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Identification and immunological characteristics of autophagy and apoptosis genes in knee osteoarthritis: A bioinformatics analysis

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Abstract

Knee osteoarthritis (KOA) is a degenerative joint disease characterized by cartilage degradation, synovial inflammation, and subchondral bone remodeling. The interplay between autophagy and apoptosis plays a critical role in chondrocyte homeostasis and disease progression. However, the shared molecular mechanisms linking these two processes to KOA pathogenesis remain incompletely understood. This study aims to identify key autophagy- and apoptosis-related genes and investigate their immunological characteristics in KOA. This study examines KOA to identify key genes associated with whole-joint components and analyze the relationship and underlying mechanisms between autophagy and apoptosis. Data from the GSE169077 dataset were extracted from the Gene Expression Omnibus database, and differential gene expression was analyzed using the edgeR model. Autophagy- and apoptosis-related genes were screened using the GeneCards database, and a protein–protein interaction network was constructed for functional pathway enrichment analysis. Further immune cell infiltration analysis was conducted, and machine learning algorithms were employed to identify two characteristic genes, *SPARC* and *BNIP3*. The results revealed 641 downregulated genes and 722 upregulated genes in KOA, with 73 overlapping genes linked to the phosphoinositide 3-kinase/protein kinase B, p53, and hypoxia-inducible factor 1 signaling pathways, and significant differences in macrophages. *SPARC* and *BNIP3* influence the progression of KOA through mechanisms such as hypoxic response, cell membrane permeability, mitochondrial dysfunction, and vascular development. The study suggests that *SPARC* and *BNIP3* could be potential targets involved in the association between KOA and both autophagy and apoptosis.

Keywords: Knee osteoarthritis; Differentially expressed genes; Machine learning; Bioinformatics; Immune infiltration

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1. Introduction

Osteoarthritis (OA) is a widespread joint disorder marked by pain, limited joint mobility, and joint deformity. Common triggers for OA onset include trauma, aging, obesity, and genetic predispositions.[1-3] Recently, the development of therapeutic agents for OA has emerged as a key research area. However, most research and clinical trials

have concentrated on specific clinical aspects, such as cartilage degeneration, subchondral bone remodeling, localized inflammation, or joint pain.[4] Newly identified drug targets display variability across different joint tissues. With the swift advancement of genomics technologies, like microarray and next-generation mRNA sequencing, bioinformatics has been extensively utilized to pinpoint crucial genes, signaling pathways, and autophagy and apoptosis mechanisms in various musculoskeletal conditions.[5-7] A multitude of studies have identified and explored potential therapeutic targets for knee OA (KOA) through bioinformatics screening. For instance, arginase 2, which is overexpressed in OA cartilage, was detected via microarray screening and further confirmed by the upregulation of matrix metalloproteinases (MMPs) that drive cartilage breakdown.[8] Activated bone turnover, neuropathy, and inflammation in KOA bone marrow lesions have also been uncovered through microarray bioinformatics.[9] Moreover, microRNA candidates with potential as OA biomarkers and therapeutic targets have been identified and validated through comprehensive paired microRNA–mRNA analysis and functional enrichment analysis.[10]

Beyond identifying potential therapeutic targets, bioinformatics-based bulk sample analysis also assists in categorizing potential OA subtypes for more precise diagnosis and personalized treatment. In recent years, considerable progress has been made in classifying potential OA subtypes using bioinformatics.[11,12] These findings have significantly enhanced the understanding of KOA and can facilitate future personalized treatments. However, most published bioinformatics studies have focused on a single type of joint component. Moreover, differentially expressed genes (DEGs) analysis across different joint components during OA progression remains limited. In 2016, approximately 5% overlap in DEGs was observed between synovial and cartilage tissues, but no further analysis was conducted on these identified overlapping DEGs.[13] Recently, another study observed a 10% overlap in DEGs between OA cartilage and subchondral bone. By comparing the overlapping DEGs between the cartilage–subchondral bone identified with previously determined OA risk genes, researchers identified interleukin (IL) 11 and cartilage adhesion-like genes as two potential therapeutic targets for OA.[14,15]

A comprehensive analysis of key DEGs and their associated pathways, as well as autophagy and apoptosis, between KOA and normal knee joints remains crucial. This study employs gene expression profiling to analyze significant differences in genes between KOA patients and normal knee joints to identify key biomarkers of abnormal gene expression and immune infiltration, thereby elucidating the pathogenic mechanisms of KOA and laying the groundwork for the development of related therapies.

2. Materials and methods

2.1. Gene expression profile in knee osteoarthritis joint tissue

The GSE169077 KOA dataset was retrieved from the Gene Expression Omnibus database, encompassing five normal knee bones and six KOA tissues. The utilized chip platform was the GPL96 (HG-U133A) Affymetrix Human Genome U133A Array.

2.2. Data processing

The R software (version 4.1.2) was employed to analyze the chips, download the matrix files, and merge the original sample arrays using the Affy package. The Mas5 algorithm was applied for background correction and quantile normalization of the dataset. Samples with substantial data loss and deviation were filtered out, and the remaining data were analyzed and adjusted. Data from five normal controls and six OA samples were annotated by eliminating duplicate probe values. Differential analysis was conducted using the edgeR model, with the results displayed via volcano and heatmap plots of DEGs. DEGs were filtered using two parameters: a *p*-value less than 0.05 and a log fold change absolute value exceeding 1.

2.3. Functional enrichment analysis

Autophagy and apoptosis-related genes were separately downloaded from the GeneCards database (https://www.genecards.org/). A Venn diagram was constructed to identify intersecting genes. Functional enrichment was performed using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The protein–protein interaction (PPI) network was created to elucidate individual protein interactions involved in various biological mechanisms, such as biological signal transduction, gene expression regulation, energy and substance metabolism, and cell cycle control. Systematic analysis of protein interactions in biological systems is crucial for understanding protein functions and their roles in specific physiological states (diseases). The STRING database (https://string-db.org/) was utilized to construct the PPI network related to DEGs, autophagy, and apoptosis genes, and the network model was visualized using Cytoscape (v3.9.1). GeneMania was employed to construct a network of first connection points for genes, predict and annotate functional pathways, and reflect annotation reliability using co-expression scores.

2.4. Estimation of immune cell infiltration

The CIBERSORTx algorithm was utilized to measure the abundance of diverse immune cell types within the samples. An immune cell marker gene annotation matrix was constructed for CIBERSORTx, and the algorithm was subsequently applied to evaluate immune cell infiltration in the dataset. Only results with a *p*-value below 0.05 were retained. A bar chart was employed to illustrate the immune cell content in the samples, while the "corrplot" package was used to generate a heatmap depicting correlations. The "ggplot" package was then leveraged to produce a box plot highlighting differences in immune cell infiltration between the OA and normal groups. Principal component analysis was performed to assess statistical significance in immune infiltration across groups and to further elucidate functional associations among immune cells.

2.5. Screening of core genes

To identify core genes associated with autophagy, apoptosis, and KOA, machine learning techniques were applied to extract features from the gene expression matrix. The dataset was divided into a training set (60%) and a validation set (40%). A 10-fold cross-validation algorithm was implemented to ensure result reliability. Models such as logistic regression, Bayesian, support vector machine, decision tree, random forest, extreme gradient boosting, and light gradient boosting machine were utilized, and their performance was evaluated using the area under the receiver operating characteristic curve and precision–recall scores.

2.6. Relationship between characteristic genes and infiltrating immune cells

To further explore the relationship between key genes and infiltrating immune cells, a clustering method was employed to analyze the association between candidate diagnostic genes and KOA-infiltrating immune cells ($p < 0.05$). The results were visualized using a heatmap.

3. Results

3.1. Identification of differentially expressed genes

Analysis of DEGs uncovered discrepancies in expression levels between upregulated (red) and downregulated genes (green) (Figure 1). The x-axis represents gene expression alterations, while the y-axis denotes statistical significance. The horizontal dashed line marks the *p*-value threshold, and the vertical dashed line indicates the gene expression change threshold. Figure 2 illustrates the distribution and interrelationships of gene expression data, constructed from the 50 most significantly upregulated and downregulated probe data. Here, blue signifies low expression and red indicates high expression. Sample category labels for normal and knee joint samples are placed at the top, with a dendrogram representing hierarchical clustering, thus reflecting the similarity among genes or samples.

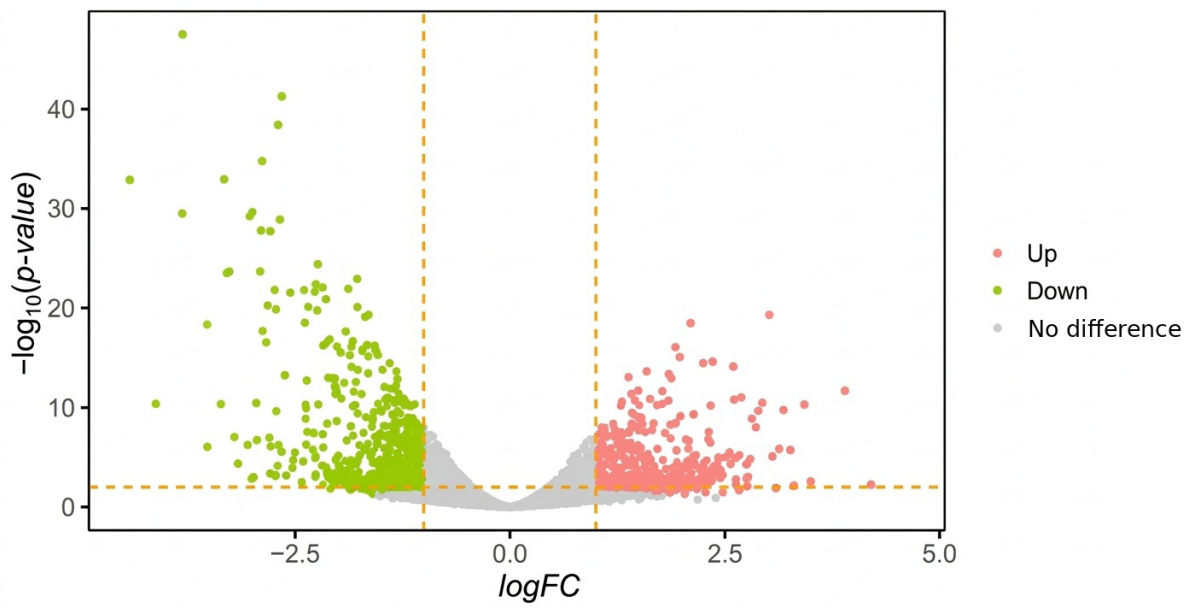


Figure 1. Volcano map

Abbreviation: FC: Fold change.

