Journal of Orthopaedic Translation 47 (2024) 50–62

Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Original Article

Targeting the senescence-related genes MAPK12 and FOS to alleviate osteoarthritis

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ARTICLE INFO

Keywords:
FOS
Hub genes
MAPK12
Osteoarthritis
Senescence

ABSTRACT

Background: The mechanism by which chondrocyte senescence aggravate OA progression has not yet been well elucidated. The aim of this study was to investigate the chondrocyte senescence related gene biosignatures in OA, and to analyze on the underlying mechanisms of senescence in OA.

Materials and methods: We intersected osteoarthritis dataset GSE82107 from GEO database and senescence dataset from CellAge database of human senescence-associated genes based on genetic manipulations experiments plus gene expression profilin, and screened out 4 overlapping genes. The hub genes were verified *in vitro* and in human OA cartilage tissues by qRT-PCR. We further confirmed the function of mitogen-activated protein kinase 12 (MAPK12) and Fos proto-oncogene (FOS) in OA *in vitro* and *in vivo* by qRT-PCR, western blotting, Edu staining, immunofluorescence, SA-β-gal staining, HE, IHC, von frey test, and hot plate.

Results: 1458 downregulated and 218 upregulated DEGs were determined from GSE82107, and 279 human senescence-associated genes were downloaded from CellAge database. After intersection assay, we screened out 4 overlapping genes, of which FOS, CYR61 and TNFSF15 were upregulated, MAPK12 was downregulated. The expression of MAPK12 was obviously downregulated, whereas the expression profiles of FOS, CYR61 and TNFSF15 were remarkedly upregulated in H_2O_2- or IL-1β-stimulated C28/I2 cells, human OA cartilage tissues, and knee cartilage of aging mice. Furthermore, both MAPK12 over-expression and FOS knock-down can promote cell proliferation and cartilage anabolism, inhibit cell senescence and cartilage catabolism, relieve joint pain in H_2O_2- or IL-1β-stimulated C28/I2 cells and mouse primary chondrocytes, destabilization of the medial meniscus (DMM) mice.

Conclusion: This study explored that MAPK12 and FOS are involved in the occurrence and development of OA through modulating chondrocyte senescence. They might be biomarkers of OA chondrocyte senescence, and provides some evidence as subsequent possible therapeutic targets for OA.

The translational potential of this article: The translation potential of this article is that we revealed MAPK12 and FOS can effectively alleviate OA by regulating chondrocyte senescence, and thus provided potential therapeutic targets for prevention or treatment of OA in the future.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease and a major cause of disability and socioeconomic burden [1,2]. Pathological changes in OA involve cartilage, bone, synovium, ligament and periarticular fat, resulting in limited joint movement [3,4]. With an aging population, the incidence of osteoarthritis is increasing every year. There is no effective prevention or treatment for OA [5], joint replacement is an effective treatment for end-stage OA [6], but still has a limited lifespan. Thus, it is especially important to prevent and treat OA at an early stage. Undoubtedly, delaying the senescence of OA joint cartilage is a very effective therapy approach.

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https://doi.org/10.1016/j.jot.2024.06.008
Received 29 December 2023; Received in revised form 7 April 2024; Accepted 3 June 2024

Cellular senescence is a stress response that causes a permanent arrest of the cell cycle [7]. There is growing evidence that aging-related changes affecting joint tissue promote the development of OA. the cellular senescence markers p16 and p21 are significantly upregulated in chondrocytes from OA patients [1,8], leading to an imbalance in chondrocyte redox homeostasis. In addition, senescent cells can also secrete various pro-inflammatory proteins, such as inflammatory cytokines and chemokines, called senescence-associated secretory phenotype (SASP) [2,9]. Senescent chondrocytes exhibit and develop a SASP that can contribute to cartilage dysfunction through paracrine action leading to senescence and dysfunction of neighboring cells. Low-level inflammation plays a role in the development of OA [10], and there is also a link between aging and inflammation. Pro-inflammatory mediators in SASP may play an important role in OA. For example, *in vitro* and *in vivo* results showed that IL6, a pro-inflammatory factor associated with aging, is an important factor in the pathogenesis of OA [11,12], which can significantly increase expression of IL-1β, TNFα and MMP13 in articular cartilage of OA patients [13,14]. However, the specific molecular mechanisms underlying the effects of cellular senescence in OA have not been fully elucidated. A better understanding of how senescence contributes to the occurrence and progression of OA could provide new ideas for clinical medication of OA.

In current study, we analyzed a previously published OA dataset containing samples from OA and non-OA individuals to identify differentially expressed genes (DEGs) associated with OA. The study further identified four hub genes by cross-analysis on this OA dataset with the human senescence gene database, which are associated with senescence in OA. Importantly, we further verified that MAPK12 and FOS may be involved in the occurrence and development of OA by regulating cellular senescence *in vitro* and *in vivo*. This study provides a possible basis for better understanding of aging and the etiology of OA, as well as SASP as a potential targeted therapeutic molecule for OA.

2. Materials and methods

2.1. OA microarray data

We searched the GEO database for “Osteoarthritis and Human” and obtained the GSE82107 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE82107) [15], which consisted of 7 normal samples and 10 OA samples, and divided them into control and OA groups.

2.2. Identification of DEGs, GO and KEGG pathway analysis

The GSE82107 dataset was analysed using “R" to filter out genes satisfying $|\log2(FC)|>1.5$ & $p < 0.05$, then volcano and heat maps were constructed. We also used Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases for clustering analysis of OA differential genes.

2.3. OA and aging-related genes

Aging related genes were obtained by searching through CellAge database of human senescence-associated genes based on genetic manipulations experiments plus gene expression profilin (https://www.genomics.senescence.info/). OA genes were intersected with aging-related genes and a Venn diagram was obtained, and the intersected genes were the OA and aging-related genes.

2.4. Cell culture

Human chondrocyte C28/I2 cells (gifted by professor Chuanju Liu from New York university) were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10 % fetal bovine serum (FBS) and 50 μg/mL penicillin-streptomycin, incubated in 5 % CO_2 and at 37 °C. Mouse primary chondrocytes were isolated from 4 days old C57 mice. After sacrificed mice, we opened knee joint capsule, then isolated cartilage into collagenase II, digested cells were cultured in F12/DMEM containing 10 % fetal bovine serum (FBS).

2.5. pcDNA3.1/myc-His-MAPK12 plasmid construction

To construct the pcDNA3.1/myc-His-MAPK12 plasmid, cDNA from human Hela cells was used as template for PCR amplification using PowerPol 2X PCR Mix with Dye (Abclonal, RK20719) (MAPK12-F: 5′-GGGCCCTCTAGACTCGAGATGAGCTCTCCGCCGCC-3′, MAPK12-R:5′-TGGAATTCTGCAGATATCCAGAGGCGTCTCCCCTTGGAG-3′). The PCR products were transformed by ligation with the double-cleaved pcDNA3.1 vector, and the single clones were picked and sent to Tsingke Biotechnology for sequencing.

2.6. Establishment of cell models

C28/I2 cells were seeded in 6-well cell plates at a density of 2×10^6 cells/well. Cells were treated with 10 ng/ml IL-1β or 400 μM H_2O_2 to establish OA or senescence model *in vitro*, then transfected with siRNA (siFOS forward: 5′-ACCUAUCUGGGUCCUUCUAUGtt-3′, siFOS reverse: 5′-CAUAGAAGGACCCAGAUAGGUtt-3′, synthesized by Tsingke Biotechnology) or pcDNA3.1-MAPK12 plasmid by Lipofectamine 2000 (ThermoFisher, 11668030) for 48 h.

