, 1:2000) at 4 °C. Membranes were probed with secondary antibody for 1 h. The results were analyzed with Image J software.

Lentiviral particles overexpressing PDGFRB or short hairpin RNA (shRNA) targeting PDGFRB or control shRNA were purchased from OBiO Technology Corp (Shanghai, China). The green fluorescent protein (GFP) gene is used as a reporter gene to detect the efficiency of infection. Cells were harvested 3 days post-

infection, which was then confirmed by WB analyses.

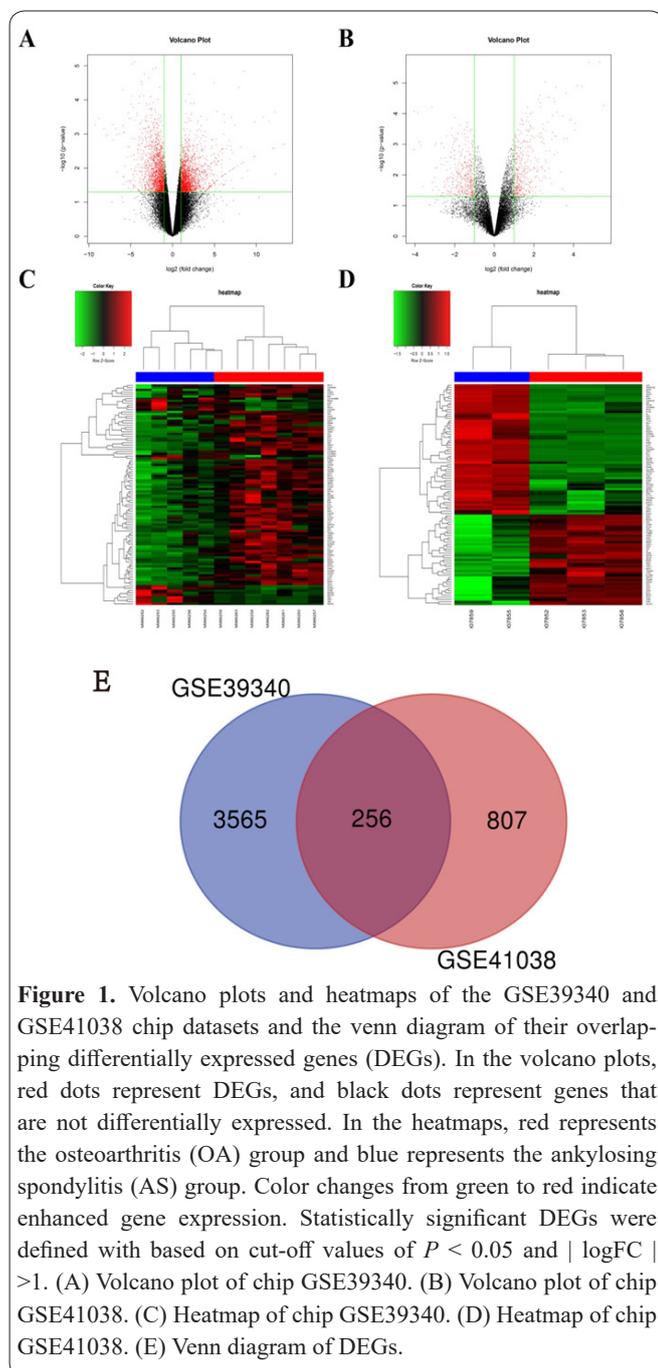
## Results

### Identification of DEGs in AS

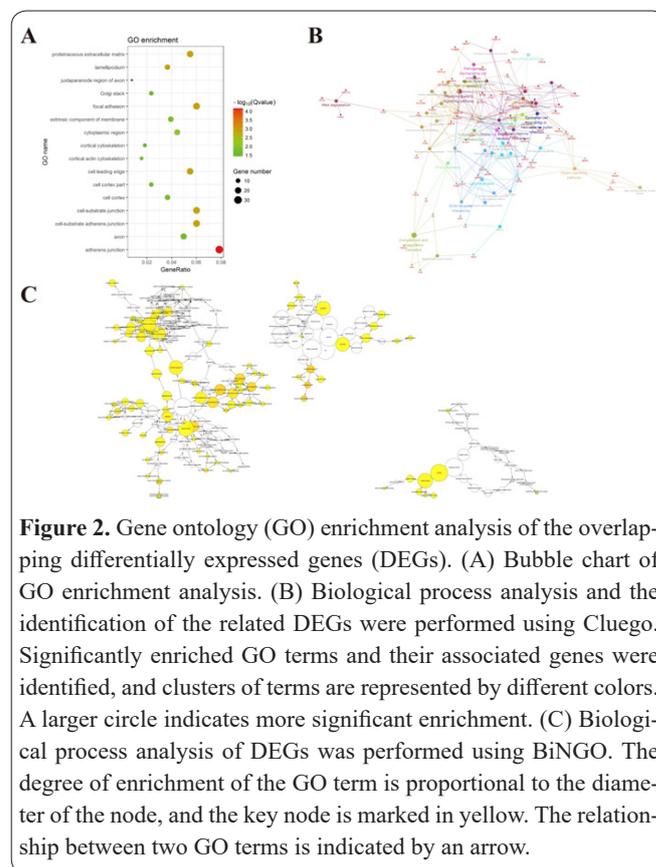
After the standardization of microarray results, a total of 4628 DEGs (3821 in GSE39340 and 1063 in GSE41038) were identified. We further constructed the volcano plots and heatmaps to reflect the expression levels of the DEGs and measure the similarities between them (Fig. 1). The overlap between the two datasets was shown in a Venn diagram (Fig. 1E). A final total of 256 DEGs, including 191 up-regulated genes and 65 down-regulated genes, was obtained.

### GO enrichment and KEGG analyses of DEGs

Using the DAVID bioinformatics resource, GO enrichment and KEGG analyses of the DEGs were performed. The top ten terms for the analyzed DEGs were listed (Table 1) and visualized in the form of Bubble charts



**Figure 1.** Volcano plots and heatmaps of the GSE39340 and GSE41038 chip datasets and the venn diagram of their overlapping differentially expressed genes (DEGs). In the volcano plots, red dots represent DEGs, and black dots represent genes that are not differentially expressed. In the heatmaps, red represents the osteoarthritis (OA) group and blue represents the ankylosing spondylitis (AS) group. Color changes from green to red indicate enhanced gene