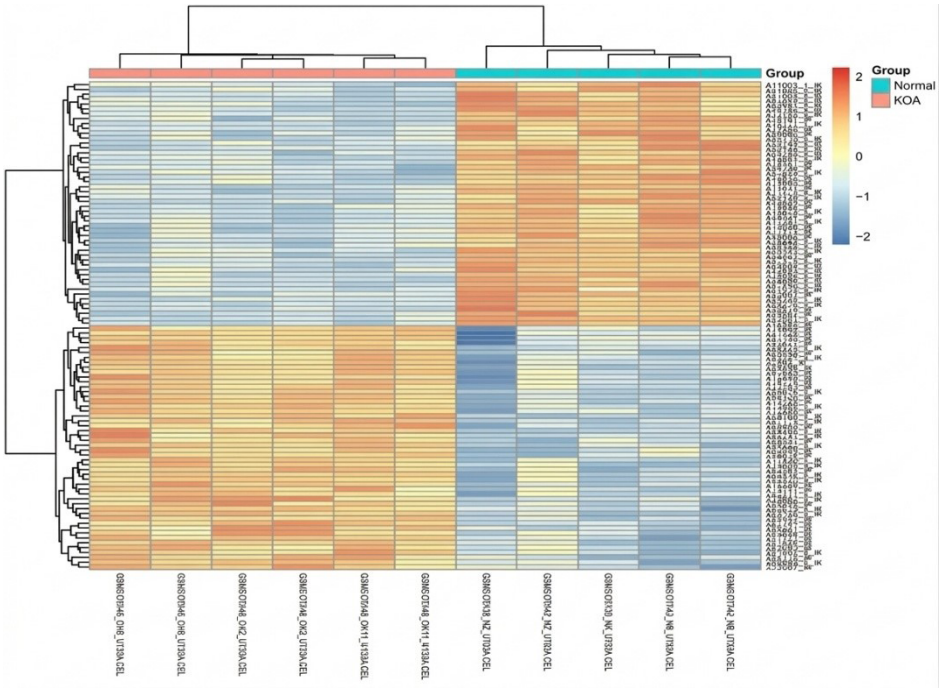


Figure 2. Heat map of differentially expressed genes. The intensity of the color corresponds to the expression (darker color indicates higher expression. Red represents upregulated genes and blue represents downregulated genes.
Abbreviation: KOA: Knee osteoarthritis.

3.2. Correlation between autophagy and apoptosis functional pathways and knee osteoarthritis

From the GeneCards database (https://www.genecards.org/), cell autophagy and apoptosis-related genes were separately retrieved, yielding 9,366 autophagy-related genes and 21,637 apoptosis-related genes. After setting the correlation score to be greater than two, 1,227 autophagy-related genes and 2,839 apoptosis-related genes remained. Concurrently, DEGs were filtered using two parameters: a *p*-value less than 0.05 and a log fold change absolute value exceeding 1. Ultimately, 1,363 DEGs were identified, including 641 downregulated genes and 722 upregulated genes. Subsequently, a Venn diagram was constructed to identify 73 overlapping genes between DEGs and autophagy and apoptosis-related genes in the database (Figure 3). Functional enrichment was conducted using the GO and KEGG databases, while the STRING database was employed to construct the PPI network (Figure 4).

Figure 5 showcases 73 intersecting genes on the left and KEGG-enriched pathways on the right. DEGs were primarily involved in the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway, p53 signaling pathway, and hypoxia-inducible factor 1 (HIF-1) signaling pathway. The line connecting a gene to a KEGG pathway signifies the association or interaction between specific genes and pathways. According to the GO enrichment results in Figure 6, the main biological processes related to DEGs were autophagy regulation, intrinsic apoptotic signaling pathways, and mitochondrial tissue regulation. Cellular components primarily consisted of outer membranes, organ outer membranes, and mitochondrial outer membranes, while the molecular functions included protein serine/threonine/tyrosine kinase activity, cytokine receptor binding, and heat shock protein binding.

3.3. Immune infiltration analysis results

Utilizing the CIBERSORTx algorithm to analyze 22 types of immune cells, examining their distribution across two tissue types, and further assessing the functional relationships among the immune cells. Figure 7 presents the distribution of the 22 immune cells in the normal and KOA groups. Red markers highlight the infiltration differences between the two groups of samples, with macrophage M0 showing a significant statistical difference, indicating a more substantial macrophage infiltration in KOA. The bar chart in Figure 8 reflects the distribution of the 22 immune cells in each sample, with the first five being normal samples and the last six being KOA samples. Figure 9 illustrates the correlation between the different types of immune cells. Red signifies positive correlation and blue represents negative correlation, with higher color

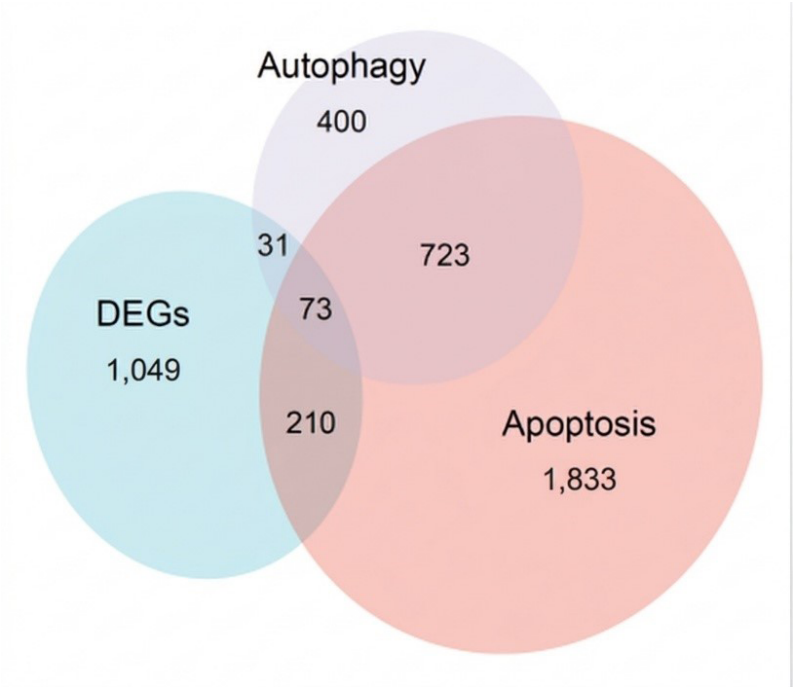


Figure 3. Venn diagram of overlapping differentially expressed genes (DEGs)