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from cells and tissues were extracted using Trizol reagent (Thermofisher, 15596018) and cDNA was reversed using RT Master Mix for qRT-PCR II (Medchemexpress, HY-K0511A) according to the reagent manufacturer’s manual for subsequent qRT-PCR analysis. GAPDH was used as an internal reference. The human and mouse primer sequences of all target genes were listed in Supplementary Tables 2 and 3

2.8. Western blotting

Cells were lysed using RIPA lysis solution (Beyotime, P0013B) containing 1 % protease inhibitor and centrifuged, then denatured by boiling with the addition of sodium dodecyl sulfate after removal of the precipitate. Subsequently, cells were separated by SDS-PAGE gel electrophoresis, transferred to polyvinylidene fluoride membranes, closed with 5 % bovine serum albumin and incubated overnight at 4 °C with the corresponding primary antibody. Then the corresponding secondary antibody was incubated for 2 h. Protein expression was detected by ECL (Affinity, KF005) chemiluminescent solution. The following primary antibodies were used: MMP13 (Proteintech, 18165-1-AP), Col2 (Proteintech, 28459-1-AP), p16 (Proteintech, 10883-1-AP), p21 (Proteintech, 10355-1-AP), ACAN (Affinity, DF7561), C-Myc-Tag (Affinity, T0052), GAPDH (Affinity, AF7021), PCNA (CST, 13110T), LMNB1 (Affinity, BF8009), ADAMTS5 (Affinity, DF13268), SOX9 (Santa Cruz, sc-166505), MAPK12 (Proteintech, 20184-1-AP), FOS (Proteintech, 66590-1-Ig), CYR61 (Proteintech, 67656-1-Ig) and TNFSF15 (Proteintech, 29899-1-AP).

2.9. Edu assay

The EdU (5-ethynyl-2′-deoxyuridine) staining was performed using EdU kit (Beyotime, C0078). Before immunostaining, the C28/I2 cells were incubated with EdU working fluid and fixed for 15 min, incubated with transparent fluid at room temperature. 500 μl Click Additive Solution were added to each well, cells were incubated in dark at room temperature. Finally, the nucleus was stained with Hoechst 33342. The images were photographed by fluorescence microscope.

2.10. SA-β-gal staining

C28/I2 cells were fixed for 15 min at room temperature, and incubated by staining fluid from SA-β-galactosidase assay kit (Beyotime, C0602) at 37 °C overnight. Senescent cells were dyed blue. The images were taken by optical microscope.

2.11. Animal experiment

8 weeks old male C57BL/6J mice were purchased from Ensiweier Inc. DMM or sham surgery were performed on the right hind knee joint of mice. MAPK12 over-expressing or FOS knock-down adenovirus was administered 10 μl by intra-articular injection at 4 weeks after surgery and twice a week for 4 weeks. Knee joint samples were collected at 8 weeks after surgery.

2.12. Hematoxylin-eosin (HE) staining

After euthanasia of the mice, the knee joints were taken and surrounding muscles were removed, fixed with 4 % paraformaldehyde at 4 °C for 48 h, decalcified with 15 % EDTA at 37 °C for 2 weeks, paraffin embedded, and sliced into 5 μm thick sections. HE staining was performed according to the kit method (Solarbio, Cat. G1121). Briefly, paraffin sections were dewaxed and hydrated for hematoxylin and eosin staining.

2.13. Safranin O/fast green staining

Knee joint of mice or human cartilage paraffin sections were stained with Safranin O-fast green stain kit (Solarbio Life Sciences, Cat. G1371) according to the manufacturer's instructions. The severity of osteoarthritis was evaluated by Osteoarthritis Research Society International (OARSI) scoring system.

2.14. Immunohistochemical staining

Knee joint of mice or human cartilage paraffin sections were digested using antigen repair solution (BOSTER, Cat. AR0026) at 37 °C for 30 min. 3 % hydrogen peroxide was incubated at room temperature for 10 min, followed by overnight incubation with corresponding primary antibody, Col2 (Proteintech, 28459-1-AP), ACAN (Affinity, DF7561), MMP13 (Proteintech, 18165-1-AP), ADAMTS5 (Affinity, DF13268), γ-H2AX (Cell Signaling Technology, 9718), IL6 (Affinity, DF6087), MAPK12 (Proteintech, 20184-1-AP), FOS (Proteintech, 66590-1-Ig), CYR61 (Proteintech, 67656-1-Ig) and TNFSF15 (Proteintech, 29899-1-AP) at 4 °C, and then immunoreactivity was detected using streptavidin-biotin assay system (ORIGENE, Cat. SP-9000). Color development was performed by DAB (ORIGENE, Cat. ZLI-9018) followed by restaining with hematoxylin (BOSTER, Cat. AR0005).

2.15. Immunofluorescence staining

C28/I2 cells or cartilage paraffin section were fixed with 4 % paraformaldehyde, then permeated with 0.5 % TritonX-100, digested with antigen repair solution (BOSTER, Cat. AR0026), incubated with 3 % hydrogen peroxide, followed by incubation with primary antibody p16 (Proteintech, 10883-1-AP), p21 (Proteintech, 10355-1-AP), or γ-H2AX (Cell Signaling Technology, 9718) to incubate the cells at 4 °C overnight. The secondary antibody reactions (Proteintech Alexa Fluor® 488, gb2AF488) were performed at room temperature for 4 h, and DAPI (Beyotime, Cat. C1006) reaction for 5 min. The images were photographed by fluorescence microscope, and quantitative analysis was conducted by Image J.

2.16. Von frey test

Following DMM surgery, mouse's soles received stimulation once a week through a von fery filament. Once the filament made a vertical contact with the skin, it gradually added more force until it twisted, at which point the amount of paw withdrawl threshould was calculated. Force of the filament was experimented with, ranging from 0.0008 g to 0.6 g, lasting for two to 3 s each time.

2.17. Hot plate

Mice were placed on a metal plate that had been pre-warmed, and the temperature was controlled while the animal's response time (s) was recorded. The animal will experience pain when the temperature hits a particular point and will react to it by pumping or licking its foot.

2.18. Statistical analysis

Our analyses were performed in the R statistical (4.0.2). Data were statistically analyzed using GraphPad Prism 9.0 software and presented as mean ± SD. An unpaired t-test was applied while comparing two groups with one variance. Multiple comparisons were carried out using one-way ANOVA test. In all experiments, p value < 0.05 was statistically significant, $****p < 0.0001$, $***p < 0.001$, $**p < 0.01$, $*p < 0.05$, ns means no significance.

3. Results

3.1. Identification of senescence-specific DEGs in osteoarthritis

We analyzed the samples in the GSE82107 dataset to obtain 1676 DEGs, including 218 up-regulated genes and 1458 down-regulated genes. The volcano plot for the visualization of DEGs were seen in Fig. 1 A. Part of DEGs were plotted in a heatmap (Fig. 1B), which indicated that SASP related genes IL6, IL-1β and MMP13 were up-regulated in OA patients. Then we performed GO enrichment analysis and KEGG analysis and the results were shown in Fig. 1C and D. Cell senescence related genes from the CellAge database were shown in Supplementary Table 1. After intersecting the GSE82107 dataset with the Cell Age database, the volcano plot and Venn diagram showed that there were 4 differentially expressed genes appearing in both OA and senescence genes, including three up-regulated genes CYR61, FOS, TNFSF15 and one down-regulated gene MAPK12 (Fig. 1A–E). Finally, we analyzed the interaction between MAPK12 or FOS and proteins related to cell proliferation, senescence, cartilage anabolism and catabolism through STRING tools (Fig. 1F), it was demonstrated that both MAPK12 or FOS might be associated with cell senescence and SASP through CDKN2A (p16), CDK2, CCND1 (CyclinD1), IL6, IL-1β, TNFα and MMP13.

