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Original Article

ITGB5 as a Potential Diagnostic Biomarker for Osteoarthritis

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Abstract

Objective: Osteoarthritis (OA) is a prevalent degenerative joint disease, especially occur in the elderly. This study aimed to uncover a novel biomarker for early diagnosis and treatment of OA. **Methods**: WGCNA, differential expression analysis and PPI network were used for screening hub genes-related to OA, utilizing the GSE55235 and GSE57218 datasets from GEO database. **Results**: Based on the data in the GEO datasets, compared to normal tissues, ITGB5 was obviously elevated in OA cartilage and synovial samples. Additionally, ROC curve results validated the diagnostic value of ITGB5 in OA. Mechanistically, transcription factors KLF4 and KLF11 could modulate ITGB5 gene transcription via binding to its promoter region, thereby affecting ITGB5 gene expression in OA tissues. GSEA results showed that ITGB5 gene was closely related to p53, wnt, TNF and T cell receptor signaling pathways, suggesting that ITGB5 may play potential roles in affecting cell apoptosis and inflammation in OA. Moreover, ITGB5 levels in OA samples was positively correlated to T helper type 1 cells, natural killer T cells, macrophages, memory CD8 T cells, activated dendritic cells. **Conclusion**: In this study, we found that ITGB5 was obviously elevated in OA samples. Moreover, ITGB5 may function as a diagnostic biomarker in OA.

Keywords: Biomarker, Diagnosis, ITGB5, Osteoarthritis

Introduction

Osteoarthritis (OA) is a prevalent degenerative joint disease[1]. OA is more likely to occur in men and women over the age of 60, and it is one of the primary causes of disability in the elderly[2]. Overweight, obesity, overuse of joints, mechanical joint injuries and old age are key risk factors in OA development[3,4]. The joints of knee, hip, hand, foot and spine can be affected by OA[5]. Seriously, OA impairs peoples' physical function and reduces patients' quality of life[6]. Currently, both surgical therapy and non-operative treatments (e.g. weight management, exercise therapy, physical therapy and oral analgesics) have been found to attenuate the symptoms of OA[7,8]. Nevertheless, the clinical outcomes remain unsatisfactory. Thus, uncovering effective biomarkers may be helpful for early diagnosis and treatment of OA.

OA is characterized by cartilage degradation, synovial inflammation, and osteophyte formation[9]. Joint cartilage and synovium play crucial roles in maintaining skeletal function and protecting the joints from damage[10]. It has been shown that multiple factors (e.g. damage-associated molecular patterns, metabolites, cytokines, and mitochondrial dysfunction) in the synovium can induce synovial inflammation[11]. Meanwhile, synovial inflammation and mechanical stress can contribute to extracellular matrix (ECM) destruction in cartilage, ultimately resulting in cartilage degradation[12]. In this study, using the datasets from GEO database, we screened differential expressed genes (DEGs) between normal and OA (OA cartilage and OA synovial) samples. The identified DEGs may be related to cartilage degradation and synovial inflammation in OA. These DEGs might be potential therapeutic targets for OA.

Integrin beta 5 (ITGB5), a member of integrins, plays

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important role in various diseases including cancer, polycystic ovary syndrome, psoriatic arthritis[13-15]. Shi et al. reported that ITGB5 could accelerate colorectal cancer progression through activating TGF-β signaling[16]. Moreover, ITGB5 could promote the proliferation of fibroblasts in keloids[17]. However, the role of ITGB5 in OA remains unclear. In the current research, utilizing weighted gene co-expression network analysis (WGCNA) and differential expression analysis, we observed dysregulation of ITGB5 in OA samples in the datasets from Gene Expression Omnibus (GEO) database. Compared to normal samples, ITGB5 was obviously elevated in OA (OA cartilage and OA synovial) samples. Moreover, receiver operating characteristic (ROC) curve analysis confirmed the potential diagnostic value of ITGB5 in OA. These results indicated that ITGB5 may serve as a diagnostic marker for OA.

Materials and methods

Data collection

The GSE57218 (mRNA expression profiles of 7 normal cartilage tissues and 33 osteoarthritic cartilage tissues), GSE114007 (mRNA expression profiles of 18 normal cartilage tissues and 20 osteoarthritic cartilage tissues), GSE55235 (mRNA expression profiles of 7 normal synovial tissues and 33 osteoarthritic synovial tissue), GSE32317 (mRNA expression profiles of 19 osteoarthritic synovial tissue) and GSE143514 (mRNA and miRNA expression profiles of 3 normal synovial tissues and 5 osteoarthritic synovial tissue) datasets were acquired from GEO (https://www.ncbi.nlm.nih.gov/geo/) database.

Identification of OA-related genes by WGCNA

The R package "WGCNA" (version1.72-1) was utilized to screen OA-related gene modules in GSE57218 dataset[18]. Top 25% genes screened by analysis of variance were used for WGCNA. Pearson's correlation analysis was used for determining the correlation coefficient between each gene. The soft-threshold β was then determined, which is important for constructing the standard of scale-free network. Next, adjacency matrix was transformed into topological overlapping matrix (TOM). Based on the TOM, hierarchical clustering was performed, and a hierarchical clustering tree was generated. The significance of gene and modules was calculated to evaluate the significance of gene and clinical information and to analyze the correlations between modules and clinical traits. Finally, key modules related to OA was obtained.

Identification of DEGs

The DEGs between normal and OA samples in the GSE55235 and GSE57218 datasets were screened by using R package "limma" (version 3.56.2) (Thresholds: |log2 FC| > 0.5 and FDR < 0.05)[19].

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses

To identify signaling pathways related to OA-related genes and DEGs, GO [including Biological Process (BP), Molecular Function (MF), Cellular Component (CC) terms] and KEGG analyses were conducted using the R package "clusterProfiler" (version 4.8.3) (Threshold: Pvalue < 0.05)[20].

Protein-protein interaction (PPI) networks

The STRING (https://string-db.org/, version 11.0) database was used for analyzing the interaction between each protein[21]. The PPI network was constructed by the STRING and visualized by the Cytoscape (version 3.7.2)[22]. Meanwhile, based on the Maximum neighborhood component (MNC) algorithm, the hub genes in the PPI networks were further screened by using the cytoHubba plug-in from the Cytoscape software.

The GeneMANIA (http://www.genemania.org) database was also used for assessing the interaction between each protein and exploring potential pathways involvement of proteins.

Prediction of the binding sites of transcription factor (TF) on the promoter region of ITGB5

The file of 1000-bp promoter sequence upstream of the start site of ITGB5 gene was obtained from UCSC (http://genome.ucsc.edu/). The transcription factor motif file was acquired from the JASPAR database (https://jaspar.genereg.net/). Subsequently, the online tool FIMO (https://meme-suite.org/meme/tools/fimo) was used for predicting the presence of transcription factor binding motifs in the region upstream of the gene promoter.

Gene set enrichment analysis (GSEA) and disease ontology (DO) analyses

Based on the median value of ITGB5 level, OA samples in the GSE114007 dataset were divided into two groups: high ITGB5 level (H-ITGB5) and low ITGB5 level (L-ITGB5) groups. DEGs between two groups were identified using R package "limma" (version 3.56.2). Meanwhile, GSEA and DO analyses were then performed on these DEGs.

Construction a miRNAs/TFs-mRNA network

A Venn diagram was used to identify differential expressed TFs (DE-TFs) between DEGs in GSE55235 dataset and 1639 TFs. Next, Pearson's correlation analysis (p<0.05 and correlation>0.6) was conducted to analyze the correlation between each DE-TF and ITGB gene. The DE-TFs found to be correlated with the ITGB5 gene was then used to build the TFs-mRNA network. Additionally, TargetScan (https://www.targetscan.org/vert_80/) and miRTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/) databases were used to predict potential common miRNAs that might

target to the ITGB5 gene. Subsequently, the miRNAs/TFs-ITGB5 network was constructed by the Cytoscape (version 3.7.2).

Prediction the relationship between ITGB5 and drugs

The Drug Gene Interaction Database (DGIdb, version 4.2.0-sha1 afd9f30b, https://dgidb.genome.wust-l.edu) was conducted to predict potential drugs related to ITGB5.

Analysis of tumor-infiltrating immune cells

The CIBERSORT software was used for analyzing the relative proportions of 22 immune cells[23]. The abundance of 28 specific immune cells was determined using the ssGSEA algorithm between H- and L-ITGB5 groups. The abundance of 64 specific immune cells was also analyzed using the R package "Xcell" (https://github.com/dviraran/xCell) between H- and L-ITGB5 groups. The correlation between ITGB5 gene expression and the level of immune cells was determined by Pearson's correlation analysis.

