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Screening and identification of key biomarkers in osteoarthritis:A comprehensive bioinformatics analysis integrating multiple datasets

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Abstract:

Objective:To identify key genes and pathways associated with the synovial inflammatory response in KOA to better study the molecular mechanism of action.

Methods:Microarray datasets (GSE55235 and GSE55457) were retrieved from the Gene Expression Omnibus (GEO). DEGs were identified using GEO2R, alongside an independent RNA-seq analysis of KOA and normal synovium. Across the three datasets, 49 overlapping DEGs (13 upregulated and 36 downregulated) were screened.GO enrichment analysis and KEGG enrichment analysis of data were performed with the help of Webgestalt and metascape. Cytoscape software was used to import the PPI network, and then

the first five percent of genes were tagged as Hub genes.

Results: Forty-nine DEGs overlapped across the three datasets, including 13 upregulated and 36 downregulated genes. Network analysis highlighted FOSL2, NR4A1, and ATF3 as candidate hub genes.

Conclusions: This study analyzed DEGs and hub genes in inflamed KOA synovium, which may help us understand the pathogenesis of KOA and provide candidate targets for diagnosis and treatment of OA.

[Keywords]: osteoarthritis; synovium; biomarker; bioinformatics analysis

Knee osteoarthritis (KOA) is currently one of the most common osteoarticular diseases. The main pathology of osteoarthritis involves cartilage degeneration, synovial cell inflammation, and subchondral bone sclerosis or the formation of new bone[1-3]. The global prevalence of KOA is 16.0%[4]. However, results of studies on the pathogenesis and treatment of KOA are suboptimal. Newly-published results show that synovial cells play a crucial role in KOA[5-7]. Meanwhile, many clinical studies have shown that the synovial inflammatory response is significantly associated with KOA symptoms such as joint pain, stiffness and cartilage damage[8-9].

Advancements in high-throughput sequencing and computational biology have revolutionized our understanding of complex diseases. An increasing body of evidence indicates that the onset and progression of KOA are driven by intricate networks of genes and signaling pathways and more and more studies have shown that the occurrence and development of KOA are related to a series of genes and signaling pathways[10]. With the help of the gene expression data recorded in the Gene Expression Omnibus (GEO) database, gene chip analysis of synovial gene expression was performed. The healthy group and the experimental group with KOA were screened, the expression status of synovial tissue DEGs in the two groups of samples was analyzed, and then selected DEGs were classified to carry out functional enrichment analysis. The protein interaction (PPI) network of the STRING database was used to screen out a number of gene species closely associated with KOA patients, which provided theoretical guidance for the clarification of the molecular mechanism of KOA pathogenesis and the screening of molecular markers.

In this study, we comprehensively profiled KOA-associated differentially expressed genes (DEGs) by leveraging multi-cohort transcriptomic datasets from the GEO database (GSE55235 and GSE55457) alongside an independent local RNA-seq cohort of human synovial tissues. Subsequent bioinformatics interrogation converged on three pivotal hub genes: FOSL2, NR4A1, and ATF3. As illustrated in our proposed roadmap (Figure 1), this work establishes a theoretical foundation for understanding KOA pathogenesis and guiding future experimental validation.

