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Identification of biomarkers related to tryptophan metabolism in osteoarthritis

Junjun Yang [a,b,*], Ping Zhou [a,b], Tangbing Xu [a,b], Kaida Bo [a,b], Chenxin Zhu [a,b], Xu Wang [a,b], Jun Chang [a,b,**]

[a] Department of Orthopaedics, The First Affiliated Hospital of Anhui Medical University, Hefei, China
[b] Department of Orthopaedics, Anhui Public Health Clinical Center, Hefei, China

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ABSTRACT

Background: OA (osteoarthritis) is a common joint disease characterized by damage to the articular cartilage and affects the entire joint tissue, with its main manifestations being joint pain, stiffness, and limited movement. Currently,we know that OA is a complex process composed of inflammatory and metabolic factors.It is reported that the occurrence and development of OA is related to the change of tryptophan metabolism.Therefore, the study of tryptophan metabolism and OA related genes is hopeful to find a new therapeutic target for OA.

Methods: Differentially expressed genes (DEGs) in GSE55235 were gained via differential expression analysis (OA samples *vs* normal samples). The tryptophan metabolic related DEGs (TMR-DEGs) were obtained by overlapping tryptophan metabolism related genes (TMRGs) and DEGs. Further, biomarkers were screening via Least absolute shrinkage and selection operator (LASSO), naive bayes (NB) and supportvector machine-recursive feature elimination (SVM-RFE) algorithm to establish a diagnostic model. Afterward, Gene Set Enrichment Analysis (GSEA) and drug prediction were performed based on diagnostic biomarkers by multiple software and databases. Eventually, expression level of biomarker public databases was verified using real-time quantitative polymerase chain reaction (RT-qPCR).

Results: Three tryptophan metabolism related biomarkers (*TDO2*, *AOX1* and *SLC3A2*) were identified in OA. GSEA analysis demonstrated that biomarkers were associated with the function of 'FoxO signaling pathway', 'spliceosome' and 'ribosome'. There were seven drugs with therapeutic potential on *TDO2* and *AOX1*. Ultimately, compared with normal group, expression of *AOX1* and *SLC3A2* in OA group remarkable lower.

Conclusion: Overall, three tryptophan metabolic related diagnostic biomarkers that associated with OA were obtained, which provided a original direction for the diagnosis and treatment of OA.

1. Introduction

OA (osteoarthritis) is a joint disease characterized by cartilage destruction and bone fragmentation caused by aging, strain, trauma and so on factors. It always occurs in weight-bearing joints and high activity joints. More than 50 % of patients with knee pain are diagnosed with OA, and the prevalence of OA is high in the middle-aged and elderly population. [1]. It causes pain, loss of function and reduced quality of life and is the leading cause of disability in older adults [2]. The cost of treatment for the disease poses a significant economic burden to patients and society. Joint damage, abnormal development of joints or limbs, altered genetic background, and working in jobs that require weight-bearing have been reported as risk factors for OA [3]. Currently, OA is often clinically diagnosed by pathography, physical examination, and characteristic OA metabolites in plasma, urine, synovial fluid and serum such as alanine and lysophosphatidylcholine [4]. Traditional treatment options include a variety of options, such as oral medications, physical therapy, lifestyle changes, physical methods, injections, and surgery. In recent years, an improved understanding of the underlying mechanisms, diagnosis and management of OA has led to many

* Corresponding author. Department of Orthopaedics, The First Affiliated Hospital of Anhui Medical University; Anhui Public Health Clinical Center, Hefei, 230000, China.
** Corresponding author. Department of Orthopaedics, The First Affiliated Hospital of Anhui Medical University; Anhui Public Health Clinical Center, Hefei, 230000, China.
E-mail addresses: 2283404561@qq.com (J. Yang), changjun_2008@hotmail.com (J. Chang).

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potential therapeutic advances [5]. Whereas, in spite of currently effective treatments and advances in research, the medical need for OA treatment remains unmet. To date, there are no effective drugs that can stop the progression of the disease [6]. In the face of this public health crisis, rigorous, high-quality OA clinical studies are urgently needed to ensure that patients receive safe and effective treatment. A growing body of research indicate that many diseases can be treated with targeted drugs [7,8]. At present, there are few reports on the therapeutic targets of OA [9]. Therefore, exploring potential biomarkers of OA and their therapeutic targets is crucial to understand the pathogenesis of OA.

Tryptophan (Trp) is one of the eight indispensable amino acids. It and its metabolites play a key role in regulating cell proliferation and maintaining cell activity [10]. It has been reported that the occurrence and development of OA are related to the change of amino acid metabolic profile. Functional amino acids, including Trp, have a variety of benefits in the treatment of OA, such as anti-oxidation [11]. Liyile et al. established the relationship between gut microbiome related Trp metabolism and diseases in OA, which showed that the change of Trp metabolism may promote the activation and synthesis of Aryl hydrocarbon receptor, and then accelerate the progress of OA [12]. However, the expression changes and biological functions of TMRGs in OA development were currently unknown.

This study purposed to recognize differentially expressed TMRGs in OA, identify key genes by using a variety of machine learning algorithms, and the potential biological pathways and molecular regulatory mechanisms were explored through multiple algorithms in the R package. The targeted drugs of key genes and molecular docking were analyzed via Autodock Vina and Pymol software. To provide a reference for the mining of potential biomarkers and the exploration of therapeutic targets in OA.

2. Materials and methods

2.1. Data acquisition

The datasets associated with OA were collected from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) (Additional file 1). GSE55235 was used as training set, which included ten Synovial tissue samples from osteoarthritic joint and ten synovial tisssue from healthy patients joints. The GSE55457, as a validation set, consisted of ten synovial membrane tissue samples from Osteoarthritis patients and ten synovial membrane tissue samples from healthy patients. Totally, 45 TMRGs were derived from MsigDB database [10].

2.2. Differential and enrichment analysis

Differentially expressed genes (DEGs) were collected between normal group and OA group in GSE55235 using 'limma' package [13]. The *p*.adj <0.05 and $|\log2FC| > 0.5$ were determined as the threshold. Further, Tryptophan metabolism related DEGs (TMR-DEGs) were obtained through overlapping DEGs and TMRGs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was established via 'clusterProfiler' [14] with $p < 0.05$ and count >1.

2.3. Protein interaction (PPI) network and machine learning methods

Spearman correlation analysis was performed between TMR-DEGs. PPI network was applied to explore the interaction between these genes through GeneMANIA database (http://genemania.org). Afterward, Least absolute shrinkage and selection operator (LASSO), naive bayes (NB) and supportvector machine-recursive feature elimination (SVM-RFE) algorithm were applied to screen important genes in GSE55235. The diagnostic biomarkers were obtained through overlapping three algorithms. Moreover, a receiver operating characteristic (ROC) curve was plotted using the pROC to evaluate the diagnostic value of the biomarkers and the model [15]. GSE55457 was regarded as an external verification set for the diagnostic value.

2.4. Gene Set Enrichment Analysis (GSEA)

GSEA was proceeded to detect the feasible KEGG pathways associated with diagnostic biomarkers through 'clusterProfiler' package [16] with *p*.adjust <0.05.

2.5. Construction of 'mRNA-transcription factors (TFs)' and 'mRNA-miRNA-circRNA' networks

The TF associated with diagnostic biomarkers were anticipated via NetworkAnalyst (http://www.networkanalyst.ca/faces/home.xhtml) and Cistrome (http://cistrome.org/db/) databases. The mRNAs and circRNAs associated with miRNAs were forecasted by Starbase database (http://starbase.sysu.edu.cn). Moreover, the results of 'mRNA-TF' and 'mRNA-miRNA-circRNA' network were optimize via Cytoscape software [17].

2.6. Potential drug prediction and molecular docking

The targeting drugs were identified through DGIdb database (https://dgidb.org/) to discover the potential therapeutic drugs for diagnostic biomarkers in OA. To evaluate the affinity of potential drugs for biomarkers, the molecular structure of the drugs was obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. Crystal structure of the target proteins were downloaded from the uniprot database. Autodock Vina (v.1.2.2) was selected for molecular docking. The energy range default was 3, and the exhaustiveness default was 8. Every ligand and receptor engages in 20 simulated docking cycles. The ideal outcome was the one with the lowest binding energy, and Pymol software (V3.4) was used to show the ideal outcome.