Statistical analysis

Wilcoxon rank sum test was used to compare the difference of gene expression and immune cell infiltration between two groups. A value of $p < 0.05$ was considered statistically significant.

Results

Identification of OA-related genes in GSE57218 dataset

To screen gene modules-related to OA, WGCNA was performed based on the data in GSE57218 dataset. The soft threshold power was set as five to construct the gene network (Figure 1A), and 11 gene modules were then identified eventually (Figure 1B). Thereafter, normal and OA samples in the GSE57218 dataset were used as the data of clinical traits. The correlation between each gene module and each sample (normal or OA sample) was then calculated (Figure 1C and 1D). Five gene modules (pink, brown, yellow, blue, red modules) were identified as OA-related gene modules (Figure 1D), there were 3436 genes in these modules.

GO and KEGG analyses were then performed on these OA-related genes. The results of KEGG analysis revealed that these genes were found to be participated in 98 pathways (e.g. oxidative phosphorylation), top 10 pathways were shown in Figure 1E. The results of GO analysis revealed that these genes were found to be related to 1267 GO-BP terms (e.g. Golgi vesicle transport and regulation of autophagy), 188 GO-MF terms (e.g. cadherin binding and electron transfer activity), 25 GO-CC terms (e.g. focal adhesion and cell-substrate junction), and top 10 pathways in GO-BP, GO-MF and GO-CC respectively were listed in Figure 1F.

Identification of hub genes-related to OA

Next, to screen DEGs between normal and OA samples, GSE55235 and GSE57218 datasets were used. A total of 1539 DEGs, including 916 upregulated genes and 623 downregulated genes, were identified in normal synovial tissues and osteoarthritic synovial tissues in GSE55235 dataset (Figure 2A). Additionally, a total of 1390 DEGs, including 572 upregulated genes and 818 downregulated genes, were identified in normal cartilage tissues and osteoarthritic cartilage tissues in GSE57218 dataset (Figure 2B). Next, 176 common genes were identified by Venn diagram from three categories: (I) 3436 OA-related genes by WGCNA, (II) 1539 DEGs in the GSE55235 dataset (III), 1390 DEGs in the GSE57218 dataset, these 176 common genes were considered as candidate OA-related hub genes (Figure 2C).

Then, GO and KEGG analyses were then performed on these 176 candidate genes. The results of KEGG analysis showed that these genes were related to 29 signaling pathways (e.g. FoxO signaling pathway, ECM-receptor interaction and TGF-beta signaling pathway) (Figure 2D). Meanwhile, GO analysis results showed that these genes were enriched in 456 GO-BP terms (e.g. bone development), 50 GO-MF terms (e.g. collagen-containing extracellular matrix) and 39 GO-CC terms (e.g. extracellular matrix structural constituent) (Figure 2E).

PPI network was then constructed using STRING database (minimum required interaction score>0.6) to uncover the interaction between each gene at the protein level. There were 80 nodes and 98 edges in the network (Figure 2F). Next, the Maximum neighborhood component (MNC) algorithm was used to evaluate the importance of nodes in the PPI network. The 15 most important genes were COL3A1, COL1A2, ITGB5, VCAN, COL5A2, SDC1, COL5A1, CCND1, SPARC, BCL6, FOXO3, SNRPA1, TNC, PRPF3 and TGFB3 (Figure 2G). Meanwhile, GeneMANIA (http://genemania.org/search/) website was used for constructing a composite gene-gene functional interaction network on these 15 genes (Figure S1). There were 486 co-expression sites, 33 co-localization sites, 46 genetic interaction sites, 209 physical interaction sites, 62 shared protein domains, 113 pathways and 19 predicted sites in the network.

Previous studies have demonstrated a correlation between dysregulated ITGB5 and inflammatory diseases (e.g. psoriatic arthritis)[15]. However, the role of ITGB5 in OA remains elusive. Thus, we explored the role ITGB5 in OA in the following analysis.

ITGB5 was obviously elevated in OA tissues

To analyze ITGB5 level in normal and OA tissues, GSE55235, GSE57218, GSE114007 and GSE143514 datasets were used. The results showed that compared to normal tissues, ITGB5 level was notably elevated in OA tissues in these four datasets (Figure 3A-D).

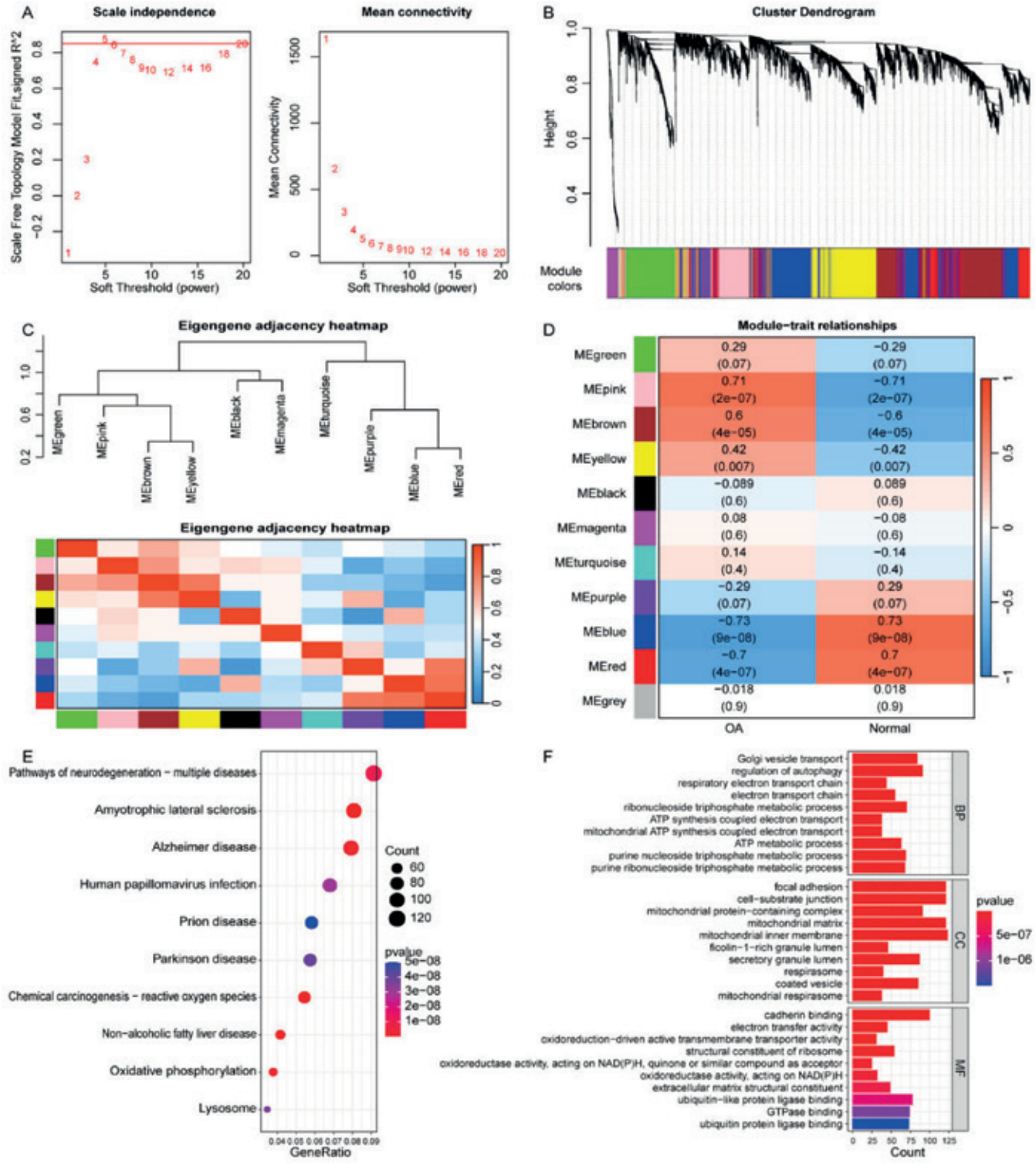

Figure 1. Identification of OA-related genes in GSE57218 dataset. (A) Screening of soft threshold β. (B) Genes are clustered into different modules. (C) Eigengene adjacency heatmap. (D) Heatmap of the correlation between modules and clinical trait of samples in GSE57218 dataset. (E) Top 10 pathways by KEGG enrichment analysis. (F) Top 10 GO-BP, top 10 GO-MF and top 10 GO-CC terms.