expression. Statistically significant DEGs were defined with based on cut-off values of  $P < 0.05$  and  $|\log_{2}FC| > 1$ . (A) Volcano plot of chip GSE39340. (B) Volcano plot of chip GSE41038. (C) Heatmap of chip GSE39340. (D) Heatmap of chip GSE41038. (E) Venn diagram of DEGs.



**Figure 2.** Gene ontology (GO) enrichment analysis of the overlapping differentially expressed genes (DEGs). (A) Bubble chart of GO enrichment analysis. (B) Biological process analysis and the identification of the related DEGs were performed using Cluego. Significantly enriched GO terms and their associated genes were identified, and clusters of terms are represented by different colors. A larger circle indicates more significant enrichment. (C) Biological process analysis of DEGs was performed using BiNGO. The degree of enrichment of the GO term is proportional to the diameter of the node, and the key node is marked in yellow. The relationship between two GO terms is indicated by an arrow.

(Fig. 2A, Fig. 3A). The most significantly enriched GO terms were adherens junction, focal adhesion, and cell-substrate adherens junction. KEGG pathway analysis revealed that the DEGs were mainly enriched for phospholipase D signaling pathway, complement and coagulation cascades, and gap junction. Among them, the three most well-matched signal pathway maps related to AS were selected and displayed using the KEGG website. They are transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling pathway, the Hippo signaling pathway, and the advanced glycation end-products-advanced glycation end-products receptor (AGE-RAGE) signaling pathway (Fig. 3B). Two Cytoscape plugins, BiNGO and Cluego, were used to visualize the relationship between these pathways and the enriched DEGs (Fig. 2B and C).