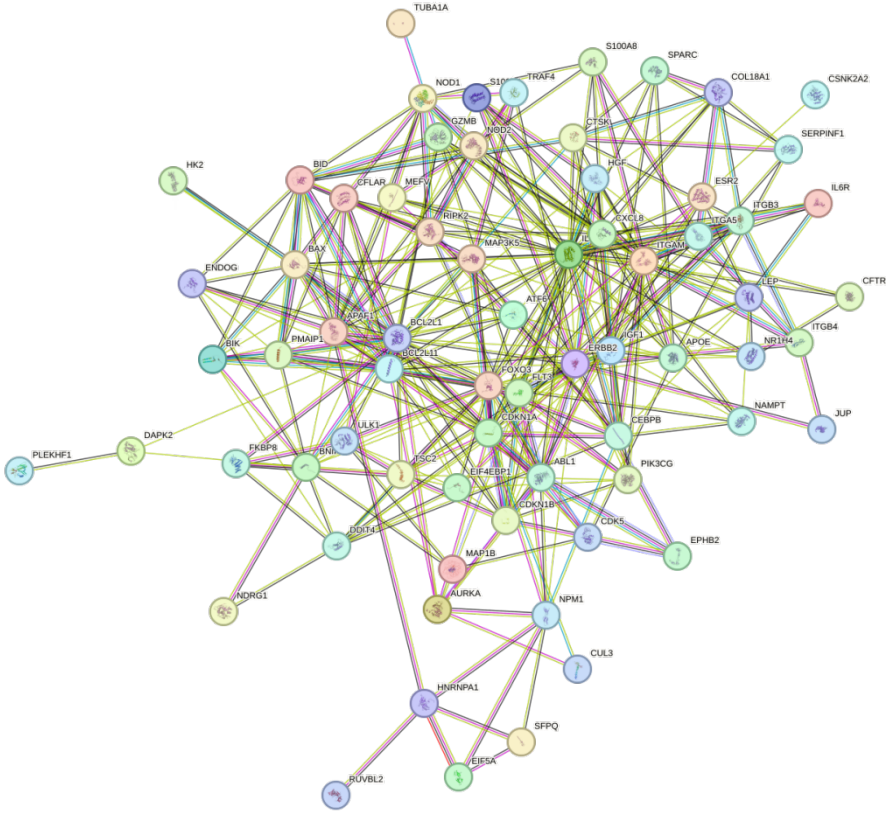


Figure 4. The protein–protein interaction network

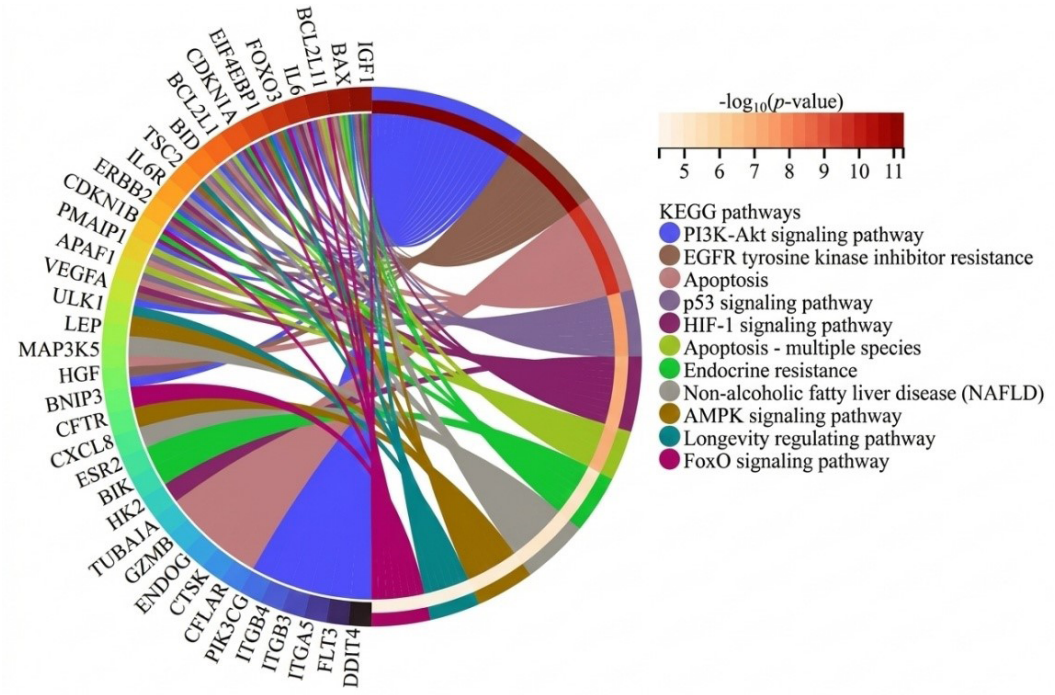


Figure 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis
Abbreviations: Akt: Protein kinase B; AMPK: AMP-activated protein kinase; EGFR: Epidermal growth factor receptor; HIF-1: Hypoxia-inducible factor 1; PI3K: Phosphoinositide 3-kinase.

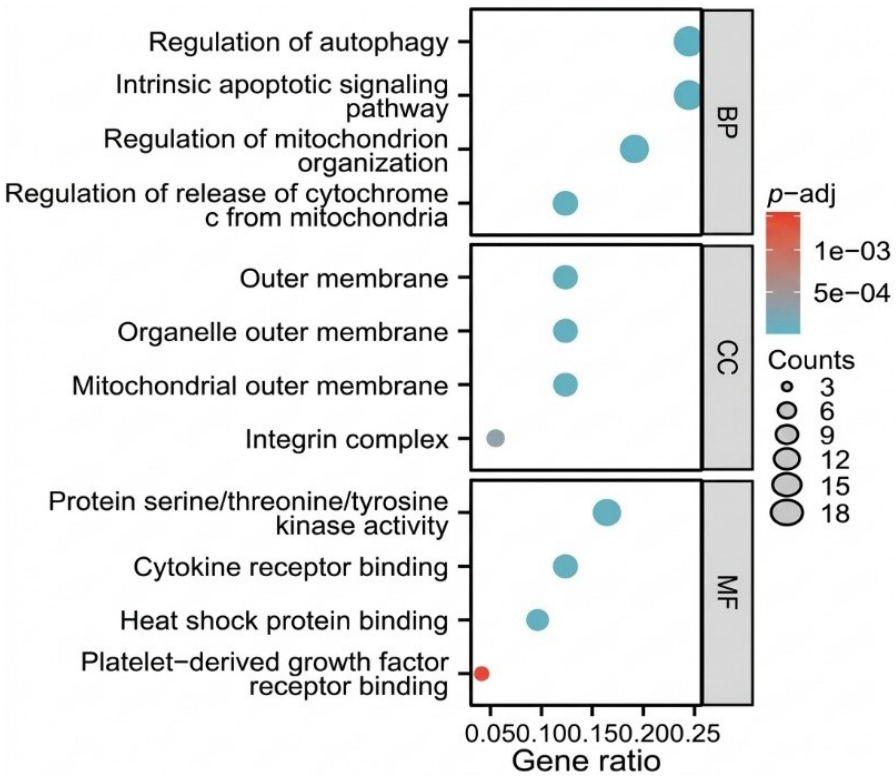


Figure 6. Gene Ontology enrichment analysis

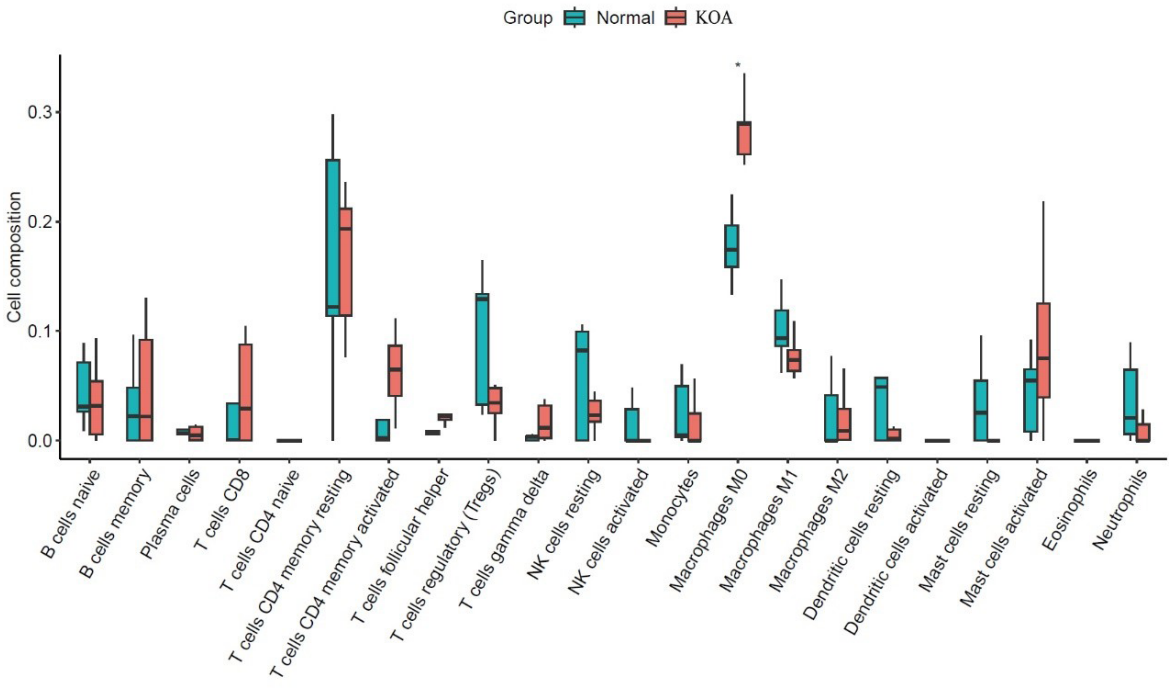


Figure 7. CIBERSORTx's violin plot of immune infiltration
Abbreviations: NK: Natural killer; KOA: Knee osteoarthritis.

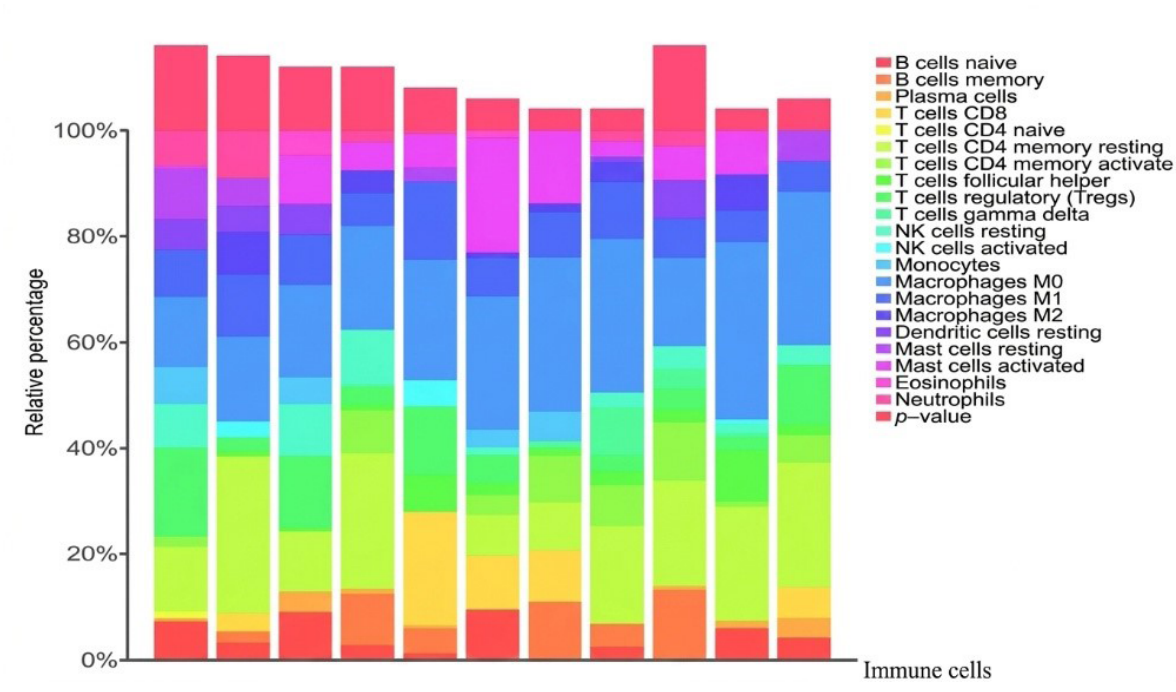


Figure 8. Bar chart of the distribution of the 22 immune cells in each sample
Abbreviation: NK: Natural killer.