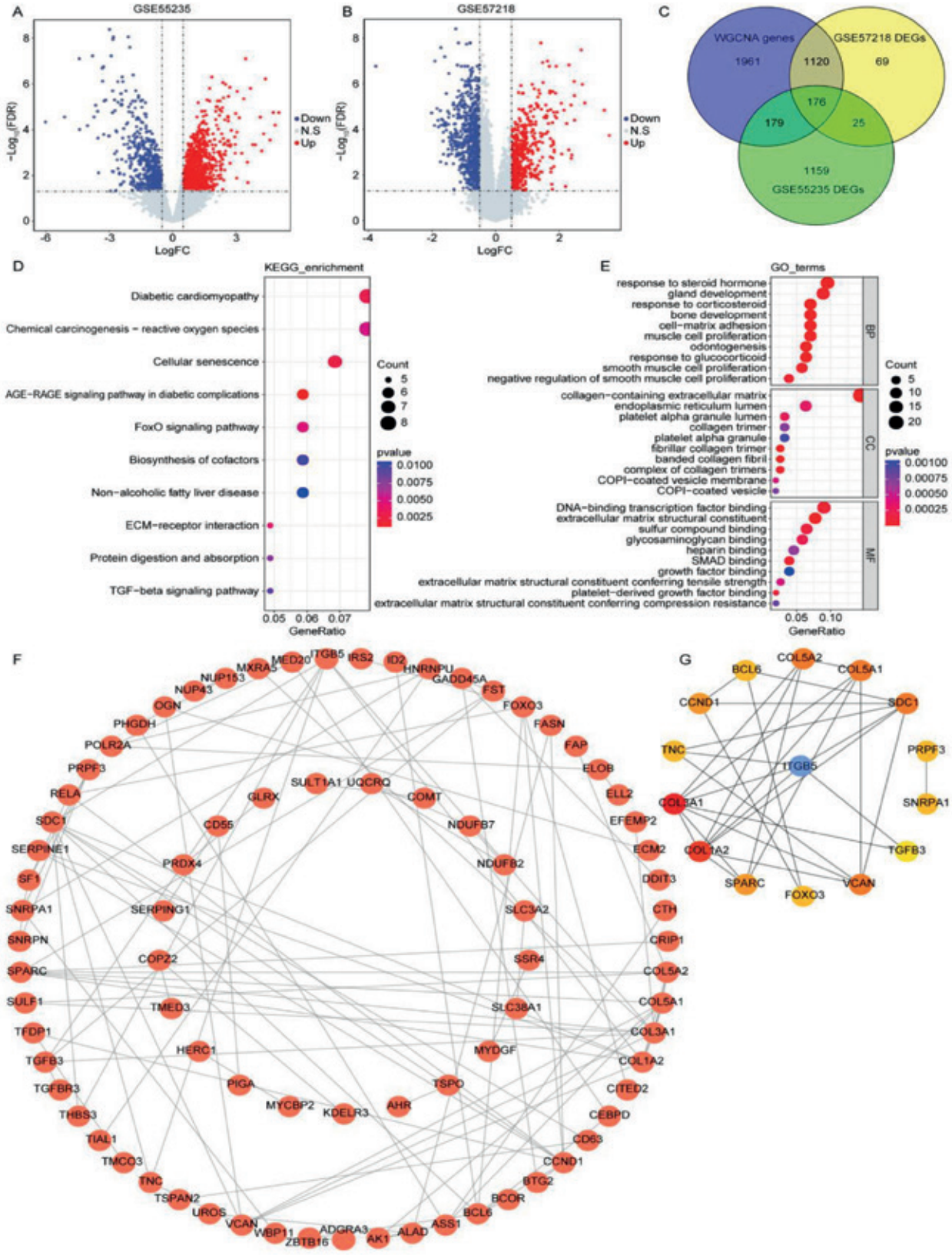

Figure 2. Identification of hub genes-related to OA. (A, B) Volcano plots of DEGs between normal and OA samples in GSE55235 and GSE57218 datasets. (C) Venn diagram identify 176 common genes from three categories: (I) OA-related genes by WGCNA, (II) DEGs in the GSE55235 dataset, (III) DEGs in the GSE57218 dataset. (D) Top 10 pathways by KEGG enrichment analysis. (E) Top 10 GO-BP, top 10 GO-MF and top 10 GO-CC terms. (F) PPI network. (G) PPI network of 15 hub genes.

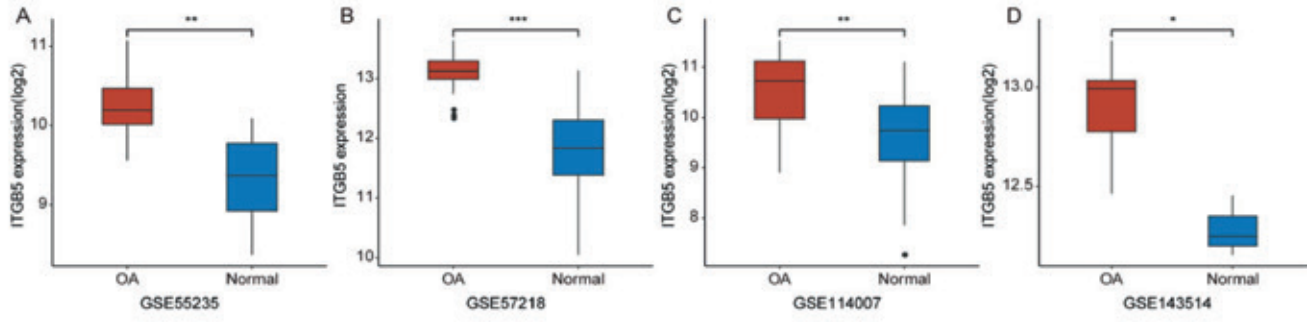

Figure 3. ITGB5 was obviously elevated in OA tissues. Box plots of ITGB5 level in normal and OA tissues in (A) GSE55235, (B) GSE57218, (C) GSE114007, (D) GSE143514 datasets.

Table 1. The correlation results of ITGB5 and TF.

TF	rho	P value
TGIF1	0.841057028	0.002294091
KLF4	0.8169419	0.003913812
YBX3	0.77968905	0.00782419
ZFHX3	-0.778381973	0.00799788
ZNF528	0.756565737	0.011315272
RELA	-0.723659051	0.017988238
VDR	-0.697243086	0.025015444
NCOA3	-0.690646711	0.027026272
ZNF668	0.683246472	0.029410688
SOX13	-0.682679568	0.029599058
MYC	0.668736715	0.034494531
KLF2	0.662986063	0.036664112
KLF11	0.658857017	0.038277551
PURA	-0.643415996	0.044734494
TSC22D1	0.632194169	0.049859159

ITGB5 could be regulated by TFs and miRNAs

To analyze the reason why ITGB5 was increased in OA tissues, we explored the TFs that bind to the promoter region upstream of ITGB5 gene firstly. In the GSE55235 dataset, there were 105 DE-TFs between normal and OA samples (Figure 4A). Thereafter, we analyzed the correlation between each DE-TF and ITGB5 gene in the GSE55235 dataset using the Pearson's correlation analysis ($p<0.05$ and correlation>0.6). A total of 15 TFs, including TGIF1, KLF4, YBX3, ZFHX3, ZNF528, RELA, VDR, NCOA3, ZNF668, SOX13, MYC, KLF2, KLF11, PURA and TSC22D1, were significantly correlated to the expression level of ITGB5 in OA (Table 1, Figure 4B-D, Figure S2A-L). Meanwhile, the network of 15 TFs and ITGB5 gene was shown in Figure 4E. We then searched TF binding sequences on the 1000-bp promoter sequence of ITGB5 gene, and uncovered that KLF2 (MA1515.1.meme) may bind to the upstream promoter region (approximately 511 bp) of ITGB5 gene, KLF11 (MA1512.1.meme) may bind to the upstream promoter region (approximately 510 bp) of ITGB5 gene, and KLF4 (MA0039.3.meme) may bind to the upstream promoter region (approximately 509 bp) of ITGB5 gene, suggesting that KLF2, KLF11 and KLF4 might bind to the promoters of ITGB5. Furthermore, analysis of KLF11 Chip-seq data in Adipose GSM1443814 dataset acquired from the Cistrome (http://cistrome.org/db/#/) database indicated a prominent binding peak on ITGB5 gene[24] (Figure 4F). Meanwhile, analysis of KLF4 Chip-seq data in Breast ENCSR265WJC_1 dataset revealed a notable binding peak on ITGB5 gene[25] (Figure 4G). These data further validated that KLF4 and KLF11 may be involved in regulating ITGB5 gene expression through binding to its promoters.

Next, to predict the potential miRNAs targeting the ITGB5 gene, TargetScan and miRTarBase databases were used. In these two databases, our analysis revealed 6 common miRNAs (hsa-let-7b-5p, hsa-miR-155-5p, hsa-miR-5688, hsa-miR-335-5p, hsa-miR-421, hsa-miR-495-3p) that may target the ITGB5 gene (Figure 4H). Additionally, the miRNAs/TFs-mRNA network of 15 TFs, 6 miRNAs and ITGB5 gene was shown in Figure 4H.

