

Leptin promotes obesity-related osteoarthritis progression via CD44/JAK2/STAT3 axis-mediated chondrocyte apoptosis

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Abstract. Osteoarthritis (OA), especially obesity-related OA, is a common joint disease in which leptin serves a notable role; however, the specific mechanism by which leptin promotes obesity-related OA progression remains unclear. In the present study, human cartilage samples confirmed the association between leptin and obesity-related OA. The Gene Expression Omnibus dataset GSE85785 was analyzed in R. Differential gene expression was screened using reverse transcription-quantitative PCR. The protein levels of CD44, apoptosis-related proteins and Janus kinase 2 (JAK2)/STAT3 pathway-related proteins were examined using western blotting. Chondrocyte apoptosis was assessed using TUNEL staining and flow cytometry. Cartilage degeneration was evaluated using H&E and Safranin O-Fast Green staining. Immunofluorescence staining and immunohistochemistry were employed to detect relevant proteins (Leptin, CD44, STAT3). The results from the present study demonstrated that leptin stimulation markedly upregulated CD44 expression, activated the JAK2/STAT3 signaling pathway and induced apoptosis in C28/I2 chondrocytes. Furthermore, the AG490 inhibitor markedly suppressed apoptosis. CD44 knockdown inhibited leptin-induced activation of the JAK2/STAT3 pathway and apoptosis in C28/I2 chondrocytes, whereas CD44 overexpression activated JAK2/STAT3 signaling and promoted apoptosis. Animal experiments further confirmed that leptin administration induced OA, as shown by histological analysis (H&E and Safranin O-Fast Green staining) revealing cartilage

degeneration and matrix loss and TUNEL staining revealed increased chondrocyte apoptosis in leptin-treated rats. In summary, the present study identified a novel mechanism by which leptin induced chondrocyte apoptosis and promoted OA through activation of the CD44-mediated JAK2/STAT3 pathway.

Introduction

Osteoarthritis (OA) is a common joint disease that affects approximately one in eight adults worldwide (1). OA is one of the main causes of chronic joint pain and limited joint movement, resulting in a considerable economic burden on society (2,3). OA is a whole-joint disease that involves pathological changes in the articular cartilage (chondrocyte apoptosis), subchondral bone (sclerosis and osteophyte formation), ligaments (degeneration) and synovium (inflammation and hyperplasia), with the knee joint being the most commonly affected joint (4). The occurrence of OA is related to various factors including age, obesity, inflammation, trauma and genetics (5). Obesity-related OA, also known as metabolic syndrome-associated OA, has recently been recognized as an independent entity (6,7). Research has indicated that obese patients are more likely to develop OA, not only because of increased mechanical load on the joints but also because of chronic inflammation and changes in adipokine secretion, which collectively promote the occurrence of OA (8,9).

Leptin is a peptide hormone secreted by white adipose cells and is involved in various physiological processes including the inflammatory response, immune regulation and bone metabolism (10,11). Research has demonstrated that most obese individuals suffer from hyperleptinemia and leptin levels are positively associated with BMI (12). Furthermore, research has indicated that high levels of leptin are expressed in the serum and synovial fluid of patients with OA. Min *et al* (13) reported that the serum leptin levels in patients with knee OA (KOA) were higher than those in controls (without KOA), and the authors also reported that leptin had diagnostic value for the incidence rate of KOA. Primrose *et al* (14) reported that high concentrations of leptin could lead to OA-like changes in the chondrocyte phenotype, including decreased SOX9, COL2A1 and ACAN expression and increased ADAMTS5 and MMP13 levels, indicating that

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leptin levels may be part of a multifactorial network leading to the development of OA.

According to research reports, the pathogenesis of OA is complex and involves multiple signaling pathways (15,16). For example, in OA, activation of the Janus kinase (JAK)/STAT signaling pathway serves a critical role in regulating chondrocyte apoptosis and the inflammatory response (15). The NF- κ B signaling pathway serves a central role in regulating inflammatory and immune responses. The activation of the NF- κ B signaling pathway is associated with upregulation of inflammatory factors such as IL-1 β and TNF- α (16). The TGF- β signaling pathway serves a role in cell proliferation and extracellular matrix synthesis, and dysregulation of the TGF- β signaling pathway may lead to cartilage degeneration (17). The Wnt/ β -catenin signaling pathway serves a role in regulating cell proliferation, differentiation and apoptosis. The activation of the Wnt/ β -catenin signaling pathway is associated with metabolic disorders in chondrocytes, such as imbalanced extracellular matrix metabolism characterized by increased MMP-13 and ADAMTS-5 and decreased aggrecan and type II collagen (18). However, the specific mechanism by which leptin causes OA is not fully understood. The present study aimed to explore the specific molecular mechanism of leptin-induced OA. We hypothesized that studying the specific molecular mechanisms of leptin in the occurrence of OA may provide novel targets for the diagnosis and treatment of OA.

Materials and methods

Human subjects. Articular cartilage specimens were obtained from 12 patients with KOA who underwent total knee arthroplasty at The Ninth Peoples Hospital of Chongqing (Chongqing, China), with approval from the Institutional Ethics Committee of The Ninth Peoples Hospital of Chongqing [Chongqing, China; approval no. 2024 (ethics review no. 008)]. Sample collection was performed between December 2024 and March 2025. Participants were stratified into non-obese (BMI <28 kg/m²; n=6) and obese (BMI $\geq$ 28 kg/m²; n=6) cohorts based on BMI criteria (6,8). The non-obese group consisted of 2 male and 4 female patients, with a mean age of 71.33 $\pm$ 3.33 years (range, 67-76 years), a mean BMI of 22.68 $\pm$ 2.75 kg/m² and a mean serum leptin level of 8.18 $\pm$ 2.83 ng/ml (range, 5.1-10.5 ng/ml). The obese group consisted of 2 male and 4 female patients, with a mean age of 71.50 $\pm$ 2.59 years (range, 68-74 years), a mean BMI of 28.98 $\pm$ 0.55 kg/m² and a mean serum leptin level of 14.51 $\pm$ 2.34 ng/ml (range, 11.3-16.7 ng/ml). Inclusion criteria were: i) Patients diagnosed with KOA according to the American College of Rheumatology criteria (2); ii) patients classified with a Kellgren-Lawrence grade III or higher (13); iii) patients scheduled for primary total knee arthroplasty; and iv) patients aged $\geq$ 60 years. Exclusion criteria were: i) Revision arthroplasty; ii) history of inflammatory arthritis or septic arthritis; iii) prior knee surgery on the affected side; and iv) malignancy or severe systemic diseases such as end-stage renal disease, severe heart failure or decompensated liver cirrhosis. Written informed consent was obtained preoperatively from all participants. The present study complied with all relevant ethical regulations and approved research protocols and was conducted in accordance with The Declaration of Helsinki. Detailed clinical characteristics of human specimens

and statistical comparison between obese and non-obese groups are presented in Tables SI and SII.

Animal models. Male Sprague-Dawley (SD) rats (6 weeks old; n=24; weight at start of study, 220 g) were purchased from Hunan Slike Jingda Experimental Animal Co., Ltd. All *in vivo* experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Southwest University (Chongqing, China; approval no. IACUC-20241223-01). All protocols complied with the National Institutes of Health Guidelines and were reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments guidelines (<https://arriveguidelines.org>) (19). SD rats were housed under standard conditions with a 12 h light/dark cycle at 22 $\pm$ 2°C and 50 $\pm$ 10% relative humidity, with free access to food and water. SD rats were anesthetized via isoflurane inhalation (4-5% for induction in 1.0 l/min O₂ until loss of righting reflex and 2.0% for maintenance in 0.8 l/min O₂ via face mask) to implant mini-osmotic pumps (Model 2006; ALZET Osmotic Pumps; ALZET LLC; DURECT corporation) into a subcutaneous pocket on the dorsal surface adjacent to the scapula. Pumps were loaded with either 1 mg/ml recombinant rat leptin (cat. no. 598-LP; R&D Systems, Inc.) in 5 mM Tris-HCl or saline in 5 mM Tris-HCl according to the manufacturers instructions. Leptin was continuously infused at 3.6 μ g/day (calculated from 0.15 μ l/h diffusion rate). For the additional treatment groups, rats receiving leptin were co-treated with either the JAK2 inhibitor AG490 (MedChemExpress; administered via daily intraperitoneal injection at 1 mg/kg body weight in a volume of 100 μ l for 16 consecutive weeks) or adeno-associated virus (AAV) encoding short hairpin RNA (shRNA) targeting CD44 (AAV-shCD44; Shanghai GeneChem; delivered via a single intra-articular injection in a volume of 30 μ l into the knee joint at the time as pump implantation). Rats were randomly divided into four experimental groups (n=6 per group): i) Saline control group; ii) leptin group; iii) leptin + AG490 group; and iv) leptin + AAV-shCD44 group. At 16 weeks after leptin administration, the rats were euthanized (150 mg/kg sodium pentobarbital was administered via intraperitoneal injection, and death was confirmed by the absence of heartbeat and respiration), and the articular cartilage (for western blotting) and knee joint specimens (for histological analyses) were collected and subjected to the respective analyses.

Enzyme-linked immunosorbent assay (ELISA) for human serum. Serum leptin concentrations were measured using a commercial human leptin ELISA kit (cat. no. ab108879; Abcam) according to the manufacturers instructions. The assay employs a sandwich ELISA format, with a leptin-specific antibody pre-coated onto the microplate. Briefly, 100 μ l of standards or serum samples were added to the appropriate wells and incubated for 2.5 h at room temperature with shaking. After washing with 1X wash buffer (diluted from the 20X wash buffer concentrate provided in the kit according to the manufacturers instructions), 100 μ l of biotinylated detection antibody was added and incubated for 1 h at room temperature with shaking. Following another washing step, 100 μ l of streptavidin-peroxidase conjugate was added and incubated for 45 min at room temperature with shaking. The plate was then washed and 100 μ l of 3,3',5,5'-tetramethylbenzidine

substrate solution was added and incubated for 30 min in the dark with shaking. The enzymatic reaction was stopped by adding 100 μ l of stop solution and the absorbance was read at 450 nm using a microplate reader.

H&E staining. The tissue samples were fixed in 4% paraformaldehyde (PFA) for 24 h at 4°C, processed using a graded ethanol series and embedded in paraffin. Serial sections (thickness, 4 μ m) were cut using a microtome. After deparaffinization in xylene and rehydration using an ethanol gradient, the sections were stained with Harris hematoxylin (3 min), differentiated in 1% acid alcohol (30 sec) and counterstained with 0.5% eosin Y (30 sec), all incubations were performed at room temperature. Following dehydration in increasing ethanol concentrations (70-100%) and xylene clearing, the slides were mounted with neutral balsam and images were captured under a Nikon Eclipse 80i light microscope (Nikon Corporation,) using NIS-Elements BR software (version 5.42; Nikon Corporation). For each section, five randomly selected fields of view were examined.

Safranin O-Fast Green staining. The tissue sections (thickness, 7 μ m), which had been fixed in 4% paraformaldehyde for 24 h at 4°C, were sequentially deparaffinized in xylene, rehydrated using a graded ethanol series (100 to 70%) and stained with 0.1% Safranin O for 5 min at room temperature. Following brief rinsing in distilled water, the sections were counterstained with 0.1% Fast Green FCF for 30 sec at room temperature. After dehydration in absolute ethanol and clearing in xylene, the slides were mounted with DPX neutral mounting medium (a mixture of distyrene, a plasticizer and xylene; Thermo Scientific; Thermo Fisher Scientific, Inc.) and images were captured under a light microscope. OARSI grading is used to assess the severity of cartilage degeneration in histological sections. The OARSI score ranges from 0 to 6, with 0.5 indicating loss of Safranin O staining without structural changes, 0 indicating normal cartilage, and 6 indicating severe cartilage deformation (17).

