The identification of key genes and Pathways in Osteoarthritis-related Inflammatory Synoviocytes by Bioinformatics Analysis

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Research Article

Keywords: Bioinformatics Analysis, Inflammatory Synoviocytes, Osteoarthritis, Differentially Expressed Genes

Posted Date: March 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-842222/v1

The identification of key genes and Pathways in Osteoarthritis-related Inflammatory Synoviocytes by Bioinformatics Analysis

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Abstract

Background: Osteoarthritis (OA) is characterized as sustained synovial inflammation and progressive cartilage degradation, in which inflammatory synoviocytes play a pivotal role. The purpose is to define key genes and relevant pathways in inflammatory synoviocytes in vivo and vitro. In the current study, the differentially expressed genes (DEGs) from GSE46750 and GSE82107 gene data were overlapped to show the intersection genes in inflammatory synoviocytes in vivo, and from GSE49604 and GSE82107 gene data were used in vitro. Meanwhile, the enrichment analyses and protein-protein interaction (PPI) were confirmed in the two overlapped DEGs, respectively. Subsequently, meta-enrichment analysis was also performed to discriminate the differences between synoviocytes in vivo and vitro. Finally, analysis of miRNA network and associated pathways in overlapped genes in vitro and vivo were also finished.

Results: A total of 36 intersection DEGs, enriched in 'cell response to oxidative stress', 'response to lipopolysaccharide (LPS)' and 'tumor necrosis factor (TNF) signaling pathway', were identified in inflammatory synoviocytes in vivo, as well as 35 overlapped DEGs, enriched in 'mineral absorption', 'response to lipopolysaccharide' and 'oxidation-reduction process', were identified in vitro. Additionally, in the meta-enrichment analysis, the results suggested that DEGs in vivo and vitro collectively enriched in 'immune response', 'response to LPS', 'chemokine signaling pathway' and 'TNF signaling pathway'. However, the 'regulation of macrophage activation', 'positive regulation of neutrophil chemotaxis', 'positive regulation of leukocyte chemotaxis' and 'leukocyte migration' were individually enriched in the DEGs from inflammatory synoviocytes in vivo. Finally, the two comparison cohorts shared five intersection genes, including C-X-C chemokine ligand (CXCL) 2, CXCL5, chloride intracellular channel (CLIC) 6, solute carrier family 7 member 2 (SLC7A2) and chromosome 15 open reading frame 48 (C15orf48), and predicted miRNAs mainly enriched in transforming growth factor (TGF)-β, Ras and Wnt signaling pathway.

Conclusions: The study demonstrated that OA-related inflammatory synoviocytes is associated with immune response, TNF signaling pathway, chemokine signaling pathway and oxidation-related process. However, compared to inflammatory model in vitro, in OA joint, the synovial inflammation is more complicated, maybe involved neutrophil recruitment, monocyte migration, and macrophage activation. Additionally, the current study also shown that some key genes CLIC6, CXCL2, CXCL5, SLC7A2 and C15orf48, may be related to the disease progression and produce effect as potential therapeutic approach in OA.

Keywords: Bioinformatics Analysis, Inflammatory Synoviocytes, Osteoarthritis, Differentially Expressed Genes

Background

Knee Osteoarthritis (OA) is a commonly encountered articular disease, which contributes to activity limitation, joint pain, and physical disability, especially among elderly people[1]. The disease is characterized as cartilage degeneration, thickening of the subchondral bone, bone deformation, and synovial inflammation[2]. Recently, emerging evidences suggested the significant role of low-grade synovial inflammation in knee OA, which was no longer defined as a simple "wear and tear" disease[3]. Clinical arthroscopic study shown that localized proliferative and inflammatory lesions in joint synovium occurred up to 50% of OA patients[4]. Additionally, other studies have also revealed that synovial inflammation was a significant predictor of OA[5], and the grade of synovitis was always coupled with the severity of cartilage damage and joint pain[6].

Synoviocytes, the resident cells in synovium, can secrete lubricant to keep joint movement and provide nutrient to maintain chondrocyte homeostasis. Pathologically, inflammatory activation of synoviocytes has been found to promote malignant pathological processes in OA[7]. In OA joint, inflammatory response and activation of synoviocytes involved multiple processes, including Toll-like receptor (TLR) activation, inflammatory factor secretion and matrix degradation product[8]. Furtherly, activated OA-related inflammatory synoviocytes initiate and maintain the chronic synovial inflammation and cartilage destruction through the secretion of interleukin (IL)-1β, tumor necrosis factor (TNF)-α and matrix metalloproteinases (MMP)[9]. Consequently, the concept of inflammatory synoviocytes leading to damaged joint and OA development has been widely recognized[10]. However, most researchers merely focused on the OA-related individual gene, which fail to comprehensively understand the crucial hub genes and relevant pathways, related to the inflammatory response in OA-related synoviocytes[11].

With the development of modern bioinformatics, it has become a useful tool to explore the intricate regulatory factors and signaling pathways in the inflammatory response of OA. Lambert et al. (2014) identified 896 differentially expressed genes (DEGs) between inflamed and natural areas in the synovium and screened out the key pathways included cartilage metabolism, inflammation, Wnt signaling and angiogenesis[12]. Nguyen et al. (2017) also identified the transcriptional regulation of synoviocyte-mediated inflammation in vitro[13]. In the current study, in order to explore the potential mechanisms of synoviocytes on the OA development, OA-related DEGs in inflammatory synoviocytes in vivo and vitro, followed by enrichment analysis and protein-protein interaction (PPI) network analysis, respectively. Meanwhile, the meta-enrichment analysis for the two OA-related DEGs lists was conducted, as well as analysis of miRNA regulatory network and related pathway in the overlapped genes were identified to initially provide certain possible therapeutic target for OA.