GSEA analysis of DEGs between H- and L-ITGB5 groups

Based on the median value of ITGB5 level, OA samples in the GSE114007 dataset were divided into H- and L-ITGB5 groups. The DEGs between two groups were identified, and the signaling pathways associated with these DEGs were analyzed by GSEA and DO analyses. The results of GSEA showed that compared to the L-ITGB5 group, DEGs in the H-ITGB5 group were notably enriched in 111 pathways, such as ECM-receptor interaction, apoptosis, T cell receptor signaling pathway, TNF signaling pathway, Wnt signaling pathway, p53 signaling pathway (Figure 5A-5E). The results of DO analysis revealed that compared to the L-ITGB5 group,

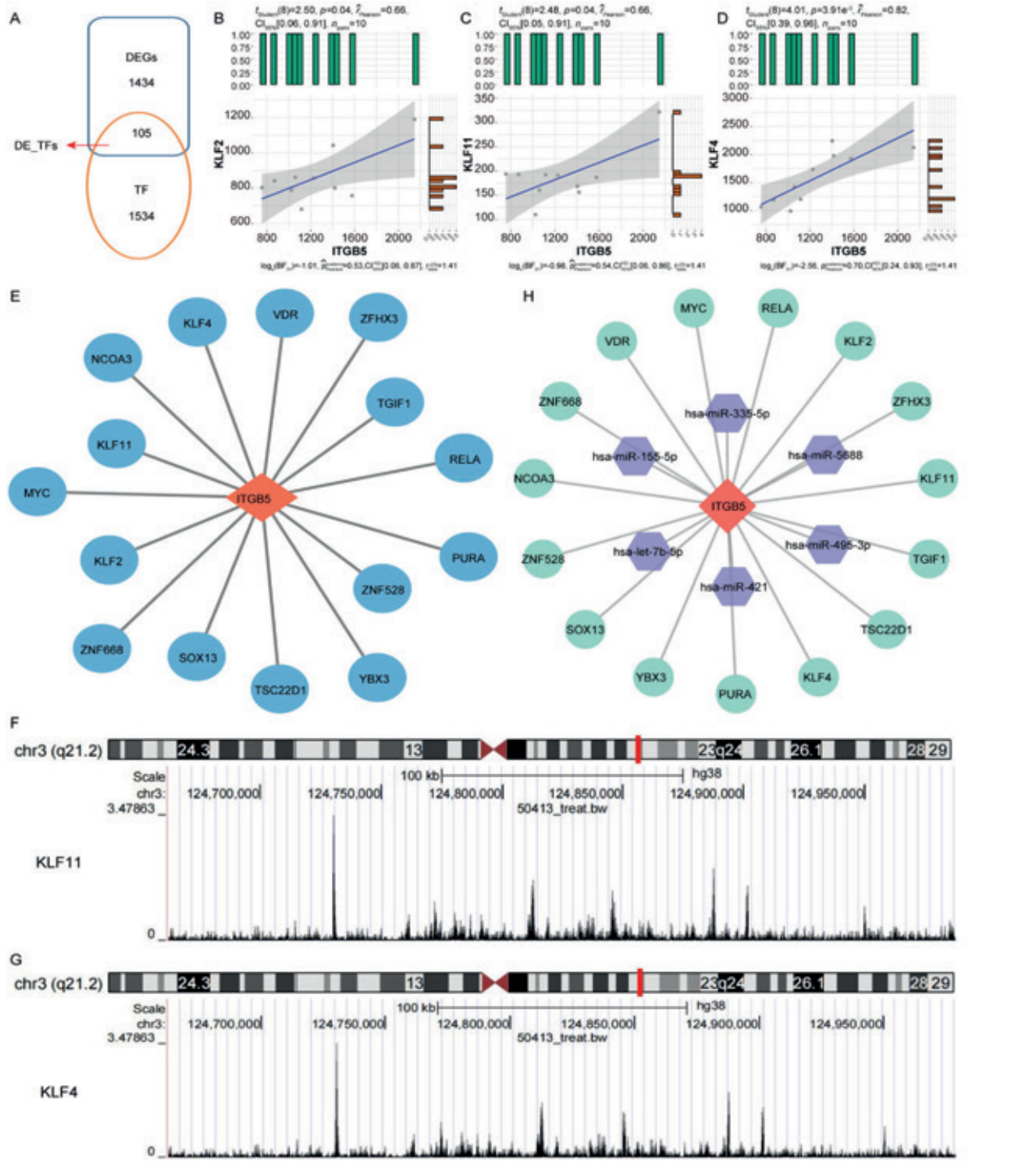

Figure 4. ITGB5 could be regulated by TFs and miRNAs. (A) Venn diagram identify 105 differential TFs (DE_TFs) between 1539 DEGs in the GSE55235 dataset and 1639 TFs. (B-D) Positive correlations between ITGB5 expression and TFs (KLF2, KLF11 and KLF4). (E) The network of ITGB5 and 15 TFs. (F) The KLF11 Chip-seq data in the Adipose GSM1443814 dataset. (G) The KLF4 Chip-seq data in the Breast ENCSR265WJC_1 dataset. (H) The network of ITGB5, 6 miRNAs and 15 TFs.

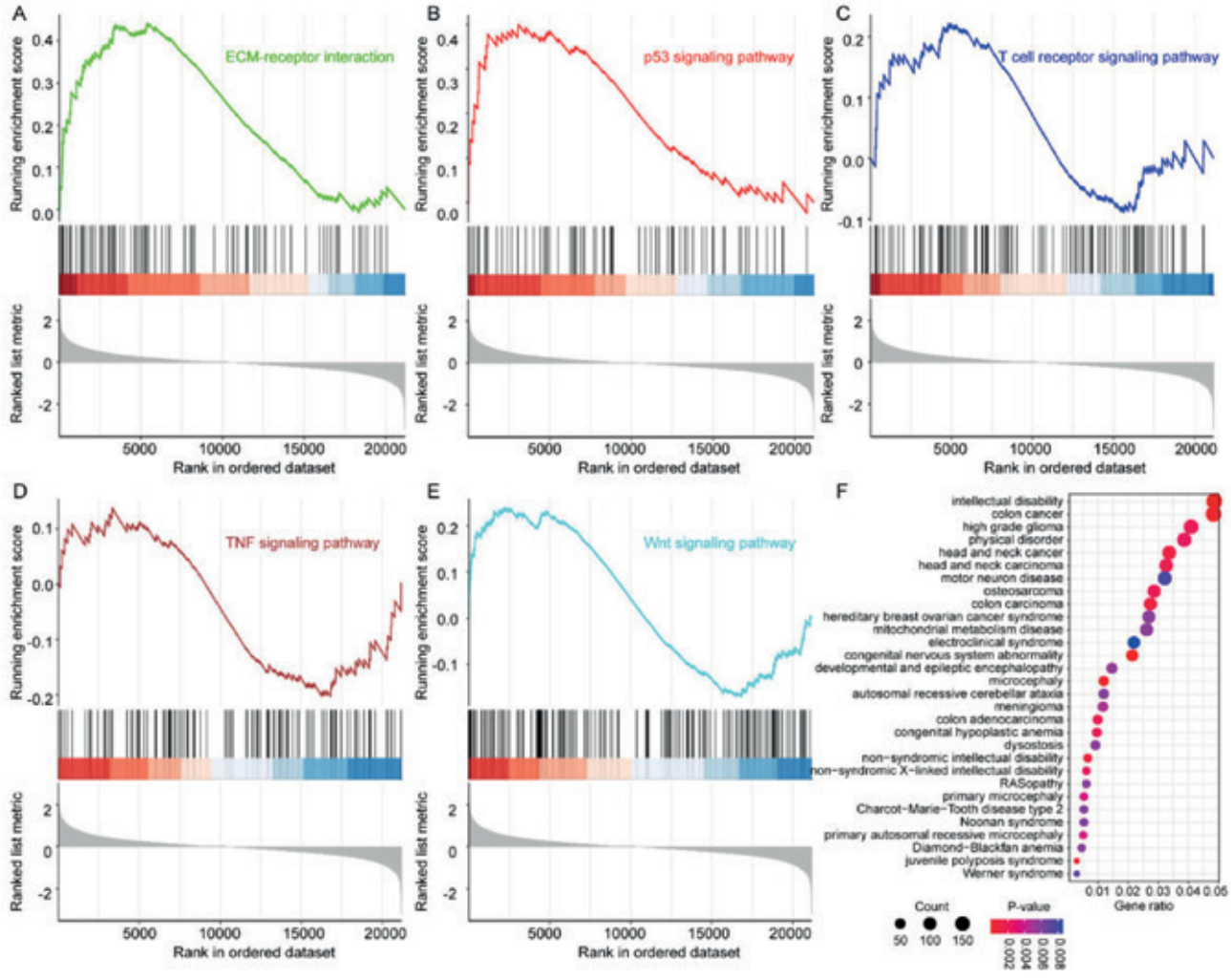

Figure 5. GSEA analysis of DEGs between H- and L-ITGB5 groups. (A, B, C, D, E) GSEA showed the enhanced activity of ECM-receptor interaction, apoptosis, T cell receptor signaling, TNF signaling, Wnt signaling, p53 signaling pathways in the H-ITGB5 group. (F) Top 30 DO analysis.

