Bioinformatics analysis to identify key genes and pathways influencing synovial inflammation in osteoarthritis

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Abstract. Osteoarthritis (OA) is a chronic arthropathy that occurs in the middle-aged and elderly population. The present study aimed to identify gene signature differences between synovial cells from OA synovial membrane with and without inflammation, and to explain the potential mechanisms involved. The differentially expressed genes (DEGs) between 12 synovial membrane with inflammation and 12 synovial membrane without inflammation from the dataset GSE46750 were identified using the Gene Expression Omnibus 2R. The DEGs were subjected to enrichment analysis, protein-protein interaction (PPI) analysis and module analysis. The analysis results were compared with text-mining results. A total of 174 DEGs were identified. Gene Ontology enrichment results demonstrated that functional molecules encoded by the DEGs primarily had extracellular location, molecular functions predominantly involving 'chemokine activity' and 'cytokine activity', and were associated with biological processes, including 'inflammatory response' and 'immune response'. The Kyoto Encyclopedia of Genes and Genomes results demonstrated that DEGS may function through pathways associated with 'rheumatoid arthritis', 'chemokine signaling pathway', 'complement and coagulation cascades', 'TNF signaling pathway', 'intestinal immune networks for IgA production', 'cytokine-cytokine receptor interaction', 'allograft rejection', 'Toll-like receptor signaling pathway' and 'antigen processing and presentation'. The top 10 hub genes [interleukin (IL)6, IL8, matrix metallopeptidase (MMP)9, colony stimulating factor 1 receptor, FOS proto-oncogene, AP1 transcription factor subunit, insulin-like growth factor 1, TYRO protein tyrosine kinase binding protein, MMP3, cluster of differentiation (CD)14 and CD163] and four gene modules were identified from the PPI network using Cytoscape. In addition, text-mining was used to identify the commonly used drugs and their targets for the treatment of OA. It was initially verified whether the results of the present study were useful for the study of OA treatment targets and pathways. The present study provided insight for the molecular mechanisms of OA synovitis. The hub genes and associated pathways derived from analysis may be targets for OA treatment. IL8 and MMP9, which were validated by text-mining, may be used as molecular targets for the OA treatment, while other hub genes require further validation.

Introduction

Osteoarthritis (OA) is one of the most common types of chronic arthritis in elderly people, and has been identified to affect ≥6-32% people worldwide (1). There are multiple risk factors for OA development, including abnormal mechanical stress, aging, obesity and genetic factors (2). Pathological features of OA primarily include articular cartilage degeneration, synovial inflammation and atypical bone formation (3). In the past, research has primarily focused on cartilage tissue and chondrocytes in the study of OA mechanisms and treatment (4,5). With the rapid development of medicine, synovial tissue and subchondral bone have additionally been investigated. Previous studies suggested that synovial tissue serves an important role in OA (6,7). As part of the joint structure, the synovial membrane may produce and regulate synovial fluid, maintain joint activity, and be adversely affected in joint diseases (8). Synovial lesions may additionally be identified in a number of joint diseases, which may serve a role in promoting the occurrence and progression of the disease (9).

Synovitis is an aseptic inflammation that occurs at the synovial membrane and is observed in rheumatoid arthritis, OA, lupus OA and gout (10). Synovitis leads to abnormal synovial fluid production and absorption, resulting in joint effusion, causing pain, swelling and other reactions (11). Previous studies observed synovitis in early OA (12), and that the severity of synovitis may be associated with different

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stages of OA (13). Fernandez-Madrid et al (14) suggested that OA synovitis is caused by the degeneration of cartilage stimulation. However, Felson et al (15) suggested that synovitis occurs not only in the early stages of OA; however, even prior to imaging. Additionally, the occurrence of synovitis may further promote cartilage degeneration, which would in turn exacerbate synovitis (11). Synovitis serves an important role in the symptoms, progression and development of OA, and is a concern for the treatment of OA.

With the development of modern biomedicine, increasing evidence suggested that the occurrence and development of OA may be mediated by a number of genes and signaling pathways (16). In order to develop clearer diagnostic criteria and more effective treatment options, it is essential to fully understand the molecular mechanism of OA. With the aim of fully understanding the gene expression alterations in OA, previous studies used DNA microarray technology to analyze gene expression profiles (17,18). The results demonstrated that molecules encoded by differentially expressed genes (DEGs) located in different cell structures and with different molecular functions (MF) were associated with different biological processes (BP) during their involvement in the disease process. The availability of bioinformatics analysis based on high-throughput technology enabled the investigation of the alterations in mRNA expression and the interaction between differential genes in OA, to provide novel insights for further in-depth OA studies.