Methods

Study Design

Fig. 1 illustrates our proposed framework of identifying key genes and pathways in OA-related inflammatory synoviocytes in vivo and in vitro from three microarray data (GSE46750, GSE49604 and GSE82107). In brief, a three-step approach was followed to perform bioinformatics analysis. Firstly, the OA-related DEGs in inflammatory synoviocytes in vivo and in vitro were screened from the three data. Subsequently, Enrichment analysis and PPI network analysis was performed in vivo and vitro, respectively. Meanwhile, the meta-enrichment analysis for the two OA-related DEGs lists was also conducted. Finally, overlapped genes were screened in the two OA-related DEGs lists, and analysis of miRNA regulatory network and related pathway in the overlapped genes were also identified to initially provide certain possible therapeutic target for OA.

Microarray data

GSE46750 gene data (GPL10558 platform) was completed by Cecile et al. In the experiment, synovial tissues were obtained from 12 knee OA patients at the time of total knee replacement. The inflammatory status of the synovial membrane was characterized according to macroscopic criteria and sorted as N/R and I. Synoviocytes were cultured separately for 7 days. Microarray gene expression profiling between cells from N/R and I areas was performed.

GSE49604 gene data (GPL10558 platform) was established by You et al. To identify molecular signatures, synoviocytes from synovial tissues of OA patients were isolated and cultured. Meanwhile, the cells were stimulated without or with IL-1β (10 ng/mL) for 24 h, and then analyzed gene expression profiles of both IL-1β- or saline-stimulated synoviocytes in vitro were identified.

GSE82107 gene data (GPL570 platform) was also obtained by Marieke et al. To identify disease characteristic genes, in the experiment, 10 synovial biopsies were obtained from OA synovium and 7 synovial samples from individuals without a joint disease. The Affymetrix Human Genome Array was used to identify DGEs in synovium of from OA patients and controls.

Identification of overlapped DEGs

The top 50 gene expressions were visualized using heat mapping through Morpheus in original microarray (https:// software.broadinstitute.org/morpheus/). The data were assigned to identify the DEGs through GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/). DEGs were identified with the criterion of p value < 0.05 and Log|FC| > 1.

The DEGs from GSE46750 and GSE82107 gene data were overlapped to show the intersection genes in OA-related inflammatory synoviocytes in vivo. The DEGs from GSE49604 and GSE82107 gene data were overlapped to show the intersection DEGs in OA-related inflammatory synoviocytes in

vitro.

Enrichment analysis for overlapped DEGs

Enrichment analyses for the DEGs was performed using DAVID 6.8 (https://david.ncifcrf.gov/) and Metascape (http://metascape.org/gp/index. html), respectively. The terms include pathway, functional set, structural complex and signature module in Metascape, and GO enrichment (biological process, cell components, molecular function) and KEGG pathway analysis in DAVID. In the current study, both single enrichment analysis and meta-enrichment analysis were performed.

Protein-protein interaction (PPI) and module analysis

PPI networks were analyzed using STRING database (http://www.string-db.org) and then the analytic results were visualized using Cytoscape 3.5 (http://www.cytoscape.org/) from the PPI network. Centiscape (Cytoscape plug-in) was used to estimate the centrality of DEGs, whose centrality degree ≥ 5 were defined as key genes. Subsequently, MCODE (Cytoscape plug-in) was used to carry out the module analysis module analysis, and the results were further analyzed for enrichment analysis.

MiRNA regulatory network and miRNA-associated pathways of the overlapped hub genes

MiRNAs interacting with ten mRNAs from the five hub genes were predicted using the miRDB database[14]. The miRNA-mRNA regulatory network was visualized using Cytoscape. The KEGG analysis for potential miRNAs was also performed using DIANA-mirPath v3.0[15].

Results

Identification of DEGs in three different data bases

In the GSE49604, 211 DEGs including 167 up- and 62 down-regulated were identified by GEO2R analysis (Fig.2a). In the GSE46750, 174 DEG including 145 up- and 29 down-regulated genes (Fig.2b), and in the GSE82107, 3404 DEGs including 2301 up- and 1103 down-regulated genes (Fig.2c) were

also identified. Additionally, thermal map was used to observe the top 30 differentially expressive genes of all samples (Fig.2d, 2e and 2f).

Identification and enrichment analysis of OA-related DEGs in inflammatory synoviocytes in vivo

Subsequently, to confirm the relation between inflammatory synoviocytes in vivo and OA, the overlap between the DEGs in the synoviocytes from inflammatory vs normal areas and those in the synovium from healthy and osteoarthritic joints were performed. In total, the two GEO datasets shared 36 intersection genes, which shown in the Fig.3a and 3b.

Overall, the majority of the DEGs mainly located in the 'extracellular space', 'extracellular exosome', and 'extracellular region', molecular functions act by 'chemokine activity', 'chemokine receptor (CXCR) receptor binding' and 'growth factor activity', and regulate biological process by 'chemokine-mediated pathway', 'response to lipopolysaccharide' and 'positive regulation of neutrophil chemotaxis'. Furthermore, the DEGs mainly involve in these pathways of "chemokine signaling pathway", "Pertussis" and "tumor necrosis factor (TNF) signaling pathway". (Fig.3c and 3d)

PPI network analysis of OA-related DEGs in inflammatory synoviocytes in vivo

In total, 17 nodes and 25 edges were identified (Fig.4a), and the hub genes were C-X-C motif chemokine ligand (CXCL) 2, CXCL5, insulin like growth factor (IGF) 1, Fos proto-oncogene, AP-1 transcription factor subunit (FOS) and Fc fragment of IgE receptor Ig (FCER1G). In total, two modules which both consist of hub genes were selected (Fig.4c). Enrichment analysis declared the module 1 and 2 may be associated with 'Chemokine receptors bind chemokines', 'myeloid leukocyte differentiation', and 'inflammatory response' (Details in the table 1).

