sciendo Revista Română de Medicină de Laborator 2025;33(1):41–50 RESEARCH ARTICLE
DOI:10.2478/rrlm-2025-0003

A diagnostic classifier for osteoarthritis constructed based on cuprotosis-related genes

Jia Xiaopeng[1], Chen Honglu[1], Li An[2]

1. Department of Orthopedics, The First People's Hospital of Yongkang, China
2. Department of Orthopedics, The Affiliated TCM Hospital of Guangzhou Medical University, China

ABSTRACT

Background: Osteoarthritis (OA), a common degenerative joint disease, is pathologically characterized by joint pain and functional limitation. Cuprotosis-related genes (CRGs) exert vital biological effects on various diseases, but their functions in OA remain largely unknown. We aimed to explore the potential role of CRGs in OA and to establish a diagnostic classifier.

Methods: The Gene Expression Omnibus database was firstly employed to collect data sets on several controls and OA samples. Batch correction was conducted using RobustRankAggreg and sva package to remove the systematic errors between different batches of sequencing. The limma package was utilized to screen differentially expressed genes, and CRGs were identified through Pearson correlation analysis.

Results: A total of 2,033 CRGs were identified after analyzing several data sets. Through Least Absolute Shrinkage and Selection Operator COX model and support vector machine-recursive feature elimination classifier, 6 crucial CRGs were finally determined, including biglycan, Ephrin-A3, leukemia inhibitory factor, natural killer cell granule protein 7, stimulator of chondrogenesis 1 and tumor necrosis factor, alpha-induced protein 3. The integrated analysis on these genes revealed that they had high prediction performance. The area under the curve was 0.772 in the training set and 0.693 in the validation set. These crucial CRGs exhibited significant correlations with the infiltration of M2 macrophages, resting mast cells and other immune cells.

Conclusions: A diagnostic classifier for OA was successfully constructed based on CRGs, and significant associations are found between crucial CRGs and immune microenvironment in OA.

Keywords: cuprotosis, diagnostic marker, immune infiltration, osteoarthritis

Received: 22 October 2024; Accepted: 17 December 2024; Published: 6 January 2025.

INTRODUCTION

Osteoarthritis (OA) is a common disease pathologically characterized by articular cartilage degeneration, chondrocyte hypertrophy and differentiation, subchondral bone thickening, synovial inflammation and osteophyte formation [1], with manifestations of joint stiffness, pain and dysfunction, which is not only a common type of arthritis, but also the primary cause of joint pain and disability [2]. OA is attributed to various factors, including age, a history of joint injury, obesity, heredity, gender and anatomical factors, but its exact causal mechanism has not been fully understood [3,4]. In clinical practice, non-steroidal anti-inflammatory drugs and intra-articular steroid injections have been extensively used to treat OA-related pain and inflammation, with limited efficacy and high toxicity [2]. Over 250 million people worldwide suffer from OA [5]. It not only exerts an impact on people's quality of life, but also imposes a heavy social and economic burden [2,6]. Currently, the early diagnosis of OA is crucial, which, however, is difficult. OA is mainly diagnosed based on radiographic examinations and clinical manifestations. However, OA is usually diagnosed only at the onset of clinical symptoms, when structural damage invariably occurs [7]. Notably, it is difficult to distinguish OA from other arthritis diseases since they share similar clinical symptoms and pathological characteristics [8]. Arthritis has such common manifestations as inflammation, deterioration of connective tissue function, pain and stiffness [9], which will not only lead to severe diagnosis confusion, but also delay the application of the best treatment. Collectively, there are now no available reliable markers with high sensitivity and specificity for early and accurate diagnosis of OA, highlighting the need for specific biomarkers.

* Corresponding author: Li An, Department of Orthopedics, The Affiliated TCM Hospital of Guangzhou Medical University, China;. E-mail: lianatcmhgmu@chi-edu.cn

Cuprotosis, a novel programmed cell death mechanism discovered recently, is defined as cell death owing to the progression of cytotoxic stress caused by protein lipidation induced by continuous accumulation of free copper element in cells [10,11]. Lipid peroxidation accumulation is a risk factor for the progression of OA. At present, studies on cuprotosis-related genes in OA are rare, and only two studies have investigated the role of CRGs in OA. Lv et al. [12] conducted a study on the role of cuprotosis-related regulatory factors in OA diagnosis and subtype, identified two molecular subtypes (family A and family B) with cuprotosis characteristics, and found that OA patients with family B characteristics had higher expression levels of interleukin 2 (IL-2), IL-4 and IL-5 together with helper T cell 1 (Th1)/Th2 imbalance in vivo. Chang et al. [13] investigated the relationship between CRGs and immune cell infiltration activity in OA in their study. However, the mechanism of cuprotosis in OA remains unclear. It is of great significance to elaborate the mechanism of action of CRGs in OA.

In this study, controls and OA samples were collected from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), and cuprotosis-related genes (CRGs) were then screened through bioinformatics methods. Thereafter, screening of key biomarkers was carried out via the machine learning algorithm, followed by construction of a genetic diagnostic classifier for OA, and immune infiltration analysis was completed to explore the correlation between it and immune cells, so as to render new strategies and directions for the treatment of OA. Finally, 6 crucial genes were identified as potential biomarkers related to cuprotosis in OA.

METHODS

Data collection

In the GEO database, relevant data were retrieved with "Osteoarthritis" as the key word. Finally, the following data sets were included into subsequent analysis: GSE12021 (consisting of 9 controls and 10 OA samples, sequencing platform: GPL96/GPL97), GSE1919 (including 5 controls and 5 OA samples, sequencing platform: GPL91), GSE29746 (including 11 controls and 11 OA samples, sequencing platform: GPL4133), GSE55235 (incorporating 10 controls and 10 OA samples, sequencing platform: GPL96), GSE55457 (including 10 controls and 10 OA samples, sequencing platform: GPL96) and GSE89408 (consisting of 28 controls and 22 OA samples, sequencing platform: GPL11154). The sample data in GSE12021, GSE1919, GSE29746, GSE55235 and GSE55457 were incorporated as a training set, and those in GSE89408 acted as a validation set. In addition, 19 CRGs were acquired for subsequent analysis with reference to the research of Tsvetkov et al. [10].

