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# Identification of genes and pathways associated with osteoarthritis by bioinformatics analyses

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**Abstract. – OBJECTIVE: This study aimed to explore the molecular mechanism of osteoarthritis (OA) development and discover underlying genes associated with OA.**

**DATA AND METHODS: Gene expression profile GSE48556 including 106 peripheral blood mononuclear cells (PBMCs) of osteoarthritis patients and 33 PBMCs of healthy controls was downloaded from the Gene Expression Omnibus database. The limma package was used to identify the differentially expressed genes (DEGs) by paired t-test. The functional enrichment analyses of DEGs was performed, followed by the construction of protein-protein interaction (PPI) network.**

**RESULTS: Total 432 DEGs including 178 up-regulated DEGs and 254 down-regulated DEGs were identified. Pathways of cytokine-cytokine receptor interaction and T cell receptor signaling pathway were significantly up-regulated in OA. Biological processes of negative regulation of transcription from RNA polymerase II promoter and negative regulation of transcription, DNA-dependent were significantly down-regulated in OA. The platelet-derived growth factor receptor, beta polypeptide (PDGFRB), interferon, gamma (IFNG), early growth response 1 (EGR1), Fas ligand (TNF superfamily, member 6) (FASLG), H3 histone, family 3B (H3.3B) (H3F3B) and so on had higher connectivity degree in the PPI networks.**

**CONCLUSIONS: DEGs of OA were mainly enriched in the pathways associated with cytokine-cytokine receptor interaction and T cell receptor signaling pathway. The DEGs such as PDGFRB, IFNG, EGR1, FASLG and H3F3B may be the potential targets for OA diagnosis and treatment.**

*Key Words:*
Osteoarthritis, Molecular mechanism, Differentially expressed genes, Pathway enrichment analysis.

## Introduction

Osteoarthritis (OA) also known as osteoarthrosis or degenerative arthritis or degenerative joint disease, is the most common form of arthritis[1]. Characteristic pathological changes of OA include articular cartilage degeneration, angiogenesis, and synovial inflammation, all of which are associated with decreased muscle strength and capsule laxitude[2]. Patients with symptomatic OA undergo a significant effect on multiple dimensions of health-relate quality of life[3]. The prevalence of OA increases with age. At present, OA is already the leading cause of disability among the elderly[4].

There are considerable studies about exploring the mechanism and therapeutic method for OA. The development and progression of OA have been considered to involve inflammation throughout the disease[5]. Increased mononuclear cell infiltration and over-expression of mediators of inflammation have been found in early OA[6]. Importantly, genetic factors have also been found to play critical roles in the OA progression. Insulin-like growth factor 1, for instance, is an important growth factor for cartilage homeostasis and has been found to reduce expression in OA patient[8]. Conversely, the anabolic response of cartilage to transforming growth factor β (TGF-β) is elevated in OA. Additionally, Mehraban et al[8] has demonstrated that cathepsin-B is up-regulated in synovial tissue during the early degenerative phase of OA. Cathepsin-B can cleave aggrecan at a site near to that of matrix metalloproteases 3 (MMP3) which is thought to be active in OA pathogenesis[9]. At present, some critical pathways have been found to be related to OA, such as tyrosine metabolism pathway 1, Wnt signaling pathway 10, circadian rhythm pathway[11]. Although progresses have been achieved about the pathogenesis of OA, the genetic mechanisms of OA are far from being understood.

In the present study, we downloaded the microarray data of GSE48556 and identified the differentially expressed genes (DEGs) between the osteoarthritis patients (OA) and healthy con-



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trols (HC) samples to explore the molecular mechanisms of OA. Besides, we performed functional enrichment analyses and protein-protein interaction (PPI) networks analysis to study and identify the DEGs and pathways for diagnosis and treatment of OA. Findings of this study may play important roles in OA genesis and may potentially serve as biomarkers in both diagnosis and prognosis of OA.

## Data and Methods

### Affymetrix Microarray Data

The microarray data of GSE48556[12] was downloaded from National Center of Biotechnology Information (NCBI) Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/), based on the platform of Illumina Human HT-12 V3.0 expression beadchip. A total of 139 samples were applied to develop array data, including 106 peripheral blood mononuclear cells (PBMCs) of OA and 33 PBMCs of HC.