3.2. Verification of senescence-specific genes expression in osteoarthritis

According to the database analysis of OA and senescence, we selected four significantly different expression proteins, including MAPK12, FOS, CYR61, TNFSF15, and measured their expression levels in OA. Firstly, we collected six human osteoarthritis lesion and non-lesion cartilage tissues, both qRT-PCR and immunohistochemical staining results revealed that mRNA or protein levels of FOS, CYR61 and TNFSF15 were downregulated, while MAPK12 was upregulated in the lesion cartilage of OA, compared with that of the non-lesion control tissues (Fig. 2A and B). Furthermore, similar results were also observed in the joint cartilage of aging mice (20 months old), compared with that of the young mice (10 weeks old) (Fig. S1). Thereafter, we established H_2O_2 induced chondrocyte senescence and IL-1β stimulated OA models *in vitro*. The results suggested that SA-β-gal levels, p16, p21, FOS, CYR61 and TNFSF15 expression were remarkedly upregulated, whereas the

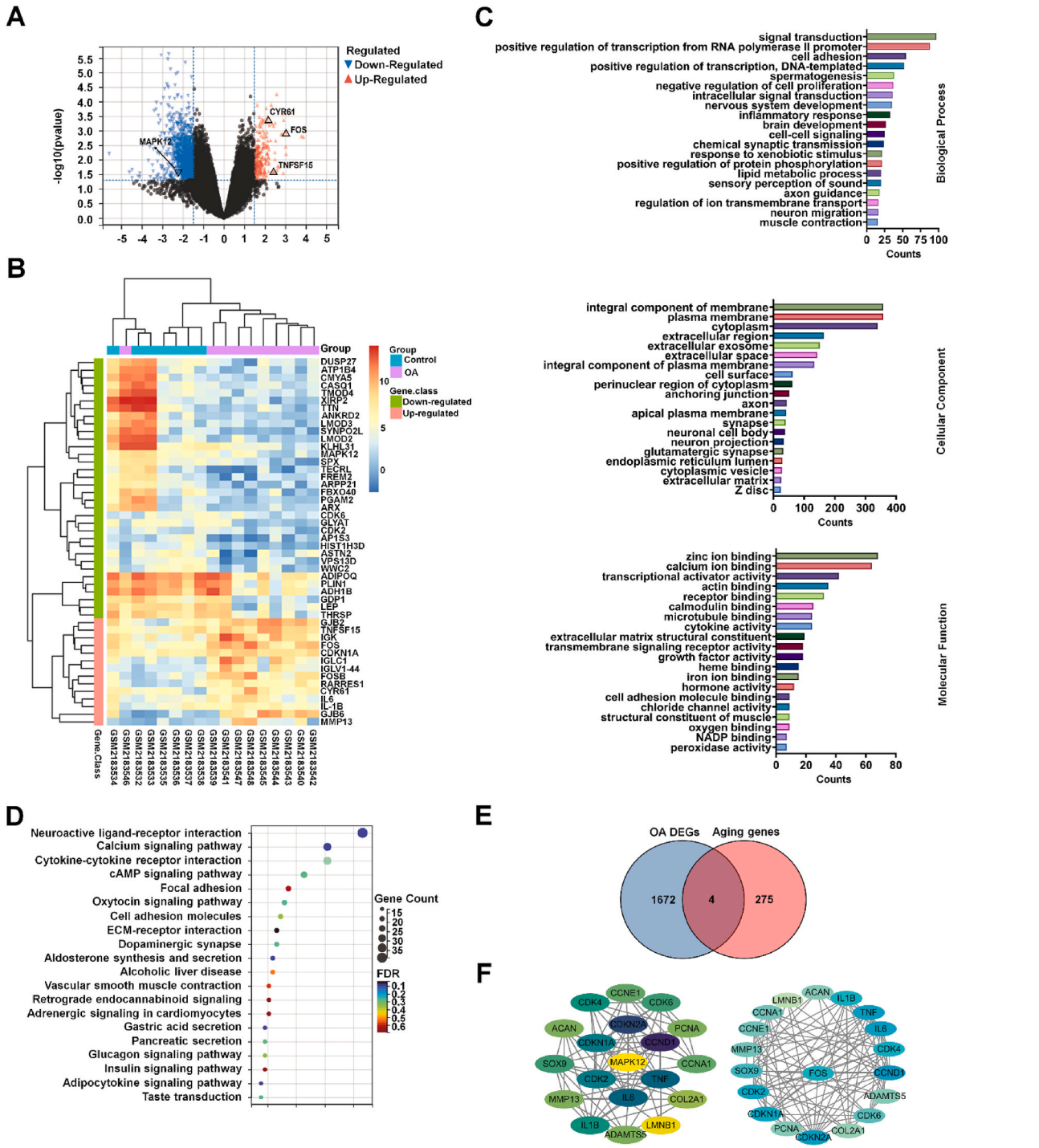

Figure 1. Identification of DEGs and screening of senescence-specific hub genes for osteoarthritis. (A) Volcano plot of DEGs for GSE82107 datasets and the screened hub senescence-specific DEGs in OA. Red denotes upregulated genes and blue denotes downregulated genes ($|\log_2 FC| \geq 1.5$ and $p.value < 0.05$). (B) The heatmap of part DEGs. As the color goes from red to blue, indicating the changing from up-regulation to down-regulation. (C) GO enrichment analysis of DEGs. (D) KEGG analysis of DEGs. (E) Venn diagram of the screened hub senescence-specific DEGs in OA. (F) Protein–protein interaction in STRING.

expression of MAPK12 was obviously downregulated in the C28/I2 cells stimulated with H_2O_2 (Figs. S2A, B, C) or IL-1β (Fig. 2C and D) compared with that of controls. These results not only confirmed the expression profiles of candidate senescence specific genes in OA, but also laid the foundation for further exploration of the mechanism of aging in OA.

3.3. *MAPK12 over-expression inhibits senescence and DNA damage induced by H_2O_2*

The expression of LMNB1, a nuclear morphologic marker, is deficient in senescent human and mouse cells [16]. It was reported that MAPK12 can be similarly used as a new type of cyclin dependent kinase (CDK) like kinase and play an important role in biological processes of various cancers by regulating cell cycle [17–19], however, the role of MAPK12 has not yet been well elucidated in cell senescence and OA. Herein, we