Identification of CRGs in OA

Because the training set was composed of sequencing data from different sequencing platforms, the systematic errors between different batches of sequencing should be removed first. In brief, batch correction analysis was conducted on the five data sets in the training set using RobustRankAggreg package (RRA, https://cran.r-project.org/web/packages/RobustRankAggreg/index.html) and sva package (https://bioconductor.org/packages/release/bioc/html/sva.html) (R language code) through RRA and Batch methods [14,15]. Then, the limma package (https://bioconductor.org/packages/release/bioc/html/limma.html) (R language code) was utilized for differential expression analysis on sequencing expression profiles of controls and OA samples in the training set, with logFC<-1 or logFC>1 and adP<0.05 as the screening thresholds, and finally the differentially expressed genes (DEGs) in OA were obtained. Thereafter, Pearson correlation analysis was implemented between DEGs in OA and the 19 CRGs, and CRGs in OA were identified with cor>0.5 and P<0.05 as the screening criteria.

Construction of OA genetic diagnostic classifier

The overlapping CRGs were obtained by intersecting the RRA group, the Batch group and the CRGs. Next, the overlapping CRGs were analyzed by Least Absolute Shrinkage and Selection Operator (LASSO) COX using the "glmnet" package (https://glmnet.stanford.edu/articles/glmnet.html) (R language code) in the training set, and the optimal model was acquired by the penalty term λ compression coefficient [16]. In addition, the "e1071" package (https://cran.r-project.org/web/packages/e1071/index.html) (R language code) was employed to establish a support vector machine-recursive feature elimination (SVM-RFE) classifier, and the feature vectors generated by SVM were used for feature selection to obtain crucial CRGs [17]. Afterwards, the intersection between feature genes extracted by the above two algorithms was utilized as biomarkers to construct a diagnostic classifier for OA. Next, a violin plot of CRG expression levels was plotted with the "ggpubr" package (https://cran.r-project.org/web/packages/ggpubr/index.html) (R language code), followed by t test with P<0.05 considered statistically significant [18]. After that, to assess the diagnostic value of biomarkers in OA, the receiver operating characteristic (ROC) curves of samples distinguished by the genetic diagnostic classifier were plotted using the "pROC" package (https://cran.r-project.org/web/packages/pROC/index.html) (R language code) for both the training set and the validation set, and the performance of the genetic diagnostic classifier was evaluated based on the area under the curve (AUC) [19].

Correlation analysis between immune cells and CRGs in OA

The "CIBERSORT" package (https://github.com/Moonerss/CIBERSORT) (R language code) was used to calculate the infiltration abundance of 22 kinds of immune cells in OA samples and controls [20]. Pearson analysis was implemented to figure out the correlation between the expression level of CRGs in the genetic diagnostic classifier for OA and the infiltration of the 22 immune cell types.

RESULTS

DEGs and CRGs identified in OA

Firstly, batch effect elimination was accomplished in the five training data sets through RRA and Batch to reduce systematic errors. Finally, 158 DEGs (|logFC>1| and adP<0.05) and 111 DEGs (|logFC>1| and adP<0.05) were identified by RRA and Batch in OA, respectively. The first 20 up-regulated DEGs and 20 down-regulated DEGs in the two methods are shown in the heat map of gene expression level (Figure 1A and B). According to Pearson correlation analysis, 2,033 CRGs were acquired from the expression profile of OA samples.

CRGs identified in OA and diagnostic classifier for OA constructed

By intersecting the RRA group, the Batch group and CRGs, 18 differently expressed CRGs were obtained (Figure 2A). The LASSO COX model displayed the smallest fitting degree when the penalty parameter λ=-0.359, and six potentially critical CRGs were acquired, namely biglycan (BGN), Ephrin-A3 (EPHA3), leukemia inhibitory factor (LIF), natural killer cell granule protein 7 (NKG7), stimulator of chondrogenesis 1 (SCRG1), and tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) (Figure 2B). The SVM-RFE model had the smallest RMSE when the eigenvalue was 16, and 16 potentially critical CRGs were obtained, namely TNFAIP3, VEGFA, SCRG1, BGN, EPHA3, LRCH1, PTN, OLR1, LIF, NKG7, NR4A2, CXCL3, NELL1, LRRC15, IL1R2 and KIAA0754 (Figure 2C). Totally six crucial CRGs (BGN, EPHA3, LIF, NKG7, SCRG1 and TNFAIP3) existed in both the LASSO COX model and the SVM-RFE model (Figure 2D). With these six crucial CRGs, a genetic diagnostic classifier for OA was constructed. The AUC value of the classifier for identifying OA samples was 0.772 [95% confidence interval (CI) = 0.666-0.865] in the training set (Figure 2E) and 0.693 (95% CI = 0.541-0.831) in the validation set (Figure 2F), suggesting good diagnostic performance of the classifier in both sets. Moreover, it was uncovered that the expression levels of BGN, EPHA3, NKG7, and SCRG1 were significantly higher in OA samples than those in controls, whereas the expression levels of LIF and TNFAIP3 were significantly lower in OA samples than those in controls (Figure 2G-L).