Table 1. Enrichment analysis of modules in overlapped DEGs of inflammatory chondrocytes in vivo

Category	Description	Count	%	Log10(P)	Log10(q)

Module 1					
Reactome Gene Sets	Chemokine receptors bind chemokines	4	100	-10.86	-6.59
GO Biological Processes	antimicrobial humoral immune response	3	75.00	-6.99	-3.67
Module 2					
GO Biological Processes	myeloid leukocyte differentiation	3	50	-4.97	-1.19
GO Biological Processes	regulation of secretion	4	66.67	-4.81	-1.19
GO Biological Processes	inflammatory response	4	66.67	-4.80	-1.19

Note. "Count" is the number of genes in the lists with membership in the given ontology term. "%" is the percentage of all of the genes that are found in the given ontology term (only input genes with at least one ontology term annotation are included in the calculation). "Log10(P)" is the p-value in log base 10. "Log10(q)" is the multi-test adjusted p-value in log base 10.

Identification and enrichment analysis of OA-related DEGs in IL-1β-treated inflammatory synoviocytes in vitro

To confirm the relation between IL-1β-induced inflammatory FLS in vitro and OA, the overlap between the DEGs in the IL-1β- or saline-treated FLS and those in the synovium from healthy and osteoarthritic joints were performed. In total, the two GEO datasets shared 35 intersection genes, which shown in the Fig.5a and 5b.

Enrichment analysis of the overlapped DEGs was also performed (Fig.5c and 5d). Overall, the majority of the DEGs mainly located in the ‘extracellular space’, molecular functions act by ‘chemokine activity’, ‘CXCR receptor binding’ and ‘hyaluronic acid binding’, and regulate biological process by ‘response to lipopolysaccharide’, ‘inflammatory response’ and ‘cell chemotaxis’. Furthermore, the DEGs mainly involve in these pathways of “Mineral absorption”, “TNF signaling pathway” and “chemokine signaling pathway”.

PPI network analysis of OA-related DEGs in IL-1β-treated inflammatory synoviocytes in vitro

According to PPI networks, we identified 17 nodes and 21 edges in Cytoscape (Fig.6a), in which the hub genes were CXCL5, C-C motif chemokine ligand (CCL) 5, prostaglandin-endoperoxide

synthase (PTGS)2, CXCL2, IL1RN, NFKB inhibitor zeta (NFKBIZ), TNF alpha induced protein (TNFAIP) 6, immediate early response (IER) 3.

Also, two modules were selected using MCODE analysis (Fig.6a), and module 1 consist of hub genes. Enrichment analysis demonstrated the module 1 and 2 may be related to 'Interleukin-10 signaling', 'response to lipopolysaccharide', ' TNF signaling pathway', and 'cellular zinc ion homeostasis' (Details in the table 2).

Table 2. Enrichment analysis of modules in overlapped DEGs of inflammatory chondrocytes in vitro

Category	Description	Count	%	Log10(P)	Log10(q)
Module 1					
Reactome Gene Sets	Interleukin-10 signaling	4	80	-10.20	-5.93
GO Biological Processes	response to lipopolysaccharide	5	100	-9.37	-5.49
GO Biological Processes	neutrophil chemotaxis	4	80	-8.84	-5.17
KEGG Pathway	TNF signaling pathway	4	80	-8.72	-5.15
Module 2					
GO Biological Processes	cellular zinc ion homeostasis	3	100	-8.52	-4.48

Note. "Count" is the number of genes in the lists with membership in the given ontology term. "%" is the percentage of all of the genes that are found in the given ontology term (only input genes with at least one ontology term annotation are included in the calculation). "Log10(P)" is the p-value in log base 10. "Log10(q)" is the multi-test adjusted p-value in log base 10.

Meta-enrichment analysis for OA-related DEGs in inflammatory synoviocytes in vitro and vivo

Meta-enrichment analysis (Figure 7a and 7b) was also performed based on two DEGs lists, which suggested that the DEGs collectively enriched in immune response, inflammatory response, response to lipopolysaccharide (GO), and TNF signaling pathway, chemokine signaling pathway (KEGG). However, the positive regulation of cell migration, negative regulation of growth and mineral absorption were individually enriched in the DEGs from the OA-related inflammatory synoviocytes in vitro, and regulation of macrophage activation, positive regulation of neutrophil chemotaxis, positive

regulation of leukocyte chemotaxis and leukocyte migration involved in inflammatory response in the DEGs from the OA-related inflammatory synoviocytes in vivo. Additionally, the two comparison cohorts shared five intersection genes, including CXCL2, CXCL5, CLIC6, solute carrier family 7 member 2 (SLC7A2) and C15orf48, which shown in the Figure 6c.

Analysis of miRNA network and associated pathways in overlapped genes in vitro and vivo

Finally, the miRNA-regulatory network in the five overlapped genes in vitro and vivo was also performed. we identified 71 predicted miRNAs, including miR-206, 27a-3p, 27b-3p and 92a-3p (Fig. 8a). Also, we found that the OA-related pathways included transforming growth factor (TGF)-β, Ras and Wnt signaling pathway (Fig. 8b).

Discussion

Knee OA has been characterized by progressive synovial inflammation and cartilage degradation, in which OA-related synoviocytes play an important role[16]. In OA joint, the activation of synoviocyte markedly involved multiple processes, which are of great importance to the OA disease progression[17]. In the current study, the synoviocytes from inflammatory area in OA synovium was identified as the inflammatory synoviocytes in vivo, and the IL-1β-treated synoviocyte was regarded as the inflammatory synoviocyte in vitro. Then the gene expression profile was analyzed in the OA-related inflammatory synoviocyte both in vitro and in vivo using multiple bioinformatics methods.

