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Development and validation of biomarkers related to PANoptosis in osteoarthritis

Y.-Y. ZHANG[1], H.-S. ZHAO[1], Y.-F. SUN[1], B.-W. LU[2], L. SUN[3]

[1]First Clinical Medical College, Shandong University of Traditional Chinese Medicine, Jinan, China
[2]College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China
[3]Department of Microorthopedics, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China

Yueyang Zhang, Yuefeng Sun and Hongsen Zhao contributed equally to this work

Abstract. – OBJECTIVE: Osteoarthritis (OA) is a high-incidence disease of the orthopedic system. However, studies on the molecular mechanisms of OA and pyroptosis, apoptosis, and necroptosis (PANoptosis) at the transcriptome level remain scarce. Therefore, this study purposed to detect biomarkers in OA and explore their relationship to the immune microenvironment.

MATERIALS AND METHODS: OA-related expression data was sourced from the Gene Expression Omnibus (GEO) database. Subsequently, differentially expressed analysis and a Venn diagram were performed to obtain differentially expressed PANoptosis-related genes (DEPGs). Furthermore, the least absolute shrinkage and selection operator (LASSO), Support Vector Machine-Recursive Feature Elimination (SVM-RFE), and random forest (RF) were implemented to screen diagnostic genes. Receiver operating characteristic (ROC) curves were performed to verify the diagnostic ability of the diagnostic genes. Next, immune infiltration analysis was performed to find the relationships between differential immune cells (OA *vs*. normal) and diagnostic genes. Finally, drug prediction analysis was also carried out, and the expression of diagnostic genes was verified in external datasets.

RESULTS: A total of 62 DEPGs were identified, which were enriched for regulating apoptotic signaling pathways, tumor necrosis factor (TNF) signaling pathways, and other related pathways. Three feature genes, nuclear factor-kappa-B inhibitor-alpha *(NFKBIA)*, RING finger protein 34 *(RNF34)*, and serine incorporator 3 *(SERINC3)* were obtained by intersecting genes obtained by the LASSO regression algorithm, SVM algorithm, and RF algorithm and showed excellent diagnostic efficacy with the Area under the curve (AUC) values of individual genes were all greater than 0.7, indicating that the model was more effective. Immuno-infiltration analysis showed that *RNF34* was positively correlated with CD56^{dim} natural killer cells, type 17 helper T cells, and *NFKBIA* was positively correlated with plasmacytoid dendritic cells. Additionally, 12 drugs were predicted by *NFKBIA*, such as gambogic acid and dioscin. In addition, *NFKBIA* and *SERINC3* were significantly downregulated, and *RNF34* was upregulated in OA samples.

CONCLUSIONS: Three genes (*NFKBIA*, *RNF34*, and *SERINC3*) related to PANoptosis, were obtained by bioinformatics analysis, which would provide a new direction for the diagnosis and treatment of OA.

Key Words:
Osteoarthritis, PANoptosis, Diagnostic genes, Immune infiltration.

Introduction

Osteoarthritis (OA) is a chronic joint disease characterized by articular cartilage degeneration, cartilage ossification, and secondary bone hyperplasia[1], which mainly involves changes in articular cartilage, subchondral bone, ligament, capsule, synovial membrane, and muscle tissue structure around the joint. More than 250 million people are affected in the world, in the future, the prevalence rate will increase due to various factors, including the population ages, obesity rates rise, and traumatic knee injury rates increase[2,3]. At present, the pathogenesis of OA is still unclear, and the treatment is mainly to relieve symptoms, including reducing pain, relieving stiffness, and maintaining joint function to improve quality of life[4]. According to the progression of the disease, the main clinical interventions include low-intensity aerobic exercise, weight control, acupuncture treatment, oral non-steroidal anti-inflammatory drugs (NSAIDs), intra-articular injection of hyaluronic acid (IA), and joint replacement in the late stage[5,6]. According to the guidelines of the International Association for the Study of Os-

Corresponding Author: Lu Sun, MD; e-mail: Sunludr@163.com

teoarthritis (OARSI)[7], topical NSAIDs are strongly recommended, but intra-articular injection, oral NSAIDs, proton pump inhibitors, and cyclooxygenase-2 (COX-2) inhibitors are conditionally recommended. The American College of Rheumatology (ACR) guidelines[8] strongly recommends gait assistance, intra-articular injections of glucocorticoids, and weight loss to treat OA. However, NSAIDs and glucocorticoids can cause many side effects, such as upper gastrointestinal bleeding and liver damage, and are prohibited from long-term use. Joint replacement is a feasible treatment option for patients with advanced OA, but rigorous preoperative examination and high surgical costs have eliminated most OA patients[9]. Therefore, it is important and necessary to explore the pathogenesis of OA and find effective treatment targets for early diagnosis, improve the quality of life of patients, and provide appropriate treatment for patients.

Pyroptosis, apoptosis, and necroptosis (PANoptosis) is a unique regulated cell death (RCD) pathway of innate immune inflammation, which is essential for activating cell apoptosis, changing ECM homeostasis, and causing inflammatory reaction[10]. The occurrence of OA is related to the degradation and synthesis imbalance of articular cartilage, extracellular matrix (ECM), and subchondral bone[11,12]. Cartilage loss, impaired chondrocyte matrix, and altered chondrocyte ECM has been shown[4,13,14] to be core evidence for the development of OA. The molecular composition of the ECM is altered in the early stage of OA, which produces catabolic factors involved in chondrocyte degradation[15]. In the process of OA development, chondrocyte PANtoptosis is a key factor in cartilage degeneration[16,17]. With the continuous apoptosis and destruction of proteoglycan, articular cartilage is eventually completely lost, resulting in direct contact between bones to cause a range of problems such as pain and restricted joint movement[18,19]. At the same time, the chondrocyte is the only cell component of cartilage, and its survival plays a decisive role in the occurrence and development of OA. There are many ways of chondrocyte death, for example, apoptosis is usually related to cell contraction, while necrotic apoptosis is related to cell swelling and cell content leakage[20]. PANoptosis, as a completely new concept, is currently arguably the most complex form of cell death, encompassing both pyroptosis, apoptosis, and necroptosis. PANoptosis has been proven[21] to be related to many physiological and pathological processes and can cause excessive inflammation, cytokine storms, and tissue and organ damage, which may be fatal. A growing body of evidence[22] highlights the comprehensive association between PANoptosis and OA. Unfortunately, it has long been thought that the pyroptosis, apoptosis, and/or necroptosis pathways operate separately. Recent studies[23] have shown that PANoptosis is an inflammatory programmed cell death regulated by the PANoptosome complex. PANoptosome is a multi-protein complex containing key proteins that activate pyrodeath, apoptosis, and programmed necrosis. In the process of PANoptosis, *NLRP3, CASP8, RIPK1/RIPK3* complex, and other cell death effect molecules can be activated simultaneously. It eventually leads to lytic death of inflammatory cells[24,25]. Previous studies[24,25] have confirmed that PANoptosome can play a regulatory role in the cell cycle and inflammatory response by affecting the formation of membrane pores of Gasdermin D (GSDMD) and Gasdermin E (GSDME) proteins, the formation and phosphorylation of mitochondrial outer membrane permeability (MOMP) and mixed spectrum kinase domain protein (MLKL). Nevertheless, it is still unknown which PANoptosis-related genes are crucial to the development of OA. Therefore, the determination of relevant biomarkers for the treatment of OA based on the potential PANoptosis-related genes involved in OA may help predict the risk of OA and carry out early diagnosis and treatment.