### Data Preprocessing and DEGs Analysis

The original array data were converted into expression measures and then performed background correction, quartile data normalization and gene ID conversion by the robust multiarray average (RMA)[13] algorithm in R affy package.

The paired t-test based on the limma package in R language was used to identify genes that were differentially expressed between OA and HC samples. Multiple testing was corrected by the Benjamini and Hochberg (BH)[14] procedure to obtain the adjusted $p$-value. Then the $\log_2$-fold change ($\log_2$FC) was calculated. The adjusted p-value $< 0.05$ and $|\log_2 FC| > 0.5$ were considered as the cutoff value for DEGs screening.

### Gene Ontology and Pathway Enrichment Analyses of DEGs

Gene ontology (GO)[15] is a tool for unification of biology which collects structured, defined and controlled vocabulary for a large scale of genes annotation. Kyoto Encyclopedia of Genes and Genomes (KEGG)[16] database is used for classification correlating gene sets into their respective pathways. The Database for Annotation, Visualization and Integrated Discovery (DAVID)[17], as a comprehensive set of functional annotation tools, has been developed for relating the functional terms with gene lists by clustering algorithm. We performed GO enrichment (p-value $< 0.05$) and KEGG pathway ($p$-value $< 0.1$ and count $> 2$) analyses of the DEGs using the DAVID online tool.

### PPI Network Construction

The Search Tool for the Retrieval of Interacting Genes (STRING)[18] database is a precomputed global resource which has been designed to evaluate the PPI information. In this paper, the STRING online tool was applied to analyze the PPI of DEGs and only those experimentally validated interactions with a combined score $> 0.4$ was selected as significant.

From the previous studies on biological network obtained, most of the PPI networks obeyed the scale-free attribution. So connectivity degree was analyzed by statistics in networks to obtain the important nodes, namely hub proteins[19] which participated in PPI relationships of the networks.

## Results

### Identification of DEGs

After data preprocessing, total of 432 genes were assessed to be differentially expressed in OA compared with the expression profiles from HC. Among these DEGs, 178 genes were up-regulated and 254 ones were down-regulated. The result has been shown in the volcano plot (Figure 1).

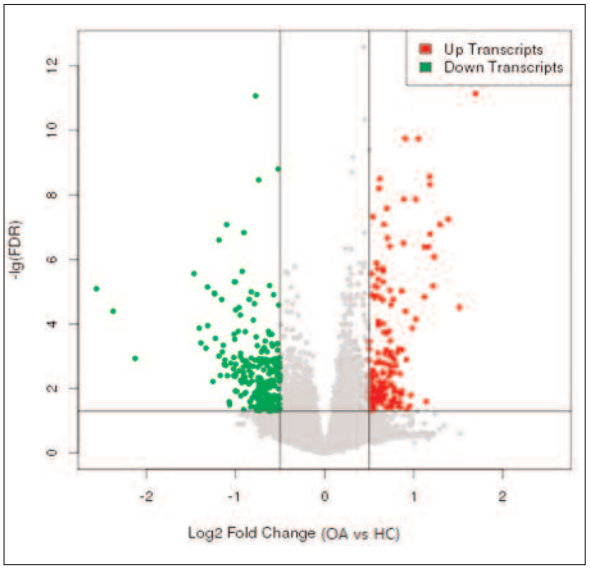


**Figure 1.** Volcano plot of microarray data. FDR stands for false discovery rate; OA stands for osteoarthritis patients; HC stands for healthy controls.

### GO and Pathway Enrichment Analysis of DEGs

After GO functional enrichment analysis, 24 biological process (BP) terms enriched by the up-regulated DEGs were obtained. The 24 BP terms were clustered into 3 functional clusterings and their functional enrichment scores were respectively 2.056, 1.758 and 1.641 (Table I). Additionally, 28 BP terms enriched by the down-regulated DEGs were obtained and their functional enrichment scores were 2.524, 2.318 and 1.726 respectively (Table II).

The significantly enriched pathways of the up-regulated DEGs and down-regulated DEGs were shown in Tables I and II respectively. The up-regulated DEGs were enriched in pathways related to cytokine-cytokine receptor interaction and T cell receptor signaling pathway (Table I) and the down-regulated DEGs were enriched in 5 pathways such as systemic lupus erythematosus and vascular smooth muscle contraction (Table II).

