Follow us @BoneJointRes

Freely available online OPEN ACCESS

BJR

CARTILAGE

Molecular crosstalk between articular cartilage, meniscus, synovium, and subchondral bone in osteoarthritis

M. Wang,
G. Tan,
H. Jiang,
A. Liu,
R. Wu,
J. Li,
Z. Sun,
Z. Lv,
W. Sun,
D. Shi

From Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China

Aims

Osteoarthritis (OA) is a common degenerative joint disease worldwide, which is characterized by articular cartilage lesions. With more understanding of the disease, OA is considered to be a disorder of the whole joint. However, molecular communication within and between tissues during the disease process is still unclear. In this study, we used transcriptome data to reveal crosstalk between different tissues in OA.

Methods

We used four groups of transcription profiles acquired from the Gene Expression Omnibus database, including articular cartilage, meniscus, synovium, and subchondral bone, to screen differentially expressed genes during OA. Potential crosstalk between tissues was depicted by ligand-receptor pairs.

Results

During OA, there were 626, 97, 1,060, and 2,330 differentially expressed genes in articular cartilage, meniscus, synovium, and subchondral bone, respectively. Gene Ontology enrichment revealed that these genes were enriched in extracellular matrix and structure organization, ossification, neutrophil degranulation, and activation at different degrees. Through ligand-receptor pairing and proteome of OA synovial fluid, we predicted ligand-receptor interactions and constructed a crosstalk atlas of the whole joint. Several interactions were reproduced by transwell experiment in chondrocytes and synovial cells, including *TNC-NT5E*, *TNC-SDC4*, *FN1-ITGA5*, and *FN1-NT5E*. After lipopolysaccharide (LPS) or interleukin (IL)-1β stimulation, the ligand expression of chondrocytes and synovial cells was upregulated, and corresponding receptors of co-culture cells were also upregulated.

Conclusion

Each tissue displayed a different expression pattern in transcriptome, demonstrating their specific roles in OA. We highlighted tissue molecular crosstalk through ligand-receptor pairs in OA pathophysiology, and generated a crosstalk atlas. Strategies to interfere with these candidate ligands and receptors may help to discover molecular targets for future OA therapy.

Cite this article: *Bone Joint Res* 2022;11(12):862–872.

Keywords: Osteoarthritis, Crosstalk, Transcriptome, Differentially expressed genes, Ligand-receptor

Article focus

- To identify differentially expressed genes in articular cartilage, meniscus, synovium, and subchondral bone during osteoarthritis.
- To clarify molecular crosstalk of different tissues.
- To investigate ligand-receptor pair findings of transcriptional analysis.

Key messages

- There were 626, 97, 1,060, and 2,330 genes differentially changed in articular cartilage, meniscus, synovium, and subchondral bone, respectively.
- Potential ligand-receptor interactions of different tissues were constructed through ligand-receptor pairings.

Correspondence should be sent to Dongquan Shi; email: shidongquan@nju.edu.cn

doi: 10.1302/2046-3758.1112.BJR-2022-0215.R1

Bone Joint Res 2022;11(12):862–872.

- Several interactions were reproduced in chondrocytes and synovial cells, including *TNC-NT5E*, *TNC-SDC4*, *FN1-ITGA5*, and *FN1-NT5E*.

Strengths and limitations

- Transcriptome analysis gives a lot of information on messenger RNA (mRNA) level.
- We established a molecular crosstalk atlas of the whole joint.
- The study was limited by the sensitivity and accuracy of the microarray and the number of samples; some low differentially expressed genes have functions which may not be screened.

Introduction

Osteoarthritis (OA) is the most common degenerative joint disease, which causes joint pain, stiffness, swelling, and limited functions.[1-3] Nearly half of people over the age of 60 years suffer from OA, which is the leading contributor to disability, while many risk factors have been associated with OA including genetic variation, ageing, sex, obesity, and injury.[4] With the process of global ageing and obesity, the burden of OA continues to increase,[5] aand there is also a lack of effective drugs to alleviate the disease.[6] OA mainly involves the hip and knee, affecting multiple tissues, and the biological and physical crosstalk between these tissues is very important. However, little is known of the crosstalk between tissues during the pathogenesis and progression of OA.

Knee OA is considered a disease of the whole joint, which involves articular cartilage, meniscus, ligament, tendon, synovium, and subchondral bone.[7] Although the main characteristic of OA is articular cartilage abrasion,[8] other tissues are also contributors to the progress of the disease. Destruction of articular cartilage releases matrix metallopeptidases (MMPs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs), and interleukins (ILs),[9] which destroy the balance of synthesis and decomposition of the extracellular matrix and further aggravate the loss of cartilage. Lesions of meniscus, inflammation of synovium, and remodelling of subchondral bone accelerate the progress of joint degeneration.[10-12] The inflammatory synovium secretes cytokines (tumour necrosis factor (TNF)-α, IL-1β),[13] which pass through synovial fluid to cartilage, and further induce the imbalance of cartilage extracellular matrix.[14] Sclerosis of subchondral bone and vascular invasion lead to the release of IL-6, transforming growth factor-β1 (TGF-β1), MMP-13, and vascular endothelial growth factor (VEGF), and diffusion of these molecules further affected articular cartilage.[15] There is still a big challenge to decipher functional interactions of tissues. Recent studies have revealed that tissue crosstalk plays a major role in the pathogenesis of OA, but the results are limited to two tissues such as bone-cartilage units,[16,17] or cartilage and synovium.[18] There is still a lack of research on crosstalk in multiple tissues.

Here, we integrated transcription profiles of articular cartilage, meniscus, synovium, and subchondral bone and proteomic data of OA synovial fluid to identify potential molecular crosstalk within and between these tissues during OA, and have provided insights for future OA diagnosis and treatment.

Methods

Transcriptome sources and quality control. We first prioritized transcriptome data, which used the same chip manufacturer or model. Transcription profiles were acquired from the Gene Expression Omnibus (GEO) database, including articular cartilage (GSE169077, normal control (NC) = 5, OA = 6), meniscus (GSE19060, NC = 3, OA = 5),[19] synovium (GSE82107, NC = 7, OA = 10),[20] and subchondral bone (GSE51588, NC = 10, OA = 40),[21] of which most of the OA tissues were at end stage of the disease (Supplementary Table i). After raw data were normalized by Robust Multiarray Average (RMA) with affy (v1.68.0; RStudio, USA) R package, probe names were converted into gene names for which the expression levels were calculated as the mean values. Articular cartilage (AC), meniscus (M), synovium (S), and subchondral bone (SB) obtained 12,399, 20,161, 20,161, and 15,248 unique genes, respectively. Cluster dendrogram and principal component analysis (PCA) were performed by hclust function, FactoMineR (v2.4, RStudio) and factoextra (v1.0.7, RStudio) with R packages.

