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Osteoporosis and Sarcopenia

Original article

Exploring molecular mechanisms underlying the pathophysiological association between knee osteoarthritis and sarcopenia

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ABSTRACT

Objectives: Accumulating evidence indicates a strong link between knee osteoarthritis (KOA) and sarcopenia. However, the mechanisms involved have not yet been elucidated. This study primarily aims to explore the molecular mechanisms that explain the connection between these 2 disorders.
Methods: The gene expression profiles for KOA and sarcopenia were obtained from the Gene Expression Omnibus database, specifically from GSE55235, GSE169077, and GSE1408. Various bioinformatics techniques were employed to identify and analyze common differentially expressed genes (DEGs) across the 3 datasets. The techniques involved the analysis of Gene Ontology and pathways to enhance understanding, examining protein-protein interaction (PPI) networks, and identifying hub genes. In addition, we constructed the network of interactions between transcription factors (TFs) and genes, the co-regulatory network of TFs and miRNAs for hub genes, and predicted potential drugs.
Results: In total, 14 common DEGs were found between KOA and sarcopenia. Detailed information on biological processes and signaling pathways of common DEGs was obtained through enrichment analysis. After performing PPI network analysis, we discovered 4 hub genes (FOXO3, BCL6, CDKN1A, and CEBPB). Subsequently, we developed coregulatory networks for these hub genes involving TF-gene and TF-miRNA interactions. Finally, we identified 10 potential chemical compounds.
Conclusions: By conducting bioinformatics analysis, our study has successfully identified common gene interaction networks between KOA and sarcopenia. The potential of these findings to offer revolutionary understanding into the common development of these 2 conditions could lead to the identification of valuable targets for therapy.

1. Introduction

Knee osteoarthritis (KOA) is a prevalent degenerative condition of the musculoskeletal system that predominantly impacts individuals in the middle-aged and older age groups. This progressive ailment gradually impairs the patient's mobility and overall well-being [1,2]. The primary pathological feature of KOA involves the breakdown and loss of articular cartilage. Different joint tissues are impacted to different extents, including remodeling of the subchondral bone, degeneration of the meniscus, weakening and looseness of ligaments, inflammation of the infrapatellar fat pad, and inflammation of the synovial membrane [2–5]. Furthermore, the presence of periarticular muscle atrophy plays a

significant role in the progression of KOA. Decreased muscle strength can alter mechanical stress, reduce joint stability, and accelerate cartilage degeneration and abnormal subchondral bone changes [6–9]. Sarcopenia, which frequently coexists with KOA [10–12], is primarily characterized by the decline in both muscle mass and strength.

The term sarcopenia was originally introduced in 1989 to describe the decline in muscle mass associated with aging. Over time, the definition of sarcopenia has evolved to encompass not only muscle mass, but also muscle strength and physical performance [13]. Similar to the KOA, sarcopenia predominantly impacts the elderly population, with its prevalence progressively rising in correlation with advancing age. According to estimates, approximately 30% of individuals aged 65 and

older were found to have sarcopenia. Moreover, the prevalence rate of sarcopenia among individuals aged 80 years and above was observed to range from 50% to 60% [14]. Consequently, the prevalence of concurrent KOA and sarcopenia among the elderly is relatively high [15,16]. Moreover, there is growing clinical evidence revealed that KOA and sarcopenia tend to coexist more frequently than expected, which was also not happened by chance [15,17–19]. Certain researchers have put forth the proposition that it would be prudent to view KOA and sarcopenia as a unified entity, referred to as sarcopenic osteoarthritis [20]. Although clinical studies have shown a strong correlation between KOA and sarcopenia and indicated that one condition can lead to the developing the other [17,20], there is a lack of evidence about pathophysiological and molecular mechanisms associated with KOA and sarcopenia.

Currently, 'bone-muscle crosstalk' is becoming a hot topic of research, which goes beyond the traditional view of pronounced mechanical interactions and suggests a biochemical interplay of communication between bones and muscles [12]. Various mechanisms have been suggested in the context of KOA and sarcopenia, such as aging, mitochondrial dysfunction, and inflammaging [20,21]. The accumulation of pro-inflammatory cytokines in the synovium is one of the drivers of KOA, such as NF kappa B (nuclear factor kappa B) and IL-6. These cytokines had also been proved to be associated with age-associated defects in tissue regeneration in mice and skeletal muscle cells by inhibiting muscle protein synthesis [20]. Another important factor in explaining the potential link between sarcopenia and osteoarthritis is the myokines and adipokines released from muscle and adipose tissue. In the presence of sarcopenia, changes in muscle and fat composition would lead to dysregulation of these factors, including myostatin, leptin, and adiponectin. These factors can further lead to an imbalance in cartilage or subchondral bone homeostasis leading to cartilage degeneration [21]. However, the significance of these findings has yet to be validated. Recently, high-throughput chip technology is gaining popularity in the research of different diseases, including drug discovery, molecular diagnosis, and classification. With the aid of bioinformatics analysis, the exploration of the common transcriptional profile of KOA and sarcopenia could reveal novel insights into the shared pathogenesis and treatments of the 2 conditions. Bioinformatics analysis was used in the study to identify common genes implicated in the development of both KOA and sarcopenia. In particular, the analysis focused on 3 datasets (GSE1428, GSE55235, and GSE169077) obtained from the Gene Expression Omnibus (GEO) repository. After identifying the common DEGs in both KOA and sarcopenia, their functions in these disorders were investigated through the enrichment analysis of gene ontology (GO) terms and pathways. To identify the hub genes, the PPI network was constructed afterwards. Ultimately, we built the network of Transcription factors (TFs)-gene regulation and TF-microRNAs (miRNA) regulation for the hub genes, and then predicted potential medications for consideration. The initial findings have unveiled common molecular pathways that contribute to the development of both KOA and sarcopenia. These results are highly anticipated to offer novel perspectives for understanding and treating these debilitating conditions. Our research workflow is depicted in Fig. 1.

2. Methods

2.1. Data collection

The datasets GSE1428, GSE55235, and GSE169077 were acquired from the freely available GEO database (www.ncbi.nlm.nih.gov/geo) [22]. The GSE1428 dataset investigated sarcopenia in skeletal muscle and consisted of microarray data collected from muscle biopsies of the vastus lateralis muscle. The research involved a group of 22 male participants, with 10 falling into the category of young individuals and 12
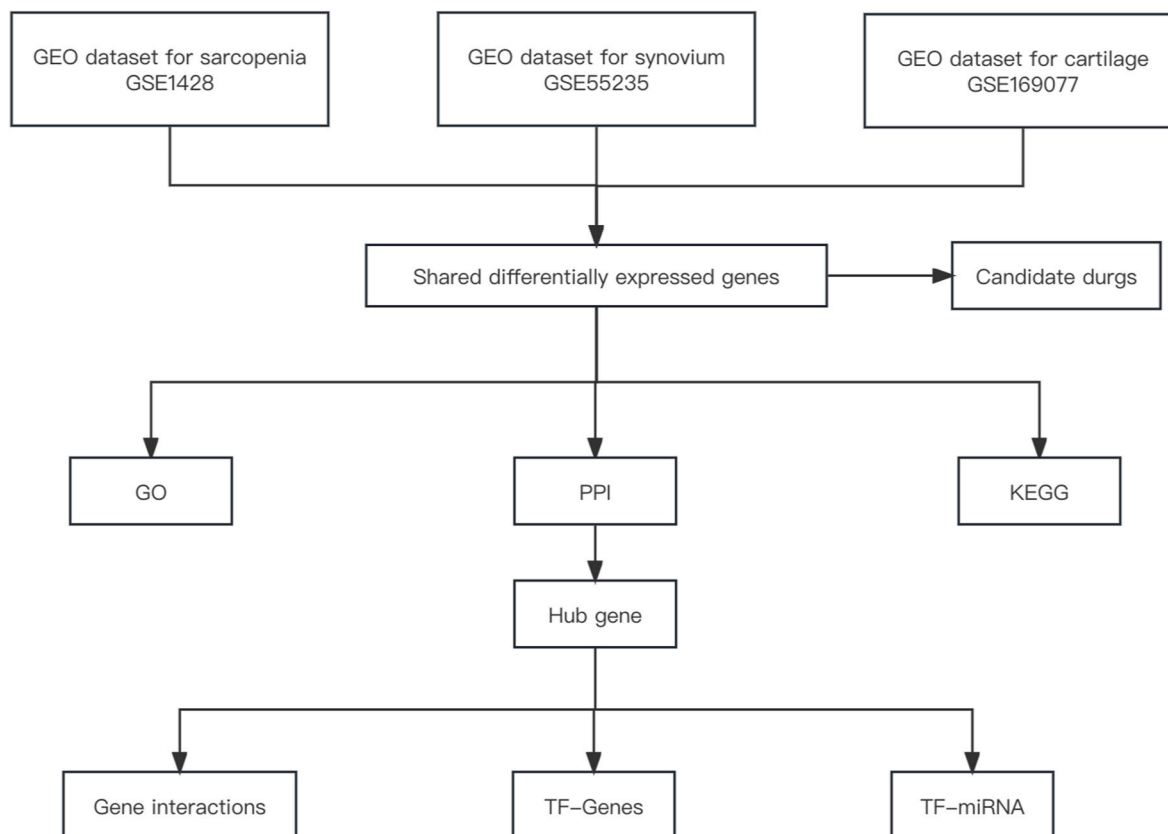

Fig. 1. Workflow diagram of current study. GEO, Gene Expression Omnibus; GO, gene ontology; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; TF, transcription factor.

belonging to the older age group [23]. To better illustrate the pathological mechanism of KOA, we utilized 2 datasets that examined the synovium and cartilage of the knee joint, respectively. The GSE55235 dataset included 10 synovial tissues obtained from a normal joint and 10 from individuals with KOA [24]. The GSE169077 dataset comprises 5 samples of normal cartilage and 6 samples of KOA cartilage (Table 1). If needed, the Robust Multi-array Average (RMA) algorithm was utilized to conduct batch correction on all datasets.