2.7. The analysis of the expression of diagnostic biomarkers

In order to confirm the expression of diagnostic biomarkers in OA, we implemented RT-qPCR. Then, five normal and five OA tissue samples were obtained from patients with their knowledge and consent from Anhui Public Health Clinical Center, and this study was licenced by the ethics committee of Anhui Public Health Clinical Center. Following the manufacturer's instructions, the total RNA from ten samples was separated using TRIzol (Ambion, Austin, USA). Total RNA was reverse-transcribed to cDNA using the first strand CDA-synthesis-kit (Servicebio, Wuhan, China) according to the producer indicator. Then, qPCR was carried out utilizing the 2xUniversal Blue SYBR Green qPCR Master Mix (Servicebio, Wuhan, China) according to the manual. The primer sequences for PCR were tabulated in Table 1. GAPDH was utilized as an internal reference gene, and the expression was calculated according to the $2-\Delta\Delta$ht method [18].

2.8. Statistical analysis

All *P* value < 0.05 was considered statistically significant.

Table 1
The primer sequences used in the real time quantitative polymerase chain reaction (RT-qPCR).

primer	sequence
TDO2 F	GTCATACAGAGCACTTCAGGGAG
TDO2 R	CATCTTCGGTATCCAGTGTCG
AOX1 F	GTTCACATTTATCTTGATGGCTCTG
AOX1 R	GACATTCGACATTGGCATTCTTA
SLC3A2 F	GAGCCTACTCGAATCCAACAAAG
SLC3A2 R	GGTAGAGTCGGAGAAGTTGAGCC
GAPDH F	CGAAGGTGGAGTCAACGGATTT
GAPDH R	ATGGGTGGAATCATATTGGAAC

3. Results

3.1. Identification of TMR-DEGs in OA

The 1600 DEGs were identified in OA, including 630 down-regulated and 970 up-regulated genes (Fig. 1A and B). Furthermore, seven TMR-DEGs associated with OA were retained by overlapping DEGs and TMRGs (Fig. 1C). Further, functional enrichment analysis was proceeded to uncover potential mechanisms for TMR-DEGs. Accordingly, 67 GO items and eight KEGG pathways were gained. We observed that

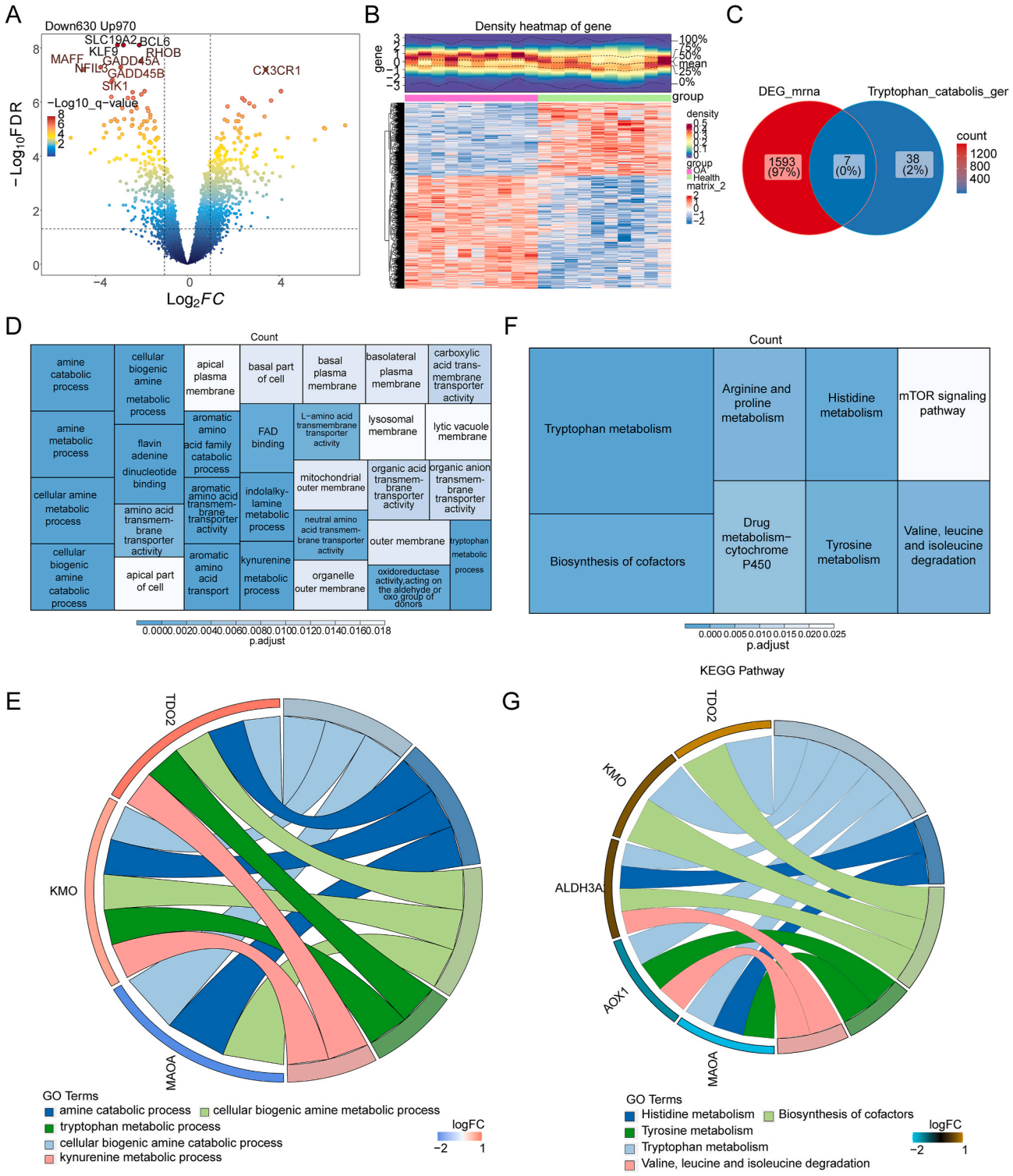

Fig. 1. Identification of the tryptophan metabolic related differentially expressed genes (TMR-DEGs) in OA. Differentially expressed genes (DEGs) normal group and OA group in GSE55235 dataset were shown by (A) volcano map, The different colour dots on the left and right sides represent 630 up- and 790 down-regulated DEGs, respectively. The heatmap (B) plot of up- and down-regulated DEGs, red and blue grids indicate up- and down-regulated DEGs, respectively. The top diagram of heat map indicates the gene expression density. (C) Venn plot for the TMR-DEGs shared by DEGs and tryptophan metabolism related genes (TMRGs). Results of (D, E) Gene Ontology (GO) and (F, G) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of TMR-DEGs.

the genes mentioned above were mainly related to the 'amine metabolic process' and the 'amine catabolic process' (Fig. 1D and E). Meanwhile, KEGG showed that DEGs were chiefly filled in the 'Tryptophan metabolism' and 'Histidine metabolism' (Fig. 1F and G).

3.2. Interaction analysis of TMR-DEGs

The correlation between the seven TMR-DEGs was shown in Fig. 2A. We found that *AOX1* was negative associated with *TDO2*, while it was positive associated with *MAOA*. The interaction between these seven TMR-DEGs was shown in Fig. 2B. We found that MAOB interacted with multiple TMR-DEGs at the same time.

3.3. Establishment of tryptophan metabolism related diagnostic model for OA

To further dig out the key genes, LASSO, NB and SVM-RFE algorithm were performed on TMR-DEGs to unearth the optima. LASSO regression analysis identified five significant genes, including *TDO2*, *AOX1*, *SLC3A2*, *SLC7A5* and *KMO* (Fig. 3A and B). Then, six feature genes were obtained via SVM-RFE, including *ALDH3A2*, *MAOA*, *SLC3A2*, *AOX1*, *SLC7A5* and *TDO2* (Fig. 3C). Meanwhile, six feature genes were obtained via NB, including *MAOA*, *TDO2*, *ALDH3A2*, *AOX1*, *SLC3A2* and *KMO* (Fig. 3D). Eventually, three tryptophan metabolism related biomarkers (*TDO2*, *AOX1* and *SLC3A2*) were obtained by intersecting the genes obtained by the three machine learning algorithms (Fig. 3E).

We establish a diagnostic model of OA composed of these diagnostic biomarkers. The AUC value of diagnostic model and biomarkers was greater than 0.7, indicating that the these genes and the model had good accuracy (Fig. 4A–C). Next, we further validated the diagnostic value in the external validation set (GSE55457) (Fig. 4B–D). The similar results was observed in GSE55457.