Correlation between immune microenvironment and CRGs in OA

The CIBERSORT algorithm was employed for analysis on the infiltration abundance of immune cells in the immune microenvironment of OA samples and controls. The results showed that the abundance of plasma cells, M2 macrophages and resting mast cells in OA samples was higher than that in controls (Figure 3A and B). Subsequently, the correlation between the six crucial CRGs in the genetic diagnostic classifier for OA and immune cells was analyzed in this study. It was revealed that the expression level of BGN had a significant positive correlation with the activity of M0 macrophages and a significant negative relation to eosinophils, plasma cells and resting dendritic cells (P<0.05) (Figure 3C). The expression level of EPHA3 was significantly positively correlated with neutrophils and resting mast cells and significantly negatively associated with eosinophils, plasma cells and resting dendritic cells (P<0.05) (Figure 3D). The LIF expression level exhibited a significantly positive correlation with naïve B cells and M1 macrophages and a significantly negative relation to resting dendritic cells, memory B cells and eosinophils (P<0.05) (Figure 3E). A significantly positive correlation was found between NKG7 expression level and T follicular helper cells, while a significantly negative correlation was detected between it and neutrophils (P<0.05) (Figure 3F). SCRG1 and memory B cells were significantly negatively correlated (P<0.05) (Figure 3G). TNFAIP3 expression level showed significantly positive associations with resting memory CD4+ T cells and memory B cells (P<0.05) (Figure 3H).

DISCUSSION

Cuprotosis is triggered by the accumulation of copper in mitochondria, leading to oxidative stress and mitochondrial dysfunction [21]. Genes involved in copper transport and metabolism can influence cuprotosis in chondrocytes, the key cells in cartilage, by disrupting copper levels and triggering cellular death pathways [22]. For example, CTR1 and SLC31A1, which encode copper influx transporters, are vital for copper uptake into cells. Dysregulation of these transporters can lead to either copper deficiency or overload, both of which can impair mitochondrial function and elevate oxidative stress [23,24]. This makes them central to the pathophysiology of cuprotosis, as copper dysregulation can enhance chondrocyte susceptibility to this form of cell death, contributing to cartilage degradation. Furthermore, genes like ATP7A, SLC25A3, CCS, and COX17, which are involved in copper handling within mitochondria, directly influence cuprotosis through maintaining mitochondrial copper homeostasis. Impaired function of these genes can exacerbate oxidative damage in cartilage, promoting the activation of cuprotosis [25,26].

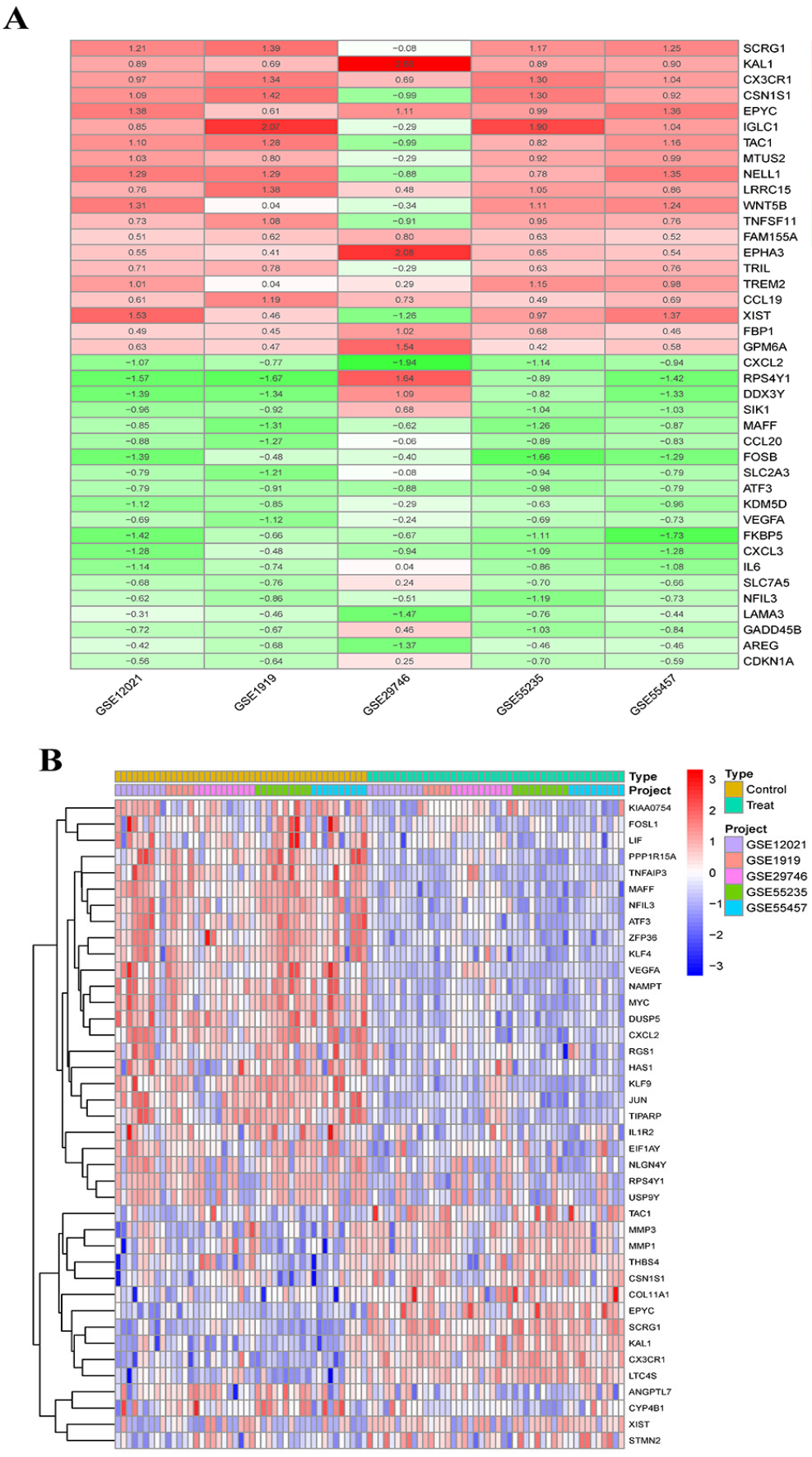

Figure 1. DEGs and CRGs identified in OA

A: Heat map of the first 20 up-regulated DEGs and 20 down-regulated DEGs based on the difference analysis after batch effect elimination by RRA. B: Heat map of the first 20 up-regulated DEGs and 20 down-regulated DEGs based on the difference analysis after batch effect elimination by Batch.

