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THE molecular mechanisms underlying synovial fibroblast differentiation in knee osteoarthritis revealed by single-cell transcriptome sequencing

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Abstract

This study aims to conduct an in-depth analysis of single-cell transcriptome data from synovial fibroblast differentiation in knee osteoarthritis (KOA), identify key target genes and underlying molecular mechanisms, and provide molecular-level theoretical support for clinical targeted therapy of KOA. The dataset (GSE176308), containing 3 KOA samples, was downloaded from the gene expression omnibus database. The R language was used to analyze key marker genes in synovial fibroblast differentiation. A series of analyses including quality control, data filtering, principal component analysis, t-distributed stochastic neighbor embedding analysis, cell trajectory mapping, gene ontology, and Kyoto encyclopedia of genes and genomes pathway analyses were performed to reveal the differentiation mechanisms of synovial fibroblasts. This dataset included 10,512 genes, 20 principal components, 8 synovial fibroblast subpopulations, 2991 marker genes, and 7 differentiation trajectories. Synovial fibroblasts were identified as early differentiated cells within the synovium. Gene ontology enrichment analysis indicated involvement in vascular regulation, ossification, wound healing, and extracellular matrix (ECM)-related functions, whereas Kyoto encyclopedia of genes and genomes analysis highlighted pathways related to ECM-receptor interaction, focal adhesion, cytoskeletal regulation, immune-related processes, and cellular metabolism. Single-cell ribonucleic acid sequencing revealed pronounced heterogeneity of synovial fibroblasts in KOA, identifying 7 differentiation trajectories and key marker genes including SPP1, TYROBP, CD74, CCL4, CCL3, APOE, and HLA-DRA. These genes and pathways are mainly involved in immune–inflammatory regulation, ECM remodeling, and cell-matrix interactions, which are central to KOA progression. Our findings provide a refined molecular framework for synovial fibroblast differentiation and suggest potential biomarkers and therapeutic targets for synovium-focused precision diagnosis and targeted intervention in KOA.

Abbreviations: BP = biological processes, ECM = extracellular matrix, FLS = fibroblast-like synoviocytes, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, KOA = knee osteoarthritis, MF = molecular functions, PCA = principal component, PCA = principal component analysis, scRNA-se = single-cell ribonucleic acid sequencing, t-SNE = t-distributed stochastic neighbor embedding.

Keywords: knee osteoarthritis, molecular mechanisms, single-cell RNA sequencing (scRNA-seq), synovial fibroblasts

1. Introduction

Knee osteoarthritis (KOA) is one of the most prevalent chronic degenerative joint diseases worldwide and a leading cause of pain, functional limitation, and disability in aging populations.[1–3] With increasing life expectancy and rising obesity rates, the global burden of KOA continues to grow, posing substantial socioeconomic and healthcare challenges. KOA is no longer regarded as a simple cartilage wear-and-tear disorder but rather as a complex, whole-joint disease involving cartilage degeneration, subchondral bone remodeling, synovial inflammation, and alterations in periarticular tissues.[4,5] Among these pathological features, synovitis has been shown to correlate strongly with pain severity, disease progression, and structural joint damage in KOA.[6]

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The authors have no conflicts of interest to disclose

The datasets generated during and/or analyzed during the current study are publicly available.

This study is a retrospective bioinformatics analysis using only publicly available data. The analyzed dataset (GSE176308) was downloaded from the Gene Expression Omnibus (GEO) database, a public repository for high-throughput functional genomic data. As the study uses de-identified, publicly accessible data without involving human subject interaction, new sample collection, or access to identifiable personal health information, and the GSE176308 dataset has undergone prior ethical review and consent procedures during original generation and deposition in line with GEO's policies, this study is exempt from additional ethical review and approval, so no further ethical approval was required.

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Synovial fibroblasts, also known as fibroblast-like synoviocytes (FLS), are the most abundant resident cells in the synovial membrane and play a central role in maintaining synovial homeostasis under physiological conditions.[7,8] In pathological states, however, synovial fibroblasts undergo phenotypic activation and contribute to chronic inflammation, extracellular matrix (ECM) remodeling, angiogenesis, and cartilage destruction through the production of cytokines, chemokines, matrix-degrading enzymes, and adhesion molecules.[9] Emerging evidence suggests that synovial fibroblasts are not a homogeneous population but instead consist of functionally distinct subpopulations with divergent roles in inflammation, tissue invasion, and repair.[10] Importantly, synovial fibroblasts are considered among the earliest differentiated and functionally responsive cell types within the synovium during joint disease progression, highlighting their potential involvement in the initiation and amplification of KOA pathology.[11]

Despite growing recognition of the importance of synovial fibroblasts in KOA, their differentiation trajectories, molecular heterogeneity, and regulatory mechanisms remain incompletely understood. Most previous studies have relied on bulk transcriptomic or histological analyses, which obscure cell-to-cell variability and fail to capture dynamic changes during fibroblast differentiation.[12,13] Consequently, key questions remain regarding how synovial fibroblasts diversify into distinct functional states, which genes drive these differentiation processes, and how such changes contribute to the inflammatory and degenerative microenvironment of the osteoarthritic joint.