In total, 36 OA-related genes in the inflammatory synoviocyte in vivo were screened. The genes mainly enriched in ‘cellular response to oxidative stress’, ‘inflammatory response’, ‘response to lipopolysaccharide’, ‘TNF signaling pathway’ and ‘chemokine signaling pathway’. Pathologically, the activation of innate immune, in particular toll-like receptor 4 (TLR4), had been shown to promote the OA disease progression. OA-related synoviocytes significantly expresses functional TLR4, and that

activation of TLR4 response to LPS (also known as TLR4 agonists) trigger inflammatory response and chemokine signaling pathway. Meanwhile, local inflammatory response along with aging and/or mechanical load can contribute to imbalance of the oxidative system and antioxidant system. The current study also shown that the DEGs enriched in the 'cellular response to oxidative stress', which suggested oxidative stress, together with inflammation, may played a potential role in OA pathology. Additionally, some hub genes such as CXCL2, CXCL5, IGF1, FOS and FCER1G, associated with 'Chemokine receptors bind chemokines', 'myeloid leukocyte differentiation', and 'inflammatory response', were also identified in our current study, which has been verified by many previous studies.

Generally, TNFα and IL-1β, the two key inflammatory cytokines in OA, have shown a marked synergism to activate the various intracellular signal pathways, which in turn trigger inflammatory response and chemokine signaling pathway in joint tissues[18]. In the current study, based on the inflammatory cell model induced by IL-1β, 35 OA-related DEGs in vitro were identified, which mainly enriched in 'TNF signaling pathway', 'response to lipopolysaccharide' and 'inflammatory response[19]. Meanwhile, some inflammatory factors including IL-1RN, PTGS2 and CXCL5 were identified as the hub gene, which furtherly verified the role of genes in the regulation of synovial inflammation. More recently, the positive relationship between bone mineral density and OA has been clarified in some clinical studies[20], conversely, other studies suggested the high rates of bone absorption may be associate with rapid OA progression[21]. The inconsistencies and areas of controversy still remain in the previous studies, however our results shown that the DEGs enriched in 'mineral absorption', which suggested the potential role of bone absorption in OA. Additionally, our results also shown the possible connection between negative regulation of G-protein coupled receptor (GPCR) protein signaling pathway and inflammatory synoviocytes. GPCR, a kind of transmembrane receptor protein transferring

234 the signals across cell membrane, involved in many pathophysiological processes in rheumatoid
235 arthritis, OA and psoriatic arthritis[22]. However, the detailed mechanism of GPCR protein signaling is
236 still unclear, additional researches are required.

237 In order to discriminate the differences of gene expression between OA-related inflammatory
238 synoviocytes in vivo and those in vitro, in our current study, meta-enrichment analysis was also
239 performed. The results showed the two DEGs lists shared the enrichments of ‘immune response’,
240 ‘inflammatory response’, ‘response to LPS’, ‘TNF signaling pathway’ and ‘chemokine signaling
241 pathway’, which suggested the immune response, including response to LPS, TNF and chemokine
242 signaling pathway are involved in the creation and development of OA both in vivo and vitro[23].
243 However, some enrichments of ‘regulation of macrophage activation’, ‘positive regulation of
244 neutrophil chemotaxis’, ‘positive regulation of leukocyte chemotaxis’ and ‘leukocyte migration
245 involved in inflammatory response’ were individually enriched in the genes from OA-related
246 inflammatory synoviocyte in vivo. In OA joint, in addition to the activation of inflammatory
247 synoviocyte, pathologic process of synovial inflammation still involved other multiple factors,
248 including neutrophil recruitment, leukocyte migration, and macrophage activation[24-26]. Synovial
249 macrophage, along with synoviocyte, resided in the synovium, also can become activated by stimuli,
250 eliciting boost the secretion of catabolic mediators such as inflammatory cytokines, proteolytic
251 enzymes, and downregulate the anabolic mediators including growth factors and anti-inflammatory
252 cytokines[27]. In addition, in OA joint, in response to chemokine signaling pathway, monocytes
253 migrated into the synovial tissue and finally differentiated into macrophage, which generated a series of
254 inflammatory responses[28]. Recently, the role of neutrophils in OA pathogenesis has been widely
255 studied. Firstly, the increased neutrophils and decreased lymphocytes have been demonstrated in some

OA animal models[29, 30]. Also, emerging evidences suggested the blood neutrophil-lymphocyte ratio has been shown to be a powerful marker in OA clinic[31]. However, the inflammatory mechanisms of neutrophils in OA remain unclear, one possible explanation may be the massive release of MMP-8 and other cytokines such as IL-1, IL-8 and TGF-β[32].

Finally, the overlapped genes, including CLIC6, CXCL2, CXCL5, SLC7A2 and C15orf48, jointly expressed in vivo and vitro was also selected. Recently, the active biologic role CXCL2 and CXCL5 in OA development following joint injury or inflammatory response was extensively identified, therefore, may be used as potential molecular biomarkers for OA[33]. Although, so far, few attentions had been paid to define the relationship between the CLIC6, SLC7A2 and C15orf48 genes and OA, these remaining overlapped genes have been reported the close connection with immune response and inflammation-associated diseases[34, 35]. Furtherly, the miRNA-regulatory network in these overlapped genes was also identified in the current study. we identified 71 predicted miRNAs, including miR-206, 27a-3p, 27b-3p and 92a-3p, which mainly enriched in TGF-β signaling pathway, Ras signaling pathway and Wnt signaling pathway. Actually, previous study has verified the increased expression of miR-27a-3p and 27b-3p in the IL-1β-stimulated OA synovial explants in vitro[36]. Also, some researchers demonstrated the miR-206 and 92a-3p may be a novel therapeutic strategy for the treatment of OA[37, 38]. Furthermore, the abnormal activation of these miRNAs-associated signaling pathways also has been shown to participate in deteriorating OA progression[39, 40]. Therefore, these important miRNAs and related pathways can be served as new targets for OA diagnosis and therapy.

Conclusions

Our results demonstrated that OA-related inflammatory synoviocytes is associated with immune response, response to LPS, TNF signaling pathway, chemokine signaling pathway and

oxidation-related process. However, compared to inflammatory model in vitro, in OA joint, the synovial inflammation is more complicated, maybe involved neutrophil recruitment, monocyte migration, and macrophage activation. Additionally, the current study also shown that some key genes and pathways, such as CLIC6, CXCL2, CXCL5, SLC7A2 and C15orf48, may be associated with OA progression.