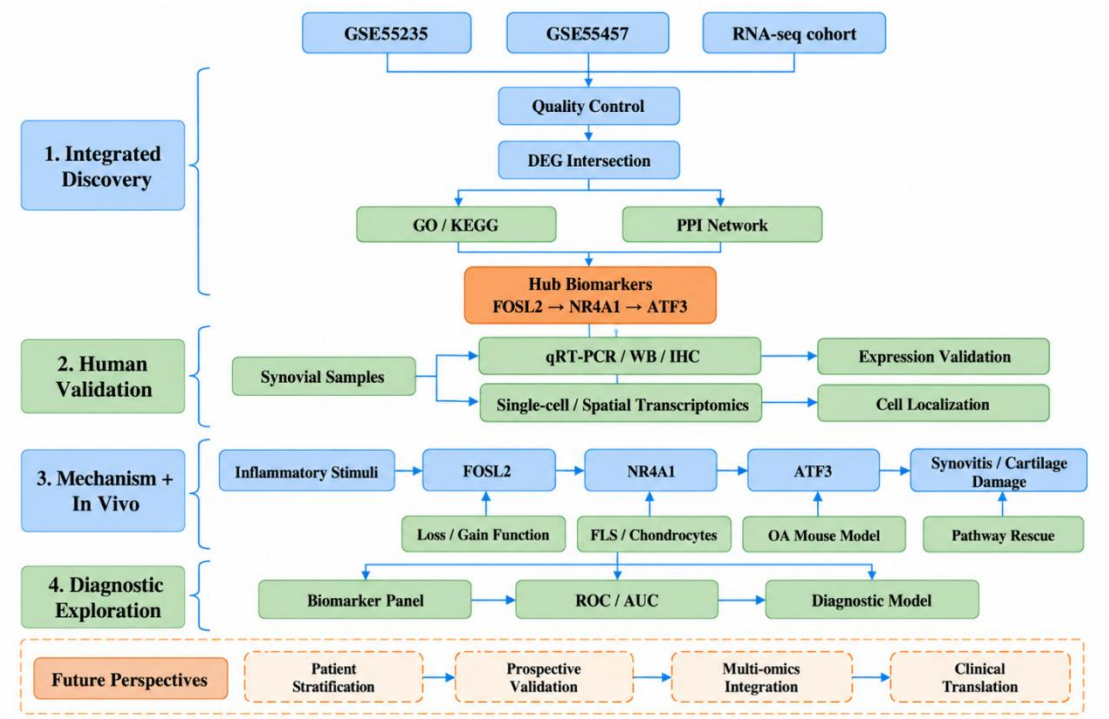


Figure 1. Schematic overview of the study design and analytical workflow.
The study comprises four main phases: (1) Integrated Discovery (transcriptomic datasets GSE55235, GSE55457, and an independent RNA-seq cohort to identify hub biomarkers FOSL2, NR4A1, and ATF3); (2) Human Validation (expression and spatial localization in synovial samples via qRT-PCR, WB, IHC, and single-cell/spatial transcriptomics); (3) Mechanism + In Vivo (loss/gain-of-function in FLS/chondrocytes, inflammatory stimuli, OA mouse models, and pathway rescue experiments); and (4) Diagnostic Exploration (biomarker panel construction, ROC/AUC evaluation, and therapeutic target identification). Orange dashed boxes indicate future perspectives, including patient stratification, prospective validation, multi-omics integration, and clinical translation. **Abbreviations:** DEG, differentially expressed gene; FLS, fibroblast-like synoviocytes; OA, osteoarthritis; ROC, receiver operating characteristic; AUC, area under the curve.

Materials and methods

1. Microarray data

Eight patients with KOA were selected as the research subjects, from among the inpatients attending Beijing Bo'ai Hospital between March 2016 and November 2018, and eight patients with knee fractures were used as controls. All experiments using human samples conformed to the guidelines set by the Declaration of Helsinki.

The current criteria for diagnosing KOA are from the diagnostic criteria reference book published by the American Association for Rheumatology Research. Inclusion criteria for the experimental group (EG) were: (1) Han nationality, born in the region, and living and working in the region for more than 20 years; (2) meeting the diagnostic criteria for KOA; (3) aged between 40 and 70 inclusive; (4) participants in this study signed an informed consent form. Exclusion criteria for the experimental group: (1) age > 70 years or < 40 years old; (2) unwilling to provide signed informed consent; (3) had long-term employment outside the local area for 5 years or more; (4) various secondary KOA; (5) inflammatory arthritis (rheumatoid arthritis, systemic lupus erythematosus, spondyloarthritis, hemophilic arthritis, gout, etc.), with a diagnosis certificate issued by a third-grade class-A hospital; (6) patients with major systemic diseases such as diabetes, heart disease, infectious diseases, mental illness or who did not cooperate. Inclusion criteria for the normal control group: (1) aged between 40 and 70 years old; (2) voluntarily