DEGs in the H-ITGB5 group were significantly enriched in 89 pathways (Figure 5F).

Composition of immune infiltrating cells between H- and L-ITGB5 groups

The correlation between ITGB5 and different cell types were then evaluated by Xcell algorithm and TISIDB database in the GSE32317 dataset. As shown in Figure 6A, ITGB5 was positively correlated to Neutrophils, Chondrocytes, Myocytes, MSC, and Th1.cells, and was negatively correlated to Endothelial cells, ly Endothelial cells, mv Endothelial cells, Adipocytes, HSC, Mesangial cells in the GSE32317 dataset using Xcell algorithm. Additionally, the data in the TISIDB database showed that ITGB5 was positively correlated to Myeloid derived suppressor cells, Type 1 T helper cells, Regulatory T cells, Natural killer T cells, Macrophages, Central memory CD8 T cells, Activated dendritic cells, and was negatively correlated to CD56bright natural killer cells and Activated CD8 T cells (Figure 6B).

Furthermore, CIBERSORT algorithm was applied to calculate the relative level of 22 immune cells in the GSE32317 dataset between H- and L-ITGB5 groups. As shown in Figure 6C, the level of follicular helper T cells was obviously reduced and M2 Macrophages level was obviously elevated in H-ITGB5 group, compared to L-ITGB5 group.

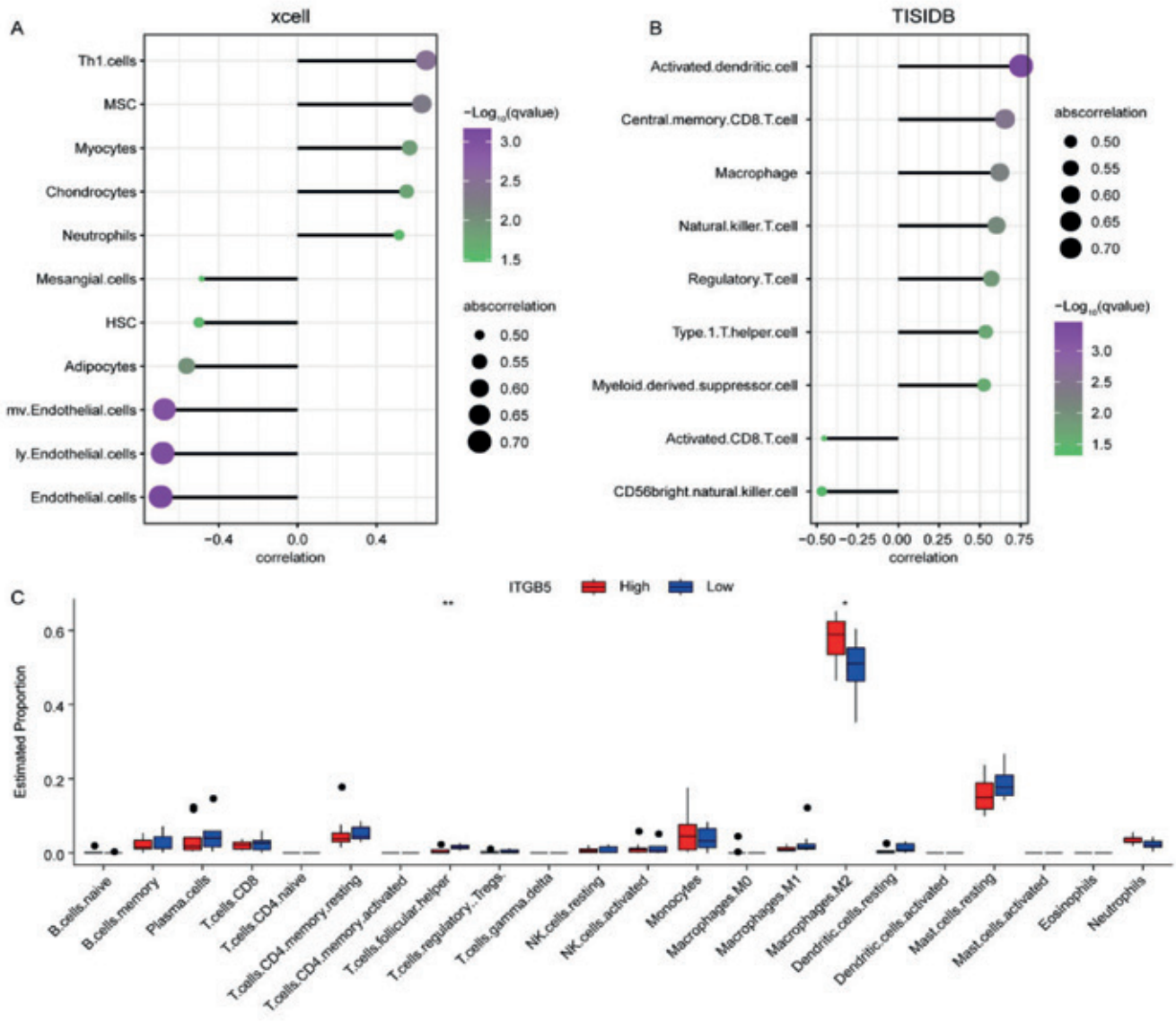

Figure 6. Composition of immune infiltrating cells between H- and L-ITGB5 groups. (A) Correlations between ITGB5 and different cells in the GSE32317 dataset by using Xcell algorithm. (B) Correlations between ITGB5 and immune cells in the GSE32317 dataset by TISIDB database. (C) Box plots of the proportions of the 22 immune cells using the CIBERSORT algorithm.

The potential diagnostic value of ITGB5 in OA

Next, to evaluate the diagnostic value of ITGB5 in OA, the ROC curve was generated. The area under the curve (AUC) value of the ROC curve was 0.918 in GSE57218 dataset, and was 0.778 in GSE114007 dataset (Figure 7A, B) These data indicated that ITGB5 had a potential diagnostic value in OA. Furthermore, the correlations between ITGB5 and different drugs were investigated using the data from DGIdb database. Figure 7C showed that ITGB5 might related to 4 drugs (e. g. cilengitide, intetumumab, abituzumab, GLPG-0187).

Discussion

Integrin beta (ITGB), a superfamily of integrins, has been found to play a role in OA[26,27]. Sun et al. found that ITGB4 level obviously reduced in OA tissues and in LPS-treated chondrocyte cells; overexpression of ITGB4 could increase cell viability and reduced cell apoptosis in LPS-treated chondrocyte cells, suggesting that ITGB4 might exert a protective role in OA[27]. Conversely, Qian et al. reported that ITGB2 was notably elevated in synovial fluid of OA patients,

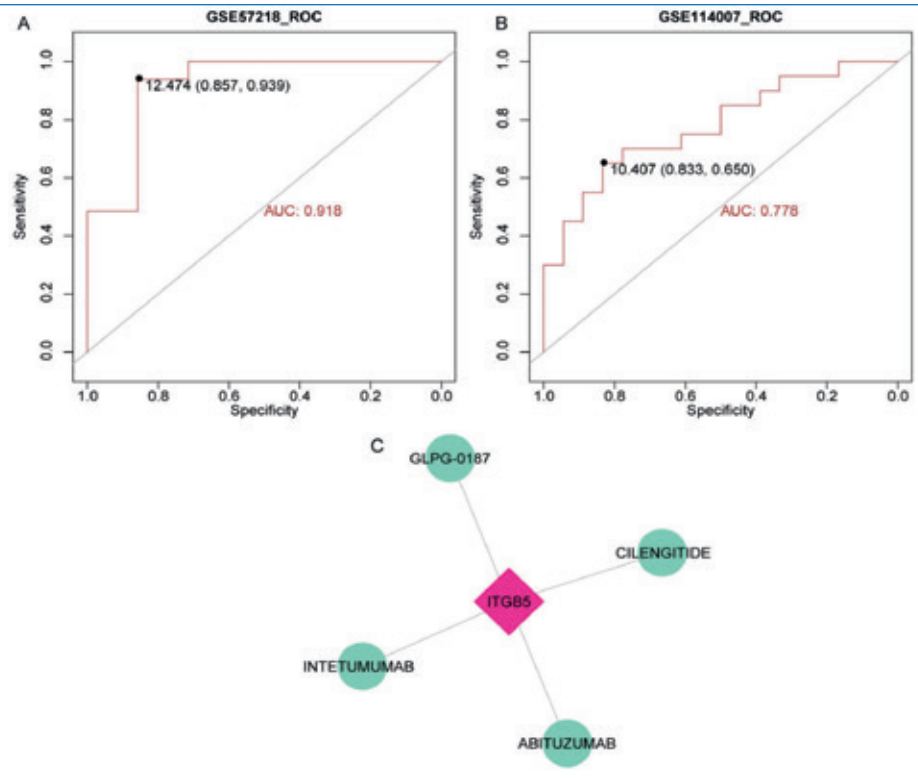

Figure 7. The potential diagnostic value of ITGB5 in OA. (A, B) ROC curves analysis for evaluating the potential diagnostic value of ITGB5 in OA in the (A) GSE57218 and (B) GSE114007 datasets. (C) The network of ITGB5 and drugs.

