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IDENTIFICATION OF KLF9 AND FOSL2 AS ENDOPLASMIC RETICULUM STRESS SIGNATURE GENES IN OSTEOARTHRITIS WITH MULTIPLE MACHINE LEARNING APPROACHES

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Abstract. Objective: This study aims to screen osteoarthritis (OA) endoplasmic reticulum (ER) stress signature genes using a machine learning approach to provide new insights and methods for OA treatment. Methods: We obtained GSE55235 and GSE98918 datasets from the gene expression omnibus (GEO) database and identified ER stress-related genes from the GeneCard database. We used R software to perform data batch correction, extract OA endoplasmic reticulum stress-related genes, and conduct differential analysis. We performed functional Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis, and gene set enrichment analysis (GSEA) on differentially expressed genes (DEGs). Additionally, we used machine learning algorithms, including Least Absolute Shrinkage and Selection Operator (LASSO) regression, SVM-RFE, and weighted gene co-expression network analysis (WGCNA), to screen OA endoplasmic reticulum stress signature genes. Human chondrocytes were selected for OA model establishment, cells without any treatment were served as the control. Results: We obtained 236 DEGs related to OA ER stress. GO and KEGG enrichment analysis showed that these genes were mainly involved in the positive regulation of leukocyte activation, collagen-containing extracellular matrix, phagosome, and other biological functions or signaling pathways. GSEA-GO analysis revealed that ER stress genes were significantly enriched in the negative regulation in metabolic processes of nucleobase-containing compounds (NES = -2.50, $P < 0.001$), while OA ER stress genes were significantly enriched in the processing and presentation of peptide antigens (NES = 2.40, $P < 0.001$). Using WGCNA analysis, LASSO regression analysis, and SVM-RFE analysis of intersection, we identified KLF9 and FOSL2 as potential OA endoplasmic reticulum stress signature genes, which were found to be more accurate as OA signature genes after validation. KLF9 expression in OA group was higher than that in control group, while FOSL2 expression was lower ($P < 0.05$). Conclusion: Machine learning and co-expression network analysis can effectively identify the genes and potential factors characteristic of ER stress in OA, which can help elucidate its pathogenesis and provide a new direction for better clinical treatment.

Keywords: Osteoarthritis, endoplasmic reticulum stress, machine learning

1 INTRODUCTION

Osteoarthritis (OA) is a chronic, degenerative joint disease characterized by cartilage degeneration, joint pain, and functional impairment. The knee joint is the one most frequently impacted by osteoarthritis (OA). The protective cartilage that covers the ends of bones in a joint begins to break down in osteoarthritis, a degenerative joint condition. With an aging society, the incidence of OA is on the rise, affecting about 1/3 of the elderly population over 65 years old who suffer from OA [1]. Furthermore,

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59–87 % of OA patients also suffer from at least one other chronic disease, especially cardiac and metabolic diseases [2]. Currently, there are no effective means to stop the progression of the disease, which results in joint pain, disability and loss of workforce. Therefore, the prevention and treatment of OA are treated as a priority in regional health policies and even recommended for attention through the revision of clinical and public policies on OA [3]. In this study, we explored the molecular mechanisms of OA to contribute to its treatment.

It is a prerequisite for OA prevention to maintain chondrocyte homeostasis, the only cell type in articular cartilage [4]. As a new area of research in OA cartilage biology, endoplasmic reticulum (ER) stress plays a key role in regulating cell survival and homeostasis [5, 6]. Previous studies have demonstated that ER stress in chondrocytes in OA is associated with cartilage degradation [7]. ER stress induces apoptosis and upregulates matrix metalloproteinase expression in chondrocytes [8], while disruption of endoplasmic reticulum homeostasis leads to the accumulation of unfolded proteins and apoptosis. Therefore, targeting ER stress can effectively delay OA degeneration. However, the molecular mechanisms associated with ER stress in OA remain unclear [9].

The development of bioinformatics technology provides an efficient, intuitive, and reliable basis for elucidating the disease's multilevel and multifaceted molecular mechanisms. Obtaining OA ER stress signature genes through bioinformatics could provide effective potential for OA treatment. In this study, we identified OA ER stress-related genes from GEO and GeneCard databases by differential expression analysis with the WGCNA, identified OA ER stress signature genes using machine learning approaches (LASSO regression, SVM-RFE), and validated them using OA meniscus dataset, and performed gene expression by real-time fluorescence quantitative PCR (RT-qPCR) detection to provide possible molecular targets for studying OA from ER stress.