### PPI network construction and module analysis

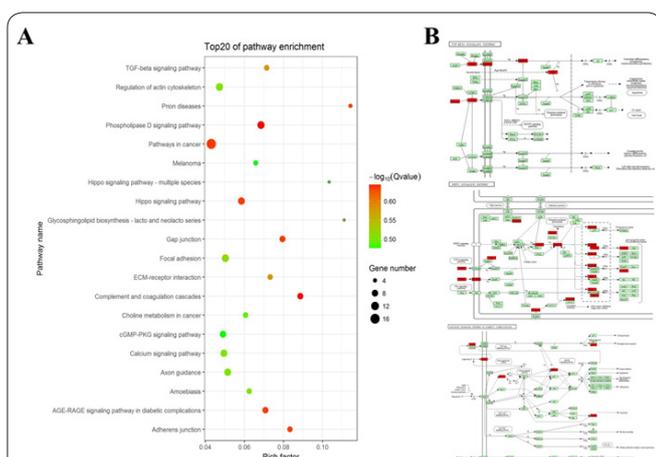
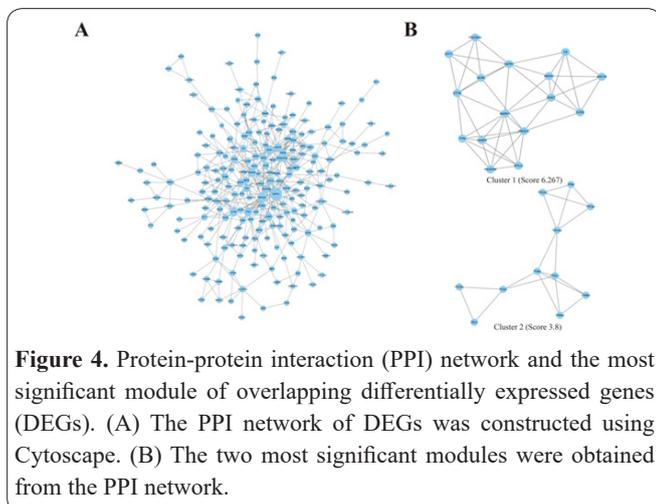
PPI network analysis of DEGs was performed using the STRING online database. Next, the data was imported into Cytoscape, and PPI networks were constructed (Fig. 4A). The two most significant modules (Score = 6.267 and 3.8) were displayed using MCODE and included 16 genes and 11 genes, respectively (Fig. 4B).

### Hub gene selection

Using the MCC algorithm, the top thirty genes were obtained, and eight of them were identified as the most important genes (Score > 150), namely mitogen-activated protein kinase 1 (*MAPK1*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (*PIK3CG*), C-X-C motif chemokine receptor type 4 (*CXCR4*), caspase 3 (*CASP3*), platelet-derived growth factor receptor beta (*PDGFRB*), KIT ligand (*KITLG*), G protein subunit gamma 7 (*GNG7*) and Proto-oncogene tyrosine-protein kinase (*FYN*); these results were similar to those obtained using MCODE, indicating that

**Table 1.** GO and KEGG pathway enrichment analysis of DEGs.

Term	Description	P-value	Count in the gene set
GO enrichment			
GO:0005912	adherens junction	0.000000192	30
GO:0005925	focal adhesion	0.00000979	23
GO:0005924	cell-substrate adherens junction	0.0000111	23
GO:0030055	cell-substrate junction	0.0000136	23
GO:0031252	cell leading edge	0.0000228	21
GO:0030027	lamellipodium	0.0000242	14
GO:0005578	proteinaceous extracellular matrix	0.0000269	21
GO:0099568	cytoplasmic region	0.000164842	17
GO:0019898	extrinsic component of membrane	0.000384338	15
GO:0005938	cell cortex	0.000466357	14
KEGG pathway enrichment			
hsa04072	Phospholipase D signaling pathway	0.001793659	10
hsa04610	Complement and coagulation cascades	0.002072128	7
hsa04540	Gap junction	0.003825516	7
hsa04520	Adherens junction	0.005816736	6
hsa04933	AGE-RAGE signaling pathway in diabetic complications	0.007285537	7
hsa05020	Prion diseases	0.007892135	4
hsa05200	Pathways in cancer	0.00847629	17
hsa04390	Hippo signaling pathway	0.00849582	9
hsa04512	ECM-receptor interaction	0.01082555	6
hsa04350	TGF-beta signaling pathway	0.012109385	6

**Figure 3.** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the overlapping differentially expressed genes (DEGs). (A) Bubble chart of KEGG pathway enrichment analysis. (B) The three most well-matched pathways related to ankylosing spondylitis (AS) are displayed as pathway maps with related DEGs marked in red.**Figure 4.** Protein-protein interaction (PPI) network and the most significant module of overlapping differentially expressed genes (DEGs). (A) The PPI network of DEGs was constructed using Cytoscape. (B) The two most significant modules were obtained from the PPI network.

with scores of  $> 100$  are listed in Table 2. Further analysis of the above genes yielded a key module of the PPI network (Fig. 5A).