Single-cell ribonucleic acid sequencing (scRNA-seq) has emerged as a powerful tool for dissecting cellular heterogeneity, identifying rare or transitional cell states, and reconstructing differentiation trajectories at single-cell resolution.[14,15] When integrated with bioinformatics analyses, scRNA-seq enables systematic identification of key regulatory genes, signaling pathways, and lineage relationships that are not accessible through conventional approaches. Recent applications of scRNA-seq in joint diseases have revealed disease-associated fibroblast subsets and immune–stromal interactions, underscoring its value for elucidating pathogenic mechanisms.[14,15] Therefore, applying scRNA-seq combined with bioinformatics analysis to synovial fibroblasts in KOA represents a rational and necessary strategy to clarify their differentiation patterns and molecular functions (MF).

In this study, we integrated scRNA-seq data with comprehensive bioinformatics analyses to investigate synovial fibroblast differentiation in KOA. By identifying key marker genes, differentiation trajectories, and enriched biological pathways, we aimed to provide new insights into the molecular mechanisms underlying synovial fibroblast-mediated joint pathology and to offer a theoretical basis for potential targeted interventions in KOA.

2. Materials and methods

2.1. Data acquisition

We downloaded dataset GSE176308 from the gene expression omnibus database. The scRNA-seq dataset GSE176308 was selected for analysis because it provides high-quality single-cell transcriptomic profiles of synovial fibroblasts from KOA patients, with sufficient sequencing depth and detailed cell annotations. Although the dataset includes a limited number of samples, it remains a valuable resource for exploring synovial fibroblast heterogeneity and differentiation at single-cell resolution, which contains scRNA-seq data from synovial fibroblasts of 3 KOA samples. The dataset uses the GPL18573 platform.

2.2. Data quality control and filtering

We conducted quality control and data filtering on the GSE176308 dataset. During quality control, duplicate genes were averaged. After the quality-control step where duplicate genes were averaged, the processed data was converted into a "Seurat" object, and the "Percentage Feature Set" function was used to calculate gene percentages, which were then used to filter the data. This helped extract information on gene features and sequencing depth. Quality control and filtering were performed using the Seurat package in R. Duplicate genes were averaged, and cells were retained based on the following criteria: cells expressing fewer than 200 genes or more than 6000 genes were excluded to remove low-quality cells and potential doublets. Cells with a mitochondrial gene percentage > 10% were filtered out to minimize the influence of stressed or dying cells. Gene expression data were normalized using the LogNormalize method with a scale factor of 10,000. Highly variable genes were identified for downstream analyses. After quality control, the filtered dataset was used for dimensionality reduction and clustering analyses.

2.3. Principal component analysis (PCA)

PCA employs orthogonal transformation to transform correlated variables into variables with no linear correlation. This method effectively identifies key dimensions and selects biologically relevant genes. Following normalization, data were scaled using Seurat ScaleData function. PCA was performed based on the highly variable genes, and the top 20 principal components with statistically significant variation ($P < .05$) were selected for downstream clustering and visualization. In this study, we performed data standardization using R, then conducted PCA to identify genes loading on each principal component, including their associated P values and the uniformity of distribution.

2.4. t-distributed stochastic neighbor embedding (t-SNE) analysis and marker genes

t-SNE is a nonlinear technique for reducing dimensionality, commonly used to visualize high-dimensional data. It maps data points from high- to low-dimensional spaces to reveal clustering patterns. In this study, we used the R language to cluster single-cell sequencing data and then visualized the results. By comparing differential expression patterns in the dataset, we aimed to identify marker genes and perform further analyses of these genes.

2.5. Cell annotation and differentiation trajectory analysis

This study used a gene expression dataset (GSE176308), which contained only synovial fibroblasts. We employed the SingleR and Monocle packages in R to annotate the cells and confirm their types. We also performed pseudotime analysis to further investigate the differentiation trajectory of synovial fibroblasts. This analysis helps reveal dynamic changes during differentiation and provides key insights for understanding the biological characteristics of synovial fibroblasts.

2.6. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis

GO and KEGG enrichment analyses were performed on synovial fibroblast marker genes to identify their functional roles and signaling pathways. Results were filtered using $P < .05$ as the cutoff, and the top 10 biological processes (BP) were plotted in a bar chart. For KEGG analysis, $P < .05$ defined significant enrichment, and the top 10 significant pathways ($P < .05$) were visualized in a bubble plot.

2.7. Statistical analysis

All statistical analyses were performed using R software (version 4.3.3). Differential gene expression analysis among synovial fibroblast clusters was conducted using the Seurat package. For each comparison, *P* values were calculated based on the Wilcoxon rank-sum test and subsequently adjusted for multiple testing using the Benjamini–Hochberg false discovery rate correction. Genes with an adjusted *P* value (adjusted $P < .05$) were considered statistically significant. For GO and KEGG enrichment analyses, *P* values were also adjusted using the Benjamini—Hochberg method, and pathways with adjusted $P < .05$ were regarded as significantly enriched. All statistical tests were 2-sided unless otherwise specified.

3. Results

3.1. Data quality control and filtering results

After performing quality control and filtering on the gene expression microarray dataset GSE176308, a significant correlation was observed between sequencing coverage and gene features. Specifically, as sequencing depth increased, the detected gene features became more abundant (Fig. 1A). The dataset included a total of 10,521 genes. From these, 2000 key genes were identified, including CXCL14, TYROBP, CCL4, CD74, SPP1, CCL4L2, PLA2G2A, CCL3, G0S2, and HLA-DRA (Fig. 1B). Furthermore, each sample's gene expression profiles and sequencing depth were analyzed to assess gene counts per single cell, gene types per single cell, and gene proportions per single cell (Fig. 1C).