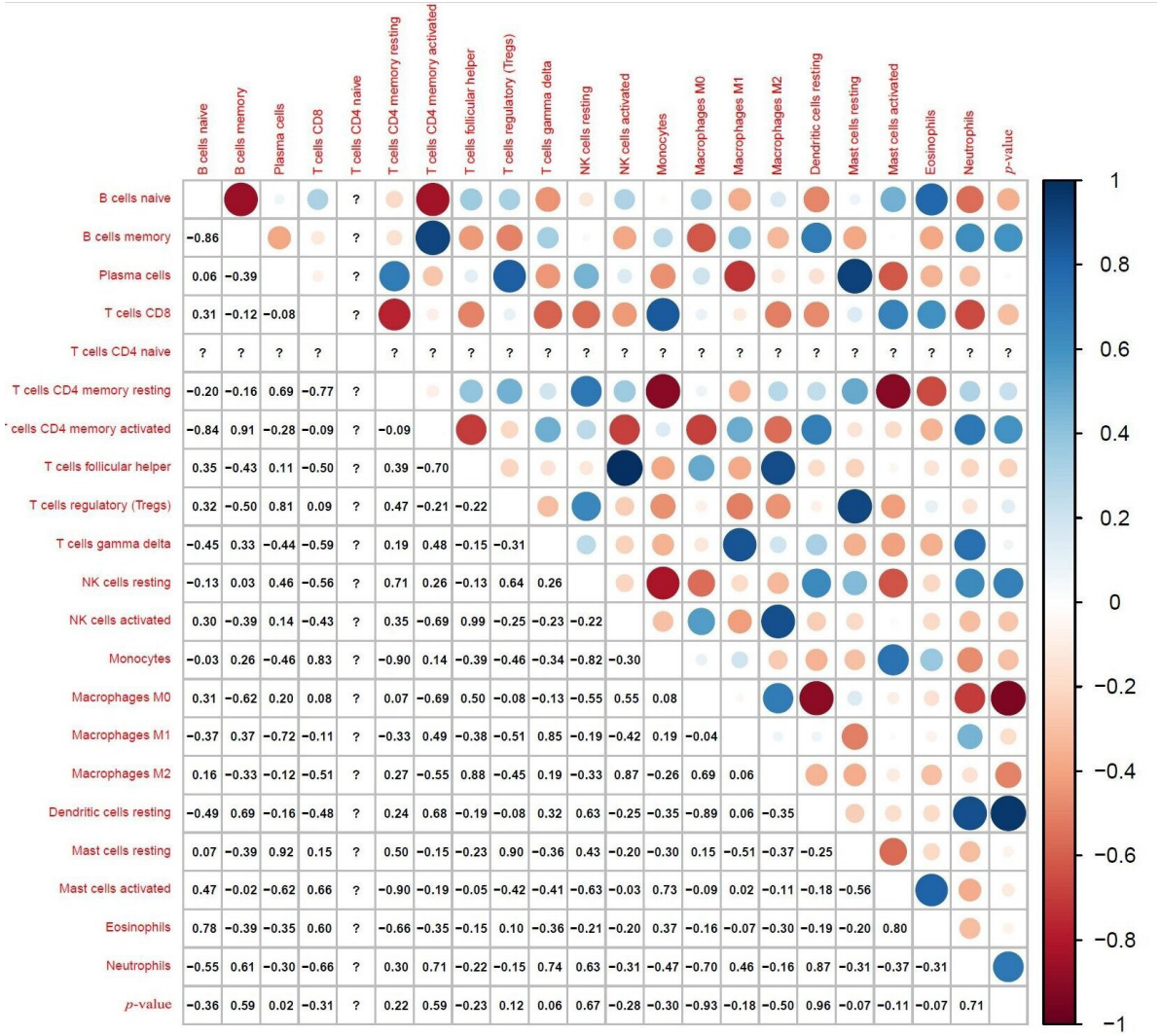


Figure 9. Heat map of immune cell correlation
Abbreviation: NK: Natural killer.

intensity indicating a stronger correlation. According to the correlation analysis between immune cells, immature B cells were positively correlated with eosinophils; memory B cells with activated T cells ($CD4^+$ memory cells); plasma cells with regulatory T cells; resting mast cells with M0 macrophages; T cells ($CD8^+$) with monocytes; follicular helper T cells with activated natural killer (NK) cells; activated NK cells with M2 macrophages; and resting dendritic cells with neutrophils. Immature B cells were negatively correlated with memory B cells; activated T cells ($CD4^{4+}$ memory cells) with immature B cells; T cells ($CD8^+$) with resting T cells ($CD4^+$ memory cells); plasma cells with M1 macrophages, resting T cells ($CD4^+$ memory cells) with monocytes; resting T cells ($CD4^+$ memory cells) with activated mast cells; resting NK cells with monocytes; and M0 macrophages with resting dendritic cells.

3.4. Determination of learning model and core gene screening

Initially, the accuracy of the learning models was compared. As shown in Figure 10, the highest accuracy was achieved by logistic regression, Bayesian, and random forest models (100%). However, this only partly reflects the model classification effectiveness, and the bias caused by the small sample size must also be considered. The results in Figure 11 demonstrate the performance of logistic regression, Bayesian, and random forest models. Figure 12 illustrates the construction of Venn diagrams for the top 15 genes based on the three best-performing models. Ultimately, two genes, *SPARC* and *BNIP3*, were deemed suitable for KOA classification in all three models, serving as potential characteristic genes for KOA.

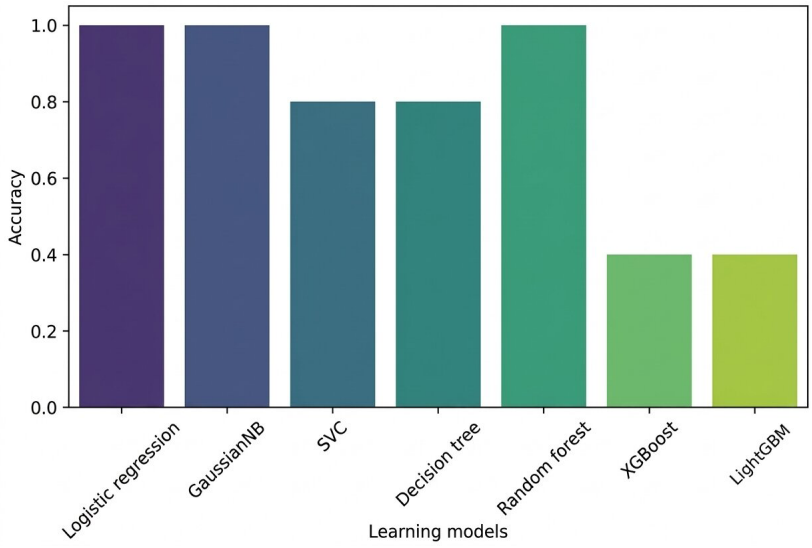


Figure 10. Comparison of the accuracy of machine learning models based on model performance on test data
Abbreviations: GBM: Gradient boosting machine; NB: Naïve Bayes; SVC: Support vector machine; XGBoost: Extreme gradient boosting.

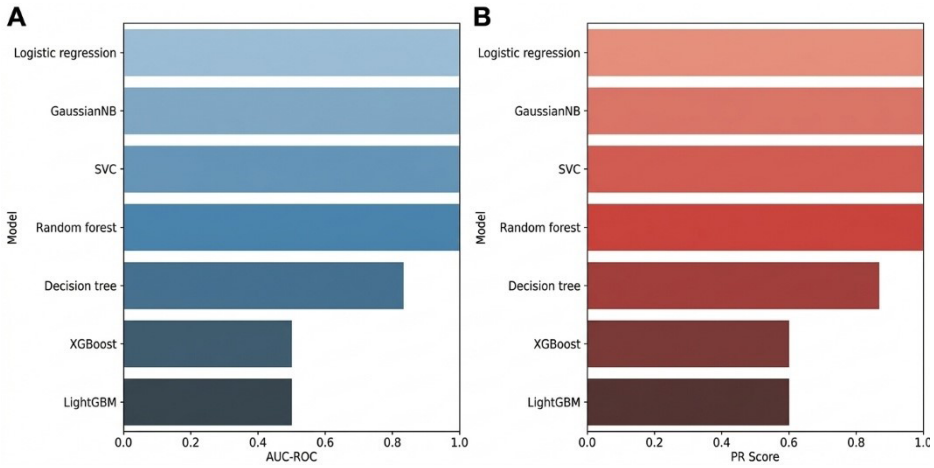


Figure 11. The performance of machine learning models. Comparison of (A) area under the receiver operating characteristic curve (AUC-ROC) and (B) precision–recall (PR) scores
Abbreviations: GBM: Gradient boosting machine; NB: Naïve Bayes; SVC: Support vector machine; XGBoost: Extreme gradient boosting.

3.5. Core gene insights

Figure 13 displays the infiltration of *SPARC* and *BNIP3* in 22 immune cells, revealing that these two genes have a relatively significant correlation with most immune cells, including $CD4^-$ T cells, monocytes, and macrophages. In Figure 14, samples were clustered into 2–10 groups using immune-related indicators, and *SPARC* and *BNIP3* genes were screened. However, no relatively significant immune subtypes were identified. Thus, the results indicate that the samples in this dataset are relatively homogeneous, with no distinct immune subtypes identified.

Figure 15 presents the network constructed with *SPARC* and *BNIP3* as the first junction genes, with functional pathway enrichment analysis indicating that these two genes contribute to KOA onset and progression by influencing hypoxia response, adjusting cell membrane permeability, causing mitochondrial dysfunction or vascular development, and other functional pathways. Physical interaction constitutes 77.64%.

