Am J Transl Res 2023;15(7):4487-4503
www.ajtr.org /ISSN:1943-8141/AJTR0143181

Original Article

The molecular link between obstructive sleep apnea and osteoarthritis: based on bioinformatics analysis and experimental validation

Xun-Shan Ren[1*], Ting Xie[2*], Huang-Ming Zhuang[1*], Tian-Run Lei[1], Fu-Ze Jiang[1], Pang-Hu Zhou[1]

[1]Department of Orthopedics, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Department of Women's Health Care, Maternal and Child Health Hospital of Hubei Province, Wuhan, Hubei, China. [*]Equal contributors.

Received March 28, 2022; Accepted June 29, 2023; Epub July 15, 2023; Published July 30, 2023

Abstract: Background: Obstructive sleep apnea (OSA) and osteoarthritis (OA) are highly prevalent and seriously affect the patient's quality of life. Patients with OSA have a high incidence of OA, however, the underlying mechanism remains unclear. Here, we investigated the molecular link between OSA and OA via bioinformatics analysis and experimental validation. Methods: We downloaded a peripheral blood monocyte microarray profile (GSE75097) for patients with OSA and two synovial microarray profiles (GSE55235 and GSE55457) for patients with OA from the Gene Expression Omnibus database. We identified OSA-associated differentially expressed genes (OSA-DEGs) in patients with OA. Additionally, we constructed protein-protein interaction networks to identify the key genes involved in OA. Immunohistochemistry was performed to verify the expression of key genes in OA rat models. RNA interference assay was performed to validate the effects of key genes on synovial cells. Gene-miRNA, gene-transcription factor, and gene-drug networks were constructed to predict the regulatory molecules and drugs for OA. Results: Fifteen OSA-DEGs screened using the threshold criteria were enriched in the tumor necrosis factor (TNF) pathway. Combining the 12 algorithms of CytoHubba, we identified JUNB, JUN, dual specificity phosphatase 1 (DUSP1), and TNF-alpha-induced protein 3 (TNFAIP3) as the key OSA-DEGs involved in OA development. Immunohistochemistry and quantitative polymerase chain reaction revealed that these key genes were downregulated in the OA synovium, promoting TNF-α expression. Therefore, OSA-DEGs, JUN, JUNB, DUSP1, and TNFAIP3 function in OA by increasing TNF-α expression. Our findings provide insights on the mechanisms underlying the effects of OSA on OA.

Keywords: Obstructive sleep apnea, osteoarthritis, GEO, TNF signaling pathway, bioinformatics

Introduction

Osteoarthritis (OA) is the leading cause of disability and decreased quality of life, especially in the elderly population. Approximately 10-15% of people aged over 60 years and almost 300 million people worldwide suffer from OA [1]. Incidence of OA increases annually with the increasing age of the global population, placing an overwhelming financial burden on society [2, 3]. OA is caused by complex risk factors, such as age, obesity, trauma, and dysregulated hormone levels [4, 5]. OA is mainly characterized by joint pain, stiffness, and decreased motion, with varying degrees of inflammation [6]. Pain is one of the most prominent manifestations of OA, and synovitis is the primary source of joint pain due to external disturbances and inadequate innervation of the joint cartilage [7]. Synovitis is observed in patients with early OA. Synovial cells release inflammatory mediators and matrix metalloproteinases that drive the development of OA. OA severity can be predicted based on the degree of synovitis [8, 9]. However, the etiology and regulatory mechanisms of synovitis are unclear.

Obstructive sleep apnea (OSA), a common sleep-related disorder characterized by repeated obstruction of the upper respiratory tract during sleep, affects approximately one billion people [10]. Risk factors for OSA include anatomical abnormalities of the upper respiratory tract, aging, obesity, genetic factors, and dys-

regulated estrogen levels. OSA is usually associated with daytime sleepiness, reduced attention, fatigue, and decreased cognitive function due to intermittent hypoxia caused by sleep apnea [11]. OSA is associated with many diseases, such as cardiovascular diseases, metabolic diseases, neurological diseases, and cancer [12]. The primary mechanism by which OSA affects other diseases is the hyperactivation of circulating inflammatory products stimulated by intermittent hypoxia, which likely affects OA progression [13].

Several studies have suggested a close association between OSA and OA. Intermittent hypoxia in OSA promotes the senescence of synovial cells and chondrocytes and activates the senescence-associated secretory phenotype [14]. Intermittent hypoxia leads to selective activation of the nuclear factor (NF)-κB inflammatory signaling pathway and the secretion of circulating inflammatory products. Therefore, OSA is often accompanied by chronic systemic low-grade inflammation. OA is considered as a systemic, chronic, and low-degree inflammatory disease with high levels of circulating inflammatory cytokines. Systemic chronic low-degree inflammation causes sarcopenic obesity, leading to the obstruction of the upper respiratory tract and exacerbation of OSA [15, 16]. In addition, progression of OA is accelerated by systemic chronic low-grade inflammation, which leads to worsened symptoms, such as synovitis and arthralgia [17]. A previous study reported that the prevalence of OSA can reach up to 66% in OA cohorts [18]. OSA shares similar risk factors with OA, such as aging, metabolic abnormalities, obesity, and dysregulated estrogen levels [19-22]. Systemic chronic low-degree inflammation, which is inevitable in OSA due to intermittent hypoxia, is also observed in OA. These shared features indicate that OSA and OA may interact via unknown inflammatory signaling pathways. Therefore, the associated signaling pathways and regulatory mechanisms should be investigated to understand the link between OSA and OA.

In this study, we identified four key OSA-associated differentially expressed genes (OSA-DEGs) via bioinformatics analysis. We also determined the expression and function of the identified key genes in vivo and in vitro. Our results indicate that the four key genes can exacerbate OA by increasing the expression of tumor necrosis factor (TNF)-α.

Materials and methods

Public datasets

Public datasets (GSE55235, GSE55457, GSE1919, and GSE75097) were obtained from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo). GSE55235, GSE55457 and GSE1919 are synovial mRNA microarray datasets from patients with OA and healthy controls, respectively. GSE75097 is a microarray dataset of peripheral blood mononuclear cells from primary snoring patients with OSA and those with moderate-to-severe OSA.

Identification of OSA-DEGs

After ID annotation and normalization of the expression matrix in the R program (version 4.1.1), DEGs were identified using the limma package (version 3.48.3) and visualized using the ggplot2 package (version 3.3.5) package, with a threshold criterion of *P*-value <0.05, |LogFC| >0.5. The online tool Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to detect OSA-DEGs.

Functional annotation of OSA-DEGs

Database for Annotation, Visualization, and Integrated Discovery (DAVID), an online bioinformatics analysis tool, was used to perform Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses as previously described [23, 24]. DAVID results were visualized using the ggplot2 package in R.

Construction of protein-protein interaction (PPI) networks and identification of the hub gene cluster

PPI networks of OSA-DEGs were constructed by using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, http://www.string-db.org/), an online tool to identify and predict interactions between proteins, and visualized using the Cytoscape software (vision 3.7.2). The CytoHubba plug-in in Cytoscape was used to study hub genes with 12 different PPI algorithms [25].

Surgical rat model of OA

Ten 8-week-old male Wistar rats were purchased from SiPeiFu Biotechnology Co., Ltd. The OA rat model was established via anterior cruciate ligament transection. In the OA group, all rats underwent ACL transaction surgery on both knees. In the sham group, the knee joint was exposed via an incision on the medial aspect of the joint capsule, and the incision was closed with braided silk sutures. Eight weeks after surgery, all rats were anesthetized with isoflurane and sacrificed. The knee joint samples were fixed with 4% paraformaldehyde, decalcified in 0.5 M EDTA, and embedded in paraffin for further study.

Animal care and experiments were in accordance with the National Research Council guide for the care and use of laboratory animals and approved by the Laboratory Animal Welfare and Ethics Committee of Renmin Hospital of Wuhan University (Approval No: 20220103A).

Histology

Paraffin-embedded tissues were cut into 6-μm-thick sections using a rotary microtome (Leica RM2016, Leica Microsystems Ltd.). Midsagittal sections were stained with hematoxylin-eosin and safranin O-fast green. Images were captured using a microscope (NIKON ECLIPSE CI, NIKON).

Immunohistochemistry

Tissue sections were subjected to antigen retrieval using a citric acid buffer. Endogenous peroxidases were quenched using 3% hydrogen peroxide at room temperature for 25 min. Non-specific protein binding was blocked by bovine serum albumin (BSA) at room temperature for 30 min. The sections were incubated with primary antibodies against JUN (1:200, A0246, Abclonal), JUNB (1:200, A5290, Abclonal), TNFAIP3 (1:200, A19128, Abclonal) and DUSP1 (1:200, A2919, Abclonal) overnight at 4°C. After washing with PBS, the sections were incubated with goat anti-rabbit IgG HRP (ab205718, Abcam) at 37°C for 50 min. DAB HRP substrate kit (K3468, DAKO) was used for dye development, and hematoxylin was used as a nuclear counterstain. Images were taken using a microscope (NIKON ECLIPSE CI, NIKON).

Synovial cell isolation and culture

To culture primary rat synovial cells, synovial tissues were isolated from 8-week-old male Wistar rats. Then, the synovial tissues were cut into pieces, placed in 0.2% collagenase II (G5029, Servicebio), and digested for 2 h at 37°C. Finally, synovial cells were centrifuged at 1200 rpm for 8 min and maintained in the complete Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 medium (PM150310B, Procell) in a humidified 37°C, 5% CO_2, and 3% O_2 atmosphere.

Immunofluorescence assay

Synovial cells were fixed in 4% paraformaldehyde for 10 min at room temperature. After washing thrice with PBS, the synovial cells were permeabilized with 0.5% Triton X-100 for 10 min and blocked with 10% BSA for 1 h at room temperature. Then, synovial cells were incubated with anti-vimentin primary antibody (1:200, A2584, Abclonal) overnight at 4°C, followed by incubation with the Cy3-conjugated secondary antibody (1:500, GB21303, Servicebio) at room temperature for 2 h. Finally, 4',6-diamidino-2-phenylindole was used as a counterstain and images were acquired using a microscope.