2 MATERIALS AND METHODS

2.1 Screening of OA Differentially Expressed Genes

The GEO database is a regularly updated information-sharing website developed by the U.S. government and includes genetic data related to various physiological or pathological states. Its main goals are to make data sharing possible, archive research results, make cross-study comparisons easier, improve validity and reproducibility, enable integrative analysis, promote the development of bioinformatics tools, and act as a learning tool. It provides free access to researchers worldwide, who can upload and access data. The GSE55235 and GSE98918 datasets were filtered by entering "Osteoarthritis" as a search term in the search field of the website. GSE55235 contains gene expression data from 10 OA patients, 10 rheumatoid patients, 10 normal joint patients. The samples of OA patients and 10 normal joint patients were selected. OA DEGs derived from synovial tissue were screened by "limma" package analysis in the R software, with $P \geq 0.05$ and ≥ 1 as output con-

ditions. GSE98918 contained gene expression data from 12 OA and 12 normal joint patients. The same method was used to obtain OA DEGs derived from meniscal tissues for subsequent validation of signature genes.

2.2 Screening for OA ER Stress-Related Genes

GeneCard is a comprehensive bioinformatics database created and maintained by a non-profit organization that provides concise genomic, proteomic, and related human gene queries. GeneCards is a comprehensive database and online resource that offers details about human genes. It is a useful resource for academics, doctors, and researchers looking for in-depth information about specific genes and their functions in health and illness. The "endoplasmic reticulum stress" was used as a keyword to filter the ER stress-related genes in this database. Then the ER stress-related genes were obtained by intersecting OA DEGs with ER stress-related genes using the R software "VennDiagram".

2.3 GO Endorsement and KEGG Pathway Enrichment Analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) are commonly used in bioinformatics for gene enrichment, which can reveal potential mechanisms at the molecular level of disease from the perspective of cellular components (CC), molecular functions (MF), biological processes (BP) and signaling pathways. Structures within a cell where gene products are active or situated are classified as cellular components in Gene Ontology. Organelles, membranes, complicated systems, the cytoskeleton, extracellular structures, the nucleus, cytoplasm, as well as tiny cells are a few examples of these parts. In Gene Ontology, groups of molecular events that result in certain biological consequences are referred to as biological processes (BP). They are arranged in a hierarchy and concentrate on functional explanations of how gene products are involved. After downloading the "clusterProfiler". Gene ontology (GO), a standardized framework used to characterize the functional characteristics of genes and gene products across many biological settings, includes Molecular Functions (MF) as a core component. "Disease Ontology Semantic and Enrichment Analysis (DOSE)", and "pathview" packages in R software, the OA ER stress-related genes were processed and annotated for subsequent analysis. Genes are categorized by GO analysis according to their roles, activities, and locations, whereas KEGG pathway analysis places genes in the context of established networks or pathways. In contrast to KEGG pathway analysis, which concentrates on intricate pathway connections and linkages, GO analysis offers a broad functional perspective.

2.4 GSEA Enrichment Analysis

GSEA analysis can sensitively identify significantly enriched gene sets and their trends by analyzing all genes in the dataset. The main goal of GSEA is to assess

if a group of predetermined genes, frequently connected to a biological pathway or function, is highly enriched at the top or bottom of a list of genes. By taking into account all of the dataset's genes and examining them in the context of pathway-level settings, Gene Set Enrichment Analysis (GSEA) efficiently pinpoints enriched gene sets and trends. GSEA uses permutation-based statistical testing, ranks genes according to modifications to expression, and displays enrichment groupings. In this study, we used the R software "clusterProfiler" for GO analysis of GSEA and "ggplot2" for visualization of the top 5 enrichment results, with FDR < 0.25 and $P < 0.05$ as the screening criteria.

2.5 Weighted Gene Co-Expression Network Analysis (WGCNA)

WGCNA is a common modular analysis technique used to screen biomarkers or drug targets for complex diseases and to identify highly synergistic gene expression matrices. The main idea underlying WGCNA is to find modules that are connected to particular traits or circumstances by classifying genes with comparable expression patterns into those modules. By performing co-expression network analysis on the corrected genes through the "WGCNA" package in R software, we can mine the genes with similar expression patterns based on the differences in gene expression patterns to obtain the module with the highest OA ER stress correlation and all the characteristic genes it contained.

2.6 LASSO Regression Analysis and SVM-RFE Analysis

LASSO regression analysis allows for both variable selection and regularization to improve the predictive accuracy and interpretability of the statistical models, while SVM-RFE analysis is a sequence backward selection algorithm based on the maximum interval principle of SVM. In machine learning and statistics, the Sequence Backward Selection (SBS) method is a feature selection method. The Sequential Feature Selection (SFS) algorithm, which is more widely used, is a version of this one. LASSO regression analysis and SVM-RFE analysis of OA ER stress genes were performed using the R software "glmnet, e1071, kernlab, caret" package. The results of WGCNA analysis, LASSO regression analysis, and SVM-RFE analysis were intersected by the Venn diagram to obtain the OA ER stress genes, the ROC curves were plotted for validation. After verifying the results again using the GSE98918 data, the test ROC is plotted.