3.2. PCA analysis results

In the study of synovial fibroblasts, we identified 20 principal components from the analysis. Statistical analysis ($P < .05$) showed significant differences among these components, making them suitable for further study (Fig. 2A). The PCA plot (PC1 vs PC2) revealed correlations among the components in the synovial fibroblast data (Fig. 2B).We then conducted a detailed analysis of the genes associated with PC1 through PC9. A principal component heatmap was generated to illustrate the distribution and clustering of these genes across the samples (Fig. 2C). The results showed significantly high expression of specific genes in the tissue samples: S100A10 in PC1, ANTXR1 in PC2, MARCKSL1 in PC3, FOS in PC4, HMGA1 in PC5, CTSK in PC6, GSN in PC7, SCARA5 in PC8, and HSPA1A in PC9. PCA revealed that the top principal components captured biologically meaningful transcriptional programs rather than technical noise. Specifically, PC1 was enriched for genes such as S100A10, which are associated with fibroblast activation and inflammatory responses, suggesting that this component reflects inflammation-related transcriptional variability. PC2 and PC3 were dominated by genes such as ANTXR1 and MARCKSL1, which are involved in ECM remodeling and cytoskeletal organization, indicating structural and migratory heterogeneity among synovial fibroblasts. In addition, PCs characterized by genes such as FOS and HSPA1A reflected stress-responsive and immediate-early gene programs. Together, these principal components highlight distinct BP underlying synovial fibroblast heterogeneity in KOA.

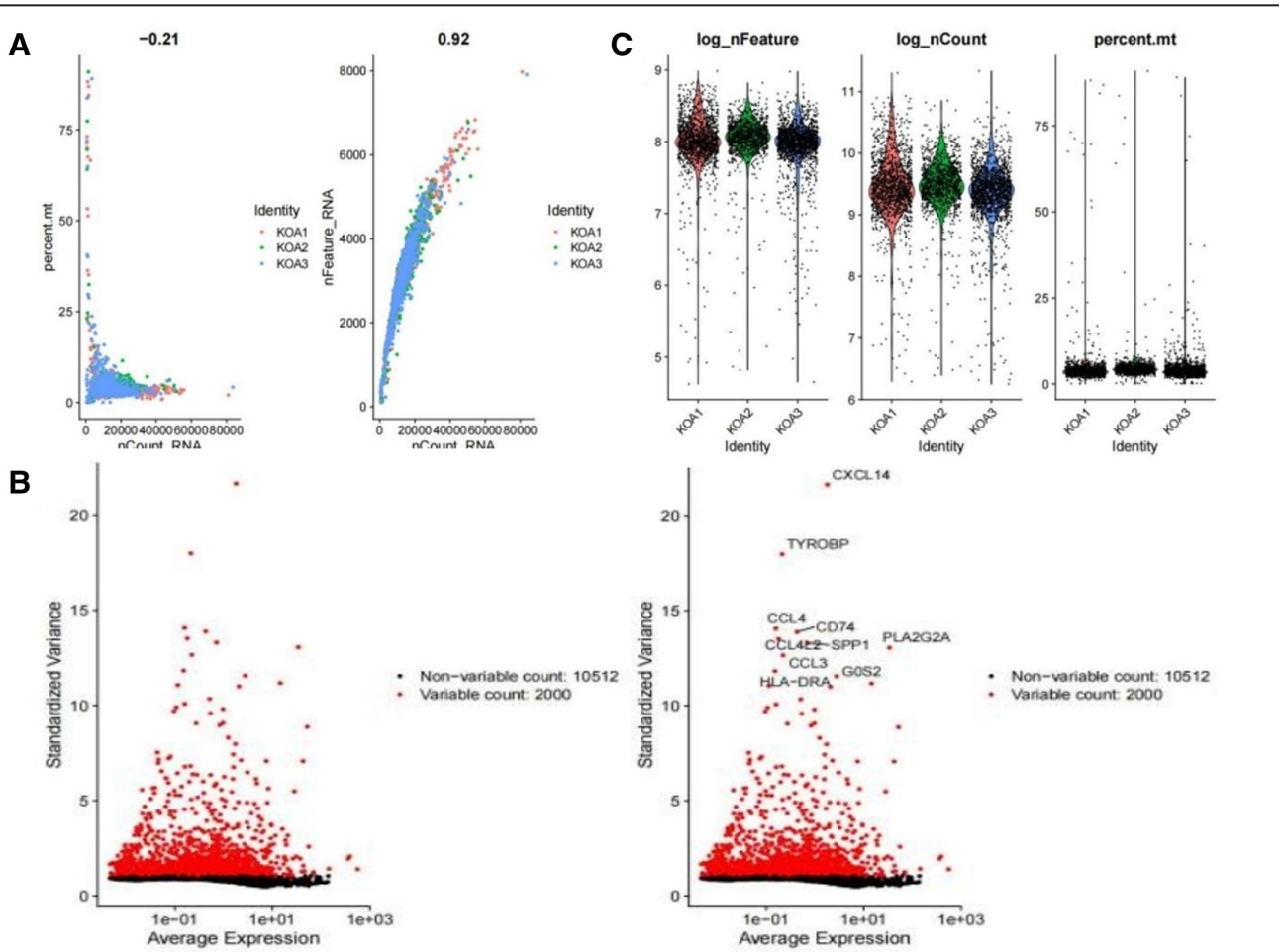

Figure 1. (A) Basic sequencing characteristics and their relationship to depth. (B) Gene expression levels from sequencing. (C) Basic sequencing characteristics and depth. KOA = knee osteoarthritis.