RNA interference

si-JUN, si-JUNB, si-TNFAIP3, si-DUSP1, and si-NC were obtained from RiboBio (Guangzhou, China). Synovial cells were transfected with siRNAs using Lipofectamine 2000, according to the manufacturer's protocol. Target sequences for RNA are listed in Table S1.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total cellular RNA was isolated using an RNA Isolation Kit, following the manufacturer's protocol. RNA concentration was measured using a NanoDrop spectrophotometer. RNA (1 μg) was reverse-transcribed using SweScript RT II Enzyme Mix. QPCR was performed using 2X Universal SYBR Green Fast qPCR Mix in a LightCycler 480 machine. β-actin was used for normalization. All primer sequences are listed in Table S2.

Construction of microRNA (miRNA)-gene and transcription factor (TF)-gene networks

miRNAs and TFs regulating the expression of OSA-DEGs were predicted using online databases. miWALK database (http://mirwalk.umm.uni-heidelberg.de/search_mirnas/) and Targetscan database (http://www.targetscan.org/vert_80/) were used to construct the miRNA-gene network, and the TF-gene network was determined using ChEA3 (https://maayanlab.cloud/chea3/) [26]. Finally, the results were visualized using the Cytoscape software.

Identification of potential drugs

Drug-gene interaction database (DGidb) (https://www.dgidb.org) was used to predict potential drugs and molecular compounds for OSA-DEGs, and the drug-gene network was analyzed and visualized using the Cytoscape software [27].

Receiver operating characteristic (ROC) curve

ROC curves were used to evaluate the predictive value of hub genes for OSA and OA based on the expression matrix and sample status of GSE55235, GSE55457, and GSE1919. ROC curves were plotted using the pROC package in R. The predictive value of hub genes was assessed based on the area under the curve (AUC) values.

Statistical analysis

All results are presented as the mean ± standard deviation (SD). For statistical analyses, two-tailed one-way analysis of variance was conducted using the GraphPad Prism software. Each experiment was repeated two or three times. Differences were considered statistically significant at $P<0.05$.

Results

Identification of OSA-DEGs between OSA and OA

Recruitment of peripheral blood mononuclear cells to synovial tissues is crucial for the inflammatory response in OA. Therefore, parsing transcriptomic signatures common to peripheral blood mononuclear cells in OSA and synovial cells in OA can aid in determining the molecular link between these two diseases. We identified 1295 DEGs in GSE55235 and 970 DEGs in GSE55457 associated with OA based on the defined criteria. Moreover, in the OSA dataset GSE75097, 466 DEGs were filtered by comparing 28 OSA samples with 20 primary snoring patients and long-term continuous positive airway pressure treatment samples. Expression profiles and DEGs are shown as heat maps and volcano plots, respectively (**Figure 1A** and **1B**). Finally, 15 OSA-DEGs, including two upregulated and 13 downregulated OSA-DEGs, shared between the synovium of OA and peripheral blood mononuclear cells in OSA were integrated using the Venn Diagram online tool (**Figure 1C**; **Table 1**).

Functional annotation of OSA-DEGs

Functional annotation analysis of the 15 OSA-DEGs was performed at a threshold of $P<0.05$. The top three terms in GO biological processes (BP), cellular components (CC), and molecular functions (MF) and KEGG are shown in the bubble chart, chord plot, and network diagram respectively (**Figure 2A-C**; **Table 2**). The top three terms related to BP among the OSA-DEGs included response to hydrogen peroxide (p-adjust: 0.004), negative regulation of protein phosphorylation (p-adjust: 0.005), and response to oxidative stress (p-adjust: 0.005). The top three terms related to CC included transcription factor complex (p-adjust: 0.002), nuclear euchromatin (p-adjust: 0.002), and euchromatin (p-adjust: 0.002). The top three terms related to MF were DNA-binding transcription activator activity, RNA polymerase II-specific (p-adjust: 7.64e-05), beta-catenin binding (p-adjust: 0.002), and transcription corepressor activity (p-adjust: 0.027). Notably, OSA-DEGs in the KEGG pathway were mainly enriched in TNF signaling pathway.

PPI network analysis and hub gene screening

PPI network, consisting of 15 OSA-DEGs, contained 15 nodes and 70 edges. OSA-DEGs were ranked by 12 algorithms in CytoHubba. JUN, JUNB, DUSP1, and TNFAIP3 were the top five OSA-DEGs in over half the algorithms (**Figure 3A** and **3B**). Therefore, these four genes were identified as the hub genes.

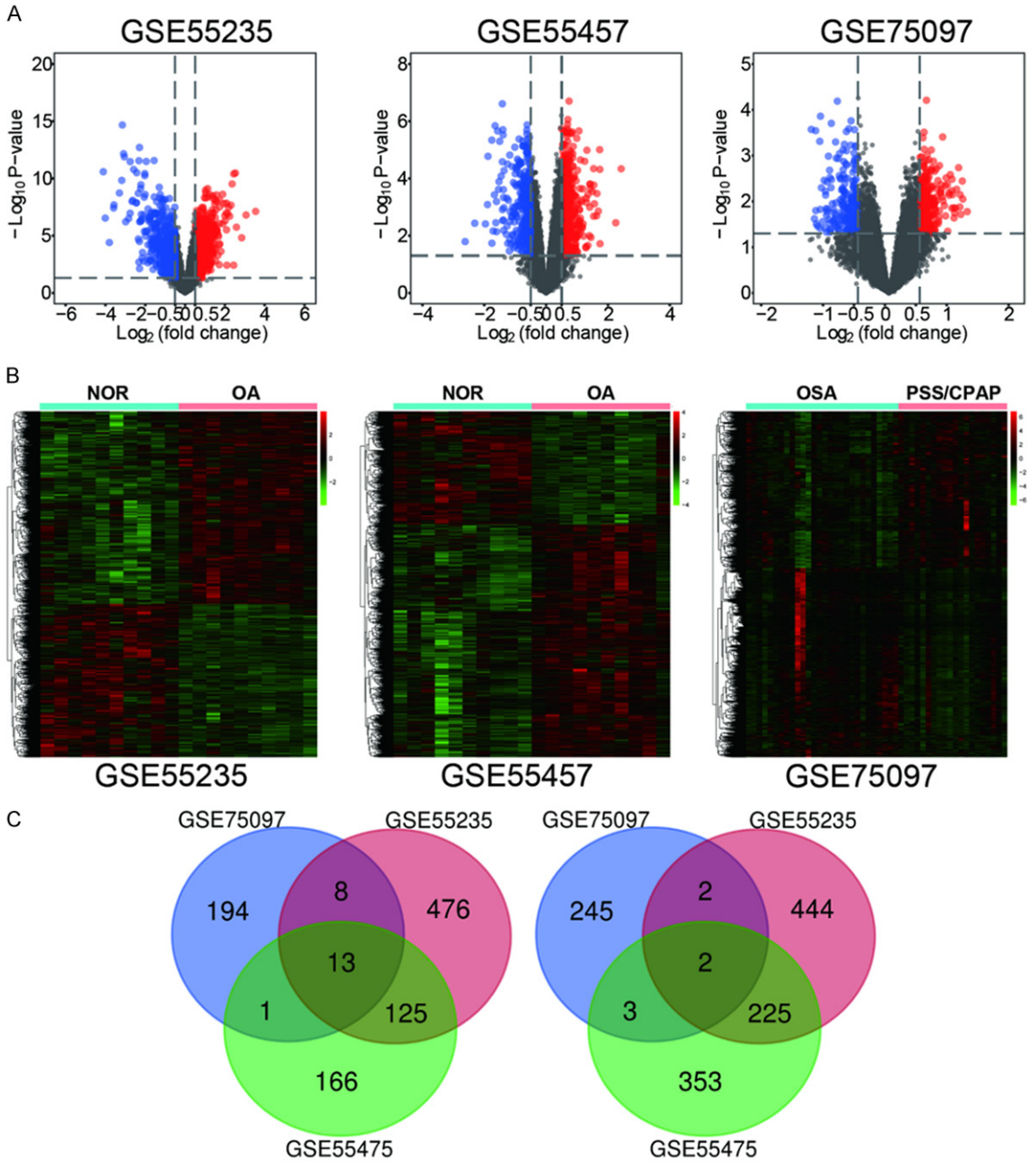

Figure 1. Differentially expressed gene in 3 datasets. A. Volcano plots of DEGs in GSE55235, GSE55457 and GSE75097, *P*-value <0.05, |LogFC| >0.5 were set as the screening threshold, red and blue points represented up-regulated genes and down-regulated genes, respectively. B. Heatmaps of DEGs in GSE55235, GSE55457, and GSE75097. C. Venn diagrams of OSA-DEGs from GSE55235, GSE55457, and GSE75097. NOR, normal sample; OA, osteoarthritis; OSA, obstructive sleep apnea; PSS/CPAP, primary snoring subject/OSA patient with long-term continuous positive airway pressure treatment.

Expression levels of the key genes in OA rats

A rat model of OA was established via anterior cruciate ligament transection. As expected, ACL transaction-mediated injury induced articular cartilage degradation and surface irregularities, confirming the successful establishment of the OA model (**Figure 4A** and **4B**). JUN, JUNB, TNFAIP3, and DUSP1 levels were downregulated in the OA synovium, consistent with the bioinformatics analysis results (**Figure 4C**).