### PPI Network Construction

Based on STRING database, total of 177 protein pairs including 58 up-regulated pairs (Figure 2) and 119 down-regulated pairs (Figure 3) were obtained. In the PPI network, there were 10 up-regulated hub genes with degree score ≥ 3 such as Fas ligand (TNF superfamily, member 6) (*FASLG*) (degree score = 9) and interferon, gamma (*IFNG*) (degree score = 8) and 25 down-regulated hub genes with degree score ≥ 4 such as early growth response 1 (*EGR1*) (degree score = 10) and H3 histone, family 3B (H3.3B) (*H3F3B*) (degree score = 7) (Table III).

**Table I.** The enriched GO terms and KEGG pathways for the up-regulated DEGs.

| Category | Term | Description | Count | *p*-value |
|---|---|---|---|---|
| Up 1 | Enrichment Score: 2.056 | | | |
| BP | GO:0051249 | Regulation of lymphocyte activation | 8 | 7.01E-04 |
| BP | GO:0002694 | Regulation of leukocyte activation | 8 | 1.37E-03 |
| BP | GO:0050865 | Regulation of cell activation | 8 | 1.86E-03 |
| BP | GO:0050863 | Regulation of T cell activation | 6 | 6.31E-03 |
| BP | GO:0051251 | Positive regulation of lymphocyte activation | 5 | 1.61E-02 |
| BP | GO:0006955 | Immune response | 14 | 2.04E-02 |
| BP | GO:0002696 | Positive regulation of leukocyte activation | 5 | 2.16E-02 |
| BP | GO:0050867 | Positive regulation of cell activation | 5 | 2.50E-02 |
| BP | GO:0002684 | Positive regulation of immune system process | 7 | 3.21E-02 |
| BP | GO:0050871 | Positive regulation of B cell activation | 3 | 4.27E-02 |
| Up 2 | Enrichment Score: 1.758 | | | |
| BP | GO:0043065 | Positive regulation of apoptosis | 12 | 3.85E-03 |
| BP | GO:0043068 | Positive regulation of programmed cell death | 12 | 4.06E-03 |
| BP | GO:0010942 | Positive regulation of cell death | 12 | 4.20E-03 |
| BP | GO:0008219 | Cell death | 14 | 2.74E-02 |
| BP | GO:0016265 | Death | 14 | 2.88E-02 |
| BP | GO:0042981 | Regulation of apoptosis | 15 | 2.97E-02 |
| BP | GO:0043067 | Regulation of programmed cell death | 15 | 3.20E-02 |
| BP | GO:0010941 | Regulation of cell death | 15 | 3.28E-02 |
| BP | GO:0006915 | Apoptosis | 12 | 3.84E-02 |
| BP | GO:0012501 | Programmed cell death | 12 | 4.20E-02 |
| Up 3 | Enrichment Score: 1.641 | | | |
| BP | GO:0042325 | Regulation of phosphorylation | 11 | 1.83E-02 |
| BP | GO:0051174 | Regulation of phosphorus metabolic process | 11 | 2.34E-02 |
| BP | GO:0019220 | Regulation of phosphate metabolic process | 11 | 2.34E-02 |
| BP | GO:0032147 | Activation of protein kinase activity | 5 | 2.73E-02 |
| KEGG | hsa04060 | Cytokine-cytokine receptor interaction | 12 | 6.07E-05 |
| KEGG | hsa04660 | T cell receptor signaling pathway | 5 | 2.54E-02 |
| KEGG | hsa04650 | Natural killer cell mediated cytotoxicity | 5 | 4.89E-02 |
| KEGG | hsa05330 | Allograft rejection | 3 | 5.26E-02 |
| KEGG | hsa04612 | Antigen processing and presentation | 4 | 5.48E-02 |

DEGs stands for differentially expressed genes; Up stands for up-regulated BP terms and pathways; GO stands for Gene ontology; BP stands for biological process; KEGG stands for Kyoto Encyclopedia of Genes and Genomes; category stands for the GO functional category; count stands for the number of enriched DEGs.