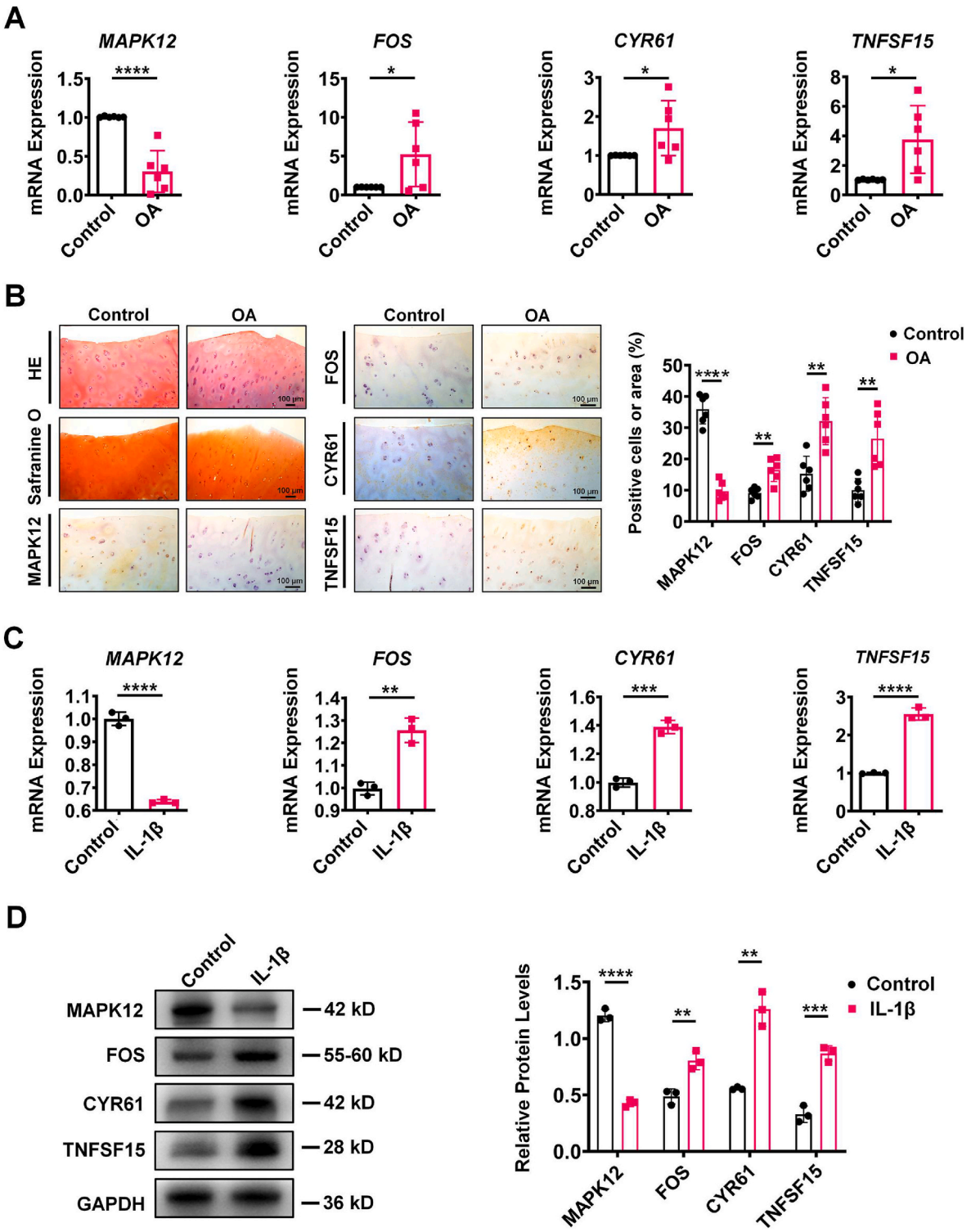

Figure 2. Verification of mRNA and protein levels of candidate senescence-specific genes in osteoarthritis. (A) The mRNA levels of *MAPK12, FOS, CYR61, TNFSF15* were investigated in human normal control and osteoarthritis (OA) cartilage tissue by qRT-PCR, n = 6. (B) HE, Safranine O staining and protein levels of MAPK12, FOS, CYR61, TNFSF15 were investigated in human normal control and osteoarthritis (OA) cartilage tissue by immunohistochemistry, n = 6. Scale bar = 100 μm. (C) The mRNA levels of *MAPK12, FOS, CYR61, TNFSF15* were investigated in human C28/I2 chondrocytes treated with 10 ng/ml IL-1β for 48h by qRT-PCR, n = 3. (D) The protein levels of MAPK12, FOS, CYR61, TNFSF15 were investigated in human C28/I2 chondrocytes treated with 10 ng/ml IL-1β for 48h by western blotting, n = 3. Statistical analysis was conducted using unpaired Student's t test. *P* values were compared between OA group and normal control group, between IL-1β group and PBS control group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, ns, no significance.

detected and found that over-expression of MAPK12 can increase expression of proliferation related genes CDK2, CDK4, CDK6, CyclinA1, CyclinD1, CyclinE1, LMNB1, PCNA (Fig. 3A–S3A, B) and cell proliferation rate (Fig. 3B) in the H_2O_2 induced C28/I2 cells compared with that of control cells. In addition, over-expression of MAPK12 can reduce expression of senescence and SASP genes, including p16, p21, catabolic associated gene MMP13, in the H_2O_2-induced C28/I2 cells compared with that of control cells (Fig. 3A–S3C). DNA damage marker γ-H2AX expression was upregulated in the H_2O_2-induced C28/I2 cells, however, over-expression of MAPK12 can remarkedly suppress γ-H2AX expression, a marker of DNA damage, compared with that of control cells (Fig. 3C). SA-β-gal staining indicated that MAPK12 over-expression can apparently relieve senescence and SASP in the H_2O_2-induced C28/I2 cells compared with that of control cells (Fig. 3D). These indicated that MAPK12 inhibits senescence and DNA damage induced by H_2O_2.

3.4. MAPK12 over-expression promotes cartilage anabolism and proliferation, inhibits cartilage catabolism and senescence stimulated by IL-1β

Meanwhile, in the *in vitro* OA microenvironment simulated by IL-1β-stimulated C28/I2 cells or mouse primary chondrocytes [20,21], we detected and found that over-expression of MAPK12 can increase expression of proliferation related genes (Fig. 4A, B, S4A, S5A, B) and reduce expression of senescence and SASP genes, including p16, p21, IL6, TNFα (Fig. 4A, B, S4B, S5B), in the IL-1β-induced chondrocytes compared with that of control cells. Furthermore, over-expression of MAPK12 can enhance expression of cartilage anabolism genes, including COL2, ACAN, SOX9, and reduce expression of cartilage catabolism genes MMP13, ADAMTS5 in the IL-1β stimulated chondrocytes (Fig. 4A, B, S4C, S5C). Moreover, EdU assays results also showed that over-expression of MAPK12 remarkedly promotes cell proliferation in the IL-1β stimulated C28/I2 cells (Fig. 4C and D).

3.5. FOS deficiency inhibits senescence and DNA damage induced by H_2O_2

FOS was reported to be up-regulated in OA [22,23], but whether it can accelerate OA process by regulating cell senescence has not been clarified. Thus we knocked down the FOS expression through siRNA approach in C28/I2 cell and detected efficiency siRNA (Fig. S6A), then we chose siFOS-2 for the follow-up experiments. It was showed that knockdown of FOS can increase expression of proliferation related genes (Fig. 5A–S6B), and reduce expression of p16, p21, MMP13 (Fig. 5A–S6C) in the H_2O_2-induced C28/I2 cells compared with that of control cells. Furthermore, EdU assays also showed that knockdown of FOS can obviously enhance the cell proliferation in the H_2O_2-induced C28/I2 cells compared with that of control cells (Fig. 5B). In addition, γ-H2AX expression was upregulated in the H_2O_2-induced C28/I2 cells, however, FOS knockdown could obviously repress its expression (Fig. 5C). SA-β-gal staining indicated that knockdown of FOS can apparently relieve the senescence in the H_2O_2-induced C28/I2 cells compared with that of control cells (Fig. 5D).

3.6. FOS deficiency promotes cartilage anabolism and proliferation, inhibits cartilage catabolism and senescence stimulated by IL-1β

At the same time, we also examined the role of FOS in the IL-1β-stimulated C28/I2 cells or mouse primary chondrocytes mimicking OA microenvironment *in vitro* [20,21], the result showed that knockdown of FOS can improve the expression of proliferation related genes (Fig. 6A, B, S7A, S8A, B), and decrease expression of p16, p21, IL6, TNFα (Fig. 6A, B, S7B, S8B) in the IL-1β-induced chondrocytes compared with that of control cells. Furthermore, FOS knockdown could increase the expression of COL2, ACAN, SOX9, and reduce expression of MMP13 in the IL-1β stimulated chondrocytes (Fig. 6A, B, S7C, S8C). Moreover, EdU assays results also showed that knockdown of FOS clearly promotes cell proliferation in the IL-1β stimulated C28/I2 cells (Fig. 6C and D).