The Gene Expression Omnibus (GEO) is a database and online resource for the gene expression of any species. The present study obtained genetic microarray dataset no. GSE46750 from GEO. The samples in GSE46750 were divided into two groups: Synovial cells with and without inflammation in OA. The two groups were compared and analyzed to identify the DEGs. Functional enrichment analysis, protein-protein interaction (PPI) networks and module analysis were conducted on the DEGs. Subsequently, text-mining of OA treatment drugs and their target genes were performed to initially validate the results. The results of the present study may enable us to recognize the effects of synovial membrane inflammation in the development of OA, and to provide certain possible OA target molecules for subsequent validation.

Materials and methods

Gene chip data. GSE46750 gene expression data (19) was obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/), which was expressed on the GPL10558 platform [(Illumina HumanHT-12 V 4.0) Bead chip; Illumina, Inc., San Diego, CA, USA]. The GSE46750 dataset samples, which were synovial cells, were derived from 12 patients with OA, specifically from those with synovial membrane with inflammation (n=12) and synovial membrane without inflammation (n=12).

Identifying DEGs. The original micro array data was analyzed through heat mapping using Morpheus (https://software.broadinstitute.org/morpheus/) to visually observe gene expression. The chip data were divided into an inflammatory synovial membrane group and a non-inflammatory synovial membrane group for analysis. GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE46750) was used to identify the

DEGs in OA synovial membrane. The criteria for a DEG was |log2 (fold change)|≥1 and P<0.05.

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. GO enrichment analysis enables the annotation of cell components (CC), MF and BP of DEGs. KEGG (http://www.genome.jp/) is a database that includes a KEGG path database for examining the path of the gene cluster and associated functions. The present study used Database for Annotation, Visualization and Integrated Discovery (DAVID 6.8; https://david.ncifcrf.gov/) (20) to perform GO enrichment analysis and KEGG pathway analysis. P<0.05 was considered to indicate a statistically significant difference.

Construction of the PPI network and module analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://www.string-db.org/) may be searched for associations between known and predicted proteins, and is commonly used to predict PPI information in molecular biology (21). The DEGs were mapped to STRING, and those with interaction scores >0.4 were selected for further examination. Cytoscape 3.5.0 (http://www.cytoscape.org/) was used to visualize the results from the PPI network. Module analysis on the PPI network results was performed using the molecular complex detection (MCODE) (22) clustering algorithm that comes with Cytoscape. Module analysis may be used to identify more connected gene groups. In addition, the module analysis results were further analyzed for function and pathway enrichment, and P<0.05 was considered to indicate a statistically significant difference.

Text-mining. Drugs commonly used for the treatment of OA were searched in The Therapeutic Target Database (https://db.idrblab.org/ttd/) (23) with the key word 'osteoarthritis'. The molecular targets of the detected drugs were identified in The Drug Gene Interaction database (DGIdb; http://dgidb.genome.wustl.edu/). These known molecules that may serve a therapeutic role in OA were compared with the present results.

Results

DEGs. Morpheus was used to observe the overall gene expression of all samples (Fig. 1), and differences in gene expression between inflammatory synovial cells (n=12) and non-inflammatory synovial cells (n=12) were identified. A total of 174 DEGs were identified by GEO2R analysis, of which 145 were upregulated and 29 were downregulated (Fig. 2).