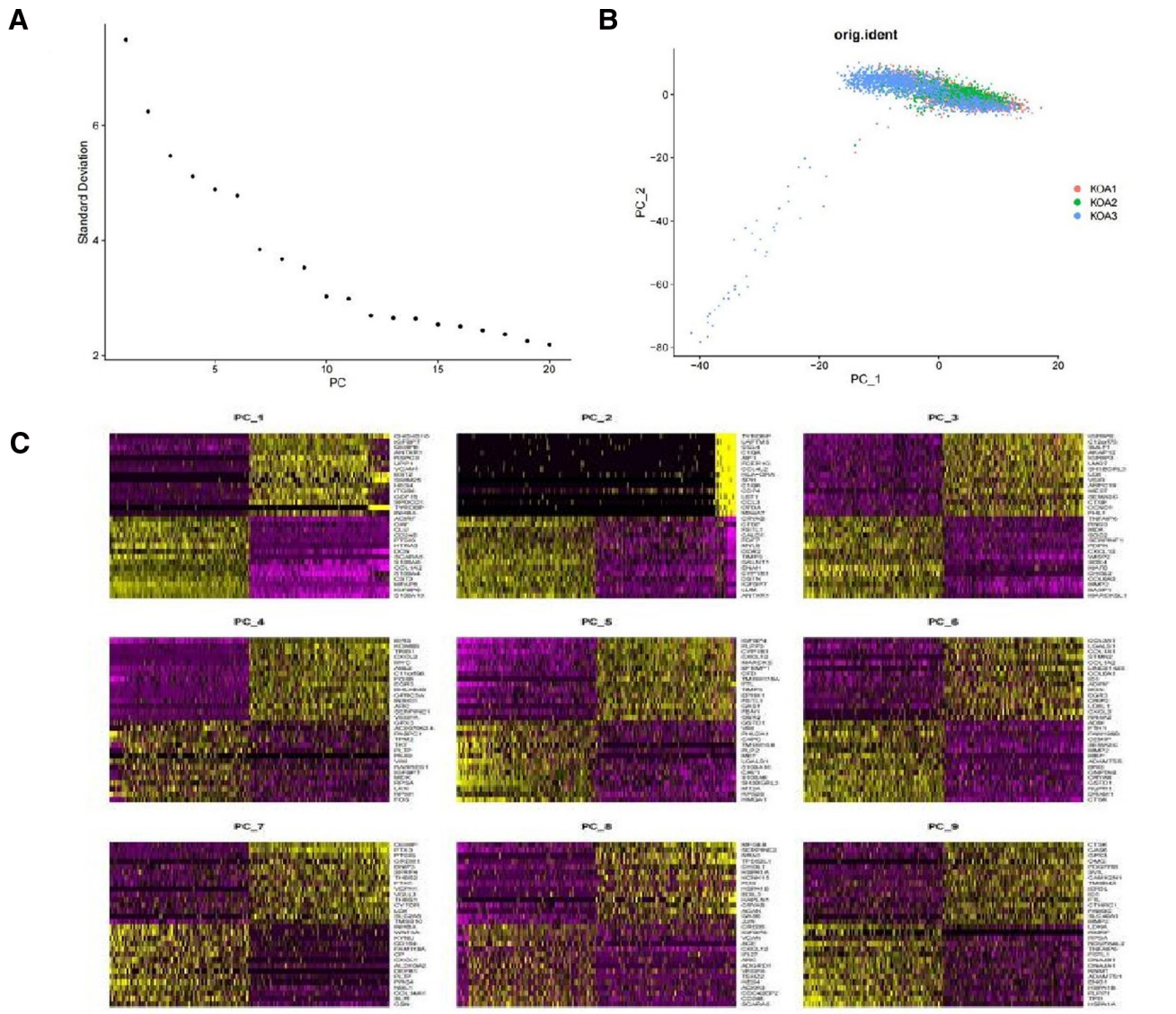

Figure 2. (A) Scree plot of PC. (B) Scatter plot of PC1 vs PC2. (C) Heatmap based on the top 9 PC. KOA = knee osteoarthritis, PC = principal component.

3.3. t-SNE clustering analysis results

We performed t-distributed stochastic neighbor embedding clustering analysis on single-cell transcriptome sequencing data, which revealed 8 distinct cell clusters color-coded to represent different components of synovial fibroblasts (Fig. 3A). Using an adjusted *P* value threshold of .05, differential analysis identified 2991 marker genes. We visualized these genes using a heatmap (Fig. 3B) and a bubble plot (Fig. 3C) to illustrate their expression patterns across the clusters. Additionally, we analyzed the expression and distribution of key genes: SPP1, TYROBP, CD74, CCL4L2, CCL4, CCL3, CCL3L1, APOE, and HLA-DRA. We found that all of these genes were highly expressed across the 8 clusters (SPP1: logFC = 5.96, *P* = .000; TYROBP: logFC = 4.33, *P* = .000; CD74: logFC = 4.33, *P* = .000; CCL4L2: logFC = 4.20, *P* = .000; CCL4: logFC = 4.13, *P* = .000; CCL3: logFC = 3.99, *P* = .000; CCL3L1: logFC = 3.93, *P* = .000; APOE: logFC = 3.64, *P* = .000; HLA-DRA: logFC = 3.62, *P* = .000) (Fig. 3D). t-SNE clustering further demonstrated transcriptional heterogeneity among synovial fibroblasts, identifying 8 distinct clusters. These clusters represent biologically distinct fibroblast subpopulations rather than arbitrary computational groupings, as evidenced by their differential expression of functionally relevant marker genes. Clusters characterized by high expression of SPP1 and TYROBP are indicative of matrix remodeling and osteogenic-associated fibroblast states, whereas clusters enriched for chemokines such as CCL3, CCL4, and CCL4L2 suggest inflammatory and immune-interacting fibroblast phenotypes. The coordinated expression of antigen-presentation–related genes (CD74, HLA-DRA) further supports functional specialization among fibroblast subsets. These results indicate that synovial fibroblasts adopt distinct functional states that may differentially contribute to inflammation, tissue remodeling, and disease progression in KOA.