Abbreviations

OA: Osteoarthritis

DEGs: differentially expressed genes

PPI: protein-protein interaction

LPS: lipopolysaccharide

TNF: tumor necrosis factor

CXCL2: C-X-C chemokine ligand 2

CXCL5: C-X-C chemokine ligand 5

CLIC6: chloride intracellular channel 6

SLC7A2: solute carrier family 7 member 2

C15orf48: chromosome 15 open reading frame 48

TGF-β: transforming growth factor-β

Ethics approval and consent to participate

This article does not contain any studies with human or animal subjects.

Consent to publish

Not applicable

Availability of data and materials

The microarray data GSE49604, GSE46750 and GSE82107 were downloaded from the GEO database in NCBI.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Funding

This research was founded by National Natural Science Foundation of China (81873329) and Natural Science Foundation of Jiangsu Province (BK20181501,SBK2020041404). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

P.W and MC.L: design study and perform the statistical analysis; XQ. S and LS J: analyze the data and prepare the figure; J.M and SJ.Yin: design the study and edit the manuscript.

Acknowledgements

The authors wish to express their gratitude to all the teachers in the Tang ZhongYing Science and Technology Building, Nanjing University of Chinese Medicine, Nanjing, China.

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Footnotes:

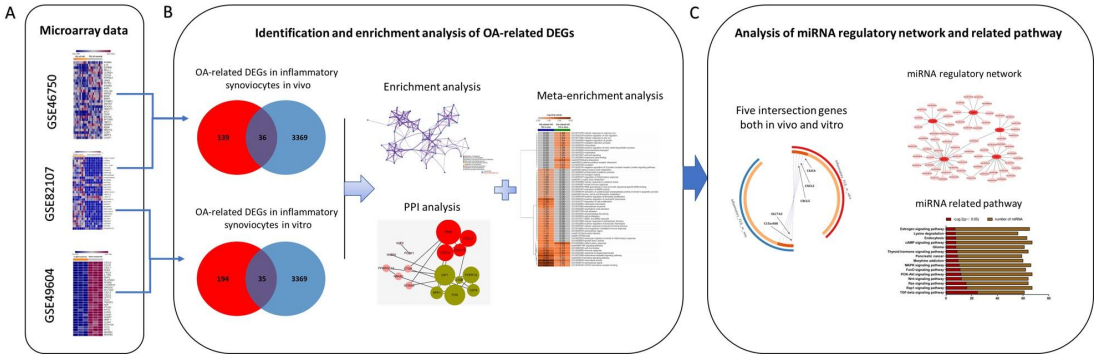


Fig.1 The framework of revealing key genes and pathways in osteoarthritis-related inflammatory synoviocytes in vivo and in vitro. (a) Microarray Data. GSE46750 gene data, in which 12 samples of synoviocytes from inflammatory vs natural areas in vivo were compared. GSE49604 gene data, in which three samples of IL-1β- and saline-treated synoviocytes in vitro were compared. GSE82107 gene data, in which 10 synovial samples from OA patient vs 7 samples from individual without a joint disease were compared. (b) Identification and enrichment analysis of OA-related DEGs. The DEGs from GSE46750 and GSE82107 gene data were overlapped to show the intersection genes in OA-related inflammatory synoviocytes in vivo. The DEGs from GSE49604 and GSE82107 gene data were overlapped to show the intersection genes in vitro. Enrichment analysis and protein-protein interaction (PPI) was performed in inflammatory synoviocytes in vivo and vitro, respectively. Subsequently, the meta-enrichment analysis for the two OA-related DEGs lists was also conducted. (c) analysis of miRNA regulatory network and related pathway in the five overlapped genes in vitro and vivo were also identified to initially provide certain possible therapeutic target for OA.