2.2. Determining shared DEGs in individuals with KOA and sarcopenia

The DEGs for the GSE1428, GSE55235, and GSE169077 datasets were successfully identified using the DESeq2 and limma packages in R, respectively [25,26]. Upon performing a t- test, the gene symbols were chosen based on meeting the criteria of a P-value below 0.05 (adjusted) and a fold change (FC) greater than 1 (log2 scale). For further analysis, the VennDiagram package in the R language was utilized to acquire shared DEGs from 3 datasets [27].

2.3. Enrichment analysis of gene ontology and pathways

To investigate the common features of DEGs in KOA and sarcopenia, Enrichr, a web-based tool for extensive gene set enrichment analysis (https://maayanlab.cloud/Enrichr/) [28], was utilized to conduct a series of enrichment analyses, encompassing gene ontology and pathways. The GO is a type of functional enrichment that is categorized into 3 separate groups: biological process, molecular function, and cellular component [29]. To obtain a thorough comprehension of the related signaling pathways, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [30], WikiPathways [31], Reactome [32], and BioPlanet [33] databases were utilized.

2.4. Construction of protein–protein interaction network and identification and analysis of hub genes

The study of biological processes at different levels of structure and function heavily relies on the creation and examination of protein-protein interaction (PPI) networks [34]. The Network Analyst platform (https://www.networkanalyst.ca) [35] and the Search Tool for the Retrieval of Interacting Genes (STRING) online database (http://string-db.org) [36] were utilized to construct the PPI network of shared DEGs in KOA and sarcopenia. Afterwards, the obtained network was visualized and incorporated into the Cytoscape platform (https://cytoscape.org/) in order to aid in the examination of protein and genetic interactions [37]. Using the degree topological algorithm, the cyto-Hubba [38] identified the hub genes with the highest degree values. Next, the co-expression network of recognized central genes was established using GeneMANIA (http://genemania.org) [39], an internet resource capable of forecasting gene interactions.

2.5. Identification of related transcription factors and miRNAs

TFs and miRNA play a crucial role in regulating gene expression at the transcription and posttranscription levels [40,41]. Understanding the regulation of gene expression in pathological conditions requires a comprehensive understanding of the regulatory transcriptional network involving TFs and miRNA [42,43]. By utilizing the Network Analyst

platform, a coregulatory network was constructed to carry out the selection of TFs and miRNA. The TF-gene regulatory network was constructed using the ENCODE database (https://www.encodeproject.org/) [44,45], which is integrated into the Networkanalyst platform. To be eligible for this study, the requirements are a signal of peak intensity that is below 500 and a regulatory potential score that is predicted to be less than 1, as determined by the BETA Minus algorithm. The TF-miRNA regulatory network was built using the RegNetwork (http://www.regnetworkweb.org) database [46], which is also integrated into the Networkanalyst platform. Relevant results were all visualized by Cytoscape.

2.6. Evaluation of candidate drugs

Predicting drug molecules was done by utilizing the Drug Signatures Database (DSigDB, http://dsigdb.tanlab.org/DSigDBv1.0/), based on the common DEGs identified in the PPIs network for sarcopenia and KOA [47]. The DSigDB database was accessed through the Enrichr platform (https://maayanlab.cloud/Enrichr/) [48], a commonly used tool for visualizing various functional details of genes. The potential medications were prioritized according to their adjusted P-values, where lower values indicated greater significance. Statistical significance was determined using a threshold of P < 0.01.

3. Results

3.1. Identification of DEGs and common genes between KOA and sarcopenia

In the GSE1428 dataset related to sarcopenia, a total of 646 DEGs were detected. Of these, 351 genes were up-regulated while 295 genes were down-regulated. In the GSE55235 dataset of synovial tissues, 985 DEGs including 511 up-regulated genes and 474 down-regulated genes were identified. Additionally, the GSE169077 dataset of cartilage revealed 358 DEGs, consisting of 158 up-regulated genes and 200 down-regulated genes. By comparing the 3 datasets GSE1428, GSE55235, and GSE169077 (BTG2, ZNF395, LOXL1, ZBTB16, TPPP3, BCL6, DDIT4, HIST2H2AA3, H1FX, CDKN1A, ADM, RPGR, FOXO3, CEBPB), a total of 14 DEGs were identified. These DEGs were represented using Venn diagrams (Fig. 2).

3.2. GO and KEGG pathway enrichment analysis

The analysis of GO enrichment of 14 common DEGs was carried out on the Enrichr platform. In the biological process, the results indicated that the common DEGs are predominantly enhanced in: cellular response to starvation, negative regulation of cellular macromolecule biosynthetic process and positive regulation of neuron death (Fig. 3A). The analysis of cellular components showed that the common DEGs were primarily located in cyclin-dependent protein kinase holoenzyme complex and nucleolus (Fig. 3B). The molecular function analysis revealed that the commonly DEGs are primarily enriched in: DNA-binding transcription repressor activity, RNA polymerase II-specific and DNA binding (Fig. 3C). For the pathway enrichment, analysis outcomes from BioPlanet (Fig. 4A), KEGG (Fig. 4B), WikiPathway (Fig. 4C) and Reactome (Fig. 4D) were gathered. The results showed transcriptional misregulation in cancer pathway and FOXO signaling pathway are

Table 1
Details of the datasets related to KOA or sarcopenia patients.

GEO	Disease type	Sample source	Samples (case vs. control)	Experiment type	Platform	Year
GSE1428	Sarcopenia	Vastus lateralis muscle	12 vs 10	Expression profiling by array	GPL96	2004
GSE55235	KOA	Synovial tissues	10 vs 10	Expression profiling by array	GPL96	2014
GSE169077	KOA	Cartilage	5 vs 5	Expression profiling by array	GPL96	2021

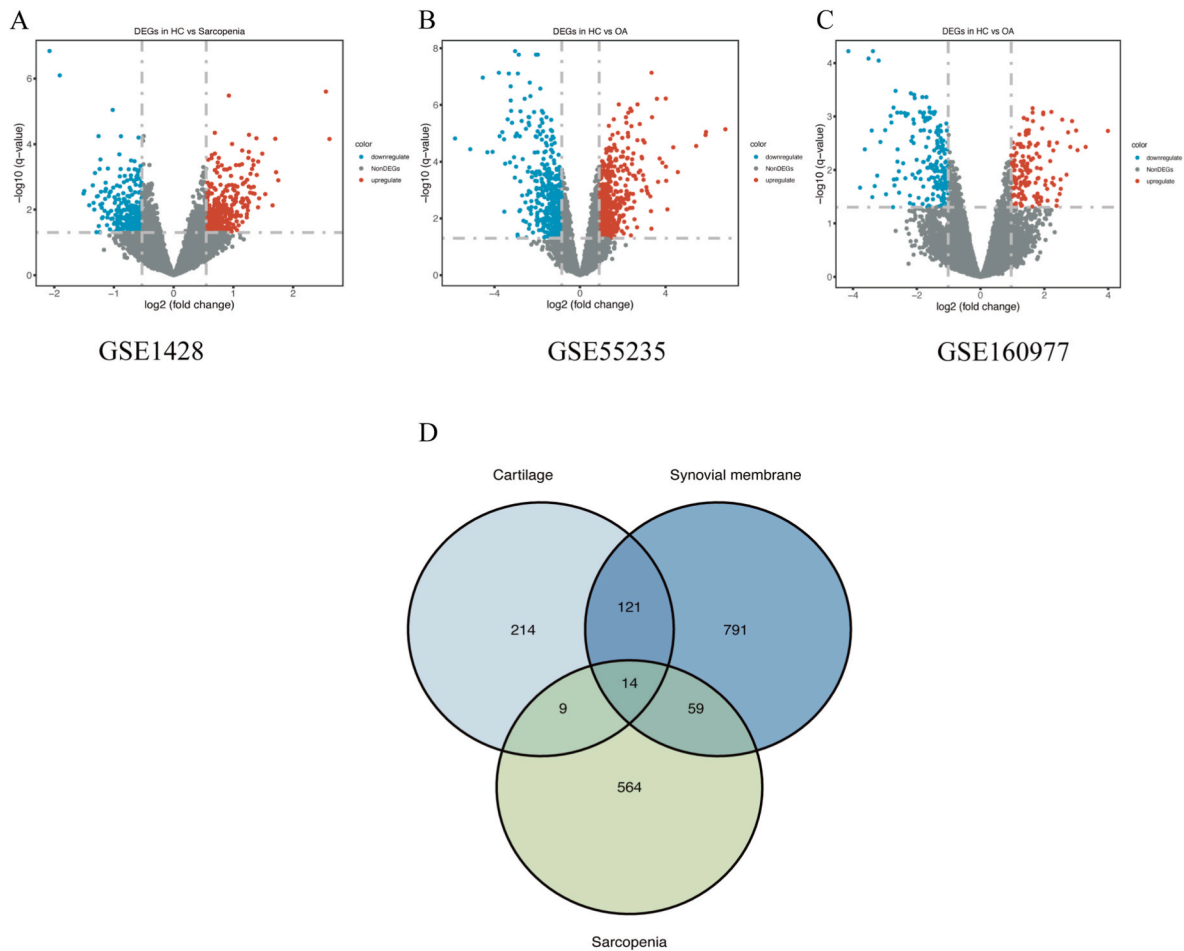KOA, knee osteoarthritis; GEO, Gene Expression Omnibus.