The development of bioinformatics and machine learning strategies has enabled the exploration of underlying mechanisms and potential biomarkers[26-28]. In this study, after obtaining the differentially expressed genes related to PANoptosis, three diagnostic genes were further excavated by three machine learning algorithms. Then, the nomogram was established based on these genes to predict the risk of OA. In addition, functional enrichment, clinical correlation, and immune infiltration analyses were implemented to explore the relationship between diagnostic genes and OA. In summary, three genes, *NFKBIA*, *RNF34*, and *SERINC3,* related to PANoptosis were screened out by bioinformatic methods, which might provide a new direction for the diagnosis and treatment of OA.

Materials and Methods

Data Source

The GSE48556 dataset and the GSE55457 dataset were sourced from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. Of these, the GSE48556 dataset includes blood mRNA expression microarray data of 106 OA and 33 normal samples. The GSE55457 data-

set includes sequencing data from synovial tissue of 10 control and 10 OA samples. These samples have clinical characteristics data such as age, disease state, gender, etc. In addition, GSE12021 (synovial tissue, 10 OA *vs*. 9 normal) and GSE55235 (synovial tissue, 10 OA *vs*. 10 normal) were utilized to validate the expressions of diagnostic genes. Moreover, 680 apoptosis-related genes (ARGs), 52 necroptosis-related genes (NRGs), 35 pyroptosis-related genes (PRGs), and 10 cuproptosis-related genes (CRGs) were obtained from published literature[29-32]. Furthermore, 34 ferroptosis-related genes (FRGs) were obtained from the FerrDb database (http://www.zhounan.org/ferrdb). These 811 genes were known as PANoptosis-related genes[33].

Acquisition of Differentially Expressed PANoptosis-Related Genes (DEPGs)

Differential analysis was performed on the GSE48556 dataset using the limma package to obtain differentially expressed genes (DEGs, $p < 0.05$ and $|Log_2FC| > 0.5$) between OA and normal samples[34]. Then, DEGs were crossed with ARGs, NRGs, PRGs, CRGs, and FRGs, respectively, and combined to obtain DEPGs. In addition, the clusterProfiler package was utilized for the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis of DEPGs ($p < 0.05$ and count > 2)[35].

Acquisition of Diagnostic Genes

In the GSE48556 dataset, we used the glmnet package, setting the parameters family to binomial and type.measure to class, to implement the least absolute shrinkage and selection operator (LASSO) logistic regression to obtain the feature genes[36]. Then, Support Vector Machine-Recursive Feature Elimination (SVM-RFE) and random forest (RF) were used to filter the feature genes, respectively[37,38]. The diagnostic genes were obtained by crossing the feature genes obtained by the three machine learning algorithms. After that, to evaluate the effectiveness of the diagnostic model, receiver operating characteristic (ROC) curves of the diagnostic model were plotted, and ROC curves were plotted separately for individual diagnostic genes to evaluate the effectiveness of the diagnostic genes in distinguishing between OA and control samples in the GSE48556 and GSE55457 datasets.

Construction of a Nomogram

The diagnostic model for diagnostic markers was constructed using the lrm function in the rms package, and a nomogram was drawn to predict the risk score of patients. In addition, the calibration curve and decision curve were plotted using the rms package and the dcurves package, respectively, to evaluate the accuracy of the nomogram.

Clinical Correlation Analysis and Single Gene Set Enrichment Analysis (GSEA)

First, this study compared the expression of diagnostic genes in different clinical features (OA *vs*. control, female *vs*. male, age ≤ 60 *vs*. age > 60). Then, the correlation coefficients of diagnostic genes with all genes were calculated and ranked, and GSEA was performed using the clusterProfiler package (available at: https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html)[39].

Immuno-Infiltration Analysis and Drug Prediction Analysis

Single-sample gene set enrichment analysis (ssGSEA) was employed to get immune cells in OA and normal samples and compared by Wilcoxon test. Next, the relationships between differential immune cells and diagnostic genes were calculated by Spearman's correlation analysis. Next, diagnostic genes were entered into the Drug-Gene Interaction Database (DGIdb, https://dgidb.org/), and a drug-gene interaction network was built. Finally, the abundance of key genes in the GSE48556, GSE55457, GSE12021, and GSE5523 datasets was further validated.

Statistical Analysis

All open databases and R packages (Vienna, Austria) were utilized to analyze and visualize this study. Comparisons between the two groups were implemented using the Wilcoxon test. The determination of significance had been detailed in the corresponding materials and methods. If there are no special instructions, $p < 0.05$ is considered to be significant.

Results

62 DEPGs and Functional Enrichment Analysis

There were 1,091 genes in the OA group compared to normal samples (Figure 1A-B). 45 differentially expressed ARGs, 7 differentially expressed NRGs, 3 PRGs, and 17 FRGs were crossed to get 62 DEPGs (Figure 1C-G). Enrichment analysis showed that 62 DEPGs were enriched to 1,096 GO functions and 33 KEGG