and might be an independent risk factor for OA[26]. These findings showed that ITGB superfamily proteins might display either anti-osteoarthritic or pro-osteoarthritic effects. In this study, according to the data in the GSE57218, GSE114007, GSE55235, and GSE143514 datasets, compared to normal tissues, ITGB5 level was remarkably elevated in osteoarthritic cartilage tissues and osteoarthritic synovial tissues. These data showed that ITGB5 might be a biomarker for OA development.

It has been shown that TFs could regulate gene expression through activating or repressing their transcription[28,29]. To better understanding the reason of abnormal expression of ITGB5 in OA, we focused on the TFs that may bind to the promoter region upstream of ITGB5 gene. Our results showed that KLF11 and KLF4 may bind to the promoters of ITGB5. KLF4 and KLF11, the members of Krüppel-like factor family, have been reported to be involved in the pathogenesis of OA[30,31]. Kawata et al. found that KLF4 could attenuate the injury of cartilage and synovium in OA mice[30]. Han et al. reported that KLF11 could attenuate oxidative stress and inhibit cell apoptosis in LPS-stimulated chondrocytes through inactivating MAPK signaling[31]. These studies showed that both KLF4 and KLF11 were reduced cartilage tissues of OA patients and could protect against OA[30,31]. Additionally, KLF4 could repress MSI2 gene transcription through targeting the promoter region of MSI2[32]. In the current research, we found that KLF4 and KLF11 could directly regulate ITGB5 gene transcription through binding to the TF binding site in the promoter of ITGB5. Given that ITGB5 level was elevated in OA tissues (Figure 3A-D), but KLF4 and KLF11 levels were reduced in OA tissues[30,31], high level of ITGB5 in OA tissues might be related to low expression levels of KLF4 or KLF11 in OA. We suspected that low expression of KLF4 or KLF11 in OA may reduce or lose the ability to suppress ITGB5 gene transcription, leading to the increased expression of ITGB5 in OA. Nonetheless, further validation is required to explore the relationship between ITGB5 and KLF4 or KLF11 in OA, which could shed more light on the crucial role of ITGB5 in OA.

Furthermore, the results of GSEA results showed that H-ITGB5 group was related to TNF, Wnt, p53 and T cell receptor signaling pathways. These pathways basically included two parts: apoptosis-related pathways (p53, wnt signaling pathways) and inflammation-related pathways (TNF and T cell receptor signaling pathways). It has been shown that chondrocyte apoptosis is a main initiator of OA, which could result in cartilage damage and ECM depletion in OA joint[33].

P53-mediated cell apoptosis plays a pathological role in OA progression[34,35]. Meanwhile, the activation of Wnt signaling could lead to chondrocyte apoptosis and extracellular matrix destruction in OA[36]. Significantly, inhibition of p53 or Wnt/β-catenin signaling could inhibit LPS-induced cell apoptosis and ECM degradation in chondrocytes[34,37-39]. Furthermore, yang et al. indicated that ITGB1 overexpression could activate the Wnt signaling in breast cancer[40], revealing a relationship between ITGB1 and Wnt signaling. Moreover, Jin et al. also found a potential relationship between p53 signaling and ITGB1[41]. Thus, we suspected that ITGB5 may be involved in OA progression by affecting chondrocyte apoptosis via p53 or Wnt/β-catenin signaling.

Furthermore, inflammation is also a major cause of OA[42]. TNF-α could induce the inflammatory response in chondrocytes and could lead to cartilage degradation in OA rats[43]. TNF-α could activate the NF-κB signaling, a crucial signaling participated in inflammation and innate immunity[44]. The inhibition of TNF-α/NF-κB signaling pathway could attenuate OA symptoms via controlling inflammatory responses[45,46]. Evidence have been shown that ITGB proteins might be involved in NF-κB-mediated inflammation response[47,48]. However, the relationship between ITGB5 and TNF-α signaling in OA remains elusive. We suspected that ITGB5 may be involved in OA progression via affecting inflammation via TNF-α signaling, which needed to be further explored.

Compared to the healthy controls, the synovium of OA patients shows an abundance population of pro-inflammatory cells such as macrophages, natural killer cells, dendritic cells, T cells, B cells, and so on[49,50]. Our results indicated that ITGB5 level in OA samples was positively correlated to T helper (Th) type 1 cells, natural killer T cells, macrophages, memory CD8 T cells, activated dendritic cells, suggested that ITGB5 may contribute to OA progression through affecting these immune cells. Previous studies have demonstrated the differentiation of CD4+ T cells into different subtypes, including pro-inflammatory Th cells (Th1, Th2, Th17) and anti-inflammatory regulatory T cells[51]. Studies have reported that Th1 cells were enriched in synovial fluid and synovial membranes of OA patients, suggesting that Th1 cells play crucial roles in OA pathogenesis[50,52]. In this study, we found that ITGB5 level in OA samples was positively correlated to Th1 cells. Thus, we suspected that ITGB5 may play a role in OA pathogenesis through elevating Th1 cells; however, future studies are needed to validate the relationship between ITGB5 and Th1 cells in OA.

Conclusion

In this study, we found that ITGB5 was obviously elevated in OA samples. Additionally, ITGB5 may potentially play a role in the progression of OA through influencing immune cell infiltration. These findings suggested that ITGB5 may serve as a diagnostic marker for OA.

Data Availability Statement

The data that support the findings of this study are openly available in Gene Expression Omnibus (GEO) at https://www.ncbi.nlm.nih.gov/geo/, reference number GSE57218, GSE114007, GSE32317, GSE143514.

Authors' contributions

SH, and WW contributed to the study conception and design, data collection and analysis. Validation was conducted by SH. The first draft of the manuscript was written by WW. JH contributed a lot during the revision by reviewing and editing the manuscript. All authors read and approved the final manuscript.

References

1. Hawker GA. Osteoarthritis is a serious disease. Clinical and experimental rheumatology 2019;37 Suppl 120(5):3-6.
2. Wang W, Niu Y, Jia Q. Physical therapy as a promising treatment for osteoarthritis: A narrative review. Frontiers in physiology 2022;13:1011407.
3. Svoboda SJ. ACL injury and posttraumatic osteoarthritis. Clin Sports Med 2014;33(4):633-40.
4. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. Clin Geriatr Med 2010;26(3):355-69.
5. O'Neill TW, McCabe PS, McBeth J. Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. Best Pract Res Clin Rheumatol 2018;32(2):312-26.
6. Collins DP, Elsouri KN, Demory Beckler M. Osteoarthritis: Can We Do Better? Cureus 2022;14(11):e31505.
7. Sinatti P, Sanchez Romero EA, Martinez-Pozas O, Villafane JH. Effects of Patient Education on Pain and Function and Its Impact on Conservative Treatment in Elderly Patients with Pain Related to Hip and Knee Osteoarthritis: A Systematic Review. International Journal of Environmental Research and Public Health 2022;19(10).
8. Richards MM, Maxwell JS, Weng L, Angelos MG, Golzarian J. Intra-articular treatment of knee osteoarthritis: from anti-inflammatories to products of regenerative medicine. The Physician and Sportsmedicine 2016; 44(2):101-8.
9. Charlier E, Deroyer C, Ciregia F, Malaise O, Neuville S, Plener Z, Malaise M, de Seny D. Chondrocyte dedifferentiation and osteoarthritis (OA). Biochem Pharmacol 2019;165:49-65.
10. Pacifici M, Koyama E, Shibukawa Y, Wu C, Tamamura Y, Enomoto-Iwamoto M, Iwamoto M. Cellular and molecular mechanisms of synovial joint and articular cartilage formation. Ann N Y Acad Sci 2006;1068:74-86.
11. Sanchez-Lopez E, Coras R, Torres A, Lane NE, Guma M. Synovial inflammation in osteoarthritis progression. Nat Rev Rheumatol 2022;18(5):258-75.
12. Choi MC, Jo J, Park J, Kang HK, Park Y. NF-kappaB Signaling Pathways in Osteoarthritic Cartilage Destruction. Cells 2019;8(7).
13. Wen X, Chen S, Chen X, Qiu H, Wang W, Zhang N, Liu