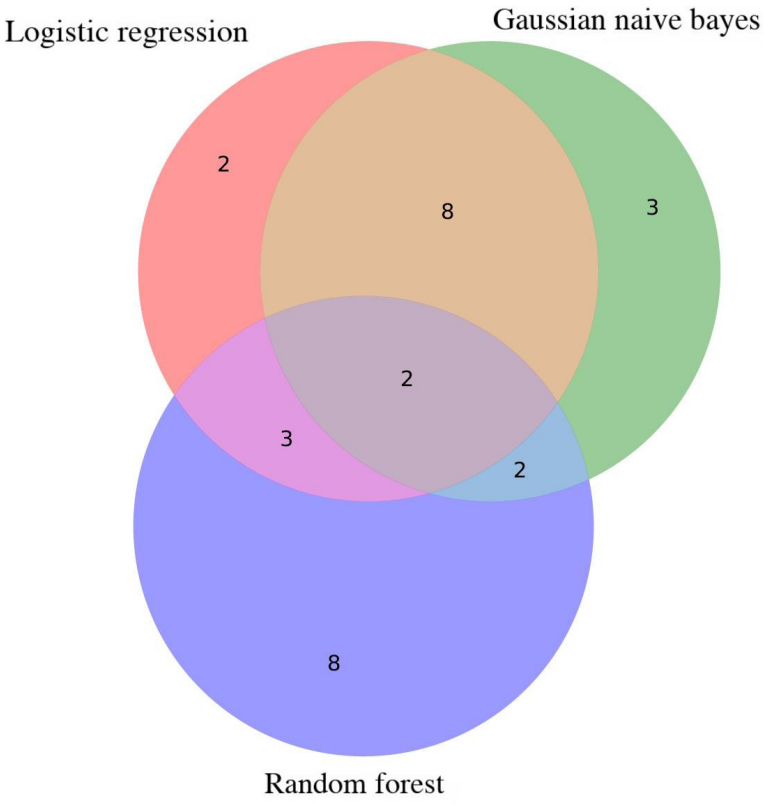


Figure 12. Venn diagram of the top 15 genes based on the three best-performing models
Abbreviation: NB: Naïve Bayes.

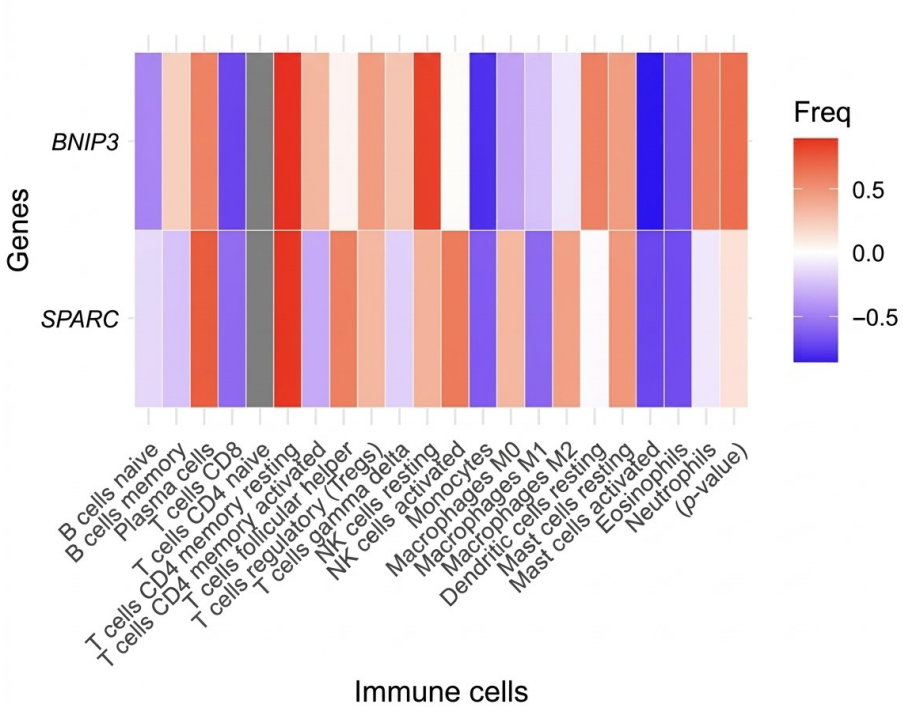


Figure 13. Gene–immune cell correlations

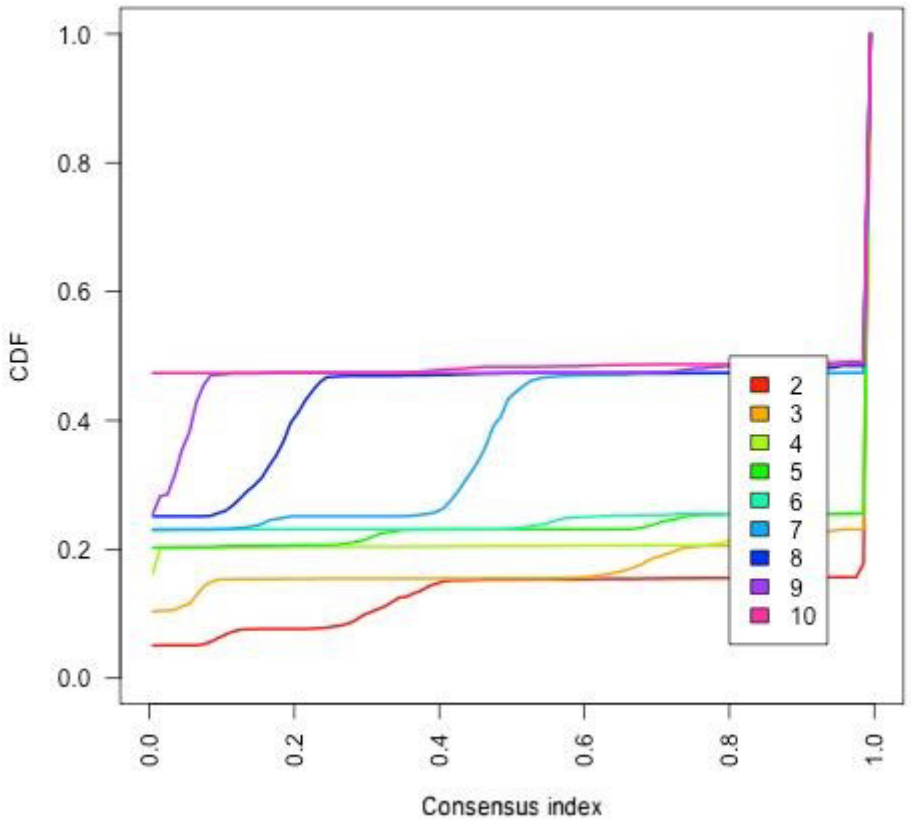


Figure 14. Consensus cumulative distribution function (CDF) plot

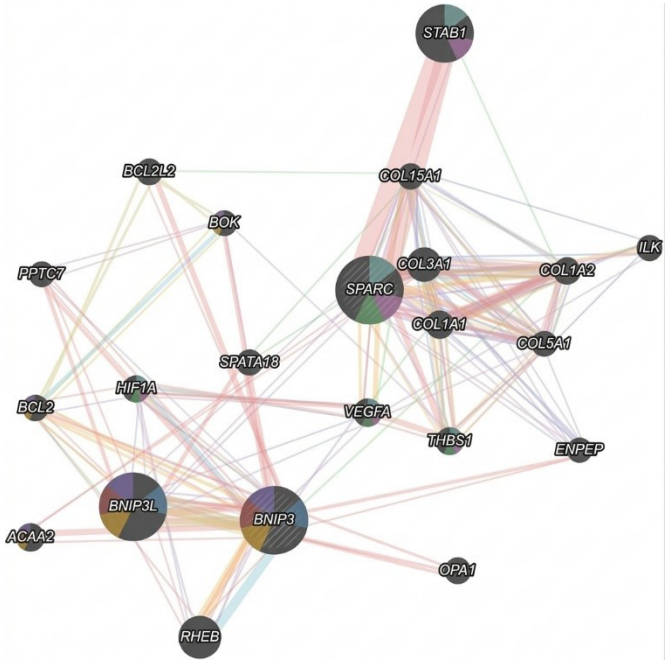


Figure 15. Gene network prediction in the GeneMania database

4. Discussion

4.1. Osteoarthritis: Molecular mechanisms and immune microenvironment

Osteoarthritis represents a degenerative joint disorder primarily involving the knee and hip articulations.[16,17] This condition affects roughly 10–15% of young adult populations and as many as 60% of older individuals.[18] The immunological milieu's molecular pathways in OA are not well characterized. Discovering novel therapeutic targets for early detection, intervention, and outcome prediction in the KOA immunological microenvironment offers substantial clinical value. KOA onset involves pro-inflammatory mediators secreted by the immune system following tissue damage or inflammation.[19] Joint inflammation manifests locally in KOA, with mechanical injury potentially prompting chondrocyte secretion of cytokines and MMPs that drive articular cartilage breakdown.[20] Consequently, immune response modulation emerges as a critical therapeutic strategy for OA management.[21]

This investigation revealed 1,363 genes showing differential expression between KOA and healthy human specimens. Ontological assessment demonstrated that autophagy modulation, intrinsic apoptosis signaling cascades, and mitochondrial homeostasis represented the predominant functional categories overlapping DEGs with autophagic and apoptotic processes. KEGG pathway mapping indicated DEG participation in PI3K/Akt, p53, and HIF-1 signaling cascades. PPI network evaluation linked activating transcription factor 6, chemokine (C-X-C motif) ligand 8, B-cell lymphoma (BCL) 2-like 1, mitogen-activated protein kinase kinase kinase 5, and Erb-B2 receptor tyrosine kinase 2 with DEGs. Given the immune cell infiltration's significance in KOA pathobiology, subsequent CIBERSORTx computational analysis demonstrated notable divergence in M0 macrophage populations between cohorts, marked by pronounced macrophage accumulation in KOA joints. Employing logistic regression, Bayesian inference, and random forest algorithms, we pinpointed *SPARC* and *BNIP3* as pivotal genes connecting autophagy and apoptosis in KOA. Expression profiles of these hub genes showed strong associations with the majority of immune cell subsets, implying potential regulation of KOA pathogenesis through interactions with these 22 immune cell types. Finally, GeneMania-based gene network modeling demonstrated that *SPARC* and *BNIP3* affect KOA onset and progression by modulating hypoxic response, altering membrane permeability, inducing mitochondrial impairment, or regulating angiogenesis, with physical interactions comprising 77.64% of these relationships. Nevertheless, precise connections between these hub genes and specific immune cell populations, along with their exact functions in KOA progression, require additional experimental validation.