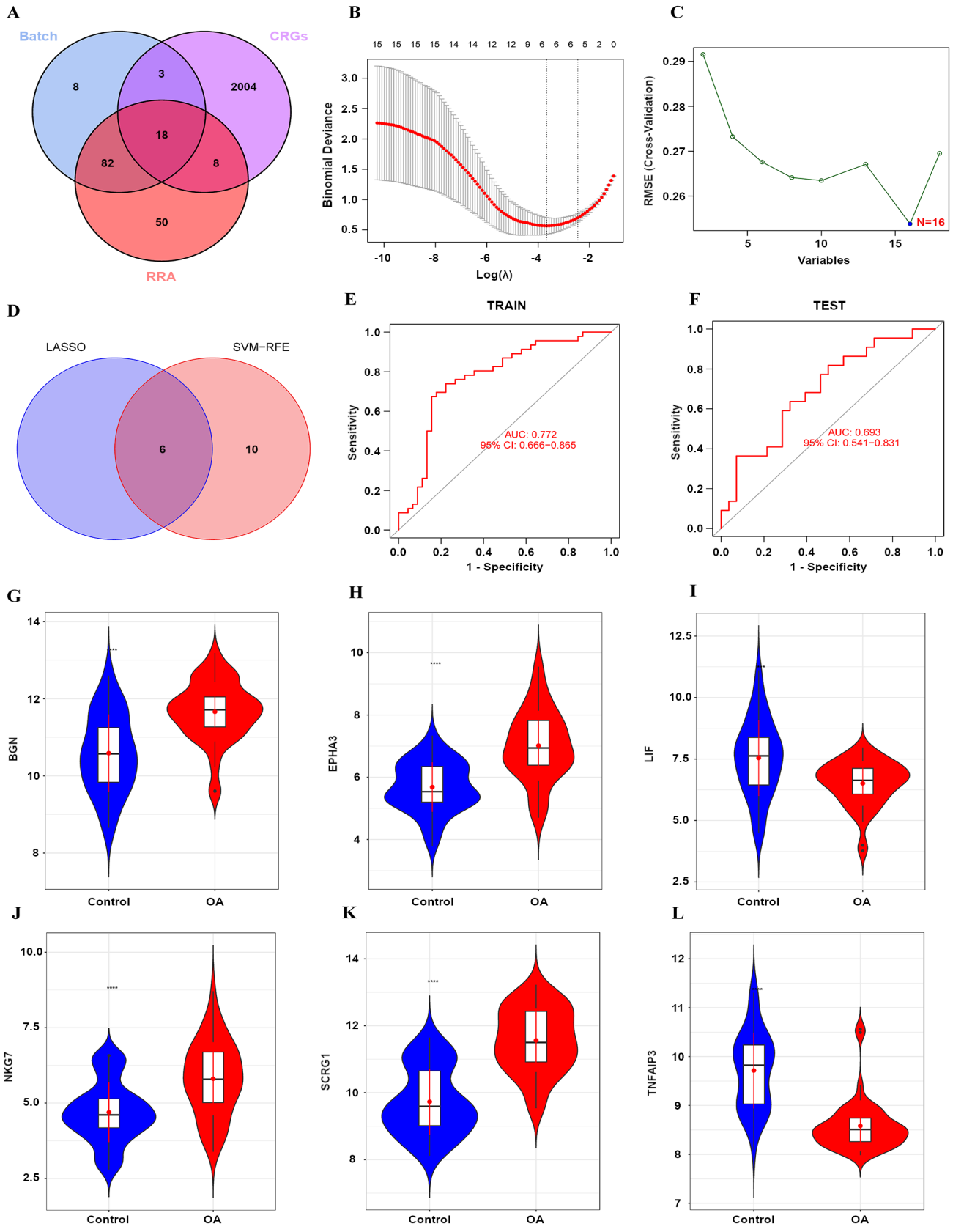

Figure 2. CRGs identified in OA and diagnostic classifier for OA constructed

A: Venn diagram showing 18 overlapping CRGs obtained by intersecting the RRA group, the Batch group and CRGs. B: CI of the best penalty parameter (λ) in LASSO COX model. C: Feature screening curve in SVM-RFE analysis. D: Overlapping CRGs in LASSO COX model and SVM-RFE model. E: ROC curve of genetic diagnostic classifier for diagnosis in the training set. F: ROC curve of genetic diagnostic classifier for diagnosis in the validation set. G: Violin plot of BGN expression level in OA samples and controls. H: Violin plot of EPHA3 expression level in OA samples and controls. I: Violin plot of LIF expression level in OA samples and controls. J: Violin plot of NKG7 expression level in OA samples and controls. K: Violin plot of SCRG1 expression level in OA samples and controls. L: Violin plot of TNFAIP3 expression level in OA samples and controls.