By querying the function of each key gene, we identified four genes that may be involved in the pathogenesis of AS: *MAPK1*, *CXCR4*, *CASP3*, and *PDGFRB*. Next, we analyzed tissue microarrays of AS and normal human knee synovial tissues through IHC to verify the expression of the identified genes. The protein expression of *MAPK1*, *CXCR4*, and *PDGFRB* was confirmed to be up-regulated in AS tissues, as shown by our bioinformatic analysis results (Fig. 5B).

### PDGFRB is upregulated and stimulates osteogenesis of fibroblasts in AS

The protein level of *PDGFRB* increased during fibroblasts osteogenesis (Fig. 6A). Lentivirus infection was successfully established (Fig. 6B). It indicated that the protein level of *PDGFRB* obviously increased in the overexpression group (Oe) and decreased in the knockdown group (Sh1 and Sh2), compared with the normal control group (NC) (Fig. 6C). Alizarin red staining indicated that *PDGFRB* overexpression enhanced the calcium deposition of fibroblasts, while *PDGFRB* knockdown showed opposite effects (Fig. 6D).

### Discussion

AS is a progressive rheumatic disease that primarily affects the axial skeleton and often spreads to the peripheral joints. The global incidence of AS is 0.1–1.4%. Advanced AS is characterized by osteoproliferation leading to irreversible bone fusion at the vertebral and sacroiliac joints, contributing to limited spinal mobility and associated pain (20). As a chronic inflammatory, progressive, and disabling disease, AS is one of the most common causes of disability and affects millions of people worldwide. Since the discovery of the AS susceptibility gene *HLA-B27*, numerous genetic variations have been discovered through various techniques (8).

these genes are critical to maintaining the stability of the network structure. The functional roles of hub genes

**Table 2.** Functional roles of hub genes with score > 100.

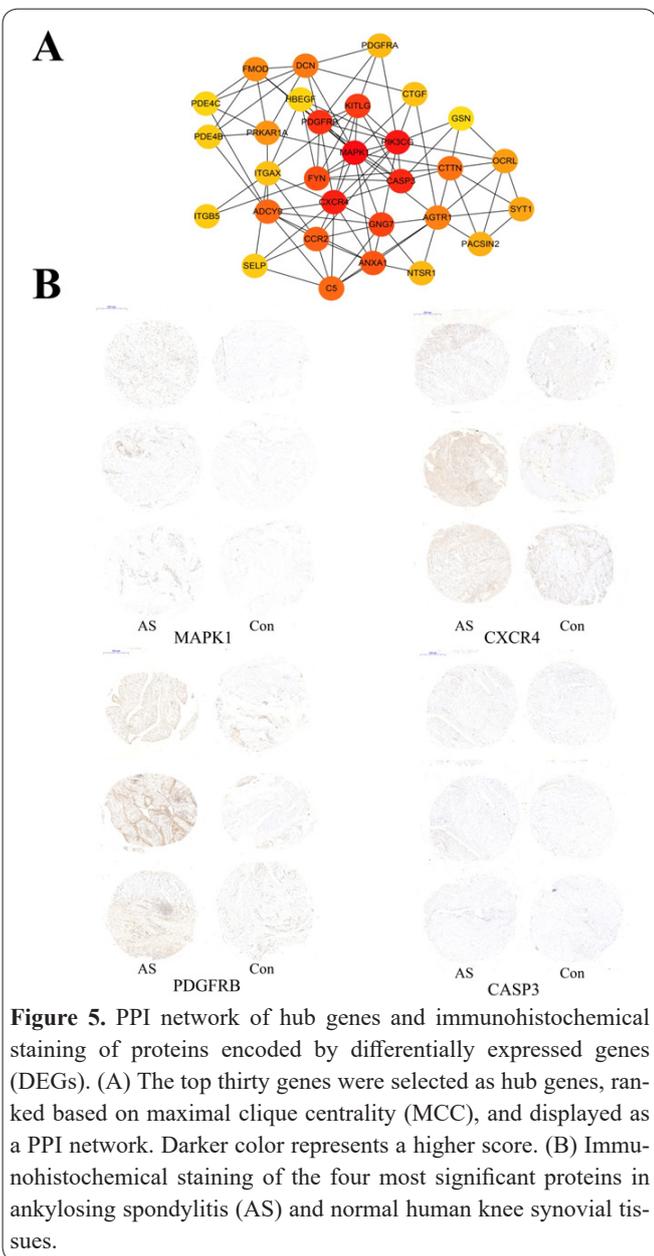