2.7 In Vitro Experiments and Polymerase Chain Reaction (PCR)

Human chondrocytes (CP-H107, Pronounced) were divided into control group (normal human chondrocytes) and OA group (OA cell model). It involves variations in the expression of genes and proteins, cell viability and shape, extracellular matrix composition, inflammatory response, cell signalling pathways, mechanical qualities,

and disease-related variables. Control group was not treated. And OA group was treated by removing the cell culture supernatant, washing twice with 1 × PBS, adding trypsin with a volume fraction of 0.25 % (containing EDTA with a volume fraction of 0.02 %) for digestion, waiting for the cells to become round and adding medium to terminate the digestion and collecting the cell suspension into a 10 mL centrifuge tube, centrifuging at 1 000 r/min for 3 min, discarding the supernatant and adding medium to resuspend the cells, preparing the cell suspension at a concentration of $2 \times 108\,L^{-1}$ for spreading treatment. The next day, when the cells were in good condition, interleukin 1 β (10 μg/L) was added for 24 h to simulate OA chondrocyte modeling. Using RT-qPCR, the expression of type II collagen was detected for model validation and mRNA expression of genes characteristic of OA endoplasmic reticulum stress was detected after successful modeling.

Total RNA was extracted from chondrocytes using Trizon Reagent (CW0580S, CWBIO), held at 50°C for 15 min, then transferred to 85°C and held for 5 s for reverse transcription reaction. A popular solution for RNA extraction from many biological materials, including cells like chondrocytes, is Trizol Reagent. It provides a yield of high-quality RNA with little degradation, allowing for downstream studies. To separate RNA, DNA, and proteins from biological materials, a solution based on phenol and guanidine isothiocyanate is known as Trizol Reagent. qPCR was performed using ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme) at at 95°C for 10 min for 1 cycle, 95°C for 10 s, 58°C for 30 s, and 72°C for 30 s under a 40-cycle reaction program. β-actin was used as an internal reference to calculate the ΔCt values for control and OA groups, respectively, and the relative expression was calculated using the 2-$\Delta\Delta$Ct method. The primer sequences are shown in Table 1.

Gene	Primer Sequence (5-3)	Fragment Length (bp)
β-actin	Forward: TGGCACCCAGCACAATGAA Reverse: CTAAGTCATAGTCCGCCTAGAAGCA	186
KLF9	Forward: AGTCCAGATGAGGATATGGGATC Reverse: TCTTTTCGGAGGCGTGTTT	163
FOSL2	Forward: AACTTTGACACCTCGTCCCG Reverse: CTGAGCCAGGCATATCTACCC	109

Table 1. qPCR primer sequences

The statistical analysis for this study was conducted using SPSS 19.0 software. All experiments were repeated three times. Data were presented using ($\pm$ s), and the differences between groups were evaluted using independent samples t-test, with $P < 0.05$ representing a significant difference.

3 RESULTS

3.1 Acquisition of OA Differentially Expressed Genes

The GSE55235 was analyzed for differential expression, and the data were validated by normalization and reproducible principal component analysis (PCA), respectively. Data analysis and machine learning both employ the dimensionality-reduction method known as Principal Component Analysis (PCA). By converting high-dimensional data into a new coordinate system and capturing the most crucial details while lowering the number of dimensions, it reduces the number of dimensions. The top 50 differential genes were selected for visual heat map processing. 618 OA DEGs were obtained (Figure 1). It entails carrying out an experiment, gathering data on gene expression, preprocessing the data, analyzing differential expression statistically, ranking genes according to significance and fold change, choosing the best genes that meet predetermined criteria, and producing a heat map to visualize expression patterns. In visual heat map processing, patterns and trends in data are graphically represented. This technique is frequently used in genomics to show how gene expression levels vary across various situations or samples.

3.2 Screening for OA ER Stress-Related Genes

After screening 6190 ER stress-related genes with "endoplasmic reticulum stress" as search keyword in the GeneCard database, we used the R software "VennDiagram" package to intersect the 618 OA DEGs of GSE55235 with 6190 ER stress-related genes, and ultimately obtained 236 OA ER stress-related genes (Figure 2).