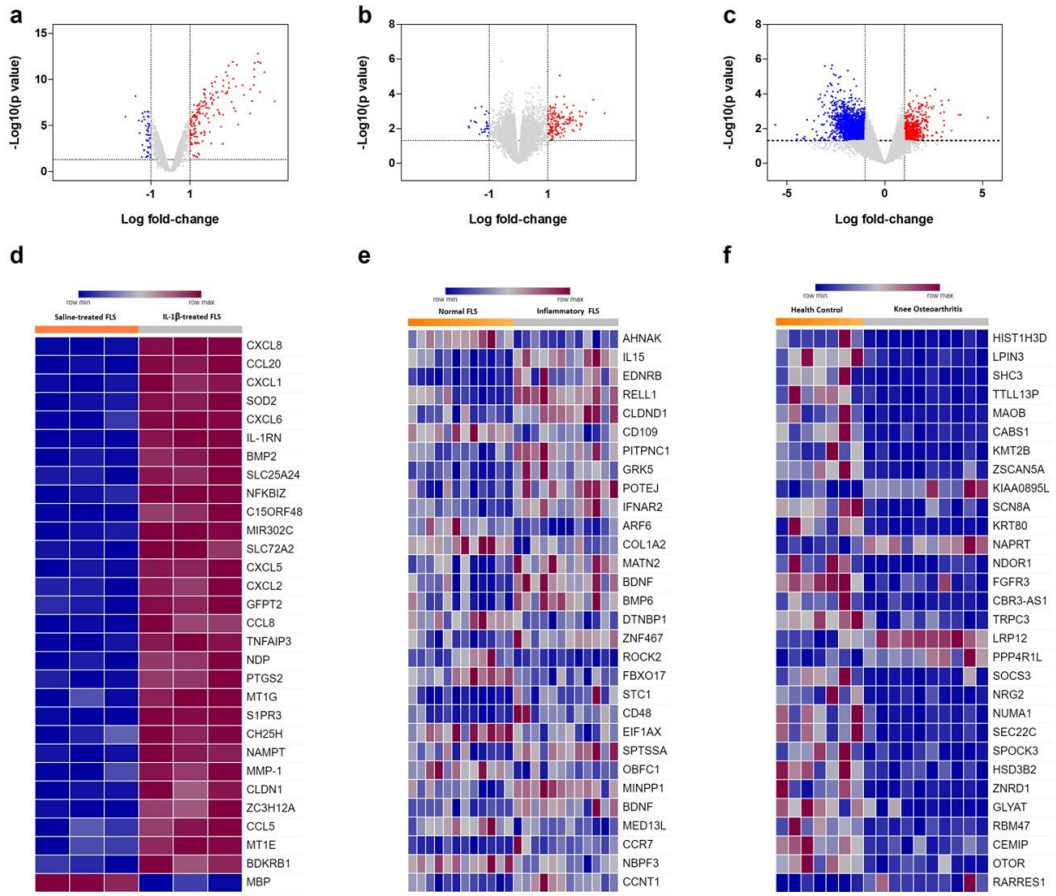


Fig.2 Identification of DEGs in three different data bases. Volcanic map of all genes in (a) IL-1β- vs saline-treated synoviocytes in vitro, (a) synoviocytes from inflammatory vs normal areas in vivo, and (c) synovial biopsies from ten patients with OA vs individuals without a joint disease. Red dots indicate upregulated genes, blue dots indicate downregulated genes and gray dots indicate genes that are not regulated. (d, e and f) Heat map of 50 genes from the three different data bases. Red indicates higher gene expression and blue indicates lower gene expression.

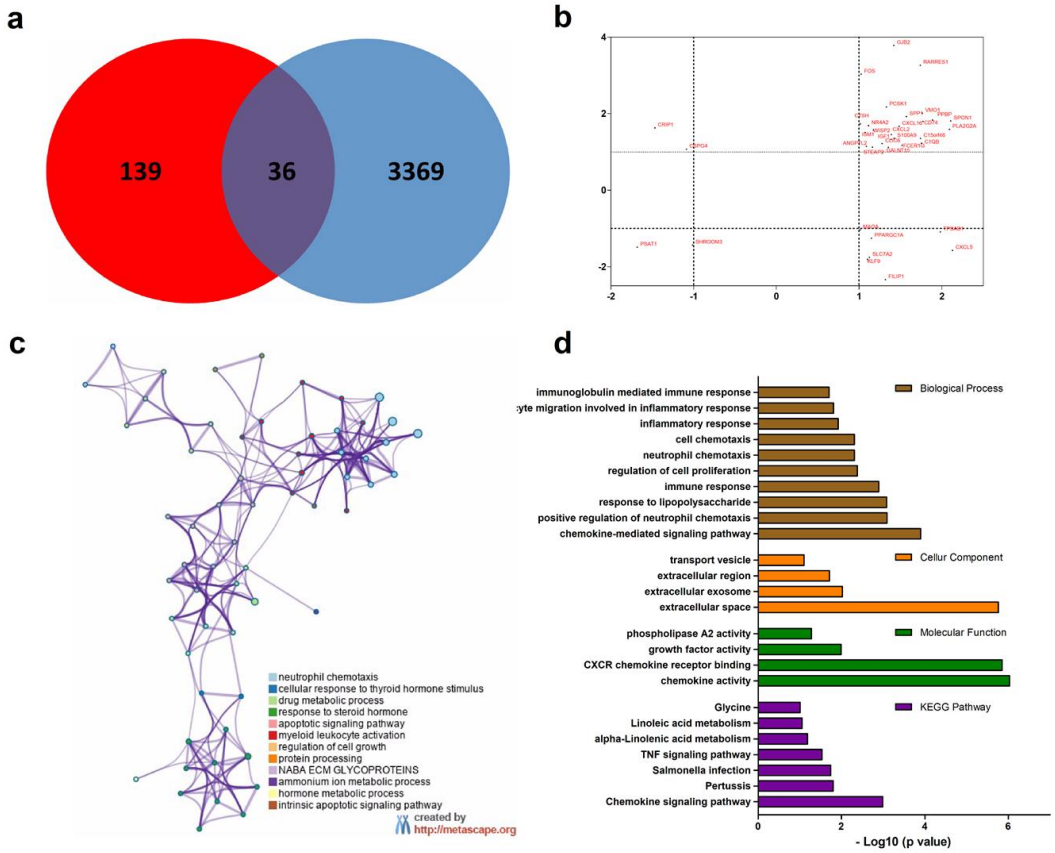
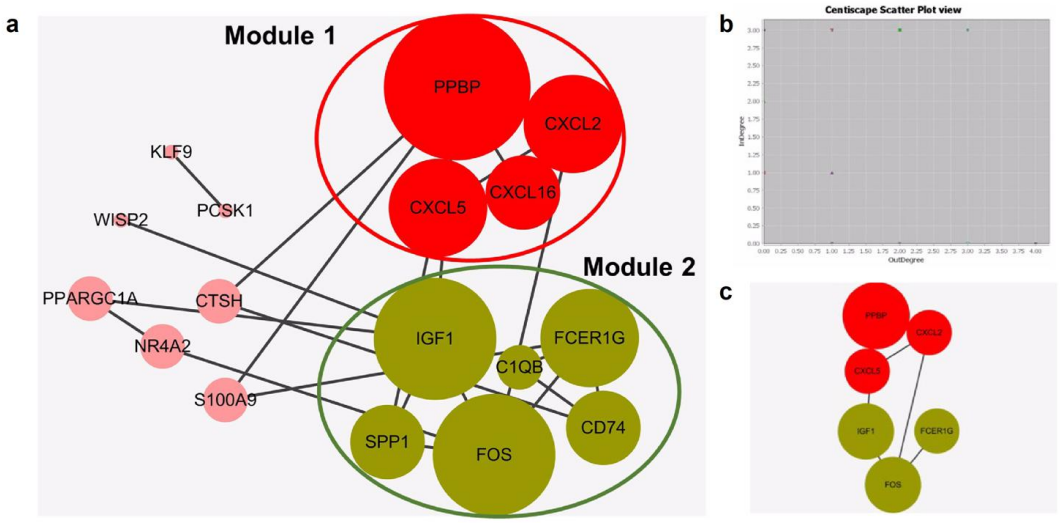


Fig.3 Identification and enrichment analysis of OA-related DEGs in inflammatory synoviocytes in vivo. (a) Venn diagram revealed that 36 common DEGs were differently expressed in the synoviocytes from inflammatory vs normal areas and the synovium from healthy and osteoarthritic joints. (b) Scatter plot view of -Log 10 (fold change). X-axis represents the synoviocytes from inflammatory vs normal areas and y-axis represents the synovium from healthy and osteoarthritic joints. (c) Enrichment analysis of OA-related DEGs in inflammatory synoviocytes in vivo, which was produced by Metascape. (d) GO enrichment and KEGG pathway analysis of OA-related DEGs in inflammatory synoviocytes in vivo, which was produced by DAVID.