Identification and analysis of differentially expressed genes. Differentially expressed genes of four tissues were filtered by the same criteria, which were 1.5-fold expression difference and p-value < 0.05. Volcano plot and heatmap were constructed by ggplot2 (v3.3.5, RStudio) and pheatmap (v1.0.12, RStudio), separately. Venn diagram was plotted by VennDiagram (v1.7.0, RStudio). Differentially expressed genes were further analyzed by Gene Ontology (GO) in terms of molecular function, cellular component, and biological process.

Construction of potential ligand-receptor interaction network. Among these differentially expressed genes, secretory ligands and corresponding receptors of respective tissues were screened by ligand-receptor database[22] based on the FANTOM5 project.[23] Firstly, ligand-receptor pairings within and between articular cartilage, meniscus, synovium, and subchondral bone were counted by RCircos (v1.2.1, RStudio). Then, potential ligand and receptor interaction networks were constructed by Cytoscape (v3.9.0, Cytoscape Team, USA). We further screened the ligands that can be secreted into synovial fluid through proteomic data of OA synovial fluid,[24] and drew the interaction map using Adobe Illustrator (Adobe, USA).

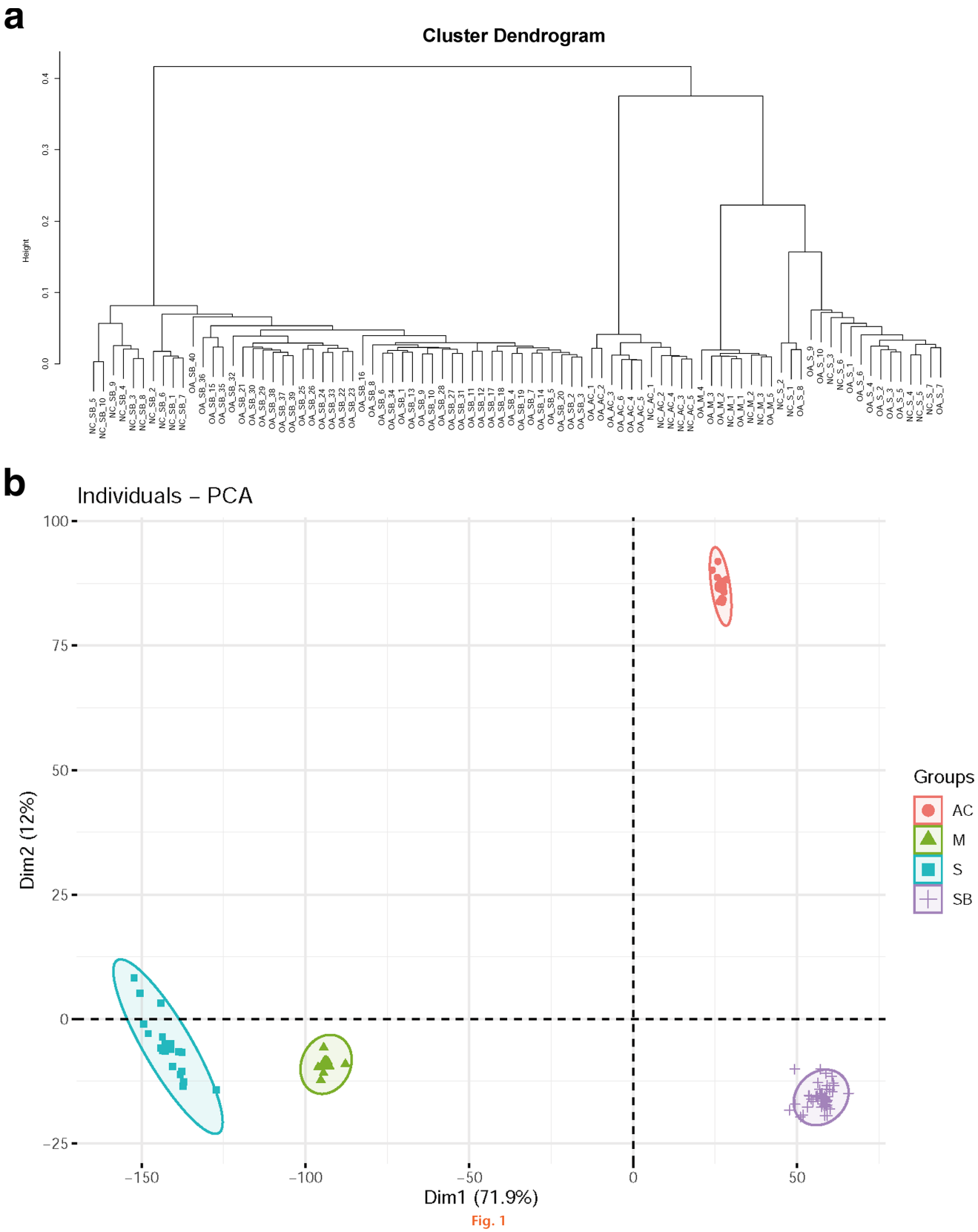
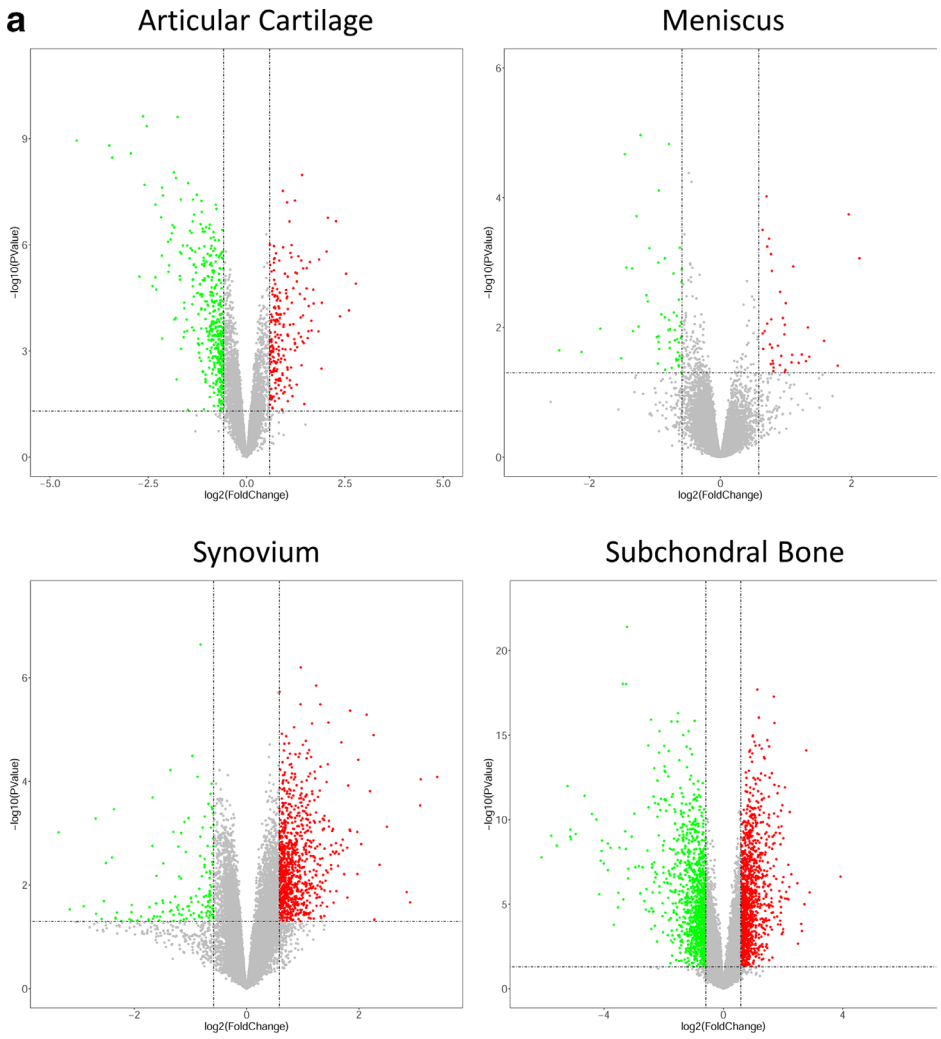