**Table II.** The enriched GO terms and KEGG pathways for the down-regulated DEGs.

| Category | Term | Description | Count | *p*-value |
|---|---|---|---|---|
| Down 1 | Enrichment Score: 2.524 | | | |
| BP | GO:0006334 | Nucleosome assembly | 7 | 7.15E-04 |
| BP | GO:0031497 | Chromatin assembly | 7 | 8.61E-04 |
| BP | GO:0065004 | Protein-DNA complex assembly | 7 | 1.09E-03 |
| BP | GO:0034728 | Nucleosome organization | 7 | 1.22E-03 |
| BP | GO:0006333 | Chromatin assembly or disassembly | 8 | 1.22E-03 |
| BP | GO:0034622 | Cellular macromolecular complex assembly | 12 | 2.62E-03 |
| BP | GO:0006323 | DNA packaging | 7 | 3.90E-03 |
| BP | GO:0034621 | Cellular macromolecular complex subunit organization | 12 | 6.23E-03 |
| BP | GO:0065003 | Macromolecular complex assembly | 16 | 2.35E-02 |
| BP | GO:0043933 | Macromolecular complex subunit organization | 16 | 3.87E-02 |
| Down 2 | Enrichment Score: 2.318 | | | |
| BP | GO:0000122 | Negative regulation of transcription from RNA polymerase II promoter | 13 | 1.60E-04 |
| BP | GO:0045892 | Negative regulation of transcription, DNA-dependent | 14 | 6.64E-04 |
| BP | GO:0051253 | Negative regulation of RNA metabolic process | 14 | 7.75E-04 |
| BP | GO:0006357 | Regulation of transcription from RNA polymerase II promoter | 21 | 1.00E-03 |
| BP | GO:0016481 | Negative regulation of transcription | 14 | 6.11E-03 |
| BP | GO:0010558 | Negative regulation of macromolecule biosynthetic process | 15 | 1.06E-02 |
| BP | GO:0010629 | Negative regulation of gene expression | 14 | 1.28E-02 |
| BP | GO:0031327 | Negative regulation of cellular biosynthetic process | 15 | 1.30E-02 |
| BP | GO:0045934 | Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 14 | 1.44E-02 |
| BP | GO:0009890 | Negative regulation of biosynthetic process | 15 | 1.53E-02 |
| BP | GO:0051172 | Ñegative regulation of nitrogen compound metabolic process | 14 | 1.59E-02 |
| BP | GO:0010605 | Negative regulation of macromolecule metabolic process | 16 | 4.92E-02 |
| Down 3 | Enrichment Score: 1.726 | | | |
| BP | GO:0006357 | Regulation of transcription from RNA polymerase II promoter | 21 | 1.00E-03 |
| BP | GO:0045944 | Positive regulation of transcription from RNA polymerase II promoter | 11 | 2.10E-02 |
| BP | GO:0045935 | Positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 15 | 2.93E-02 |
| BP | GO:0051173 | Ppositive regulation of nitrogen compound metabolic process | 15 | 3.68E-02 |
| BP | GO:0045893 | Positive regulation of transcription, DNA-dependent | 12 | 4.28E-02 |
| BP | GO:0051254 | Positive regulation of RNA metabolic process | 12 | 4.49E-02 |
| KEGG | hsa05322 | Systemic lupus erythematosus | 6 | 1.06E-02 |
| KEGG | hsa04270 | Vascular smooth muscle contraction | 6 | 1.74E-02 |
| KEGG | hsa04621 | NOD-like receptor signaling pathway | 4 | 5.12E-02 |
| KEGG | hsa00230 | Purine metabolism | 6 | 5.57E-02 |
| KEGG | hsa04722 | Neurotrophin signaling pathway | 5 | 8.68E-02 |

DEGs stands for differentially expressed genes; Down stands for down-regulated BP terms and pathways; GO stands for Gene ontology; BP stands for biological process; KEGG stands for Kyoto Encyclopedia of Genes and Genomes; category stands for the GO functional category; count stands for the number of enriched DEGs.

## Discussion

OA is a slowly progressive rheumatic disease observed mainly in elderly people[20]. Its etiologies involve biomechanical, biochemical, and genetic factors, all of which may contribute to the OA lesion in cartilage by disrupting chondrocyte-matrix associations and altering metabolic responses in the chondrocyte[21]. In the present study, the analysis of gene expression profiling revealed the abnormally expressed genes associated with OA and enabled the identification of targets for therapeutic strategy. In this study, a total of 432 DEGs were identified between OA and HC samples.