GO term enrichment analysis. Fig. 3 presents the results of the GO analysis. The majority of the DEGs were determined to be located in the 'extracellular region', the 'plasma membrane' and the 'vesicle'. The DEGs were demonstrated to exert molecular functions by regulating 'chemokine activity', 'chemokine receptor binding', 'growth factor activity' and other 'cytokine activity'. Furthermore, the DEGs may regulate the OA membrane inflammatory response by involvement in the regulation of 'inflammatory response', 'immune response', 'response to lipopolysaccharide' and 'chemotaxis'.
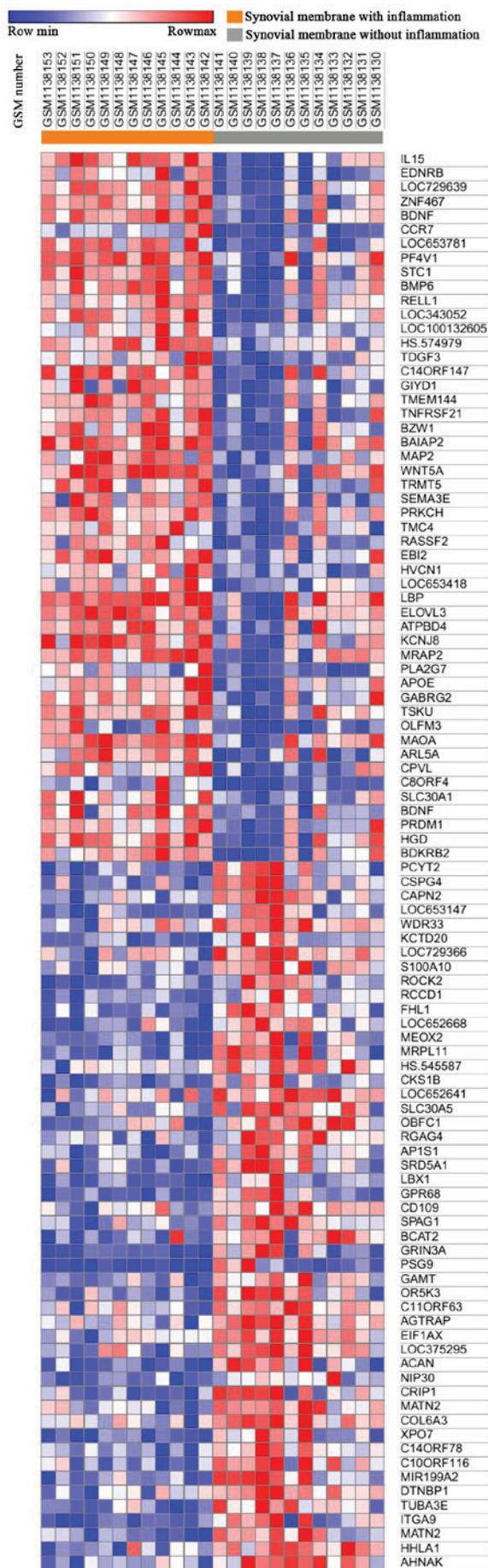
Figure 1. Heat map of 100 genes from all samples. Red indicates higher gene expression and blue indicates lower gene expression.
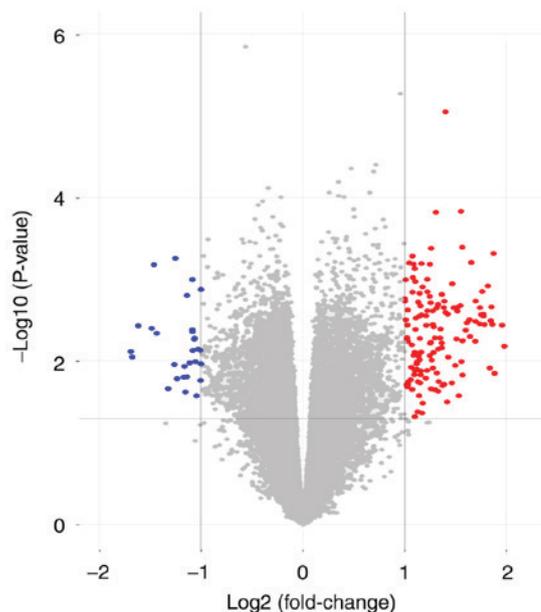
Figure 2. Volcanic map of all genes. Red dots indicate upregulated genes, blue dots indicate downregulated genes and gray dots indicate genes that are not regulated.

KEGG pathway analysis. The KEGG analysis results (Fig. 4) suggested that DEGs, through pathways, including 'chemokine signaling pathway', 'complement and coagulation cascades', 'TNF signaling pathway', 'intestinal immune network for IgA production', 'cytokine-cytokine receptor interaction', 'Toll-like receptor signaling pathway' and 'antigen processing and presentation', serve a role in regulating the synovial inflammation of OA.