No.	score	Gene symbol	Full name	Function
1	345	MAPK1	mitogen-activated protein kinase 1	MAPK1 acts as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation, and development.
2	300	PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma	PIK3CG activates signaling cascades involved in cell growth, survival, proliferation, motility, and morphology.
3	278	CXCR4	C-X-C motif chemokine receptor 4	CXCR4 can bind bacterial lipopolysaccharide (LPS) and mediate LPS-induced inflammatory response, including TNF secretion by monocytes.
4	270	CASP3	caspase 3	Caspases are involved in the signaling pathways of apoptosis, necrosis, and inflammation.
5	265	PDGFRB	Platelet-Derived Growth Factor Receptor Beta	PDGFRB has roles in the regulation of many biological processes including embryonic development, angiogenesis, cell proliferation, and differentiation.
6	252	KITLG	KIT ligand	KITLG plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration and function, and melanogenesis.
7	164	GNG7	G protein subunit gamma 7	GNG7 plays a role in the regulation of adenylyl cyclase signaling in certain regions of the brain, and the formation or stabilization of a G protein heterotrimer.
8	152	FYN	Fyn proto-oncogene	FYN is implicated in the control of cell growth.
9	144	ANXA1	annexin A1	ANXA1 plays an important role in the innate immune response as an effector of glucocorticoid-mediated responses and regulator of the inflammatory process.
10	138	CCR2	C-C motif chemokine receptor 2	CCR2 is a receptor for monocyte chemoattractant protein-1, a chemokine that specifically mediates monocyte chemotaxis.
11	134	C5	Complement C5	C5 is a component of the complement system, a part of the innate immune system that plays an important role in inflammation, host homeostasis, and host defense against pathogens.
12	134	ADCY9	adenylate cyclase 9	ADCY9 catalyzes the formation of the signaling molecule cAMP in response to the activation of G protein-coupled receptors.