Immunohistochemistry (IHC). Paraffin-embedded tissue sections (thickness, 4 μ m) were deparaffinized in xylene (two changes, 15 min each), rehydrated through a descending ethanol series (100, 95, and 70% ethanol, each for 5 min) and rinsed in distilled water for 5 min. The sections were then subjected to antigen retrieval in 10 mM sodium citrate buffer (pH 6.0) at 95°C for 20 min using a microwave processor. After antigen retrieval, the sections were washed with PBS (pH 7.4) three times for 5 min each. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min at room temperature, followed by incubation with 5% normal goat serum (cat. no. ab7481; Abcam) in PBS for 20 min at room temperature to reduce non-specific binding. The sections were then incubated with primary antibodies overnight at 4°C in a humidified chamber. After being washed with PBS (three times for 5 min each), the sections were treated with an HRP-conjugated goat anti-rabbit secondary antibody (cat. no. SA00001-2; dilution, 1:200; Proteintech Group, Inc.) for 30 min at 37°C. Diaminobenzidine was applied for 3 min for chromogenic detection, followed by counterstaining with Mayers hematoxylin for 30 sec at room temperature. Finally,

the sections were dehydrated using a graded ethanol series (70, 95 and 100%), cleared in xylene and imaged under a light microscope. The primary antibodies used were as follows: Leptin (cat. no. 17436-1-AP; dilution, 1:200; Proteintech Group, Inc.) and CD44 (cat. no. ab16728; dilution, 1:200; Abcam).

Data acquisition. Gene expression data were retrieved from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo>) by searching using the key words 'Leptin' and 'Osteoarthritis' (Medical Subject Headings Terms) (20). The dataset GSE85785 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE85785>) was selected for subsequent analysis (21). Data normalization was performed in R (version 4.5.0; <https://www.r-project.org>) using the robust multiarray average method (22). Differentially expressed genes (DEGs) were identified using the 'limma' package (version 3.62.2; <https://bioconductor.org/packages/3.20/bioc/html/limma.html>) (adjusted $P < 0.01$ and $\log_2$ fold change > 1), and volcano plots were generated with 'ggplot2' to visualize the DEGs. To further explore the biological significance of the gene expression profiles, gene set enrichment analysis (GSEA) (22) was performed using the clusterProfiler package (version 4.12.0; <https://bioconductor.org/packages/clusterProfiler/>) in R.

Cell culture and treatment. The C28/I2 human chondrocyte cell line was purchased from the American Type Culture Collection and incubated with 90% Dulbeccos modified Eagles medium/nutrient mixture F-12 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin solution (Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂. When the cells reached 80-90% confluence, the cells were digested with 0.25% trypsin containing EDTA and then subcultured. In some experimental groups (PBS, Leptin and Leptin+AG490), the cells were stimulated with 80 ng/ml leptin (PeproTech, Inc.; Thermo Fisher Scientific, Inc.) for 24 h, followed by a 2-h stimulation with or without treatment with 10 μ M JAK2/STAT3 inhibitor AG490 (MedChemExpress). All incubations were performed at 37°C with 5% CO₂. In addition, a separate control group was included in which cells were treated with AG490 alone (10 μ M for 2 h) under the same conditions. Unstimulated cells were used as the control group.

Cell transfection. shRNAs targeting CD44 (sh-CD44) with the target sequences of shRNA#1 (5-ATGGACTCCAGT CATAGTATA-3), shRNA#2 (5-GGACCAATTACCATA ACTAT-3) and shRNA#3 (5-CCTCCCAGTATGACACAT ATT-3), the non-targeting scrambled negative control shRNA (sh-NC) with the target sequence of 5-CCTAAGGTTAAG TCGCCCTCG-3 (sh-CD44 and sh-NC were cloned into the GV493 vector), the CD44 overexpression plasmid (OE-CD44), and its negative control plasmid (OE-NC) (OE-CD44 and OE-NC were cloned into the GV657 vector) were obtained from Shanghai GeneChem Co., Ltd., and transfected into the C28/I2 human chondrocyte cell line. The plasmid vector was transfected into the C28/I2 human chondrocyte cell line using E-trans detection reagent (Shanghai GeneChem) at 2 μ g of plasmid per well (6-well plate) according to the manufacturers instructions. After 48 h transfection, the C28/I2 chondrocytes

Table I. Primers for reverse transcription-quantitative PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
CD38	AAGTTGCCATTAGCTCCCCC	ATGAGTCCCGAAAGCAGCAA
CD44	CAGCTCATACCAGCCATCCA	TGGGGTGTGAGATTGGGTTG
IL-6	CCACCGGGAACGAAAGAGAA	GAGAAGGCAACTGGACCGAA
IL-1 β	TCGCCAGTGAAATGATGGCT	GGTCGGAGATTTCGTAGCTGG
BAX	TTTCCGAGTGGCAGCTG	CAAAGTAGAAAAGGGCGACAAC
BCL-2	GGATGCCTTTGTGGAAGTGT	CACTTGTGGCTCAGATAGGC
Caspase-3	AGAACTGGACTGTGGCATTG	TAACCAGGTGCTGTGGAGTA
GAPDH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA

were stimulated with leptin (80 ng/ml) for 24 h at 37°C with 5% CO₂ and then used for further analysis.

Cell Counting Kit-8 (CCK-8) assay. A CCK-8 (Biosharp Life Sciences) assay was used to evaluate cell viability. C28/I2 chondrocytes were seeded at a density of 5,000 cells per well in a 96-well plate and allowed to adhere overnight in a culture incubator. The culture medium was subsequently replaced with fresh medium containing different concentrations of leptin (10, 20, 40, 80, 160, 240 and 320 ng/ml). After a 24-h incubation (23), CCK-8 solution was added to each well. Following a 1-h incubation, the optical density values were measured at 450 nm using a microplate reader.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from C28/I2 chondrocytes using the Quick RNA Extraction Kit (cat. no. AG21024; Hunan Accurate Bio-Medical Technology Co., Ltd.) according to the manufacturers instructions. The RNA was subsequently reverse transcribed into cDNA using the Evo M-MLV RT Mix Kit (Hunan Accurate Bio-Medical Technology Co., Ltd.) according to the manufacturers instructions. mRNA expression was detected using a Premix SYBR qPCR Kit (cat. no. AG11702; Hunan Accurate Bio-Medical Technology Co., Ltd.). The qPCR thermocycling conditions were as follows: Initial denaturation at 95°C for 30 sec (1 cycle), followed by 40 cycles of denaturation at 95°C for 5 sec and annealing/extension at 60°C for 30 sec. GAPDH was used as an endogenous control to normalize the mRNA expression levels. The relative expression levels of target genes were calculated using the 2^{- $\Delta\Delta C_q$} method (24). The primers used for each gene are listed in Table I.

Flow cytometry. Apoptosis was detected using an annexin V-FITC/PI apoptosis detection kit (Wuhan Servicebio Technology Co., Ltd.). The procedure was as follows: C28/I2 chondrocytes were inoculated into a 6-well plate at a density of 5x10⁵ cells/well. The cells were then divided into experimental groups and stimulated with 80 ng/ml leptin for 24 h, either alone or in combination with 10 μ M AG490 (2 h), sh-CD44 transfection or OE-CD44 transfection, at 37°C with 5% CO₂. The cells were then digested with trypsin without EDTA, mixed with the cell culture supernatant and collected using centrifugation at 500 x g for 5 min at 4°C. The cells were washed twice with precooled PBS. The samples were subsequently gently

suspended in precooled 1X binding buffer and the cell concentration was adjusted to 5x10⁵ cells/ml. Annexin V-FITC (5 μ l) and PI (5 μ l) were added to 100 μ l of cell suspension and the mixture was incubated at room temperature for 8-10 min in the dark. Subsequently, 400 μ l precooled 1X binding buffer was added and detection was performed using a CytoFLEX flow cytometer (Beckman Coulter, Inc.). Data were analyzed using FlowJo software (version 10.8.1; FlowJo, LLC). Apoptosis rate (%)=early apoptosis rate (%) + late apoptosis rate (%).

Immunofluorescence staining. The C28/I2 human chondrocyte cells were cultured on glass coverslips and fixed with 4% PFA for 15 min at room temperature, followed by three 5-min washes with PBS. Permeabilization was performed with 0.1% Triton X-100 in PBS for 10 min at room temperature. After blocking with 5% BSA (Proteintech Group, Inc.) for 30 min at room temperature, the cells were incubated with a CD44 primary antibody (dilution, 1:200; cat. no. 84854-5-RR; Proteintech Group, Inc.) in a humidified chamber overnight at 4°C. Following three washes with PBS (5 min each), the samples were incubated with CoraLite[®]488-conjugated Goat Anti-Rabbit IgG (H+L) secondary antibodies (dilution, 1:500; cat. no. SA00013-2; Proteintech Group, Inc.) for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI for 5 min at room temperature and the cells were imaged under a fluorescence microscope.

TUNEL staining. Paraffin-embedded tissue sections (4 μ m thick) or PFA-fixed cells (fixed with 4% PFA for 15 min at room temperature) were permeabilized with 0.1% Triton X-100 for 10 min at room temperature. After three washes with PBS (5 min each), the samples were incubated with the TUNEL reaction mixture (CoraLite[®] Plus 488 TUNEL Apoptosis Detection Kit; cat. no. PF00006; Proteintech Group, Inc.) for 1 h at 37°C in a humidified chamber protected from light. Following three washes with PBS (5 min each), the nuclei were counterstained with DAPI (1 μ g/ml) for 5 min at room temperature and the samples were mounted with ProLong Gold Antifade Mountant (cat. no. P36930; Invitrogen; Thermo Fisher Scientific, Inc.), and images were captured under a fluorescence microscope in five random fields of view.