Analysis of hub genes and modules. Following the analysis based on the PPI networks, 122 nodes (DEGs) and 444 edges (interactions between DEGs) were identified in Cytoscape (Fig. 5). The genes with higher scores were the hub genes, as the genes of higher degree may be associated with OA. The top 10 hub genes were interleukin (IL)6, IL8, matrix metal-lopeptidase (MMP)9, colony stimulating factor 1 receptor (CSF1R), FOS proto-oncogene, AP1 transcription factor subunit (FOS), insulin-like growth factor 1 (IGF1), TYRO protein tyrosine kinase binding protein (TYROBP), MMP3, cluster of differentiation (CD) 14 and CD163. A total of four modules were selected through MCODE analysis (Fig. 5). Enrichment analysis demonstrated that modules 1 and 2 may be associated with 'cytokine-cytokine receptor interactions', 'NOD-like receptor signaling pathways' and 'toll-like receptor signaling pathways' (Table I).

Common therapeutic drugs and their targets. The drugs commonly used in the treatment of OA included lumira-coxib, rofecoxib, guggulsterone, nepafenac, glucosamine, diclofenac, valdecoxib, naproxen, tiaprofenic acid, celecoxib, tolmetin, etoricoxib, tenoxicam, salsalate and mefenamic acid (Fig. 6). The target genes of these drugs were identified in DGIdb, which included IL8, MMP9, IL10, BCL2 associated X, apoptosis regulator, cyclin dependent kinase inhibitor 1B (Fig. 6).
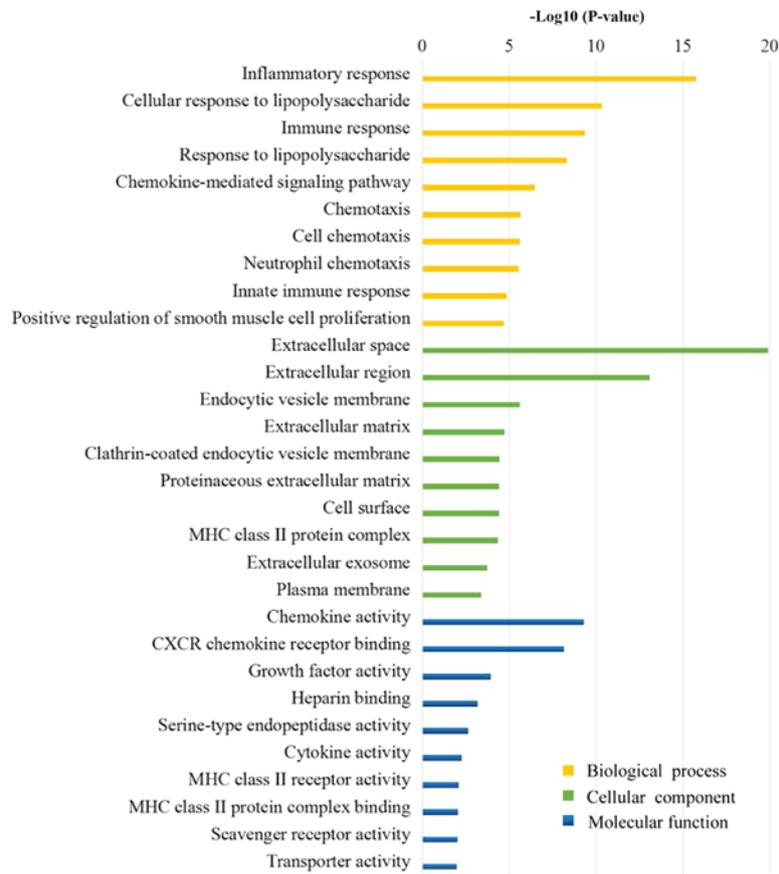
Figure 3. The top 10 biological processes, cellular component and molecular function analysis in differentially expressed genes between non-inflammatory synovial cells and inflammatory synovial cells in osteoarthritis.
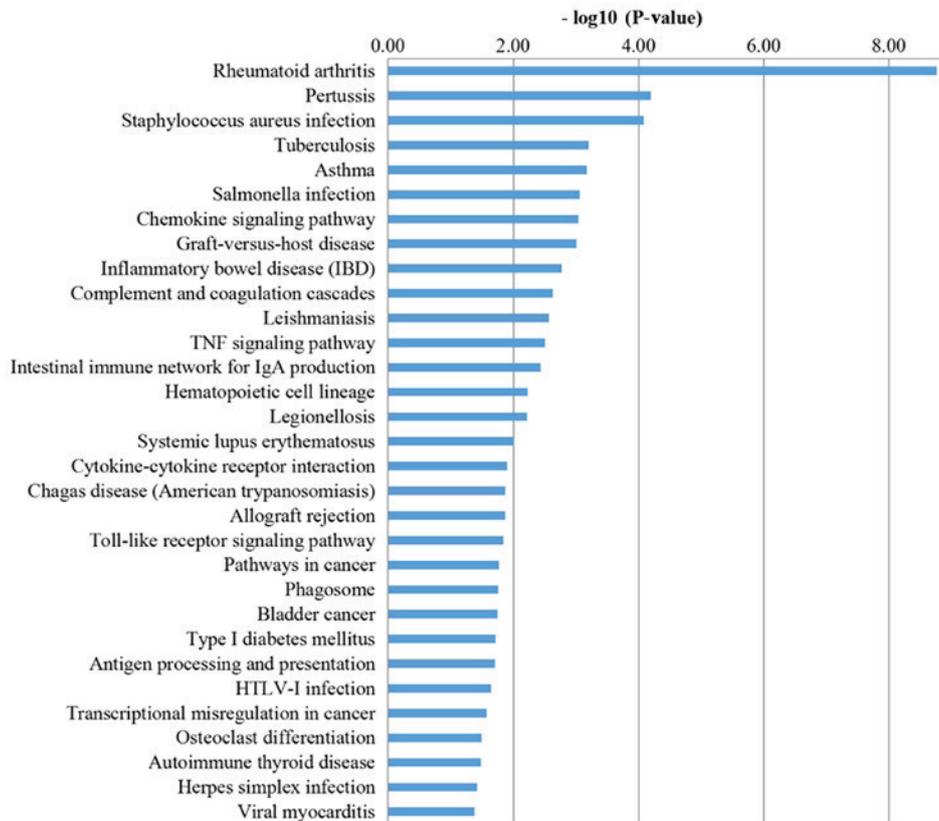
Figure 4. Kyoto Encyclopedia of Genes and Genomes pathways in differentially expressed genes between non-inflammatory synovial cells and inflammatory synovial cells in osteoarthritis.
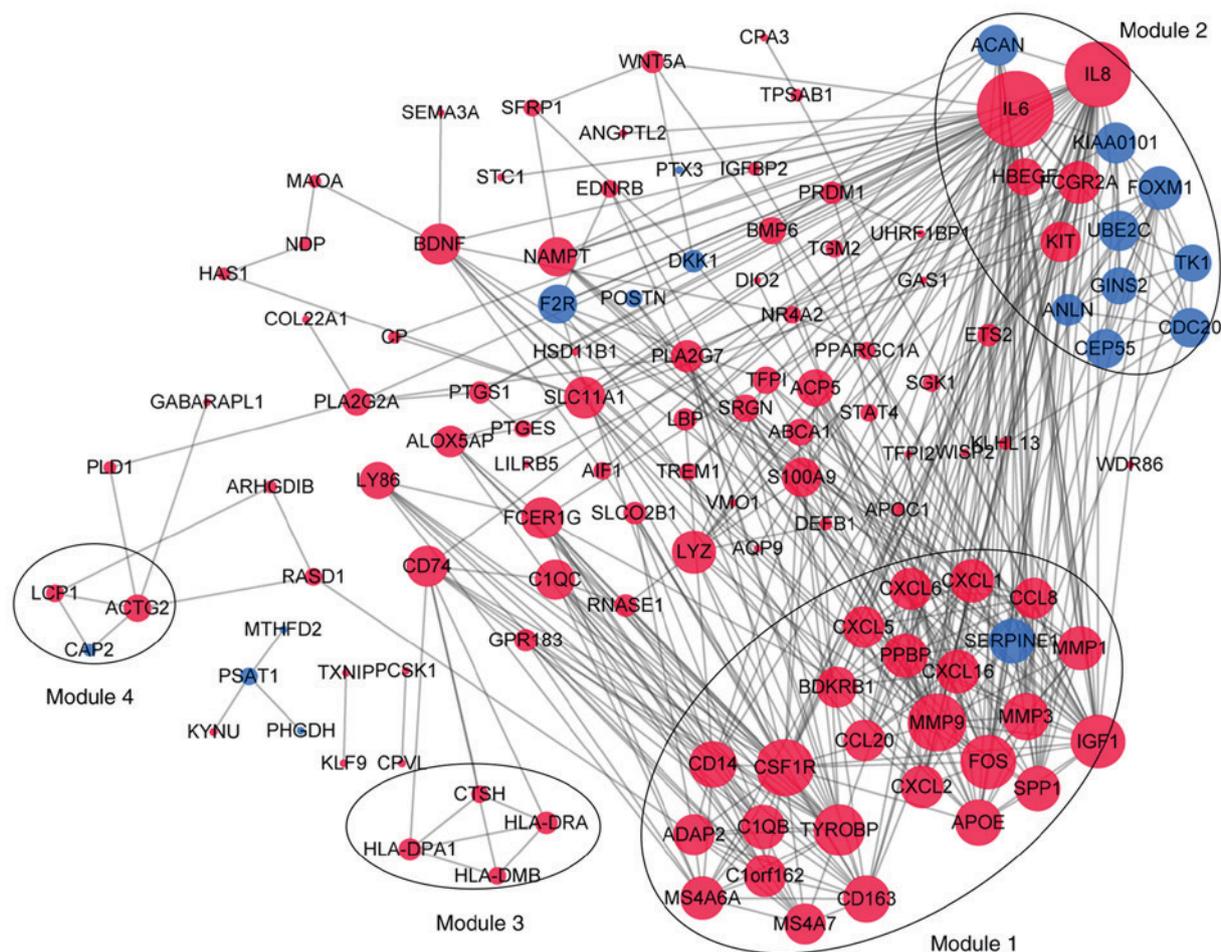
Figure 5. Protein-protein interaction network constructed using Cytoscape. Sizes of dots are proportional to the score. Red dots indicate upregulation, blue dots indicate downregulation and gray edges indicate protein interactions.