3.3 GO and KEGG Enrichment Analysis

GO and KEGG enrichment analysis of 236 OA ER stress-related genes by the R software Bioconductor package revealed that the genes were significantly enriched mainly in the extracellular matrix, proteoglycan binding, glycosaminoglycan binding and other biological functions (Figure 3, Table 2). Protein cores and ligands on cell surfaces or in the extracellular matrix interact during proteoglycan binding. It aids in the formation of the matrix, cell adhesion, signalling, and tissue structure.

3.4 GSEA Enrichment Analysis

GSEA analysis of the dataset genes by R software and GSEA-GO analysis revealed that ER stress genes were significantly enriched in the negative regulation of metabolic processes of nucleobase-containing compounds (NES = -2.50, $P < 0.001$), chromatin components (NES = -2.33, $P < 0.001$), sequence-specific DNA binding of cis-regulatory regions (NES = -2.36, $P < 0.001$), sequence-specific

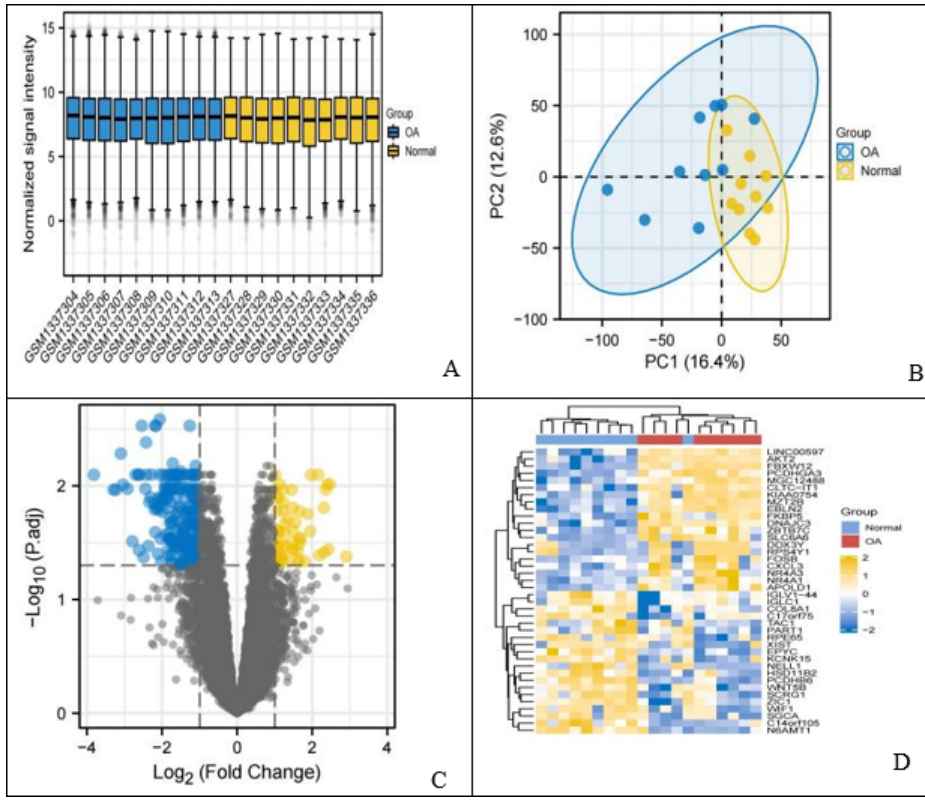

Figure 1. A) Normalized calibration plot of data from 20 samples, blue for normal joint patients, yellow for OA patients. B) Volcano plot of OA differential genes, yellow for upregulated genes, blue for down-regulated genes. C) Validation plot of repeatability principal component analysis. The denser the scatter of the same kind, the higher the repeatability. Yellow for OA specimens, blue for normal joint specimens. D) The top 50 OA differential genes visualized heat map, yellow is high expression, blue is low expression. Blue signifies healthy joints, whereas yellow denotes increased expression in OA. 'D' stands for the top 50 OA differential genes. The heat map reveals molecular differences between OA and healthy joints, as well as expression trends, differential genes, and expression patterns.

DNA binding (NES = −2.38, P < 0.001) and transcriptional regulatory activity (NES = −2.34, P < 0.001); OA ER stress genes were significantly enriched in peptide antigen processing and presentation (NES = 2.40, P < 0.001), antigen receptor-mediated signaling pathways (NES = 2.39, P < 0.001), collagen metabolic processes (NES = 2.40, P < 0.001), intracellular membranes (NES = 2.51, P < 0.001) and intrinsic components of the plasma membrane (NES = 2.51, P < 0.001) (Figure 4).