Fig. 1

Quality control of all transcriptional data. a) Cluster dendrogram and b) principal component analysis (PCA) of normal and osteoarthritis articular cartilage, meniscus, synovium, and subchondral bone. The red circle represents articular cartilage, the green triangle represents meniscus, the cyan square represents synovium, and the purple plus sign represents subchondral bone. AC, articular cartilage; M, meniscus; NC, negative control; S, synovium; SB, subchondral bone.



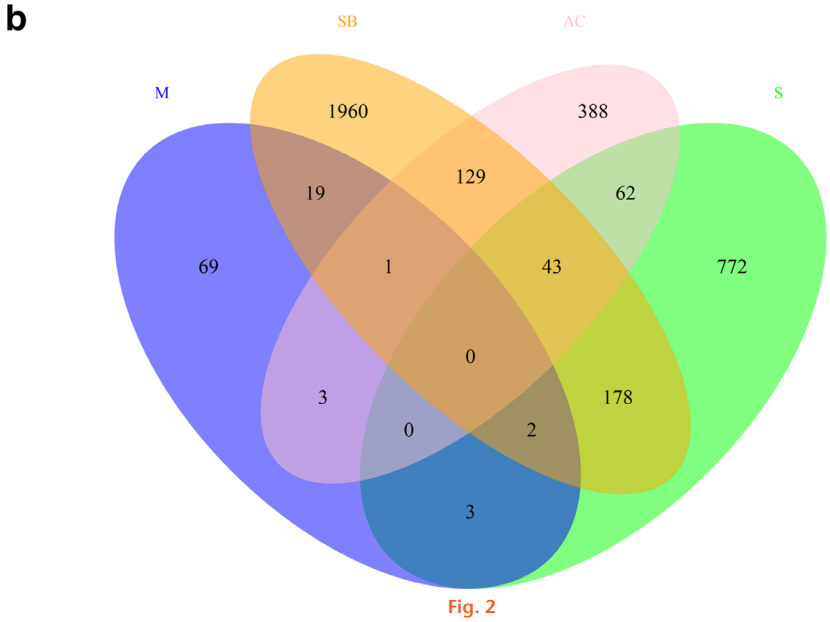
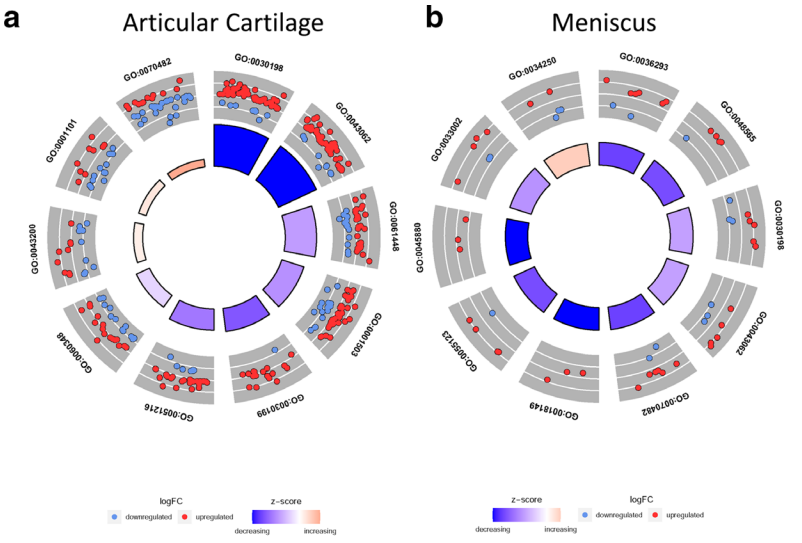


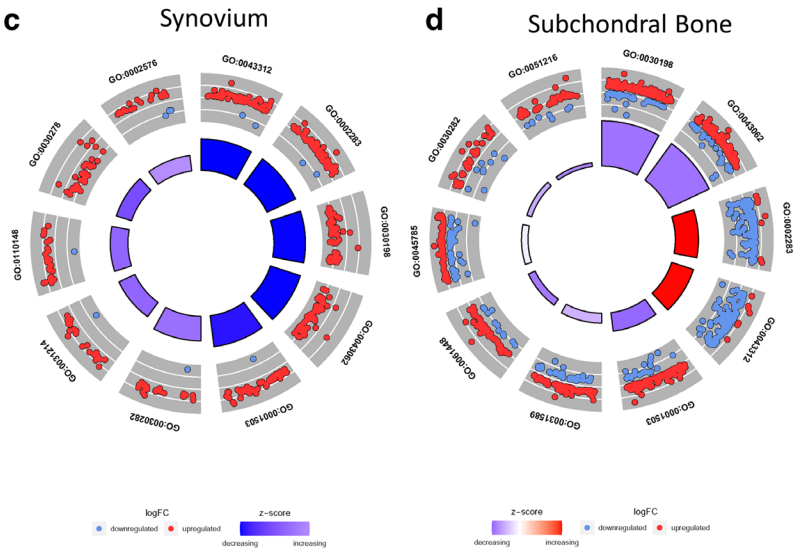
Fig. 2

Statistics of differentially expressed genes in all tissues. a) Volcano plot. Red dots indicate upregulated genes and green dots indicate downregulated genes. b) Venn diagram of differentially expressed genes in the four tissues. AC, articular cartilage; M, meniscus; S, synovium; SB, subchondral bone.