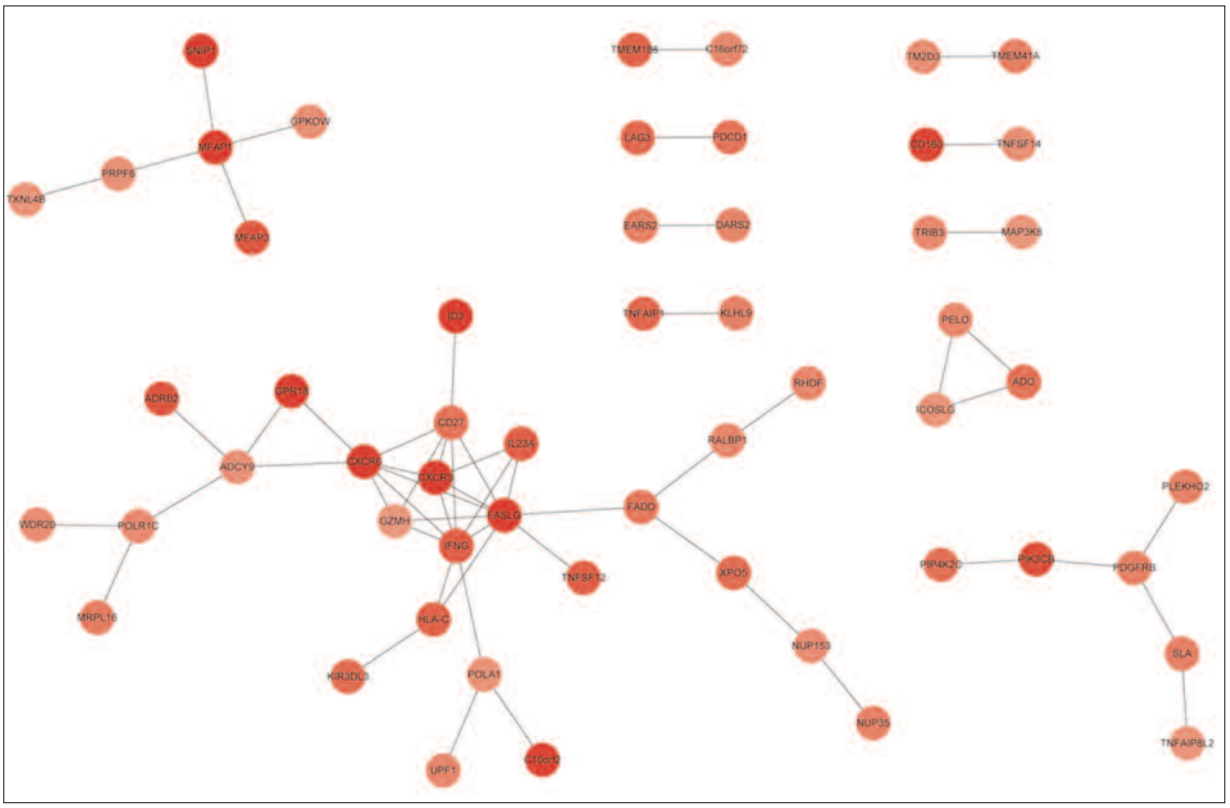


**Figure 2.** The constructed protein-protein interaction network of up-regulated DEGs. Node stands for the protein (gene); edge stands for the interaction of proteins.

The up-regulated DEGs were enriched in pathways of cytokine-cytokine receptor interaction and T cell receptor signaling pathway, while the down-regulated DEGs were enriched in BP terms related to negative regulation of transcription. In addition, in the PPI network, *PDGFRB*, *IFNG*, *EGR1*, *FASLG* and *H3F3B* had higher connectivity degree. The results suggested that these genes and pathways may play important roles in the progression of OA.