3.4. Functional enrichment analysis

To further investigate the feasible roles of *TDO2*, *AOX1* and *SLC3A2* in OA, we performed single-gene GSEA on diagnostic biomarkers. KEGG results indicated that 'spliceosome', 'ribosome pathway' and 'FoxO signaling pathway' were enriched in the groups with high-expression of *TDO2*, *AOX1* and *SLC3A2* (Fig. 5A–C).

3.5. Analysis of regulatory network

The 26 TFs were identified from the intersection of NetworkAnalyst

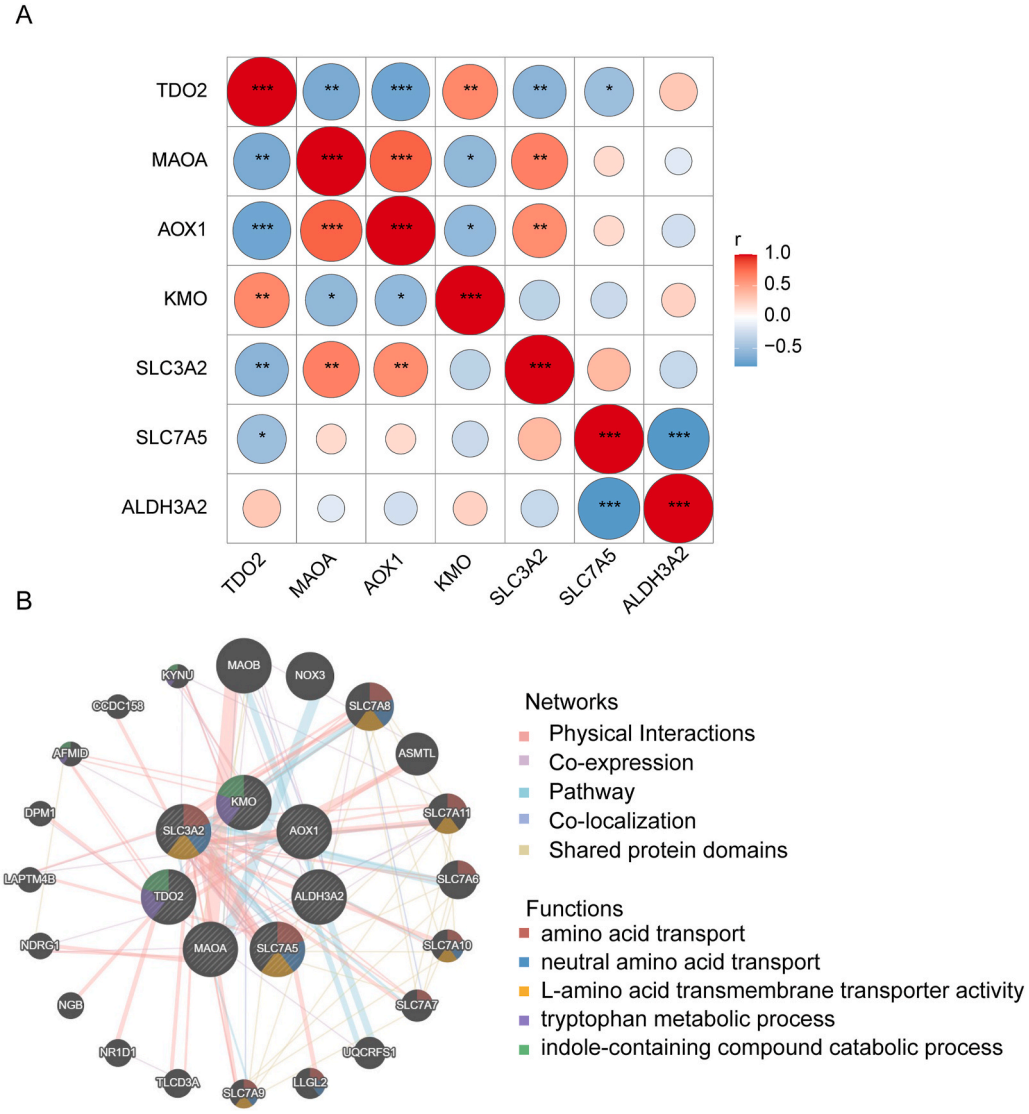

Fig. 2. Interaction analysis of seven TMR-DEGs. (A) The correlation heatmap among seven TMR-DEGs, red represents positive correlation, blue represents negative correlation (B) Protein-protein interactions (PPI) network of seven TMR-DEGs. Functional associations between targets were investigated using GeneMANIAGenes.

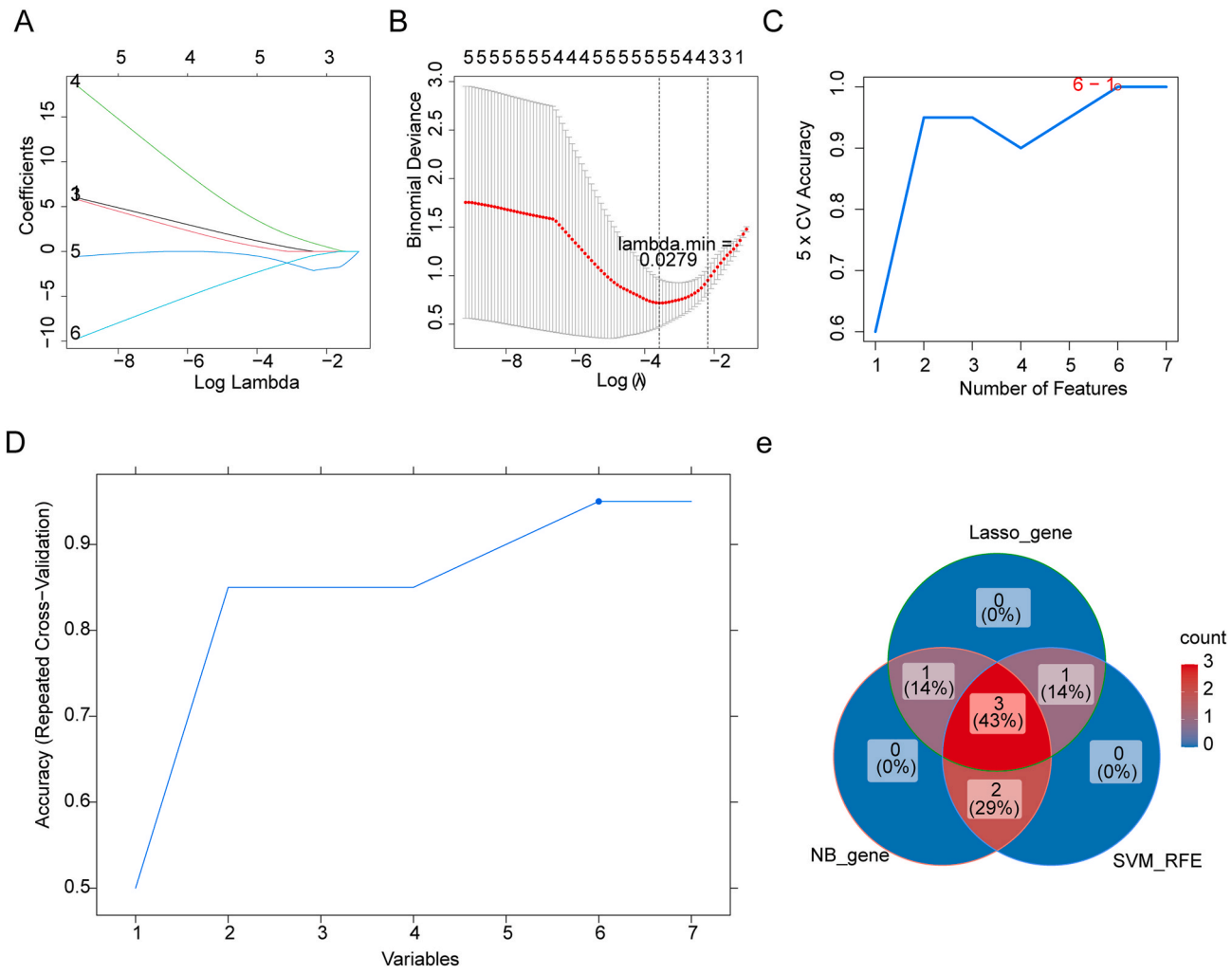

Fig. 3. Screening of tryptophan metabolism related diagnostic genes in OA. (A) Five significant genes were identified in the least absolute shrinkage and selection operator (LASSO) model. The relationship between lambda values and corresbonding cross-validated error rate was plotted to subject the optimal lambda for the LASSO model. (B) Cross-validation for tuning parameter selection in the LASSO model. The plot illustrates the trajectory of each OA-related feature's coefficient in the LASSO coefficient profiles as the lambda value changes in the LASSO algorithm. (C) The relationship between generalization error and feature number in support vector machine-recursive feature elimination (SVM-RFE), and the error rate of this mode is the lowest when the feature number reaches 6. (D) The relationship between prediction accuracy and various number in naive Bayes (NB), and the accuracy of this mode is the highest when the feature number reaches 6. (E) Venn diagram of three tryptophan metabolism related biomarkers shared by three machine learning algorithms.