Discussion

At present, factors, including heredity, age and mechanical alterations, are involved in the complex pathogenesis of OA (24). The pathogenesis remains unclear, and thus, the treatment of OA primarily relieves symptoms and no specific treatment has been identified. Microarray and sequencing technology that provide expression levels of thousands of genes in humans, have been widely used to predict the potential therapeutic targets for diseases. A thorough understanding of the molecular mechanisms of OA may provide insight for diagnosis and treatment. In the present study, 174 DEGs were identified between synovial membrane with and without inflammation in OA, among which, 145 genes were upregulated and 29 were downregulated. Synovial inflammation has been identified as one of the pathological manifestations of the development and progression of OA (25). These DEGs were involved in the regulation of synovial inflammation. In the present study, an enrichment analysis was conducted to further understand the regulatory roles of DEGs in OA.

The top 10 CC terms of the DEGs demonstrated that they are primarily located in the 'extracellular region', 'plasma membrane', 'vesicles membrane'. The roles of DEGs in MF are to activate 'cytokine activity', promote 'chemokine receptor binding', and regulate 'receptor binding' and 'phospholipid

binding'. The top 10 BP terms involved in DEGs are primarily 'inflammatory response', 'immune response' and 'cell-cell signaling'. The KEGG pathways involved in synovial inflammation development of OA by DEGs include 'chemokine signaling pathway', 'complement and coagulation cascades', 'TNF signaling pathway', 'cytokine-cytokine receptor interaction', 'allograft rejection', 'Toll-like receptor signaling pathway' and 'antigen processing and presentation'. Previous studies suggested that immune responses, including complement activation, cytokines and immune cell populations are involved in the occurrence and development of OA (26-28). Additionally, previous studies suggested that cytokines are involved in the pathological process of OA, and cause inflammatory reactions and pain (29,30). A previous study demonstrated that the occurrence of coagulation cascades in the synovium was observed in joint diseases, including OA, and the associated molecules were more highly expressed in inflammatory arthritis (31).

PPI networks containing the DEGs were additionally constructed, and the top 10 hub genes were IL6, IL8, MMP9, CSF1R, FOS, IGF1, TYROBP, MMP3, CD14 and CD163. IL6 and IL8 primarily regulate immune responses and inflammatory responses (32). Previous studies demonstrated that IL6 and IL8 expression levels were increased in OA synovial tissues (33,34). The role of MMP9 is primarily to

Table I. Enriched pathways of modules 1-3.