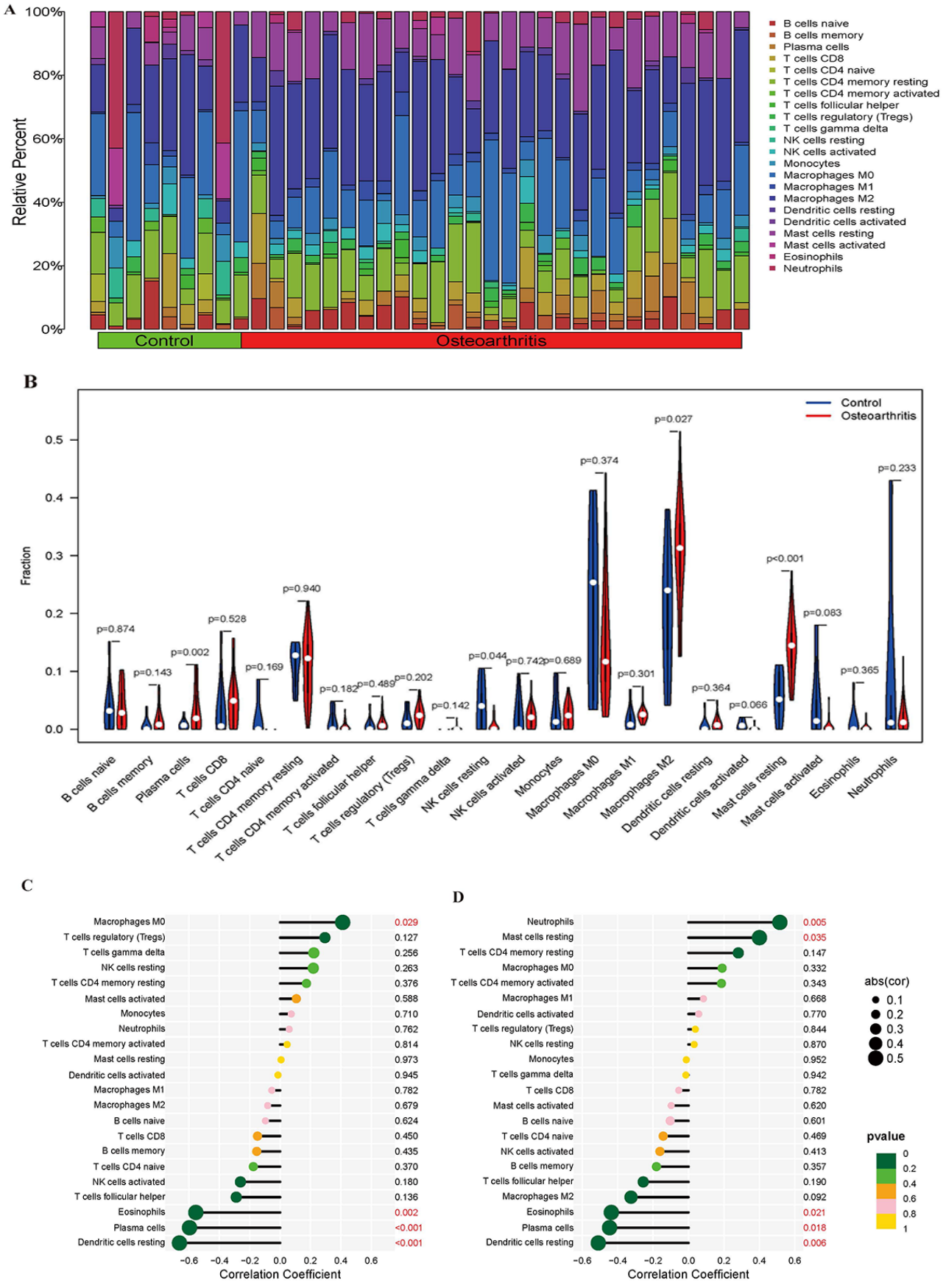

Figure 3. Correlation between CRGs and immune microenvironment in OA

A: Accumulation diagram of the infiltration abundance ratio of 22 immune cells in OA samples and controls. B: Violin plot showing the infiltration level of 22 immune cells in OA samples and controls. C: Lollipop chart of correlation between BGN and infiltration abundance of 22 immune cells. D: Lollipop chart of correlation between EPHA3 and infiltration abundance of 22 immune cells.