and Cistrome databases (Fig. 6A). The 'mRNA-TF' network was established to explore the regulatory mechanism of *TDO2*, *AOX1* and *SLC3A2* (Fig. 6B). There were complex interrelationships in this network, in which SIN3A was associated with *AOX1*. ZNF384 might regulate the expression of TDO2. Meanwhile, 'mRNA-miRNA-circRNA' network was established, which had three mRNAs, seven miRNAs and fifteen circRNAs. In the network, hsa-miR-495–3p affected the expression of *TDO2*, and hsa-miR-31–5p regulated the expression of *AOX1* (Fig. 6C). The hsa circ 0075 924 might affected the expression of *SLC3A2* through hsa-miR-490–3p.

3.6. Biomarkers-drug interaction network

We explored the potential therapeutic drugs for *TDO2*, *AOX1* and *SLC3A2*. There were seven drugs with therapeutic potential on *TDO2* and *AOX1* (Fig. 7A). Drugs targeting *TDO2* was DEXAMETHASONE and INSULIN (Fig. 7B). Drugs targeting *AOX1* was ALLOPURINOL, AZATHIOPRINE, FEBUXOSTAT, ISOVANILLIN and MENADIONE (Fig. 7C). Molecular docking results suggested that the binding energies of these drugs to their biomarkers were less than −5 (Fig. 7D–Table 2).

3.7. Expression validation of the diagnostic genes

The expression level of *AOX1*, *SLC3A2* and *TDO2* was analyzed in the GSE55235 dataset (Fig. 8A). At the transcriptional level, compared with the normal group, *AOX1* and *SLC3A2* had a lower expression in OA group. While *TDO2* showed the opposite expression trend. Further, expression trend was verified by RT-qPCR experiments (Fig. 8B). This was consistent with the results of the public database.

4. Discussion

OA is the most familiar joint disease, which is characterized by complex structural changes in the knee joint, leading to joint pain, stiffness, physical dysfunction and even disability, which brings a huge burden to social and economic health [19,20]. Moreover, there are few studies on the expression changes and biological functions of TMRGs in development of OA. Therefore, the elucidation of TMRGs related to OA is of great significance for OA treatment. This bioinformatics analysis study for the first time used different machine learning algorithms to obtain three tryptophan metabolism-related diagnostic biomarkers (*TDO2*, *AOX1* and *SLC3A2*) associated with OA based on OA

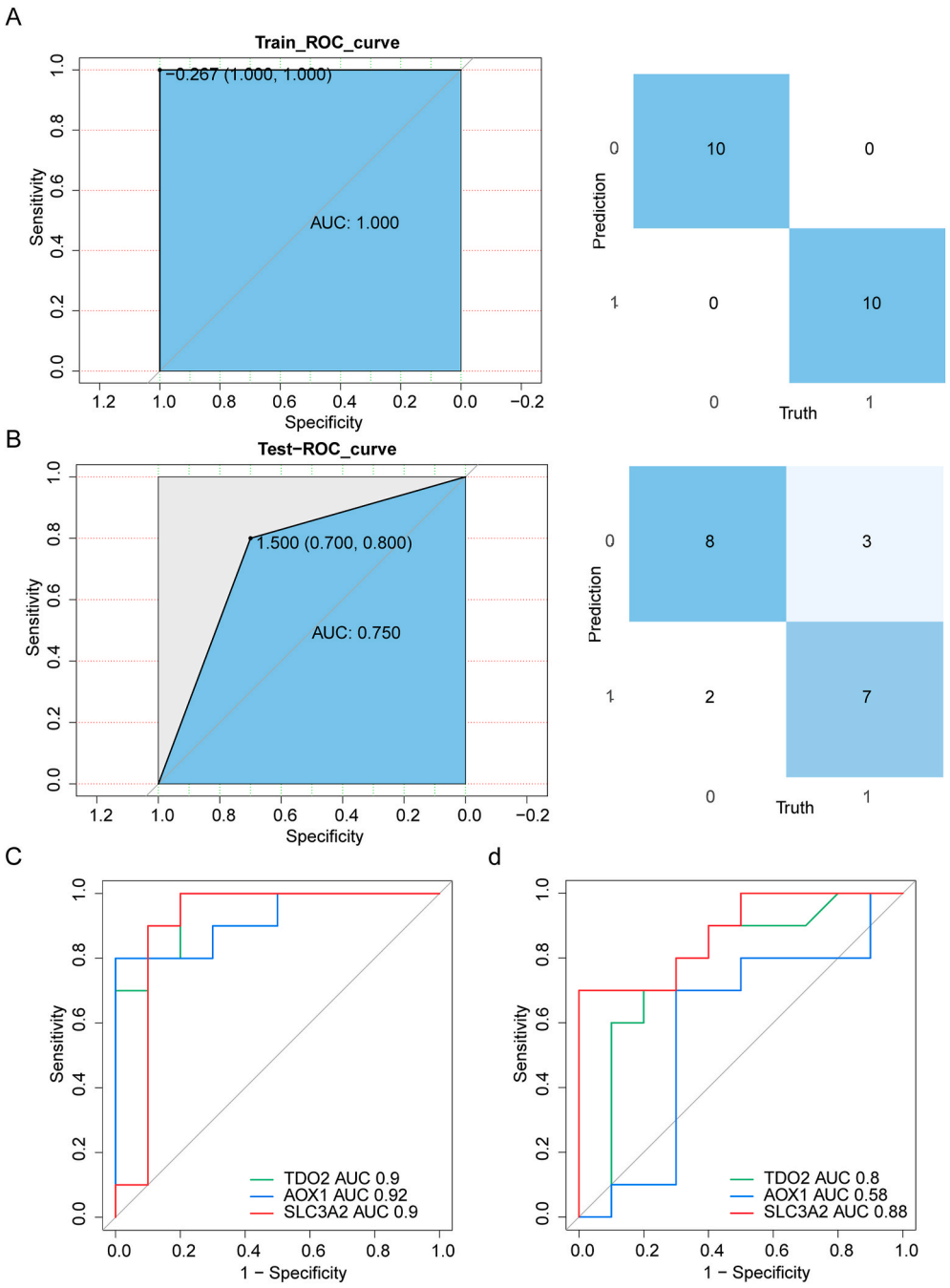

Fig. 4. Construction of the tryptophan metabolism related diagnostic model for OA. (A) Receiver operating characteristic curve (ROC) curve and prediction matrix of the diagnostic model in train set (GSE55235) for OA. (B) ROC curve and prediction matrix of the diagnostic model in test set (GSE55457) for OA. (c,d) ROC curve of single diagnostic genes for predicting OA in train set (GSE55235) (C) and test set (GSE55457) (D).

transcription data from two large public GEO databases. The OA prediction model was constructed and verified. Seven effective drugs related to *TDO2* and *AOX1* were found in DGIDB database, and the binding information between the drugs and the corresponding proteins of the genes was analyzed. The results showed that the expression levels of these three core genes were significantly different between the normal group and the disease group. Among them, *TDO2* has an up-regulated role in the disease, while *AOX1* and *SLC3A2* played a down-regulated role in the development of the disease. In addition, the OA prediction model we constructed showed high predictive validity on the validation set. Overall, we have obtained the diagnostic biomarkers related to tryptophan metabolism in OA, which lays a better outlook for the diagnosis and treatment of OA.