Fig. 2. Volcano diagram and Venn diagram. (A) The volcano map of GSE1428. (B) The volcano map of GSE55235. (C) The volcano map of GSE160977. Upregulated genes are colored in red; downregulated genes are colored in green. (D) The 3 datasets showed an overlap of 14 DEGs. HC, healthy control; OA, osteoarthritis.

significantly assembled in the KEGG pathway database.

3.3. Protein–protein interaction analysis and network construction

The set of 14 common DEGs was utilized as input in both the Network Analyst platform and the STRING online database. The resulting file from this analysis was subsequently imported into Cytoscape for the purpose of visual representation. The PPI network comprising commonly shared genes is composed of 53 nodes and 108 edges. The cytoHubba plugin was utilized to identify the hub DEGs in the PPI network, namely FOXO3, BCL6, CDKN1A, and CEBPB, based on their degree (Fig. 5). The biological functions of the hub DEGs were investigated using a complex gene interaction network constructed based on data from the GeneMANIA database. The network consisted of various types of interactions, including co-expression (77.64%), physical interactions (8.01%), co-localization (3.63%), predicted interactions (5.37%), and pathway associations (1.88%) (Fig. 6). A total of 20 genes, which were found to be associated with the 4 hub DEGs, were identified. The findings of this study indicate that these genes are primarily involved in the regulation of cyclin-dependent protein kinase activity, negative regulation of cell population proliferation, negative regulation of mitotic cell cycle, positive regulation of transcription by RNA polymerase II, negative regulation of protein phosphorylation, transcription regulator complex and negative regulation of growth.

3.4. TF-gene interaction network and TF-miRNA coregulatory network

The NetworkAnalyst web tool was utilized to generate a TF-gene

interaction network consisting of 4 hub DEGs. There were 318 edges and 201 nodes in the network. Among them, CDKN1A is regulated by 105 TF genes, CEBPB is regulated by 86 genes, FOXO3 is regulated by 85 genes and BCL6 is regulated by 43 genes. It was observed that a total of 87 TFs exhibit regulation over multiple hub genes within the network. This finding provides evidence for the extensive interaction between TFs and hub genes. Among them, DMAP1, NRF1, FOXJ2, ATF3, MAZ regulate all 4 hub genes (Fig. 7). The construction of the TF-miRNA co-regulatory network was carried out using the NetworkAnalyst platform (Fig. 8). The network consisted of a total of 67 nodes and 142 edges. Within this network, 23 miRNAs and 40 TF genes were found to interact with hub genes. Within the miRNAs, has-miR-302a shows the highest degree of 3, which simultaneous regulates CDKN1A, FOXO3 and BCL6.

3.5. Prediction of candidate drugs

Using the DSigDB database, the Enrichr platform was utilized to identify potential pharmaceutical candidates. Table 2 presented the adjusted P-value, which is used to determine the extraction of the top 10 potential chemical compounds. The findings of the study indicated that strophanthidin PC3 UP and cicloheximide PC3 UP exhibited the highest degree of gene interaction among the various drug molecules examined.

4. Discussion

The KOA and sarcopenia are common musculoskeletal diseases which both occur predominantly in middle-aged and elderly population. With the increasing aging of the population, the incidence rates will

A

cellular response to starvation (GO:0009267)

negative regulation of cellular macromolecule biosynthetic process (GO:2000113)

positive regulation of neuron death (GO:1901216)

negative regulation of mitotic cell cycle (GO:0045930)

positive regulation of fat cell differentiation (GO:0045600)

DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest (GO:0006977)

regulation of DNA-templated transcription, initiation (GO:2000142)

regulation of transcription initiation from RNA polymerase II promoter (GO:0060260)

mitotic G1 DNA damage checkpoint signaling (GO:0031571)

negative regulation of transcription by RNA polymerase II (GO:0000122)

B

cyclin-dependent protein kinase holoenzyme complex (GO:0000307)

nucleus (GO:0005634)

serine/threonine protein kinase complex (GO:1902554)

sperm flagellum (GO:0036126)

9+2 motile cilium (GO:0097729)

intracellular membrane-bounded organelle (GO:0043231)

mitochondrial outer membrane (GO:0005741)

nucleolus (GO:0005730)

nuclear lumen (GO:0031981)

organelle outer membrane (GO:0031968)

C

DNA-binding transcription repressor activity, RNA polymerase II-specific (GO:0001227)

DNA binding (GO:0003677)

RNA polymerase II cis-regulatory region sequence-specific DNA binding (GO:0000978)

cis-regulatory region sequence-specific DNA binding (GO:0000987)

DNA-binding transcription activator activity, RNA polymerase II-specific (GO:0001228)

RNA polymerase II transcription regulatory region sequence-specific DNA binding (GO:0000977)

transcription cis-regulatory region binding (GO:0000976)

cyclin-dependent protein serine/threonine kinase inhibitor activity (GO:0004861)

transcription regulatory region nucleic acid binding (GO:0001067)

double-stranded DNA binding (GO:0003690)

Fig. 3. The top 10 GO terms of common genes between KOA and sarcopenia. (A) Biological Processes, (B) Cellular Component, (C) Molecular Function. GO, gene ontology; KOA, knee osteoarthritis.
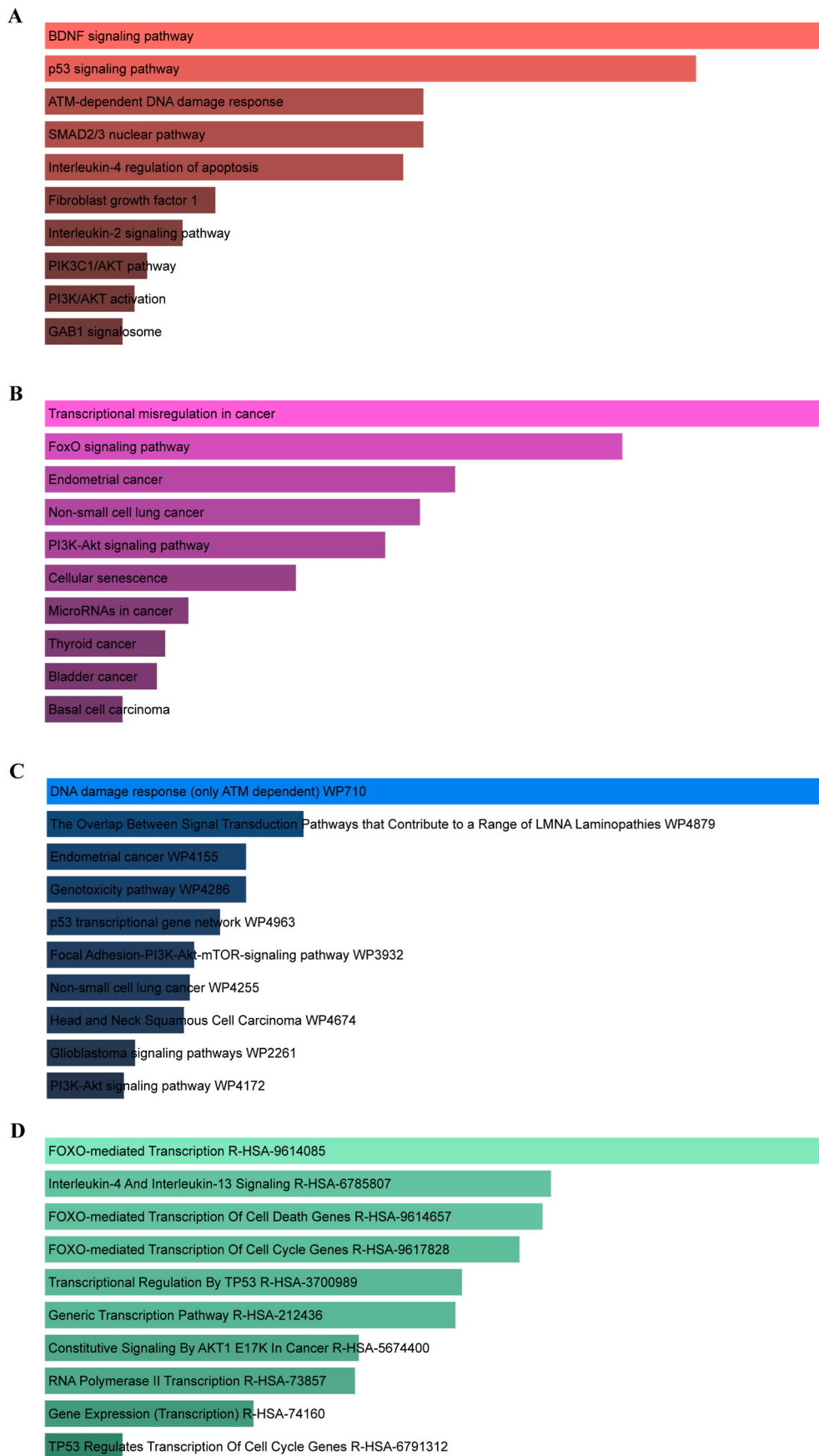
Fig. 4. The top 10 pathway enrichment analysis of common genes between KOA and sarcopenia. (A) BioplanetPathway, (B) KEGG Human Pathway, (C) Wiki-pathway, (D) ReactomePathway. KOA, knee osteoarthritis; KEGG, Kyoto Encyclopedia of Genes and Genomes.
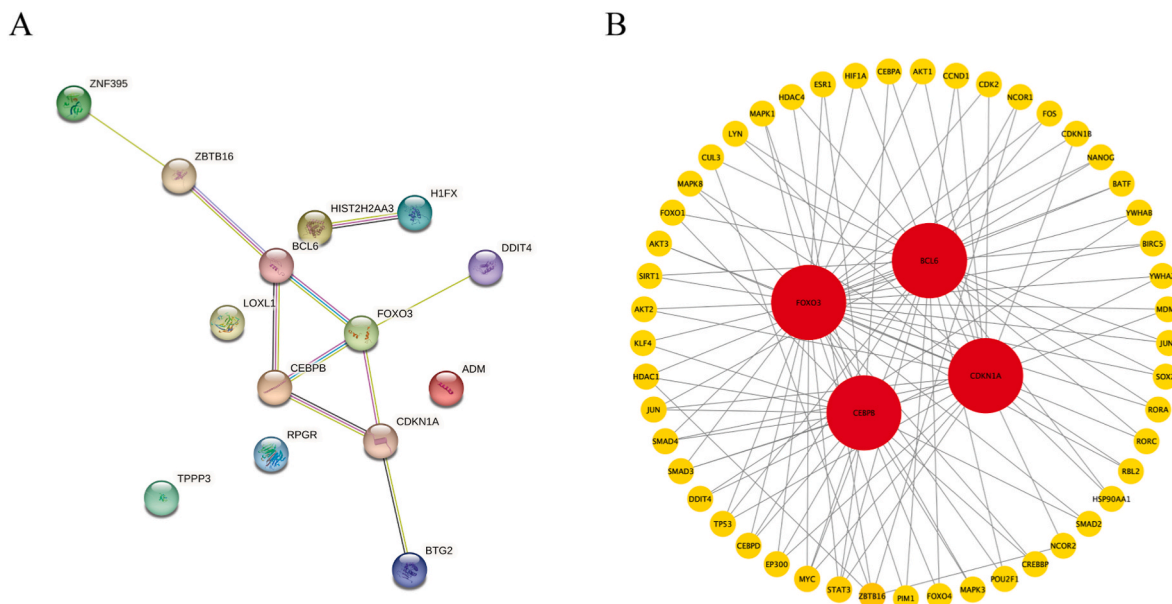
A