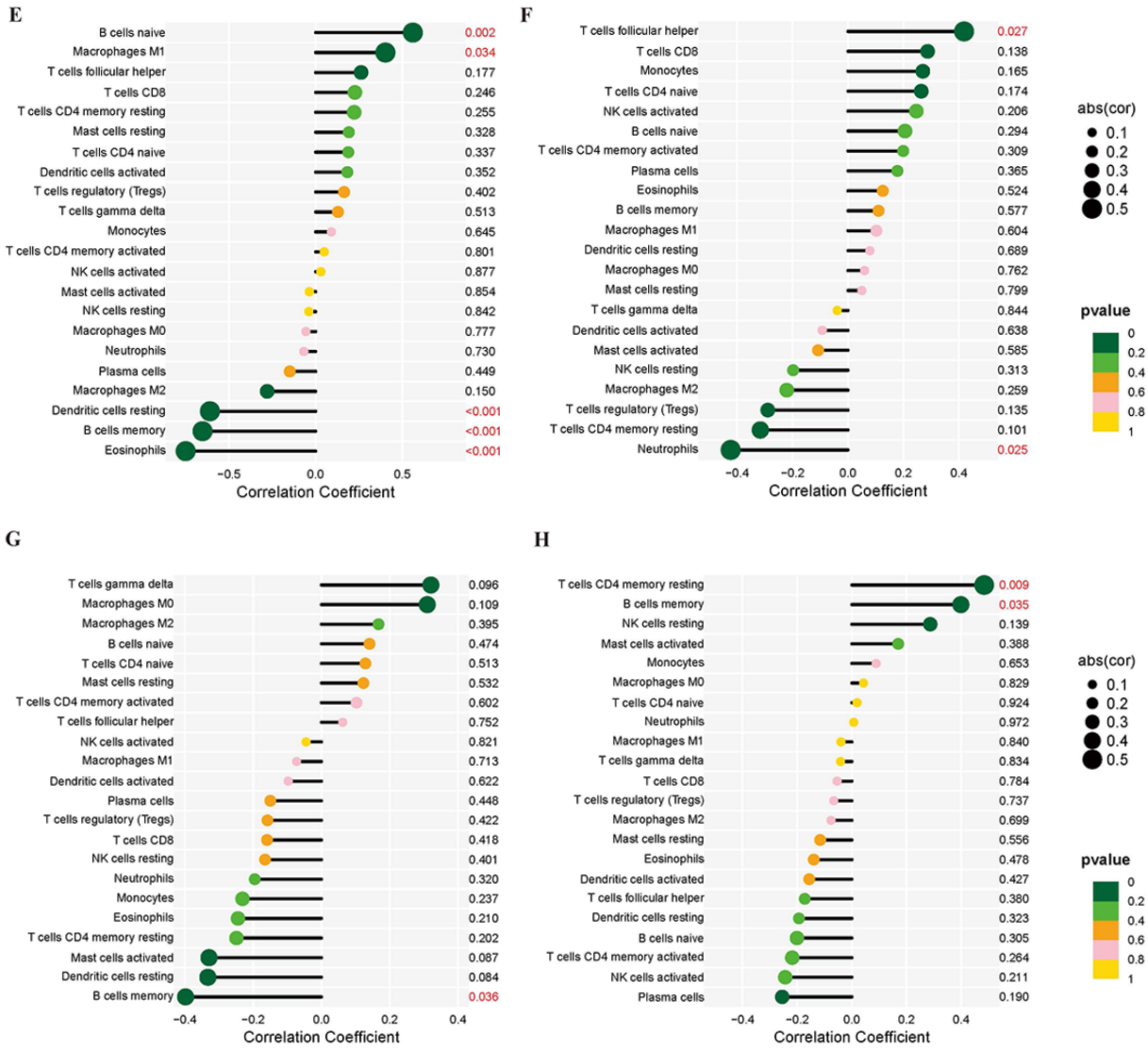

Figure 3 (continued). Correlation between CRGs and immune microenvironment in OA

E: Lollipop chart of correlation between LIF and infiltration abundance of 22 immune cells. F: Lollipop chart of correlation between NKG7 and infiltration abundance of 22 immune cells. G: Lollipop chart of correlation between SCRG1 and infiltration abundance of 22 immune cells. H: Lollipop chart of correlation between TNFAIP3 and infiltration abundance of 22 immune cells.

Thus, identifying the genes related with cuprotosis may provide a clear link between copper homeostasis and OA progression, showing how copper dysregulation contributes directly to the degeneration of joint tissues. In the present study, therefore, CRGs were identified to understand the relationship between cuprotosis and OA mechanism. Moreover, immune infiltration analysis was conducted to investigate the potential relationship between CRGs and immune cell infiltration.

By comparing two algorithms, six CRGs (BGN, EPHA3, LIF, NKG7, SCRG1, and TNFAIP3) were identified, and the genetic diagnostic classifier for OA composed of these six genes displayed good diagnostic performance. BGN is expressed in chondrocyte progenitor cells owing to the stimulation of TGF-β3 and EGF, which may lead to degeneration and regeneration in the advanced stage of OA [27]. This is in line with the result of the present study, that is, the expression level of BGN was higher in OA samples than that in controls. Song et al. [28] found that EPHA3 was a diagnostic marker of OA, EPHA3 was significantly up-regulated in OA samples, and quercetin might be able to treat OA by interacting with EPHA3. NKG7 serves as a vital player in regulating lymphocyte exocytosis and inflammatory responses of various diseases. It aggravates infection-induced cellular inflammation by affecting CD4+ and CD8+ T cells. Besides, its expression in natural killer cells and CD8+ T cells is very important for controlling cancer progression and metastasis through promotion of CD107a translocation and target cell killing. In the case of infections, NKG7 has an influence on the activation of CD4+ T cells, highlighting that NKG7 acts as a promising therapeutic target in different immune responses [29]. In the present study, the results revealed that NKG7 had an increased

expression in OA samples. However, research on the role of NKG7 in OA has not been conducted at present, and exploration on the function of NKG7 is required. SCRG1, as a diagnostic marker of OA, plays a vital role in the development and progression of diseases by jointly regulating immune-related pathways through the interaction of related proteins [30]. Additionally, SCRG1 is involved in the cell growth inhibition and differentiation in DEX-dependent chondrogenesis, mainly by stimulating the chondrogenesis of C3H10T1/2 cells, and has an elevated expression in cartilage in vitro [31]. Ma et al. [32] found that DHA attenutated the inhibition of sclerosing protein by reducing LIF secreted by osteoclasts, thus weakening abnormal bone remodeling, repressing angiogenesis in subchondral bone, and further hindering the progression of OA. TNFAIP3 secreted by articular cartilage stem cells can impede osteoclasts and improve the abnormal remodeling of subchondral bone in OA [33]. The above studies indicate that BGN, EPHA3, LIF, SCRG1, and TNFAIP3 are potential therapeutic targets of OA. In this study, the genetic diagnostic classifier based on six CRGs showed good diagnostic performance (AUC=0.772 in the training set, AUC=0.693 in the validation set). These results denote that these genes may be biological markers of cuprotosis in OA.