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455 **Fig.4 PPI network analysis of OA-related DEGs in inflammatory synoviocytes in vivo.** (a) PPI

456 network of OA-related DEGs in inflammatory synoviocytes in vivo constructed using Cytoscape. Sizes

457 of dots are proportional to the score and gray edges indicate protein interactions. (b) Scatter plot view

458 of centrality degree. X-axis represent centrality outdegree and y-axis represent centrality indegree. (c)

459 PPI network of hub genes (centrality degree≥5) constructed using Cytoscape. Sizes of dots are

460 proportional to the score and gray edges indicate protein interactions.

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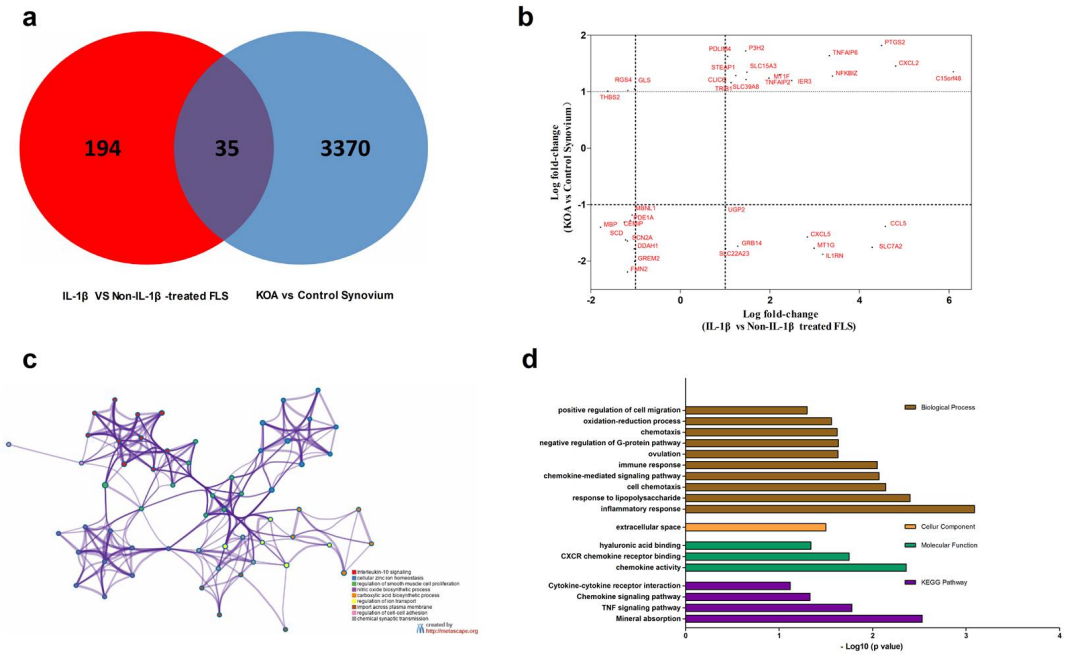

Fig.5 Identification and enrichment analysis of OA-related DEGs in IL-1β-treated synoviocytes in vitro. (a) Venn diagram revealed that 36 common DEGs were differently expressed in in IL-1β- vs saline-treated synoviocytes in vitro and the synovium from healthy and osteoarthritic joints. (b) Scatter plot view of -Log 10 (fold change). X-axis represents in IL-1β- vs saline-treated synoviocytes and y-axis represents the synovium from healthy and osteoarthritic joints. (c) Enrichment analysis of OA-related DEGs in inflammatory synoviocytes in vitro, which was produced by Metascape. (d) GO enrichment and KEGG pathway analysis of OA-related DEGs in inflammatory synoviocytes in vitro, which was produced by DAVID.