Table 1. List of OSA-DEGs

OSA-DEGs	Genes names
Up-regulated	HLTF, TBL1XR1
Down-regulated	CD163, PPP1R15A, NR4A2, JUNB, NFIL3, KLF4, DUSP1, BCL6, JUN, MAFF, TNFAIP3, SAT1, GADD45B

Abbreviations: OSA-DEGs, OSA-associated differentially expressed genes; HLTF, helicase like transcription factor; TBL1XR1, TBL1X/Y related 1; PPP1R15A, protein phosphatase 1, regulatory subunit 15A; NR4A2, nuclear receptor subfamily 4 group A member 2; JUNB, Jun B proto-oncogene; NFIL3, nuclear factor interleukin 3 regulated; KLF4, KLF transcription factor 4; DUSP1, dual specificity phosphatase 1; BCL6, B cell leukemia/lymphoma 6; JUN, Jun proto-oncogene; MAFF, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F; TNFAIP3, TNF alpha induced protein 3; SAT1, spermidine/spermine N1-acetyltransferase 1; GADD45B, growth arrest and DNA damage inducible beta.

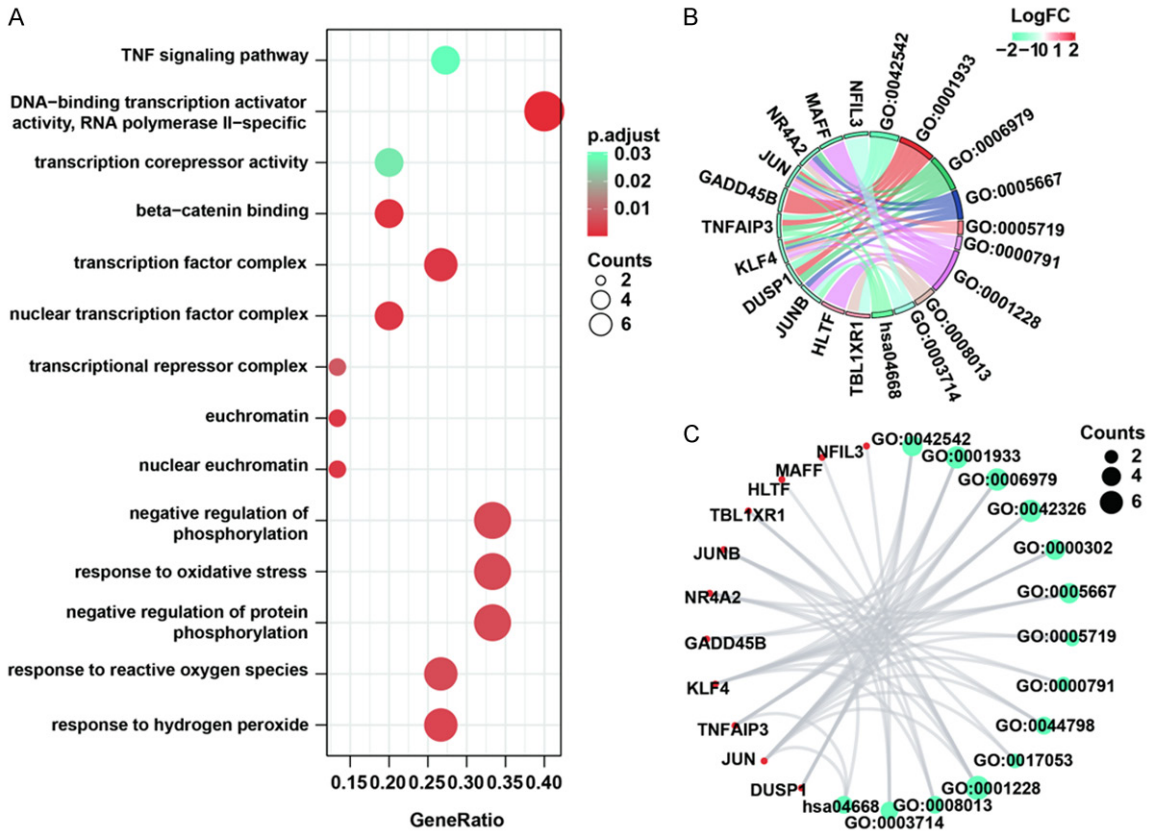

Figure 2. Gene Ontology and KEGG enrichment analysis of OSA-DEGs. A. Bubble chart showed the terms of BP (biological process), MF (molecular function), CC (cell composition) in the GO and KEGG pathway. The x-axis label and y-axis label represent the GeneRatio and the names of terms, respectively. B. Chord plot showed the relationship between each OSA-DEGs and terms. Fold change values of OSA-DEGs were mapped by color scale. C. The network diagram showed the connection of each OSA-DEGs to GO and KEGG terms. HLTF, helicase like transcription factor; TBL1XR1, TBL1X/Y related 1; PPP1R15A, protein phosphatase 1, regulatory subunit 15A; NR4A2, nuclear receptor subfamily 4 group A member 2; JUNB, Jun B proto-oncogene; NFIL3, nuclear factor interleukin 3 regulated; KLF4, KLF transcription factor 4; DUSP1, dual specificity phosphatase 1; BCL6, B cell leukemia/lymphoma 6; JUN, Jun proto-oncogene; MAFF, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F; TNFAIP3, TNF alpha induced protein 3; SAT1, spermidine/spermine N1-acetyltransferase 1; GADD45B, growth arrest and DNA damage inducible beta.

Effects of the key genes on synovial cells

Primary synovial cells from rats were isolated and cultured to confirm the effects of the four key genes on synovial cells. Immunofluorescence assay revealed that the isolated synovial cells expressed vimentin, a biomarker of synovial cells, consistent with the results of previ-

Table 2. GO terms and KEGG enrichment analysis of OSA-DEGs

	Terms	P.adjust	Count
BP	response to hydrogen peroxide	0.004	4
	negative regulation of protein phosphorylation	0.005	5
	response to oxidative stress	0.005	5
	negative regulation of phosphorylation	0.005	5
	response to reactive oxygen species	0.005	4
CC	transcription factor complex	0.002	4
	nuclear euchromatin	0.002	2
	euchromatin	0.002	2
	nuclear transcription factor complex	0.002	3
	transcriptional repressor complex	0.008	2
MF	DNA-binding transcription activator activity, RNA polymerase II-specific	7.64E-05	6
	beta-catenin binding	0.002	3
	transcription corepressor activity	0.037	3
KEGG	TNF signaling pathway	0.031	3

Abbreviations: GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; BP, biological processes; CC, cellular components; MF, molecular function; TNF, tumor necrosis factor.

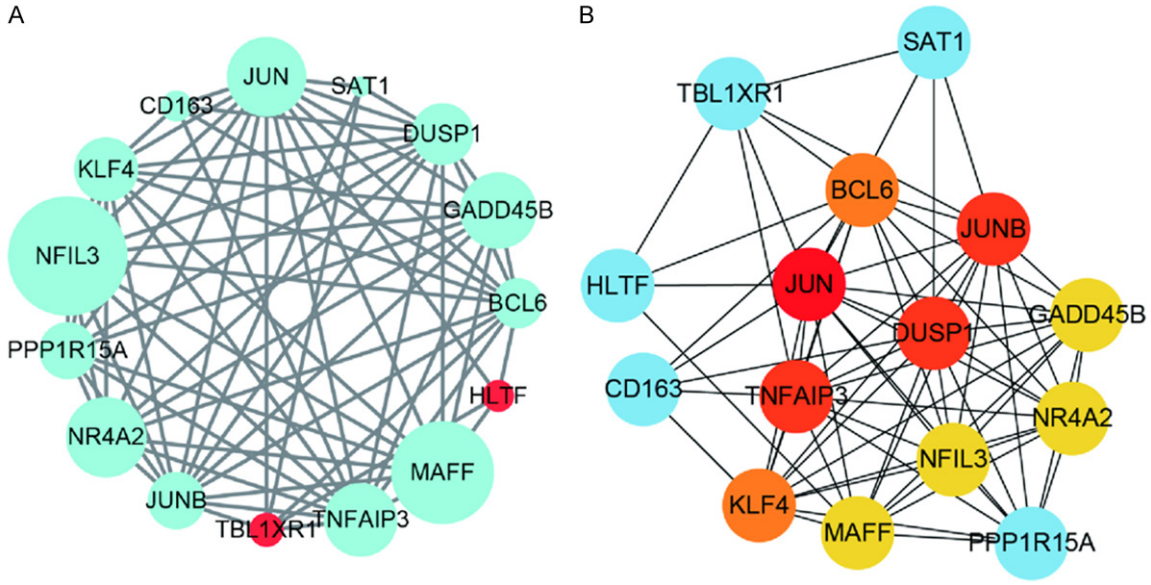

Figure 3. PPI network analysis of OSA-DEGs. A. PPI network of OSA-DEGs, green dots represented down-regulated genes, red dots represented up-regulated genes, and the size represented LogFC. B. The subnetwork based on the MCC algorithm in cytohubba, red color represents high rank. HLTF, helicase like transcription factor; TBL1XR1, TBL1X/Y related 1; PPP1R15A, protein phosphatase 1, regulatory subunit 15A; NR4A2, nuclear receptor subfamily 4 group A member 2; JUNB, Jun B proto-oncogene; NFIL3, nuclear factor interleukin 3 regulated; KLF4, KLF transcription factor 4; DUSP1, dual specificity phosphatase 1; BCL6, B cell leukemia/lymphoma 6; JUN, Jun proto-oncogene; MAFF, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F; TNFAIP3, TNF alpha induced protein 3; SAT1, spermidine/spermine N1-acetyltransferase 1; GADD45B, growth arrest and DNA damage inducible beta.

ous studies (**Figure 5A**). Subsequently, an RNA interference assay was performed to knock down the expression of JUN, JUNB, TNFAIP3, and DUSP1 in synovial cells (**Figure 5B**). Twenty-four hours after the transfection of siRNA into synovial cells, a significant decrease in the mRNA levels of JUN, JUNB, TNFAIP3, and DUSP1 was observed via qRT-PCR. As the