ID	Description
GO:0030198	extracellular matrix organization
GO:0043062	extracellular structure organization
GO:0061448	connective tissue development
GO:0001503	ossification
GO:0030199	collagen fibril organization
GO:0051216	cartilage development
GO:0060348	bone development
GO:0043200	response to amino acid
GO:0001101	response to acid chemical
GO:0070482	response to oxygen levels

ID	Description
GO:0036293	response to decreased oxygen levels
GO:0048565	digestive tract development
GO:0030198	extracellular matrix organization
GO:0043062	extracellular structure organization
GO:0070482	response to oxygen levels
GO:0018149	peptide cross-linking
GO:0055123	digestive system development
GO:0045880	positive regulation of smoothened signaling pathway
GO:0033002	muscle cell proliferation
GO:0034250	positive regulation of cellular amide metabolic process



ID	Description
GO:0043312	neutrophil degranulation
GO:0002283	neutrophil activation involved in immune response
GO:0030198	extracellular matrix organization
GO:0043062	extracellular structure organization
GO:0001503	ossification
GO:0030282	bone mineralization
GO:0031214	biomineral tissue development
GO:0110148	biomineralization
GO:0030278	regulation of ossification
GO:0002576	platelet degranulation

ID	Description
GO:0030198	extracellular matrix organization
GO:0043062	extracellular structure organization
GO:0002283	neutrophil activation involved in immune response
GO:0043312	neutrophil degranulation
GO:0001503	ossification
GO:0031589	cell-substrate adhesion
GO:0061448	connective tissue development
GO:0045785	positive regulation of cell adhesion
GO:0030282	bone mineralization
GO:0051216	cartilage development

Fig. 3

Gene enrichment of biological process in a) articular cartilage, b) meniscus, c) synovium, and d) subchondral bone. The outer layer is marked with the IDs of Gene Ontology (GO). The red and blue dots in the middle layer represent upregulated and downregulated genes, respectively. The sector in the inner layer represents *z*-score, which indicates the significance of gene enrichment. The following table shows the specific GO ID description.

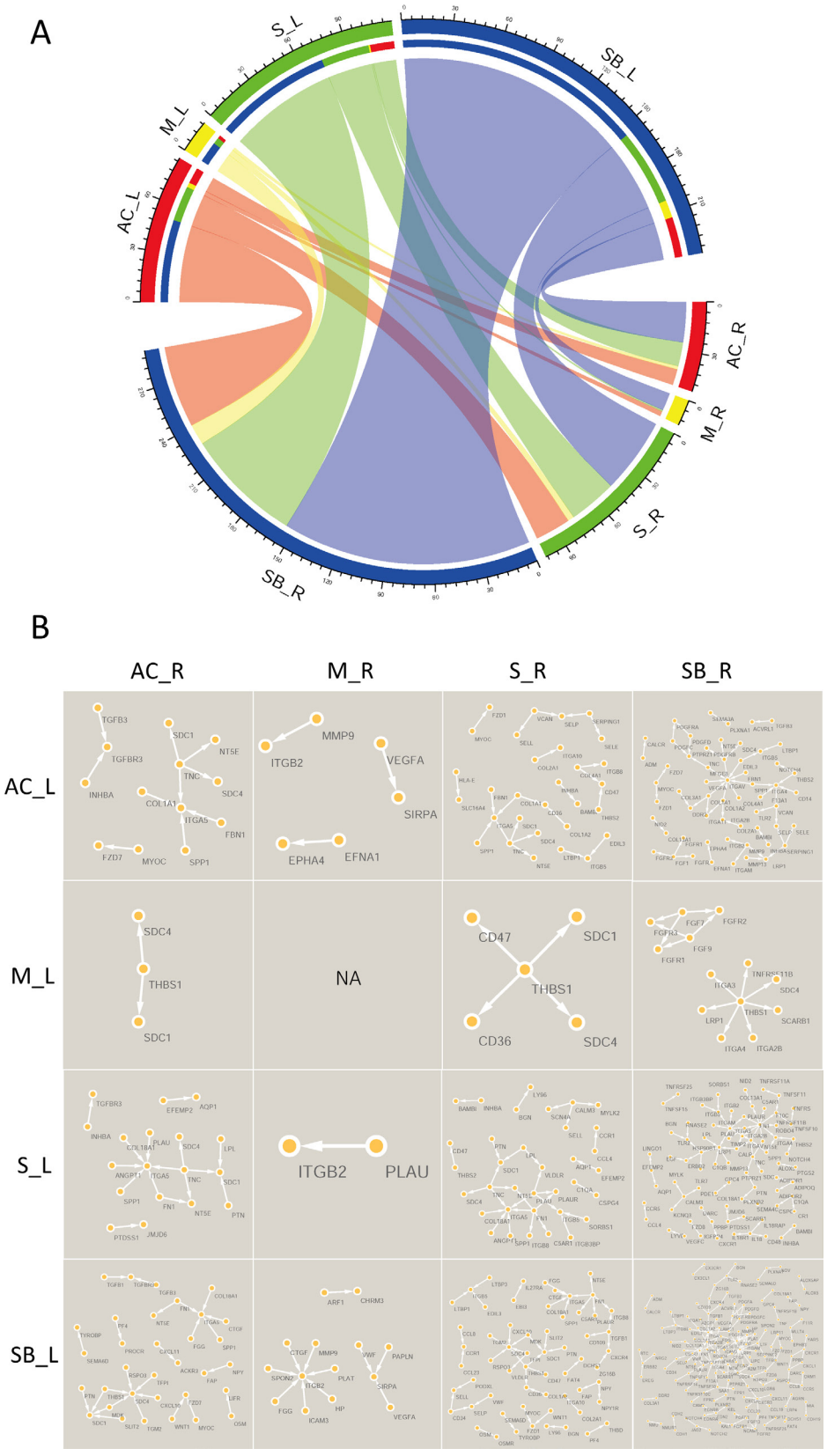


Fig. 4

Ligand-receptor pairings in different tissues. a) Statistics of ligand-receptor pairing in each tissue. Red represents articular cartilage (AC), yellow represents meniscus (M), green represents synovium (S), and blue represents subchondral bone (SB). b) Network of ligand and receptor interactions in four tissues. L, ligand; R, receptor; N/A, not applicable.