B

Fig. 5. PPI network of common genes among KOA and sarcopenia. A: PPI network output from STRING online database. B: PPI network output from Network Analyst platform. The highlighted 4 hub genes, based on their degree, are FOXO3, BCL6, CDKN1A, and CEBPB. The analyzed network holds 53 nodes and 108 edges. PPI, protein-protein interaction; KOA, knee osteoarthritis; STRING, Search Tool for the Retrieval of Interacting Genes.

increase gradually, which imposed a heavy burden on society. And the coexsiting of two diseases can make the situation worse. Although numerous studies revealed that KOA and sarcopenia are significantly intercorrelated, few studies focus on the shared genetic mechanism. The objective of this study was to discover the common DEGs linked to both KOA and sarcopenia. Following the identification of 14 common DEGs between KOA and sarcopenia, a sequence of bioinformatics investigations was carried out. Identifying these shared DEGs could potentially improve our comprehension of the underlying mechanisms and therapeutic targets in KOA and sarcopenia.

The process of detecting GO terms utilized the 14 identified common DEGs. The most significant GO biological process terms include cellular response to starvation, negative regulation of cellular macromolecule biosynthetic process, positive regulation of neuron death, negative regulation of mitotic cell cycle, and positive regulation of fat cell differentiation. Among them, cellular response to starvation and negative regulation of cellular macromolecule biosynthetic process represents the nutrient deficiencies in KOA and sarcopenia [49,50]. Especially the micronutrients such as vitamin K, vitamin D and magnesium, have already been shown to play an important role in KOA and sarcopenia [51–53]. Consequently, nutritional interventions are the fundamental and important actions for these 2 conditions [54,55]. The positive regulation of adipocyte differentiation could potentially serve as a significant mechanism for elucidating the interplay between KOA and sarcopenia. Sarcopenia is closely associated with fat infiltration in muscle, while the infrapatellar fat pad also contributes to pathophysiological processes in KOA [3,56]. The primary GO terms within the molecular function category are as follows: DNA-binding transcription repressor activity, RNA polymerase II-specific; DNA binding; and RNA polymerase II *cis*-regulatory region sequence-specific DNA binding. RNA polymerase II is a multiprotein complex that transcribes DNA into precursors of messenger RNA (mRNA) and miRNA [57]. The molecular function of these common genes indicated that the dysregulation of mRNA or miRNA is present in both KOA and sarcopenia, but further study is needed to determine which one specifically. The cyclin-dependent protein kinase holoenzyme complex and nucleus are among the highest-ranking cellular components. In mammals, the cell cycle regulatory network is primarily controlled by a set of 20 kinases known as cyclin-dependent protein kinases (CDKs) [58]. It has been

previously discovered that the proliferation of myosatellite cells and chondrocytes is significantly influenced by several CDKs [59,60].

By analyzing the 14 common DEGs, we obtained the identification of the KEGG pathway to find similar pathways between KOA and sarcopenia. Top 10 KEGG pathways included: transcriptional misregulation in cancer, FOXO signaling pathway, endometrial cancer, non-small cell lung cancer, PI3K-AKT signaling pathway, cellular senescence, microRNAs in cancer, thyroid cancer, bladder cancer, and basal cell carcinoma. The forkhead box O (FOXO) family of transcription factors play an important role in cell proliferation, apoptosis, differentiation and resistance to oxidative stress by shuttling in and out of the nucleus [61–64]. The PI3K-AKT pathway is a cellular signaling pathway that facilitates cellular processes such as metabolism, cell division, cell viability and growth, and blood vessel formation upon receiving signals from outside the cell [65,66]. Both of these pathways play a crucial role in preserving cellular balance and are implicated in the development of KOA and sarcopenia. The relaxation of the FOXO and PI3K-AKT signaling pathway plays a role in reducing cell survival under oxidative stress, promoting the presence of cartilage-degrading enzymes in KOA, and disrupting muscle protein turnover in sarcopenia [62,63, 67–69]. Currently, the FOXO and PI3K/AKT pathways are promising therapeutic targets in both KOA and sarcopenia which had already drawn the attention of researchers [61,68–71]. In the interim, findings derived from BioPlanet indicate that the gene pathways that exhibit the highest level of interaction are the BDNF signaling pathway and the p53 signaling pathway. The utilization of WikiPathway and Reactome databases independently yields the identification of two distinct pathways: DNA damage response (only ATM dependent) WP710 and FOXO-mediated transcription R-HSA-9614085.

Based on the analysis of the PPI network, the proteins FOXO3, BCL6, CDKN1A, and CEBPB have been identified as hub proteins due to their significantly high degrees. Three genes have been identified as being associated with the pathological mechanism of KOA and sarcopenia. FOXO3 is a member of FOXO family of transcription factors, which have an important role both in KOA and sarcopenia as described above [54, 63,72]. In particular, FOXO3 can induce atrophy of muscle cells through activation of Atrogin-1, but it is also important for self-renewal of myosatellite cells in adult muscle regeneration [67,73]. While FOXO3 regulates apoptosis and extracellular matrix metabolism in
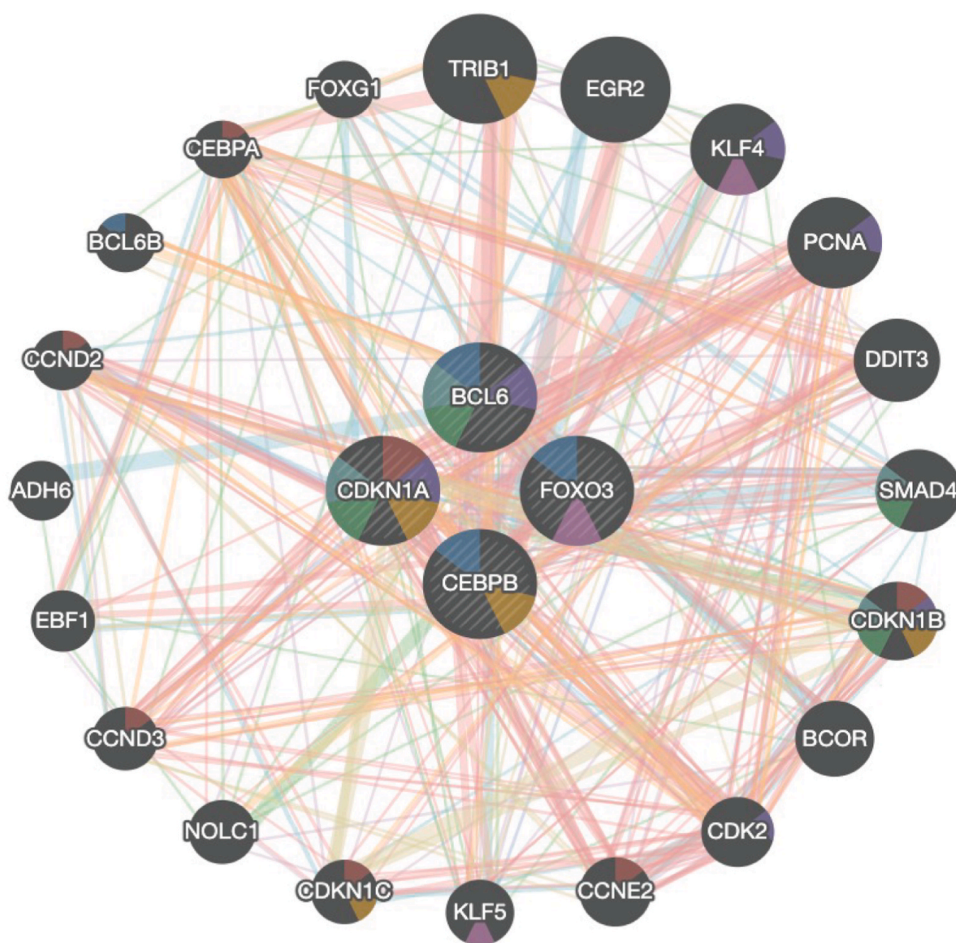
Fig. 6. Analysis of 4 hub genes and their co-expressed genes in co-expression physical interactions, co-localization, predicted interactions, and pathway associations using GeneMANIA.