W, Wang T, Ding X, Zhang L. ITGB5 promotes innate radiation resistance in pancreatic adenocarcinoma by promoting DNA damage repair and the MEK/ERK signaling pathway. Front Oncol 2022;12:887068.
14. Du C, Chen X. Transcriptome Profiling of Oocytes at the Germinal Vesicle Stage from Women from Mongolia with Polycystic Ovary Syndrome. Int J Gen Med 2021; 14:4469-78.
15. Cretu D, Liang K, Saraon P, Batruch I, Diamandis EP, Chandran V. Quantitative tandem mass-spectrometry of skin tissue reveals putative psoriatic arthritis biomarkers. Clin Proteomics 2015;12(1):1.
16. Shi W, He J, Huang Y, Zeng Z, Feng Z, Xu H, Nie Y. Integrin beta5 enhances the malignancy of human colorectal cancer by increasing the TGF-beta signaling. Anti-cancer Drugs 2021;32(7):717-26.
17. Yang T, Chen M, Ma K, Fu X. [The effects of down-regulated ITGB5 expression on the proliferation of keloid fibroblasts]. Zhonghua Zheng Xing Wai Ke Za Zhi 2017;33(1):49-52.
18. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 2008;9:559.
19. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43(7):e47.
20. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16(5):284-7.
21. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019; 47(D1):D607-D13.
22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13(11):2498-504.
23. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015;12(5):453-7.
24. Loft A, Forss I, Siersbaek MS, Schmidt SF, Larsen AS, Madsen JG, Pisani DF, Nielsen R, Aagaard MM, Mathison A, Neville MJ, Urrutia R, Karpe F, Amri EZ, Mandrup S. Browning of human adipocytes requires KLF11 and reprogramming of PPARgamma superenhancers. Genes Dev 2015;29(1):7-22.
25. Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, Hilton JA, Jain K, Baymuradov UK, Narayanan AK, Onate KC, Graham K, Miyasato SR, Dreszer TR, Strattan JS, Jolanki O, Tanaka FY, Cherry JM. The Encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res 2018;46(D1):D794-D801.
26. Qian W, Li Z. Expression and diagnostic significance of integrin beta-2 in synovial fluid of patients with osteoarthritis. Journal of orthopaedic surgery (Hong Kong) 2023;31(2):10225536221147213.
27. Sun L, Zheng W, Liu QD, Ge L. Valproic Acid Protects Chondrocytes from LPS-Stimulated Damage via Regulating miR-302d-3p/ITGB4 Axis and Mediating the PI3K-AKT Signaling Pathway. Frontiers in Molecular Biosciences 2021;8:633315.
28. Park M, Kang KW, Kim JW. The role and application of transcriptional repressors in cancer treatment. Arch Pharm Res 2023;46(1):1-17.
29. Sladek TL. E2F transcription factor action, regulation and possible role in human cancer. Cell Proliferation 1997;30(3-4):97-105.
30. Kawata M, Teramura T, Ordoukhanian P, Head SR, Natarajan P, Sundaresan A, Olmer M, Asahara H, Lotz MK. Kruppel-like factor-4 and Kruppel-like factor-2 are important regulators of joint tissue cells and protect against tissue destruction and inflammation in osteoarthritis. Ann Rheum Dis. 2022.
31. Han F, Jiang H, Qu W, Rui YJ. KLF11 protects chondrocytes via inhibiting p38 MAPK signaling pathway. Eur Rev Med Pharmacol Sci 2020;24(12):6505-16.
32. Luo DD, Zhao F. KLF4 suppresses the proliferation and metastasis of NSCLC cells via inhibition of MSI2 and regulation of the JAK/STAT3 signaling pathway. Transl Oncol 2022;22:101396.
33. Wang BW, Jiang Y, Yao ZL, Chen PS, Yu B, Wang SN. Aucubin Protects Chondrocytes Against IL-1beta-Induced Apoptosis In Vitro And Inhibits Osteoarthritis In Mice Model. Drug Des Devel Ther 2019;13:3529-38.
34. Xu M, Feng M, Peng H, Qian Z, Zhao L, Wu S. Epigenetic regulation of chondrocyte hypertrophy and apoptosis through Sirt1/P53/P21 pathway in surgery-induced osteoarthritis. Biochem Biophys Res Commun 2020; 528(1):179-85.
35. Hashimoto S, Nishiyama T, Hayashi S, Fujishiro T, Takebe K, Kanzaki N, Kuroda R, Kurosaka M. Role of p53 in human chondrocyte apoptosis in response to shear strain. Arthritis and Rheumatism 2009;60(8):2340-9.
36. Li X, Han Y, Li G, Zhang Y, Wang J, Feng C. Role of Wnt signaling pathway in joint development and cartilage degeneration. Front Cell Dev Biol 2023;11:1181619.
37. Gong Z, Wang Y, Li L, Li X, Qiu B, Hu Y. Cardamonin alleviates chondrocytes inflammation and cartilage degradation of osteoarthritis by inhibiting ferroptosis via p53 pathway. Food Chem Toxicol 2023;174:113644.
38. Hou L, Shi H, Wang M, Liu J, Liu G. MicroRNA-497-5p attenuates IL-1beta-induced cartilage matrix degradation in chondrocytes via Wnt/beta-catenin signal pathway. International Journal of Clinical and Experimental Pathology 2019;12(8):3108-18.
39. Zhang LB, Man ZT, Li W, Zhang W, Wang XQ, Sun S. Calcitonin protects chondrocytes from lipopolysaccharide-induced apoptosis and inflammatory

response through MAPK/Wnt/NF-kappaB pathways. Mol Immunol 2017;87:249-57.
40. Yang J, Hou Y, Zhou M, Wen S, Zhou J, Xu L, Tang X, Du YE, Hu P, Liu M. Twist induces epithelial-mesenchymal transition and cell motility in breast cancer via ITGB1-FAK/ILK signaling axis and its associated downstream network. The International Journal of Biochemistry & Cell Biology 2016;71:62-71.
41. Jin G, Wang K, Liu Y, Liu X, Zhang X, Zhang H. Proteomic Level Changes on Treatment in MCF-7/DDP Breast Cancer Drug- Resistant Cells. Anticancer Agents Med Chem 2020;20(6):687-99.
42. Lieberthal J, Sambamurthy N, Scanzello CR. Inflammation in joint injury and post-traumatic osteoarthritis. Osteoarthritis Cartilage 2015;23(11):1825-34.
43. Han G, Zhang Y, Li H. The Combination Treatment of Curcumin and Probucol Protects Chondrocytes from TNF-alpha Induced Inflammation by Enhancing Autophagy and Reducing Apoptosis via the PI3K-Akt-mTOR Pathway. Oxid Med Cell Longev 2021;2021:5558066.
44. Chen M, Chen Z, Huang D, Sun C, Xie J, Chen T, Zhao X, Huang Y, Li D, Wu B, Wu D. Myricetin inhibits TNF-alpha-induced inflammation in A549 cells via the SIRT1/NF-kappaB pathway. Pulm Pharmacol Ther 2020;65:102000.
45. Wang C, Gao Y, Zhang Z, Chi Q, Liu Y, Yang L, Xu K. Safflower yellow alleviates osteoarthritis and prevents inflammation by inhibiting PGE2 release and regulating NF-kappaB/SIRT1/AMPK signaling pathways. Phytomedicine: international journal of phytotherapy and Phytopharmacology 2020;78:153305.
46. Chen Z, Lin CX, Song B, Li CC, Qiu JX, Li SX, Lin SP, Luo WQ, Fu Y, Fang GB, Wei-Ping L, Saw PE, Ding Y. Spermidine activates RIP1 deubiquitination to inhibit TNF-alpha-induced NF-kappaB/p65 signaling pathway in osteoarthritis. Cell Death Dis 2020;11(7):503.
47. Yang C, Lan R, Wang X, Zhao Q, Li X, Bi J, Wang J, Yang G, Lin Y, Liu J, Yin G. Integrin beta3, a RACK1 interacting protein, is critical for porcine reproductive and respiratory syndrome virus infection and NF-kappaB activation in Marc-145 cells. Virus Res 2020;282:197956.
48. Yee NK, Hamerman JA. beta(2) integrins inhibit TLR responses by regulating NF-kappaB pathway and p38 MAPK activation. European Journal of Immunology 2013;43(3):779-92.
49. Nedunchezhiyan U, Varughese I, Sun AR, Wu X, Crawford R, Prasadam I. Obesity, Inflammation, and Immune System in Osteoarthritis. Front Immunol 2022;13:907750.
50. Li YS, Luo W, Zhu SA, Lei GH. T Cells in Osteoarthritis: Alterations and Beyond. Front Immunol 2017;8:356.
51. Sekiya T, Yoshimura A. In Vitro Th Differentiation Protocol. Methods in molecular biology (Clifton, NJ) 2016;1344:183-91.
52. Yamada H, Nakashima Y, Okazaki K, Mawatari T, Fukushi J, Oyamada A, Fujimura K, Iwamoto Y, Yoshikai Y. Preferential accumulation of activated Th1 cells not only in rheumatoid arthritis but also in osteoarthritis joints. J Rheumatol 2011;38(8):1569-75.