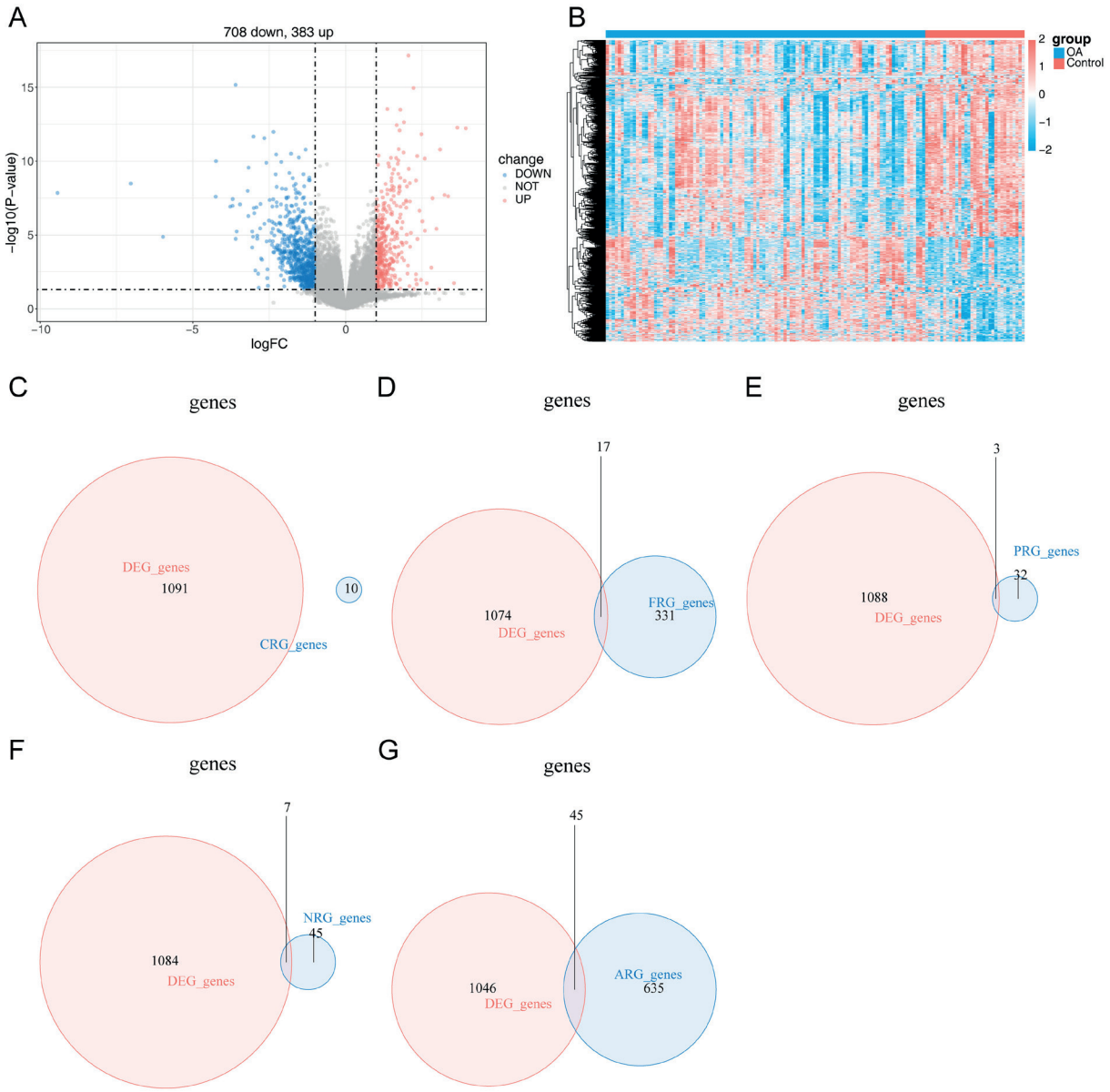

Figure 1. Differential expression analysis. **A**, The volcano plot of 1091 DEGs in the osteoarthritis group compared to normal samples. **B**, The expression heatmap of DEGs in osteoarthritis and normal samples. DEGs, differentially expressed genes. Identification of 62 DEPGs. **C**, Venn diagram of DEGs and 10 CRGs. **D**, Venn diagram of 17 intersected genes between DEGs and FRGs. **E**, Venn diagram of 3 intersected genes between DEGs and PRCs. **F**, Venn diagram of 7 intersected genes between DEGs and NRGs. **G**, Venn diagram of 45 intersected genes between DEGs and ARGs. CRGs, cuproptosis-related genes; ARGs, apoptosis-related genes; NRGs, necroptosis-related genes; PRGs, pyroptosis-related genes; FRGs, ferroptosis-related genes; DEPGs, differentially expressed PANoptosis-related genes; DEGs, differentially expressed genes.

pathways; for example, GO pathways, such as the regulation of apoptotic signaling pathway, regulation of intrinsic apoptotic signaling pathway, and KEGG pathways, such as apoptosis, tumor necrosis factor-alpha (TNF) signaling pathway, and nod-like receptor signaling pathway (Figure 2A-B).

NFKBIA, RNF34, and SERINC3 Were Diagnostic Genes

In the LASSO regression analysis, 12 feature genes were screened for a lambda.min of 0.0223: *IL1B*, *XBP1*, *GZMB*, *NFKBIA*, *PTRH2*, *CTNNA1*, *CX3CR1*, *E2F2*, *ERN1*, *PLEKHF1*, *RNF34*, *SERINC3* (Figure 3A). 54 feature genes were screened

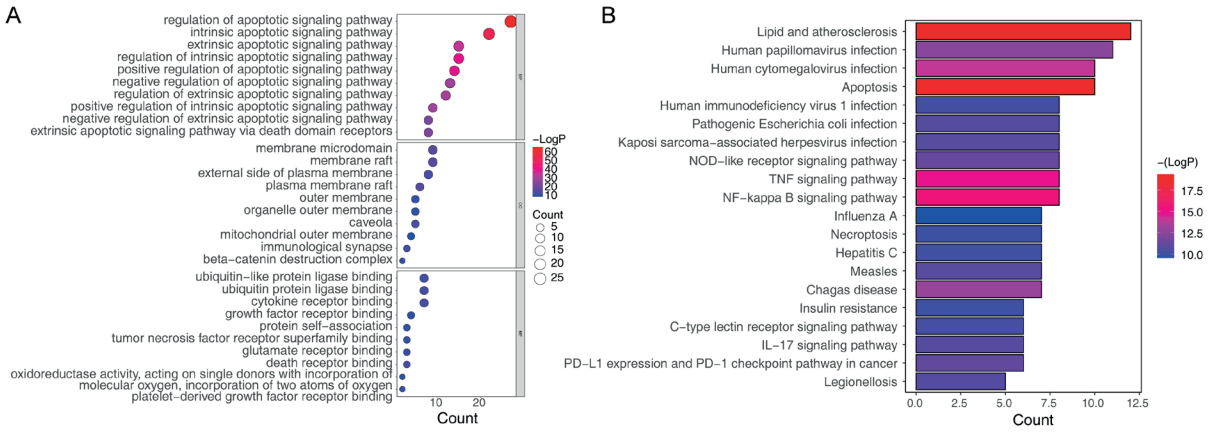

Figure 2. Functional enrichment analysis of DEPGs. **A**, Bubble plot of top 30 GO terms enriched for DEPGs. **B**, Bar plot of top 20 KEGG pathways enriched for DEPGs. GO: Gene Ontology; BP: biological progress, CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEPGs, differentially expressed PANoptosis-related genes.

by the SVM-RFE algorithm (Figure 3B). 8 feature genes (*RNF34*, *SERINC3*, *GSK3B*, *NFKBIA*, *PLEKHF1*, *FBXW7*, *PIAS4*, and *FADD*) were obtained by the RF algorithm (Figure 3C-D). A total of three diagnostic genes were obtained after crossover, namely *NFKBIA*, *RNF34*, and *SERINC3* (Figure 3E). The area under the curve (AUC) values of the diagnostic model in the GSE48556