chondrocytes [72]. CDKN1A, also known as p21, encodes a potent CDK inhibitor which binds to and inhibits the activity of CDK2 or CDK4 and thus functions as a regulator of cell cycle [74]. Although numerous studies have shown that CDKN1A is a key gene in KOA through bioinformatic analysis [75–78], but the true role of it remains obscure. The low expression or knocking down CDKN1A resulted in an enhancement of chondrogenic differentiation, which could even lead to the cartilage regeneration [79]. However, some studies also indicated that the low expression of CDKN1A was associated with the proliferation of fibroblast-like synoviocytes which is one of the manifestations of KOA [80]. In sarcopenia, CDKN1A contribute to the age-associated decrease in satellite cell proliferation, which causes the muscle atrophy [81]. CEBPB is an important transcription factor regulating the expression of genes involved in immune and inflammatory responses [82,83]. In KOA, CEBPB induces chondrocyte apoptosis by regulating the expression of

MMP13 [84]. While in the sarcopenia, the role of CEBPB is less described, but it has been demonstrated to play a central role in adipose differentiation [85]. As to the BCL6, a gene encodes a zinc finger transcription factor, there is a lack of studies documenting the involvement of it in KOA or sarcopenia, underscoring the significance of further investigation in this area. According to GeneMANIA database, the hub genes primarily govern the regulation of cyclin-dependent protein kinase activity, which in turn plays a crucial role in controlling the cell cycle and cellular proliferation. In line with previous studies, the proliferation of myosatellite cells and chondrocytes is an important pathogenesis and therapeutic target for KOA and sarcopenia [86–89].

TFs and miRNAs both have a crucial impact on the development of numerous diseases through their co-regulation of gene expression [43, 90]. Through our analysis, we have identified the TFs and miRNA that may serve as shared causes and potential treatment targets for KOA and
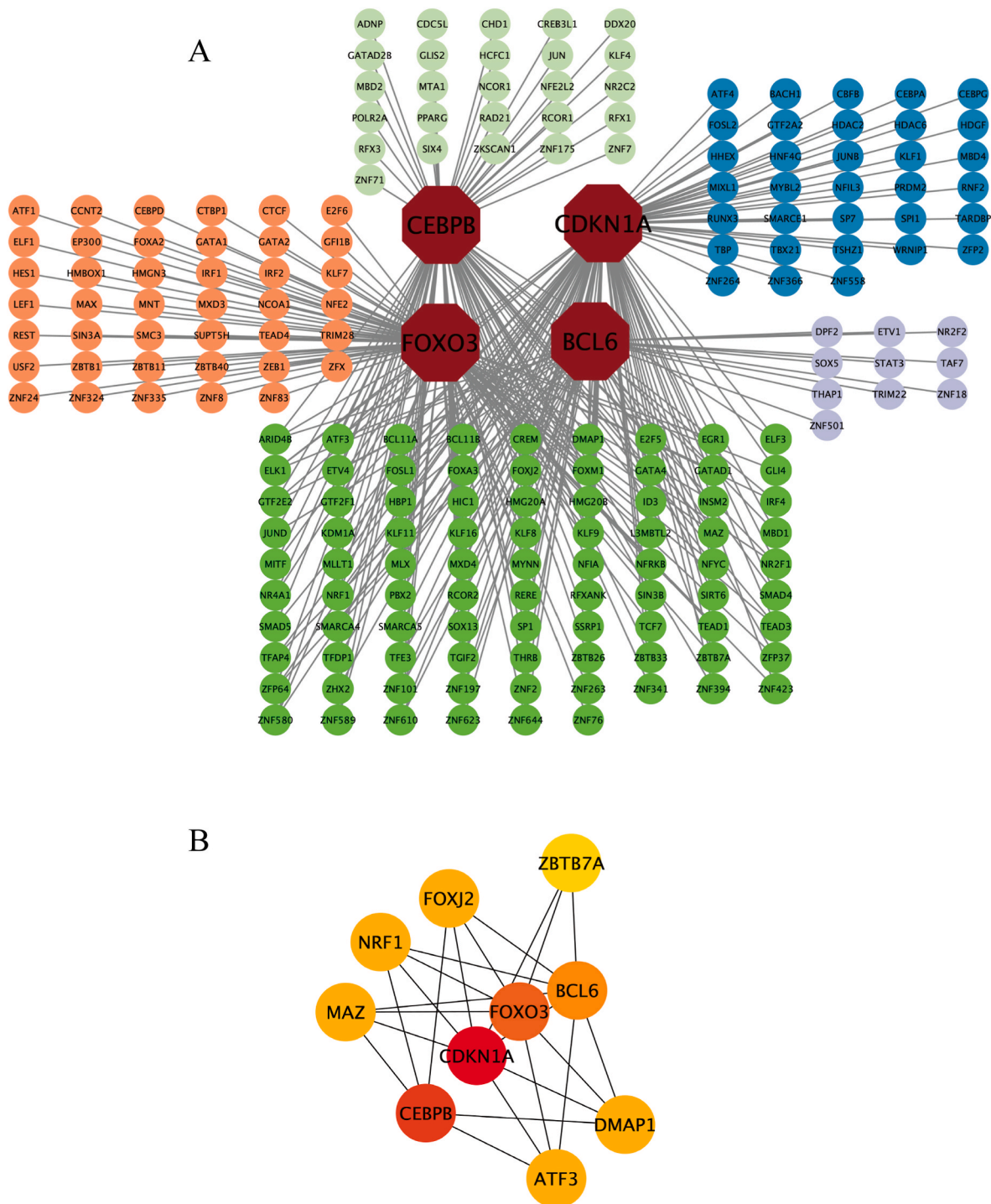
Fig. 7. Network for TF-gene interaction with hub genes. (A) The highlighted red color node represents the hub genes and other nodes represent TFs. There were 318 edges and 201 nodes in the network. Among them, CDKN1A is regulated by 105 TF genes, CEBPB is regulated by 86 genes, FOXO3 is regulated by 85 genes and BCL6 is regulated by 43 genes. It was observed that a total of 87 TFs exhibit regulation over multiple hub genes within the network. (B) A significant gene clustering module via Cytoscape. Six TFs closely related to the hub genes were identified. TF, transcription factor.

sarcopenia. In TF-gene interaction network, CDKN1A exhibits a significantly elevated rate of interaction with other transcription factor genes. Additionally, among the regulators, namely DMAP1, NRF1, FOXJ2, ATF3, and MAZ, there is evidence of interaction with all 4 hub genes. In the TF-miRNA coregulatory network, EGR1 and has-miR-302a show the highest degree in TFs and miRNAs, respectively. Among the TFs, ATF3, NRF1 and EGR1 have already been shown to be involved in KOA and sarcopenia [91–94]. EGR1 (early growth response 1) facilitates the