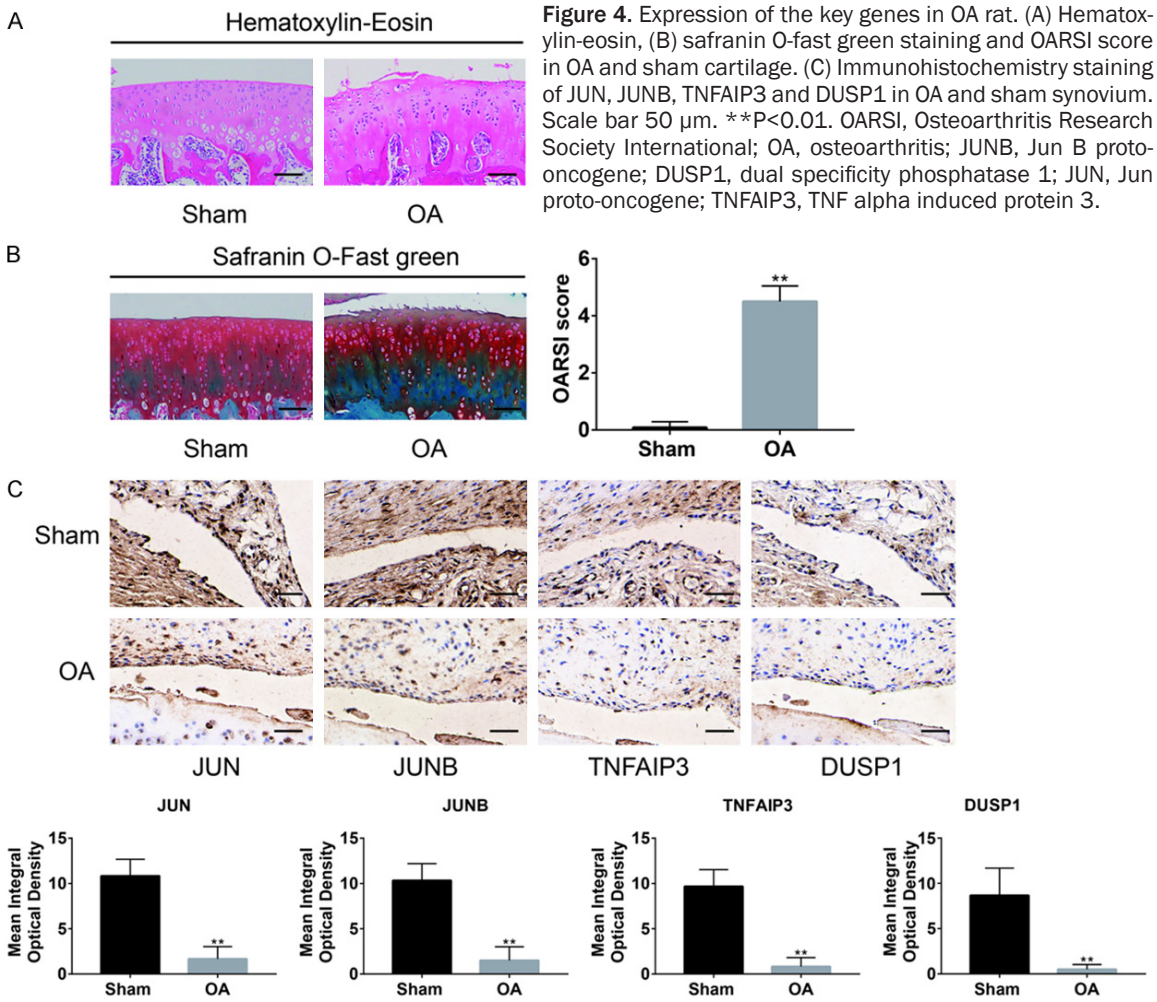

Figure 4. Expression of the key genes in OA rat. (A) Hematoxylin-eosin, (B) safranin O-fast green staining and OARSI score in OA and sham cartilage. (C) Immunohistochemistry staining of JUN, JUNB, TNFAIP3 and DUSP1 in OA and sham synovium. Scale bar 50 μm. **P<0.01. OARSI, Osteoarthritis Research Society International; OA, osteoarthritis; JUNB, Jun B proto-oncogene; DUSP1, dual specificity phosphatase 1; JUN, Jun proto-oncogene; TNFAIP3, TNF alpha induced protein 3.

OSA-DEGs were mainly enriched in the TNF signaling pathway, we measured the expression of TNFα, the main effector molecule of the TNF signaling pathway, using similar experiments. TNF-α levels were significantly increased in synovial cells after the knockdown of JUN, JUNB, TNFAIP3, and DUSP1 (**Figure 5C**).

Construction of miRNA-gene and TF-gene networks

OSA-DEGs were modulated by 61 miRNAs and 50 TFs (**Figure 6A** and **6B**). TBL1XR1 was modulated by 39 miRNAs. MAFF and TNFAIP3 were modulated by nine and five miRNAs, respectively. In the TF-gene network, OSA-DEGs were modulated by a large number of TFs. JUNB, GADD45B, and MAFF were modulated by 48 TFs, NR4A2 by 47 TFs, and TNFAIP3 and JUN by 46 TFs. Moreover, JUN, NFIL3, KLF4, and JUNB acted as TFs and regulated the expression levels of other OSA-DEGs.

Potential drug analysis

As shown in the drug-gene network, 55 drugs had potential effects on OSA-DEGs (**Figure 7**). JUN was affected by 44 drugs, including bupropion hydrochloride, and was found to be the most crucial target. Furthermore, albuterol, vasopressin, and hydroxyurea regulated DUSP1 expression. Methotrexate regulated TNFAIP3 expression. Insulin and dexamethasone regulated JUNB expression.

Diagnostic values of hub genes

Diagnostic values of hub genes were assessed using ROC curves and the corresponding AUC values. AUC values for DUSP1, TNFAIP3, JUNB, and JUN in the GSE55235 dataset, AUC values for DUSP1, TNFAIP3, JUNB, and JUN were 0.98 (*p*-value <0.0001, CL: 0.9329-1), 1 (*p*-value <0.0001, CL: 1-1), 0.98 (*p*-value <0.0001, CL: 0.9329-1) and 0.99 (*p*-value

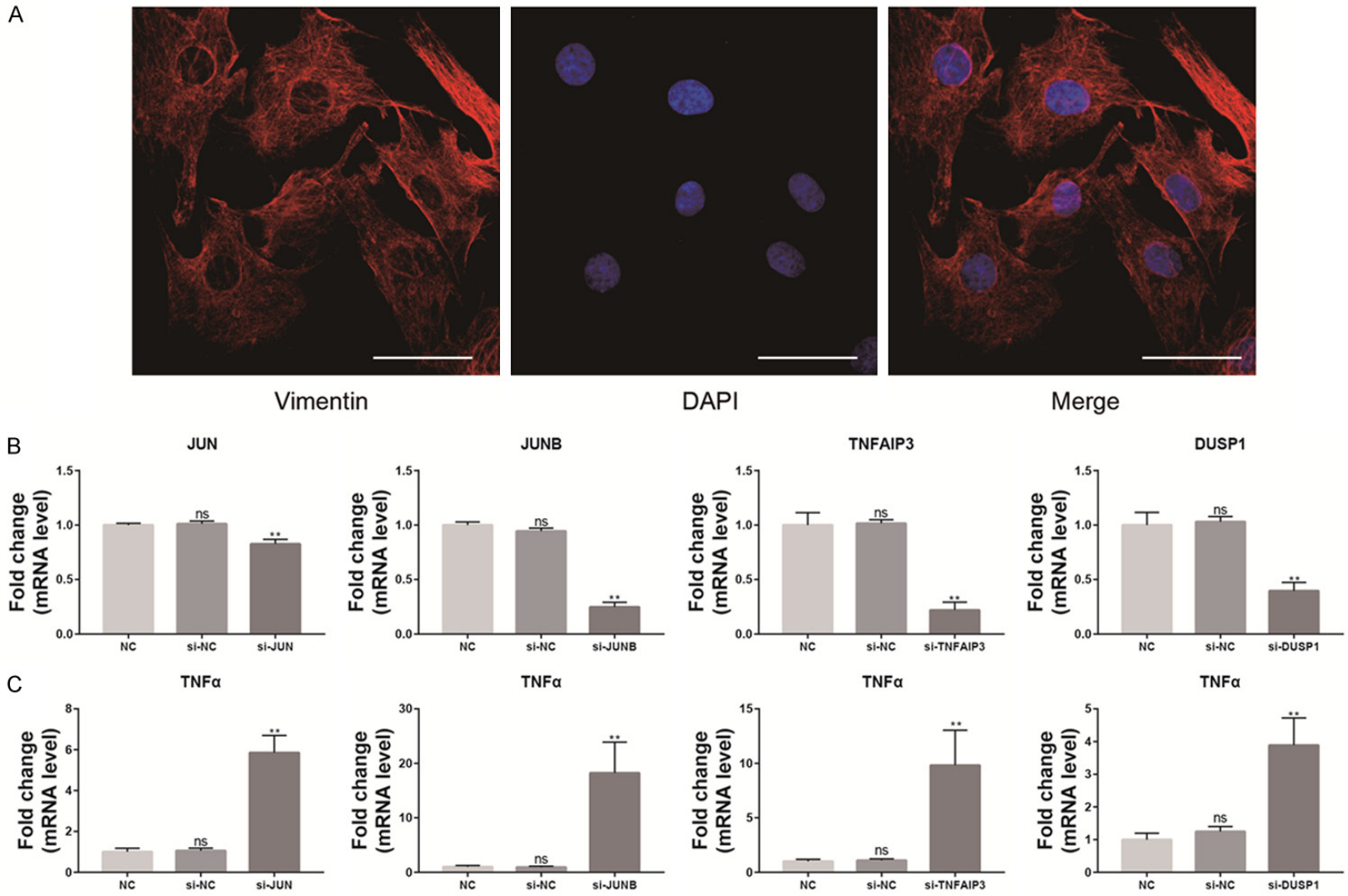

Figure 5. Effects of the key genes in synovial cells. A. Representative imaging of immunofluorescence staining. Scale bar 100 μm. B, C. RT-qPCR analysis of JUN, JUNB, TNFAIP3, DUSP1 and TNFα. Data represented the mean ± SD from three independent experiments. **P<0.01 versus NC; ns, not statistically significant. JUNB, Jun B proto-oncogene; DUSP1, dual specificity phosphatase 1; JUN, Jun proto-oncogene; TNFAIP3, TNF alpha induced protein 3; TNF, tumor necrosis factor.