Figure 2. Intersection of OA-related genes and ER stress

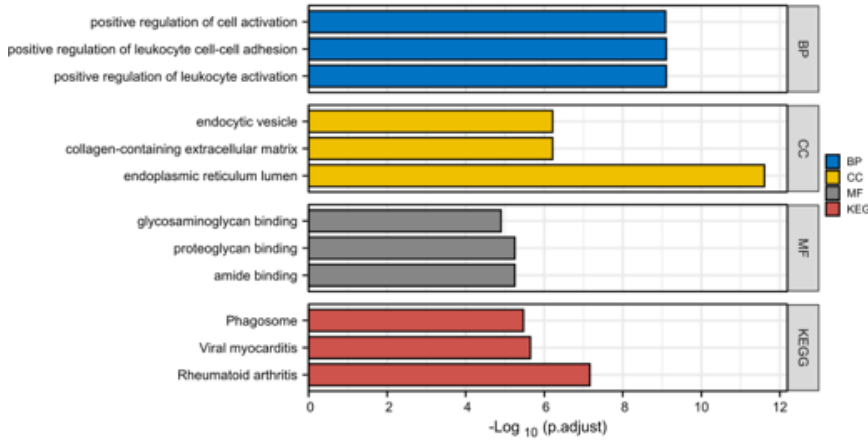

Figure 3. Analysis of GO and KEGG enrichment

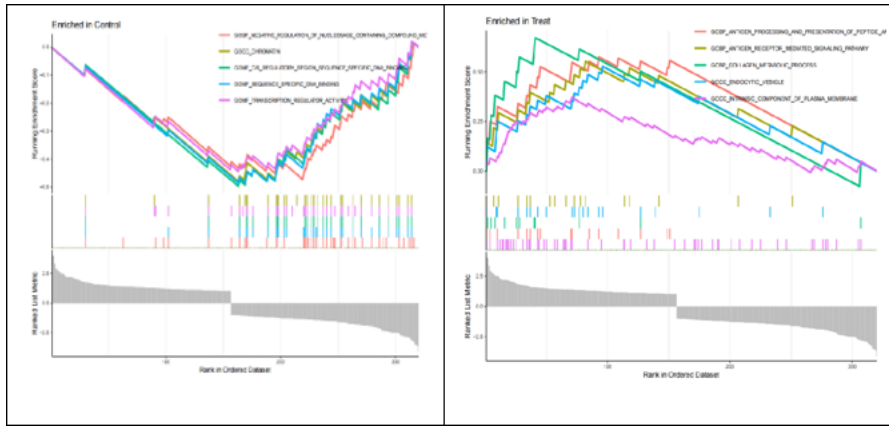

Figure 4. Analysis of GSEA enrichment

Ontology	ID	Description	GeneRatio	p.adjust
BP	GO:0002696	positive regulation of leukocyte activation	27/232	< 0.01
BP	GO:1903039	positive regulation of leukocyte cell-cell adhesion	21/232	< 0.01
BP	GO:0050867	positive regulation of cell activation	27/232	< 0.01
CC	GO:0005788	endoplasmic reticulum lumen	26/236	< 0.01
CC	GO:0062023	collagen-containing extracellular matrix	22/236	< 0.01
CC	GO:0030139	endocytic vesicle	19/236	< 0.01
MF	GO:0033218	amide binding	21/234	< 0.01
MF	GO:0043394	proteoglycan binding	8/234	< 0.01
MF	GO:0005539	glycosaminoglycan binding	16/234	< 0.01
KEGG	hsa05323	Rheumatoid arthritis	15/159	< 0.01
KEGG	hsa05416	Viral myocarditis	11/159	< 0.01
KEGG	hsa04145	Phagosome	16/159	< 0.01

Note: BP, biological process; CC, cellular component; MF, biological function; KEGG, pathway enrichment.

Table 2. GO and KEGG enrichment analysis results

3.5 WGCNA Analysis

WGCNA analysis of the calibration genes was performed using R software, and the hierarchical clustering map of the samples was drawn by calculating the correlation between samples. Meanwhile, three gene modules (grey module, blue module, turquoise module) were obtained using the algorithm of the dynamic shear tree, in which grey module contained CDKAL1 gene, blue module contained EIF2AK3, KDELR3, PPP1R15A, ATF3, and CEBPB, and the turquoise module contained PPP1R15A, CLU, IL6, CXCL8 and ATF3 (Figure 5). Genes with weak correlations are shown in the grey module, significantly co-expressed genes linked to certain functions are shown in the blue module, and diversified genes that may play several roles are shown in the turquoise module.