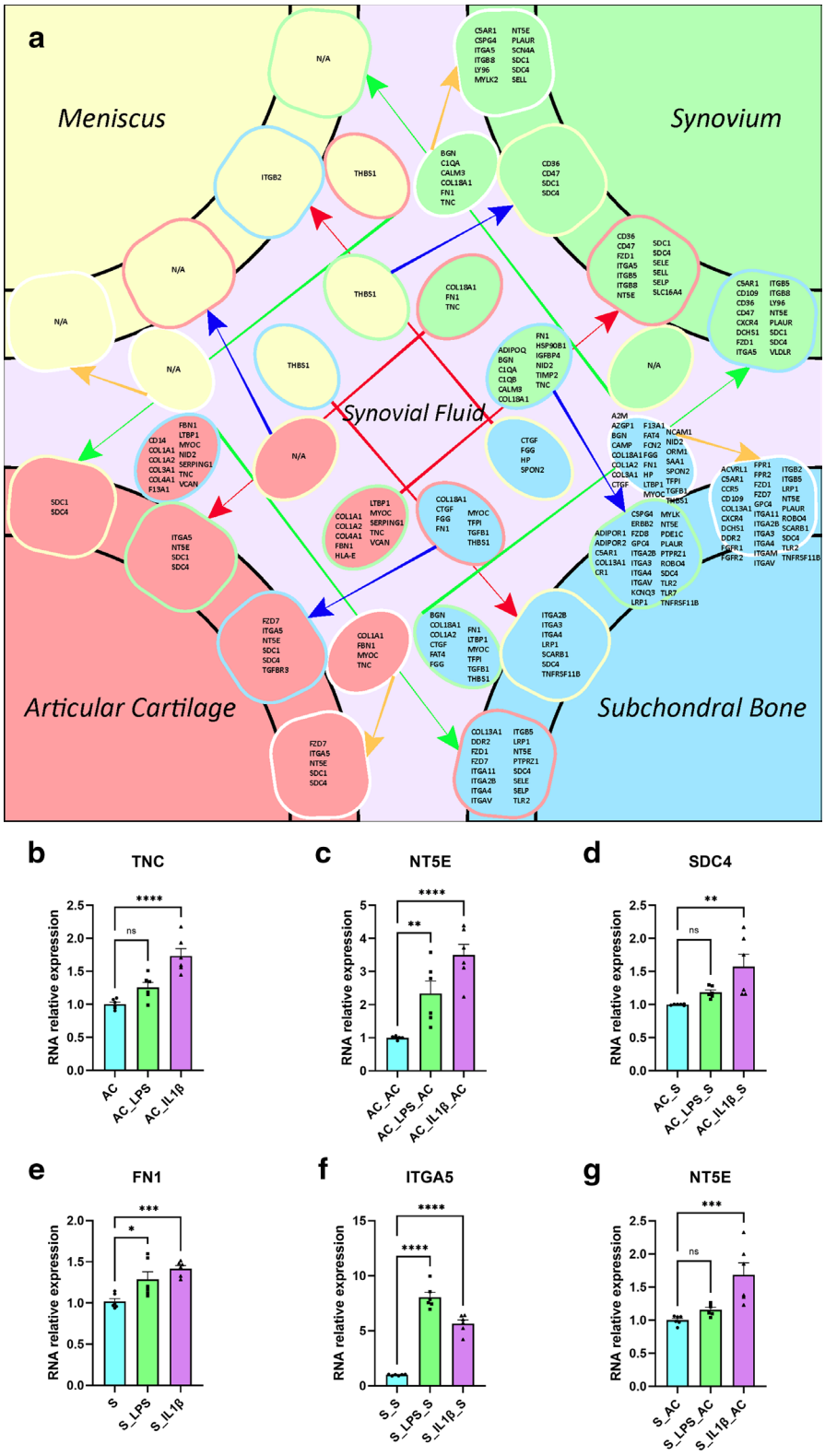


Fig. 5

Potential molecular crosstalk within and between tissues through synovial fluid. a) Crosstalk atlas of the whole joint during osteoarthritis (OA). Ellipses represent ligands and squares represent receptors. N/A, not applicable. b) and e) Relative expression of tenascin-C (TNC) in chondrocytes and fibronectin 1 (FN1) in synovial cells after lipopolysaccharide (LPS) and interleukin (IL)-1β stimulation. c) and d) Relative expression of NT5E in chondrocytes and SDC4 in synovial cells after co-culture of LPS and IL-1β stimulated chondrocytes. f) and g) Relative expression of ITGA5 in synovial cells and NT5E in chondrocytes after co-culture of LPS and IL-1β stimulated synovial cells. Data are presented as the mean (standard error of the mean (SEM)). *Statistical significance; ns, no statistical significance. AC, articular cartilage; M, meniscus; N/A, not applicable; S, synovium; SB, subchondral bone.

Reproduction of ligand-receptor interaction. Human articular cartilage and synovium samples were collected from two male OA patients (aged 63 and 75 years) who underwent total knee arthroplasty. Cartilage and synovium samples were dissociated with 0.2% collagenase II and I (Gibco, Thermo Fisher Scientific, USA), separately. After incubation for six hours at 37°C, cells were filtered and cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Thermo Fisher Scientific, USA) with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin-streptomycin (Gibco). Next, 2×10^5 cells were seeded into each well in a 12-well plate and transwell plate. Chondrocytes and synovial cells in transwell plate were stimulated with 10 ng/ml of IL-1β (Proteintech, Fisher Scientific, USA) and 100 ng/ml of lipopolysaccharide (LPS) (Millipore Sigma, USA) for 12 hours, respectively, and non-stimulated cells were used as controls. Stimulated and non-stimulated chondrocytes in transwell plate were co-cultured with chondrocytes and synovial cells in 12-well plate, respectively. Stimulated and non-stimulated synovial cells in transwell plate were co-cultured with synovial cells and chondrocytes in a 12-well plate, respectively. After 48 hours of co-culture, the RNAs of all cells in the 12-well plate were extracted with RNA-quick Purification Kit (ES Science, China), and reverse-transcribed by HiScript III RT SuperMix for qPCR (Vazyme, China). Quantitative polymerase chain reaction (qPCR) was conducted with ChamQ SYBR Color qPCR Master Mix (Vazyme), and performed on LightCycler480 (Roche, Switzerland).

Statistical analysis. All data are shown as means (standard error of the mean (SEM)), and statistical analysis was performed by independent-samples *t*-tests on GraphPad Prism 9 (GraphPad Software, USA).

Results

Transcriptome data process and analysis. We produced a boxplot which showed that the signal levels of all raw data were uneven, especially in articular cartilage. After RMA normalization, probe signals were uniformed, and the medians were displayed at the same level (Supplementary Figure a). Cluster dendrogram and PCA analysis showed that articular cartilage (AC), meniscus (M), synovium (S), and subchondral bone (SB) were clustered separately, which indicates that the source of these tissue samples was reliable (Figures 1a and 1b). Normal and OA articular cartilage were gathered respectively, as were the meniscus and subchondral bone (Figure 1a).