A, Module 1

Pathway	FDR	Genes
Cytokine-cytokine receptor interaction	2.80×10^{-9}	CCL20, CCL8, CSF1R, CXCL1, CXCL16, CXCL2, CXCL5, CXCL6, PPBP
Rheumatoid arthritis	2.80×10^{-9}	CCL20, CXCL1, CXCL5, CXCL6, FOS, MMP1, MMP3
Chemokine signaling pathway	3.57×10^{-9}	CCL20, CCL8, CXCL1, CXCL16, CXCL2, CXCL5, CXCL6, PPBP
TNF signaling pathway	3.57×10^{-9}	CCL20, CXCL1, CXCL2, CXCL5, FOS, MMP3, MMP9
Pertussis	1.35×10^{-6}	C1QB, CD14, CXCL5, CXCL6, FOS
Transcriptional misregulation in cancer	8.55×10^{-5}	CD14, CSF1R, IGF1, MMP3, MMP9
Salmonella infection	0.000131	CD14, CXCL1, CXCL2, FOS
Legionellosis	0.00145	CD14, CXCL1, CXCL2
Pathways in cancer	0.00145	CSF1R, FOS, IGF1, MMP1, MMP9
Complement and coagulation cascades	0.00248	BDKRB1, C1QB, SERPINE1
Chagas disease (American trypanosomiasis)	0.00628	C1QB, FOS, SERPINE1
Toll-like receptor signaling pathway	0.00669	CD14, FOS, SPP1
Osteoclast differentiation	0.011	CSF1R, FOS, TYROBP
Bladder cancer	0.0206	MMP1, MMP9
NOD-like receptor signaling pathway	0.0439	CXCL1, CXCL2

B, Module 2

Pathway	FDR	Genes
Cytokine-cytokine receptor interaction	0.0431	IL6, IL8, KIT
NOD-like receptor signaling pathway	0.0431	IL6, IL8
Hematopoietic cell lineage	0.0431	IL6, KIT
Epithelial cell signaling in Helicobacter pylori infection	0.0431	HBEGF, IL8
Salmonella infection	0.0431	IL6, IL8
Pertussis	0.0431	IL6, IL8
Legionellosis	0.0431	IL6, IL8
Malaria	0.0431	IL6, IL8
Pathways in cancer	0.0431	IL6, IL8, KIT
Rheumatoid arthritis	0.0431	IL6, IL8
Toll-like receptor signaling pathway	0.0494	IL6, IL8
Chagas disease (American trypanosomiasis)	0.0494	IL6, IL8
Amoebiasis	0.0494	IL6, IL8

C, Module 3

Pathway	FDR	Genes
Intestinal immune network for IgA production	0.00149	HLA-DPA1, HLA-DRA
Type I diabetes mellitus	0.00149	HLA-DPA1, HLA-DRA
Staphylococcus aureus infection	0.00149	HLA-DPA1, HLA-DRA
Asthma	0.00149	HLA-DPA1, HLA-DRA
Autoimmune thyroid disease	0.00149	HLA-DPA1, HLA-DRA
Allograft rejection	0.00149	HLA-DPA1, HLA-DRA
Graft-versus-host disease	0.00149	HLA-DPA1, HLA-DRA
Viral myocarditis	0.00152	HLA-DPA1, HLA-DRA
Inflammatory bowel disease	0.00172	HLA-DPA1, HLA-DRA
Antigen processing and presentation	0.00175	HLA-DPA1, HLA-DRA
Leishmaniasis	0.00175	HLA-DPA1, HLA-DRA

Table I. Continued.

C, Module 3

Pathway	FDR	Genes
Rheumatoid arthritis	0.00244	HLA-DPA1, HLA-DRA
Systemic lupus erythematosus	0.00275	HLA-DPA1, HLA-DRA
Toxoplasmosis	0.00363	HLA-DPA1, HLA-DRA
Cell adhesion molecules	0.00499	HLA-DPA1, HLA-DRA
Phagosome	0.0051	HLA-DPA1, HLA-DRA
Tuberculosis	0.00645	HLA-DPA1, HLA-DRA
Influenza A	0.00645	HLA-DPA1, HLA-DRA
Herpes simplex infection	0.00645	HLA-DPA1, HLA-DRA
Epstein-Barr virus infection	0.00722	HLA-DPA1, HLA-DRA
HTLV-I infection	0.012	HLA-DPA1, HLA-DRA