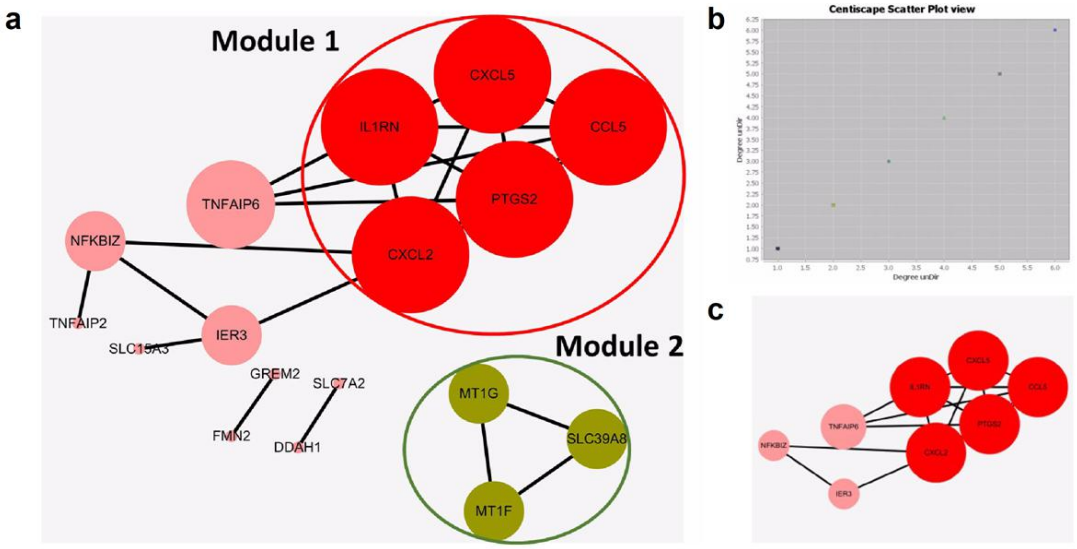

Fig.6 PPI network analysis of OA-related DEGs in IL-1β-treated synoviocytes in vitro. (a) PPI network of OA-related DEGs in IL-1β-treated synoviocytes in vitro. constructed using Cytoscape. Sizes of dots are proportional to the score and gray edges indicate protein interactions. (b) Scatter plot view of centrality degree. X-axis represents centrality outdegree and y-axis represents centrality indegree. (c) PPI network of hub genes (centrality degree≥5) constructed using Cytoscape. Sizes of dots are proportional to the score and gray edges indicate protein interactions.

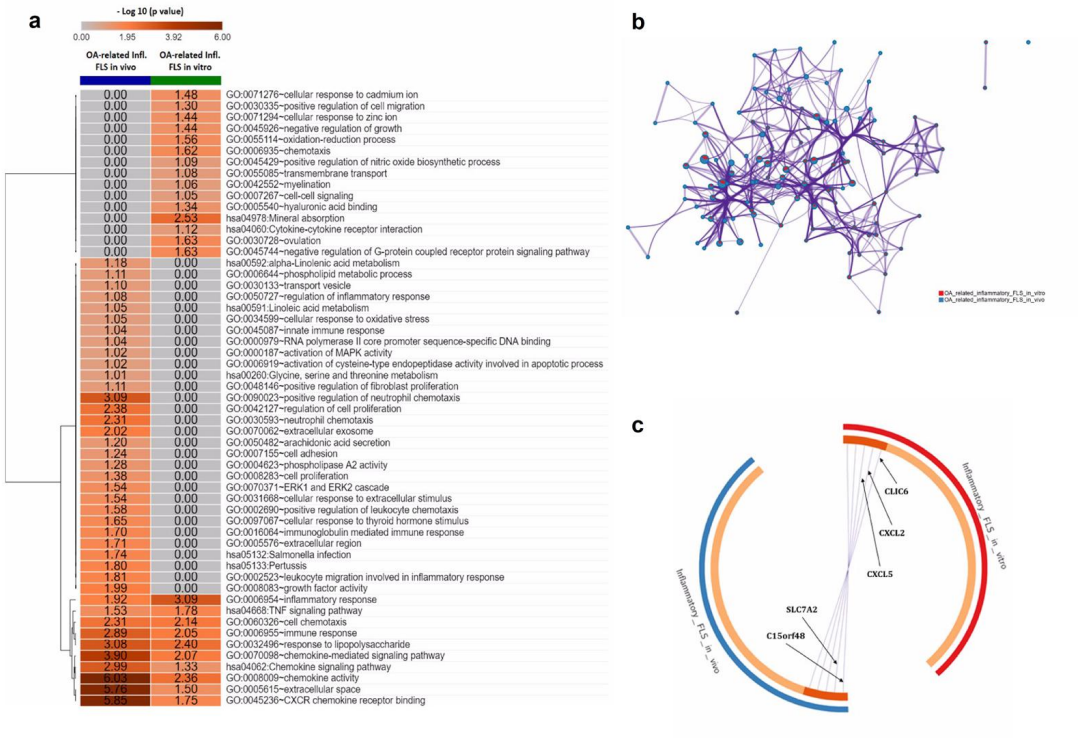

Fig.7 Meta-enrichment analysis for OA-related DEGs in inflammatory synoviocytes in vivo and vitro. (a) Heatmap of enrichments across two gene lists, colored by p values. (b) Network of enriched terms are colored by cluster ID and nodes share the same cluster are typically close to each other. (c) Overlap among gene lists at the gene level, where purple curves link identical genes and blue curves link genes belong to the same enriched ontology term. The inner circle represents gene lists, where hits are arranged along the arc. Genes hit multiple lists are colored in dark orange, and genes unique to a list are shown in light orange.

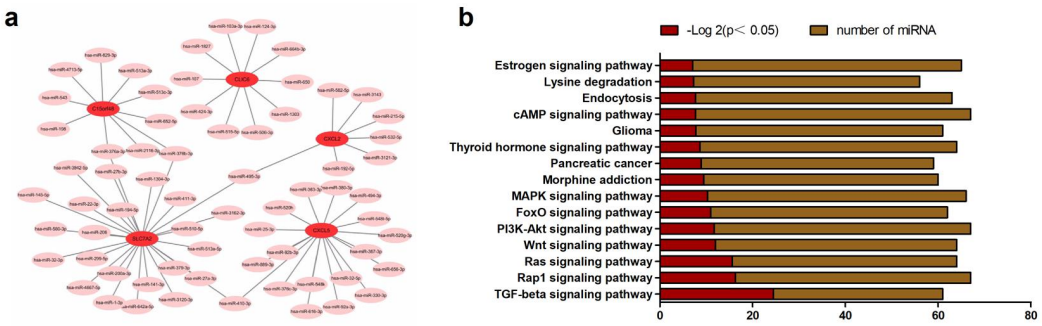

Fig.8 MiRNA–mRNA regulatory networks in hub overlapped genes. (a) MiRNA–mRNA (CLIC6, CXCL2, CXCL5, SLC7A2 and C15orf48) regulatory networks. Red nodes denote genes from sub-networks and purple nodes denote miRNAs; red edges denote mRNAs-mRNAs interaction relationship and blue edges denote miRNAs-mRNAs interaction relationship. (b) Bar graph for KEGG pathway enrichment analysis of the predicted miRNAs.