4.2. Molecular mechanisms of *SPARC* and *BNIP3* in knee osteoarthritis

Secreted protein acidic and rich in cysteine (SPARC), also known as BM-40 and osteonectin, is involved in various biological processes such as cell adhesion, migration, proliferation, and apoptosis, particularly in developing and remodeling tissues. Adipocytes are the primary source of SPARC, which inhibits adipogenesis and promotes fibrosis in adipose tissue.[22,23] Studies have shown that SPARC secretion in the superficial and middle zones of KOA cartilage is associated with obesity and diabetes, indicating a link between metabolic disorders and KOA pathology.[24,25] SPARC regulates cell–extracellular matrix interactions and mechanical signaling by binding to receptors, such as integrin αvβ3, αvβ5, and αvβ6.[26] In articular cartilage, SPARC modulates chondrocyte metabolic balance by affecting the expression of MMPs and tissue inhibitors of metalloproteinases, thereby regulating cartilage matrix degradation and synthesis. Additionally, SPARC interacts with the transforming growth factor-beta signaling pathway to regulate chondrocyte differentiation and apoptosis. Research indicates that *SPARC* expression is upregulated in the cartilage of KOA patients, likely exacerbating KOA pathology by promoting cartilage matrix degradation and chondrocyte apoptosis.

B-cell lymphoma 2-interacting protein 3 (BNIP3) is a pro-apoptotic member of the BCL-2 family of apoptosis proteins. BNIP3 participates in multiple cellular signaling pathways and mediates various cellular functions, including autophagy and apoptosis. Under hypoxic conditions, *BNIP3* expression is significantly upregulated. By interacting with BCL-2 family proteins, it increases mitochondrial outer membrane permeability, releasing cytochrome C and thus activating the caspase cascade to induce apoptosis. Moreover, BNIP3 is involved in autophagy, regulating autophagosome formation and degradation by binding to autophagy-related proteins such as LC3. In articular cartilage, abnormal *BNIP3* expression may be associated with chondrocyte hypoxic stress and apoptosis, thereby influencing KOA occurrence and progression. Studies have shown that *BNIP3* expression is increased in the cartilage of KOA patients, likely exacerbating cartilage damage by promoting chondrocyte apoptosis and autophagy.[27] Reports indicate that *BNIP3* overexpression significantly inhibits cartilage cell inflammation caused by endotoxin stimulation, reducing the secretion of pro-inflammatory cytokines, such as

tumor necrosis factor (TNF), IL-1β, and IL-6. This, in turn, alleviates chondrocyte apoptosis. Additionally, *BNIP3* overexpression induces the upregulation of autophagy-related protein expression, suggesting that it may mitigate endotoxin-induced cartilage cell inflammatory damage by activating autophagy.[28] High methylation of *BNIP3* has been reported in various diseases,[29,30] and our findings suggest that *BNIP3* may also be involved in chondrocyte apoptosis, leading to cartilage degeneration.

4.3. Signaling pathways related to autophagy and apoptosis in knee osteoarthritis

Autophagy is a cellular self-degradation mechanism that forms double-membrane-bound autophagosomes. These structures enclose damaged organelles and proteins and transport them to lysosomes for degradation. Autophagy plays a crucial role in maintaining cellular homeostasis, clearing damaged organelles and proteins, and regulating cellular metabolism. Numerous signaling pathways are involved in the autophagy process, including the mammalian target of rapamycin, AMP-activated protein kinase, and PI3K/Akt signaling pathways. In KOA, autophagy dysfunction may lead to chondrocyte damage and death. Studies have found that the expression levels of autophagy-related proteins (e.g., LC3, Beclin-1) are significantly elevated in the cartilage cells of KOA patients, indicating that autophagy is activated in KOA. Autophagy activation may alleviate chondrocyte stress responses by clearing damaged organelles and proteins. However, excessive autophagy can damage chondrocytes and even cause cell death, thereby accelerating the pathological process of KOA.[26]

Apoptosis is a programmed cell death process that maintains tissue homeostasis by regulating cell survival and death. This process involves multiple signaling pathways, including the mitochondrial pathway, death receptor pathway, and endoplasmic reticulum stress pathway. The mitochondrial pathway is one of the primary apoptosis routes. It controls mitochondrial outer membrane permeability by regulating BCL-2 family protein expression, releasing cytochrome C, and activating the caspase cascade to induce apoptosis. In KOA, apoptosis dysfunction may result in chondrocyte damage and death. Research has shown that the expression levels of apoptosis-related proteins (e.g., caspase-3, caspase-9) are significantly upregulated in the cartilage cells of KOA patients, indicating that apoptosis is activated in KOA. Apoptosis activation may help reduce cartilage matrix degradation by clearing damaged chondrocytes. However, excessive apoptosis can lead to massive chondrocyte death, thereby accelerating the pathological process of KOA.

Autophagy and apoptosis have a complex interplay in cellular stress responses. On one hand, autophagy can alleviate cellular stress by clearing damaged organelles and proteins, thereby inhibiting apoptosis. On the other hand, excessive autophagy may cause cellular energy metabolic disturbances, thereby activating apoptotic pathways. In KOA, the interplay between autophagy and apoptosis may influence the pathological process of KOA by regulating chondrocyte metabolism and stress responses. Studies have found that the expression levels of autophagy- and apoptosis-related proteins are significantly upregulated in the cartilage cells of KOA patients, indicating that both autophagy and apoptosis are activated in KOA. Autophagy activation may help reduce cellular stress and inhibit apoptosis by clearing damaged organelles and proteins. However, excessive autophagy can lead to cellular energy metabolic disturbances and activate apoptotic pathways. Additionally, apoptosis activation may affect autophagy levels by regulating intracellular signaling pathways. For example, BCL-2 family proteins are involved in apoptosis regulation and interact with Beclin-1 to modulate autophagy levels.[31]

4.4. Correlation between knee osteoarthritis and immune cells

4.4.1. Neutrophils

Neutrophils are the "pioneers" of the innate immune system, possessing multiple functions, such as promoting inflammation, phagocytosing pathogens, and combating infections. In collagenase-induced mouse arthritis models, neutrophils were among the first cells to migrate to the synovium and initiate synovial inflammation.[32] In OA mouse models, depleting neutrophils significantly slowed the progression of OA. Mechanistically, neutrophils release inflammatory mediators, such as IL-6, TNF-α, and neutrophil elastase, thereby exacerbating joint inflammation and participating in cartilage damage.

4.4.2. Macrophages

Macrophages are a heterogeneous group of cells that play crucial roles in maintaining homeostasis, phagocytosing particles, and regulating immunity. Macrophages are divided into classically activated-inflammatory macrophages (M1) and alternatively activated-anti-inflammatory macrophages (M2). In the synovial tissue of OA, macrophages are one of the most abundant inflammatory cells, primarily polarized to the M1 phenotype.[33]

The aggregation, activation, and polarization of macrophages are regulated by chemokines, various intra-articular substances, and cells. The CCL2/CCR2 chemokine

axis is a key factor mediating macrophage aggregation in OA.[34] Intra-articular substances, such as cartilage debris, basic calcium phosphate crystals, and plasma proteins, can activate pattern recognition receptors, like Toll-like receptors, through damage-associated molecular patterns, thereby promoting macrophage activation.[35] Synovial fibroblasts, Th17 cells, and chondrocytes can induce macrophage aggregation, activation, and polarization through different pathways. Specifically, Yes-associated protein 1-mediated enhanced glycolysis in synovial fibroblasts promotes macrophage aggregation and M1 polarization;[36] exosomes from inflammatory synovial fibroblasts enhance macrophage glycolysis to promote M1 polarization;[37] Th17 cells promote macrophage aggregation and M1 polarization via IL-17/monocyte chemoattractant protein 1;[38] chondrocytes promote macrophage IL-1β production through the release of exosome-like vesicles.[39]

Macrophages contribute to OA pathological changes through several mechanisms:

(i) Amplifying synovial inflammation: After removing macrophages from OA synovium, macrophage-derived inflammatory factors IL-1 and TNF-α are significantly reduced, along with a significant decrease in inflammatory factors IL-6 and IL-8 secreted by synovial fibroblasts, and levels of MMP-1 and MMP-3. This indicates that inflammatory factors released by macrophages play a key role in driving synovial inflammation. The reduced efferocytosis function of macrophages leads to the accumulation of synovial apoptotic cells that should be phagocytosed, and the release of their contents activates the immune system, thereby promoting inflammatory responses.
(ii) Promoting cartilage damage: After promoting M1 polarization through genetic engineering, the degree of cartilage damage in OA mice was exacerbated, while promoting M2 polarization alleviated this phenotype. Direct release of various inflammatory factors and matrix-degrading enzymes triggered cartilage inflammation and chondrocyte apoptosis. Activation of the β-catenin pathway in chondrocytes by R-spondin-2, cartilage senescence mediated by IL-18 or exosomes, and inhibition of mesenchymal stem cell chondrogenesis are all essential mechanisms by which M1 macrophages cause cartilage damage.
(iii) Abnormal subchondral bone remodeling: M1 macrophages inhibit osteocyte maturation by suppressing the Notch signaling pathway both *in vitro* and *in vivo*. Immature osteocytes further lead to abnormal bone mineralization, thereby causing subchondral bone remodeling abnormalities.
(iv) Osteophyte formation: After clearing macrophages, osteophyte formation in OA is significantly reduced. Transforming growth factor-beta and bone morphogenetic proteins 2/4 are key signaling molecules mediating osteophyte formation by macrophages.
(v) Inducing neuronal connections: In OA, macrophages highly express IL-1β and TNF-α. Synovial macrophages and synovial fibroblasts stimulated by IL-1β and TNF-α exhibit high expression of nerve growth factor. After clearing macrophages in arthritic mice, nerve growth factor expression in synovial tissue was significantly reduced. This indicates that macrophage-derived IL-1β and TNF-α regulate nerve growth factor expression in synovial tissue, inducing neuronal ingrowth and providing a pathological basis for OA pain.