3.7. MAPK12 over-expression and FOS deficiency inhibit chondrocyte senescence, relieve osteoarthritis and joint pain in DMM mice

To determine the specific roles of MAPK12 and FOS in osteoarthritis pathophysiology, we used 8-weeks-old wild type male C57BL/6J mice to establish a DMM model *in vivo*, then the adenovirus of over-expression MAPK12 (OE-MAPK12) or siFOS were injected into the knee cavity twice a week for 4 weeks. At 8 weeks post-DMM surgery, HE and Safranine O results showed that mice of the OE-control and si-control groups exhibit increased articular cartilage degeneration, meniscal lesions and synovial hyperplasia, while OE-MAPK12 and siFOS effectively rescued the cartilage damage, meniscal injury and synovitis (Fig. 7A), which was further validated by analysis of Osteoarthritis Research Society International (OARSI), meniscus total scores and synovitis score (Fig. 7B). Furthermore, the addition of MAPK12 or deficiency of FOS rescued the inhibition of cartilage anabolism and promotion of catabolism induced by DMM surgery, with increased Col2, ACAN and decreased MMP13, Adamts5 expression in OE-MAPK12 and siFOS groups, compared to the OE-control and si-control groups (Fig. 7C and D). Meanwhile, both von frey test (Fig. 7E) and hot plate (Fig. 7F) results showed the joint pain were relieved to a large extent in the OE-MAPK12 and siFOS mice at 6 and 8 weeks post-DMM surgery. As expected, the decreased expression of senescence-related markers p16, p21, γ-H2AX and IL6 in the articular cartilage tissue of OE-MAPK12 and siFOS mice, displayed the anti-senescence effect of MAPK12 over-expression and FOS deficiency in OA (Fig. 8A, B, C, D, E). Taken together, these observations indicated that MAPK12 and FOS play important roles in chondrocyte senescence and osteoarthritis development and progression.

4. Discussion

With the acceleration of the aging process, the incidence of OA is on the rise, which has increasingly become a major problem affecting the quality of life of the elderly. Research shows that among people over 50 years old, OA can lead to long-term disability, and the ultimate mortality rate is as high as 53 % [24]. Osteoarthritis is the most common chronic degenerative joint disease caused by various factors, including articular cartilage fibrosis, chapping and loss of articular cartilage, with cartilage damage and joint pain as the main symptom [25]. Usually, the lesions can also involve the whole joint, thus causing inflammation of the synovial membrane and infrapatellar fat pad. New findings demonstrated that aging is the largest risk factor for most chronic diseases, including osteoarthritis [26]. However, the role and exact mechanism on how chondrocyte senescence to exacerbate OA progression has not been well elucidated.

Cell senescence is a kind of cell state that is highly dynamic, multi-step process, during which the properties of senescent cells continuously evolve and diversify in specific physiological processes. In the process of senescence, the ability of proliferation and differentiation as well as the physiological functions of cells will gradually decline [27]. Rb phosphorylation is reported to be necessary to remove transcriptional target inhibition and promote cell cycle progression. However, p21 and p16, as an inhibitor of CyclinE/CDK2, CyclinD/CDK4, 6 complexes respectively, can accumulate in senescent cells, thereby promoting the continuous activation of Rb family proteins, eventually leading to senescence and irreversible cell cycle arrest during G0/G1 and S phases. The down-regulation of Lmnb1 is another important feature of cell senescence, and the expression of Lmnb1 was absent in senescent human and mouse cells [16]. Senescence is also significantly involved in the development and progression of osteoarthritis. The number of individual articular chondrocytes in adulthood is stable and hardly increase, so chondrocytes are more susceptible to external stimuli

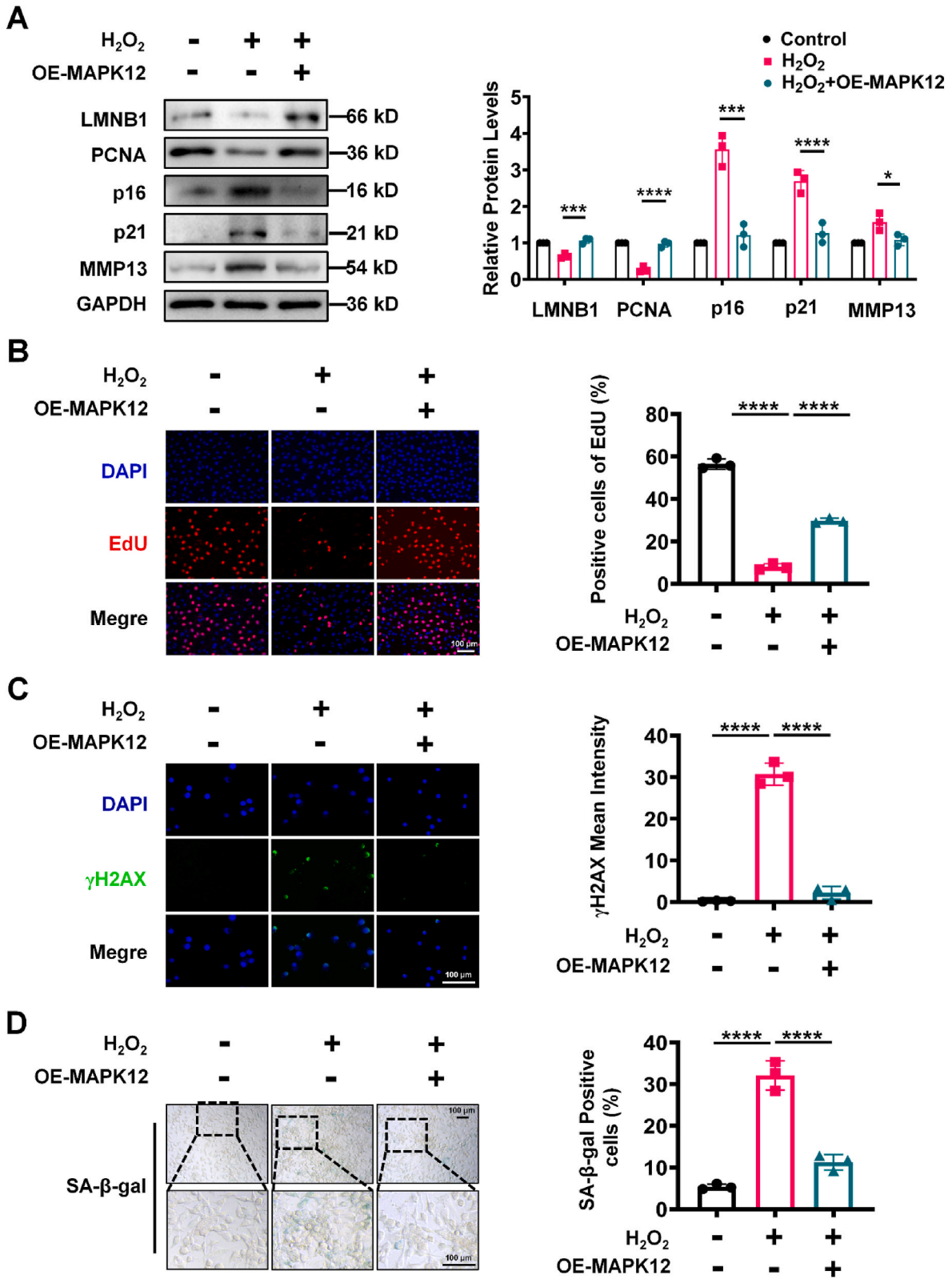

Figure 3. MAPK12 over-expression inhibits senescence and DNA damage of C28/I2 cells induced by H_2O_2. (A) The protein levels of LMNB1, PCNA, p16, p21, MMP13 were investigated in C28/I2 cells by western blotting, n = 3. (B) EdU staining was adopted for cell proliferation capability of C28/I2 cells, n = 3. Scale bar = 100 μm. (C) The protein level of γ-H2AX was investigated in C28/I2 cells by immunofluorescence, n = 3. Scale bar = 100 μm. (D) SA-β-gal staining was adopted for senescence of C28/I2 cells, n = 3. Scale bar = 100 μm. Statistical analysis was conducted using one-way ANOVA. *P* values were compared between OE-MAPK12 group or control group and H_2O_2 group. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, ns, no significance.