Western blotting and antibodies. Articular cartilage from SD rats was snap-frozen in liquid N₂, pulverized under cryogenic conditions and lysed in RIPA buffer for 30 min

at 4°C. C28/I2 chondrocytes were stimulated according to experimental groups: Control (no stimulation), 80 ng/ml leptin for 24 h alone, 80 ng/ml leptin for 24 h followed by 10 μ M AG490 for 2 h, sh-CD44 transfection followed by 80 ng/ml leptin for 24 h or OE-CD44 transfection followed by 80 ng/ml leptin for 24 h, at 37°C with 5% CO₂. Total protein was extracted by lysing the cells on ice for 30 min with RIPA buffer (Biosharp Life Sciences) containing 1% PMSF, and the protein concentration was measured using a BCA assay kit (Biosharp Life Sciences). Equal amounts of protein samples (15–30 μ g per lane) were separated via 10 or 12.5% SDS-PAGE and then transferred to PVDF membranes (MilliporeSigma). The membranes were blocked with 5% skim milk for 1 h at room temperature, washed three times with TBS with containing 0.1% Tween-20 and then incubated with primary antibodies overnight at 4°C. The membranes were washed three times and then incubated with the HRP-conjugated goat anti-rabbit IgG (H+L) secondary antibody (dilution, 1:5,000; cat. no. SA00001-2; Proteintech Group, Inc.) for 1 h at room temperature. All protein bands were detected using an enhanced chemiluminescence detection kit (Beyotime Biotechnology) and a ChemiDoc imaging system (Bio-Rad Laboratories, Inc.). The antibodies used were as follows: BAX (dilution, 1:2,000; cat. no. 50599-2-Ig; Proteintech Group, Inc.), BCL-2 (dilution, 1:1,000; cat. no. T40056; Abmart Pharmaceutical Technology Co., Ltd.), caspase-3 (dilution, 1:3,000; cat. no. 19677-1-AP; Proteintech Group, Inc.), cleaved caspase-3 (dilution, 1:3,000; cat. no. 25128-1-AP; Proteintech Group, Inc.), CD44 (dilution, 1:2,000; cat. no. 30854-1-AP; Proteintech Group, Inc.), JAK2 (dilution, 1:1,000; cat. no. T55287; Abmart Pharmaceutical Technology Co., Ltd.), p-JAK2 (dilution, 1:500; cat. no. T56570; Abmart Pharmaceutical Technology Co., Ltd.), STAT3 (dilution, 1:2,000; cat. no. 10253-2-AP; Proteintech Group, Inc.), p-STAT3 (dilution, 1:1,000; cat. no. 28945-1-AP; Proteintech Group, Inc.), leptin receptor isoform B (Ob-Rb; dilution, 1:500; cat. no. 20966-1-AP; Proteintech Group, Inc.) and β -actin (dilution, 1:4,000; cat. no. 20536-1-AP; Proteintech Group, Inc.).

Statistical analysis. Bioinformatics analysis was conducted using R (version 4.5.0; <https://www.r-project.org>). All statistical analyses were conducted using GraphPad Prism 10 (Dotmatics). The data are presented as the mean $\pm$ standard deviation. An unpaired Students t-test was performed for comparisons of two groups. One-way ANOVA was used for comparisons of >2 groups, followed by Tukeys HSD post hoc test. For categorical or discontinuous data, the Mann-Whitney U test was performed for two-group comparisons and the Kruskal-Wallis test with Dunns post hoc test was used for ≥ 3 groups. For categorical frequency data, Fisher's exact test was applied. The numbers of independent experiments and events, along with the statistical details and methods, are provided in the relevant figure legends. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Significant increase in leptin expression in obesity-related OA. Histopathological analysis revealed characteristic OA alterations in both groups. H&E staining revealed reduced

cellularity of the articular chondrocytes, accompanied by marked membrane rupture and vacuolization (Fig. 1A). Safranin O-Fast Green staining showed reduced Safranin O (red) staining, indicating proteoglycan loss, and increased Fast Green (green) staining, together with surface fibrillation and clefts, confirming notable cartilage matrix degradation, verifying OA progression in both groups (Fig. 1A and B); the OARSI scores were 4–5 in the non-obese group and 4–6 in the obese group, with no significant difference between the two groups. Notably, IHC analysis revealed a significantly elevated leptin-positive rate in obesity-related OA samples compared with non-obesity-related OA controls (Fig. 1A and B). The clinical characteristics of the 12 participants, including age, BMI, KL grade and serum leptin concentrations measured using ELISA, are summarized in Table SI and SII.

DEGs and enrichment analysis. To explore the mechanism of leptin in OA, transcriptome sequencing data from GEO (GSE85785) were first analyzed. In total, analysis of the GSE85785 dataset identified 1,462 DEGs, including 773 upregulated genes and 689 downregulated genes (Table SIII). Furthermore, volcano plots depicting the DEGs were constructed (Fig. 2A). GSEA revealed significant enrichment of gene sets related to the JAK2/STAT3 signaling pathway and apoptosis in the leptin group (Fig. 2B and C; Table SIV). The DEGs from the two groups were subsequently intersected with genes related to apoptosis and the JAK2/STAT3 signaling pathway, ultimately identifying four genes (CD38, CD44, IL-6, IL-1 β) for further analysis (Fig. 2D).

Leptin concentration and key genes (CD44). The IC₅₀ of leptin in C28/I2 chondrocytes was determined using a CCK-8 assay (Fig. 3A) and the corresponding concentration (80 ng/ml) was subsequently used for further research. Using RT-qPCR, CD44 was identified as the most significantly different gene amongst the four genes (Fig. 3B). To further validate the optimal leptin concentration for CD44 expression, RT-qPCR analysis was performed at various leptin concentrations and the results confirmed that CD44 exhibited the most pronounced differential expression compared with the control at 80 ng/ml (Fig. 3C). Consistently, western blotting further demonstrated that CD44 protein expression was significantly altered at 20, 40 and 80 ng/ml compared with the control, with the most pronounced change at 80 ng/ml (Fig. 3D and E). Based on these findings, 80 ng/ml was selected as the optimal concentration for all subsequent experiments.

Leptin promotes CD44 expression, activates the JAK2/STAT3 signaling pathway and induces apoptosis in C28/I2 chondrocytes. To investigate the effect of leptin on C28/I2 chondrocytes, the cells were treated with leptin (80 ng/ml) for 24 h prior to further analysis. Western blotting revealed that leptin significantly increased the protein expression level of CD44 in C28/I2 chondrocytes (Fig. 4A and B). RT-qPCR revealed that leptin significantly increased the transcription levels of BAX and reduced those of BCL-2 and caspase-3 in C28/I2 chondrocytes (Fig. 4C). Immunofluorescence staining revealed that leptin significantly increased the protein expression levels of CD44 in C28/I2 chondrocytes (Fig. 4D and E). Western blotting revealed that leptin significantly increased the

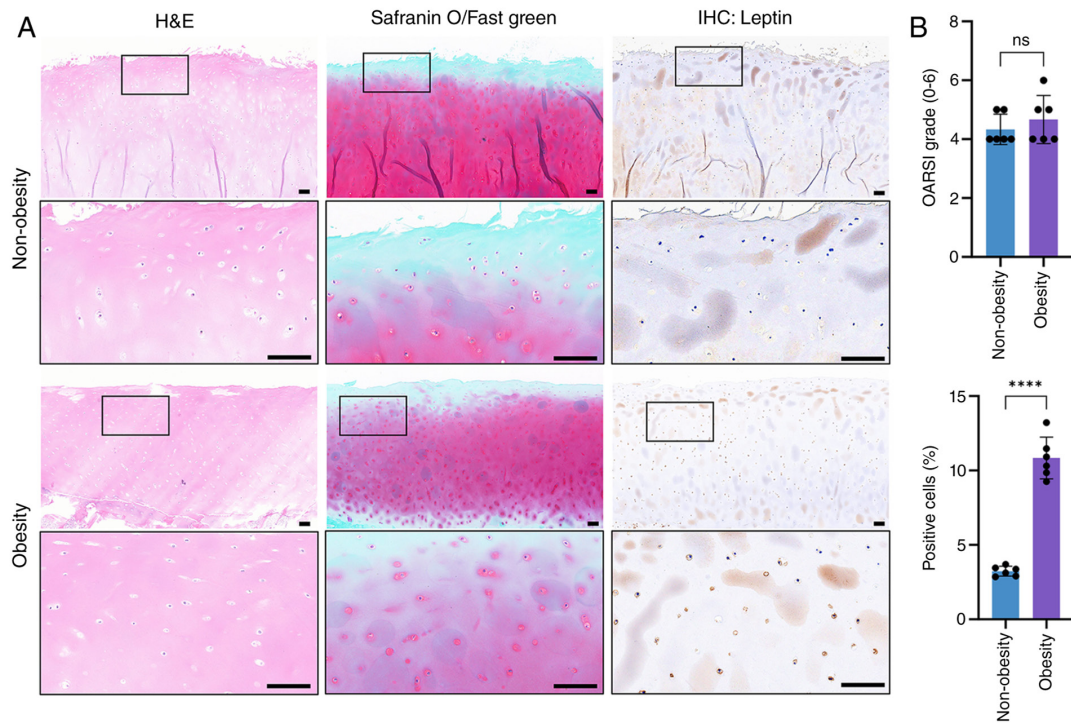


Figure 1. Leptin expression is significantly elevated in obesity-related OA. (A) H&E staining and Safranin O-fast green staining showed cartilage surface fibrillation/tearing, cartilage thinning, and chondrocyte disorganization in both groups. (B) OARSI scores showed no significant difference between groups. IHC showed significantly more leptin-positive cells in obesity-related OA than in non-obesity-related OA. Black boxes in Fig. 1A mark magnified regions; lower panels show high-magnification views. Scale bar, 100 μ m. ****P<0.0001. ns, not significant; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International.

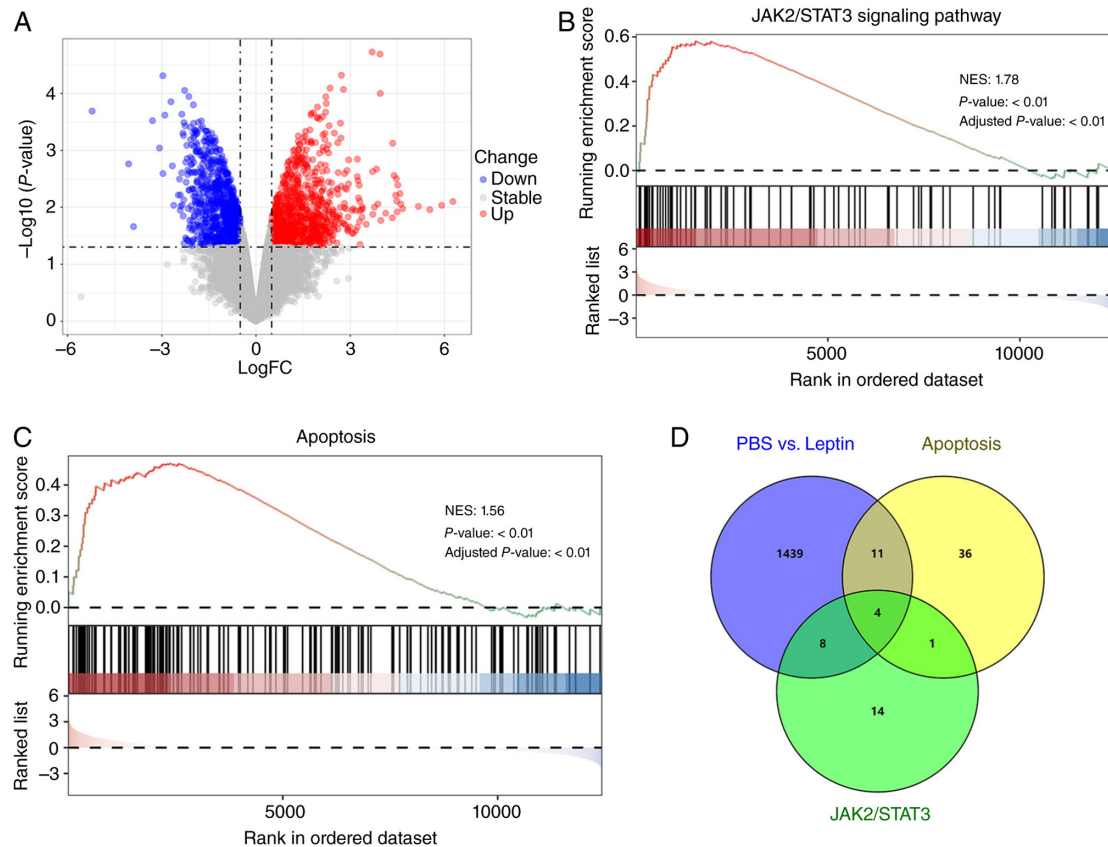


Figure 2. DEGs and enrichment analysis results. (A) Volcano plot showing differentially expressed genes between the PBS-treated and leptin-treated groups. (B) GSEA enrichment of the JAK2/STAT3 signaling pathway in published transcriptomics data from GSE85785. (C) GSEA enrichment of apoptosis in published transcriptomics data from GSE85785. (D) Venn diagram displaying the determination of 4 key genes (CD38, CD44, IL-6 and IL-1 β). DEGS, differentially expressed genes; JAK2, Janus kinase 2; GSEA, gene set enrichment analysis; NES, normalized enrichment score.