participated in this research and signed the informed consent; (3) no history of knee arthritis, knee KL Grading < 2. Exclusion criteria for normal control group: (1) age >70 years or <40 years old; (2) unwilling to provide signed informed consent. This experiment adopted a voluntary democratic approach, and the participants in the healthy group should be aged 62.5 ± 6.3 years old, requiring a total of three males and five females. The age of the affected group should be between 65.7 ± 4.6 years, and four males and four females were required. In addition to the information mentioned above, all other characteristics of the two groups should remain the same, in order to ensure that there were no statistically-significant differences in the final data between the two groups. Knee joint synovial tissue was collected from both the experimental group and the control group, and RNA was immediately extracted and stored in a -80°C freezer. Before the start of the study, the study was explained to all patients and the informed consent was signed. The consent of the local hospital ethics committee (2017-075-1) was obtained before this project was carried out. A total of 1,025 differentially-expressed genes were detected by RNAseq. (log FC >1 and adj. *P*-value <0.05 were considered statistically significant.).

All gene-related data were downloaded from

GEO (http://www.ncbi.nlm.nih.gov/geo). (Affymetrix GPL96, Affymetrix Human Genome U133A Array). The three datasets consisted of 28 KOA synovial samples and 28 normal samples, of which the GSE55235 datasets contained 10 synovial tissues of healthy joints and 10 synovial tissues of KOA joints, and the GSE55457 datasets also contained 10 synovial tissues of healthy joints and 10 synovial tissues of KOA joints.

2. Identification of DEGs

The synovial tissue of each dataset sample was analyzed using GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r), which is a web tool of GEO, and DEGs were finally screened. According to the adjusted *P*-values (adj. *P*) < 0.01, log FC (fold change) > 2 screen DEGs in synovial tissue of KOA and healthy controls in the three datasets. The DEGs of the three groups of data were analyzed using the online database (http://bioinformatics. psb.ugent. be/ webtools/ Venn).

3. GO functional enrichment and KEGG pathway enrichment analyses of DEGs.

Webgestalt (http://www.webgestalt.org)[11][13] is an online biological information database that translates gene lists into biological insights. Metascape (https://metascape.org/) is a web-based portal designed to provide a comprehensive gene list annotation and analysis resource for experimental biologists[14]. KEGG is a comprehensive database that integrates genomic, chemical functional, and systems information. One of the features and advantages of this database is the ability to correlate a fully sequenced catalog of genomic information with higher-level cellular and ecosystem functions[15].

With the increasing complexity and scale of biological research, GO analysis can provide unified description and proof of gene products, and is the most comprehensive annotation tool for gene function and products[16]. In order to be able to analyze the specific functions of DEGs, we also used online databases such as Webgestalt and Metascape to perform biological analysis tests on samples. KEGG pathway visualization was performed using the Pathview online tool[17].

4. PPI network construction and Hub gene selection

Understanding the roles and mechanisms that exist between different proteins is of great significance to our understanding of the mechanisms of disease development and progression[18]. We selected DEGs with an interaction score > 0.4 for visualization in Cytoscape software[19]. Submodule analysis using the MCODE plugin was used to identify more connected genomes[20]. The standard settings were as follows: MCODE scores > 5, degree cut-off = 2, node score cut-off = 0.2, Max depth = 100 and k-score = 2.

Results

1. DEGs in synovial tissue of healthy controls and KOA patients

Totals of 786 and 1,048 DEGs were identified from the gene chip datasets GSE55457 and GSE55235, respectively. In the EG group 1,025 DEGs were identified. From the results shown in Figure 2：49 overlapping DEGs were found to be completely contained within all three datasets. In addition, it can be clearly seen that the synovial tissue provided by the KOA group and the normal group contains 13 upregulated genes and 36 downregulated genes.