3.4. Cell annotation and cell trajectory analysis results

The SingleR package in R was used to annotate the 8 identified cell components, revealing that all were synovial fibroblasts, consistent with the study's objectives (Fig. 4A). Pseudotime analysis further revealed 7 differentiation pathways in synovial fibroblasts (Fig. 4B). The synovial fibroblast cluster was homogeneous and the first to differentiate. Literature suggests synovial fibroblasts can differentiate into proliferative, invasive, hypertrophic, and regulatory subtypes under the influence of key regulatory factors, as reported in.[16–18]

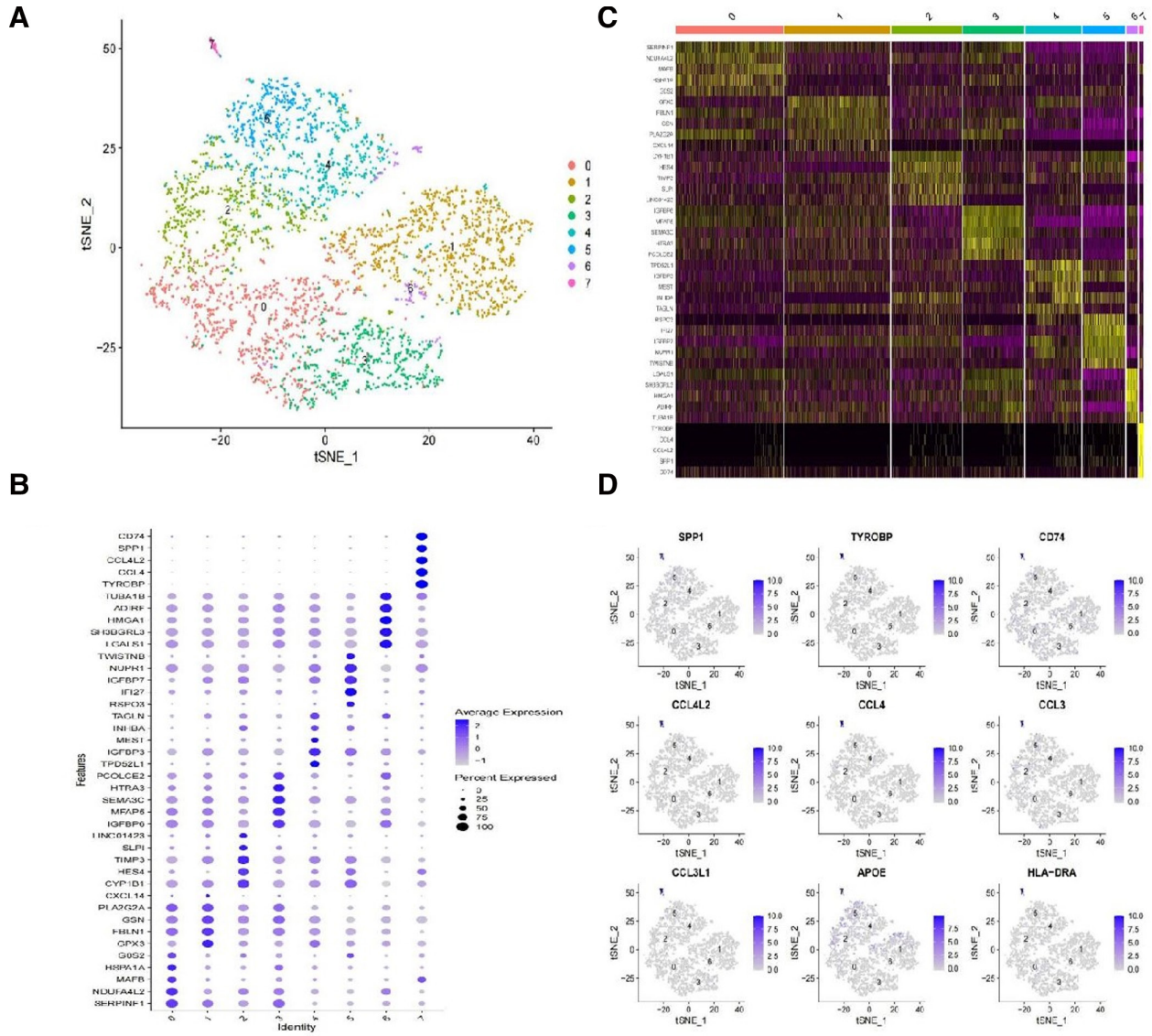

Figure 3. (A) Synovial fibroblast composition distribution. (B) Marker gene bubble plot. (C) Marker gene heatmap. (D) Key gene expression/distribution. t-SNE = t-distributed stochastic neighbor embedding.