4.4.3. Mast cells

When specific antigens activate mast cell surface receptors, mast cells undergo degranulation, releasing various inflammatory mediators, such as histamine and tryptase, which serve to aggregate inflammatory cells and regulate immune responses. In the synovial tissue of OA, the number of infiltrating mast cells is significantly increased.[40] Activated mast cells exhibit enhanced degranulation and increased release of histamine and tryptase.[41] In mice with mast cell knockout, the progression of OA is alleviated, suggesting that activated mast cells play a significant role in promoting OA pathological changes.

Immunoglobulins have been confirmed as key factors mediating mast cell activation in OA.[42] After activation, mast cells release inflammatory mediators, such as histamine, tryptase, and TNF-α, to exacerbate synovial and cartilage inflammation and induce cartilage damage. Histamine can cause the upregulation of MMP-3 and MMP-13 in chondrocytes.[43] Tryptase can induce chondrocyte apoptosis, reduce the level of proteoglycan 4, lead to decreased joint fluid, worsen inflammation, and thereby cause cartilage degradation.[44] Mast cells are also found in OA osteophytes, and their role in inflammation amplification and subchondral bone remodeling warrants further study.

4.4.4. Natural killer cells

Natural killer cells are a group of antigen-independent cytotoxic cells. Based on CD56 expression, NK cells can be divided into the $CD56^{dim}$ subtype, which is primarily cytotoxic, and the $CD56^{bright}$ subtype, which mainly secretes cytokines. In OA, the number of infiltrating NK cells increases, and they are significantly activated.[45] Depleting NK cells can reduce the pathological damage of OA in mice. The chemotaxis of neutrophils via the

CXCR3/CXCL10 axis is one of the mechanisms underlying NK cell aggregation in OA. In OA, infiltrating NK cells are of the $CD56^{bright}$ subtype and exhibit stronger secretion functions of interferon and pro-inflammatory granzyme A. This implies that in OA, the role of NK cells is more about regulating other immune cells.[46]

4.4.5. Other innate immune cells

Although dendritic cells, eosinophils, and basophils account for a relatively small proportion of synovial inflammatory cells, and related research is limited, these findings still warrant attention. In OA synovium, the number of infiltrating dendritic cells increased.[47] OA-related basic calcium phosphate crystals can activate dendritic cells. Dendritic cells derived from the knee joint cavity can promote inflammation i*n vitro* through Toll-like receptor-dependent pathways. After pharmacological inhibition of dendritic cells, T cells in OA exhibited inhibitory effects, and joint inflammation was reduced.[48]

Based on these findings, KOA is primarily associated with immune cell activation. KOA is characterized by mitochondrial dysfunction and autophagy regulation.[49] This study stratified samples into different clusters, where samples within each cluster exhibited similar molecular patterns across transcriptomic, proteomic, and other molecular features, achieving the goal of molecular subtyping in large cohorts.[50] Subsequent clustering analysis of the two core genes into 2–10 clusters, along with consistent clustering and immune analyses, confirmed the absence of significant heterogeneity and no differential expression of related genes.

Subsequent investigations should probe more thoroughly into the precise functional roles of these genes within the OA immunological milieu. Several constraints characterize the current analysis. Primarily, the scarcity of publicly available KOA transcriptomic datasets constrained the cohort dimensions, limiting representation of population heterogeneity. Additionally, machine learning predictions exhibited conservative tendencies, while clustering outputs demonstrated substantial variability. Upcoming studies ought to incorporate expanded patient cohorts alongside single-cell resolution approaches. Complementary histological assessments (lipidomics, metabolomics) and experimental validations would further augment insight into disease pathobiology. These results additionally imply that OA pathogenesis involves immunological activation, chemokine-driven signaling cascades, and inflammatory processes, thereby advancing understanding of disease evolution. Such discoveries will contribute to clarifying the immune regulatory framework governing KOA and inform improved therapeutic strategies. Nevertheless, the biological activities of hub genes, their relationships with immune cell infiltration, and the prognostic relevance of infiltration patterns in KOA advancement necessitate additional experimental exploration.

5. Conclusion

This investigation offers a thorough understanding of the pathophysiological processes driving KOA and its relationship to the immunological landscape. Through delineation of DEGs and examination of autophagic and apoptotic cascades, we underscore the probable functions of *SPARC* and *BNIP3* as pivotal molecules regulating disease advancement. The crosstalk between these programmed cell death mechanisms, together with substantial contributions from neutrophils, macrophages, mast cells, and NK cells, illustrates the intricate nature of pathogenesis. Our results indicate that modulating these networks and immune components may yield innovative treatment approaches for clinical intervention.

Notwithstanding these noteworthy findings, several constraints exist, notably the limited patient cohort stemming from scarce publicly available datasets and the cautious approach inherent in algorithmic forecasts. Subsequent investigations ought to prioritize expanded populations and single-cell resolution profiling to better capture disease variability. Moreover, additional exploration is warranted to clarify the precise functions of critical molecules, such as *SPARC* and *BNIP3*, their crosstalk with leukocyte subsets, and the implications of cellular infiltration patterns in pathological progression. Such endeavors will enhance comprehension of this disorder and facilitate the development of improved therapeutic modalities.

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Conflict of interest

The authors declare they have no competing interests.

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Availability of data

All data analyzed have been presented in the paper.

References

1. Sandell LJ. Etiology of osteoarthritis: genetics and synovial joint development. *Nat Rev Rheumatol.* 2012;8(2):77-89.

   doi: 10.1038/nrrheum.2011.199

2. Chen D, Shen J, Zhao W, *et al*. Osteoarthritis: toward a comprehensive understanding of pathological mechanism. *Bone Res.* 2017;5:16044.

   doi: 10.1038/boneres.2016.44

3. Chang L, Liu A, Xu J, *et al*. TDP-43 maintains chondrocyte homeostasis and alleviates cartilage degradation in osteoarthritis. *Osteoarthr Cartil.* 2021;29(7):1036-1047.

   doi: 10.1016/j.joca.2021.03.015

4. Latourte A, Kloppenburg M, Richette P. Emerging pharmaceutical therapies for osteoarthritis. *Nat Rev Rheumatol.* 2020;16(12):673-688.

   doi: 10.1038/s41584-020-00518-6

5. Kang YJ, Yoo JI, Baek KW. Differential gene expression profile by RNA sequencing study of elderly osteoporotic hip fracture patients with sarcopenia. *J Orthop Translat.* 2021;29:10-18.

   doi: 10.1016/j.jot.2021.04.009

6. Li Y, Pan D, Liu S, *et al*. Identification of circ-FAM169A sponges miR-583 involved in the regulation of intervertebral disc degeneration. *J Orthop Translat.* 2020;26:121-131.

   doi: 10.1016/j.jot.2020.07.007

7. Umeno J, Matsumoto T, Fuyuno Y, Esaki M, Torisu T. SLCO2A1 gene is the causal gene for both primary hypertrophic osteoarthropathy and hereditary chronic enteropathy. *J Orthop Translat.* 2021;28:10-11.

   doi: 10.1016/j.jot.2020.12.005

8. Choi WS, Yang JI, Kim W, *et al*. Critical role for arginase II in osteoarthritis pathogenesis. *Ann Rheum Dis.* 2019;78(3):421-428.

   doi: 10.1136/annrheumdis-2018-214282

9. Kuttapitiya A, Assi L, Laing K, *et al*. Microarray analysis of bone marrow lesions in osteoarthritis demonstrates upregulation of genes implicated in osteochondral turnover, neurogenesis and inflammation. *Ann Rheum Dis.* 2017;76(10):1764-1773.

   doi: 10.1136/annrheumdis-2017-211396

10. Kung LHW, Ravi V, Rowley L, *et al*. Cartilage MicroRNA Dysregulation During the Onset and Progression of Mouse Osteoarthritis Is Independent of Aggrecanolysis and Overlaps With Candidates From End-Stage Human Disease. *Arthritis Rheumatol.* 2018;70(3):383-395.

    doi: 10.1002/art.40378

11. Soul J, Dunn SL, Anand S, *et al*. Stratification of knee osteoarthritis: two major patient subgroups identified by genome-wide expression analysis of articular cartilage. *Ann Rheum Dis.* 2018;77(3):423.

    doi: 10.1136/annrheumdis-2017-212603

12. Yuan C, Pan Z, Zhao K, *et al*. Classification of four distinct osteoarthritis subtypes with a knee joint tissue transcriptome atlas. *Bone Res.* 2020;8(1):38.

    doi: 10.1038/s41413-020-00109-x

13. Park R, Ji JD. Unique gene expression profile in osteoarthritis synovium compared with cartilage: analysis of publicly accessible microarray datasets. *Rheumatol Int.* 2016;36(6):819-827.

    doi: 10.1007/s00296-016-3451-1

14. Styrkarsdottir U, Lund SH, Thorleifsson G, *et al*. Meta-analysis of Icelandic and UK data sets identifies missense variants in SMO, IL11, COL11A1 and 13 more new loci associated with osteoarthritis. *Nat Genet.* 2018;50(12):1681-1687.