Cytokines which are produced in joint tissues and released into the synovial fluid regulate a broad range of inflammatory processes[22]. Many of these factors are necessary for normal homeostasis and keep at low levels, but in OA their balance may be disturbed[21]. Kokkonen et al[23] also reported that many cytokines are expressed and functionally actived in the synovial tissue when the disease develops. In this study, the cytokine-cytokine receptor interaction pathway was found enriched with several up-regulated DEGs such as *PDGFRB*. As a cell surface tyrosine kinase receptor, *PDGFRB* plays an essential role in the regulation of embryonic development, cell proliferation, survival, differentiation, chemotaxis and migration[24]. PDGFRB has been detected in fibroblast-like cells in the inflamed synovial membrane[25]. Remmers et al[26] suggested that PDGFRB over-expressed significantly in the synovial membrane of rheumatoid arthritis (RA) and OA patients. In addition, T cell mediated immune response to some antigens is present in the target organs of autoimmune diseases[27]. Study[28] has reported that T cells are responsible for the initiation and perpetuation of ongoing synovial inflammation, which may lead to cartilage and joint destruction brought about by some enzymes[29]. Oligoclonal expansion of T cells was reported in the synovial tissue of RA[30]. Sakkas et al[31] also suggested that T cells plays a significant role in the pathogenesis of RA. In this study, BP term associated with regulation of T cell activation and pathway of T cell receptor signaling pathway were enriched by up-regulated DEGs such as *IFNG* which was a hub gene with higher connectivity degree of 8. IFNG is a soluble cytokine of T cell with immunoregulatory, antiviral and anti-tumor properties as well as a potent activator of macrophages[32]. Previous study[33] has found that IFNG and its specific receptor could

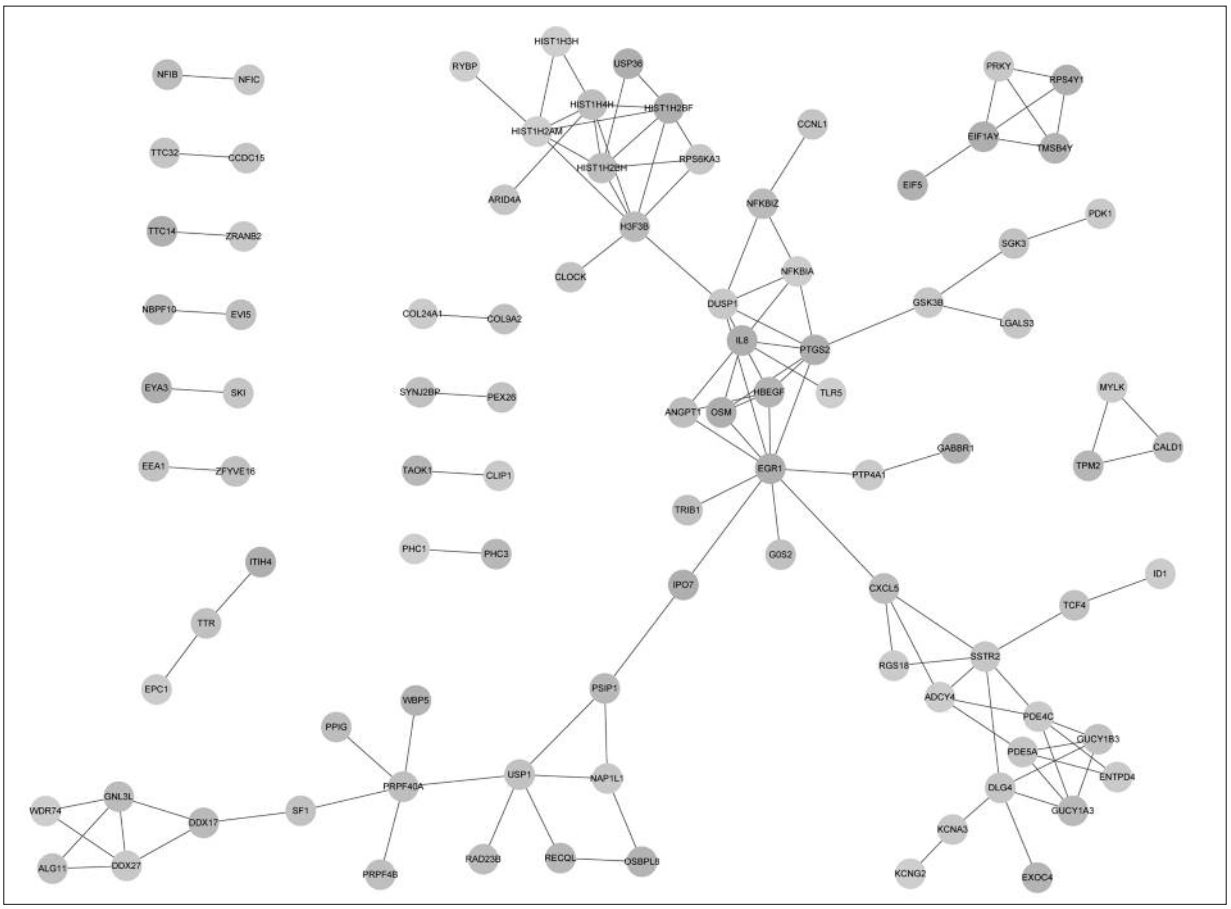


**Figure 3.** The constructed protein-protein interaction network of down-regulated DEGs. Node stands for the protein (gene); edge stands for the interaction of proteins.

be detected in synovial tissue specimens of patients with OA and RA. Recently, Doodes et al[34] found that IFNG has several proinflammatory properties which contribute to inflammation in arthritis. As a result, DEGs related to the pathways of cytokine-cytokine receptor interaction and T cell receptor signaling pathway may be used as potential targets for OA treatment.