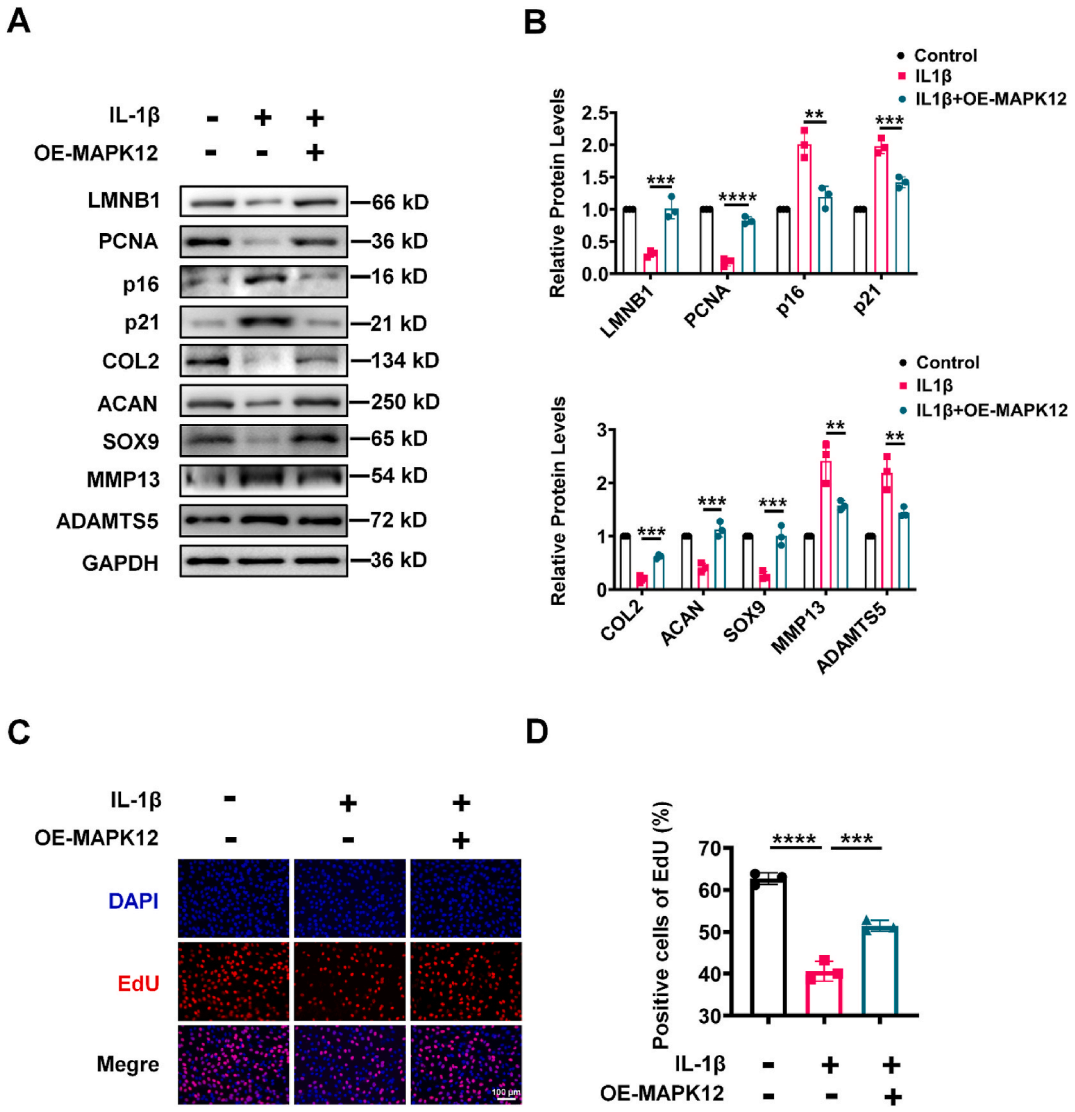

Figure 4. Over-expression of MAPK12 promotes cartilage anabolism and proliferation, inhibits cartilage catabolism and senescence of IL-1β stimulated C28/I2 cells. (A, B) The protein levels and quantification of LMNB1, PCNA, p16, p21, COL2, ACAN, SOX9, MMP13, ADAMTS5 were investigated in C28/I2 cells by western blotting, n = 3. (C, D) Representative image and quantification of EdU staining adopted for cell proliferation ability of C28/I2 cells, n = 3. Scale bar = 100 μm. Statistical analysis was conducted using one-way ANOVA. *P* values were compared between OE-MAPK12 group or control group and IL-1β group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, ns, no significance.

such as ultraviolet radiation, oxidative damage and chronic inflammation, which will induce cellular senescence of chondrocytes [28]. Pathological research results of OA show that articular cartilage injury is the most important pathological change, mainly manifested as degradation of articular cartilage, apoptosis of chondrocytes, and progressive degradation of extracellular matrix (ECM) [17].

Research showed that chondrocytes senescence related β-Galactosidase (SA-β-Gal) level were positively correlated with the severity of the degree of knee articular lesions [29]. Cellular senescence also exhibits proliferative arrest and hallmark features such as senescence associated secretory phenotype (SASP). SASP is of critical concern in mediating several of the pathophysiological effects of senescent cells. During aging chondrocyte, SASP factors can exert their effects in both autocrine and paracrine fashion. The main components of SASP include pro-inflammatory cytokines, growth modulators, matrix metalloproteinases (MMPs), chemokines, extracellular matrix components, proteases, and angiogenic factors, etc. Cytokine, such as IL-1α and IL6, serve in a cell-autonomous manner to modulate the senescent state. Firstly, SASP can change the cellular environment in chondrocytes, which will lead to osteoarthritis. Senescent chondrocytes secrete some SASPs, which will lead to increased expression of catabolic cytokines such as IL-1β, IL6, TNFα etc., and these catabolic factors can further promote the secretion of metal matrix proteins (MMPs), including MMP1, MMP13 [30]. And MMP13 is considered as the core of irreversible degradation of cartilage type II collagen lattice in OA [31]. These factors may affect the inflammatory microenvironment, when the inflammation lasts longer, it would be harmful. Chronic inflammation leads to the senescence of healthy adjacent cells, degradation of ECM, synovitis [32]. Secondly, senescent chondrocytes lose their responsiveness to growth factors. The ability of growth factors to promote cell proliferation or synthesis will significantly decrease in senescent chondrocytes. In aged mouse cartilage tissue and primary human chondrocytes, p16 expression were significantly upregulated [33]. Therefore, a better understanding of chondrocytes senescence and OA, would be of great importance to relieve OA.

Through algorithm, we identified four candidate senescence related genes for osteoarthritis, including MAPK12, FOS, CYR61 and TNFSF15. Firstly, we confirmed that in human OA cartilage tissues MAPK12