There were 626, 97, 1,060, and 2,330 differentially expressed genes in AC, M, S, and SB of normal versus OA samples (Figure 2a). We produced a volcano plot which showed that the numbers of upregulated and downregulated genes in M and SB were relatively average, while there were more downregulated genes in OA AC tissues (N_up = 221, N_down = 405) and more upregulated genes in OA S tissues (N_up = 923, N_down = 137, Figure 2a). In addition, the number of differentially expressed genes in meniscus was the lowest (N = 97), while this was highest in subchondral bone (N = 2,330). There were no common differentially expressed genes in four tissues, but there were several common genes in three or two tissues (Figure 2b). For example, 43 genes were changed in AC, S, and SB tissues, which indicates that they may have similar functions in different tissues during OA.

Gene Ontology enrichment in different tissues. GO biological process analysis showed that genes in AC were mostly enriched in extracellular matrix and structure organization, connective tissue development, and ossification (Figure 3a). Genes in M were mostly enriched in response to decreased oxygen levels, digestive tract development, extracellular matrix, and structure organization (Figure 3b). Genes in S were mostly enriched in neutrophil degranulation, neutrophil activation involved in immune response, and extracellular matrix and structure organization (Figure 3c). Genes in SB were mostly enriched in extracellular matrix and structure organization, neutrophil degranulation, and neutrophil activation involved in immune response (Figure 3d). These results were in line with cartilage destruction, synovial inflammation, and subchondral bone sclerosis in OA pathology. Interestingly, neutrophil degranulation and neutrophil activation involved in immune response enriched genes in S and SB showed opposite expression trends during OA, as most genes in S have increased expression, while most genes in SB have decreased expression (Figures 3c and 3d). Also, extracellular matrix and structure organization enriched genes displayed different patterns in AC, S, and SB, as all genes in S had increased expression while some genes in AC and SB had decreased expression (Figures 3a, 3c, and 3d). This indicates that cartilage, synovium, and subchondral bone may play different roles in the OA process. In terms of cellular component, genes in AC, S, and SB were all enriched in collagen-containing extracellular matrix, while genes in M were enriched in postsynaptic specialization (Supplementary Figure b). In molecular function, genes in AC, S, and SB were all enriched in extracellular matrix structural constituent, while genes in M were enriched in amide binding (Supplementary Figure b).

Construction of potential ligand-receptor interaction network. Firstly, we filtered differentially expressed ligands and receptors in each tissue through the ligand-receptor database. There were 55 ligands and 23 receptors with altered expression in AC during OA, seven ligands and nine receptors in M, 64 ligands and 51 receptors in S, and 148 ligands and 152 receptors in SB. For these ligands and receptors, we gathered statistics on each tissue according to their pairings (Figure 4a). The results showed many potential molecular crosstalks within and between tissues (Figure 4b, Supplementary Table ii). For comparison within each tissue, subchondral bone had the most pairings, including extracellular matrix proteins (collagen type I alpha 2 chain (COL1A2), collagen type II alpha 1 chain (COL2A1), fibronectin 1 (FN1)), while meniscus had no pairings. For comparison between tissues, ligands in articular cartilage and meniscus

had the most pairings with receptors in subchondral bone, including collagens, MMPs, and fibroblast growth factors (FGFs). Ligands in synovium had the most pairings with receptors in articular cartilage and subchondral bone, including extracellular matrix related proteins (tenascin-C (TNC), FN1). Ligands in subchondral bone had the most pairings with receptors in articular cartilage and synovium, including inflammation related proteins (TGF-β1, thrombospondin 1 (THBS1)).

Reproduction of tissue crosstalk in chondrocytes and synovial cells. Using the proteomic data of OA synovial fluid,[24] we screened the proteins that can be secreted into synovial fluid and their paired receptors (Figure 5a). FN1, TNC, and TGF-β1 may play central roles in the communication of various tissues during OA. FN1 and TNC could bind integrins to activate p38 mitogen-activated protein kinase (MAPK) signalling pathway, and TGF-β1 could bind TGFBRs to activate NFκB signalling pathway to promote OA progression.

Several ligand-receptor pairs were selected to perform cell transwell verification. First, *TNC* (AC ligand) and *FN1* (S ligand) in transcriptome analysis showed increased expression during OA (Supplementary Figures ca and cc), and the expression of their corresponding receptors *NT5E*, *SDC4*, and *ITGA5* also increased (Supplementary Figures cb and cd). Then, we used LPS or IL-1β to stimulate chondrocytes and synovial cells to mimic the state of OA. The expression of inflammation related genes (*IL-1β*, *IL-6*, and prostaglandin-endoperoxide synthase 2 (*PTGS2*)) increased, and extracellular matrix degradation related genes (*MMP1* and *MMP3*) also increased after stimulation (Supplementary Figures da and db), of which IL-1β induced inflammation is stronger than LPS. *TNC* expression was upregulated in chondrocytes after stimulation (Figure 5b), and the stimulated cells were regarded as signal giver. Then, we used transwell experiment to co-culture AC (stimulated)–AC (non-stimulated) and AC (stimulated)–S (non-stimulated), separately. Results showed that the corresponding receptor *NT5E* in chondrocytes and *SDC4* in synovial cells, which serves as signal receiver, was also upregulated (Figures 5c and 5d). As for synovial cells, *FN1* showed an increased expression after stimulation, and we co-cultured S (stimulated)–S (non-stimulated) and S (stimulated)–AC (non-stimulated) (Figure 5e). Its receptor *ITGA5* in synovial cells and *NT5E* in chondrocytes also showed an increased expression (Figures 5f and 5g). The inflammatory synovium and cartilage not only communicate with themselves but also with each other. These results confirm that molecular crosstalks occur within and between tissues during the process of OA.

Discussion

Transcriptomic analysis gives a lot of information, which plays an important role in OA research. We integrated transcription profiles of articular cartilage, meniscus, synovium, and subchondral bone to identify possible molecular crosstalk. We also reproduced some of the results on human primary cells, which supplement the studies of pathological communication in OA tissues.