3.6 LASSO Regression Analysis and SVM-RFE Analysis

LASSO regression analysis and SVM-RFE analysis of OA ER stress-related genes were performed in R software. Eight OA ER stress signature genes (KLF9, FOSL2, RHOB, CEBPD, DDIT4, ADM, TNFSF11, PPIC) were screened by LASSO re-

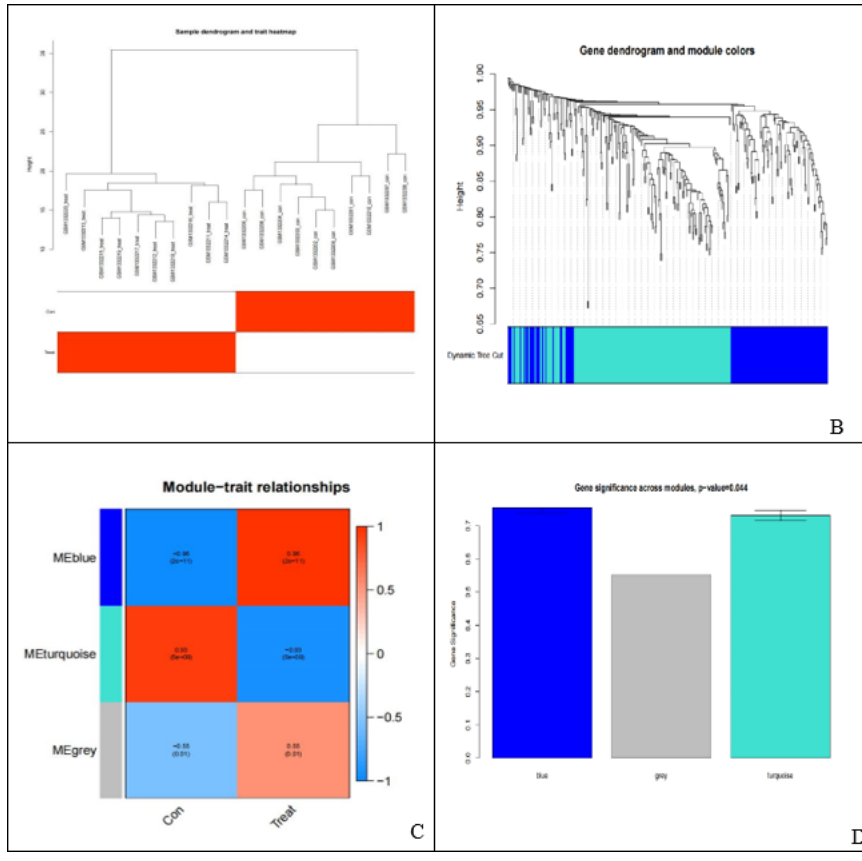

Figure 5. The WCGAN analysis results

gression analysis. The two OA ER stress signature genes (KLF9 and FOSL2) were screened by SVM-RFE analysis. The main objective is to determine which genes are most pertinent and useful for classifying or predicting osteoarthritis in light of ER stress-related mechanisms. By the intersection of WGCNA analysis, LASSO regression analysis and SVM-RFE analysis was performed using the R software "Veen" package, KLF9 and FOSL2 were obtained. The signature genes were validated using the GSE98918 dataset, and in experimental group, KLF9 expression was found to be higher than control group ($P = 0.67$), but the difference was not statistically significant; FOSL2 expression was lower than control group ($P < 0.05$), and the difference was statistically significant. ROC curves were further applied to determine the KLF9 and FOSL2 as disease signature genes The study showed that ROC: AUC = 1, both of which were greater than 0.5, indicating that the accuracy of KLF9 and FOSL2 as disease signature genes were high (Figure 6).