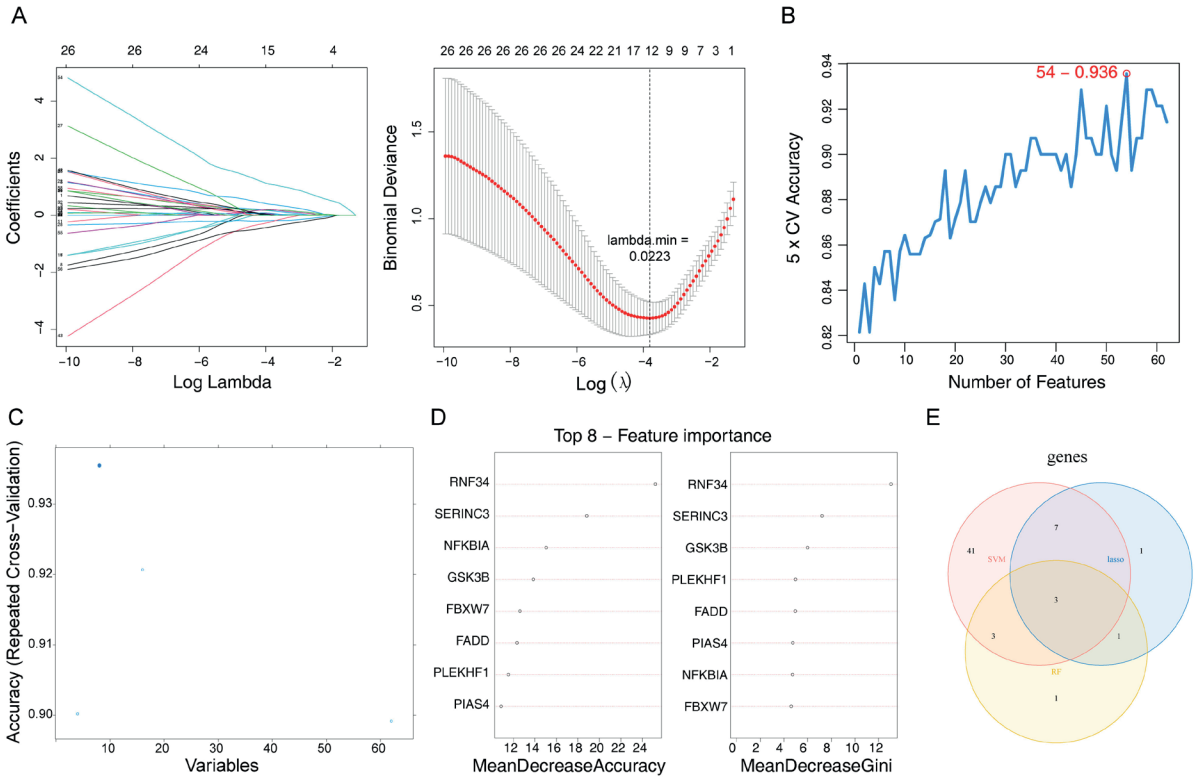

Figure 3. Confirmation of three diagnostic genes. **A**, LASSO regression analysis and cross-validation based on 62 DEPGs. **B**, 54 feature genes were identified using SVM-RFE algorithm. **C**, Cross-validation for tuning the parameter selection in the RF algorithm. **D**, 8 feature genes were obtained by the RF algorithm. **E**, Venn diagram of 3 diagnostic biomarkers by intersecting the results of SVM-RFE, LASSO regression, and RF algorithms. LASSO, least absolute shrinkage, and selection operator; SVM-RFE, Support Vector Machine-Recursive Feature Elimination; RF, random forest; DEPGs, differentially expressed PANoptosis-related genes.

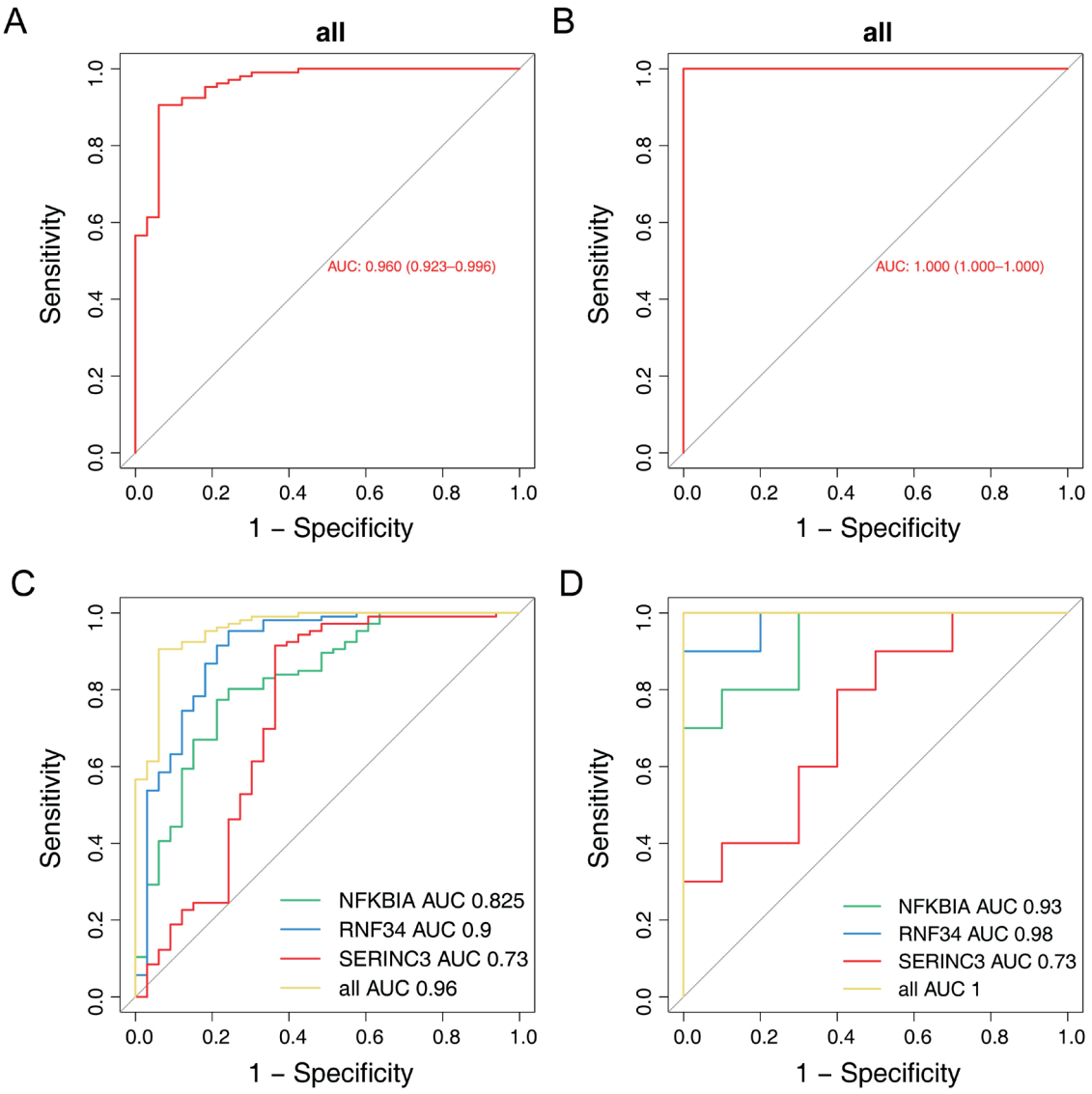

Figure 4. Evaluation of the diagnostic values of three biomarkers. **A**, ROC curve of the diagnostic model in the GSE48556 dataset. **B**, ROC curve of the diagnostic model in the GSE55457 dataset. **C**, ROC curves of three biomarkers in the GSE48556 dataset. **D**, ROC curves of three biomarkers in the GSE55457 dataset. ROC, receiver operating characteristic.

and GSE55457 were 0.960 and 1, respectively, indicating the good discriminatory efficacy of the model (Figure 4A, 5B). In addition, the AUC values of all three diagnostic genes were greater than 0.7 (Figure 4C-D).