The tryptophan-2, 3-dioxygenase2 (*TDO2*) was located at 8p11. 21, encoding 10 exons, a total of 81 437 nucleotides, which was a heme-containing dioxygenase, which has the enzymatic activity of decomposing tryptophan and was related to inflammation-related diseases [21]. Studies have shown that the level of *TDO2* in patients with OA was remarkably higher than these without arthritis. Further analysis found that the level of *TDO2* in knee joint synovial membrane and synovial fluid of OA patients was positively correlated with clinical manifestations, KL grade and proinflammatory cytokine levels [22]. Recently, the expression of *TDO2* has been reported to be highly upregulated in joint tissues of rheumatoid arthritis patients and rats with help-induced arthritis [23]. In this study, we found similar results, among the three core genes, only *TDO2* was highly expressed in the disease tissues, and

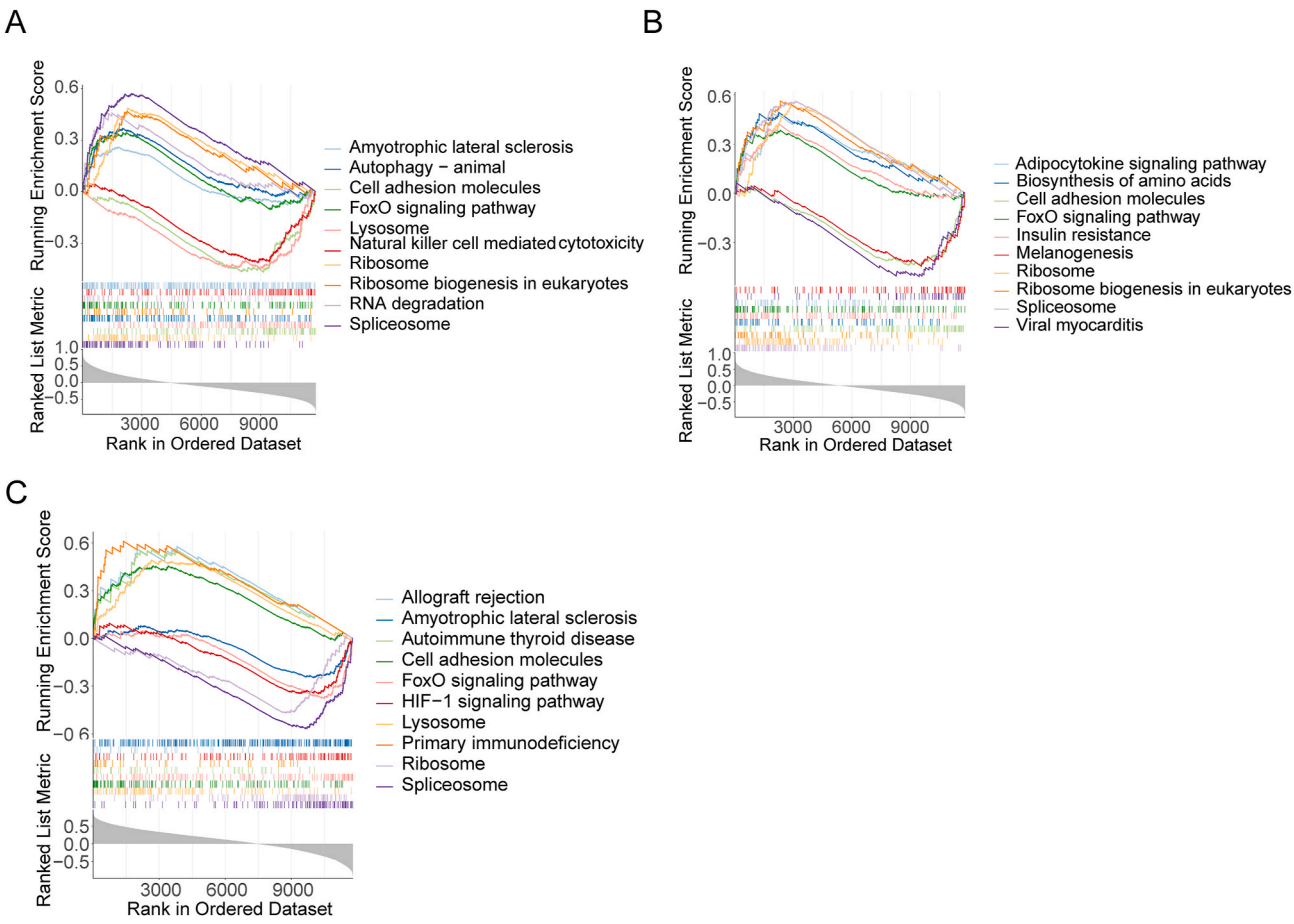

Fig. 5. Gene Set Enrichment Analysis (GSEA) of three diagnostic biomarkers. (A) *TDO2*, (B) *AOX1* and (C) *SLC3A2*. The top 10 enriched items were demonstrated by the lines in each figure.

we applied *TDO2* to the prediction model, which provided a simple method for the diagnosis of arthritis. Alcohol oxidase gene I (*AOX1*) was an alcohol oxidase with a wide range of substrates, including a variety of aromatic heterocycles and aldehydes. Beyond that, it has been implicated in the bioactivation of prodrugs, the regulation of oxygen homeostasis in vivo, the production of nitric oxide, and lipogenesis. [24]. Our study found that *AOX1* was strongly expressed in the normal patient group, but the reliability of this core gene was not supported by the verification of gene expression in the GEO external validation dataset. The relationship between *AOX1* and the occurrence and progression of arthritis needs to be further studied. Solute carrier family 3 member 2 (*SLC3A2*), also known as 4F2hc, CD98hc, was an 85 kDa type II transmembrane glycoprotein [25]. It usually acted as a chaperone and heterodimerises with some amino acid transporters (e.g. SLC7A5, SLC7A11) to play a role at the plasma membrane [26]. A bioinformatics analysis study found that *SLC3A2* was related to ferroptosis related functions, and the expression of *SLC3A2* was remarkably down-regulated in the cartilage damage of knee OA patients, and the expression of *SLC3A2* was also down-regulated in the cartilage damage area of OA patients. The study also elaborated that the clinical factors such as K-L grade, obesity grade and BMI [27]. Some scholars have proposed that the mechanism of *SLC3A2* in OA might be that when cystine was inhibited, the uptake of cystine was limited, thereby blocking the manufacture of GSH, leading to a decrease in cellular antioxidant capacity and promoting ferroptosis, which leaded to irreversible degeneration of joint structure and finally arthritis [28]. *SLC3A2* deficiency was found to promote ferroptosis by upregulating the expression of mTOR and P70S6K in another study of laryngeal cancer. [29]. In a study of vascular smooth muscle cell apoptosis, it was found that HCMV-miR-US33–5p binds to the 1′-untranslated region of EPAS3 to inhibit its expression. This results in the inhibition of SLC3A2 expression, which ultimately promotes cell apoptosis and inhibits cell proliferation. [30]. Previous studies have shown that down-regulation of *SLC3A2* inhibited cell proliferation and increases cellular oxidative senescence, which may play a role in joint development.

In previous studies, the binding energy between effective molecule and SARS-CoV-2 M^{pro} protein was calculated by density functional tight binding (DFTB), and the free-energy surfaces/thermodynamics of large biochemical systems was accurately and efficiently predicted using GPU-based DFTB approach [31,32]. Although DFTB was used to understand large biomolecular systems, the molecular docking was performed in this study. The predicted drugs were DEXAMETHASONE; INSULIN; MENADIONE; AZATHIOPRINE; FEBUXOSTAT; ALLOPURINOL and ISOVANILLIN, among which AOX1 had the best docking with FEBUXOSTAT, followed by TDO2 and DEXAMETHASONE drug, AOX1 and MENADIONE drug. This was consistent with a study that FEBUXOSTAT can significantly relieve the symptoms and reduce the inflammatory response in patients with knee arthritis [33], but FEBUXOSTAT was often used in the treatment of gout and gouty arthritis in clinical practice [34]. The appropriate dose of DEXAMETHASONE has been widely accepted for the treatment of arthritis [35,36]. Previous studies have shown that MENADIONE drugs played a positive role in anti-oxidation of joint structure and cartilage recovery, thereby preventing the progression of arthritis [37]. The current literature review suggests that the results of the present study confirm and extend previously known results, and that these findings provide promising evidence and potentially attractive directions for future research into new targets for OA diagnosis and immunotherapy.