Extensive research has been performed to attempt to link these variations to the major clinical symptoms of AS—inflammation and ossification. The use of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) inhibitors such as infliximab and etanercept has been shown to reduce pain in some AS patients (21, 22). However, the pain and disability caused by AS can only be treated to provide short-term relief and cannot be cured; much remains to be elucidated to identify its inductive factors and underlying mechanisms (23, 24). Nowadays, microarray technology and bioinformatics analysis may be a good help for us to discover more key genes associated with the pathogenesis and new targets for drugs of AS.

In this study, two mRNA microarray datasets were analyzed to identify DEGs between AS and OA tissues. A total of 256 DEGs were screened and identified, including 191 up-regulated genes and 65 down-regulated genes. After a browse of all DEGs, it is worth noting that several TNF- and interleukin (IL)-related receptors were detected as DEGs, such as *TNFRSF4*, *IL21R*, and *TIMD4*. In recent years, many new members of the TNF family have been identified (25). The expression of these genes and their receptors were recently shown to be elevated in AS patients compared to those in healthy controls, which might depend on HLA-B27 status (26).

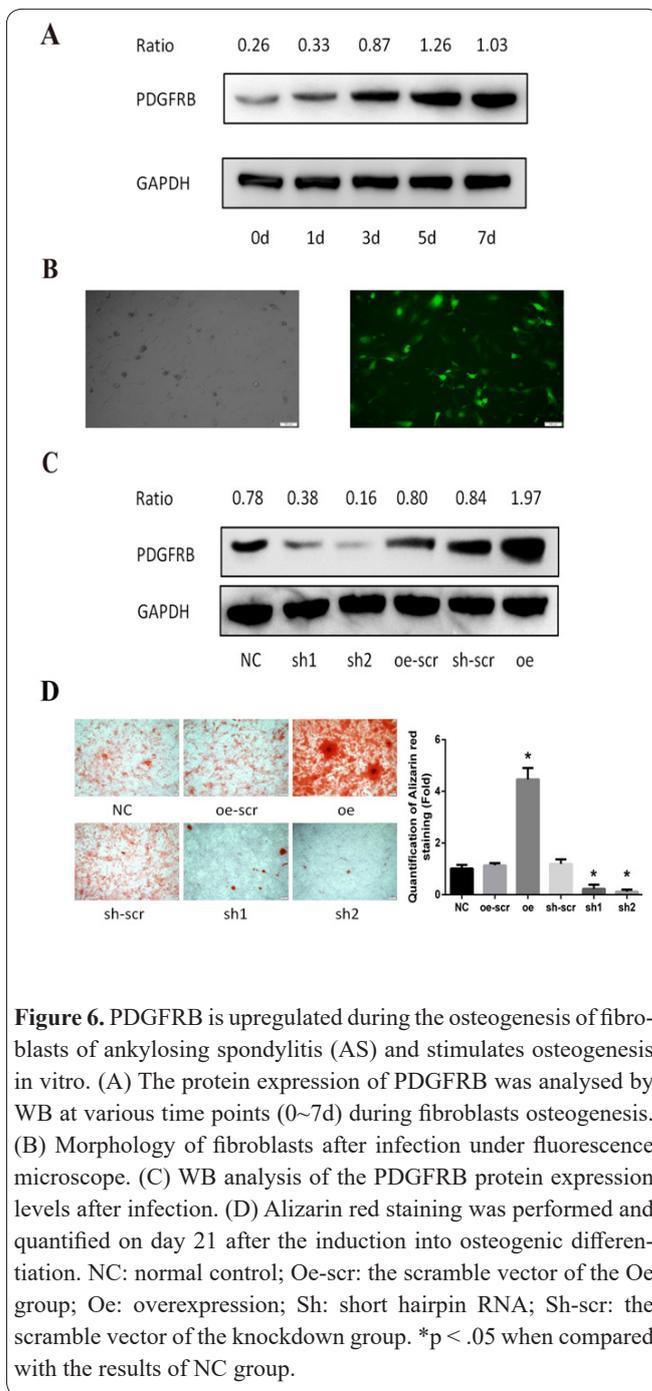
The development of AS likely depends on a highly sensitive immune response caused by genetic susceptibility genes as *HLA-B27*.

GO enrichment and KEGG analyses of the DEGs were then performed. The DEGs were mainly enriched for adherens junction, focal adhesion, phospholipase D signaling pathway, and complement and coagulation cascades. KEGG pathway analysis identified three signal pathways involved in the pathogenesis of AS: the TGF- $\beta$  signaling pathway, the Hippo signaling pathway, and the AGE-RAGE signaling pathway. Wang *et al.* (27) reported that TGF- $\beta$ 1 and connective tissue growth factor (CTGF) were up-regulated in the cytoplasm of inflammatory cells in pannus and bone marrow in sacroiliac tissue samples of patients with AS compared to that in the healthy controls. RAGE was found to be expressed not only by monocytes but also in synovial fibroblasts (28). Based on the above results, Barbara *et al.* (29) speculated that  $\beta$ 2 microglobulin ( $\beta_2m$ ), which is modified by AGE, may be accumulated in the synovia of individuals with AS-associated *HLA-B27* subtypes, indicating the potential role of RAGE. The above hypothesis was consistent with our results. Recently, a growing number of studies have identified Hippo to play a role in inflammatory and immunolo-



gical processes (30). Moreover, as a key DEG in the Hippo signaling pathway, *YAP* is known to promote inflammation (31). Thus, we speculated that *YAP* may have participated in the development of inflammation in AS as a key protein in the Hippo pathway. GO enrichment analysis using Cluego revealed that the three spots representing AS-related signaling pathways were spatially close and that there were many common DEGs between them. This suggests that these pathways are both involved in the progression of AS. In addition, developmental processes and bone development lead to ossification, which is functionally related to the clinical phenotype of AS. Furthermore, the calcium signaling pathway was identified as another important pathway, which indicates that calcium metabolism disorders may play a role in the process of ossification in AS.