    doi: 10.1038/s41588-018-0247-0

15. Tuerlings M, van Hoolwerff M, Houtman E, *et al*. RNA Sequencing Reveals Interacting Key Determinants of Osteoarthritis Acting in Subchondral Bone and Articular Cartilage: Identification of IL11 and CHADL as Attractive Treatment Targets. *Arthritis Rheumatol.* 2021;73(5):789-799.

    doi: 10.1002/art.41600

16. Bowman S, Awad ME, Hamrick MW, Hunter M, Fulzele S. Recent advances in hyaluronic acid based therapy for osteoarthritis. *Clin Transl Med.* 2018;7(1):6.

    doi: 10.1186/s40169-017-0180-3

17. Rosshirt N, Hagmann S, Tripel E, *et al*. A predominant Th1 polarization is present in synovial fluid of end-stage osteoarthritic knee joints: analysis of peripheral blood, synovial fluid and synovial membrane. *Clin Exp Immunol.* 2019;195(3):395-406.

    doi: 10.1111/cei.13230

18. Jafri MA, Kalamegam G, Abbas M, *et al*. Deciphering the Association of Cytokines, Chemokines, and Growth Factors

in Chondrogenic Differentiation of Human Bone Marrow Mesenchymal Stem Cells Using an ex vivo Osteochondral Culture System. *Front Cell Dev Biol.* 2020;7:380.

doi: 10.3389/fcell.2019.00380

19. Schaible HG. Nociceptive neurons detect cytokines in arthritis. *Arthritis Res Ther.* 2014;16(5):470.

doi: 10.1186/s13075-014-0470-8

20. Kadam UT, Blagojevic M, Belcher J. Statin use and clinical osteoarthritis in the general population: a longitudinal study. *J Gen Intern Med.* 2013;28(7):943-949.

doi: 10.1007/s11606-013-2382-8

21. Lin R, Deng C, Li X, *et al*. Copper-incorporated bioactive glass-ceramics inducing anti-inflammatory phenotype and regeneration of cartilage/bone interface. *Theranostics.* 2019;9(21):6300-6313.

doi: 10.7150/thno.36120

22. Kos K, Wong S, Tan B, *et al*. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. *Diabetes.* 2009;58(8):1780-1788.

doi: 10.2337/db09-0211

23. Lee DE, Kehlenbrink S, Lee H, Hawkins M, Yudkin JS. Getting the message across: mechanisms of physiological cross talk by adipose tissue. *Am J Physiol Endocrinol Metab.* 2009;296(6):E1210-E1229.

doi: 10.1152/ajpendo.00015.2009

24. Kos K, Wilding JP. SPARC: a key player in the pathologies associated with obesity and diabetes. *Nat Rev Endocrinol.* 2010;6(4):225-235.

doi: 10.1038/nrendo.2010.18

25. Nakamura S, Kamihagi K, Satakeda H, *et al*. Enhancement of SPARC (osteonectin) synthesis in arthritic cartilage. Increased levels in synovial fluids from patients with rheumatoid arthritis and regulation by growth factors and cytokines in chondrocyte cultures. *Arthritis Rheum.* 1996;39(4):539-551.

doi: 10.1002/art.1780390402

26. Ham SM, Song MJ, Yoon HS, Lee DH, Chung JH, Lee ST. SPARC Is Highly Expressed in Young Skin and Promotes Extracellular Matrix Integrity in Fibroblasts via the TGF-β Signaling Pathway. *Int J Mol Sci.* 2023;24(15):12179.

doi: 10.3390/ijms241512179

27. Loeser RF. Molecular mechanisms of cartilage destruction: mechanics, inflammatory mediators, and aging collide. *Arthritis Rheum.* 2006;54(5):1357-1360.

doi: 10.1002/art.21813

28. Ma Z, Wang D, Weng J, Zhang S, Zhang Y. BNIP3 decreases the LPS-induced inflammation and apoptosis of chondrocytes by promoting the development of autophagy. *J Orthop Surg Res.* 2020;15(1):284.

doi: 10.1186/s13018-020-01791-7

29. Brown R. The bcl-2 family of proteins. *Br Med Bull.* 1997;53(3):466-477.

doi: 10.1093/oxfordjournals.bmb.a011624

30. Veron V, Marandel L, Liu J, *et al*. DNA methylation of the promoter region of bnip3 and bnip3l genes induced by metabolic programming. *BMC Genom.* 2018;19(1):677.

doi: 10.1186/s12864-018-5048-4

31. Musumeci G, Castrogiovanni P, Trovato FM, *et al*. Biomarkers of Chondrocyte Apoptosis and Autophagy in Osteoarthritis. *Int J Mol Sci.* 2015;16(9):20560-20575.

doi: 10.3390/ijms160920560

32. Benigni G, Dimitrova P, Antonangeli F, *et al*. CXCR3/CXCL10 Axis Regulates Neutrophil-NK Cell Cross-Talk Determining the Severity of Experimental Osteoarthritis. *J Immunol.* 2017;198(5):2115-2124.

doi: 10.4049/jimmunol.1601359

33. Zhang H, Cai D, Bai X. Macrophages regulate the progression of osteoarthritis. *Osteoarthr Cartil.* 2020;28(5):555-561.

doi: 10.1016/j.joca.2020.01.007

34. Raghu H, Lepus CM, Wang Q, *et al*. CCL2/CCR2, but not CCL5/CCR5, mediates monocyte recruitment, inflammation and cartilage destruction in osteoarthritis. *Ann Rheum Dis.* 2017;76(5):914-922.

doi: 10.1136/annrheumdis-2016-210426

35. Hamasaki M, Terkawi MA, Onodera T, *et al*. Transcriptional profiling of murine macrophages stimulated with cartilage fragments revealed a strategy for treatment of progressive osteoarthritis. *Sci Rep.* 2020;10(1):7558.

doi: 10.1038/s41598-020-64515-1

36. Yang J, Li S, Li Z, *et al*. Targeting YAP1-regulated Glycolysis in Fibroblast-Like Synoviocytes Impairs Macrophage Infiltration to Ameliorate Diabetic Osteoarthritis Progression. *Adv Sci.* 2024;11(5):e2304617.

doi: 10.1002/advs.202304617

37. Liu B, Xian Y, Chen X, *et al*. Inflammatory Fibroblast-Like Synoviocyte-Derived Exosomes Aggravate Osteoarthritis via Enhancing Macrophage Glycolysis. *Adv Sci.* 2024;11(14):e2307338.

doi: 10.1002/advs.202470078

38. Hsieh SL, Yang SY, Lin CY, *et al*. MCP-1 controls IL-17-promoted monocyte migration and M1 polarization in osteoarthritis. *Int Immunopharmacol.* 2024;132:112016.

doi: 10.1016/j.intimp.2024.112016

39. Ni Z, Kuang L, Chen H, *et al*. The exosome-like vesicles

from osteoarthritic chondrocyte enhanced mature IL-1β production of macrophages and aggravated synovitis in osteoarthritis. *Cell Death Dis.* 2019;10(7):522.

doi: 10.1038/s41419-019-1739-2

40. de Lange-Brokaar BJ, Kloppenburg M, Andersen SN, *et al*. Characterization of synovial mast cells in knee osteoarthritis: association with clinical parameters. *Osteoarthr Cartil.* 2016;24(4):664-671.

doi: 10.1016/j.joca.2015.11.011

41. Buckley MG, Walters C, Wong WM, *et al*. Mast cell activation in arthritis: detection of alpha- and beta-tryptase, histamine and eosinophil cationic protein in synovial fluid. *Clin Sci.* 1997;93(4):363-370.

doi: 10.1042/cs0930363

42. Wang Q, Lepus CM, Raghu H, *et al*. IgE-mediated mast cell activation promotes inflammation and cartilage destruction in osteoarthritis. *eLife.* 2019;8:e39905.

doi: 10.7554/eLife.39905

43. Tetlow LC, Woolley DE. Histamine stimulates matrix metalloproteinase-3 and -13 production by human articular chondrocytes in vitro. *Ann Rheum Dis.* 2002;61(8):737-740.

doi: 10.1136/ard.61.8.737

44. Das N, de Almeida LGN, Derakhshani A, *et al*. Tryptase β regulation of joint lubrication and inflammation via proteoglycan-4 in osteoarthritis. *Nat Commun.* 2023;14(1):1910.

doi: 10.1038/s41467-023-37598-3

45. Kriegova E, Manukyan G, Mikulkova Z, *et al*. Gender-related differences observed among immune cells in synovial fluid in knee osteoarthritis. *Osteoarthr Cartil.* 2018;26(9):1247-1256.

doi: 10.1016/j.joca.2018.04.016

46. Wang H, Zeng Y, Zhang M, *et al*. CD56brightCD16- natural killer cells are shifted toward an IFN-γ-promoting phenotype with reduced regulatory capacity in osteoarthritis. *Hum Immunol.* 2019;80(10):871-877.

doi: 10.1016/j.humimm.2019.07.283

47. Qiu G, Zhong S, Xie J, *et al*. Expanded CD1c+CD163+ DC3 Population in Synovial Tissues Is Associated with Disease Progression of Osteoarthritis. *J Immunol Res.* 2022;2022:9634073.

doi: 10.1155/2022/9634073

48. Wang G, Zhang J, Fang Y, Cao W, Xu B, Chen X. Stimulation of tolerogenic dendritic cells using dexamethasone and 1,25-dihydroxyvitamin D3 represses autologous T cell activation and chondrocyte inflammation. *Exp Ther Med.* 2019;17(1):679-688.

doi: 10.3892/etm.2018.7036

49. Chen P, Zheng L, Wang Y, *et al*. Desktop-stereolithography 3D printing of a radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration. *Theranostics.* 2019;9(9):2439-2459.

doi: 10.7150/thno.31017

50. Stewart PA, Welsh EA, Slebos RJC, *et al*. Proteogenomic landscape of squamous cell lung cancer. *Nat Commun.* 2019;10(1):3578.

doi: 10.1038/s41467-019-11452-x