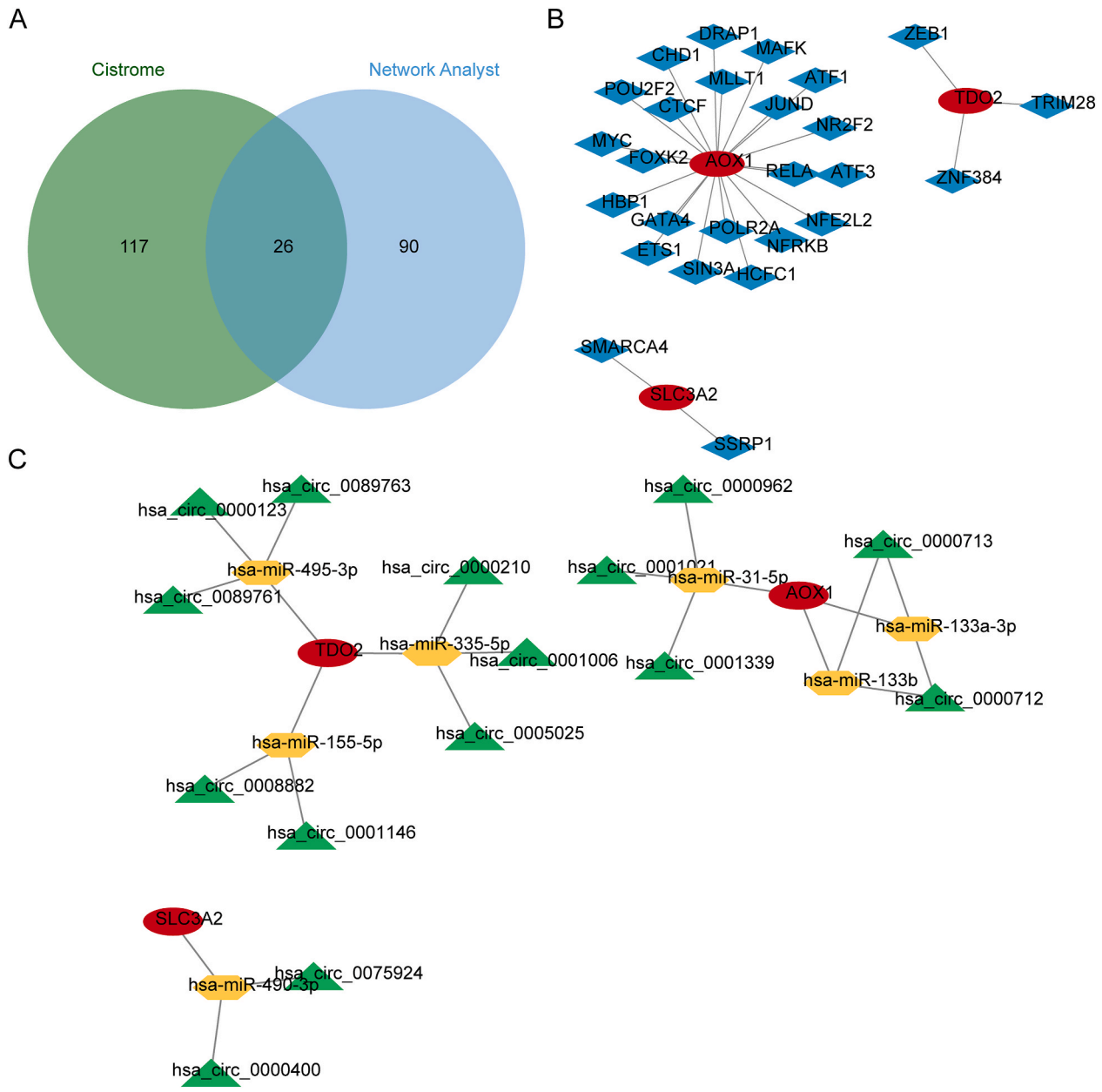

Fig. 6. Analysis of the regulatory network of three diagnostic biomarkers. (A) Venn diagram for the common transcription factors (TF) from the NetworkAnalyst and Cistrome databases. (B) The mRNA-TF network targeting diagnostic biomarkers, red represents biomarker, blue represents TF. (C) The mRNA-miRNA-circRNA network of diagnostic biomarkers, red represents biomarker, yellow represents miRNA, and green represents circRNA.

Based on the GEO public database data, this study intends to mine the key biomarkers in OA through a series of bioinformatics methods, and construct a stable and reliable prediction model, which provides a theoretical foundation for the diagnosis, treatment and pathogenesis of OA. Nevertheless, this study has certain limitations. Firstly, these data used in the current work were obtained from public databases and the reliability of the data could not be verified. Second, the sample size included in the validation of core gene expression was small, and more studies with larger sample sizes are needed to probe differences in *AOX1* levels between different sub-types of OA. The difference in AOX1 was found to be non-significantly differentially expressed in the OA and control groups as verified by qRT-PCR, which may be due to the variability of the samples. However, due to time and resource constraints, we were unable to conduct relevant experiments immediately. In future studies, we will include more experiments to investigate the specific mechanism. Finally, although we observed differential gene expression between OA and normal groups, in vivo and animal studies were not performed to further investigate the underlying mechanisms.

Funding

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Ethics approval

This study was conducted according to the tenets of the Declaration of Helsinki. Approval was granted by the ethics committee of Anhui Public Health Clinical Center.

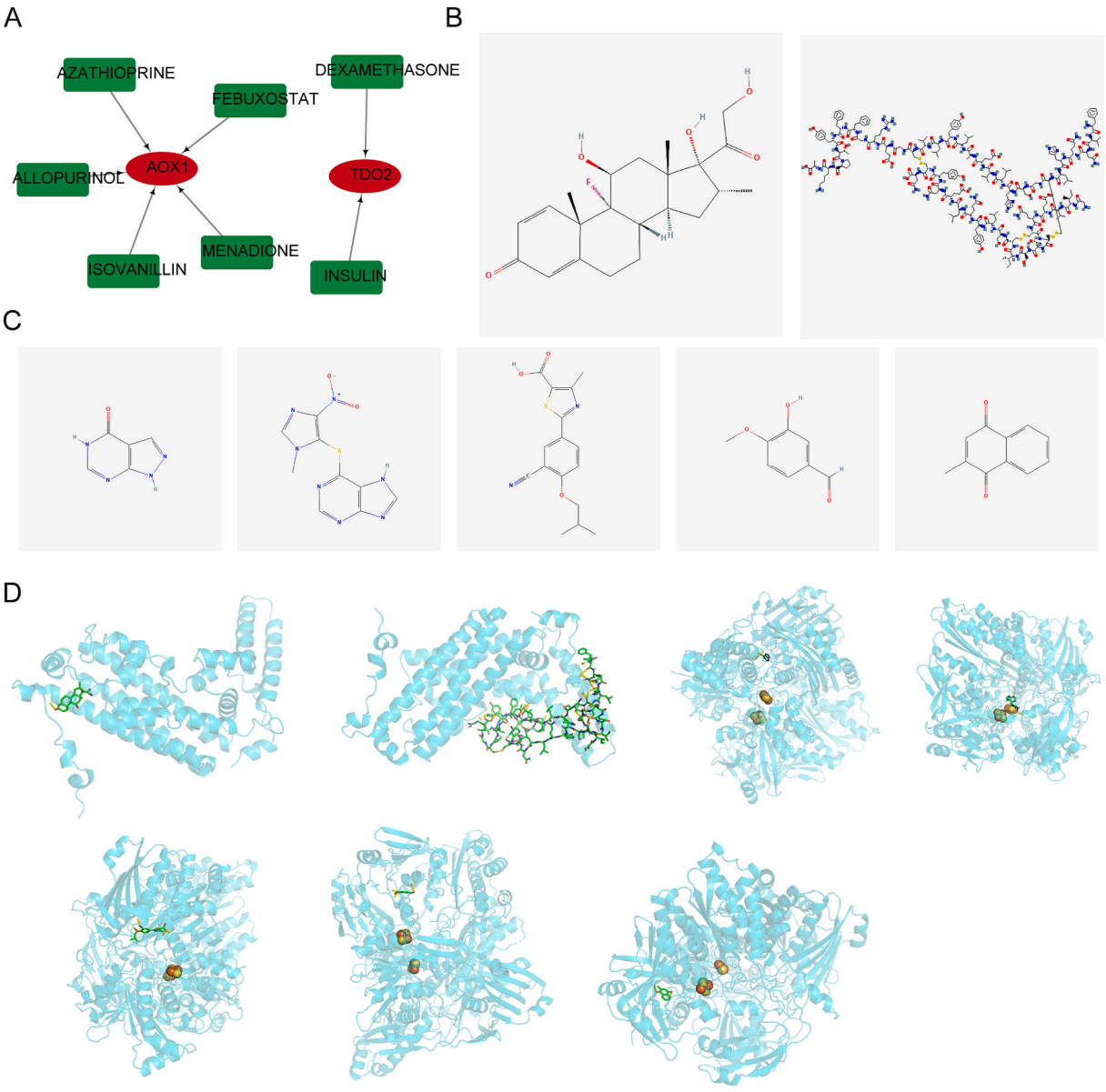

Fig. 7. Drug prediction of three diagnostic biomarkers. (A) The biomarkers-drug interaction network using DGIdb database. red represents biomarker, green represents drug targets. (B) The crystal structure of the two drug targets targeting *TDO2*. (C) The crystal structure of the five drug targets targeting *AOX1*. (D) Molecular docking results of corresponding therapeutic drugs targeting *TDO2* and *AOX1*.