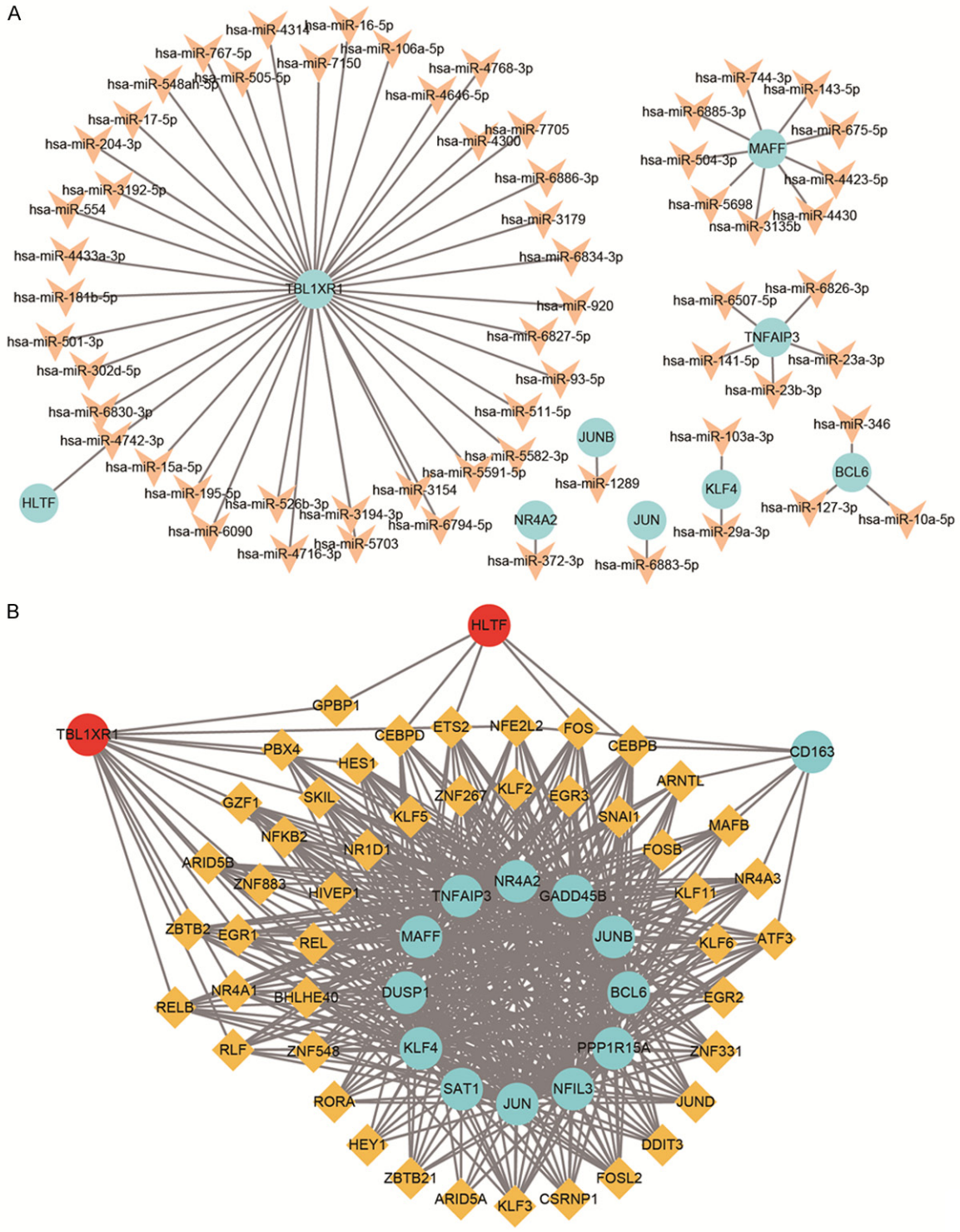

Figure 6. The miRNA-gene and TF-gene networks of OSA-DEGs. A. miRNA-gene network. B. TF-gene network. Star shape nodes represented miRNA, diamond nodes represented TF, circle nodes represented OSA-DEGs, up-regulated genes were red, and down-regulated genes were green. HLTF, helicase like transcription factor; TBL1XR1, TBL1X/Y related 1; PPP1R15A, protein phosphatase 1, regulatory subunit 15A; NR4A2, nuclear receptor subfamily 4 group A member 2; JUNB, Jun B proto-oncogene; NFIL3, nuclear factor interleukin 3 regulated; KLF4, KLF transcription factor 4; DUSP1, dual specificity phosphatase 1; BCL6, B cell leukemia/lymphoma 6; JUN, Jun proto-oncogene; MAFF, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F; TNFAIP3, TNF alpha induced protein 3; SAT1, spermidine/spermine N1-acetyltransferase 1; GADD45B, growth arrest and DNA damage inducible beta.

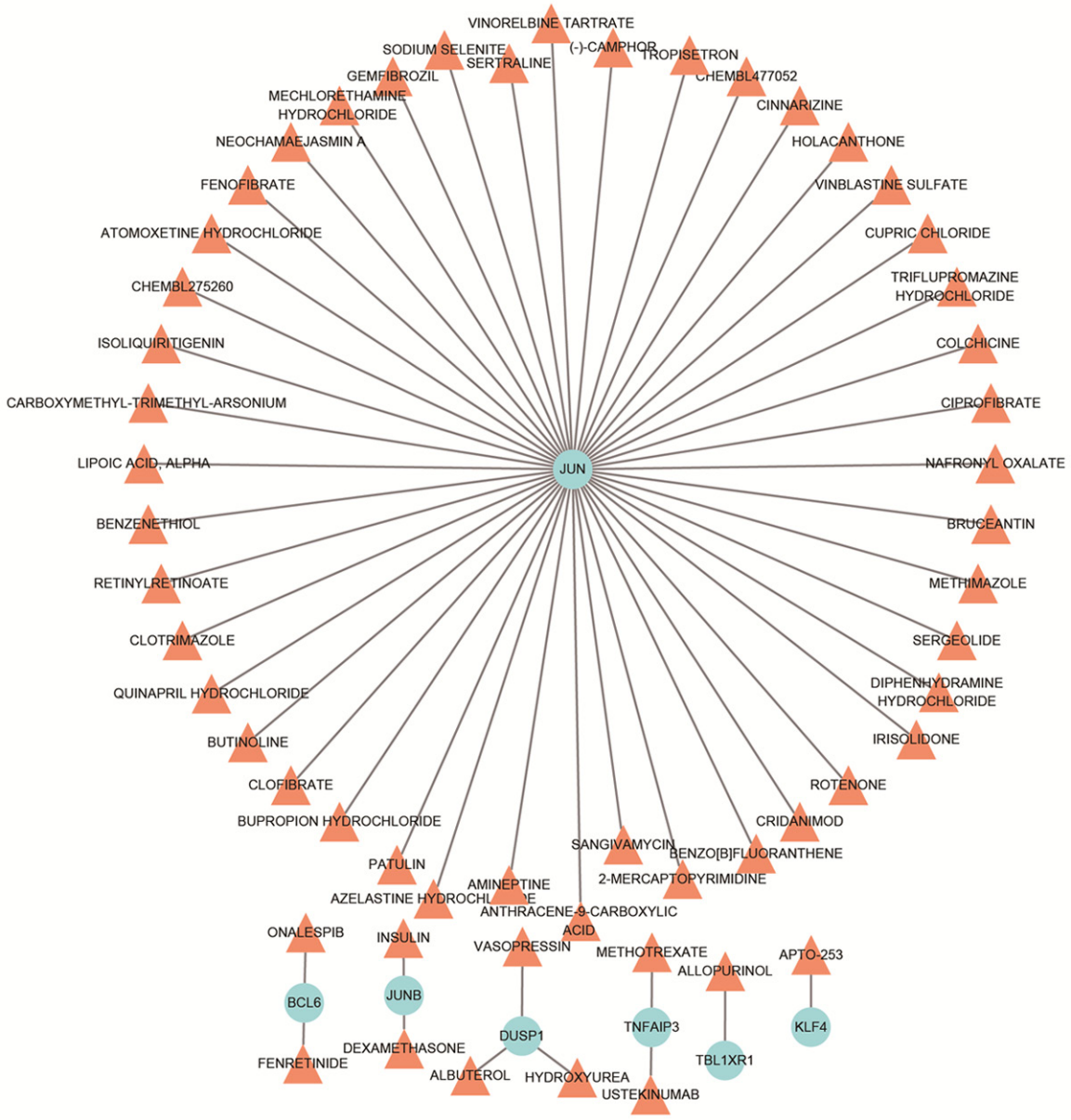

Figure 7. The drug-gene network of OSA-DEGs. Triangles nodes represented drugs, circle nodes represented OSA-DEGs, up-regulated genes were red, and down-regulated genes were green. TBL1XR1, TBL1X/Y related 1; JUNB, Jun B proto-oncogene; KLF4, KLF transcription factor 4; DUSP1, dual specificity phosphatase 1; BCL6, B cell leukemia/lymphoma 6; JUN, Jun proto-oncogene; TNFAIP3, TNF alpha induced protein 3.