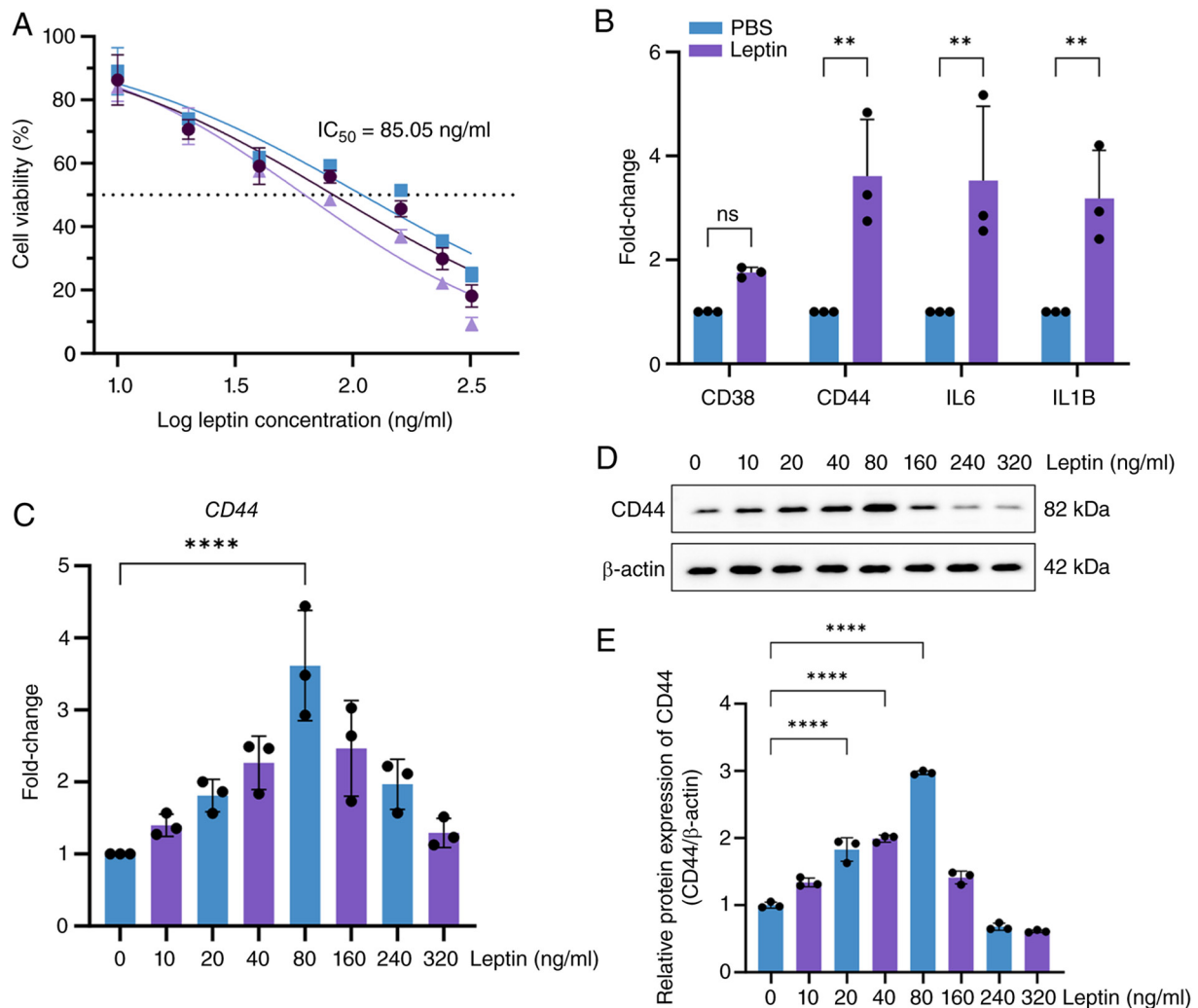


Figure 3. Screening for leptin concentration and identification of key genes. (A) Cell Counting Kit-8 assay results demonstrated that the IC_{50} of leptin was 85.05 ng/ml. (B) RT-qPCR quantification indicated a significant increase in CD44 expression in C28/I2 chondrocytes upon leptin stimulation (80 ng/ml). (C) RT-qPCR analysis of CD44 expression at various leptin concentrations. (D) Western blotting and (E) semi-quantification of CD44 protein expression at various leptin concentrations. ** $P < 0.01$ and **** $P < 0.0001$. ns, not significant. RT-qPCR, Reverse transcription-quantitative PCR.

protein expression levels of BAX and cleaved caspase-3, and reduced the protein expression levels of BCL-2 and caspase-3, in C28/I2 chondrocytes (Fig. 4F and G). TUNEL staining revealed that leptin significantly induced apoptosis in C28/I2 chondrocytes (Fig. 4H and I). Furthermore, the flow cytometry results revealed that, compared with the control group, leptin significantly increased the rate of C28/I2 chondrocyte apoptosis (Fig. 4K and L). Western blotting revealed that leptin significantly increased the protein levels of p-JAK2 and p-STAT3 in C28/I2 chondrocytes, indicating activation of the JAK2/STAT3 signaling pathway (Fig. 4J and M). Collectively, these results indicate that leptin promotes C28/I2 chondrocyte apoptosis and increases CD44 expression, accompanied by activation of the JAK2/STAT3 signaling pathway, suggesting that leptin may contribute to chondrocyte loss and cartilage degeneration in OA.

Leptin induces C28/I2 chondrocyte apoptosis via the JAK2/STAT3 signaling pathway. To verify the relationship between leptin and the JAK2-STAT3 signaling pathway, AG490, a specific inhibitor of JAK2-STAT3, was used to

treat C28/I2 chondrocytes. Numerous studies have confirmed that the JAK2/STAT3 signaling pathway can be blocked by AG490 (25-28). In the present study, the protein levels of BAX and cleaved caspase-3 were lower in C28/I2 chondrocytes stimulated with leptin and treated with AG490 than in those without AG490 treatment (Fig. 5A and B). BCL-2 and caspase-3 protein expression was upregulated after leptin stimulation in the presence of AG490 (Fig. 5A and B). TUNEL staining and flow cytometry further confirmed that AG490 markedly attenuated leptin-induced apoptosis in C28/I2 chondrocytes (Fig. 5C-F). Collectively, these findings indicated that leptin promoted apoptosis in C28/I2 chondrocytes via activation of the JAK2/STAT3 pathway.

CD44 knockdown inhibits the JAK2/STAT3 signaling pathway and suppresses apoptosis. To elucidate the role of CD44 in OA progression, CD44 expression was downregulated in C28/I2 chondrocytes via sh-CD44 transfection. Compared with that in the sh-NC group, CD44 expression in C28/I2 chondrocytes was significantly downregulated upon transfection with sh-CD44 #1, sh-CD44 #2 or sh-CD44 #3, with sh-CD44 #1 exhibiting

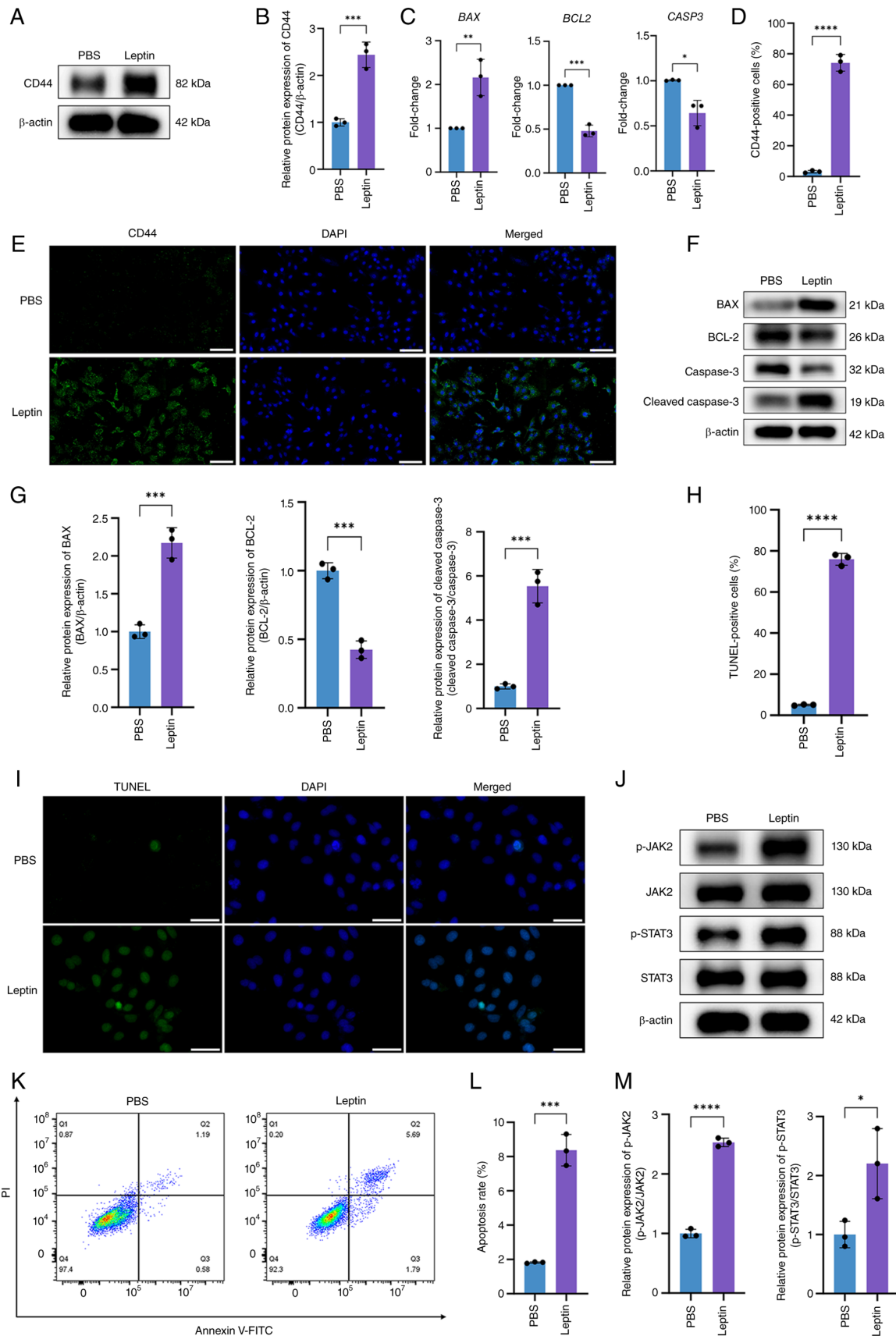


Figure 4. Leptin promotes CD44 expression, activates the JAK2/STAT3 signaling pathway and induces apoptosis in C28/I2 chondrocytes. (A) Western blotting and (B) semi-quantification of CD44 in C28/I2 chondrocytes upon leptin stimulation. B-actin served as a loading control. (C) Reverse transcription-quantitative PCR analysis of changes in BAX, BCL-2 and caspase-3 mRNA abundance upon leptin stimulation. (D) Quantification of CD44 upon leptin stimulation and (E) immunofluorescence detection (scale bar, 100 μ m). (F) Western blotting and (G) semi-quantification of apoptosis-related proteins (BAX, BCL-2, caspase-3 and cleaved caspase-3) upon leptin stimulation. (H) Quantification of apoptosis upon leptin stimulation and (I) TUNEL staining detection (scale bar, 50 μ m). (K) Flow cytometric assessment of apoptotic rate and (L) quantification of apoptosis upon leptin stimulation. (J) Western blotting and (M) semi-quantification of p-JAK2, JAK2, p-STAT3 and STAT3 upon leptin stimulation. * P <0.05, ** P <0.01, *** P <0.001 and **** P <0.0001. JAK2, Janus kinase 2; p-, phosphorylated.