Table 2
Molecular docking results of potential therapeutic drugs for *TDO2* and *AOX1*.

Gene	Drug	Binding Energy(kcal/mol)
TDO2	DEXAMETHASONE	−7.4
TDO2	INSULIN	−5.6
AOX1	MENADIONE	−7.4
AOX1	AZATHIOPRINE	−6.9
AOX1	FEBUXOSTAT	−8.1
AOX1	ALLOPURINOL	−6.4
AOX1	ISOVANILLIN	−5.6

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Not applicable.

CRediT authorship contribution statement

Junjun Yang: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Formal analysis, Data curation. **Ping Zhou:** Data curation. **Tangbing Xu:** Methodology. **Kaida Bo:** Validation, Methodology, Data curation. **Chenxin Zhu:** Software. **Xu Wang:** Formal analysis, Data curation. **Jun Chang:** Writing – review & editing.

Declaration of competing interest

Authors declare that they have no competing interests.

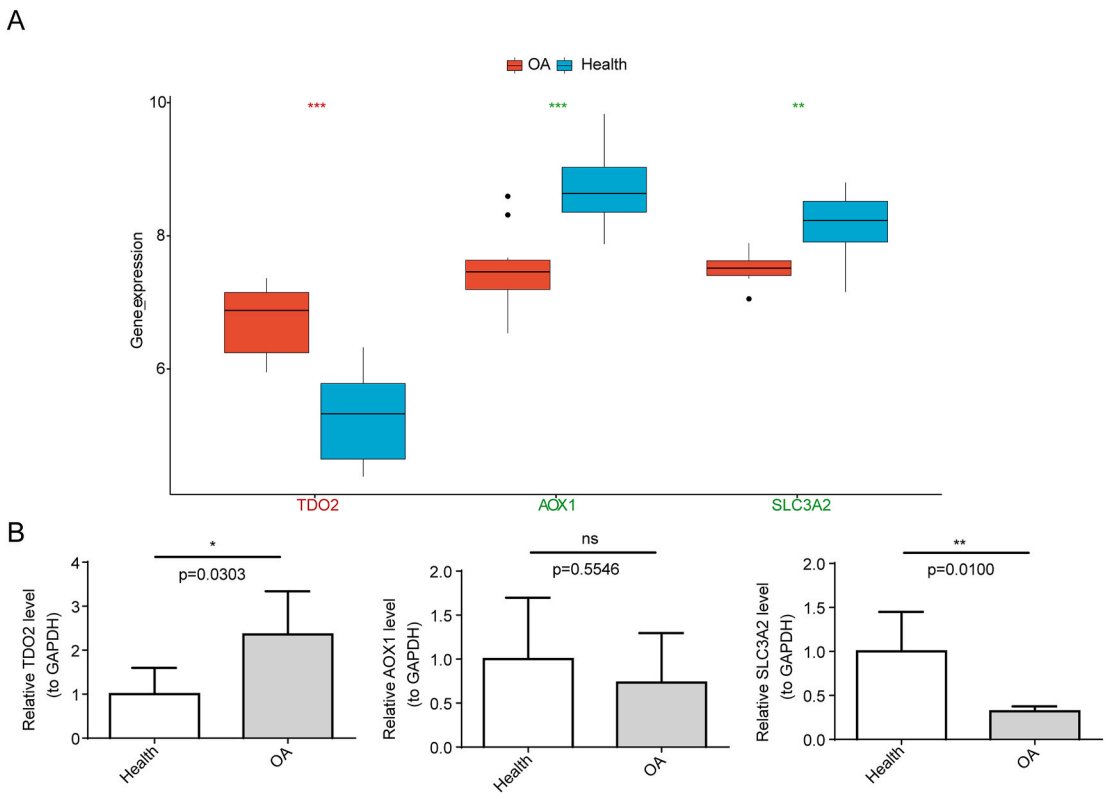

Fig. 8. Verifying of the expression level of three diagnostic biomarkers. (A) The expression level of diagnostic biomarkers in the GSE55235 datasets. (B) The expression of diagnostic biomarkers in the clinical OA and normal tissues using quantitative real-time fluorescence PCR (qRT-PCR). $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101763.

References

[1] W.B. Xu, V. Kotheeranurak, H.L. Zhang, J.Y. Feng, J.W. Liu, C.M. Chen, G.X. Lin, G. Rui, Identification of the circRNA-miRNA-mRNA regulatory network in osteoarthritis using bioinformatics analysis, Front. Genet. 13 (2022) 994163, https://doi.org/10.3389/fgene.2022.994163.

[2] L.A. Mandl, Osteoarthritis year in review 2018: clinical, Osteoarthritis Cartilage 27 (2019) 359–364, https://doi.org/10.1016/j.joca.2018.11.001.

[3] X. Hu, S. Ni, K. Zhao, J. Qian, Y. Duan, Bioinformatics-led discovery of osteoarthritis biomarkers and inflammatory infiltrates, Front. Immunol. 13 (2022) 871008, https://doi.org/10.3389/fimmu.2022.871008.

[4] P.M. Van Pevenage, J.T. Birchmier, R.K. June, Utilizing metabolomics to identify potential biomarkers and perturbed metabolic pathways in osteoarthritis: a systematic review, Semin. Arthritis Rheum. 59 (2023) 152163, https://doi.org/10.1016/j.semarthrit.2023.152163.

[5] S. Jang, K. Lee, J.H. Ju, Recent updates of diagnosis, pathophysiology, and treatment on osteoarthritis of the knee, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22052619.

[6] E. Charlier, C. Deroyer, F. Ciregia, O. Malaise, S. Neuville, Z. Plener, M. Malaise, D. de Seny, Chondrocyte dedifferentiation and osteoarthritis (OA), Biochem. Pharmacol. 165 (2019) 49–65, https://doi.org/10.1016/j.bcp.2019.02.036.

[7] Y. Qian, Y. Gong, Z. Fan, G. Luo, Q. Huang, S. Deng, H. Cheng, K. Jin, Q. Ni, X. Yu, C. Liu, Molecular alterations and targeted therapy in pancreatic ductal adenocarcinoma, J. Hematol. Oncol. 13 (2020) 130, https://doi.org/10.1186/s13045-020-00958-3.

[8] A.C. Tan, D.S.W. Tan, Targeted therapies for lung cancer patients with oncogenic driver molecular alterations, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 40 (2022) 611–625, https://doi.org/10.1200/jco.21.01626.

[9] Q. Yao, X. Wu, C. Tao, W. Gong, M. Chen, M. Qu, Y. Zhong, T. He, S. Chen, G. Xiao, Osteoarthritis: pathogenic signaling pathways and therapeutic targets, Signal Transduct. Targeted Ther. 8 (2023) 56, https://doi.org/10.1038/s41392-023-01330-w.

[10] C. Xue, X. Gu, Y. Zhao, J. Jia, Q. Zheng, Y. Su, Z. Bao, J. Lu, L. Li, Prediction of hepatocellular carcinoma prognosis and immunotherapeutic effects based on tryptophan metabolism-related genes, Cancer Cell Int. 22 (2022) 308, https://doi.org/10.1186/s12935-022-02730-8.