We also observed that the down-regulated DEGs of *EGR1* which had the highest connectiv-

**Table III.** The statistical results of connectivity degree of top 8 hub genes in protein-protein interaction network.

| Gene | Degree | adj. *p* value | Gene | Degree | adj. *p* value |
|---|---|---|---|---|---|
| Up-regulated DEGs | | | | | |
| FASLG | 9 | 4.06E-07 | CXCR3 | 5 | 1.48E-05 |
| IFNG | 8 | 1.56E-02 | MFAP1 | 4 | 7.27E-12 |
| CXCR6 | 7 | 6.72E-06 | ADCY9 | 4 | 1.86E-02 |
| CD27 | 6 | 2.85E-03 | GZMH | 4 | 3.68E-02 |
| Down-regulated DEGs | | | | | |
| EGR1 | 10 | 4.04E-05 | SSTR2 | 6 | 1.69E-02 |
| H3F3B | 7 | 8.59E-12 | HIST1H2BH | 6 | 1.38E-02 |
| PTGS2 | 7 | 1.78E-03 | HIST1H2AM | 6 | 2.06E-02 |
| DUSP1 | 6 | 2.47E-02 | HIST1H2BF | 6 | 2.18E-04 |

DEGs stands for differentially expressed genes and adj. *p*. value stands for adjusted *p*-value.

ity degree participated in several GO BP terms such as negative regulation of transcription from RNA polymerase II promoter and negative regulation of transcription, DNA-dependent. EGR1 is an immediate early gene which acts as a nuclear coupler of early cytoplasmic events to long-term alterations in gene expression[35]. EGR1 has been shown to stimulate the expression of TGF-β which is a member of a gene family involved in proliferation and differentiation of cartilage chondrocytes[36]. Importantly, EGR1 mRNA is found significantly down-regulated in cartilage of OA[37]. Although the present evidence of direct association between the two BP terms above and OA progression are rare, these BP terms and their enriched gene *EGR1* may be critical for OA progression based on our results.

In the PPI network, we also found *FASLG* and *H3F3B* were hub genes with high connectivity degree. The protein encoded by *FASLG* is the ligand for fas cell surface death receptor (FAS). The interaction between FAS and this ligand is critical in triggering apoptosis of several kinds of cells[38]. FASLG is an important molecule for the regulation of apoptosis of chondrocytes in OA cartilage. FASLG expression is found elevated in OA cartilage compared with normal cartilage[39]. Additionally, Hashimoto et al[40] has confirmed that FASLG could induce apoptosis in chondrocytes and found a significant amount of FASLG in synovial fluids of patients with OA. In this study, the hub gene, *H3F3B*, was found down-regulated in the PPI network. H3F3B belongs to histone family. Histones are basic nuclear proteins which are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes[41]. A previous study[42] identified two members of the histone family, H3F3B and histone cluster 2, H2aa3, to be down-regulated in OA chondrocytes relative to healthy control samples. Taken together, these data support the hypothesis that *FASLG* and *H3F3B* may be a candidate molecular marker associated with OA.

## Conclusions

Our data provide a comprehensive bioinformatics analysis of DEGs, BP terms and pathway which may be involved in OA. The findings in current study may contribute to our understanding of the underlying molecular mechanisms of OA. DEGs such as *PDGFRB*, *IFNG*, *EGR1*, *FASLG* and *H3F3B* and their related BP terms and pathways such as cytokine-cytokine receptor interaction and T cell receptor signaling pathway have the potential to be used as targets for OA diagnosis and treatment.

In the process of data analysis, only a few cases of OA and HC samples based on one platform were used for the analyses. This may cause a high rate of false positive result. Further genetic and experimental studies with larger sample size are still needed to confirm our observation.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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