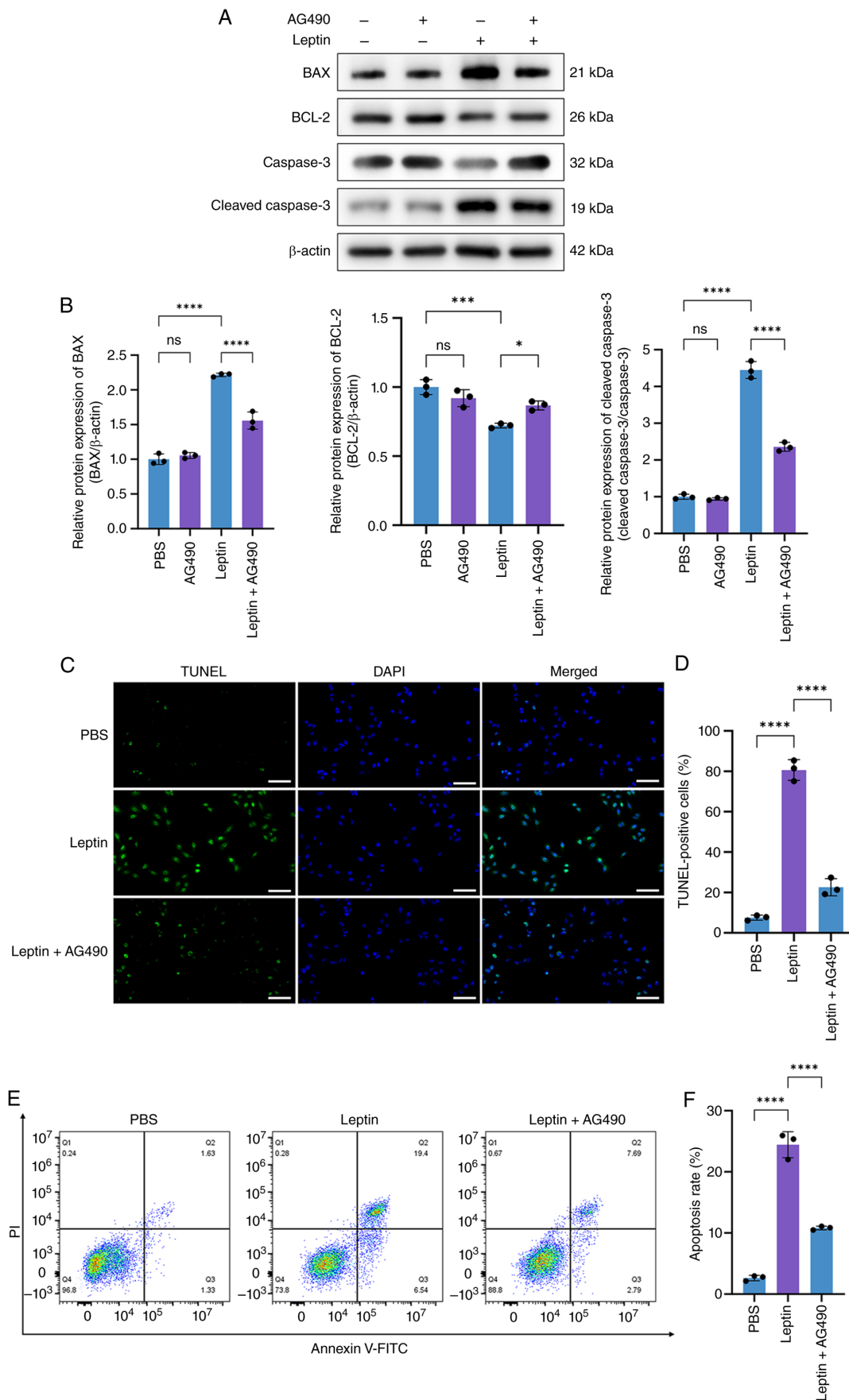


Figure 5. Leptin induces apoptosis, which is attenuated by the JAK2 inhibitor AG490. (A) Western blotting and (B) semi-quantification of apoptosis-related proteins (BAX, BCL-2, caspase-3 and cleaved caspase-3) in C28/I2 chondrocytes treated with leptin and either with or without AG490. (C) TUNEL staining and (D) quantification of apoptosis in C28/I2 chondrocytes treated with leptin in the presence or absence of AG490. (scale bar, 100 μ m). (E) Flow cytometric detection of apoptosis in C28/I2 chondrocytes treated with PBS, leptin, or leptin + AG490. (F) Quantification of the apoptotic rate. * $P < 0.05$, *** $P < 0.001$, and **** $P < 0.0001$. ns, not significant.

the highest knockdown efficiency (Fig. S1A-C). Western blotting revealed that CD44 knockdown significantly reduced leptin-induced apoptosis (Fig. 6A and B). Flow cytometry further corroborated these findings (Fig. 6C and D). Western blotting revealed that CD44 knockdown significantly reduced leptin-induced activation of the JAK2/STAT3 signaling pathway (Fig. 6E and F). Immunofluorescence staining showed representative images of decreased STAT3 nuclear translocation (Fig. 6G). These results indicated that CD44 is a key gene involved in leptin-induced apoptosis of C28/I2 chondrocytes through the JAK2/STAT3 signaling pathway.

CD44 overexpression activates the JAK2/STAT3 signaling pathway and induces apoptosis. To elucidate the role of CD44 in OA progression, CD44 expression was overexpressed in C28/I2 chondrocytes via OE-CD44 transfection. Compared with that in the OE-NC group, CD44 expression in C28/I2 chondrocytes was significantly upregulated following transfection with OE-CD44 (Fig. S1D-F). Western blotting revealed that CD44 overexpression significantly enhanced apoptosis in both the PBS and leptin groups (Fig. 7A and B). Flow cytometry further corroborated these findings (Fig. 7C and D). Western blotting revealed that CD44 overexpression significantly enhanced activation of the JAK2/STAT3 signaling pathway in both the PBS and leptin groups (Fig. 7E and F). These findings provided further validation for the earlier conclusions that CD44 is a key gene involved in leptin-induced apoptosis of C28/I2 chondrocytes through the JAK2/STAT3 signaling pathway. Leptin activates the JAK2/STAT3 signaling pathway through its canonical receptor Ob-Rb, a well-established pathway mediating chondrocyte apoptosis in OA (21). To determine whether CD44 influences JAK2/STAT3 activation through regulation of Ob-Rb, Ob-Rb protein expression was examined following CD44 knockdown or overexpression. Neither CD44 overexpression nor knockdown significantly affected Ob-Rb expression, indicating that CD44 regulates JAK2/STAT3 pathway activation and chondrocyte apoptosis independently of Ob-Rb (Fig. 7G and H).

Leptin promotes CD44 expression, activates the JAK2/STAT3 signaling pathway, induces chondrocyte apoptosis and exacerbates OA in vivo. To investigate the role of CD44 in leptin-induced OA *in vivo*, CD44 expression was knocked down in rat knee joints via intra-articular injection of AAV-shCD44. Successful knockdown of CD44 in articular cartilage was confirmed using RT-qPCR and Western blotting (Fig. S1G-I). Western blotting revealed that leptin activated the JAK2/STAT3 signaling pathway, while CD44 knockdown significantly attenuated leptin-induced activation of the JAK2/STAT3 pathway (Fig. 8A and B). H&E and Safranin O-Fast Green staining showed that leptin promoted KOA development in rats, as control joints had smooth surfaces and uniform Safranin O staining, whereas leptin-treated joints showed reduced Safranin O staining, surface fibrillation/clefting, cartilage thinning and disorganized chondrocytes, all characteristic of KOA; AG490 did not significantly ameliorate leptin-induced cartilage degeneration, whereas CD44 knockdown significantly ameliorated subchondral bone and synovium changes but not cartilage degeneration (Fig. 8C and D). IHC revealed that leptin

increased the CD44-positive cell rate in rat chondrocytes (Fig. 8C and D). TUNEL staining demonstrated that leptin induced chondrocyte apoptosis, which was markedly reduced by AG490 treatment or CD44 knockdown (Fig. 8E and F). These results collectively indicated that leptin promoted CD44 expression, activated the JAK2/STAT3 signaling pathway, induced chondrocyte apoptosis and exacerbated OA *in vivo*.

Discussion

The present study revealed the role of leptin in the progression of OA and identified CD44 as a key gene mediating leptin-induced KOA progression. The present study also demonstrated the regulatory role of leptin in apoptosis of C28/I2 chondrocytes. Mechanistically, leptin promoted high CD44 expression and the phosphorylation of JAK2. In leptin-treated chondrocytes, increased CD44 expression was accompanied by JAK2/STAT3 activation, indicated by increased p-JAK2 and p-STAT3, and by altered expression of apoptosis-related proteins, including BAX, BCL-2, caspase-3, and cleaved caspase-3, suggesting that the CD44/JAK2/STAT3 axis contributes to chondrocyte apoptosis (Fig. 9). Therefore, the present findings revealed the specific mechanism of action of leptin in the progression of OA and suggested that leptin may be a potential therapeutic target in patients with obesity-related OA. Clinically, serum leptin levels were significantly higher in the obese group than in the non-obese group, suggesting that elevated leptin levels are closely associated with obesity and the severity of cartilage degeneration in patients with OA.

The main feature of OA is cartilage degeneration and chondrocytes are the main cell type of articular cartilage (29). Chondrocyte apoptosis is a key factor in the pathogenesis of OA (30,31). CD44 is a multifunctional cell surface glycoprotein that serves roles in various biological processes, including migration, inflammation, immune response, apoptosis and cartilage matrix remodeling. Studies have demonstrated that CD44 is involved in the regulation of apoptosis and is associated with OA (32,33). The present study revealed that after C28/I2 chondrocytes were treated with leptin, the expression level of CD44 was significantly upregulated. Additionally, significant changes in the expression levels of apoptosis-related proteins (BAX, BCL-2, caspase-3 and cleaved caspase-3) were observed. Flow cytometry was used to analyze apoptosis markers (Annexin V-FITC and PI) within the cell population and a significant increase in the percentage of apoptotic cells was identified following leptin stimulation of C28/I2 chondrocytes. Taken together, these data from the present study suggested that leptin stimulation triggered apoptosis in C28/I2 chondrocytes.