First, the gene expression patterns of various tissues during OA were different. In the cluster dendrogram, an obvious distinction can be found between normal tissue and OA tissue (Figure 1a). However, synovial tissue could not be distinguished, which may be due to some normal synovial patients having mild symptoms of OA or the inflammatory reaction of normal tissue itself. Meniscus had the least differentially expressed genes (n = 97), while subchondral bone had the most (n = 2,330). This was limited by the number of samples, while on the other hand, subchondral bone was much more affected than meniscus during the process of OA. For articular cartilage and synovium, it showed the opposite number of upregulated and downregulated genes. There were more genes downregulated in articular cartilage and more genes upregulated in synovium. This was consistent with the physiological and pathological results of continuous loss of articular cartilage, thickening of synovium, and continuous secretion of synovial fluid during the process of OA.[25] The study was limited by the sensitivity and accuracy of the microarray and the number of samples, and some low differentially expressed genes have functions that may not be screened.[26] However, there were some common genes that were differentially expressed in two or three tissues, such as *SPP1*, *OGN*, *NID2*, *NT5E*, and *SDC4*. These genes may play similar functions in the pathological process of different tissues.

Second, gene enrichment analysis showed that in four tissues, most genes were all enriched in extracellular matrix and structure organization. This indicated that the change of extracellular matrix is the most common feature of pathological changes in OA. Also, many studies have demonstrated that the catabolism of extracellular matrix is a key factor in the progression of OA.[27,28] The unique characteristic of each tissue was that genes in articular cartilage were related to tissue development, genes in meniscus were related to metabolism, and genes in synovium and subchondral bone were related to neutrophils. This was in accordance with the pathological process of OA that blood vessels are absent in articular cartilage and meniscus, while vascular invasion occurs in synovium and subchondral bone during OA.[29,30]

Third, ligands and receptors with pairing were screened, while those which did not have paired ligands or receptors could be missed. Consistent with the GO results, we found that many extracellular matrix related proteins appeared in the molecular crosstalk map, of which ligands included collagens and some non-collagen proteins (fibronectin, fibrillin, and tenascin), as well as versican. In addition, their corresponding receptors included syndecans, integrins, and selectins. Multiple reports have revealed that these ligands and receptors are involved in the OA process. TNC concentration in human synovial fluid correlates with progression of knee OA, and intra-articular injections of TNC could prevent cartilage degeneration in mouse OA models.[31,32] The classic FN1 is associated with human OA in genome-wide association

study (GWAS) study, and conditional knockout mice of the fibronectin in articular cartilage aggravates OA.[33,34]

Finally, a signal crosstalk atlas was constructed based on secreted ligands and corresponding receptors of different tissues by combining human synovial fluid proteomic data. We further reproduced the communication between synovium and articular cartilage on human primary cells. We hope that this signal atlas will contribute to the development of OA diagnosis and treatment in the future.

However, there are several limitations to the current results. First, as the samples were derived from patients with late-stage OA, the differentially expressed genes in early OA could not be observed, so the results have limited impact on the study of molecular events in early OA. Second, while fewer genes had expression changes in OA meniscus, the identification of a common target in all tissues was affected. We also selected genes that change in most tissues for validation. Moreover, our results focused on ligand-receptor interactions, and many other unpaired molecules which may play an important role would be missed. Finally, limited by the available amputation patient samples, we used cells from OA patients and used IL-1β stimulation to simulate the OA model to verify the results of bioinformatic findings.

In conclusion, we highlighted the role of tissue molecular crosstalk through ligand-receptor pairs in OA pathophysiology. Results showed that articular cartilage, meniscus, synovium, and subchondral bone displayed different expression patterns in transcriptome, which indicates that each tissue has a specific role in OA. Moreover, we reproduced transcriptome findings of articular cartilage and synovium crosstalk through transwell experiment in human chondrocytes and synovial cells. *TNC* and *FN1* were upregulated ligands in most tissues during OA, and the expression of their corresponding receptors *NT5E*, *SDC4*, and *ITGA5* also increased. Exploring their downstream targets will be more effective for OA diagnosis and clinical treatment.

Supplementary material

Tables showing patient characteristics and ligand and receptor pairing within and between tissues. Figures showing: boxplot of all transcriptional profiles; Gene Ontology enrichment of articular cartilage, meniscus, synovium, and subchondral bone; heatmap of ligands and receptors in articular cartilage and synovium; and relative expression of interleukin (IL)-1β, IL-6, PTGS2, matrix metallopeptidase 1 (MMP1), and MMP3 in chondrocytes and synovial cells after lipopolysaccharide (LPS) and IL-1β stimulation.