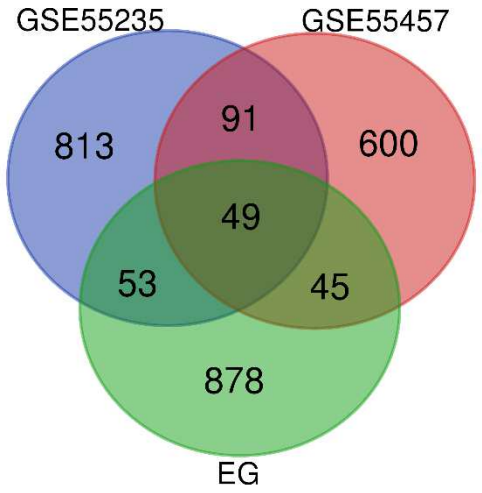


Figure 2. Venn diagram. DEGs were selected with a fold change > 2 and *P*-value < 0.01 among the RNA expression profiling sets GSE55235, GSE55457 and EG. The three datasets showed an overlap of 49 genes.

2. GO and KEGG enrichment analyses of DEGs.

The GO database results showed that DEGs involved in biological

processes (BPs) were changing in many areas. These included cell metabolism, multicellular reproduction, and cell development, where obvious enrichment could be seen. Changes in molecular function (MF) were concentrated in protein-binding regions, including nucleic acid binding and iron binding. In addition, Figure 3 shows that the location of the changes in the cellular composition (CC) of DEGs were in the cell membrane and nucleus. Figure 4 shows the results of GO and KEGG data analysis, and the DEGs affected catalytic activity, which in turn controlled the differentiation of epithelial cells. The specific enrichment network and related pathways are detailed in Figures 5 and 6.

3. Network construction and Hub gene selection

Figure 6A shows the PPI network structure of DEGs. It can be seen that a total of three genes were identified as central genes, with a degree of more than 10%. Figure 6B lists the names of these important genes, NR4A1, FOSL2, and ATF3.

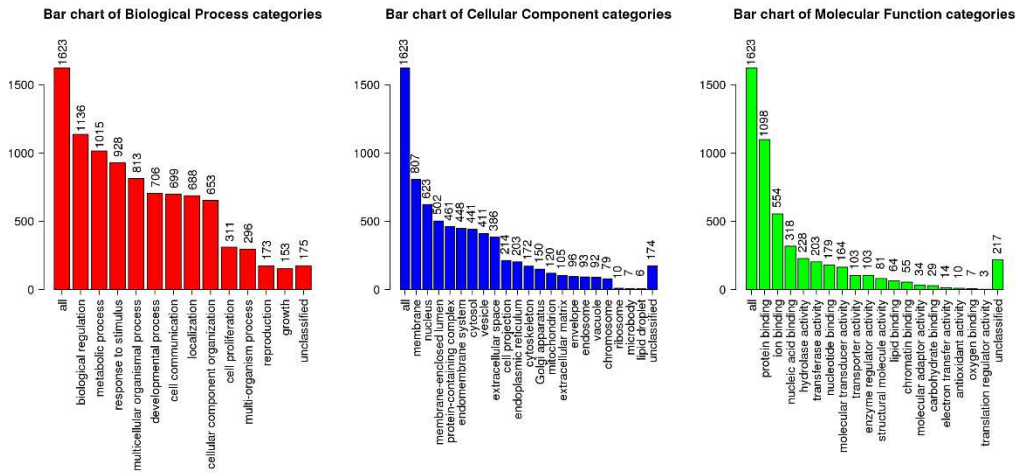


Figure 4. The results of GO analysis with DEGs obtained from patients with KOA. In the figure, the biological processes that occur in cells, the cellular composition structure, and cell molecules are distinguished by different colors. Red represents biological processes, blue represents cellular components, and green represents molecular function. The color bar height indicates the number of IDs.