Furthermore, the infiltration abundance of immune cells in OA samples was also analyzed, and it was discovered that the abundance of M2 macrophages and resting mast cells was higher in OA samples than that in controls. Research revealed that reducing the infiltration of M1 macrophages and promoting the infiltration of M2 macrophages in the synovium can suppress the injury of subchondral bone and reduce the injury of articular cartilage [34]. Macrophage activation is not only the premise of over-expression of matrix metalloproteinases, but also an important step before the up-regulation of cytokines and subsequent degradation and destruction of cartilage [35,36]. In addition, Heng et al. [37] pointed out that the increased abundance of resting mast cells was related to the pathogenesis of OA. In the case that OA, M2 macrophages are generally considered to exert anti-inflammatory and tissue repair effects, which is conducive to inhibiting inflammation and facilitating the healing of damaged tissues. In the case of OA, however, an elevation in the abundance of M2 macrophages may indicate a complicated situation. M2 macrophages are helpful to alleviate inflammation, but their high abundance may reflect the continuous inflammation and need of tissue repair process in the disease, signifying the chronic and potentially active state of the disease. This may also suggest that the normal immune regulation mechanism is trying to control the tissue damage and inflammation caused by OA [38]. Hence, M2 macrophages at a high level may reflect both the severity of pathological state and the body's natural response to disease process.

CONCLUSIONS

In conclusion, crucial genes were firstly screened by LASSO COX and SVM-RFE models, including BGN, EPHA3, LIF, NKG7, SCRG1, and TNFAIP3. Then, a genetic diagnostic classifier for OA was established with these genes as biomarkers, which displayed high prediction accuracy. These findings render new insights into the molecular mechanism of OA and potential targets for the development of therapeutic strategies. However, there are still shortcomings in this study. First of all, the bioinformatics analysis conducted in this study was based on previous data, and the genetic diagnostic classifier for OA constructed in this study still needs to be validated for the diagnostic potential by multi-center clinical trials with a large sample size. Finally, CRGs were identified in OA, but their functions were not clarified through cell experiments and animal experiments.

ABBREVIATIONS

CRG- cuprotosis-related gene

DEG- differentially expressed gene

GEO- Gene Expression Omnibus database

IL- interleukin

OA- osteoarthritis

AUTHORS' CONTRIBUTION

Study design: JX. Data collection: CH and LA. Data analysis: JX and CH. Writing: LA. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

None to declare.

Publisher's Note: The Editorial Office stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Li K, Zhang Y, Zhang Y, Jiang W, Shen J, Xu S, et al. Tyrosine kinase Fyn promotes osteoarthritis by activating the β-catenin pathway. Ann Rheum Dis. 2018;77(6):935-43. DOI: 10.1136/annrheumdis-2017-212658
2. Mathiessen A, Conaghan PG. Synovitis in osteoarthritis: current understanding with therapeutic implications. Arthritis Res Ther. 2017;19(1):18. DOI: 10.1186/s13075-017-1229-9

3. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. Nat Rev Rheumatol. 2016;12(7):412-20. DOI: 10.1038/nrrheum.2016.65
4. Prieto-Alhambra D, Judge A, Javaid MK, Cooper C, Diez-Perez A, Arden NK. Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. Ann Rheum Dis. 2014;73(9):1659-64. DOI: 10.1136/annrheumdis-2013-203355
5. Carlson AK, Rawle RA, Wallace CW, Brooks EG, Adams E, Greenwood MC, et al. Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. Osteoarthritis Cartilage. 2019;27(8):1174-84. DOI: 10.1016/j.joca.2019.04.007
6. Zhai G, Randell EW, Rahman P. Metabolomics of osteoarthritis: emerging novel markers and their potential clinical utility. Rheumatology (Oxford). 2018;57(12):2087-95. DOI: 10.1093/rheumatology/kex497
7. Carlson AK, Rawle RA, Adams E, Greenwood MC, Bothner B, June RK. Application of global metabolomic profiling of synovial fluid for osteoarthritis biomarkers. Biochem Biophys Res Commun. 2018;499(2):182-8. DOI: 10.1016/j.bbrc.2018.03.117
8. Jiang M, Chen T, Feng H, Zhang Y, Li L, Zhao A, et al. Serum metabolic signatures of four types of human arthritis. J Proteome Res. 2013;12(8):3769-79. DOI: 10.1021/pr400415a
9. Shim EJ, Hahm BJ, Go DJ, Lee KM, Noh HL, Park SH, et al. Modeling quality of life in patients with rheumatic diseases: the role of pain catastrophizing, fear-avoidance beliefs, physical disability, and depression. Disabil Rehabil. 2018;40(13):1509-16. DOI: 10.1080/09638288.2017.1300691
10. Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. Science. 2022;375(6586):1254-61. DOI: 10.1126/science.abf0529
11. Chen L, Min J, Wang F. Copper homeostasis and cuproptosis in health and disease. Signal Transduct Target Ther. 2022 ;7(1):378. DOI: 10.1038/s41392-022-01229-y
12. Lv Z, Yang YX, Li J, Fei Y, Guo H, Sun Z, et al. Molecular Classification of Knee Osteoarthritis. Front Cell Dev Biol. 2021;9:725568. DOI: 10.3389/fcell.2021.725568
13. Chang B, Hu Z, Chen L, Jin Z, Yang Y. Development and validation of cuproptosis-related genes in synovitis during osteoarthritis progress. Front Immunol. 2023;14:1090596. DOI: 10.3389/fimmu.2023.1090596
14. Li Z, Feng J, Zhong J, Lu M, Gao X, Zhang Y. Screening of the Key Genes and Signalling Pathways for Diabetic Nephropathy Using Bioinformatics Analysis. Front Endocrinol. 2022;13:864407. DOI: 10.3389/fendo.2022.864407
15. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics. 2012;28(6):882-3. DOI: 10.1093/bioinformatics/bts034
16. Simon N, Friedman J, Hastie T, Tibshirani R. Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent. J Stat Softw. 2011;39(5):1-13. DOI: 10.18637/jss.v039.i05
17. Shi H, Yuan X, Liu G, Fan W. Identifying and Validating GSTM5 as an Immunogenic Gene in Diabetic Foot Ulcer Using Bioinformatics and Machine Learning. J Inflamm Res. 2023;16:6241-56. DOI: 10.2147/JIR.S442388
18. Cheng Q, Chen X, Wu H, Du Y. Three hematologic/immune system-specific expressed genes are considered as the potential biomarkers for the diagnosis of early rheumatoid arthritis through bioinformatics analysis. J Transl Med. 2021;19(1):18. DOI: 10.1186/s12967-020-02689-y
19. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77. DOI: 10.1186/1471-2105-12-77
20. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling Tumor Infiltrating Immune Cells with CIBERSORT. Methods Mol Biol. 2018;1711:243-59. DOI: 10.1007/978-1-4939-7493-1_12
21. Yang Z, Feng R, Zhao H. Cuproptosis and Cu: a new paradigm in cellular death and their role in non-cancerous diseases. Apoptosis. 2024;29:1330-60. DOI: 10.1007/s10495-024-01993-y
22. Liu S, Pan Y, Li T, Zou M, Liu W, Li Q, et al. The role of regulated programmed cell death in osteoarthritis: from pathogenesis to therapy. Int J Mol Sci. 2023;24(6):5364. DOI: 10.3390/ijms24065364
23. Su Y, Zhang X, Li S, Xie W, Guo J. Emerging Roles of the Copper-CTR1 Axis in Tumorigenesis. Mol Cancer Res. 2022;20(9):1339-53. DOI: 10.1158/1541-7786.MCR-22-0056
24. Dame C, Horn D, Schomburg L, Grünhagen J, Chillon TS, Tietze A, et al. Fatal congenital copper transport defect caused by a homozygous likely pathogenic variant of SLC31A1. Clin Genet. 2023;103(5):585-9. DOI: 10.1111/cge.14289
25. Cobine PA, Moore SA, Leary SC. Getting out what you put in: Copper in mitochondria and its impacts on human disease. Biochim Biophys Acta Mol Cell Res. 2021;1868(1):118867. DOI: 10.1016/j.bbamcr.2020.118867
26. Ruiz LM, Libedinsky A, Elorza AA. Role of copper on mitochondrial function and metabolism. Front Mol Biosci. 2021;8:711227. DOI: 10.3389/fmolb.2021.711227
27. Janssen JN, Batschkus S, Schimmel S, Bode C, Schminke B, Miosge N. The Influence of TGF-β3, EGF, and BGN on SOX9 and RUNX2 Expression in Human Chondrogenic Progenitor Cells. J Histochem Cytochem. 2019;67(2):117-27. DOI: 10.1369/0022155418811645
28. Song S, Yu J. Identification of the shared genes in type 2 diabetes mellitus and osteoarthritis and the role of quercetin. J Cell Mol Med. 2024;28(4):e18127. DOI: 10.1111/jcmm.18127
29. Ng SS, De Labastida Rivera F, Yan J, Corvino D, Das I, Zhang P, et al. The NK cell granule protein NKG7 regulates cytotoxic granule exocytosis and inflammation. Nat Immunol. 2020;21(10):1205-18. DOI: 10.1038/s41590-020-0758-6
30. Liu G, He G, Zhang J, Zhang Z, Wang L. Identification of SCRG1 as a Potential Therapeutic Target for Human Synovial Inflammation. Front Immunol. 2022;13:893301. DOI: 10.3389/fimmu.2022.893301
31. Ochi K, Derfoul A, Tuan RS. A predominantly articular cartilage-associated gene, SCRG1, is induced by glucocorticoid and stimulates chondrogenesis in vitro. Osteoarthritis Cartilage. 2006;14(1):30-8. DOI: 10.1016/j.joca.2005.07.015
32. Ma L, Zhao X, Liu Y, Wu J, Yang X, Jin Q. Dihydroartemisinin attenuates osteoarthritis by inhibiting abnormal bone remodeling and angiogenesis in subchondral bone. Int J Mol Med. 2021;47(3):04855. DOI: 10.3892/ijmm.2021.4855

33. Li ZL, Li XT, Hao RC, Wang FY, Wang YX, Zhao ZD, et al. Human osteoarthritic articular cartilage stem cells suppress osteoclasts and improve subchondral bone remodeling in experimental knee osteoarthritis partially by releasing TNFAIP3. Stem Cell Res Ther. 2023;14(1):253. DOI: 10.1186/s13287-023-03411-7

34. Zhou F, Mei J, Han X, Li H, Yang S, Wang M, et al. Kinsenoside attenuates osteoarthritis by repolarizing macrophages through inactivating NF-κB/MAPK signaling and protecting chondrocytes. Acta Pharm Sin B. 2019;9(5):973-85. DOI: 10.1016/j.apsb.2019.01.015

35. Blom AB, van Lent PL, Libregts S, Holthuysen AE, van der Kraan PM, van Rooijen N, et al. Crucial role of macrophages in matrix metalloproteinase-mediated cartilage destruction during experimental osteoarthritis: involvement of matrix metalloproteinase 3. Arthritis Rheum. 2007;56(1):147-57. DOI: 10.1002/art.22337

36. Haseeb A, Haqqi TM. Immunopathogenesis of osteoarthritis. Clin Immunol. 2013;146(3):185-96. DOI: 10.1016/j.clim.2012.12.011

37. Heng H, Liu J, Hu M, Li D, Su W, Li J. WDR43 is a potential diagnostic biomarker and therapeutic target for osteoarthritis complicated with Parkinson's disease. Front Cell Neurosci. 2022;16:1013745. DOI: 10.3389/fncel.2022.1013745

38. Xie J, Huang Z, Pei F. [Role and progress of innate immunity in pathogenesis of osteoarthritis]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2019;33(3):370-6. DOI: 10.7507/1002-1892.201810068