deterioration and enlargement of cartilage by triggering the Krüppel--like factor 5 and β-catenin signaling in KOA [95], while also hindering the ability to eliminate excess reactive oxygen species in muscle [95,96]. While the role of the DMAP1, FOXJ2 and MAZ in KOA and sarcopenia need to be investigated in future research. As to the has-miR-302a, it can promote inflammatory cytokine release and matrix metalloproteinase production and ultimately leads to cartilage degeneration in KOA [97]. Meanwhile, has-miR-302a is also tightly related to the mitochondrial
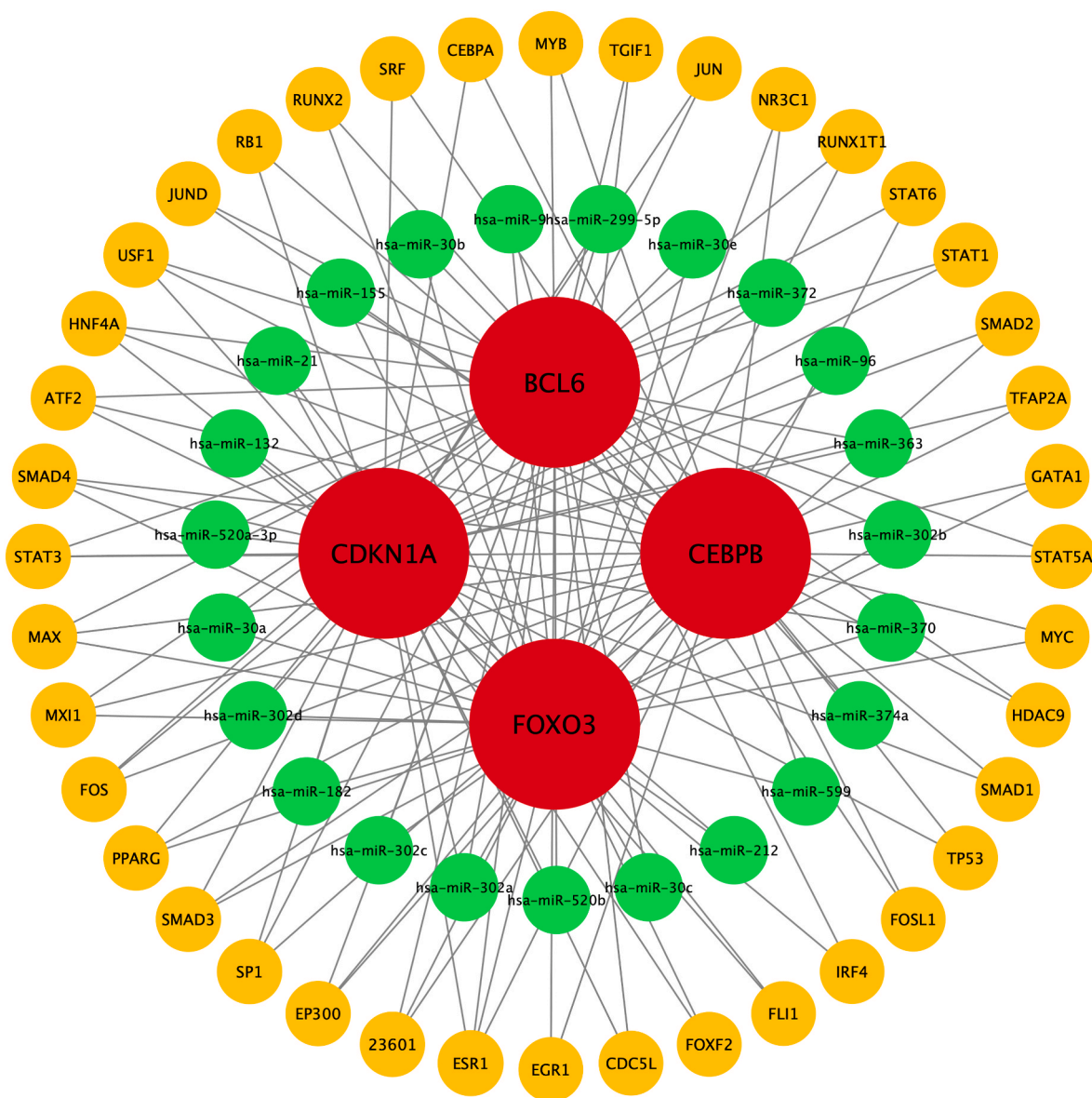
Fig. 8. The network presents the TF-miRNA coregulatory network via NetworkAnalyst platform. The network consists of 67 nodes and 142 edges including 40 TF-genes, 23 miRNA and 4 hub genes. The nodes in red color are the hub genes, a yellow node represents TF-genes and other nodes indicate miRNAs. Within the miRNAs, has-miR-302a shows the highest degree of 3, which simultaneous regulates CDKN1A, FOXO3 and BCL6. TF, transcription factor.

function and glycolysis of the skeletal muscle [98].

Through the utilization of the DSigDB database, 14 common DEGs were employed in order to forecast potential pharmaceutical agents. Subsequently, the 10 most noteworthy drugs were identified and brought to attention. Among them, various cardiac glycoside compounds were identified, such as strophanthidin, lanatoside, digitoxigenin and digoxin. Cardenolides have a direct effect on strengthening myocardial contractility, whether they have the same effect on other muscles or sarcopenia has not been reported. However, the therapeutic role of cardenolides in osteoarthritis has been reported in several studies in the last 2 years [99,100]. Wang et al. [100] found that digoxin activates chondrocyte differentiation and anabolism to protect against KOA. The repurposing digoxin for KOA further validates the reliability of our analysis, and the repurposing cardenolides for sarcopenia is another promising direction, which need more in-depth researches. Cicloheximide is one of inhibitors of protein synthesis, which could inhibit starvation-induced autophagy through mTORC1 activation [101,102]. As shown in GO biological process, the common genes mainly were enriched in cellular response to starvation. It appears that in KOA and

sarcopenia, there is an unexpected autophagy induced by starvation. Parthenolide, an active compound derived from chrysanthemum's leaves and flowers, possesses significant properties in reducing inflammation, alleviating migraines, and combating cancer. According to recent research, parthenolide has been found to regulate mitophagy caused by oxidative stress and safeguard myoblasts from apoptosis. This compound also exhibits similar effects on chondrocytes [103,104].

Previous research has investigated the fundamental genes or pathways that are linked to KOA and sarcopenia, respectively. Nevertheless, the molecular mechanisms that are common to both of these conditions have yet to be investigated. The current study aimed to investigate and ascertain the shared DEGs, hub genes, TFs, and miRNAs associated with KOA and sarcopenia. This study represents the first attempt to identify these factors, providing valuable insights into the shared underlying mechanisms and potential therapeutic strategies for both conditions. However, our study also has some limitations. First, only 3 expression profiles were included in this study. The small number of samples may make the results less convincing. Meanwhile, in the patients of KOA datasets, the status of sarcopenia was not stated, but this does not affect

Table 2
Prediction of top 10 candidate drugs for KOA and sarcopenia.

Term	P-value	Adjusted P-value	Combined Score	Gene
Strophanthidin PC3 UP	2.95E-12	2.68E-09	2074.55077	HIST2H2AA3; BTG2; CDKN1A; CEBPB; BCL6; DDIT4; ADM; H1FX; FOXO3
Cicloheximide PC3 UP	2.50E-09	1.13E-06	1047.3028	HIST2H2AA3; CDKN1A; CEBPB; BCL6; ADM; H1FX; FOXO3
Lanatoside C PC3 UP	4.46E-09	1.14E-06	932.017895	HIST2H2AA3; BTG2; CDKN1A; BCL6; ADM; H1FX; FOXO3
Parthenolide MCF7 UP	5.01E-09	1.14E-06	1305.88193	HIST2H2AA3; BTG2; CDKN1A; CEBPB; DDIT4; ADM
Ciclopirox MCF7 UP	6.34E-09	1.15E-06	2128.25475	BTG2; ZNF395; CDKN1A; DDIT4; ADM
Digitoxigenin PC3 UP	1.36E-08	1.76E-06	742.421699	HIST2H2AA3; BTG2; CDKN1A; CEBPB; BCL6; ADM; H1FX
Digoxin PC3 UP	1.36E-08	1.76E-06	742.421699	HIST2H2AA3; BTG2; CDKN1A; BCL6; ADM; H1FX; FOXO3
Cephaeline PC3 UP	2.09E-08	2.37E-06	679.459889	HIST2H2AA3; CDKN1A; CEBPB; BCL6; DDIT4; ADM; H1FX
Mefloquine MCF7 UP	3.31E-08	3.11E-06	1376.21726	HIST2H2AA3; CEBPB; BCL6; DDIT4; FOXO3
Anisomycin PC3 UP	3.81E-08	3.11E-06	488.12565	HIST2H2AA3; BTG2; CDKN1A; CEBPB; BCL6; ADM; H1FX; FOXO3

KOA, knee osteoarthritis.

our results as we take the intersection of the 2 diseases; Second, given that our study relies solely on bioinformatics analysis without any clinical validation, it is imperative to conduct further verification of the functionality of hub genes using an in vitro model. This aspect will be the primary focus of our future research, along with assessing the safety and efficacy of candidate drugs.

5. Conclusions

In summary, we have successfully identified common and hub DEGs and elucidated the shared molecular mechanisms underlying KOA and sarcopenia using various bioinformatics methodologies. This study offers a potential avenue for further exploration into the shared pathogenic mechanisms and treatment approaches for KOA and sarcopenia.

CRediT author statement

Jiyong Yang: Conceptualization, Methodology, Writing - Original Draft. **Tao Jiang**: Formal analysis, Software, Data Curation. **Guangming Xu**: Investigation, Resources, Visualization. **Shuai Wang**: Validation, Resources, Visualization. **Wengang Liu**: Supervision, Writing - Review & Editing, Project administration, Funding acquisition. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

Data availability statement

The datasets presented in this study can be found in online repositories (https://www.ncbi.nlm.nih.gov/geo/).

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

References

[1] Roemer FW, Kwoh CK, Fujii T, Hannon MJ, Boudreau RM, Hunter DJ, et al. From early radiographic knee osteoarthritis to joint arthroplasty: determinants of structural progression and symptoms. Arthritis Care Res 2018;70:1778–86.

[2] Abramoff B, Caldera FE. Osteoarthritis pathology, diagnosis, and treatment options. Med Clin 2019;104:293–311.

[3] Klein-Wieringa IR, Kloppenburg M, Bastiaansen-Jenniskens YM, Yusuf E, Kwekkeboom JC, El-Bannoudi H, et al. The infrapatellar fat pad of patients with osteoarthritis has an inflammatory phenotype. Ann Rheum Dis 2011;70:851.

[4] Yang J, Liu W. The Role of AIM2 Inflammasome in knee osteoarthritis. J Inflamm Res 2022;15:6453–561.