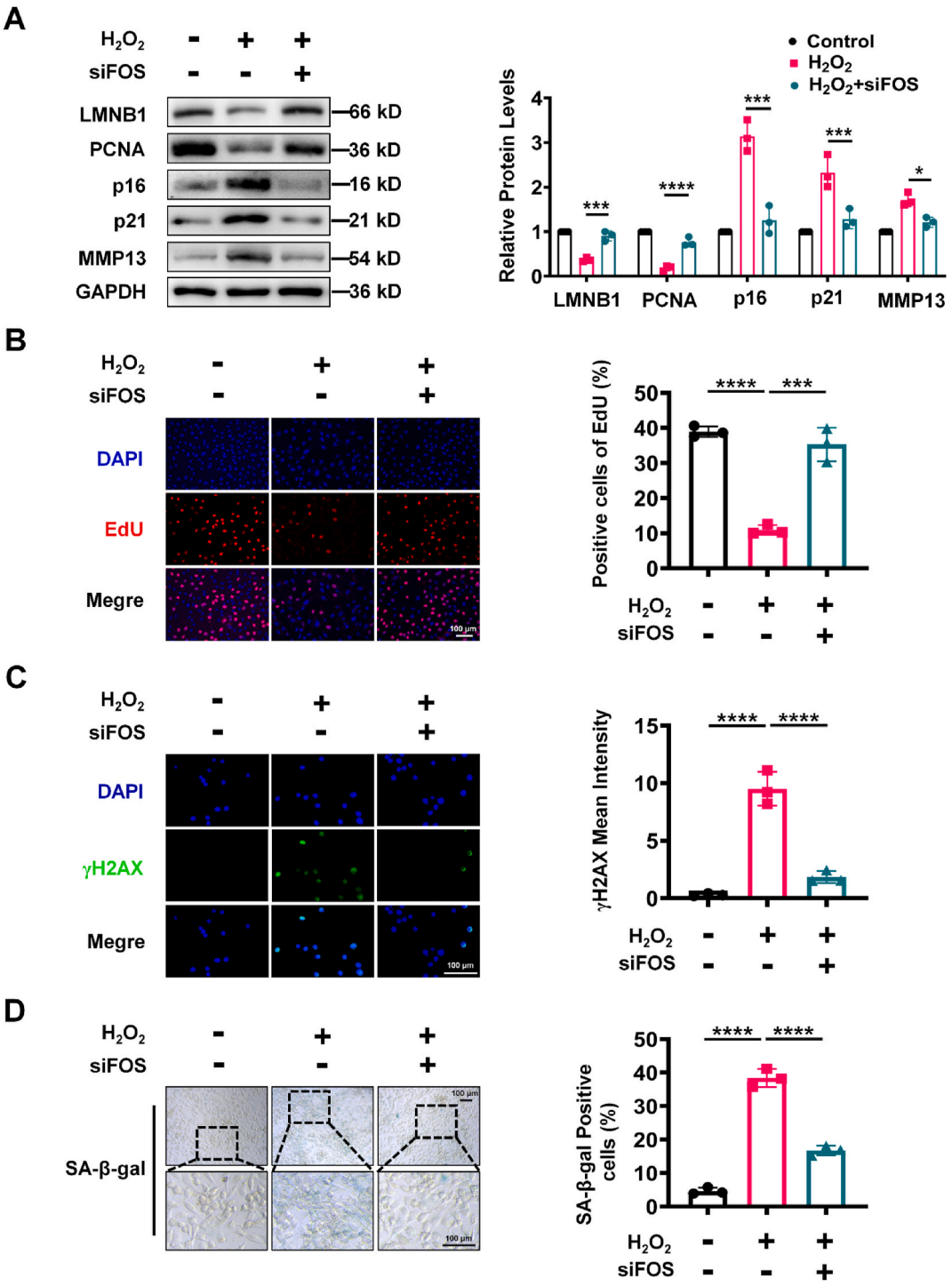

Figure 5. FOS knockdown inhibits senescence and DNA damage of H_2O_2 induced C28/I2 cells. (A) The protein levels of LMNB1, PCNA, p16, p21, MMP13 were investigated in C28/I2 cells by western blotting, n = 3. (B) EdU staining was adopted for cell proliferation capability of C28/I2 cells, n = 3. Scale bar = 100 μm. (C) The protein level of γ-H2AX was investigated in C28/I2 cells by immunofluorescence. Scale bar = 100 μm. (D) SA-β-gal staining was adopted for senescence of C28/I2 cells, n = 3. Scale bar = 100 μm. Statistical analysis was conducted using one-way ANOVA. *P* values were compared between siFOS group or control group and H_2O_2 group. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, ns, no significance.

expressions were remarkably downregulated, FOS, CYR61 and TNFSF15 expressions were obviously upregulated. Next, we further verified the roles and functions of these four candidate genes in the H_2O_2-induced cells and IL-1β stimulated C28/I2 cells and found FOS, CYR61, TNFSF15 expressions were upregulated, MAPK12 expressions were down-regulated *in vitro* chondrocyte senescence and OA models. These indicated that the four senescence-specific hub genes might modulate the occurrence and progression of OA by regulating senescence.

MAPK12, also known as p38γ, is described to contribute local phosphorylation in the nucleus [34]. Previously, MAPK12 plays an important role in regulating cell cycle of various cancers. For instance, MAPK12 can promote the proliferation and movement, inhibit the apoptosis of esophageal squamous cell carcinoma (ESCC) cells *in vitro* [35]. In colorectal cancer, MAPK12 deletion could result in decreased

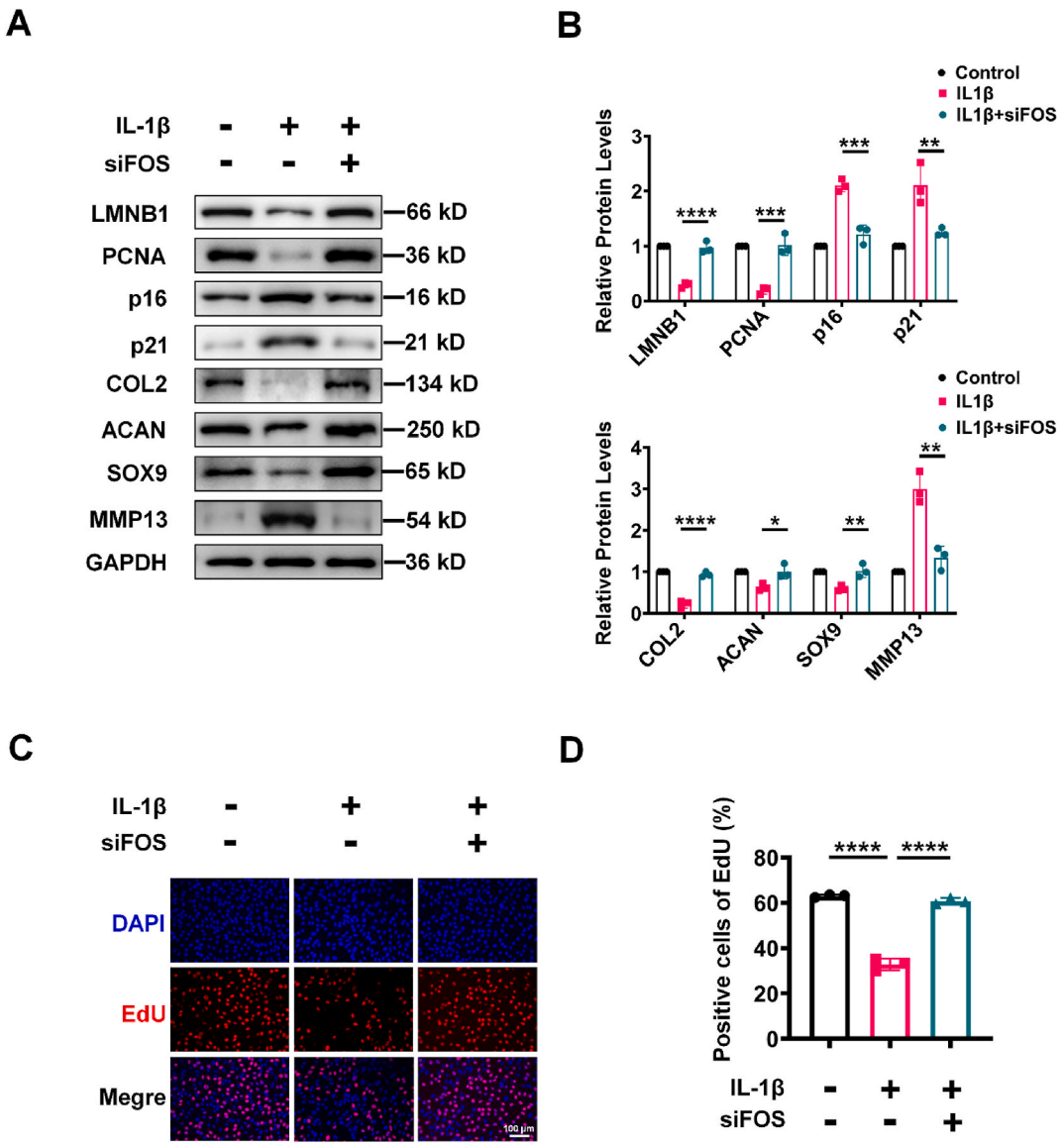

Figure 6. FOS knockdown promotes cartilage anabolism and proliferation, inhibits cartilage catabolism and senescence of IL-1β stimulated C28/I2 cells. (A, B) The protein levels and quantification of LMNB1, PCNA, p16, p21, COL2, ACAN, SOX9, MMP13 were investigated in C28/I2 cells by western blotting, n = 3. (C, D) Representative image and quantification of EdU staining adopted for cell proliferation ability of C28/I2 cells, n = 3. Scale bar = 100 μm. Statistical analysis was conducted using one-way ANOVA. *P* values were compared between siFOS group or control group and IL-1β group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns, no significance.