3.5. GO and KEGG pathway enrichment analysis results

GO is a standardized framework of gene annotation terms used to identify and classify biological features in high-throughput genomic or transcriptomic data. Using R-based tools, we performed GO enrichment analysis on marker genes and filtered them using a significance cutoff of $P < .05$. These genes were enriched in BP (e.g., vascular regulation, ossification, and wound healing). They also correlated with cellular components, including collagen-rich ECM and endoplasmic reticulum lumen, as well as MF such as glycosaminoglycan binding and heparin binding (Fig. 5A). Furthermore, KEGG analysis showed these genes were enriched in pathways such as ECM-receptor interaction, ribosome, protein digestion and absorption, Pathways in cancer, focal adhesion, actin cytoskeleton regulation, phagosome, and oxidative phosphorylation (Fig. 5B). KEGG pathway analysis revealed enrichment of several pathways with clear biological relevance to KOA. Although the term "Pathways in cancer" was identified, this does not indicate oncogenic transformation in KOA. Rather, this pathway encompasses fundamental cellular processes, including cell proliferation, survival signaling, migration, and ECM remodeling, which are also central to synovial fibroblast activation and synovial hyperplasia observed in osteoarthritis. Importantly, the enrichment of ECM–receptor interaction and focal adhesion pathways highlights the critical role of cell-matrix communication in KOA pathogenesis. These pathways regulate fibroblast adhesion, migration, and mechanotransduction, thereby influencing synovial fibrosis and cartilage degeneration. In addition, pathways related to actin cytoskeleton regulation further support the involvement of cytoskeletal remodeling in fibroblast activation and joint tissue remodeling. Collectively, these enriched pathways provide mechanistic insights into how synovial fibroblasts contribute to inflammation and structural deterioration in KOA.

4. Discussion

KOA is a degenerative disease characterized by chronic inflammatory responses and knee joint damage. As the condition progresses, it can spread from a single joint to affect multiple joints.[19–21] Among various pathogenic mechanisms, the abnormal immune responses in the joint (triggered by cells like B cells, T cells, macrophages, and synovial fibroblasts) are key factors. Chronic inflammation and synovial hyperplasia also contribute to the progression and persistence of osteoarthritis.[22–25] Among

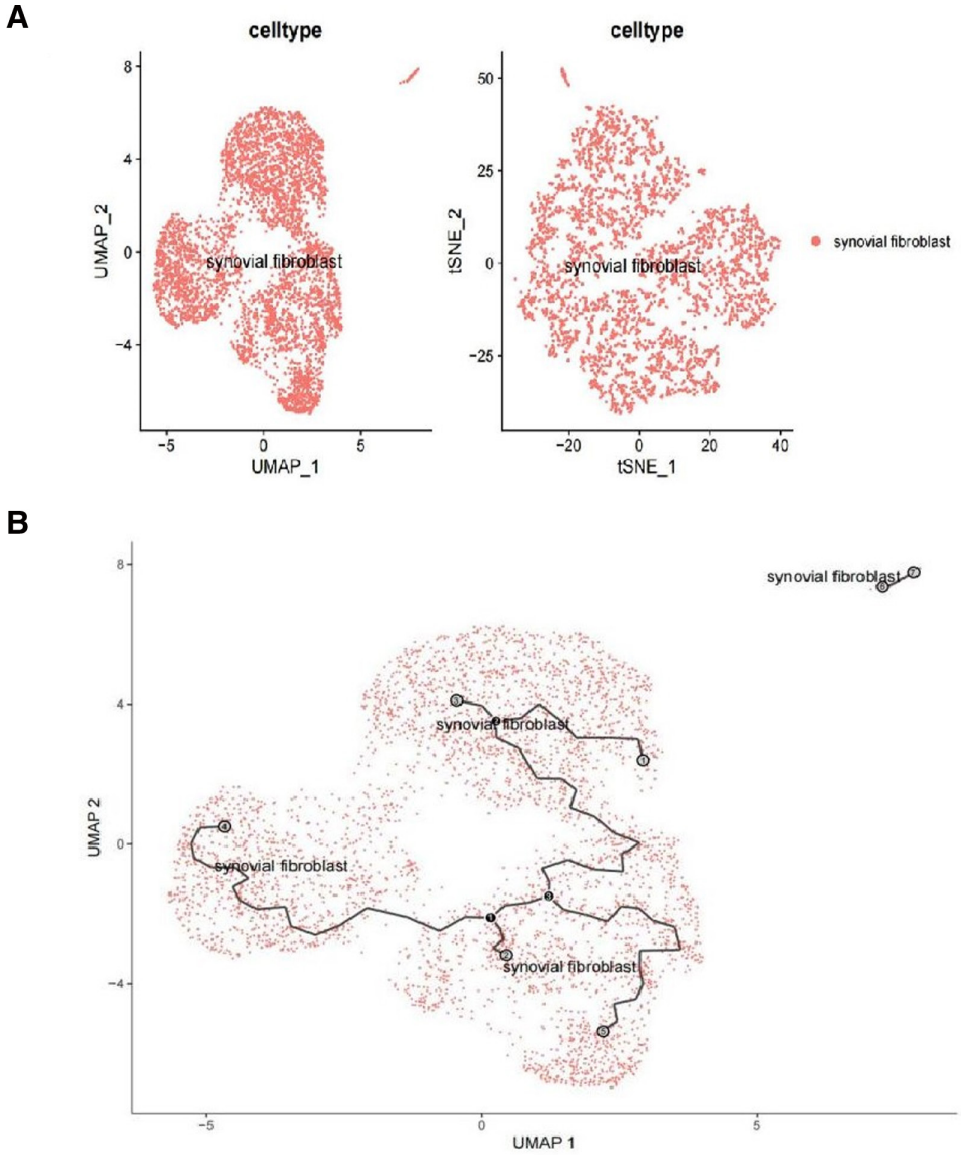

Figure 4. (A) Labeling of synovial fibroblast subpopulations. (B) Differentiation trajectory of synovial fibroblasts.