[5] Lane NE, Brandt K, Hawker G, Peeva E, Schreyer E, Tsuji W, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. Osteoarthritis Cartilage 2011;19:478–82.

[6] Ruhdorfer A, Wirth W, Eckstein F. Association of knee pain with a reduction in thigh muscle strength – a cross-sectional analysis including 4553 osteoarthritis initiative participants. Osteoarthritis Cartilage 2017;25:658–66.

[7] Yamauchi K, Suzuki S, Kato C, Kato T. Atrophy of individual thigh muscles measured by MRI in older adults with knee osteoarthritis: a cross-sectional study. Ann Phys Rehabilitation Medicine 2020;63:38–45.

[8] Espinosa SE, Costello KE, Souza RB, Kumar D. Lower knee extensor and flexor strength is associated with varus thrust in people with knee osteoarthritis. J Biomech 2020;107:109865.

[9] Bokaeian HR, Bakhtiary AH, Mirmohammadkhani M, Moghimi J. Quadriceps strengthening exercises may not change pain and function in knee osteoarthritis. J Bodyw Mov Ther 2018;22:528–33.

[10] Kim H-J, Hong Y-H. Age-related low skeletal muscle mass correlates with joint space narrowing in knee osteoarthritis in a South Korean population: a cross-sectional, case-control study. J Yeungnam Med Sci 2022;39:285–93.

[11] Veronese N, Stefanac S, Koyanagi A, Al-Daghri NM, Sabico S, Cooper C, et al. Lower limb muscle strength and muscle mass are associated with incident symptomatic knee osteoarthritis: a longitudinal cohort study. Front Endocrinol 2021;12:804560.

[12] Chung SM, Hyun MH, Lee E, Seo HS. Novel effects of sarcopenic osteoarthritis on metabolic syndrome, insulin resistance, osteoporosis, and bone fracture: the national survey. Osteoporos Int 2016;27:2447–57.

[13] Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr 1997;127:990S–1S.

[14] Chen L-K, Liu L-K, Woo J, Assantachai P, Auyeung T-W, Bahyah KS, et al. Sarcopenia in asia: consensus report of the asian working group for sarcopenia. J Am Med Dir Assoc 2014;15:95–101.

[15] Aslan SG, Genç H. Sarcopenia in knee osteoarthritis: the association with clinical and sonographic findings, physical function, and nutrition. Ir J Medical Sci 2023;92(3):1231–9.

[16] Vlietstra L, Stebbings S, Meredith-Jones K, Abbott JH, Treharne GJ, Waters DL. Sarcopenia in osteoarthritis and rheumatoid arthritis: the association with self-reported fatigue, physical function and obesity. PLoS One 2019;14:e0217462.

[17] Misra D, Fielding RA, Felson DT, Niu J, Brown C, Nevitt M, et al. Risk of knee osteoarthritis with obesity, sarcopenic obesity, and sarcopenia. Arthritis Rheumatol 2019;71:232–7.

[18] Vlietstra L, Meredith-Jones K, Stebbings S, Abbott JH, Treharne GJ, Waters DL. Sarcopenic obesity is more prevalent in osteoarthritis than rheumatoid arthritis: are different processes involved? Rheumatology 2017;56:1816–8.

[19] Park D, Park CJ, Jung M-G, Choi YH, Park K-S, Koh HS. High prevalence of sarcopenia in Asian female patients awaiting primary total knee arthroplasty: application of updated diagnostic tools from the Asian working group for sarcopenia. J Orthop Surg 2022;30:10225536221113034.

[20] Veronese N, Punzi L, Sieber C, Bauer J, Reginster JY, Maggi S, et al. Sarcopenic osteoarthritis: a new entity in geriatric medicine? Eur Geriatr Med 2018;9:141–8.

[21] Tagliaferri C, Wittrant Y, Davicco M-J, Walrand S, Coxam V. Muscle and bone, two interconnected tissues. Ageing Res Rev 2015;21:55–70.

[22] Barrett T, Troup DB, Wilhite SE, Ledoux P, Evangelista C, Kim IF, et al. NCBI GEO: archive for functional genomics data sets—10 years on. Nucleic Acids Res 2011;39:D1005–10.

[23] Giresi PG, Stevenson EJ, Theilhaber J, Koncarevic A, Parkington J, Fielding RA, et al. Identification of a molecular signature of sarcopenia. Physiol Genom 2005; 21:253–63.

[24] Woetzel D, Huber R, Kupfer P, Pohlers D, Pfaff M, Driesch D, et al. Identification of rheumatoid arthritis and osteoarthritis patients by transcriptome-based rule set generation. Arthritis Res Ther 2014;16:R84.

[25] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.

[26] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.

[27] Chen H, Boutros PC. VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinf 2011;12:35.

[28] Doms A, Schroeder M. GoPubMed: exploring PubMed with the gene ontology. Nucleic Acids Res 2005;33:W783–6.

[29] Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016;44:W90–7.

[30] Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res 2021;49. D545-D551.

[31] Martens M, Ammar A, Riutta A, Waagmeester A, Slenter DN, Hanspers K, et al. WikiPathways: connecting communities. Nucleic Acids Res 2021;49:D613–21.

[32] Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, et al. The reactome pathway knowledgebase. Nucleic Acids Res 2019;48:D498–503.

[33] Huang R, Grishagin I, Wang Y, Zhao T, Greene J, Obenauer JC, et al. The NCATS BioPlanet – an integrated platform for exploring the universe of cellular signaling pathways for toxicology, systems biology, and chemical genomics. Front Pharmacol 2019;10:445.

[34] Pagel P, Kovac S, Oesterheld M, Brauner B, Dunger-Kaltenbach I, Frishman G, et al. The MIPS mammalian protein–protein interaction database. Bioinformatics 2005;21:832–4.

[35] Xia J, Gill EE, Hancock REW. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat Protoc 2015;10:823–44.

[36] Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 2021;49:D605-D612.

[37] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498–504.

[38] Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014;8:S11.

[39] Franz M, Rodriguez H, Lopes C, Zuberi K, Montojo J, Bader GD, et al. GeneMANIA update 2018. Nucleic Acids Res 2018;46:W60–4.

[40] Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, et al. The human transcription factors. Cell 2018;172:650–65.

[41] Bushati N, Cohen SM. microRNA Functions. Annu Rev Cell Dev Biol 2007;23: 175–205.

[42] Hobert O. Common logic of transcription factor and microRNA action. Trends Biochem Sci 2004;29:462–8.

[43] Zhou Y, Ferguson J, Chang JT, Kluger Y. Inter- and intra-combinatorial regulation by transcription factors and microRNAs. BMC Genom 2007;8:396.

[44] de Souza N. The ENCODE project. Nat Methods 2012;9:1046.

[45] Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, et al. The Encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res 2018;46:D794–801.

[46] Liu Z-P, Wu C, Miao H, Wu H. RegNetwork: an integrated database of transcriptional and post-transcriptional regulatory networks in human and mouse. Database 2015;2015:bav095.

[47] Yoo M, Shin J, Kim J, Ryall KA, Lee K, Lee S, et al. DSigDB: drug signatures database for gene set analysis. Bioinformatics 2015;31:3069–71.

[48] Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinf 2013;14:128.

[49] Ganapathy A, Nieves JW. Nutrition and sarcopenia—what do we know? Nutrients 2020;12:1755.

[50] Wu X, Liyanage C, Plan M, Stark T, McCubbin T, Barrero RA, et al. Dysregulated energy metabolism impairs chondrocyte function in osteoarthritis. Osteoarthr Cartilage; 2023; 31:613–626.

[51] Thomas S, Browne H, Mobasheri A, Rayman MP. What is the evidence for a role for diet and nutrition in osteoarthritis? Rheumatology 2018;57:iv61–74.

[52] Chin K-Y. The Relationship between Vitamin K and Osteoarthritis: a review of current evidence. Nutrients 2020;12:1208.

[53] Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the provide study: a randomized, double-blind, placebo-controlled trial. J Am Med Dir Assoc 2015;16:740–7.

[54] Liu P, Hu Y, Wang M, Kang L. Nutrient weight against sarcopenia: regulation of the IGF-1/PI3K/Akt/FOXO pathway in quinoa metabolites. Curr Opin Pharmacol 2021;61:136–41.

[55] Park CY. Vitamin D in the prevention and treatment of osteoarthritis: from clinical interventions to cellular evidence. Nutrients 2019;11:243.

[56] Lee D, Kuroki T, Nagai T, Kawano K, Higa K, Kurogi S, et al. Sarcopenia, ectopic fat infiltration into the lumbar paravertebral muscles, and lumbo-pelvic deformity in older adults undergoing lumbar surgery. Spine 2022;47:46–57.

[57] Taatjes DJ. RNA polymerase II transcription. J Mol Biol 2021;433:167037.

[58] Malumbres M. Cyclin-dependent kinases. Genome Biol 2014;15:122.

[59] Kobayashi Y, Tanaka T, Mulati M, Ochi H, Sato S, Kaldis P, et al. Cyclin-dependent kinase 1 is essential for muscle regeneration and overload muscle fiber hypertrophy. Front Cell Dev Biol 2020;8:564581.

[60] Machida S, Booth FW. Increased nuclear proteins in muscle satellite cells in aged animals as compared to young growing animals. Exp Gerontol 2004;39:1521–5.

[61] Lee S, Dong HH. FoxO integration of insulin signaling with glucose and lipid metabolism. J Endocrinol 2017;233:67–79.