<0.0001, CL: 0.9623-1) respectively (**Figure 8A**). In the GSE55457 dataset, AUC values for DUSP1, TNFAIP3, JUNB, and JUN were 0.96 (*p*-value <0.0001, CL: 0.8861-1), 0.92 (*p*-value <0.0001, CL: 0.810-1) and 0.95, 0.85 (*p*-value <0.0001 respectively, CL: 0.6784-1), 0.95 (*p*-value <0.0001, CL: 0.8648-1) (**Figure 8B**). The AUC for JUN, JUNB, TNFAIP3, and DUSP1 in GSE1919 were 0.96 (CL: 0.849-1.000), 0.800 (CL: 0.472-1.000), 1 (CL: 1-1), 0.920 (CL: 0.736-1.000) (**Figure 8C**). These results suggest that the identified hub genes possess good diagnostic values for OA, and to a certain extent, for OSA. Finally, we verified the expression levels of these hub genes using GSE55235, GSE55457 and GSE1919 datasets for external validation. Consistent with previous results, DUSP1, TNFAIP3, JUNB, and JUN levels were downregulated in patients with OSA and OSA (**Figure 8D-F**).

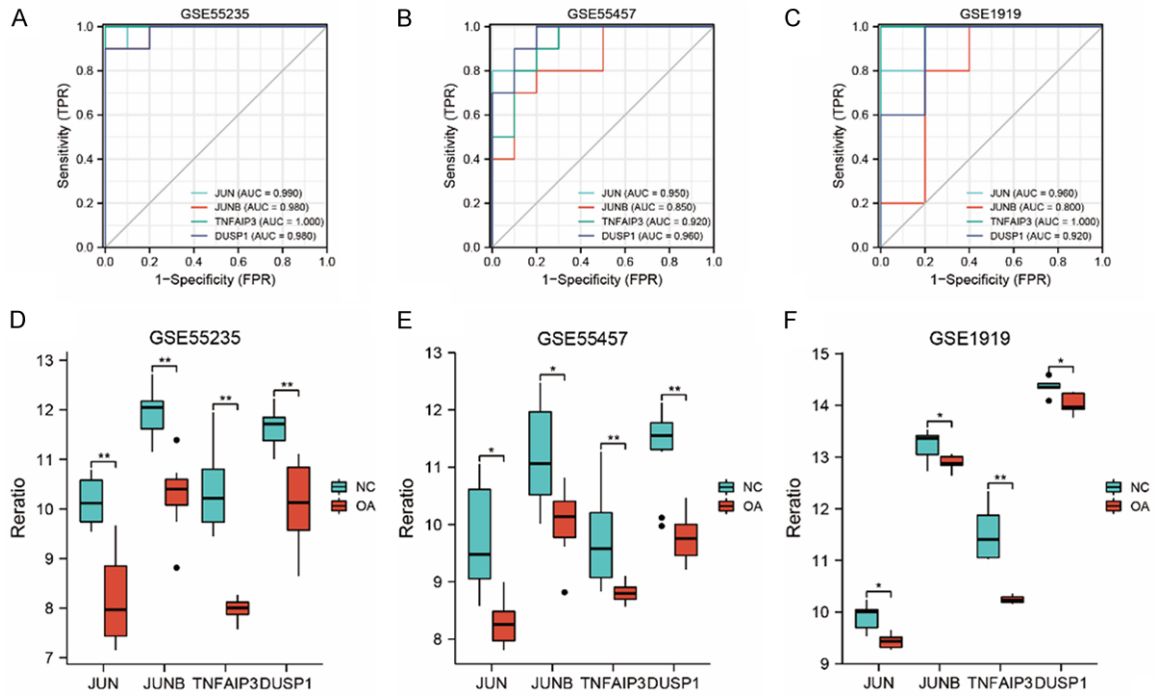

Figure 8. Predictive value and expression level of 4 key genes in OA. A-C. Analysis with ROC curve in 4 key genes. D-F. The expression level of 4 key genes. *P<0.05, **P<0.01. NC, negative control; OA, osteoarthritis.

Discussion

OA and OSA are highly prevalent and share similar pathophysiological features, such as localized or circulatory low-grade inflammation. Epidemiological studies have shown that patients with OSA exhibit a high incidence of severe OA symptoms. Zhang et al. reported that intermittent hypoxia, a significant feature of OSA, is a vital trigger for the cartilage and synovial tissue to produce a senescence-associated secretory phenotype (SASP) and catabolic and anabolic imbalances. Therefore, exploring the molecular mechanisms by which OSA affects OA progression is necessary for the prevention and treatment of OA [12].

We downloaded mRNA microarray data from the peripheral blood monocytes of patients with OSA and from synovial cells pf patients with OA. OSA-DEGs were analyzed to identify the common molecular features between OSA and OA using bioinformatics methods. GO and KEGG enrichment analyses of OSA-DEGs revealed that OSA-DEGs were mainly enriched in the TNF signaling pathway, consistent with the pathological progression of OSA and OA. TNF is produced by various cells, exerting its effects by binding to TNFR1/2, activating the downstream NF-κB and mitogen-activated protein kinase (MAPK) pathway. They regulate the expression levels of downstream genes, such as c-jun, JNK, and ERK, thereby promoting immune cell differentiation and activation and playing important roles in anti-infection and tumor defense responses [28]. In middle-aged and elderly populations, the TNF signaling pathway is involved in some pathological processes, such as chronic systemic inflammatory response and autoimmunity [29-31]. Circulating TNF signaling pathway is hyperactivated in patients with OSA due to intermittent hypoxia from apnea. Disorders of circadian hormones in patients with OSA may also be essential factors for the activation of the TNF signaling pathway [32]. Activation of the circulating TNF signaling pathway increases the risk of many diseases, such as cardiovascular and metabolic diseases [33, 34]. Furthermore, activation of the circulating TNF signaling pathway promotes muscle dysfunction and increases the risk of upper airway dysfunction [35].

TNF signaling pathway also plays an essential role in the pathological progression of OA. Circulating TNF signaling pathway is activated

in patients with OA [36]. Peripheral blood monocytes and synovial cells can secrete TNF-α. TNF-α binds to TNFR1 on the surface of synovial cells in patients with OA to inhibit the synthesis of proteoglycan, connexin, and type 2 collagen and promote the expression levels of MMP1, MMP3, and MMP13, resulting in cartilage anabolic and catabolic imbalance [37-39]. TNF-α can also induce the expression of pro-inflammatory cytokines, such as IL-6, and chemokines, such as IL-8, causing an inflammatory cascade that further aggravates anabolic and catabolic imbalances [40, 41]. Moreover, TNF can upregulate the expression of prostaglandin E2, which binds to the prostaglandin receptor EP and is the leading cause of inflammatory pain in OA [42, 43]. In addition, TNF-α can promote the production of reactive oxygen species, dominated by NO and superoxide anions, further leading to cartilage degradation. Our BP results from the GO enrichment analysis are consistent with the above findings [44]. Thus, the TNF signaling pathway may mediate the interaction between OSA and OA.

We subsequently performed PPI network analysis of OSA-DEGs and screened four hub genes that were downregulated in both diseases using the CytoHubba algorithm. JUN and JUNB belong to the Jun-family. Activator protein-1 (AP-1) component regulates transcription by forming an AP-1 complex by dimerizing with the AP-1-family of proteins. Low AP-1 protein expression promotes the secretion of TNF-α. JunB/c-Jun are essential physiological regulators of TNF-α shedding by directly activating the converting enzyme inhibitor, tissue inhibitor of metalloproteinase-3, thus showing high expression of skin and circulating TNF-α in c-Jun/JunB-deficient mice, leading to inflammatory skin diseases [45]. High expression levels were also observed in the dendritic cells of c-Jun/JunB-deficient mice [46]. Another component of the AP-1 protein dimer, the Fos protein, exerts similar anti-inflammatory effects. Overexpression of the Fos protein inhibits the expression of inflammatory cytokine TNF-α in mice, thereby promoting fracture repair. In OA, overexpression of AP-1 protein in adipose-derived stromal cells showed better therapeutic effects [47, 48]. In summary, low expression of JUNB and JUN in patients with OSA and OA may be responsible for the activation of the TNF signaling pathway.

TNFAIP3, also known as A20, is a ubiquitin-dependent regulator of immune homeostasis [49]. TNFAIP3 is a critical anti-inflammatory mediator that regulates the ubiquitin state of the molecules through its dual enzymatic activity, inhibiting the MAPK and NF-κB pathway activation and antagonizing the cytotoxicity of the TNF signaling pathway [50]. TNFAIP3 inhibits TNF signaling pathway function and release of chemokines and inflammatory cytokines in airway smooth muscles [50]. In OA synovial cells, TNFAIP3 works as an NF-κB antagonist to inhibit the release of the inflammatory factors, IL-6 and IL-8 [51].

DUSP1 is a threonine-tyrosine dual-specificity phosphatase that plays a crucial role in inflammatory responses and chronic metabolic diseases [52]. Activities of the MAPK and NF-κB signaling pathways and expression levels of pro-inflammatory cytokines, such as TNF-α, IL1β, and IL-1α, were enhanced in DUSP1-deficient mice [53]. In OSA, DUSP1 suppresses MAPK activity and reduces IH-induced oxidative stress by inducing SOD enzyme expression [54]. Here, the protective role of DUSP1 in OA synovial cells was confirmed. Overexpression of DUSP1 inhibited MAPK pathway in OA synovial cells and reduced the expression levels of inflammatory cytokines and MMPs [55]. DUSP1 knockdown increased the inflammatory response to allergens and production of IL-6 and IL-8 in airway epithelial cells [56]. DUSP1-deficient mice exhibited high inflammation during dust exposure [57]. In this study, we observed the downregulation of JUN, JUNB, TNFAIP3, and DUSP1 levels in OA synovial tissues. Consistent with previous studies, increased expression of TNF was observed in synovial cells with JUN, JUNB, TNFAIP3, or DUSP1 knockdown. Therefore, these four OSA-associated genes may mediate the effects of OSA on OA via the TNF signaling pathway.

miRNAs, with tissue- and developmental stage-specific expression patterns, influence tissue development and homeostasis by regulating gene expression and are involved in the pathogenesis of various diseases [58]. Altered circular miRNAs in patients with OSA increase the expression levels of inflammatory genes, such as TNF-α, in monocytes, increasing the risk of other diseases, such as cardiovascular diseases [59, 60]. miRNAs also participate in the syn-

thesis of the cartilage matrix and synovitis in OA [61, 62]. We analyzed potential miRNAs and TFs involved in the regulation of OSA-DEGs. TBL1XR1 is an OSA-DEG regulated by most miRNAs that is involved in the growth and metastasis of multiple tumors. However, its roles and diagnostic values in OSA and OA remain unclear [63]. Here, expression levels of hub genes JUN, JUNB, and TNFAIP3 were regulated by the corresponding miRNAs. These miRNAs are likely upstream factors of hub gene downregulation that function as biomarkers. Hub genes JUNB, JUN, and TNFAIP3 are regulated by most TFs, and JUNB and JUN also regulate other OSA-DEGs as TFs. This regulatory network underscores the crucial role of the JUN family proteins in the interactions between OSA and OA.