synovial cells, FLS, which are the most numerous, are regarded as the primary effector cells in synovial physiological and pathological changes. They mediate the adhesion to, invasion of, and degradation of articular cartilage and bone.[25,26] The activation of FLS is mainly reflected in the multilayered structure and hyperplastic characteristics of the synovial tissue, which not only indicates an imbalance between cell proliferation and apoptosis but also involves the migration of these cells into the articular cartilage directly in contact with the lining layer, as well as into distal cartilage and bone.[27] Using single-cell transcriptomics, this study investigates the gene expression and cellular differentiation mechanisms of synovial fibroblasts, aiming to better understand the protective and repair mechanisms of synovial tissue and provide new insights for the treatment of KOA.

This study employed scRNA-seq transcriptome analysis and bioinformatics to identify 10,521 genes expressed in synovial fibroblasts, including 2000 key genes such as SPP1, TYROBP, CD74, CCL4L2, CCL4, CCL3, CCL3L1, APOE, and HLA-DRA. SPP1 is an ECM adhesion molecule involved in bone mineralization, immune response, tumor metastasis, inflammation, and angiogenesis.[28] Single-cell transcriptome analysis revealed several key genes, including SPP1 and TYROBP, that were highly expressed across synovial fibroblast subclusters and closely associated with differentiation trajectories, suggesting their potential involvement in the pathogenesis of KOA. SPP1 (osteopontin) is a multifunctional ECM-associated glycoprotein that plays critical roles in immune regulation, angiogenesis, tissue remodeling, and mineralization. Previous studies have demonstrated that SPP1 expression is significantly upregulated in osteoarthritic cartilage and synovium, where it correlates with angiogenic activity, cellular senescence, and disease severity.[29,30] Notably, recent single-cell studies identified an SPP1^{+} chondrocyte subpopulation with enhanced inflammatory and matrix-remodeling properties in human ostroarthritis cartilage.[29] Consistent with these findings, our results extend the role of SPP1 to synovial fibroblasts, indicating that SPP1 may participate in fibroblast-mediated ECM remodeling and inflammatory amplification within the synovial microenvironment during KOA progression. TYROBP (DAP12) is an immune adaptor protein containing an immunoreceptor tyrosine-based activation motif and is essential for signal transduction in innate immune cells, particularly macrophages and osteoclasts.[31,32] TYROBP-mediated signaling has been shown to regulate immune cell activation, polarization, and survival, thereby contributing to chronic inflammatory responses.[33,34]

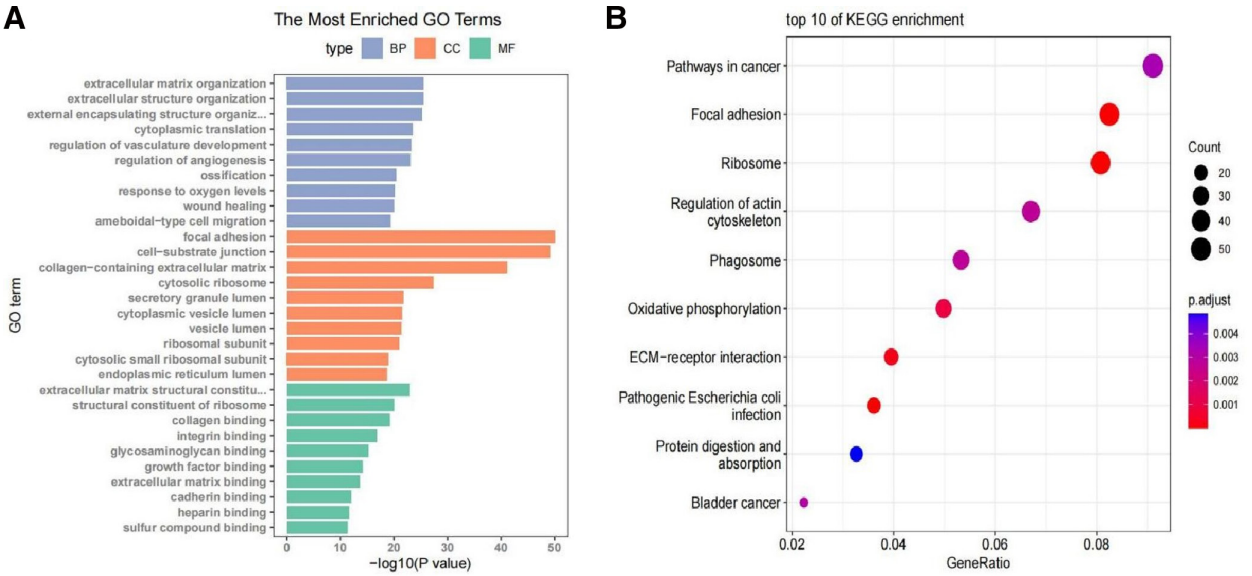

Figure 5. (A) GO enrichment analysis. (B) KEGG enrichment analysis. ECM = extracellular matrix, GO = gene oncology, KEGG = Kyoto encyclopedia of genes and genomes.