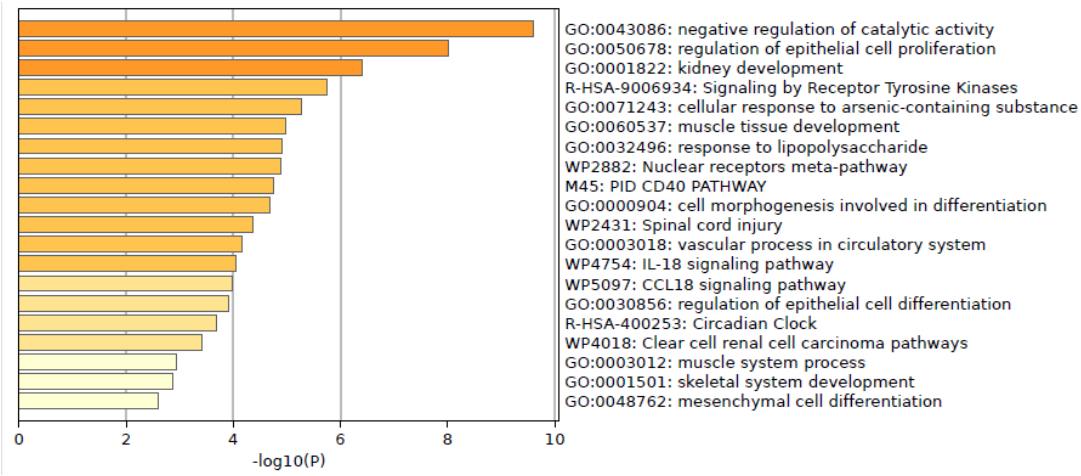


Figure 4. Results of DEG enrichment analysis by GO/KEGG were obtained from patients with KOA.



A B

Figure 5. Network of enriched terms: In part (A) nodes are colored according to the cluster ID, so nodes that share the same ID are generally closer. In part (B) the color is based on the *P*-value, so whether it contains more genes can be seen according to the *P*-value.

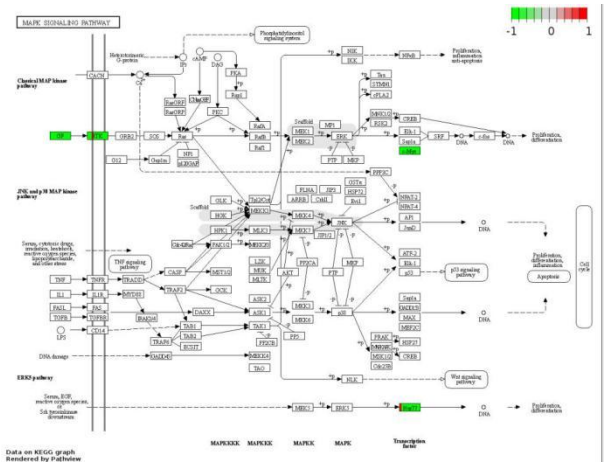
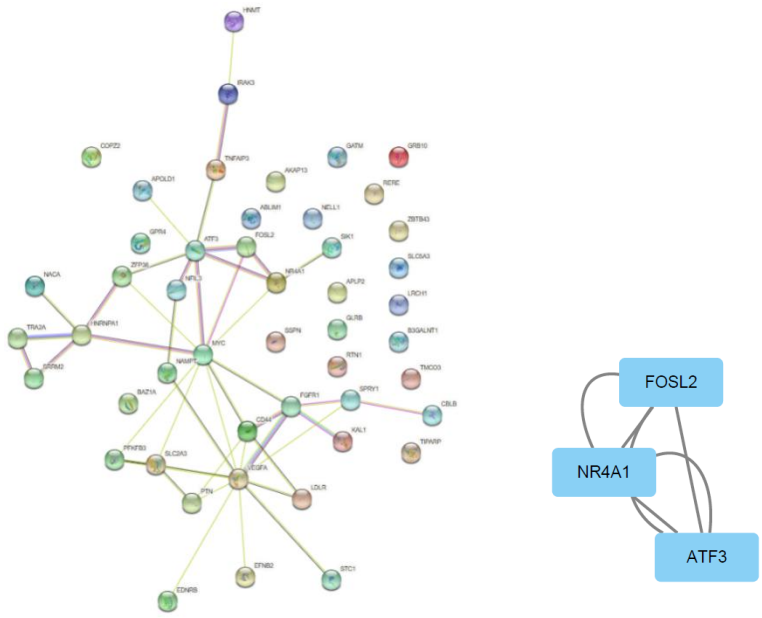


Figure 6. Pathway and color of DEG protein expression.