DGIdb database was used to search for potential drugs and molecular compounds targeting OSA-DEGs. JUN is a crucial drug target and 44 drugs and their molecular compounds affect JUN expression. JUN-targeting drugs, such as bupropion hydrochloride, butinoline, and clotrimazole, antagonize the TNF signaling pathway-induced inflammatory response in different tissues [64-66]. Remaining hub genes (JUNB, TNFAIP3, and DUSP1) are the targets of dexamethasone, methotrexate, and albuterol, respectively, which are anti-inflammatory drugs used to inhibit autoimmunity. These drugs may be effective treatment modalities for OSA and OA.

Our study has some limitations. First, in addition to the molecular linkages, other features of OSA that may affect OA, such as obesity, were not evaluated in this study and should be assessed in future studies. In addition, we validated the expression levels and effects of key genes only in the OA model, not in OSA due to the difficulty of sample acquisition. Therefore, further clinical studies are necessary to address these limitations.

Conclusion

In this study, we demonstrated the molecular links between OSA and OA via bioinformatics analysis and experimental validation. We found that four key OSA-associated genes, JUN, JUNB, DUSP1, and TNFAIP3, mediated the activation of the TNF signaling pathway and were involved in the development of OA. This study provides new insights into the underlying mechanisms of OA that may aid in its treatment.

Acknowledgements

Thanks are due to Bin Li, Wen-Yi Jin and Chang-Geng Xu for assistance with the data analysis and valuable instruction. This work was supported by the Hubei Provincial key research and development program (No. 2021BCA147), the Cross-Innovation Talent Program of Renmin Hospital of Wuhan University (No. JCRCFZ-2022-019), and the Fundamental Research Funds for the Central Universities (No. 2042023kf0224).

Disclosure of conflict of interest

None.

Address correspondence to: Pang-Hu Zhou, Department of Orthopedics, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China. E-mail: zhoupanghu@126.com

References

[1] Ni Z, Zhou S, Li S, Kuang L, Chen H, Luo X, Ouyang J, He M, Du X and Chen L. Exosomes: roles and therapeutic potential in osteoarthritis. Bone Res 2020; 8: 25.

[2] Kloppenburg M and Berenbaum F. Osteoarthritis year in review 2019: epidemiology and therapy. Osteoarthritis Cartilage 2020; 28: 242-248.

[3] Deshpande BR, Katz JN, Solomon DH, Yelin EH, Hunter DJ, Messier SP, Suter LG and Losina E. Number of persons with symptomatic knee osteoarthritis in the us: impact of race and ethnicity, age, sex, and obesity. Arthritis Care Res (Hoboken) 2016; 68: 1743-1750.

[4] Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, Kington RS, Lane NE, Nevitt MC, Zhang Y, Sowers M, McAlindon T, Spector TD, Poole AR, Yanovski SZ, Ateshian G, Sharma L, Buckwalter JA, Brandt KD and Fries JF. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000; 133: 635-646.

[5] Loeser RF, Goldring SR, Scanzello CR and Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 2012; 64: 1697-1707.

[6] Li C, Zheng Z, Ha P, Jiang W, Berthiaume EA, Lee S, Mills Z, Pan H, Chen EC, Jiang J, Culiat CT, Zhang X, Ting K and Soo C. Neural EGFL like 1 as a potential pro-chondrogenic, anti-inflammatory dual-functional disease-modifying

osteoarthritis drug. Biomaterials 2020; 226: 119541.
[7] Dieppe PA and Lohmander LS. Pathogenesis and management of pain in osteoarthritis. Lancet 2005; 365: 965-973.
[8] Baker K, Grainger A, Niu J, Clancy M, Guermazi A, Crema M, Hughes L, Buckwalter J, Wooley A, Nevitt M and Felson DT. Relation of synovitis to knee pain using contrast-enhanced MRIs. Ann Rheum Dis 2010; 69: 1779-1783.
[9] Glyn-Jones S, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H and Carr AJ. Osteoarthritis. Lancet 2015; 386: 376-387.
[10] Sauer C, Schluter B, Hinz R and Gesch D. Childhood obstructive sleep apnea syndrome: an interdisciplinary approach: a prospective epidemiological study of 4,318 five-and-a-half-year-old children. J Orofac Orthop 2012; 73: 342-358.
[11] Sateia MJ. International classification of sleep disorders-third edition highlights and modifications. Chest 2014; 146: 1387-1394.
[12] Gaspar LS, Sousa C, Alvaro AR, Cavadas C and Mendes AF. Common risk factors and therapeutic targets in obstructive sleep apnea and osteoarthritis: an unexpectable link? Pharmacol Res 2021; 164: 105369.
[13] Ryan S, Taylor CT and McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation 2005; 112: 2660-2667.
[14] Zhang Y, Zhou S, Cai W, Han G, Li J, Chen M and Li H. Hypoxia/reoxygenation activates the JNK pathway and accelerates synovial senescence. Mol Med Rep 2020; 22: 265-276.
[15] Ruan G, Xu J, Wang K, Wu J, Zhu Q, Ren J, Bian F, Chang B, Bai X, Han W and Ding C. Associations between knee structural measures, circulating inflammatory factors and MMP13 in patients with knee osteoarthritis. Osteoarthritis Cartilage 2018; 26: 1063-1069.
[16] Piovezan RD, Hirotsu C, Moizinho R, Souza HD, D'Almeida V, Tufik S and Poyares D. Associations between sleep conditions and body composition states: results of the EPISONO study. J Cachexia Sarcopenia Muscle 2019; 10: 962-973.
[17] Goldring MB and Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011; 23: 471-478.
[18] Taylor SS, Hughes JM, Coffman CJ, Jeffreys AS, Ulmer CS, Oddone EZ, Bosworth HB, Yancy WS Jr and Allen KD. Prevalence of and characteristics associated with insomnia and obstructive sleep apnea among veterans with knee and hip osteoarthritis. BMC Musculoskelet Disord 2018; 19: 79.
[19] Huang T, Lin BM, Markt SC, Stampfer MJ, Laden F, Hu FB, Tworoger SS and Redline S. Sex differences in the associations of obstructive sleep apnoea with epidemiological factors. Eur Respir J 2018; 51: 1702421.
[20] Peppard PE and Hagen EW. The last 25 years of obstructive sleep apnea epidemiology-and the next 25? Am J Respir Crit Care Med 2018; 197: 310-312.
[21] Hishikawa Y. International classification of sleep disorders. Jap J Neuropsychopharmacol 1996; 18: 65-72.
[22] Silverwood V, Blagojevic-Bucknall M, Jinks C, Jordan JL, Protheroe J and Jordan KP. Current evidence on risk factors for knee osteoarthritis in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage 2015; 23: 507-515.
[23] Gene Ontology Consortium. The Gene Ontology (GO) project in 2006. Nucleic Acids Res 2006; 34: D322-326.
[24] Kanehisa M and Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000; 28: 27-30.
[25] Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014; 8 Suppl 4: S11.
[26] Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz ML, Utti V, Jagodnik KM, Kropiwnicki E, Wang ZC and Ma'ayan A. ChEA3: transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Res 2019; 47: W212-W224.
[27] Cotto KC, Wagner AH, Feng YY, Kiwala S, Coffman AC, Spies G, Wollam A, Spies NC, Griffith OL and Griffith M. DGIdb 3.0: a redesign and expansion of the drug-gene interaction database. Nucleic Acids Res 2018; 46: D1068-D1073.
[28] Idriss HT and Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc Res Tech 2000; 50: 184-195.
[29] Clohisy JC, Teitelbaum S, Chen S, Erdmann JM and Abu-Amer Y. Tumor necrosis factor-alpha mediates polymethylmethacrylate particle-induced NF-kappaB activation in osteoclast precursor cells. J Orthop Res 2002; 20: 174-181.
[30] Osta B, Benedetti G and Miossec P. Classical and paradoxical effects of TNF-alpha on bone homeostasis. Front Immunol 2014; 5: 48.
[31] Balkwill F. TNF-alpha in promotion and progression of cancer. Cancer Metastasis Rev 2006; 25: 409-416.
[32] Kheirandish-Gozal L and Gozal D. Obstructive sleep apnea and inflammation: proof of concept based on two illustrative cytokines. Int J Mol Sci 2019; 20: 459.
[33] Quercioli A, Mach F and Montecucco F. Inflammation accelerates atherosclerotic processes