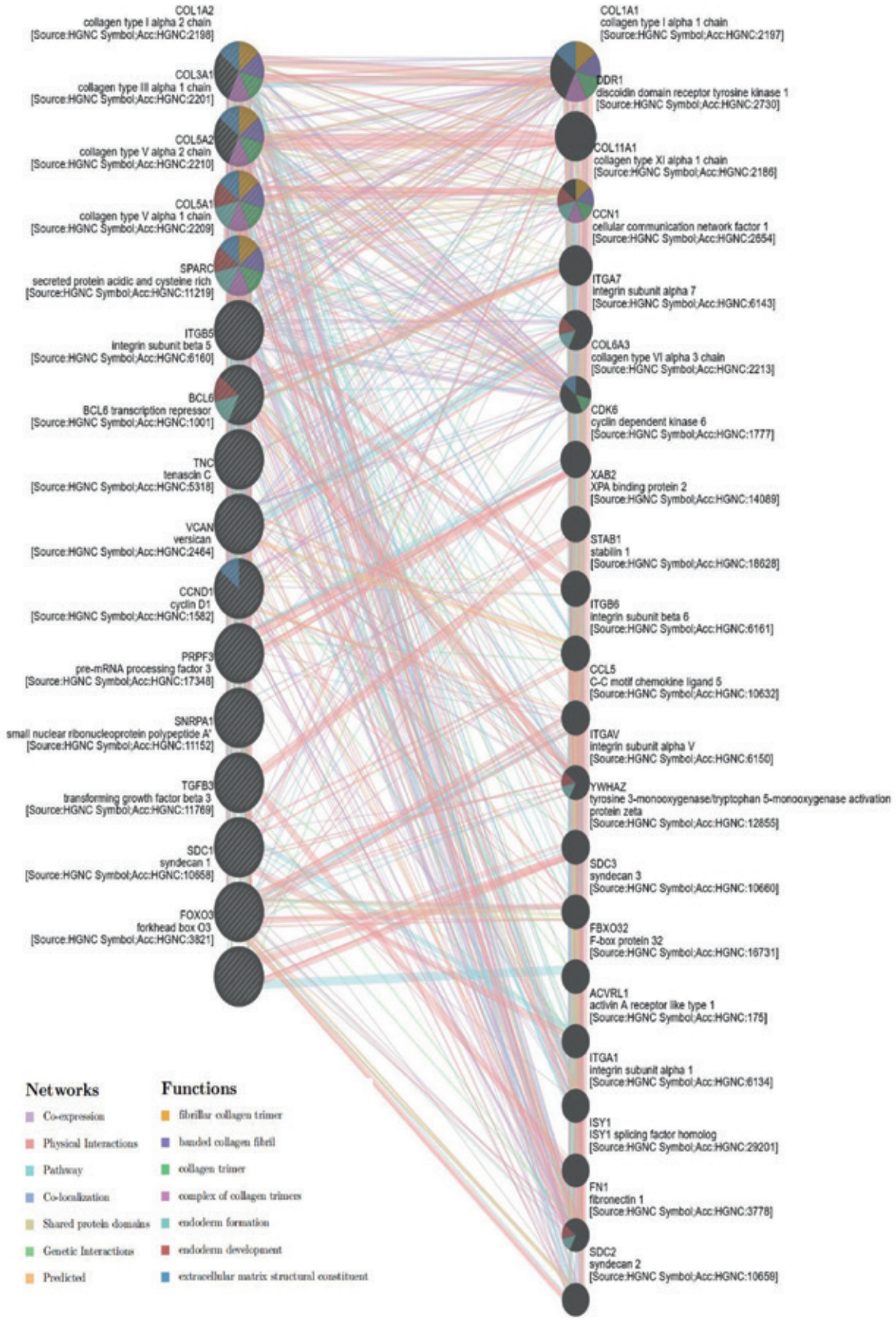

Figure S1. PPI network by GENEMANIA.

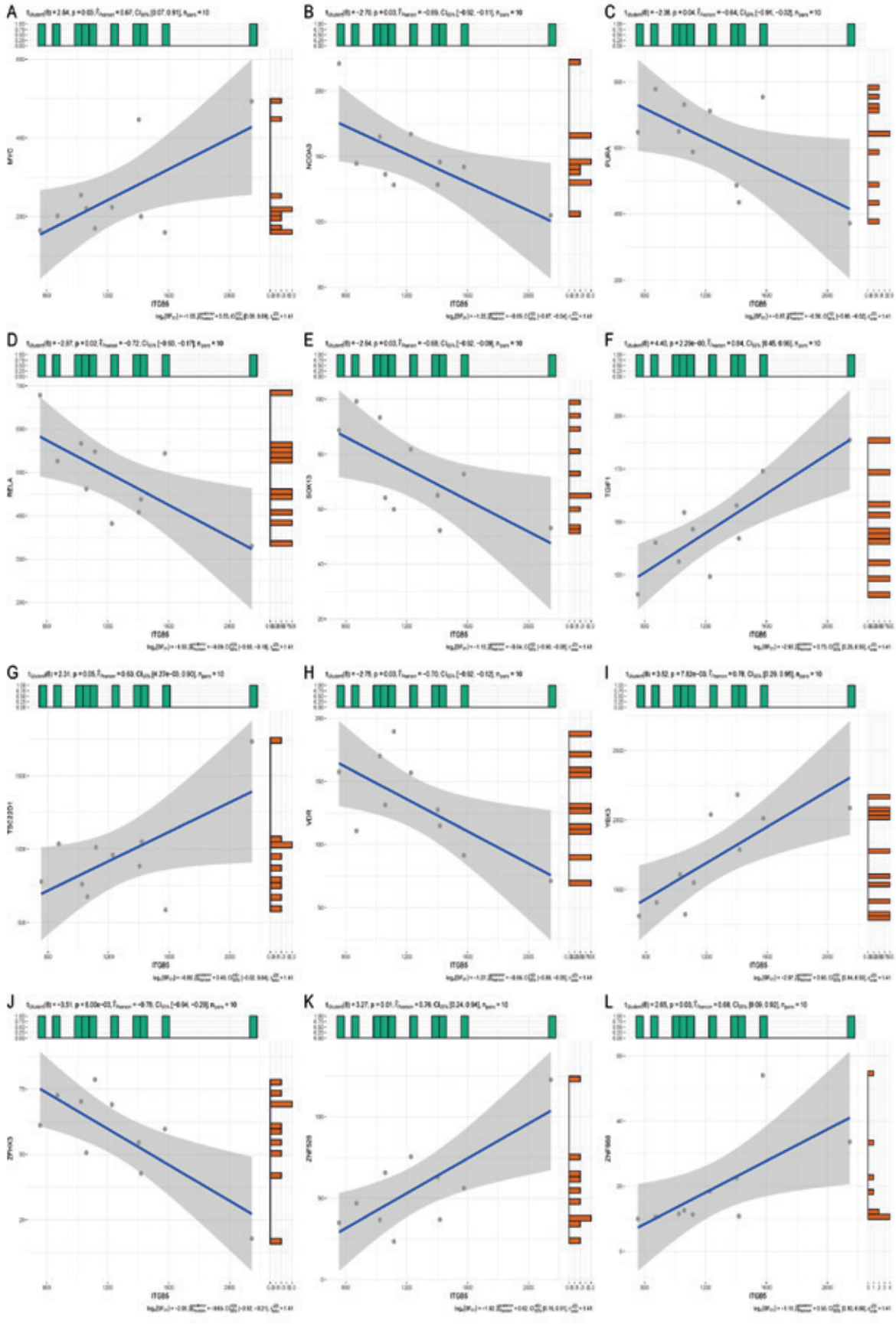

Figure S2. Correlations between ITGB5 expression and TFs.