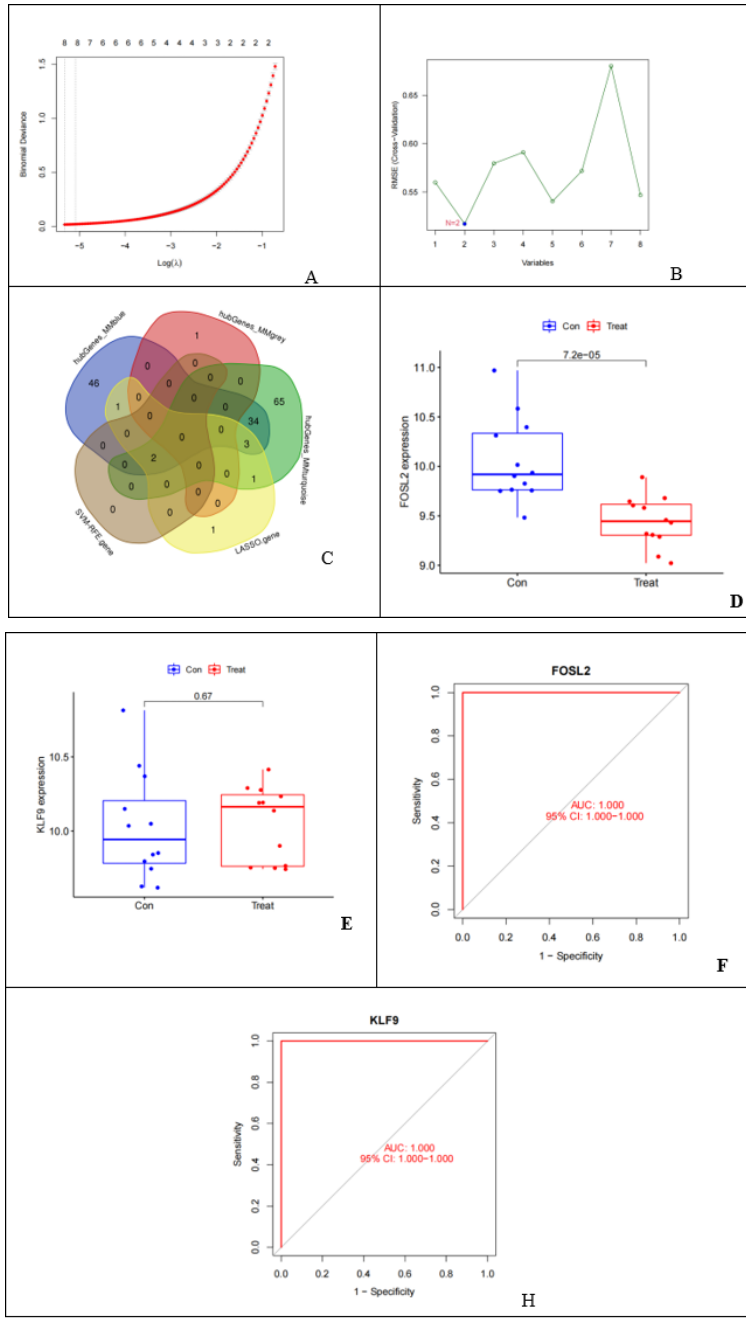

Figure 6. LASSO plot, SVM-RFE plot, intersection of different algorithms and ROC curve plot

3.7 In Vitro Cellular Gene Expression Validation

RT-qPCR results showed that KLF9 ($P = 0.046$) and FOSL2 ($P = 0.008$) were significantly different ($P < 0.05$) in the control and OA groups (Figure 7).

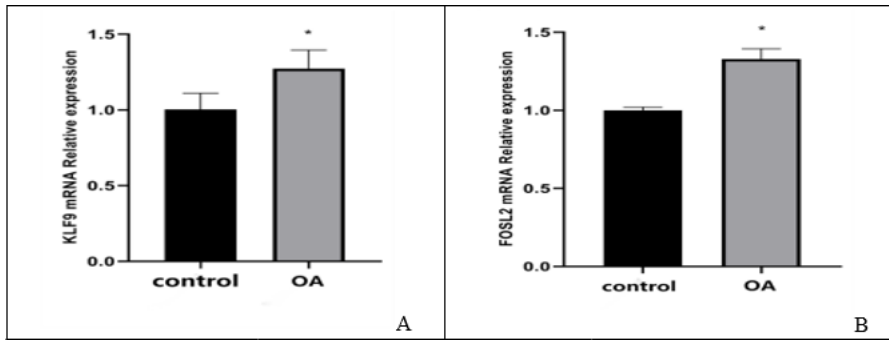

Figure 7. Relative expression of KDELR3 mRNA in two groups of chondrocytes by qPCR

4 DISCUSSION

OA is the most common joint disease worldwide and is characterized by degenerative destruction of articular cartilage. But the pathogenesis of OA remains unclear. Some studies have confirmed the involvement of ER stress in OA progression [10] and even suggested that ER stress-induced chondrocyte death is a contributing factor to OA cartilage degeneration. Modulating this signaling pathway may be a potential therapeutic strategy for OA [11]. Nevertheless, the mechanism of action remains unknown. Therefore, based on bioinformatics and machine learning approaches, we have identified key genes and their associated features of ER stress in OA to provide a basis for further exploring the pathogenesis of OA from the perspective of ER stress.