in Obstructive Sleep Apnea Syndrome (OSAS). Sleep Breath 2010; 14: 261-269.
[34] Cooke AA, Connaughton RM, Lyons CL, McMorrow AM and Roche HM. Fatty acids and chronic low grade inflammation associated with obesity and the metabolic syndrome. Eur J Pharmacol 2016; 785: 207-214.
[35] Li X, Moody MR, Engel D, Walker S, Clubb FJ Jr, Sivasubramanian N, Mann DL and Reid MB. Cardiac-specific overexpression of tumor necrosis factor-alpha causes oxidative stress and contractile dysfunction in mouse diaphragm. Circulation 2000; 102: 1690-1696.
[36] Ansari MY, Ahmad N and Haqqi TM. Oxidative stress and inflammation in osteoarthritis pathogenesis: role of polyphenols. Biomed Pharmacother 2020; 129: 110452.
[37] Saklatvala J. Tumor-necrosis-factor-alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 1986; 322: 547-549.
[38] Lefebvre V, Peetersjoris C and Vaes G. Modulation by interleukin-1 and tumor necrosis factor-alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophys Acta 1990; 1052: 366-378.
[39] Reboul P, Pelletier JP, Tardif G, Cloutier JM and Martel-Pelletier J. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. J Clin Invest 1996; 97: 2011-2019.
[40] Guerne PA, Carson DA and Lotz M. IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth-factors, and hormones in vitro. J Immunol 1990; 144: 499-505.
[41] Lotz M, Terkeltaub R and Villiger PM. Cartilage and joint inflammation. Regulation of IL-8 expression by human articular chondrocytes. J Immunol 1992; 148: 466-473.
[42] Lee AS, Ellman MB, Yan D, Kroin JS, Cole BJ, van Wijnen AJ and Im HJ. A current review of molecular mechanisms regarding osteoarthritis and pain. Gene 2013; 527: 440-447.
[43] Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP and Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 2011; 7: 33-42.
[44] Afonso V, Champy R, Mitrovic D, Collin P and Lomri A. Reactive oxygen species and superoxide dismutases: role in joint diseases. Joint Bone Spine 2007; 74: 324-329.
[45] Guinea-Viniegra J, Zenz R, Scheuch H, Hnisz D, Holcmann M, Bakiri L, Schonthaler HB, Sibilia M and Wagner EF. TNF alpha shedding and epidermal inflammation are controlled by Jun proteins. Genes Dev 2009; 23: 2663-2674.
[46] Novoszel P, Drobits B, Holcmann M, Fernandes CS, Tschismarov R, Derdak S, Decker T, Wagner EF and Sibilia M. The AP-1 transcription factors c-Jun and JunB are essential for CD8 alpha conventional dendritic cell identity. Cell Death Differ 2021; 28: 2404-2420.
[47] Yamaguchi T, Takada Y, Maruyama K, Shimoda K, Arai Y, Nango N, Kosaki N, Takaishi H, Toyama Y and Matsuo K. Fra-1/AP-1 impairs inflammatory responses and chondrogenesis in fracture healing. J Bone Miner Res 2009; 24: 2056-2065.
[48] Schwabe K, Garcia M, Ubieta K, Hannemann N, Herbort B, Luther J, Noel D, Jorgensen C, Casteilla L, David JP, Stock M, Herrmann M, Schett G and Bozec A. Inhibition of osteoarthritis by adipose-derived stromal cells overexpressing Fra-1 in mice. Arthritis Rheumatol 2016; 68: 138-151.
[49] Malynn BA and Ma A. A20: a multifunctional tool for regulating immunity and preventing disease. Cell Immunol 2019; 340: 103914.
[50] Britt RD Jr, Thompson MA, Sasse S, Pabelick CM, Gerber AN and Prakash YS. Th1 cytokines TNF-alpha and IFN-gamma promote corticosteroid resistance in developing human airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 2019; 316: L71-L81.
[51] Yun Z, Peng HZ, Wang W, Ma Q, Chen J, Wang XL and Ma BA. A20 inhibits the release of inflammatory cytokines by suppressing the activation of the nuclear factor-kappa B pathway in osteoarthritic fibroblast-like synoviocytes. Biochem Biophys Res Commun 2019; 508: 877-881.
[52] Sheng JQ, Li HY, Dai Q, Lu C, Xu M, Zhang JS and Feng JX. DUSP1 recuses diabetic nephropathy via repressing JNK-Mff-mitochondrial fission pathways. J Cell Physiol 2019; 234: 3043-3057.
[53] Goel S, Saheb Sharif-Askari F, Saheb Sharif Askari N, Madkhana B, Alwaa AM, Mahboub B, Zakeri AM, Ratemi E, Hamoudi R, Hamid Q and Halwani R. SARS-CoV-2 Switches 'on' MAPK and NFkappaB signaling via the reduction of nuclear DUSP1 and DUSP5 expression. Front Pharmacol 2021; 12: 631879.
[54] Hoffmann MS, Singh P, Wolk R, Narkiewicz K and Somers VK. Obstructive sleep apnea and intermittent hypoxia increase expression of dual specificity phosphatase 1. Atherosclerosis 2013; 231: 378-383.
[55] Peng HZ, Yun Z, Wang W and Ma BA. Dual specificity phosphatase 1 has a protective role in osteoarthritis fibroblast-like synoviocytes via inhibition of the MAPK signaling pathway. Mol Med Rep 2017; 16: 8441-8447.
[56] Golebski K, van Egmond D, de Groot EJ, Roschmann KI, Fokkens WJ and van Drunen CM. EGR-1 and DUSP-1 are important negative

regulators of pro-allergic responses in airway epithelium. Mol Immunol 2015; 65: 43-50.
[57] Zhang J, Chen Y, Namani A, Elshaer M, Jiang Z, Shi H, Tang X and Wang XJ. Comparative transcriptome analysis reveals Dusp1 as a critical regulator of inflammatory response to fly ash particle exposure in mouse. Ecotoxicol Environ Saf 2020; 190: 110116.
[58] Soifer HS, Rossi JJ and Saetrom P. MicroRNAs in disease and potential therapeutic applications. Mol Ther 2007; 15: 2070-2079.
[59] Chen YC, Hsu PY, Su MC, Chin CH, Liou CW, Wang TY, Lin YY, Lee CP, Lin MC and Hsiao CC. miR-21-5p under-expression in patients with obstructive sleep apnea modulates intermittent hypoxia with re-oxygenation-induced-cell apoptosis and cytotoxicity by targeting pro-inflammatory TNF-alpha-TLR4 signaling. Int J Mol Sci 2020; 21: 999.
[60] Du Y, Wang X, Li L, Hao W, Zhang H, Li Y, Qin Y, Nie S, Christopher TA, Lopez BL, Lau WB, Wang Y, Ma XL and Wei Y. miRNA-mediated suppression of a cardioprotective cardiokine as a novel mechanism exacerbating post-MI remodeling by sleep breathing disorders. Circ Res 2020; 126: 212-228.
[61] Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno-Kudo H and Asahara H. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev 2010; 24: 1173-1185.
[62] Pauley KM, Satoh M, Chan AL, Bubb MR, Reeves WH and Chan EK. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Ther 2008; 10: R101.
[63] Venturutti L, Teater M, Zhai A, Chadburn A, Babiker L, Kim D, Beguelin W, Lee TC, Kim Y, Chin CR, Yewdell WT, Raught B, Phillip JM, Jiang Y, Staudt LM, Green MR, Chaudhuri J, Elemento O, Farinha P, Weng AP, Nissen MD, Steidl C, Morin RD, Scott DW, Prive GG and Melnick AM. TBL1XR1 mutations drive extranodal lymphoma by inducing a pro-tumorigenic memory fate. Cell 2020; 182: 297-316.
[64] Thapa D, Lee JS, Park SY, Bae YH, Bae SK, Kwon JB, Kim KJ, Kwak MK, Park YJ, Choi HG and Kim JA. Clotrimazole ameliorates intestinal inflammation and abnormal angiogenesis by inhibiting interleukin-8 expression through a nuclear factor-kappa B-dependent manner. J Pharmacol Exp Ther 2008; 327: 353-364.
[65] Wu W, Liu L, Zhu H, Sun Y, Wu Y, Liao H, Gui Y, Li L, Liu L, Sun F and Lin H. Butyrolactone-I, an efficient alpha-glucosidase inhibitor, improves type 2 diabetes with potent TNF-alpha-lowering properties through modulating gut microbiota in db/db mice. FASEB J 2019; 33: 12616-12629.
[66] Camara-Lemarroy CR, Guzman-de la Garza FJ, Cordero-Perez P, Alarcon-Galvan G, Ibarra-Hernandez JM, Munoz-Espinosa LE and Fernandez-Garza NE. Bupropion reduces the inflammatory response and intestinal injury due to ischemia-reperfusion. Transplant Proc 2013; 45: 2502-2505.

Table S1. Target sequences of siRNA

siRNA name	Target sequences
si-r-JUN	GGCACAGCTTAAACAGAAA
si-r-JUNB	GCATCAAAGTGGAGCGAAA
si-r-TNFAIP3	GGAGTGGACTTCAGTACAA
si-r-DUSP1	GCTCCACTCAAGTCTTCTT

Abbreviations: JUNB, Jun B proto-oncogene; DUSP1, dual specificity phosphatase 1; JUN, Jun proto-oncogene; TNFAIP3, TNF alpha induced protein 3.

Table S2. Primer sequences used in this study

Gene name	F/R	Sequences 5'-3'
β-actin	F	TGCTATGTTGCCCTAGACTTCG
	R	GTTGGCATAGAGGTCTTTACGG
JUN	F	CCCCATCGACATGGAGTCTC
	R	TGGCACCCACTGTTAACGTG
JUNB	F	GGGGCAGGCAGCTACTTTTC
	R	CATCTTGTGCAGGTCGTCCA
TNFAIP3	F	ACGGCACGACTCACCTGATC
	R	CTGTTTCCTCGGGGTTCCAG
DUSP1	F	TCCGGATGCAGCTCCTGTAG
	R	GCACAAACACCCTTCCTCCA
TNFα	F	TCCAGAACTCCAGGCGGT
	R	TTGGTGGTTTGCTACGAC

Abbreviations: JUNB, Jun B proto-oncogene; DUSP1, dual specificity phosphatase 1; JUN, Jun proto-oncogene; TNFAIP3, TNF alpha induced protein 3; TNF, tumor necrosis factor.