In osteoarthritic joints, synovial macrophage–fibroblast crosstalk is a key driver of synovitis and cartilage degeneration. Our identification of TYROBP as a highly expressed gene in synovial fibroblast populations suggests a previously underappreciated role for immune-related signaling pathways in fibroblast differentiation and function. This finding aligns with prior bioinformatics and immunological studies implicating TYROBP-associated pathways in inflammatory joint diseases,[35,36] while further highlighting synovial fibroblasts as active participants in immune modulation rather than passive structural cells. Taken together, these observations suggest that key genes such as SPP1 and TYROBP may act as molecular hubs linking synovial fibroblast differentiation, immune activation, and ECM remodeling in KOA. Compared with previous bulk transcriptomic studies, our single-cell approach provides higher-resolution evidence that these genes are broadly expressed across fibroblast subpopulations and are dynamically involved in differentiation trajectories. This not only corroborates existing literature but also offers new insights into the cell type-specific mechanisms underlying synovial pathology in KOA, thereby supporting their potential value as therapeutic targets. CD74, a type II transmembrane protein, is essential for macrophage migration inhibitory factor in activating NF-κB downstream signaling receptors and regulating bone mass in mice.[37] Other studies indicate that interleukin-1β treatment upregulates HLA-DRA expression in normal human chondrocytes.[38] Additionally, CCL4 regulates chondrocyte apoptosis and ROS levels during KOA progression.[39]

GO enrichment analysis revealed that genes in synovial fibroblasts are associated with multiple BP, including vascular regulation, ossification, and wound healing; cellular components such as the collagen-containing ECM and the endoplasmic reticulum lumen; as well as MF like heparin binding and glycosaminoglycan binding. Among these, the development of synovial fibroblasts and connective tissue is vital for synovial repair, while joint degeneration is closely related to ECM degradation.[7] Chondrocytes are the predominant cells in articular cartilage and contribute to cartilage maintenance.[40] Heparin-binding secretory peptides enhance chondrocyte aggregation and proliferation by stimulating ECM synthesis, reducing matrix metalloproteinase activity, and inducing inhibitor production, thereby promoting cartilage formation.[41] Proteoglycans with attached glycosaminoglycan chains are present on chondrocyte surfaces and within the surrounding cartilage matrix, allowing these molecules to mediate cell-cell and cell-matrix interactions.[42,43] This study offers novel insights into synovial protection and repair from cellular, biological, and molecular perspectives.

KEGG analysis revealed its association with signaling pathways such as cancer-related pathways, ribosome, protein digestion and absorption, actin cytoskeleton regulation, phagosome, focal adhesion, ECM-receptor interaction, and oxidative phosphorylation. Among these, the ECM is a key regulator of tissue structure and homeostasis. The ECM delivers both chemical and mechanical signals to cells, regulating cellular phenotypes and responses to coordinate tissue function. Matrix molecules interconnect to form a functional network that dynamically interacts with cell surface receptors on resident cells. Cell-matrix interactions are vital for cellular and tissue function, and the development of various diseases, including fibrosis and osteoarthritis, is linked to ECM receptors.[44,45] Moreover, studies indicate that astragaloside IV enhances chondrocyte proliferation by upregulating ECM-receptor interaction, thereby preventing articular cartilage degeneration.[46] Focal adhesion bridges the cytoskeleton and ECM, playing a key role in cell signaling through integrin-mediated cell-ECM adhesion, orchestrating mechanotransduction, cell migration, cell cycle progression, proliferation, differentiation, growth, and repair.[47,48] Meanwhile, ribosomes are involved in biogenesis, assembly, maturation, and translation processes, which may enhance chondrocyte repair.[49,50] The enrichment of cancer-related signaling modules further underscores that synovial fibroblasts in KOA share invasive and hyperplastic features with activated fibroblasts in other chronic inflammatory diseases, supporting the concept of fibroblast-driven tissue remodeling rather than malignant transformation. Furthermore, in KOA, cytoskeletal integrity drives KOA progression, characterized by excessive apoptosis and senescence of chondrocytes.[51]

This study used single-cell transcriptome sequencing analysis combined with bioinformatics techniques to explore the mechanisms underlying the differentiation of synovial fibroblasts in KOA. The findings revealed that synovial fibroblasts can differentiate into various types and possess multiple differentiation pathways. Regulating key genes (SPP1, TYROBP, CD74, CCL4L2, CCL4, CCL3, CCL3L1, APOE, and HLA-DRA) can intervene in the differentiation of synovial fibroblasts, which plays a crucial role in protecting and repairing the synovium. However, several limitations of this study should be

acknowledged. First, the analysis was based on a single publicly available scRNA-seq dataset comprising only 3 KOA samples, which may limit the robustness and generalizability of the results. Second, the findings were not experimentally validated, and thus causal relationships between the identified marker genes, pathways, and synovial fibroblast differentiation cannot be definitively established.

Future studies should aim to validate these findings in larger, independent KOA cohorts, including scRNA-seq datasets with increased sample size and clinical heterogeneity. Integration of multicenter datasets may further improve statistical power and generalizability. In addition, experimental validation using in vitro and in vivo models (such as gene perturbation, immunohistochemistry, and functional assays) will be essential to confirm the roles of key genes (e.g., SPP1, TYROBP, and CD74) and pathways in synovial fibroblast activation and KOA progression. Such efforts will help translate single-cell discoveries into clinically relevant biomarkers and therapeutic targets.

Author contributions

Conceptualization: Kaiwei Zhang.
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Investigation: Ji Fei.
Methodology: Haozhu Chen.
Supervision: Kaiwei Zhang.
Validation: Ji Fei.
Writing – original draft: Haozhu Chen.
Writing – review & editing: Haozhu Chen, Kaiwei Zhang.

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