Construction and Evaluation of a Nomogram

NFKBIA, *RNF34*, and *SERINC3* constitute the nomogram to predict the risk of death in cases (Figure 5A). The slope of the calibration curve was close to 1 (Figure 5B). The benefit rates of the nomogram were higher in all the decision curves than in the other models, indicating the better effect of the nomogram (Figure 5C).

Clinical Correlation Analysis and GSEA

All three diagnostic genes were significantly different between OA and control groups (Figure 6A). *NFKBIA* and *RNF34* were also differentially expressed between the sexes. Moreover, *RNF34* was higher in patients with > 60 (Figure 6B). GSEA showed that *NFKBIA* and *RNF34* were related to platelet activation. *RNF34* and *SERINC3* were enriched in the noncoding RNA

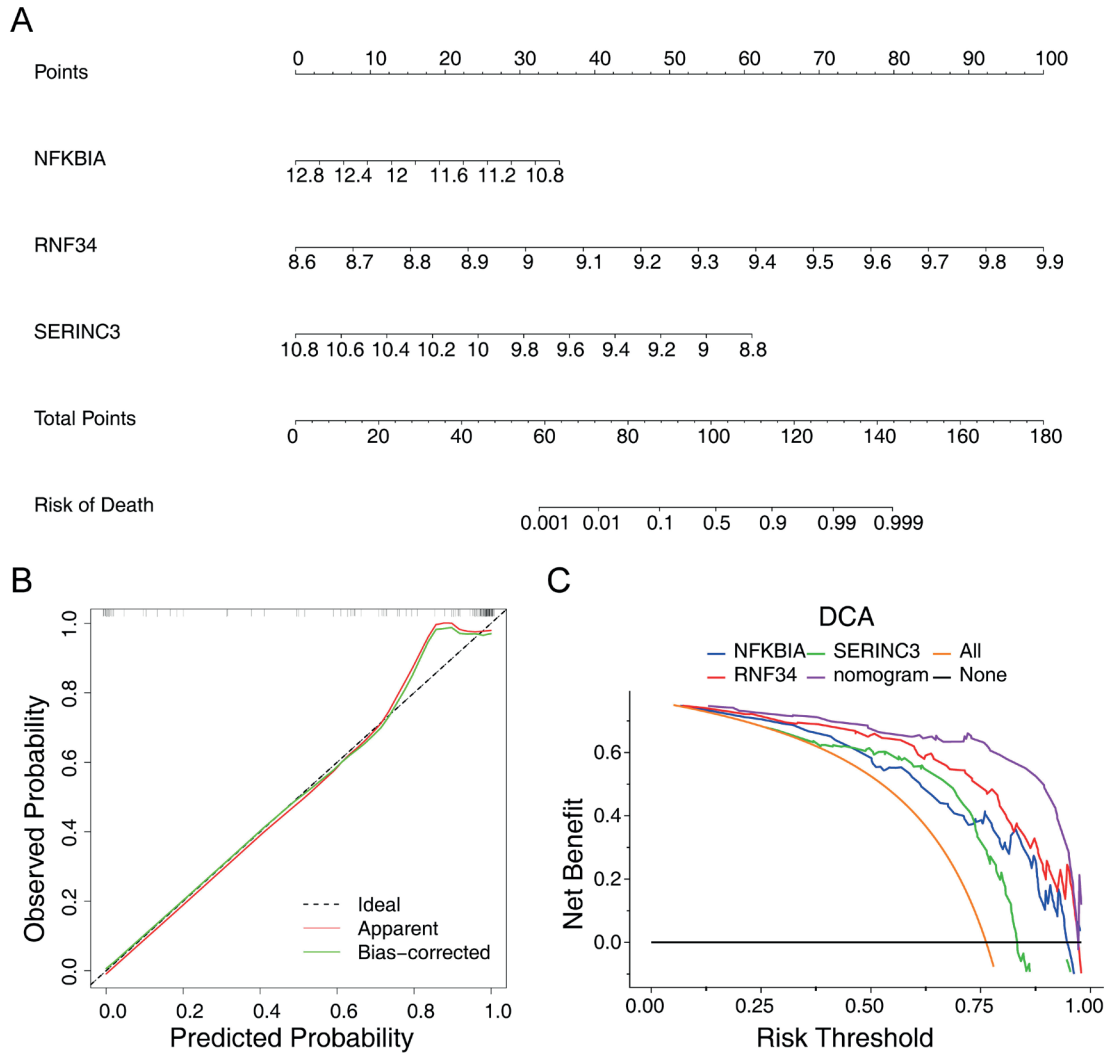

Figure 5. Development and validation of a nomogram. **A**, A diagnostic nomogram was developed based on three biomarkers. **B**, Calibration plot of the nomogram. **C**, DCA curve of the nomogram. DCA, decision curves.

(ncRNA) metabolic process and nucleocytoplasmic transport and ECM-receptor interaction. Moreover, *SERINC3* was associated with autophagy, cell cycle, valine, leucine and isoleucine degradation (**Supplementary Figure 1A-C**).

Immuno-Infiltration Analysis and Drug Prediction Analysis

Heat maps of 28 immune cells in OA and control groups were shown in Figure 7A. $CD56^{dim}$ natural killer cell, Central memory CD8 T cell, myeloid-derived suppressor cell (MDSC), Plasmacytoid dendritic cell, follicular helper T cell, and Type 17 helper T cell were significantly different between OA and normal samples (Figure 7B). In addition, *RNF34* was positively related to $CD56^{dim}$ natural killer cell, Type 17 helper T cell, and *NFKBIA* was positively collated with plasmacytoid dendritic cell. *SERINC3* had a negative correlation with follicular helper T cell, MDSC, and Central memory CD8 T cell (Figure 7C). Moreover, *NFKBIA* predicted 12 drugs, such as gambogic acid, dimethylwedelolactone, peperomin E, and dioscin, etc. (Figure 8A). Finally, in the GSE48556 dataset, *NFKBIA* and *SERINC3* were down-regulated, and *RNF34* was up-regulated in OA (Figure 8B). The expressions of *NFKBIA* and *RNF34* were verified in the GSE55457, GSE12021, and GSE55235 datasets (Figure 8C-E).