References

1. **Sanchez-Lopez E**, **Coras R**, **Torres A**, **Lane NE**, **Guma M**. Synovial inflammation in osteoarthritis progression. *Nat Rev Rheumatol*. 2022;18(5):258–275.
2. **Liu-Bryan R**, **Terkeltaub R**. Emerging regulators of the inflammatory process in osteoarthritis. *Nat Rev Rheumatol*. 2015;11(1):35–44.
3. **Goldring SR**, **Goldring MB**. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage-bone crosstalk. *Nat Rev Rheumatol*. 2016;12(11):632–644.
4. **Palazzo C**, **Nguyen C**, **Lefevre-Colau M-M**, **Rannou F**, **Poiraudeau S**. Risk factors and burden of osteoarthritis. *Ann Phys Rehabil Med*. 2016;59(3):134–138.
5. **Safiri S**, **Kolahi A-A**, **Smith E**, **et al**. Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the Global Burden of Disease Study 2017. *Ann Rheum Dis*. 2020;79(6):819–828.
6. **Im G-I**. Current status of regenerative medicine in osteoarthritis. *Bone Joint Res*. 2021;10(2):134–136.
7. **Chen D**. Osteoarthritis: A complicated joint disease requiring extensive studies with multiple approaches. *J Orthop Translat*. 2022;32:130.
8. **Heinegård D**, **Saxne T**. The role of the cartilage matrix in osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):50–56.
9. **Jenei-Lanzl Z**, **Meurer A**, **Zaucke F**. Interleukin-1β signaling in osteoarthritis - chondrocytes in focus. *Cell Signal*. 2019;53:212–223.
10. **Badlani JT**, **Borrero C**, **Golla S**, **Harner CD**, **Irrgang JJ**. The effects of meniscus injury on the development of knee osteoarthritis: data from the osteoarthritis initiative. *Am J Sports Med*. 2013;41(6):1238–1244.
11. **de Lange-Brokaar BJE**, **Ioan-Facsinay A**, **van Osch GJVM**, **et al**. Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. *Osteoarthritis Cartilage*. 2012;20(12):1484–1499.
12. **Li G**, **Yin J**, **Gao J**, **et al**. Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. *Arthritis Res Ther*. 2013;15(6):223.
13. **Kapoor M**, **Martel-Pelletier J**, **Lajeunesse D**, **Pelletier J-P**, **Fahmi H**. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):33–42.
14. **Jayadev C**, **Hulley P**, **Swales C**, **et al**. Synovial fluid fingerprinting in end-stage knee osteoarthritis: a novel biomarker concept. *Bone Joint Res*. 2020;9(9):623–632.
15. **Lories RJ**, **Luyten FP**. The bone-cartilage unit in osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):43–49.
16. **Yuan XL**, **Meng HY**, **Wang YC**, **et al**. Bone-cartilage interface crosstalk in osteoarthritis: potential pathways and future therapeutic strategies. *Osteoarthritis Cartilage*. 2014;22(8):1077–1089.
17. **Qin H-J**, **Xu T**, **Wu H-T**, **et al**. SDF-1/CXCR4 axis coordinates crosstalk between subchondral bone and articular cartilage in osteoarthritis pathogenesis. *Bone*. 2019;125:140–150.
18. **Chou C-H**, **Jain V**, **Gibson J**, **et al**. Synovial cell cross-talk with cartilage plays a major role in the pathogenesis of osteoarthritis. *Sci Rep*. 2020;10(1):10868.
19. **Sun Y**, **Mauerhan DR**, **Honeycutt PR**, **et al**. Analysis of meniscal degeneration and meniscal gene expression. *BMC Musculoskelet Disord*. 2010;11:19.
20. **Broeren MGA**, **de Vries M**, **Bennink MB**, **et al**. Functional tissue analysis reveals successful cryopreservation of human osteoarthritic synovium. *PLoS One*. 2016;11(11):e0167076.
21. **Chou C-H**, **Wu C-C**, **Song I-W**, **et al**. Genome-wide expression profiles of subchondral bone in osteoarthritis. *Arthritis Res Ther*. 2013;15(6):R190.
22. **Ramilowski JA**, **Goldberg T**, **Harshbarger J**, **et al**. A draft network of ligand-receptor-mediated multicellular signalling in human. *Nat Commun*. 2015;6:1–12.
23. **No authors listed**. FANTOM (Functional ANnoTation Of the Mammalian genome). Riken. 2014. https://fantom.gsc.riken.jp/5/suppl/Ramilowski_et_al_2015/ (date last accessed 27 October 2022).
24. **Balakrishnan L**, **Nirujogi RS**, **Ahmad S**, **et al**. Proteomic analysis of human osteoarthritis synovial fluid. *Clin Proteomics*. 2014;11(1):1–13.
25. **Jin H**, **Jiang S**, **Wang R**, **Zhang Y**, **Dong J**, **Li Y**. Mechanistic insight into the roles of integrins in osteoarthritis. *Front Cell Dev Biol*. 2021;9:693484.
26. **Bolón-Canedo V**, **Alonso-Betanzos A**, **López-de-Ullibarri I**, **Cao, R**. Challenges and future trends for microarray analysis. In: *Microarray Bioinformatics*. Springer, 2019: 283–293.
27. **Rahmati M**, **Nalesso G**, **Mobasheri A**, **Mozafari M**. Aging and osteoarthritis: Central role of the extracellular matrix. *Ageing Res Rev*. 2017;40:20–30.
28. **Guilak F**, **Nims RJ**, **Dicks A**, **Wu CL**, **Meulenbelt I**. Osteoarthritis as a disease of the cartilage pericellular matrix. *Matrix Biol*. 2018;71–72:40–50.
29. **Ashraf S**, **Walsh DA**. Angiogenesis in osteoarthritis. *Curr Opin Rheumatol*. 2008;20(5):573–580.
30. **Ni R**, **Guo XE**, **Yan C**, **Wen C**. Hemodynamic stress shapes subchondral bone in osteoarthritis: An emerging hypothesis. *J Orthop Translat*. 2022;32:85–90.
31. **Hasegawa M**, **Hirata H**, **Sudo A**, **et al**. Tenascin-C concentration in synovial fluid correlates with radiographic progression of knee osteoarthritis. *J Rheumatol*. 2004;31(10):2021–2026.

32. **Matsui Y**, **Hasegawa M**, **Iino T**, **Imanaka-Yoshida K**, **Yoshida T**, **Sudo A**. Tenascin-C prevents articular cartilage degeneration in murine osteoarthritis models. *Cartilage*. 2018;9(1):80–88.
33. **Yang H-Y**, **Su S-L**, **Peng Y-J**, **et al**. An intron polymorphism of the fibronectin gene is associated with end-stage knee osteoarthritis in a Han Chinese population: two independent case-control studies. *BMC Musculoskelet Disord*. 2014;15:1–9.
34. **Almonte-Becerril M**, **Gimeno-LLuch I**, **Villarroya O**, **Benito-Jardón M**, **Kouri JB**, **Costell M**. Genetic abrogation of the fibronectin-$\alpha 5\beta 1$ integrin interaction in articular cartilage aggravates osteoarthritis in mice. *PLoS One*. 2018;13(6):e0198559.

Author information:
- M. Wang, PhD, Postdoctoral Researcher
- G. Tan, MD, Student
- H. Jiang, MD, Student
- A. Liu, PhD, Postdoctoral Researcher
- R. Wu, MD, Research Assistant
- J. Li, PhD, Student
- Z. Sun, PhD, Student
- Z. Lv, PhD, Student
- D. Shi, PhD, Professor
  State Key Laboratory of Pharmaceutical Biotechnology, Division of Sports Medicine and Adult Reconstructive Surgery, Department of Orthopedic Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China.
- W. Sun, PhD, Orthopaedic Surgeon, Department of Orthopedics, The Affiliated Jiangyin Hospital of Southeast University Medical College, Wuxi, China.

Author contributions:
- M. Wang: Data curation, Software, Visualization, Writing – original draft.
- G. Tan: Methodology.
- H. Jiang: Methodology.
- A. Liu: Writing – review & editing.
- R. Wu: Writing – review & editing.
- J. Li: Validation.
- Z. Sun: Validation.
- Z. Lv: Validation.
- W. Sun: Validation.
- D. Shi: Conceptualization, Funding acquisition, Project administration.

Funding statement:
- The authors disclose receipt of the following financial or material support for the research, authorship, and/or publication of this article: this work was supported by National Key R&D Program of China (2018YFC1105904), Key Program of NSFC (81730067), National Science Foundation of China (81772335, 81941009, 81802196, 82172481), Natural Science Foundation of Jiangsu Province, China (BK20180127), and Jiangsu Provincial Key Medical Talent Foundation, Six Talent Peaks Project of Jiangsu Province (WSW-079).

ICMJE COI statement:
- The authors declare no competing interests.

Acknowledgements:
- The authors acknowledge the support from members of the Division of Sports Medicine and Adult Reconstructive Surgery, Department of Orthopedic Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School.

Ethical review statement:
- The use of human articular cartilage and synovium was approved by the Ethical Committee of Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School (2020-156-01), and obtained by verbal consent of all patients.

Open access funding
- The authors report that they received open access funding for their manuscript from the National Science Foundation of China (82172481).