7A 7B

Figure 7A The protein–protein interaction network was analyzed using string. 7B Hub genes were constructed using Cytoscape

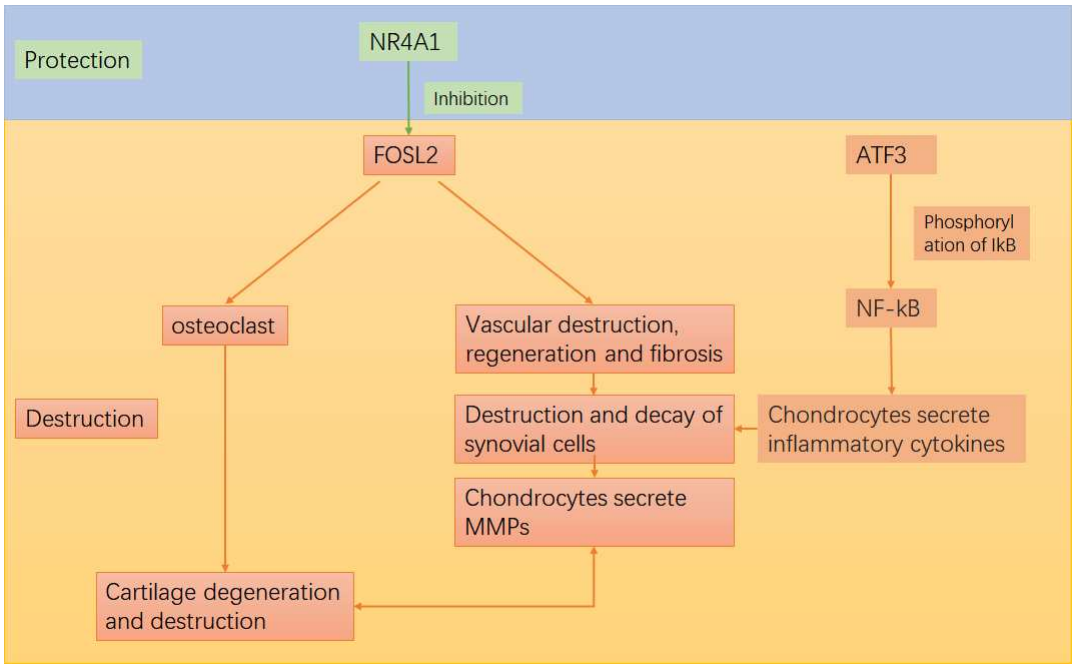


Figure 8： proposed mechanism figure to the main findings of the study.

Discussion

KOA is a musculoskeletal disease with a high risk of disability. At present, conservative treatment such as drug therapy is often used in the early stage of the disease, but there is no effective treatment plan except for joint replacement in the late stage[21][23]. The key to treatment is the identification of early non-dominant KOA. Molecular analysis of the pathological mechanism of KOA is

the basis of individualized treatment[24]. Therefore, we focused on the molecular mechanism of KOA, hoping to provide a feasible solution for its treatment.

During the progression of KOA, the functional properties and structure of articular cartilage gradually change[25], due to processes such as articular cartilage degeneration, subchondral bone sclerosis, synovial inflammation, etc. [26]. KOA is a disease involving progressive degeneration of the entire joint, with symptoms of synovitis accompanying the entire disease process. The relationship between synovitis and structural progression in osteoarthritis is well documented[27][30], but in previous studies, there was less research on the synovium. Therefore, exploring the pathogenesis of synovitis may provide a new perspective for discovering the etiology of KOA and identifying potential new treatments.

Three datasets, GSE55235, GSE55457 and GE group, were included in this study. The microarray datasets GSE55235 and GSE55457 were downloaded from the GEO database. RNAseq was performed using samples from KOA and normal synovium (Experimental Group, EG). A total of 49 DEGs, comprising 36 downregulated genes and 13 upregulated genes, were identified in the above three datasets. In order to better understand the pathogenesis of synovitis symptoms, Webgestalt and metascape were used to enrich the data. From the results of GO analysis, DEGs are reflected in cell metabolism, multi-cell reproduction, and cell development. From the perspective of molecular function results, the enrichment changes are mainly reflected in the process of protein production, nucleic acid composition and iron synthesis. From the perspective of composition changes, these are reflected in the cell membrane and nucleus.