The JAK2/STAT3 signaling pathway serves a critical role in regulating cell proliferation, differentiation and apoptosis. Zhao *et al* (34) and Zhao *et al* (35) reported that blocking the JAK2/STAT3 signaling pathway with the inhibitor AG490 ameliorated cartilage damage in OA and that AG490 alleviated cartilage destruction in a rat model of OA. AG490, a JAK2 inhibitor (26-28), effectively attenuated the JAK2/STAT3 signaling pathway and significantly reduced leptin-induced chondrocyte apoptosis in the present study, further suggesting that leptin regulates apoptosis through activation of the JAK2/STAT3 signaling pathway. Although previous studies have established

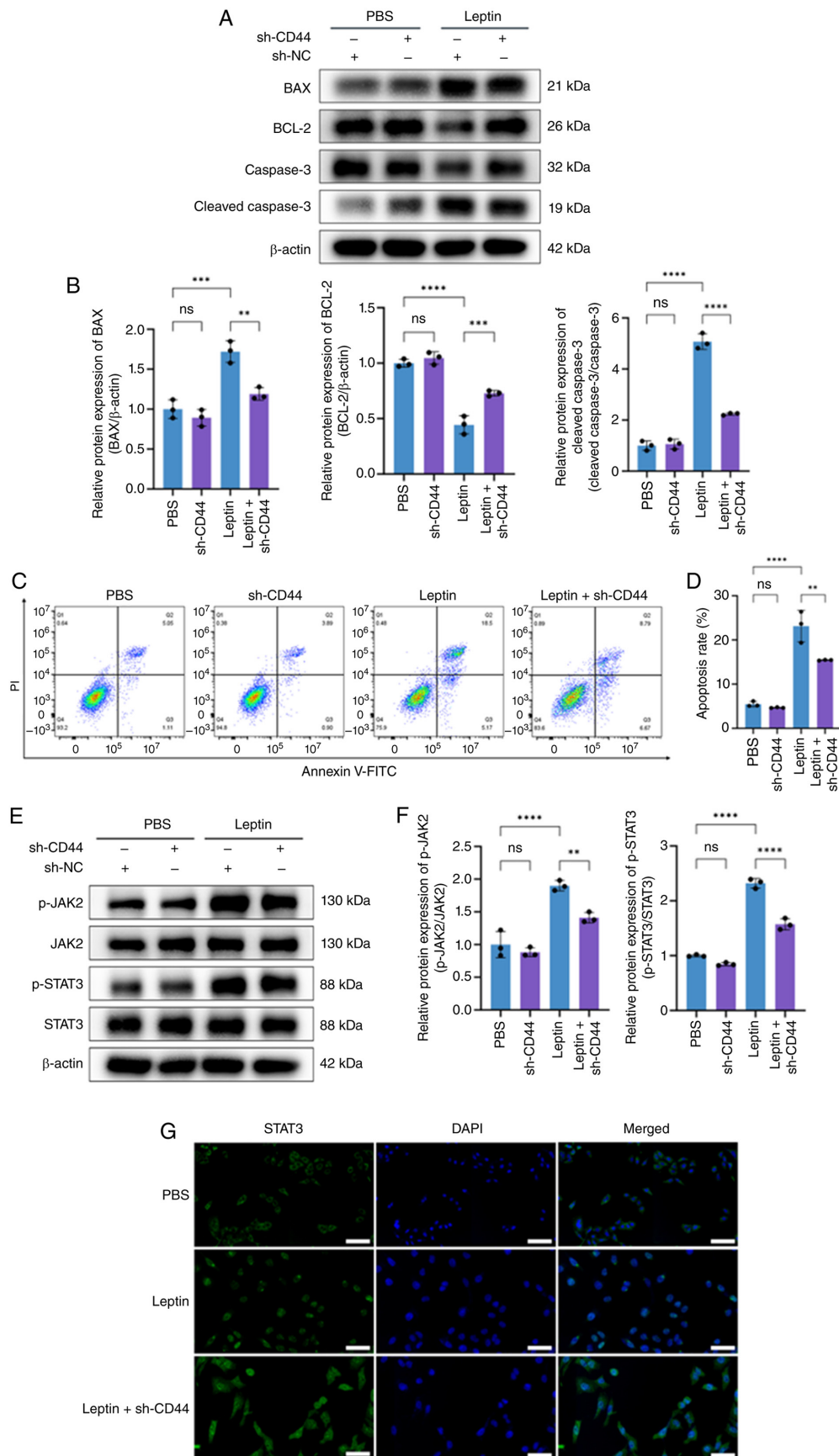


Figure 6. Leptin promotes chondrocyte apoptosis by stimulating the JAK2/STAT3 signaling pathway through CD44. (A) Western blotting and (B) semi-quantification of apoptosis-related proteins (BAX, BCL-2, caspase-3 and cleaved caspase-3) in C28/I2 chondrocytes from PBS and leptin groups following CD44 knockdown. (C) Flow cytometric detection of apoptosis in C28/I2 chondrocytes treated with PBS, sh-CD44, leptin, or leptin + sh-CD44. (D) Quantification of the apoptotic rate. (E) Western blotting and (F) semi-quantification of JAK2/STAT3 pathway-related proteins (p-JAK2, JAK2, p-STAT3 and STAT3) following CD44 knockdown. (G) Immunofluorescence examination of STAT3 nuclear translocation in the leptin-treated group following CD44 knockdown (scale bar, 50 μ m). **P<0.01, ***P<0.001 and ****P<0.0001. JAK2, Janus kinase 2; ns, not significant; p-, phosphorylated.

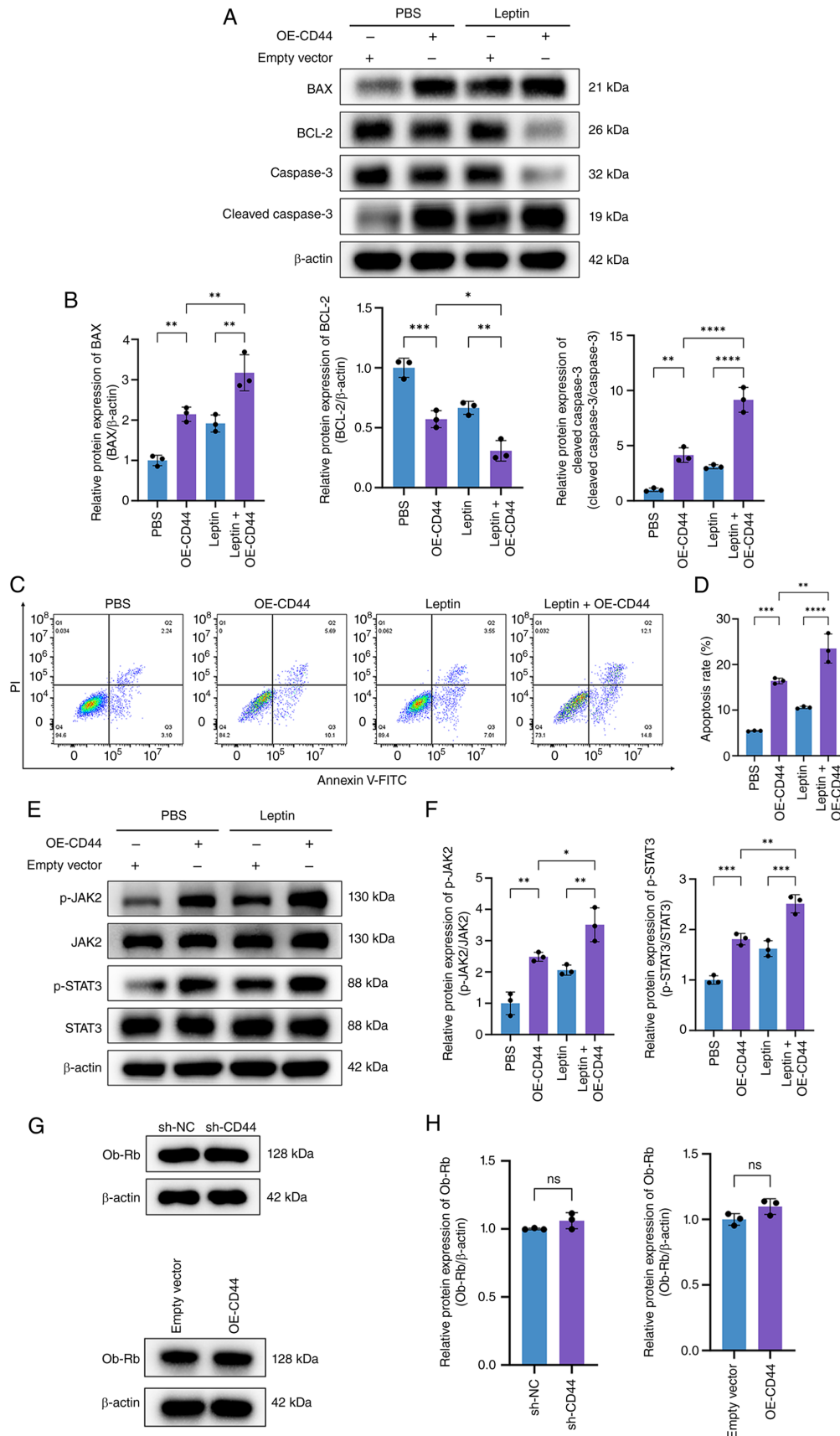


Figure 7. CD44 overexpression exacerbates leptin-induced chondrocyte apoptosis and JAK2/STAT3 activation. (A) Western blotting and (B) semi-quantification of apoptosis-related proteins (BAX, BCL-2, caspase-3 and cleaved caspase-3) in C28/I2 chondrocytes from PBS and leptin groups following CD44 overexpression. (C) Flow cytometric detection of apoptosis in C28/I2 chondrocytes treated with PBS, OE-CD44, leptin, or leptin + OE-CD44. (D) Quantification of the apoptotic rate. (E) Western blotting and (F) semi-quantification of JAK2/STAT3 pathway-related proteins (p-JAK2, JAK2, p-STAT3 and STAT3) following CD44 overexpression. (G) Western blotting analysis of Ob-Rb protein expression following CD44 knockdown or overexpression. (H) Quantification of Ob-Rb protein expression from the Western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. JAK2, Janus kinase 2; ns, not significant; Ob-Rb, leptin receptor isoform B; p-, phosphorylated.