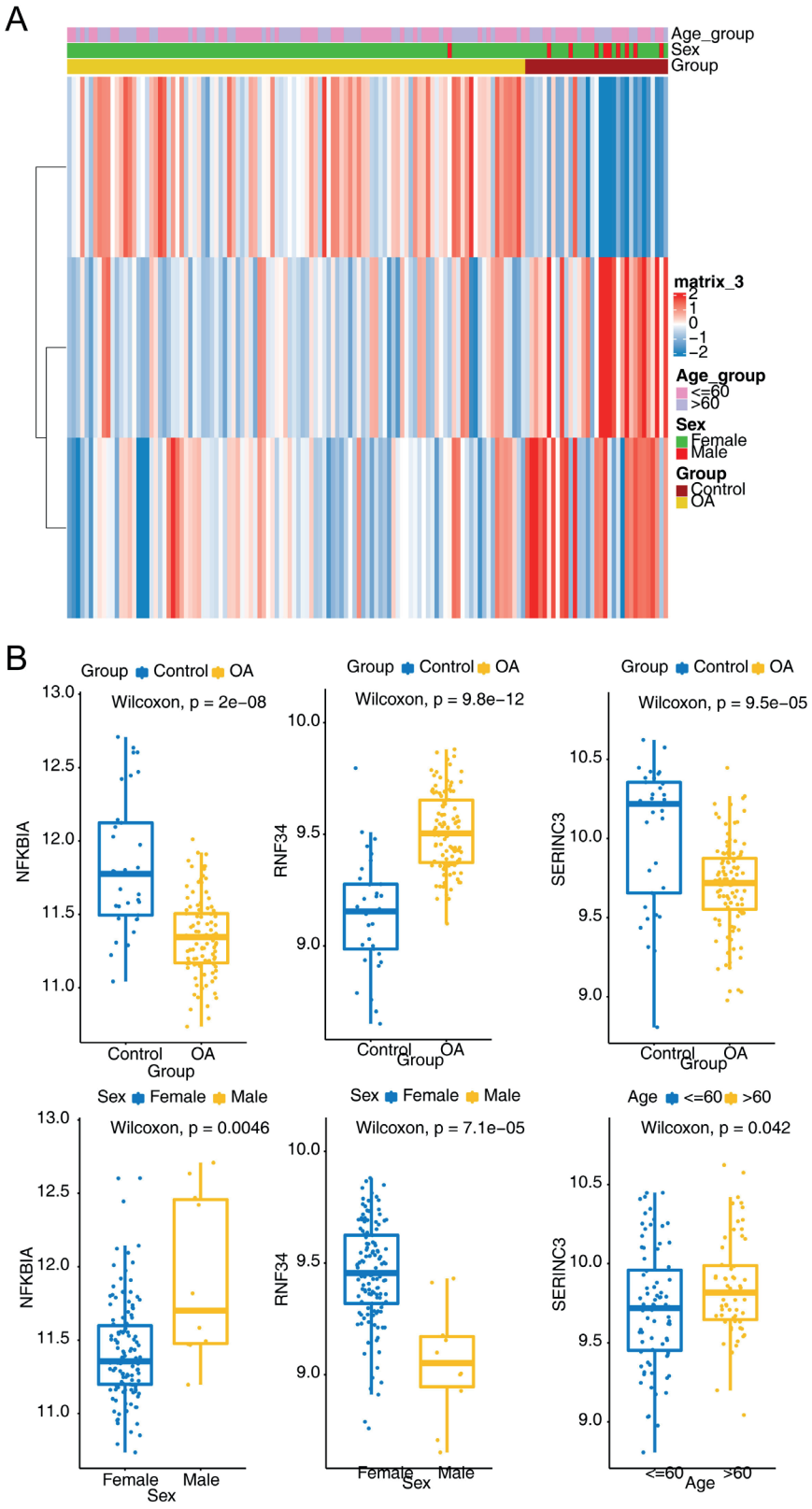

Figure 6. Correlation analysis of clinical traits and three biomarkers. **A**, Heatmap of the correlations between the expression of three biomarkers and clinic features. **B**, Box plots of the correlations of three biomarkers with the group, age, and sex of osteoarthritis.

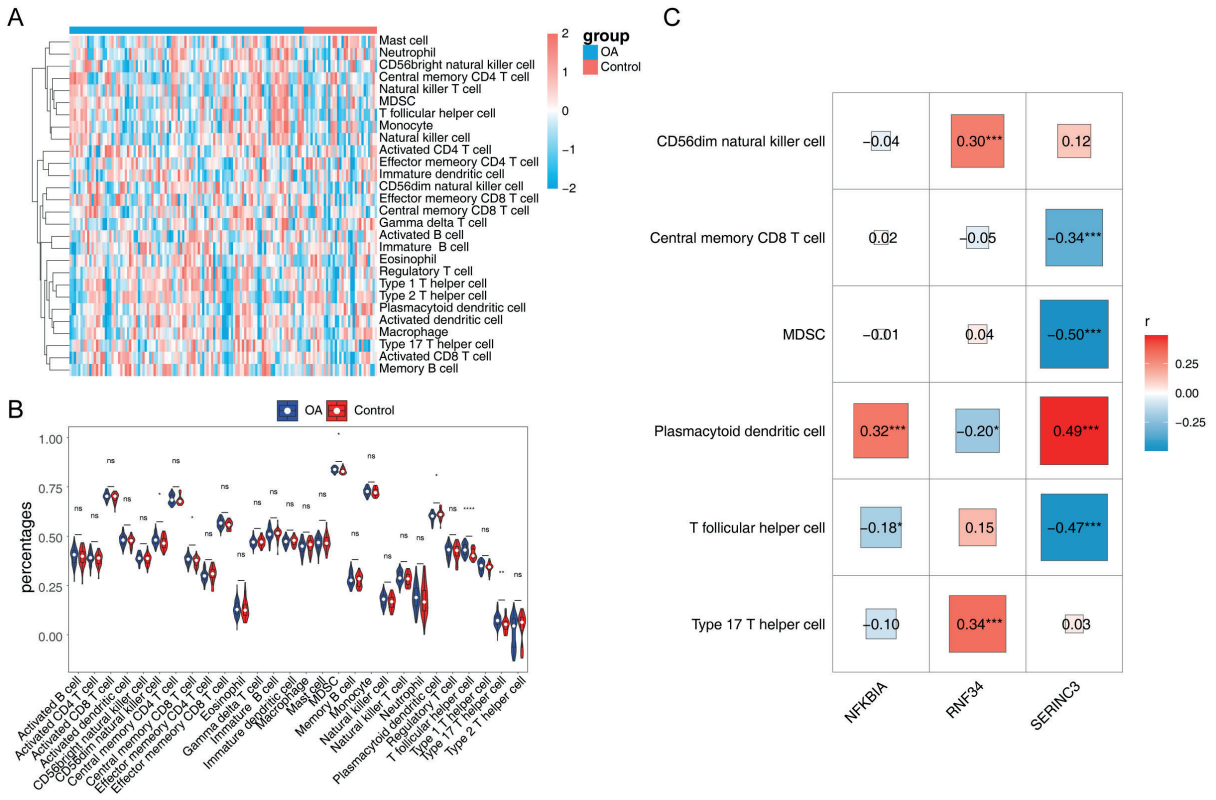

Figure 7. Immuno-infiltration analysis. **A**, Heatmap of the proportion of 28 immune cells in osteoarthritis and control groups. **B**, The percentages of 28 immune cells in osteoarthritis and control samples. ns, no significant difference; *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$. **C**, The correlation of immune cells and three biomarkers. *, $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$.