[11] Y. Li, W. Xiao, W. Luo, C. Zeng, Z. Deng, W. Ren, G. Wu, G. Lei, Alterations of amino acid metabolism in osteoarthritis: its implications for nutrition and health, Amino Acids 48 (2016) 907–914, https://doi.org/10.1007/s00726-015-2168-x.

[12] L. Chen, Z. Huang, Q. Li, C. Chen, Y. Luo, P. Kang, Activated intestinal microbiome-associated tryptophan metabolism upregulates aryl hydrocarbon receptor to promote osteoarthritis in a rat model, Int. Immunopharm. 118 (2023) 110020, https://doi.org/10.1016/j.intimp.2023.110020.

[13] A. Colaprico, T.C. Silva, C. Olsen, L. Garofano, C. Cava, D. Garolini, T.S. Sabedot, T. M. Malta, S.M. Pagnotta, I. Castiglioni, M. Ceccarelli, G. Bontempi, H. Noushmehr, TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data, Nucleic Acids Res. 44 (2016) e71, https://doi.org/10.1093/nar/gkv1507.

[14] G. Yu, L.G. Wang, Y. Han, Q.Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS A J. Integr. Biol. 16 (2012) 284–287, https://doi.org/10.1089/omi.2011.0118.

[15] X. Robin, N. Turck, A. Hainard, N. Tiberti, F. Lisacek, J.C. Sanchez, M. Müller, pROC: an open-source package for R and S+ to analyze and compare ROC curves, BMC Bioinf. 12 (2011) 77, https://doi.org/10.1186/1471-2105-12-77.

[16] L. Kumar, E.F. M, Mfuzz: a software package for soft clustering of microarray data, Bioinformation 2 (2007) 5–7, https://doi.org/10.6026/97320630002005.

[17] P. Liu, H. Xu, Y. Shi, L. Deng, X. Chen, Potential molecular mechanisms of plantain in the treatment of gout and hyperuricemia based on network pharmacology, Evid. base Compl. Alternative Med. : eCAM 2020 (2020) 3023127, https://doi.org/10.1155/2020/3023127.

[18] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.

[19] X.L. Zhang, J. Yang, L. Yang, J.G. Liu, X.Y. Cai, W.M. Fan, X.Q. Yun, J.Z. Ma, X. S. Weng, Efficacy and safety of zhuanggu joint capsules in combination with celecoxib in knee osteoarthritis: a multi-center, randomized, double-blind, double-dummy, and parallel controlled trial, Chin. Med. J. 129 (2016) 891–897, https://doi.org/10.4103/0366-6999.179789.

[20] J. Wu, K. Wang, J. Xu, G. Ruan, Q. Zhu, J. Cai, J. Ren, S. Zheng, Z. Zhu, P. Otahal, C. Ding, Associations between serum ghrelin and knee symptoms, joint structures and cartilage or bone biomarkers in patients with knee osteoarthritis,

Osteoarthritis Cartilage 25 (2017) 1428–1435, https://doi.org/10.1016/j.joca.2017.05.015.
[21] H.J. Ball, H.J. Yuasa, C.J. Austin, S. Weiser, N.H. Hunt, Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway, Int. J. Biochem. Cell Biol. 41 (2009) 467–471, https://doi.org/10.1016/j.biocel.2008.01.005.
[22] G. Rong, T. Zhang, Y. Xu, Z. Zhang, B. Gui, K. Hu, J. Zhang, Z. Tang, C. Shen, High levels of TDO2 in relation to pro-inflammatory cytokines in synovium and synovial fluid of patients with osteoarthritis, BMC Muscoskel. Disord. 23 (2022) 604, https://doi.org/10.1186/s12891-022-05567-4.
[23] Y. Chang, P. Han, Y. Wang, C. Jia, B. Zhang, Y. Zhao, S. Li, S. Li, X. Wang, X. Yang, W. Wei, Tryptophan 2,3-dioxygenase 2 plays a key role in regulating the activation of fibroblast-like synoviocytes in autoimmune arthritis, Br. J. Pharmacol. 179 (2022) 3024–3042, https://doi.org/10.1111/bph.15787.
[24] L. Xiong, Y. Feng, W. Hu, J. Tan, S. Li, H. Wang, Expression of AOX1 predicts prognosis of clear cell renal cell carcinoma, Front. Genet. 12 (2021) 683173, https://doi.org/10.3389/fgene.2021.683173.
[25] J. Fort, L.R. de la Ballina, H.E. Burghardt, C. Ferrer-Costa, J. Turnay, C. Ferrer-Orta, I. Usón, A. Zorzano, J. Fernández-Recio, M. Orozco, M.A. Lizarbe, I. Fita, M. Palacín, The structure of human 4F2hc ectodomain provides a model for homodimerization and electrostatic interaction with plasma membrane, J. Biol. Chem. 282 (2007) 31444–31452, https://doi.org/10.1074/jbc.M704524200.
[26] D. Fotiadis, Y. Kanai, M. Palacín, The SLC3 and SLC7 families of amino acid transporters, Mol. Aspect. Med. 34 (2013) 139–158, https://doi.org/10.1016/j.mam.2012.10.007.
[27] H. Liu, Z. Deng, B. Yu, H. Liu, Z. Yang, A. Zeng, M. Fu, Identification of SLC3A2 as a potential therapeutic target of osteoarthritis involved in ferroptosis by integrating bioinformatics, clinical factors and experiments, Cells 11 (2022), https://doi.org/10.3390/cells11213430.
[28] S.J. Dixon, D.N. Patel, M. Welsch, R. Skouta, E.D. Lee, M. Hayano, A.G. Thomas, C. E. Gleason, N.P. Tatonetti, B.S. Slusher, B.R. Stockwell, Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis, Elife 3 (2014) e02523, https://doi.org/10.7554/eLife.02523.
[29] F. Wu, G. Xiong, Z. Chen, C. Lei, Q. Liu, Y. Bai, SLC3A2 inhibits ferroptosis in laryngeal carcinoma via mTOR pathway, Hereditas 159 (2022) 6, https://doi.org/10.1186/s41065-022-00225-0.
[30] J. Dong, S. Li, Z. Lu, P. Du, G. Liu, M. Li, C. Ma, J. Zhou, J. Bao, HCMV-miR-US33-5p promotes apoptosis of aortic vascular smooth muscle cells by targeting EPAS1/SLC3A2 pathway, Cell. Mol. Biol. Lett. 27 (2022) 40, https://doi.org/10.1186/s11658-022-00340-w.
[31] N. Sepay, S. Chakrabarti, M. Afzal, A. Alarifi, D. Mal, Identification of 4-acrylamido-N-(pyridazin-3-yl)benzamide as anti-COVID-19 compound: a DFTB, molecular docking, and molecular dynamics study, RSC Adv. 12 (2022) 24178–24186, https://doi.org/10.1039/d2ra04333e.
[32] A. Kumar, P.R. Arantes, A. Saha, G. Palermo, B.M. Wong, GPU-enhanced DFTB metadynamics for efficiently predicting free energies of biochemical systems, Molecules 28 (2023), https://doi.org/10.3390/molecules28031277.
[33] Q. Geng, H. Zhang, Y. Cui, Q. Wei, S. Wang, Febuxostat mitigates IL-18-induced inflammatory response and reduction of extracellular matrix gene, Am. J. Tourism Res. 13 (2021) 979–987.
[34] E. Pascual, F. Sivera, U. Yasothan, P. Kirkpatrick, Febuxostat, Nat. Rev. Drug Discov. 8 (2009) 191–192, https://doi.org/10.1038/nrd2831.
[35] C. Wernecke, H.J. Braun, J.L. Dragoo, The effect of intra-articular corticosteroids on articular cartilage: a systematic review, Orthopaedic journal of sports medicine 3 (2015), https://doi.org/10.1177/2325967115581163, 2325967115581163.
[36] W. Fang, F. Yang, W. Li, Q. Hu, W. Chen, M. Yang, J. Chen, L. Qiu, Dexamethasone microspheres and celecoxib microcrystals loaded into injectable gels for enhanced knee osteoarthritis therapy, Int. J. Pharm. 622 (2022) 121802, https://doi.org/10.1016/j.ijpharm.2022.121802.
[37] S. Zhu, D. Makosa, B. Miller, T.M. Griffin, Glutathione as a mediator of cartilage oxidative stress resistance and resilience during aging and osteoarthritis, Connect. Tissue Res. 61 (2020) 34–47, https://doi.org/10.1080/03008207.2019.1665035.