FDR, false discovery rate; TNF, tumor necrosis factor; NOD, nucleotide-binding oligomerization domain; IgA, immunoglobulin A; HTLV-I, human T-lymphotrophic like virus I; CCL, C-C motif chemokine ligand; CSF1R, colony stimulating factor 1 receptor; CXCL, C-X-C motif chemokine ligand; PPBP, pro-platelet basic protein; FOS, FOS proto-oncogene, AP-1 transcription factor subunit; MMP, matrix metallopeptidase; C1QB, complement C1q B chain; CD14, CD14 molecule; IGF1, insulin like growth factor 1; BDKRB1, bradykinin receptor 1; SERPINE1, serpin family E member 1; SPP1, secrete phosphoprotein 1; TYROBP, TYRO protein tyrosine kinase binding protein; IL, interleukin; KIT, KIT proto-oncogene receptor tyrosine kinase; HBEGF, heparin binding EGF like growth factor; HLA-DPA1, major histocompatibility complex, class II, DP α 1; HLA-DRA, major histocompatibility complex, class II, DR α.

Figure 6. Commonly used drugs for the treatment of osteoarthritis and their target genes. The yellow triangles indicate drugs and the blue diamonds indicate the target genes.

degrade and remodel the extracellular matrix, and the role of MMP3 is to degrade extracellular matrix proteins (35). The results of the present study were consistent with the results of previous studies, which demonstrated high expression of MMP9 and MMP3 in OA synovial tissues (36,37). CSF1R encodes a receptor protein for colony stimulating factor 1, which mediates the majority of the biological effects of this cytokine (38). A previous study revealed macrophage-CSF and macrophage-CSF and granulocyte-CSF are involved in the inflammatory response in a rheumatoid arthritis mouse model (39). FOS is a nuclear protein transcription factor that regulates the growth, division, differentiation, proliferation and apoptosis of cells (40). Compared with normal human synovial tissues, immunohistochemistry demonstrated strong staining of c-FOS in OA synovial tissues (41). IGF1 encodes an active protein peptide substance that promotes cell growth and is essential for sugar, lipid, protein metabolism and inorganic salt metabolism (42). A previous study demonstrated that the IGF1 content in OA synovial fluid was twice that in normal fluid and that all forms of IGF1 were highly concentrated in the cartilage of OA, which may affect the progression of the disease by regulating the anabolic metabolism of cartilage macromolecules (43). TYROBP is involved in immune and inflammatory reactions (44). A previous study demonstrated that TYROBP was more highly expressed in OA synovial tissues compared with healthy synovial tissues (45). A previous clinical study demonstrated that the expression levels of CD14 and CD163 in the serum and joint fluid were increased in patients with OA, and were directly proportional to the activation of macrophages, joint space, osteophytes and the severity of pain (46). CD14, as a co-receptor, was involved in the signaling pathway of lipopolysaccharide binding and may trigger inflammatory activation of synovial cells by stimulating toll-like receptors (47). CD163 is a marker for cells from the monocyte/macrophage lineage and is also hemoglobin scavenger receptor, with pro- and anti-inflammatory effects (48). Comparing the results of the present study with the text-mining results, IL8 and MMP9 were identified as useful therapeutic targets for OA.

Module analysis demonstrated that the development of OA synovitis is associated with 'cytokine-cytokine receptor interactions', 'NOD-like receptor signaling pathway', 'Toll-like receptor signaling pathway'. Previous studies have demonstrated that the occurrence and development of OA were associated with a number of cytokines, leading to synovial inflammation, cartilage damage and osteophyte production (30,49). The present study demonstrated that OA synovial inflammation is associated with 'NOD-like receptor signaling pathway', 'Toll-like receptor signaling pathway' and 'TNF signaling pathway', and OA progression is additionally associated with 'osteoclast differentiation'.

In the present study, bioinformatics analysis was performed to investigate synovial inflammation. The DEGs identification and in-depth analysis may help to identify useful targets and pathways in OA pathogenesis. These results may provide insight for the study of OA therapeutic targets. A limitation of the present study was that the screened genes and pathways were not experimentally validated, which may become a focus of future studies.

Availability of data and materials

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

Authors' contributions

XL and JL conceived and designed the study. GW, ZZ and YH collected the expression data and screened for the differentially expressed genes. JC, CF and JY analyzed and interpreted the data. JL wrote the manuscript. GW and XL reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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