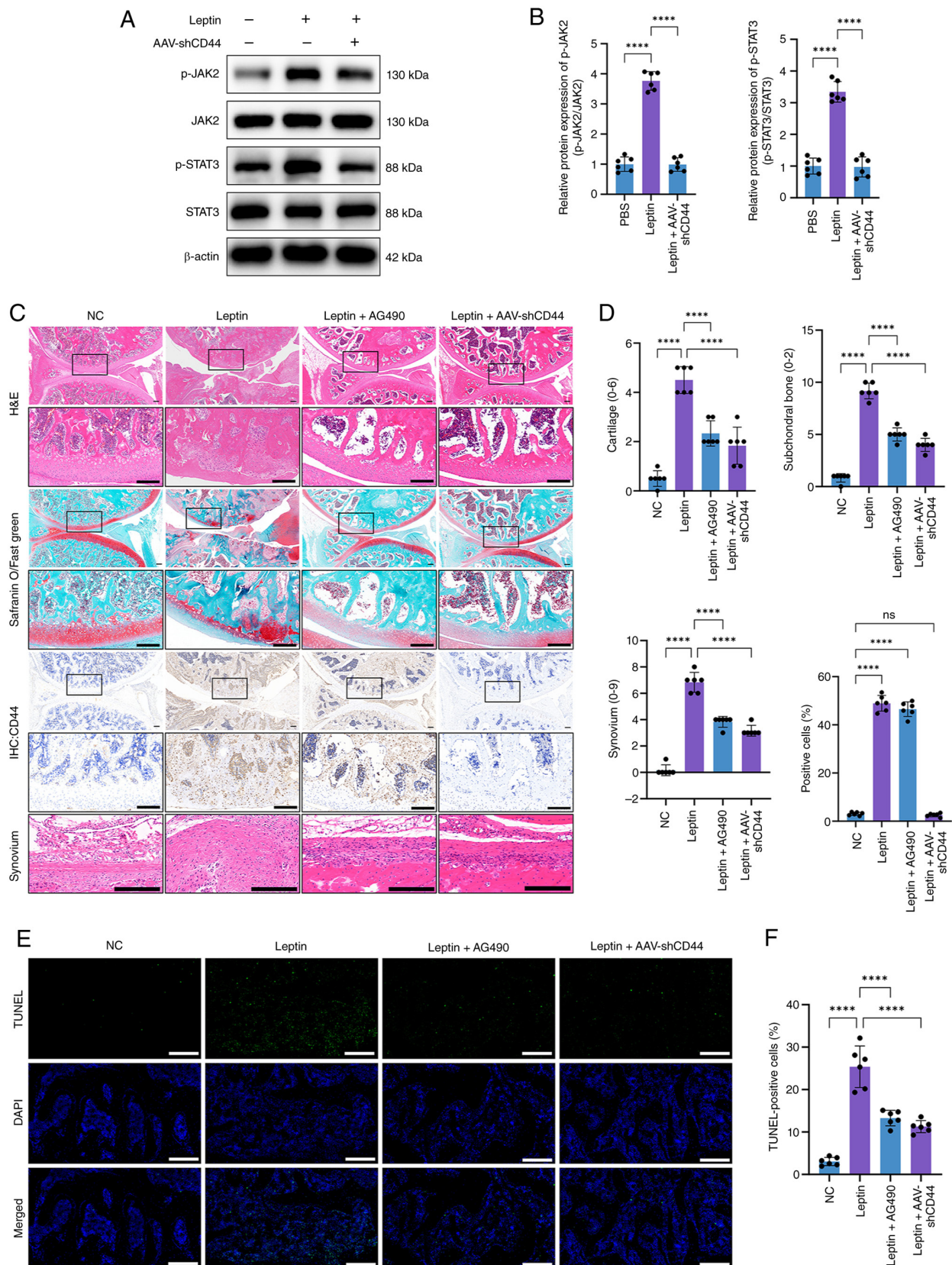


Figure 8. Leptin promotes CD44 expression, activates the JAK2/STAT3 signaling pathway, induces chondrocyte apoptosis and exacerbates osteoarthritis *in vivo*. (A) Western blotting and (B) semi-quantification of JAK2/STAT3 pathway-related proteins (p-JAK2, JAK2, p-STAT3 and STAT3) in rat chondrocytes. (C) H&E and Safranin O-Fast Green staining images of articular cartilage, subchondral bone and synovium, as well as IHC staining for CD44 in articular cartilage across the four groups. These images show cartilage degeneration, characterized by surface fibrillation, cartilage thinning, chondrocyte disorganization, reduced Safranin O staining (indicating proteoglycan loss) and subchondral bone remodeling and synovial hyperplasia. The black rectangular boxes mark the magnified regions, and the lower panels show high-magnification views (scale bar, 200 μ m). (D) Quantification of cartilage degeneration, subchondral bone remodeling, synovium inflammation/hyperplasia and the percentage of CD44-positive cells in articular cartilage across the four groups. (E) TUNEL staining detection and (F) quantification of apoptosis in rat cartilage tissue (scale bar, 100 μ m). **** $P < 0.0001$. JAK2, Janus kinase 2; ns, not significant; shRNA, short hairpin RNA; p-, phosphorylated.

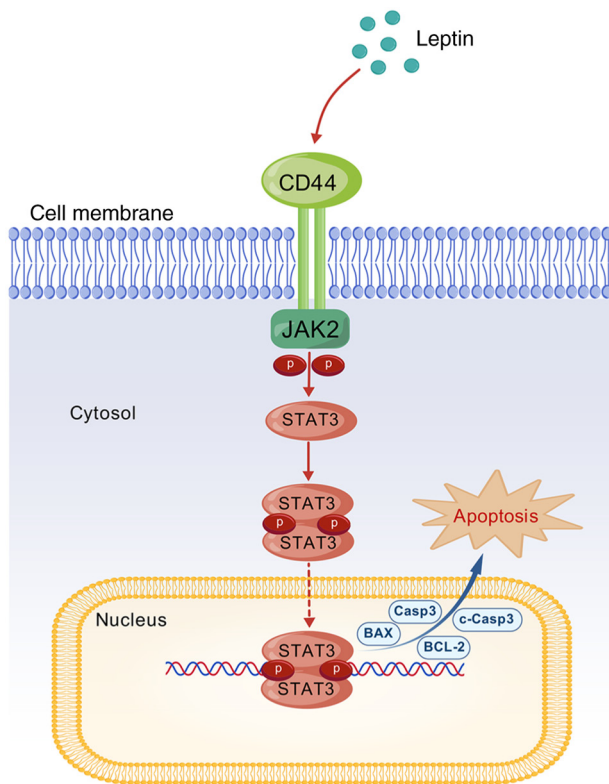


Figure 9. A model of the mechanism of leptin in osteoarthritis. Following leptin stimulation, CD44 expression increases, activating the JAK2/STAT3 signaling pathway. Overactivation of this pathway induces chondrocyte apoptosis, ultimately promoting osteoarthritis progression. Casp3, caspase-3; c-Casp3, cleaved caspase-3; JAK2, Janus kinase 2.

the involvement of JAK2/STAT3 in OA pathogenesis, most have focused on inflammatory cytokine-mediated pathway activation; whether metabolic signals can directly activate the JAK2/STAT3 signaling pathway remains unclear. The present study suggested that leptin, as an adipokine, directly activated the JAK2/STAT3 signaling pathway in chondrocytes, thereby establishing a direct mechanistic link between obesity-related metabolic signals and OA progression.

CD44 is a cell surface molecule that is associated with multiple signaling pathways, including the JAK2/STAT3 signaling pathway. Guo *et al* (36) reported that CD44 and its downstream JAK2/STAT3 signaling pathway served roles in the formation of fibrotic scars after spinal cord injury. Ni *et al* (37) reported that, in cystitis, the interaction between CD44 and the JAK2/STAT3 signaling pathway was associated with inhibited apoptosis, as indicated by increased expression of the anti-apoptotic proteins Mcl-1 and Bcl-xL. Prajapati and Kumar (38) reported that the absence of microRNA-6844 reduced the expression levels of stemness and other tumor markers in cancer stem-like cells via the CD44/JAK2/STAT3 axis, suggesting a mechanistic link between CD44 and the JAK2/STAT3 signaling pathway. The results from the present study demonstrated that CD44 knockdown prior to leptin stimulation significantly attenuated JAK2/STAT3 activation and inhibited apoptosis, whereas CD44 knockdown in the absence of leptin had no significant effect on JAK2/STAT3 pathway activity or apoptosis, likely due to the low basal expression level of CD44 in normal

chondrocytes (32). By contrast, CD44 overexpression induced marked changes in apoptosis-related and JAK2/STAT3 pathway-related proteins in both the PBS and leptin groups. Specifically, it increased p-JAK2, p-STAT3, BAX and cleaved caspase-3 and decreased BCL-2. These changes indicate that even without exogenous leptin, CD44 overexpression is sufficient to activate JAK2/STAT3 signaling and promote apoptosis, while leptin further enhances these effects. Based on the CD44 knockdown results, these findings suggest that CD44 is an upstream regulator of JAK2/STAT3-mediated chondrocyte apoptosis. Collectively, these findings suggest that leptin induces chondrocyte apoptosis through the CD44/JAK2/STAT3 axis. The canonical leptin receptor Ob-Rb is widely recognized as the primary mediator of leptin signaling (39-41); however, whether CD44 activates the JAK2/STAT3 pathway through Ob-Rb remains unknown to the best of our knowledge. The results from the present study demonstrated that neither CD44 overexpression nor knockdown significantly affected Ob-Rb expression, indicating that CD44 regulates JAK2/STAT3 pathway activation and chondrocyte apoptosis independently of Ob-Rb. Previous studies have confirmed the involvement of the CD44/JAK2/STAT3 axis in various pathological conditions, including spinal cord injury (36), cystitis cystica and glandularis (37) and breast cancer (38); however, its role in leptin-mediated chondrocyte apoptosis has remained unclear. The findings from the present study suggest that CD44 may function as an upstream regulator of JAK2/STAT3 signaling, potentially acting in parallel with, rather than exclusively through, Ob-Rb, as CD44 overexpression or knockdown did not significantly alter Ob-Rb expression. These observations provide new insights into the receptor dependency of leptin-induced chondrocyte apoptosis and raise the possibility that CD44 serves as an independent mediator in obesity-related OA pathogenesis, a role that has not been previously reported to the best of our knowledge.

The *in vivo* experiments in the present study further validated the aforementioned findings. Western blotting demonstrated that leptin treatment activated the JAK2/STAT3 signaling pathway in rat knee articular cartilage, whereas CD44 knockdown significantly inhibited this activation. IHC revealed that leptin upregulated CD44 expression in cartilage tissue. Furthermore, H&E and Safranin O-Fast Green staining, together with histological scoring, demonstrated that leptin treatment induced typical OA-like pathological changes in rat knee joints, characterized by articular cartilage structural disruption, matrix loss and reduced chondrocyte cellularity, accompanied by aberrant subchondral bone remodeling and synovial hyperplasia. Notably, AG490 did not significantly ameliorate leptin-induced cartilage degeneration, whereas CD44 knockdown significantly ameliorated subchondral bone remodeling and synovial hyperplasia but did not significantly ameliorate cartilage degeneration. TUNEL staining further demonstrated that chondrocyte apoptosis was markedly increased in the leptin group, whereas AG490 or CD44 knockdown effectively reduced the number of apoptotic cells, further confirming the pro-apoptotic role of leptin in cartilage degradation through the CD44/JAK2/STAT3 pathway. Collectively, these *in vivo* results were consistent with the *in vitro* observations, together validating the functional relevance of the CD44/JAK2/STAT3 pathway in leptin-driven OA pathogenesis.