Discussion

PANoptosis is the current research direction to explore new biomarkers and therapeutic targets, but the current research is still incomplete. In this study, we identified 3 characteristic genes associated with PANoptosis that inspired our understanding of the relationship between PANoptosis and OA. At the same time, we searched for new treatment options by immune infiltration analysis, which may help to discover new biomarkers and therapeutic targets. In this study, we first screened DEPGs between OA samples and normal samples from the GSE48556 dataset, among which 383 differentially up-regulated genes and 708 differentially down-regulated genes were detected. After the intersection of genes related to the action of DEGs and PANoptosis, 62 differentially expressed genes of OA related to the action of PANoptosis were obtained.

Subsequently, GO and KEGG enrichment analyses were performed on these 62 DEPGs to investigate the potential biological function of these differentially expressed DEPGs. GO, and KEGG pathway analysis showed that these DEGs were mainly enriched in the apoptosis signaling pathway, nuclear factor kappa B (NF-κB) signaling pathway, intrinsic apoptosis signaling pathway, TNF signaling pathway, human cytomegalovirus infection, lipid, and atherosclerosis. It is well known that the chondrocyte cycle and the degree of inflammatory response play a decisive role in the progression of OA. Studies[40,41] have shown that interferon gene stimulating factor STING, hypoxia-inducible factor 2 alpha (HIF-2α) and catabolic enzyme can be regulated through the NF-κB signaling pathway, thus delaying PANoptosis of chondrocytes and improving ECM metabolism of chondrocytes. In addition, TNF-α has been shown[42,43] to be closely related to the proliferation and differentiation of osteoblasts (OB) and the activation of fibroblast-like synovial cells (FLS), thus participating in the pathogenesis of OA. Sevin et al[44] suggested that hypercholesterolemia could cause atherosclerotic cartilage injury by inducing lipid oxidation and deposition. It

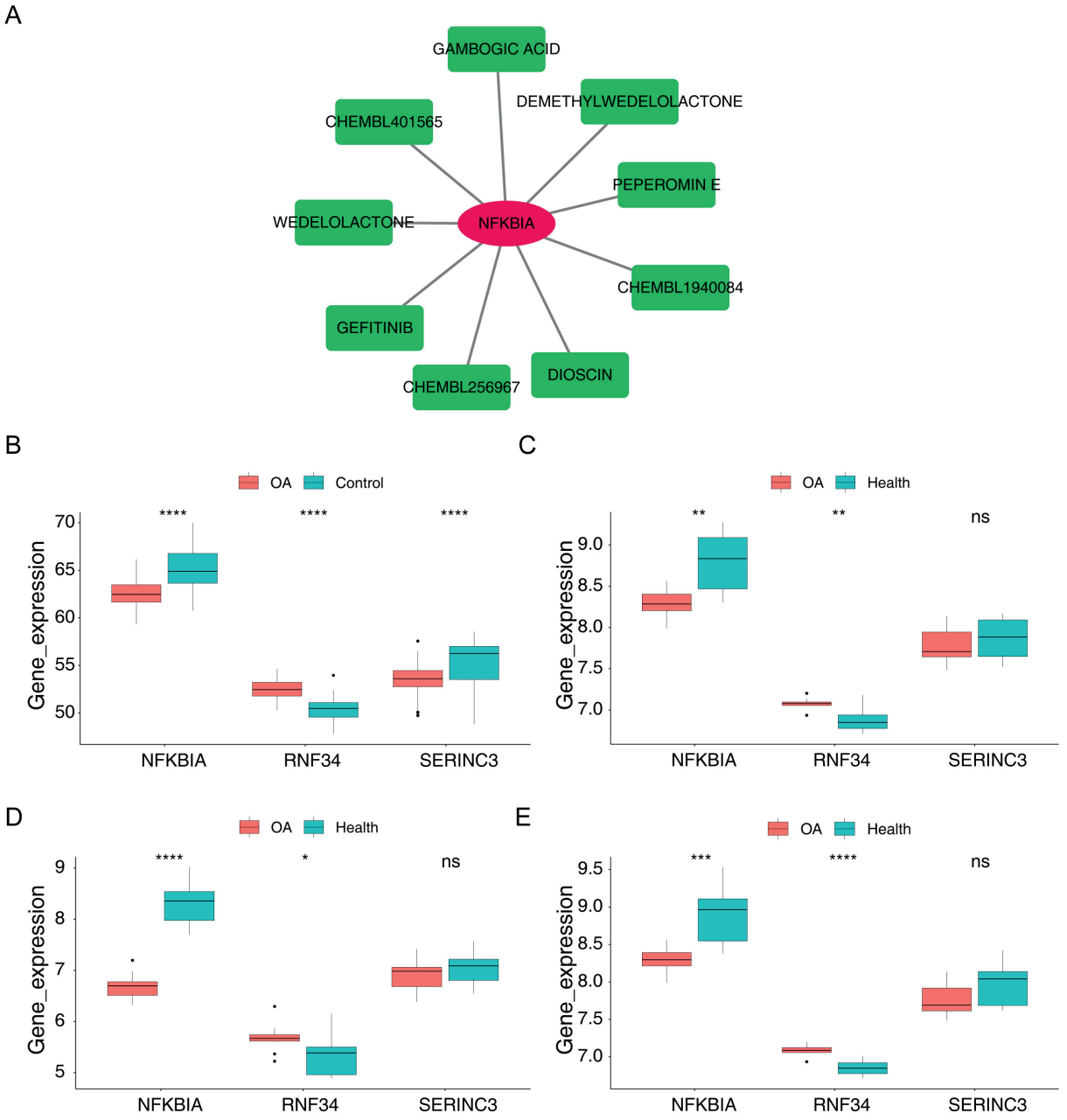

Figure 8. **A**, Drug-gene interaction network. Validation of the expression of three biomarkers. **B**, The expression of three biomarkers between osteoarthritis and control samples in the GSE48556 dataset. **C**, Validation of the expression of three biomarkers between osteoarthritis and control samples in the GSE55457 dataset. **D**, Validation of the expression of three biomarkers between osteoarthritis and control samples in the GSE12021 dataset. **E**, Validation of the expression of three biomarkers between osteoarthritis and control samples in the GSE55235 dataset.

is worth noting that high serum cholesterol levels are closely related to the accumulation of lipids in chondrocytes and the severity of OA, suggesting that OA may be a complication of hypercholesterolemia[45].

In order to further evaluate the prognostic significance of these genes, we used LASSO regression to screen out 12 potential DEPGs associated with OA and obtained three genes associated with the survival prognosis of patients with OA, namely *NFKBIA, RNF34,* and *SERINC3*. Current research evidence[46,47] suggests that *NFKBIA* prevents PANoptosis in cells by inhibiting the accumulation of substrates such as metalloproteinases (MMP) and ADAMT. When *NFKBIA* is inhibited, it leads to the accumulation of degrading proteases in cells,

aggravates intraarticular inflammatory responses, and induces PANoptosis in chondrocytes[48,49]. However, Sivertsen et al[50] found that *NFKBIA* could induce the transcription of bone morphogenetic protein (BMP), promote the differentiation of specific mesenchymal cells, and thus promote the proliferation of chondrocytes and osteoblasts. Whitty et al[51] found in the experiment that pBMP-2 and pBMP-7 could not only promote chondrocyte proliferation but also synthesize the main component of extracellular matrix glycosaminglycans (GAGs). Since the pathogenesis of OA is still unclear, the above evidence[51] has suggested that *NFKBIA*, one of the genes involved in the action of PANoptosis, may be related to inflammatory response, chondrocyte survival cycle, and cartilage ECM homeostasis. Although our results show that *NFKBIA*, as a downregulated gene in the OA group, may delay the development of OA, so far, it is not known whether *NFKBIA* plays a positive or negative role in OA, which can be used as a new target for the study of the pathogenesis of OA.