[62] Matsuzaki T, Alvarez-Garcia O, Mokuda S, Nagira K, Olmer M, Gamini R, et al. FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. Sci Transl Med 2018;10:3135–43.

[63] Akasaki Y, Alvarez-Garcia O, Saito M, Caramés B, Iwamoto Y, Lotz MK. FoxO Transcription factors support oxidative stress resistance in human chondrocytes. Arthritis Rheumatol 2014;66:3349–58.

[64] Murtaza G, Khan AK, Rashid R, Muneer S, Hasan SMF, Chen J. FOXO transcriptional factors and long-term living. Oxid Med Cell Longev 2017;2017: 3494289.

[65] Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis 2004;9:667–76.

[66] Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. Nat Rev Clin Oncol 2013;10:143–53.

[67] Gopinath SD, Webb AE, Brunet A, Rando TA. FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal. Stem Cell Rep 2014;2: 414–26.

[68] Guo X, Pan X, Wu J, Li Y, Nie N. Calycosin prevents IL-1β-induced articular chondrocyte damage in osteoarthritis through regulating the PI3K/AKT/FoxO1 pathway. Vitro Cell Dev Biology 2022;58:491–502.

[69] Feng L, Li B, Xi Y, Cai M, Tian Z. Aerobic exercise and resistance exercise alleviate skeletal muscle atrophy through IGF-1/IGF-1R-PI3K/Akt pathway in mice with myocardial infarction. Am J Physiol Cell Physiol 2022;322:164–76.

[70] Lee KI, Choi S, Matsuzaki T, Alvarez-Garcia O, Olmer M, Grogan SP, et al. FOXO1 and FOXO3 transcription factors have unique functions in meniscus development and homeostasis during aging and osteoarthritis. Proc Natl Acad Sci USA 2020; 117:3135–43.

[71] Zhao C, Li X, Sun G, Liu P, Kong K, Chen X, et al. CircFOXO3 protects against osteoarthritis by targeting its parental gene FOXO3 and activating PI3K/AKT-mediated autophagy. Cell Death Dis 2022;13:932.

[72] Akasaki Y, Hasegawa A, Saito M, Asahara H, Iwamoto Y, Lotz MK. Dysregulated FOXO transcription factors in articular cartilage in aging and osteoarthritis. Osteoarthritis Cartilage 2014;22:162–70.

[73] Vitadello M, Sorge M, Percivalle E, Germinario E, Danieli-Betto D, Turco E, et al. Loss of melusin is a novel, neuronal NO synthase/FoxO3-independent master switch of unloading-induced muscle atrophy. J Cachexia Sarcopenia Muscle 2020;11:802–19.

[74] Engeland K. Cell cycle regulation: p53-p21-RB signaling. Cell Death Differ 2022; 29:946–60.

[75] Xia L, Gong N. Identification and verification of ferroptosis-related genes in the synovial tissue of osteoarthritis using bioinformatics analysis. Front Mol Biosci 2022;9:992044.

[76] Shi S, Wan F, Zhou Z, Tao R, Lu Y, Zhou M, et al. Identification of key regulators responsible for dysregulated networks in osteoarthritis by large-scale expression analysis. J Orthop Surg Res 2021;16:259.

[77] 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.

[78] Zhang X, Yuan Z, Cui S. Identifying candidate genes involved in osteoarthritis through bioinformatics analysis. Clin Exp Rheumatol 2015;34:282–90.

[79] Bertram KL, Narendran N, Tailor P, Jablonski C, Leonard C, Irvine E, et al. 17-DMAG regulates p21 expression to induce chondrogenesis in vitro and in vivo. Dis Model Mech 2018;11:dmm033662.

[80] Gang G, Xu H, Si L, Zhu X, Yu T, Jiang Z, et al. Treatment effect of CDKN1A on rheumatoid arthritis by mediating proliferation and invasion of fibroblast-like synoviocytes cells. Clin Exp Immunol 2018;194:220–30.

[81] Xu Q, Li J, Wu Y, Zhou W, Xu Z. Colorectal cancer chemotherapy drug bevacizumab may induce muscle atrophy through CDKN1A and TIMP4. Front Oncol 2022;12:897495.

[82] Kinoshita S, Akira S, Kishimoto T. A member of the C/EBP family, NF-IL6 beta, forms a heterodimer and transcriptionally synergizes with NF-IL6. Proc Natl Acad Sci USA 1992;89:1473–6.

[83] Chinery R, Brockman JA, Dransfield DT, Coffey RJ. Antioxidant-induced nuclear translocation of CCAAT/Enhancer-binding Protein β a critical role for protein kinase a-mediated phosphorylation of Ser299. J Biol Chem 1997;272:30356–61.

[84] Hirata M, Kugimiya F, Fukai A, Saito T, Yano F, Ikeda T, et al. C/EBPβ and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2α as the inducer in chondrocytes. Hum Mol Genet 2012;21:1111–23.

[85] Guo L, Li X, Tang Q-Q. Transcriptional regulation of adipocyte differentiation: a central role for CCAAT/Enhancer-binding Protein (C/EBP) β. J Biol Chem 2015; 290:755–61.

[86] Charlier E, Deroyer C, Ciregia F, Malaise O, Neuville S, Plener Z, et al. Chondrocyte dedifferentiation and osteoarthritis (OA). Biochem Pharmacol 2019; 165:49–65.

[87] Harrell CR, Markovic BS, Fellabaum C, Arsenijevic A, Volarevic V. Mesenchymal stem cell-based therapy of osteoarthritis: current knowledge and future perspectives. Biomed Pharmacother 2019;109:2318–26.

[88] Santilli V, Bernetti A, Mangone M, Paoloni M. Clinical definition of sarcopenia. Clin Cases Miner Bone Metabolism 2014;11:177–80.

[89] Sinclair M, Gow PJ, Grossmann M, Angus PW. Review article: sarcopenia in cirrhosis – aetiology, implications and potential therapeutic interventions. Aliment Pharm Therap 2016;43:765–77.

[90] Zhang H-M, Kuang S, Xiong X, Gao T, Liu C, Guo A-Y. Transcription factor and microRNA co-regulatory loops: important regulatory motifs in biological processes and diseases. Briefings Bioinf 2015;16:45–58.

[91] Zhang S, Yang F, Huang Y, He L, Li Y, Wan YCE, et al. ATF3 induction prevents precocious activation of skeletal muscle stem cell by regulating H2B expression. Nat Commun 2023;14:4978.

[92] Iezaki T, Ozaki K, Fukasawa K, Inoue M, Kitajima S, Muneta T, et al. ATF3 deficiency in chondrocytes alleviates osteoarthritis development. J Pathol 2016; 239:426–37.

[93] Liu Z, Wang H, Hou Y, Yang Y, Jia J, Wu J, et al. CNC-bZIP protein NFE2L1 regulates osteoclast differentiation in antioxidant-dependent and independent manners. Redox Biol 2021;48:102180.

[94] Picca A, Calvani R, Bossola M, Allocca E, Menghi A, Pesce V, et al. Update on mitochondria and muscle aging: all wrong roads lead to sarcopenia. Biol Chem 2018;399:421–36.

[95] Sun X, Huang H, Pan X, Li S, Xie Z, Ma Y, et al. EGR1 promotes the cartilage degeneration and hypertrophy by activating the Krüppel-like factor 5 and β-catenin signaling. Biochim Biophys Acta, Mol Basis Dis 2019;1865:2490–503.

[96] Pagel J-I, Deindl E. Disease progression mediated by egr-1 associated signaling in response to oxidative stress. Int J Mol Sci 2012;13:13104–17.

[97] Jin Y, Chen X, Gao ZY, Liu K, Hou Y, Zheng J. The role of miR-320a and IL-1β in human chondrocyte degradation. Bone Joint Res 2017;6:196–203.

[98] Dahlmans D, Houzelle A, Andreux P, Jörgensen JA, Wang X, Windt LJ de, et al. An unbiased silencing screen in muscle cells identifies miR-320a, miR-150, miR-196b, and miR-34c as regulators of skeletal muscle mitochondrial metabolism. Mol Metabol 2017;6:1429–42.

[99] Reddy D, Ghosh P, Kumavath R. Strophanthidin attenuates MAPK, PI3K/AKT/mTOR, and wnt/β-catenin signaling pathways in human cancers. Front Oncol 2020;9:1469.

[100] Jia H, Duan L, Yu P, Zhou Y, Liu R, Wang H. Digoxin ameliorates joint inflammatory microenvironment by downregulating synovial macrophage M1-like-polarization and its-derived exosomal miR-146b-5p/Usp3&Sox5 axis. Int Immunopharm 2022;111:109135.

[101] Wang K, Ding X, Jiang N, Zeng C, Wu J, Cai X, et al. Digoxin targets low density lipoprotein receptor-related protein 4 and protects against osteoarthritis. Ann Rheum Dis 2022;81:544–55.

[102] Watanabe-Asano T, Kuma A, Mizushima N. Cycloheximide inhibits starvation-induced autophagy through mTORC1 activation. Biochem Bioph Res Co 2014; 445:334–9.

[103] Mao W, Zhu Z. Parthenolide inhibits hydrogen peroxide-induced osteoblast apoptosis. Mol Med Rep 2018;17:8369–76.

[104] Ren Y, Li Y, Lv J, Guo X, Zhang J, Zhou D, et al. Parthenolide regulates oxidative stress-induced mitophagy and suppresses apoptosis through p53 signaling pathway in C2C12 myoblasts. J Cell Biochem 2019;120:15695–708.