Next, we selected the top thirty DEGs as hub genes. Among them, *MAPK1*, *CXCR4*, *CASP3*, and *PDGFRB* were the most highly ranked, as they were highly expressed and showed functions closely related to AS. The *MAPK1* gene encodes a member of the MAPK family. MAPKs, also known as extracellular signal-regulated kinases (ERKs), act as central signal factors controlling multiple biochemical signals and are involved in



**Figure 6.** PDGFRB is upregulated during the osteogenesis of fibroblasts of ankylosing spondylitis (AS) and stimulates osteogenesis in vitro. (A) The protein expression of PDGFRB was analysed by WB at various time points (0~7d) during fibroblasts osteogenesis. (B) Morphology of fibroblasts after infection under fluorescence microscope. (C) WB analysis of the PDGFRB protein expression levels after infection. (D) Alizarin red staining was performed and quantified on day 21 after the induction into osteogenic differentiation. NC: normal control; Oe-scr: the scramble vector of the Oe group; Oe: overexpression; Sh: short hairpin RNA; Sh-scr: the scramble vector of the knockdown group. \**p* < .05 when compared with the results of NC group.

a wide variety of cellular processes such as proliferation, differentiation, transcription regulation, and development (32). Xie *et al.* (33, 34) reported that ERK-1/2 is overactivated during the osteogenic differentiation of AS-mesenchymal stem cells (MSCs), and blocking the ERK-1/2 pathway in AS-MSCs using the inhibitor U0126 reversed the abnormal osteogenic differentiation process. According to Li *et al.* (35), the two pathways most strongly associated with AS were the MAPK signaling pathway and the toll-like receptor (TLR) signaling pathway in the inflammatory environment. *CXCR4* encodes a CXC chemokine receptor specific for stromal cell-derived factor-1 located on the cell surface, which acts with the CD4 protein to support HIV entry into cells; it is also highly expressed in breast cancer cells (36). He *et al.* (37) discovered that inhibition of *CXCR4* prevents the proliferation and osteogenic potential of fibroblasts from ankylosing spondylitis, indicating that *CXCR4* may play a key role in the development and perpetuation of joint stiffness. Our results were

consistent with these previous reports. Caspases (cysteine aspartate proteases) are involved in the signaling pathways of leading to apoptosis, necrosis, and inflammation. Faouzi *et al.* (38) reported that treatment with anti-Fas antibodies induced the expression of hepatic chemokines and promoted inflammation through a caspase-3-dependent pathway. Furthermore, the inhibition of caspase was shown to have anti-inflammatory effects (39). This suggests that CASP3 may be involved in the inflammation of AS. PDGFRB is a catalytic receptor with intracellular tyrosine kinase activity that plays a role in the regulation of many biological processes, including embryonic development, angiogenesis, cell proliferation, and differentiation. It also induces the phosphorylation of PIK3R1, leading to the activation of the AKT1 signaling pathway and creating a binding site for growth factor receptor-bound protein 2 (GRB2), resulting in the activation of MAPK1/ERK2 and/or MAPK3/ERK1(40). As an upstream signaling molecule, drugs targeting PDGFRB may help treat AS.

We performed IHC of AS and normal human knee synovial tissue chips to verify our results. Among the four most significantly hub genes, MAPK1, CXCR4, and PDGFRB were shown to be up-regulated in AS and thus may play important roles in AS progression.

As mentioned above, there have been many reports showing that MAPK1 and CXCR4 are involved in the pathogenesis of AS, while the function of PDGFRB in AS remains unclear. Fig. 2B shows that PDGFRB is associated with the calcium signaling pathway supporting our hypothesis that PDGFRB may contribute to dysregulated calcium metabolism leading to ossification. Davies *et al.* (41) identified that PDGF, the ligand of PDGFRB, is a potent osteoinductive factor in a model of tissue-engineered skeletal muscle. We have further demonstrated that PDGFRB can promote osteogenic differentiation of fibroblasts in AS by means of lentiviral infection and alizarin red staining. Cumulatively, these findings suggest that PDGFRB may be involved in AS pathogenesis by affecting osteogenic differentiation.

In conclusion, this study identified some important biological processes and pathways, including adherens junction, focal adhesion, cell-substrate adherens junction, TGF- $\beta$  signaling pathway, the Hippo signaling pathway, and the AGE-RAGE signaling pathway. Three hub genes MAPK1, CXCR4, and PDGFRB were detected highly expressed *in situ*. Especially, PDGFRB was proved to stimulate osteogenesis in AS, which offers the opportunity to become a target receptor for drugs.

### Acknowledgements

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### Compliance with ethical standards

Informed consent was obtained from all individual participants included in the study. The study was approved by the Local Ethics Committee of Changhai Hospital.

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