We screened OA DEGs and ER stress-related genes from GEO and GeneCard databases, respectively, and obtained 236 OA ER stress-related genes after intersection. Then, GO and KEGG analyses revealed that they were significantly enriched mainly in biological functions related to collagen-containing extracellular matrix, proteoglycan binding, and glycosaminoglycan binding. Chondrocyte ER stress is one of the most important aspects affecting OA cartilage degeneration [12, 13] with the extracellular matrix playing an important role in this process [14]. It may result from various cellular stresses such as hypoxia, oxidative stress, or accumulation of glycosylation end products that affect the synthesis and secretion of extracellular matrix, leading to the deposition of protein aggregates in the endoplasmic reticulum, ultimately inducing ER stress [15]. The endoplasmic reticulum, an organelle with important biological functions within cells, is subject to significant ER

stress during articular cartilage degeneration [16, 17]. Core proteoglycan, a typical proteoglycan, is an important raw material for the collagen binding process and plays an important role in the pathogenesis of OA. A prospective study found [18] that serum levels of core proteoglycan were positively correlated with WOMAC scores in OA patients, suggesting that proteoglycan is one of the important risk factors for the development of OA. Chondroitin sulfate, a typical representative of glycosaminoglycans, is commonly found in cartilage and extracellular matrix [19]. By safeguarding cartilage, lowering inflammation, easing pain, increasing synovial fluid characteristics, and maybe slowing the advancement of the illness, chondroitin sulfate is essential in controlling the symptoms of osteoarthritis (OA). Its many advantages make it a crucial part of managing OA since they help with pain alleviation and better joint function. Due to its anti-inflammatory activity, chondroitin sulfate plays a therapeutic role in symptomatic OA. Its anti-stress properties also play an important role in maintaining the structural integrity of cartilage, delaying cartilage degeneration, and reducing cartilage degeneration symptoms in OA patients [20].

Further GSEA analysis of ER stress-related genes in OA showed their significant enrichment in peptide antigen processing and presentation, antigen receptor-mediated signaling pathways. This suggests OA ER stress may be closely related to antigen or organismal immune response. It was previously believed that OA was only attributed to type II collagen and proteoglycan degradation which led to cartilage destruction. However, in recent years, many scholars have agreed that B and T lymphocyte populations are important components of antigen-specific lymphocytes involved in innate and adaptive immune responses, which can cause inflammatory responses and thus exacerbate OA progression [21, 22].

By performing WGCNA analysis, LASSO regression, and SVM-RFE analysis on OA ER stress-related genes, we finally screened KLF9 and FOSL2 as OA ER stress signature genes. KLF9, as a member of the KLF family, was predicted by BioShin to also as an OA signature gene [23]. KLF9 plays an important role in the oxidative stress response. It is affected by Nrf2 stimulation, which can increase KLF9-dependent ROS and eventually lead to cartilage degradation [24]. In addition, KLF9 induces TMEM38B, ITPR1 expression of endoplasmic reticulum calcium stores, which promotes the release of additional calcium from endoplasmic reticulum calcium and exacerbates ER stress and cell death [25]. FOSL2 is a key regulator of leptin expression in adipocytes. When deficient in humans, the risk of obesity increases, and its expression is also suppressed in patients with early OA when chondrocytes are in an early hypertrophic state; this is similar to the findings of Xie et al. [26]. However, no direct regulation of OA by KLF9 and FOSL2 from an ER stress perspective has been reported.

Our study has utilized bioinformatics, machine learning, and RT-qPCR to validate KLF9 and FOSL2 as OA ER stress signature genes. However, there are some limitations that should be acknowledged. The available data for bioinformatics analysis was not sufficient, which could have led to potential biases in the results. In addition, only in vitro cell experiments were carried out in this study, and later in

vivo animal experiments are required to verify the results to make the results more credible.

5 CONCLUSION

According to the study's findings, many machine learning techniques were used to identify the genes KLF9 and FOSL2 as major endoplasmic reticulum (ER) stress signature genes in osteoarthritis (OA). These genes may be used as biomarkers and therapeutic targets since they are connected to OA and ER stress. The study's conclusions aid in understanding the molecular underpinnings of OA and provide information on the development of the condition and prospective therapies.

Declaration

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval and consent to participate: This study does not involve medical ethics approval and consent to participate.

Consent for publication: All authors of this study have agreed to be published in your journal.

Competing interests: No competing interests in this study.

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Authors' contributions: All authors contributed to the study conception and design. Changshen Yuan and Wenfei Xu: conception and design of the study and funding acquisition. Wenfei Xu, Changshen Yuan, Chunyu Ming, Yanhong Li and Qijie Mei: data acquisition, bioinformatics analysis and drafting and critical revision of the manuscript. Wenfei Xu, Duan Kan, Jinrong Guo, Qi Hu and Chao Zeng: visualization and validation. All authors contributed to the article and approved the submitted version.

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Availability of data and material: The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

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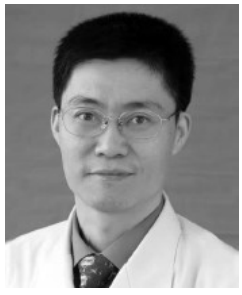

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