Overall, the present study revealed a specific molecular mechanism of leptin-induced OA, namely that leptin promotes chondrocyte apoptosis via the CD44/JAK2/STAT3 signaling axis, and highlighted the role of CD44 in mediating chondrocyte phenotype changes in patients with OA. The novelty of the present study lies in identifying the CD44/JAK2/STAT3 signaling axis as a key mediator of leptin-induced chondrocyte apoptosis, a role that, to the best of our knowledge, has not been previously established in the context of obesity-related OA. While the present study offered insights, it had several limitations. First, the sample size of the bioinformatics analysis dataset (GSE85785) was relatively small, which may have resulted in incomplete bioinformatics findings. However, relevant public databases were examined and no other eligible dataset was identified to be currently available. This constitutes a limitation and a future direction of the present study. Second, the pathogenesis of obesity-related OA is complex, involving factors such as adipokine imbalance (such as adiponectin, resistin, visfatin and chemerin), proinflammatory cytokines (such as TNF- α , IL-6 and IL-1 β), matrix-degrading enzymes (such as MMPs and ADAMTS) and metabolic and oxidative stress; the degeneration of chondrocytes induced by leptin cannot fully simulate the progression observed in patients with obesity-related OA. Third, the present results indicated that although the use of AG490 inhibitors or the knockdown of CD44 could inhibit chondrocyte apoptosis, the inhibition was only partial, suggesting that leptin may engage additional pro-apoptotic pathways to cooperate with the CD44/JAK2/STAT3 axis in driving OA pathogenesis. However, to the best of our knowledge, in-depth experimental exploration of alternative pro-apoptotic pathways that cooperate with the CD44/JAK2/STAT3 axis has not yet been conducted. Fourth, the number of human cartilage samples analyzed in the present study were relatively small ($n=12$, with 6 obese and 6 non-obese patients), which may limit the statistical power and generalizability of the clinical observations; future studies with larger and more diverse patient cohorts are warranted to validate and extend the findings from the present study.

In conclusion, the present study demonstrated that leptin induced chondrocyte apoptosis by activating the JAK2/STAT3 signaling pathway via CD44, contributing to OA development. These findings suggest that CD44, JAK2 and STAT3 may serve as potential therapeutic targets for the treatment of obesity-related OA.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HQ conceptualized the study, reviewed and edited the manuscript and contributed to developing the methodology and performing the experiments. HQ and CZ performed the bioinformatics analyses. HQ, CZ, XZ and CC performed data analysis. HQ, YZ and ZD wrote, reviewed and edited the original manuscript. YZ and ZD made contributions to the conception and design of the study, supervised the experiments and participated in the acquisition, analysis and interpretation of data. YZ and ZD provided supervision and acquired funding for the present study. HQ, YZ and ZD confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All human experiments have been approved by the Ethics Committee of The Ninth People's Hospital of Chongqing [Chongqing, China; approval no. 2024 (ethics review no. 008)] and written informed consent was obtained preoperatively from all participants. All animal experiments have been approved by the Institutional Animal Care and Use Committee of Southwest University (approval no. IACUC-20241223-01) and conducted in accordance with institutional and national guidelines for animal care.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Liu Z, Xie W, Li H, Liu X, Lu Y, Lu B, Deng Z and Li Y: Novel perspectives on leptin in osteoarthritis: Focus on aging. *Genes Dis* 11: 101159, 2024.
2. Katz JN, Arant KR and Loeser RF: Diagnosis and treatment of hip and knee osteoarthritis: A review. *JAMA* 325: 568-578, 2021.
3. Jang S, Lee K and Ju JH: Recent updates of diagnosis, pathophysiology, and treatment on osteoarthritis of the knee. *Int J Mol Sci* 22: 2619, 2021.
4. Zhu S, Qu W and He C: Evaluation and management of knee osteoarthritis. *J Evid Based Med* 17: 675-687, 2024.
5. Yan M, Zhang J, Yang H and Sun Y: The role of leptin in osteoarthritis. *Medicine (Baltimore)* 97: e0257, 2018.
6. Shumnalieva R, Kotov G, Ermencheva P and Monov S: Pathogenic mechanisms and therapeutic approaches in obesity-related knee osteoarthritis. *Biomedicines* 12: 9, 2023.
7. Jansen NEJ, Molendijk E, Schiphof D, van Meurs JBJ, Oei EHG, van Middelkoop M and Bierma-Zeinstra SMA: Metabolic syndrome and the progression of knee osteoarthritis on MRI. *Osteoarthritis Cartilage* 31: 647-655, 2023.
8. Chen L, Zheng JY, Li G, Yuan J, Ebert JR, Li H, Papadimitriou J, Wang Q, Wood D, Jones CW and Zheng M: Pathogenesis and clinical management of obesity-related knee osteoarthritis: Impact of mechanical loading. *J Orthop Translat* 24: 66-75, 2020.
9. Raud B, Gay C, Guiguet-Auclair C, Bonnin A, Gerbaud L, Pereira B, Duclos M, Boirie Y and Coudeyre E: Level of obesity is directly associated with the clinical and functional consequences of knee osteoarthritis. *Sci Rep* 10: 3601, 2020.
10. Dardeno TA, Chou SH, Moon HS, Chamberland JP, Fiorenza CG and Mantzoros CS: Leptin in human physiology and therapeutics. *Front Neuroendocrinol* 31: 377-393, 2010.

11. Reid IR, Baldock PA and Cornish J: Effects of leptin on the skeleton. *Endocr Rev* 39: 938-959, 2018.
12. de Luis DA, Perez Castrillón JL and Dueñas A: Leptin and obesity. *Minerva Med* 100: 229-236, 2009.
13. Min S, Shi T, Han X, Chen D, Xu Z, Shi D, Teng H and Jiang Q: Serum levels of leptin, osteopontin, and sclerostin in patients with and without knee osteoarthritis. *Clin Rheumatol* 40: 287-294, 2021.
14. Primrose JG, Jain L, Bolam SM, Monk AP, Munro JT, Dalbeth N and Poulsen RC: Concentration-dependent effects of leptin on osteoarthritis-associated changes in phenotype of human chondrocytes. *Connect Tissue Res* 64: 457-468, 2023.
15. Chen B, Ning K, Sun ML and Zhang XA: Regulation and therapy, the role of JAK2/STAT3 signaling pathway in OA: A systematic review. *Cell Commun Signal* 21: 67, 2023.
16. Yao Q, Wu X, Tao C, Gong W, Chen M, Qu M, Zhong Y, He T, Chen S and Xiao G: Osteoarthritis: Pathogenic signaling pathways and therapeutic targets. *Signal Transduct Target Ther* 8: 56, 2023.
17. Ge Q, Shi Z, Zou KA, Ying J, Chen J, Yuan W, Wang W, Xiao L, Lin X, Chen D, *et al*: Protein phosphatase PPM1A inhibition attenuates osteoarthritis via regulating TGF- β /Smad2 signaling in chondrocytes. *JCI Insight* 8: e166688, 2023.
18. Zhou Y, Wang T, Hamilton JL and Chen D: Wnt/ β -catenin signaling in osteoarthritis and in other forms of arthritis. *Curr Rheumatol Rep* 19: 53, 2017.
19. Kilkenny C, Browne WJ, Cuthill IC, Emerson M and Altman DG: Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biol* 8: e1000412, 2010.
20. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, *et al*: NCBI GEO: Archive for functional genomics data sets-update. *Nucleic Acids Res* 41: D991-DE995, 2013.
21. Liu Z, Fan Q, Shen C, Li H, Ding J, Jin F, Sha L and Zhang Z: Leptin-mediated gene expression profiling exhibits new candidate genes associated with osteoarthritis in rat. *Gene Expr Omnibus (GEO)*: GSE85785, 2016.
22. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP: Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102: 15545-15550, 2005.
23. Jiang M, He J, Sun Y, Dong X, Yao J, Gu H and Liu L: Leptin induced TLR4 expression via the JAK2-STAT3 pathway in obesity-related osteoarthritis. *Oxid Med Cell Longev* 2021: 7385160, 2021.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
25. Zhou Y, Sun Y, Hou W, Ma L, Tao Y, Li D, Xu C, Bao J and Fan W: The JAK2/STAT3 pathway inhibitor, AG490, suppresses the abnormal behavior of keloid fibroblasts *in vitro*. *Int J Mol Med* 46: 191-200, 2020.
26. Zhang J, Liu C and You G: AG490, a JAK2-specific inhibitor, downregulates the expression and activity of organic anion transporter-3. *J Pharmacol Sci* 136: 142-148, 2018.
27. Fan L and Zhou L: AG490 protects cerebral ischemia/reperfusion injury via inhibiting the JAK2/3 signaling pathway. *Brain Behav* 11: e01911, 2021.
28. Wang YY, Lin SY, Chang CY, Wu CC, Chen WY, Liao SL, Chen YF, Wang WY and Chen CJ: Jak2 inhibitor AG490 improved poststroke central and peripheral inflammation and metabolic abnormalities in a rat model of ischemic stroke. *Antioxidants (Basel)* 10: 1958, 2021.
29. Liu Y, Duan M, Zhang D and Xie J: The role of mechano growth factor in chondrocytes and cartilage defects: A concise review. *Acta Biochim Biophys Sin (Shanghai)* 55: 701-712, 2023.
30. Hwang HS and Kim HA: Chondrocyte apoptosis in the pathogenesis of osteoarthritis. *Int J Mol Sci* 16: 26035-26054, 2015.
31. Kong H, Han JJ, Dmitrii G and Zhang XA: Phytochemicals against osteoarthritis by inhibiting apoptosis. *Molecules* 29: 1487, 2024.
32. Liu Q, Zeng H, Yuan Y, Wang Z, Wu Z and Luo W: Osteopontin inhibits osteoarthritis progression via the OPN/CD44/PI3K signal axis. *Genes Dis* 9: 128-139, 2020.
33. Chiang YF, Huang KC, Chen HY, Hamdy NM, Huang TC, Chang HY, Shieh TM, Huang YJ and Hsia SM: Hinokitiol inhibits breast cancer cells *in vitro* stemness-progression and self-renewal with apoptosis and autophagy modulation via the CD44/Nanog/SOX2/Oct4 pathway. *Int J Mol Sci* 25: 3904, 2024.
34. Zhao C, Zhao L, Zhou Y, Feng Y, Li N and Wang K: Artesunate ameliorates osteoarthritis cartilage damage by upregulating MTA1 expression and promoting the transcriptional activation of LXA4 to suppress the JAK2/STAT3 signaling pathway. *Hum Mol Genet* 32: 1324-1333, 2023.
35. Zhao Z, Bi B, Cheng G, Zhao Y, Wu H, Zheng M and Cao Z: Melatonin ameliorates osteoarthritis rat cartilage injury by inhibiting matrix metalloproteinases and JAK2/STAT3 signaling pathway. *Inflammopharmacology* 31: 359-368, 2023.
36. Guo J, Yang T, Zhang W, Yu K, Xu X, Li W, Song L, Gu X, Cao R and Cui S: Inhibition of CD44 suppresses the formation of fibrotic scar after spinal cord injury via the JAK2/STAT3 signaling pathway. *iScience* 27: 108935, 2024.
37. Ni Y, Zhao S, Yin X, Wang H, Guang Q, Hu G, Yang Y, Jiao S and Shi B: Intravesicular administration of sodium hyaluronate ameliorates the inflammation and cell proliferation of cystitis cystica et glandularis involving interleukin-6/JAK2/Stat3 signaling pathway. *Sci Rep* 7: 15892, 2017.
38. Prajapati KS and Kumar S: Loss of miR-6844 alters stemness/self-renewal and cancer hallmark(s) markers through CD44-JAK2-STAT3 signaling axis in breast cancer stem-like cells. *J Cell Biochem* 124: 1186-1202, 2023.
39. Peelman F, Zabeau L, Moharana K, Savvides SN and Tavernier J: 20 Years of leptin: Insights into signaling assemblies of the leptin receptor. *J Endocrinol* 223: T9-T23, 2014.
40. Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, Gojobori T and Isenovic ER: Leptin and obesity: Role and clinical implication. *Front Endocrinol (Lausanne)* 12: 585887, 2021.
41. Münzberg H and Morrison CD: Structure, production and signaling of leptin. *Metabolism* 64: 13-23, 2015.



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