In this article, by constructing the PPI network of DEGs and performing visual analysis through Cytoscape, Hub genes were identified according to the degrees of freedom. The results showed that: FOSL2, NR4A1, and ATF3 have the highest degrees of freedom, with degrees $\geqslant$ 10. Previous studies have shown the association of single nucleotide polymorphisms (SNPs) within the FOSL2 promoter with the risk of Crohn's disease, ulcerative colitis, asthma, hay fever, and eczema[31], and additional research suggests that Fosl2 may play a role in the control of inflammatory diseases[32]. FOSL2 has been implicated in chondrocyte differentiation, metabolism, stress response and survival in articular cartilage[33].Moreover,it has been reported that FOSL2 potentially plays a key role in knee osteoarthritis pathogenesis and correlates with immune cell infiltration[32]. However, there is no relevant evidence for the role of the FOSL2 gene in the synovium. According to another study, NR4A1 is involved in the apoptosis of chondrocytes in osteoarthritis[33]. In addition,NR4A1 can improve the level of intraarticular inflammatory factors,by inhibiting the NF-κB signal pathway, thereby reducing intraarticular inflammation and cartilage degeneration, and it plays a protective role in OA[34][35].Atf3 is implicated in the pathogenesis of KOA through modulation of inflammatory cytokine expression in chondrocytes[36].ATF3 modulates Akt signaling, a pathway that may mediate

its role in OA development[37]. In parallel, Atf3+ senescent chondrocytes have been identified as a key pathogenic population in the aging meniscus, driving degeneration via ANGPTL4 signaling. Targeting Atf3+ cells or ANGPTL4 may therefore offer new therapeutic strategies for age-related meniscus degeneration and arthritis[38][39].All three hub genes have been preliminarily investigated in synovial tissue, but their mechanism of action needs to be further verified.

This study has several limitations. First, the modest local cohort (n=8) and the use of fracture controls may introduce trauma-induced inflammatory bias, necessitating larger multicenter validation. Second, the current findings are primarily bioinformatics-based. As illustrated in the roadmap (Figure 1), experimental validation—including expression profiling (qRT-PCR/WB/IHC), single-cell/spatial transcriptomics, and in vitro/in vivo mechanistic assays (FLS/chondrocytes, OA mouse models)—remains to be performed. Consequently, the proposed mechanisms (Figure 8) are largely hypothetical. Finally, prospective clinical studies are required to evaluate the diagnostic and translational potential of these hub genes.

In summary, integrated analysis of three synovial transcriptomic datasets identified 49 shared DEGs and prioritized FOSL2, NR4A1, and ATF3 for follow-up work. Independent validation and mechanistic experiments are needed before these candidates can be considered biomarkers or therapeutic targets for KOA.

Data Availability

The microarray data used to support the findings of this study can be downloaded from theGSE55235 and GSE55457 datasets (https://www.ncbi.nlm.nih.gov/geo). The processed data are available from the corresponding author upon request.The experiments with human samples conformed to the guidelines set by the Declaration of Helsinki.

Ethical Approval

This study was approved by the Beijing Bo’ai Hospital Clinical Trial Organization Ethics Committee (no. 2017-075-1).

Conflicts of Interest

All authors declare that they have no conflicts of interest.All the authors agree to submit this manuscript to Scientific Reports.

Authors’ Contributions

Dejian Zhang and Jiangfeng Zhou contributed equally to this work. Dejian Zhang conducted the search, collected and extracted the data, and wrote the manuscript. Jiangfeng Zhou interpreted the results, and wrote the manuscript.

Haoran Qiao, Shengfei Luo and Lixi Zhao extracted and analyzed the data. Hongwei Min provided feedback on the manuscript.

Declarations

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