Ring finger protein 34 (*RNF34*) is an E3 ubiquitin ligase that not only inhibits angiogenesis and remodeling, skeletal muscle function, and energy metabolism but also promotes oxidative stress response[52,53]. Inflammation and oxidative stress have been proven[54,55] to play a key role in the development of OA. Le Bourhis et al[56] found that *RNF34* could participate in inhibiting apoptosis by ubiquitinating Caspase 8/10. In addition, *RNF34* directly encodes RING finger domain proteins targeting the degradation of nucleotide-binding oligomerization domain protein 1 (NOD1), thereby down-regulating the expression of cytokines associated with inflammatory responses. In conclusion, the *RNF34* protein is involved in cell apoptosis and inflammatory response of one of PANoptosis, providing a theoretical basis for our results. Our results indicate that *RNF34* is an up-regulated gene in the OA group. Unfortunately, the specific role of *RNF34* in OA has not been discovered so far, which can be used as a fresh idea in future research.

As a distinct family of proteins, Serine-binding protein 3 (*SERINC3*) is highly conserved in eukaryotes due to its minimal homology[57]. Zeng et al[58] found that *SERINC3* can promote the production of type I interferon (IFNs) and activate the NF-κB inflammatory signaling pathway. However, there are few studies on *SERINC3* in OA. Previous studies[59] have shown that *SERINC3* is involved in many physiological and pathological environments, including cellular PANoptosis and inflammatory response. Since the onset of OA is closely related to PANoptosis and inflammatory response, we speculate that the high expression of *SERINC3* may aggravate OA, which can be used as a brand-new target for further research.

In order to accurately assess the activity of immune cells in the microenvironment of diseased tissues, immune-infiltration analysis was performed, in which MDSC and monocytes accounted for the largest proportion of abundance. ssGSEA results show that $CD56^{dim}$ natural killer cell, central memory CD8 T cell, MDSC, plasmacytoid dendritic cell, T follicular helper cell, Type17 helper cell immune cell score was different between the disease and the control group, reflecting the difference in immune function between the disease and normal samples. Dendritic cells (DCS), derived from MDSC, are the most powerful antigen-presenting cells in the human body. An animal experiment[60] confirmed that the over-expression of DC was closely related to the severity of intraarticular inflammation. In addition, pDC, as the main producer of IFN-I, can directly kill irregular cells and activate NK cells to induce cell apoptosis[61-63], thus accelerating the aging of chondrocytes and PANoptosis[64,65]. Alahdal et al[66] also proposed a new immunotherapy for intraarticular injection of tolerant pDC to control the progression of OA inflammation. Th17 cells mainly secrete IL-17a, TNF-α, and other cytokines to aggravate the inflammatory response in OA patients[67,68]. Ohtori et al[69] also confirmed that the TNF-α inhibitor etanercept can effectively inhibit the progression of OA inflammation. Meanwhile, Th17 can also cause odd bone resorption and subchondral heterotopic ossification[70,71], and accelerate the degeneration of articular cartilage[72]. Monasterio et al[73] found that T follicular helper cells can cause a dynamic imbalance of Th1/Th2 cells, accelerate inflammatory response, and trigger activation of apoptosis-related pathways. In conclusion, intraarticular inflammatory response and chondrocyte PANoptosis were positively correlated with OA progression.

Subsequently, we included age and gender as variables to construct a diagnostic model with three biomarkers and predicted the survival probability of OA patients. The results showed that the three genes were distinct in the disease group, *NFKBIA, RNF34,* and *SERINC3* genes were distinctive in age, and *RNF34* and *NFKBIA* were different in sex. As it is known, advanced age is one of the most important risk factors for OA. Its incidence and prevalence increase significantly with the increase of age, among which, the prevalence

of OA among people over 40 years old is about 22%, one-third of the elderly suffer from OA, and the proportion of females is higher than males[74].

Finally, we entered three core genes into the DGIDB database and predicted 12 drugs through *NFKBIA*. Previous studies[75,76] have confirmed that Wedelolactone can promote osteoblast proliferation and inhibit the inflammatory response. A recent new study[77] has shown that Wedelolactone can also inhibit the enhancer of zeste homolog 2 (EZH2) and promote chondrogenic differentiation of MSCs by activating the FOXO1 signaling pathway. This is consistent with the results of our study. Wedelolactone can improve PANoptosis of cartilage tissue in patients with OA and is expected to reverse the irreversible damage of cartilage tissue in OA. Gambogic acid can inhibit the expression of chemokine receptor 4 (*CXCR4*) in chondrocytes to reduce PANoptosis in chondrocytes and delay the process of OA[78]. Due to the poor prognosis, high variability, and unpredictability of OA progression, effective risk classification and treatment strategies are needed to develop personalized targeted therapies. Our study links the pathogenesis of OA with the action of PANoptosis based on bio-information technology, which is innovative in OA-related studies so far and provides a good theoretical basis for OA. However, there are still some shortcomings in our study. First, our study is a retrospective study based on the samples from public databases, and the sample size needs to be further expanded. Then, the findings in our study need to be further validated by experiments. The efficacy of targeted therapeutic agents for OA predicted by the database needs to be further explored in the clinical setting. In addition, the molecular mechanisms of three genes (*NFKBIA*, *RNF34*, and *SERINC3*) needs to be further explored in OA, and this is the focus of our next research.

Conclusions

In conclusion, we identified three key genes (*NFKBIA*, *RNF34*, and *SERINC3*) related to PANoptosis, which had great predictive value in OA. The diagnostic model based on these three genes showed excellent diagnostic and predictive performance. Additionally, the results of immune infiltration revealed that differential immune cells between OA and normal samples might be linked to the occurrence and development of OA, especially T cells. Our results will provide a new theoretical basis for studying the role of PANoptosis in OA and provide new targets for treating OA patients.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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Ethics Approval

Not applicable.

Informed Consent

Not applicable.

Availability of Data and Materials

The datasets generated during the current study are available in the Gene expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/, accession codes: GSE55457, GSE48556) repository.

Authors' Contributions

We declare that this work was done by the authors named in this article, and the authors will bear all liabilities claims relating to the content of this article.

ORCID ID

Yueyang Zhang: 0000-0002-2889-4806
Yuefeng Sun: 0009-0000-6181-4209
Hongsen Zhao: 0009-0003-0839-0234
Bowen Lu: 0009-0001-6224-9317
Lu Sun: 0009-0004-4303-8276.

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