Rb phosphorylation, CyclinE1 and CyclinA level [17]. MAPK12 silence in the pancreatic duct epithelial cells (KPC) lead to blocking of cell cycle from G1 to S phase, which was associated with lower expressions of CDK6 and p-Rb [18]. p38MAPK and phosphoinositide-3-kinase (PI3K) pathway are reported to be involved in the development and regulation of SASP. Different components of SASP can control aging states by modelating or transmitting SASP through autocrine or paracrine signaling pathways. It is dynamically and temporally, regulated at multiple different levels such as secretion, mRNA stability and translation, chromatin modification and transcription [36,37]. Previous research revealed that p38γ activation reduced MMP13 production in human chondrocytes [38]. Nevertheless, the role of MAPK12 regulating chondrocyte senescence in OA has not been reported. Particularly, we paid close attention to the effect of MAPK12 in OA.

On the other hand, FOS (Fos proto-oncogene) has been implicated as regulators of cell proliferation, differentiation, apoptosis and transformation. Previous research reports that FOS was significantly over-expressed in OA [22,23]. The specific inhibition of Fos repressed the transactivation of downstream MMPs and inflammatory cytokines, thus preventing cartilage destruction [39]. Over-expression of FOS promoted expression of cartilage catabolism gene ADAMTS5 induced by IL-1β in human chondrocytes [40]. However, whether and how FOS affects the occurrence and development of OA by regulating cell senescence has not been known yet. In the present study, we found that over-expression of MAPK12 or siFOS can promote expression of cell proliferation markers CDK2, CDK4, CDK6, CyclinA1, CyclinD1, CyclinE1, LMNB1, PCNA, as well as cell proliferation rate, inhibit expression of cell senescence and SASP markers p16, p21, MMP13, SA-β-Gal level in the H_2O_2-induced senescence model, thus blocking cell cycle arrest, DNA damage and cell senescence. And we further confirmed that either over-expression of MAPK12 or knockdown of FOS can remarkedly promote proliferation genes and cartilage anabolism gene, including CDK2, CDK4, CDK6, CyclinA1, CyclinD1, CyclinE1, LMNB1, PCNA, Col2, ACAN, SOX9, whereas clearly inhibit senescence, SASP and cartilage catabolism genes, such as p16, p21, IL6, TNFα, MMP13, ADAMTS5 *in vitro* OA model. Furthermore, *in vivo* DMM model, we found MAPK12

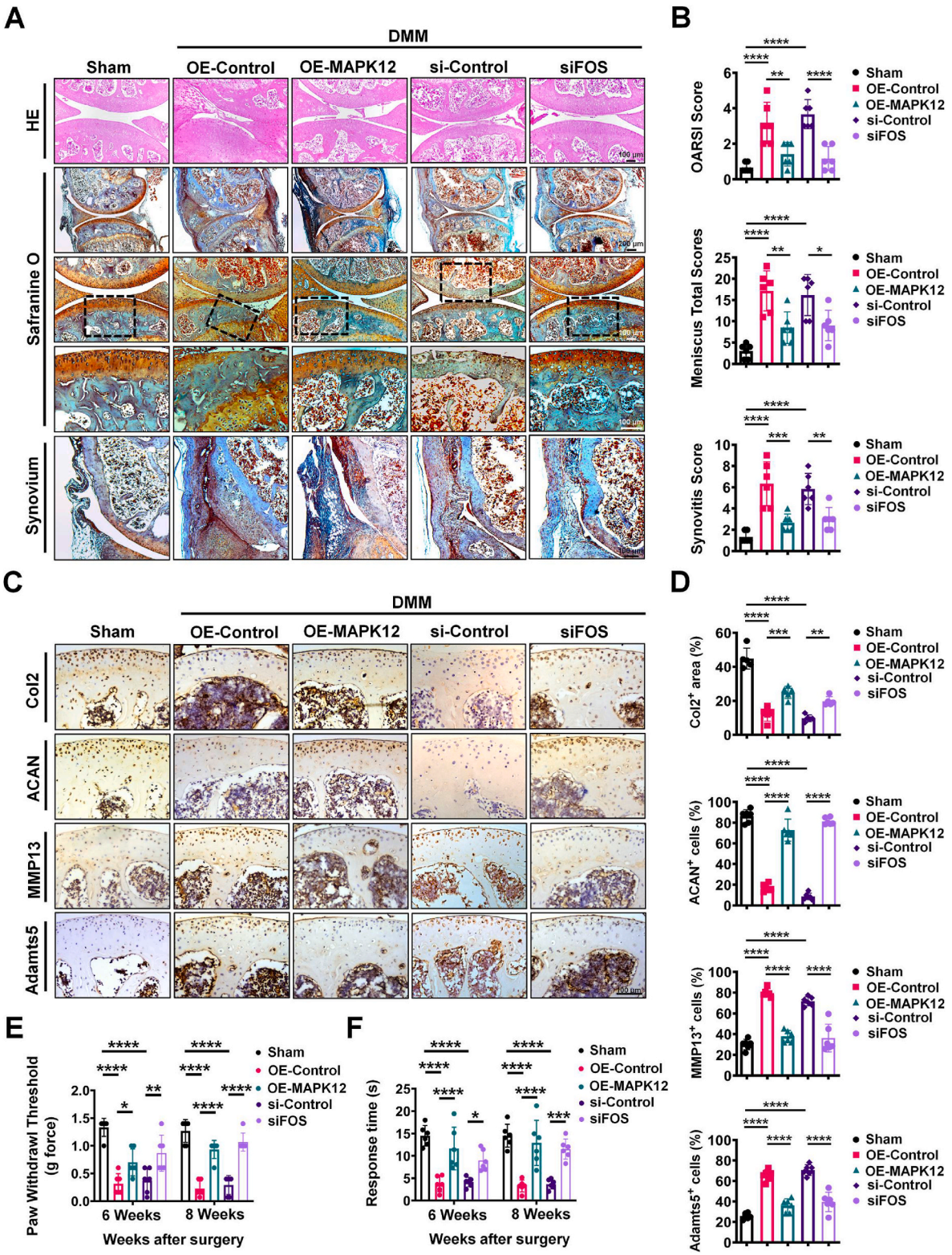

Figure 7. MAPK12 over-expression and FOS deficiency relieve osteoarthritis and joint pain in OA. (A) Representative images of HE, Safranine O staining and synovium in mice knee joint with or without MAPK12 over-expression or FOS knock-down adenovirus, n = 6. (B) OARSI score, meniscus total scores and synovitis score of mice knee joint with or without MAPK12 over-expression or FOS knock-down adenovirus, n = 6. (C, D) Representative images and quantification of Col2, ACAN, MMP13, Adamts5 protein levels in the knee cartilage of mice by immunohistochemistry, n = 6. Scale bar = 100 μm. (E) Paw withdrawl threshold of mice hind paw was measured using von frey filaments at 6 and 8 weeks post-DMM surgery, n = 6. (F) Response time of mice hind paw retraction or licking was recorded using hot plate at 6 and 8 weeks post-DMM surgery, n = 6. *P* values were compared between OE-MAPK12 and OE-control group, between siFOS group and si-control group, between OE-control or si-control group and sham group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, ns, no significance.

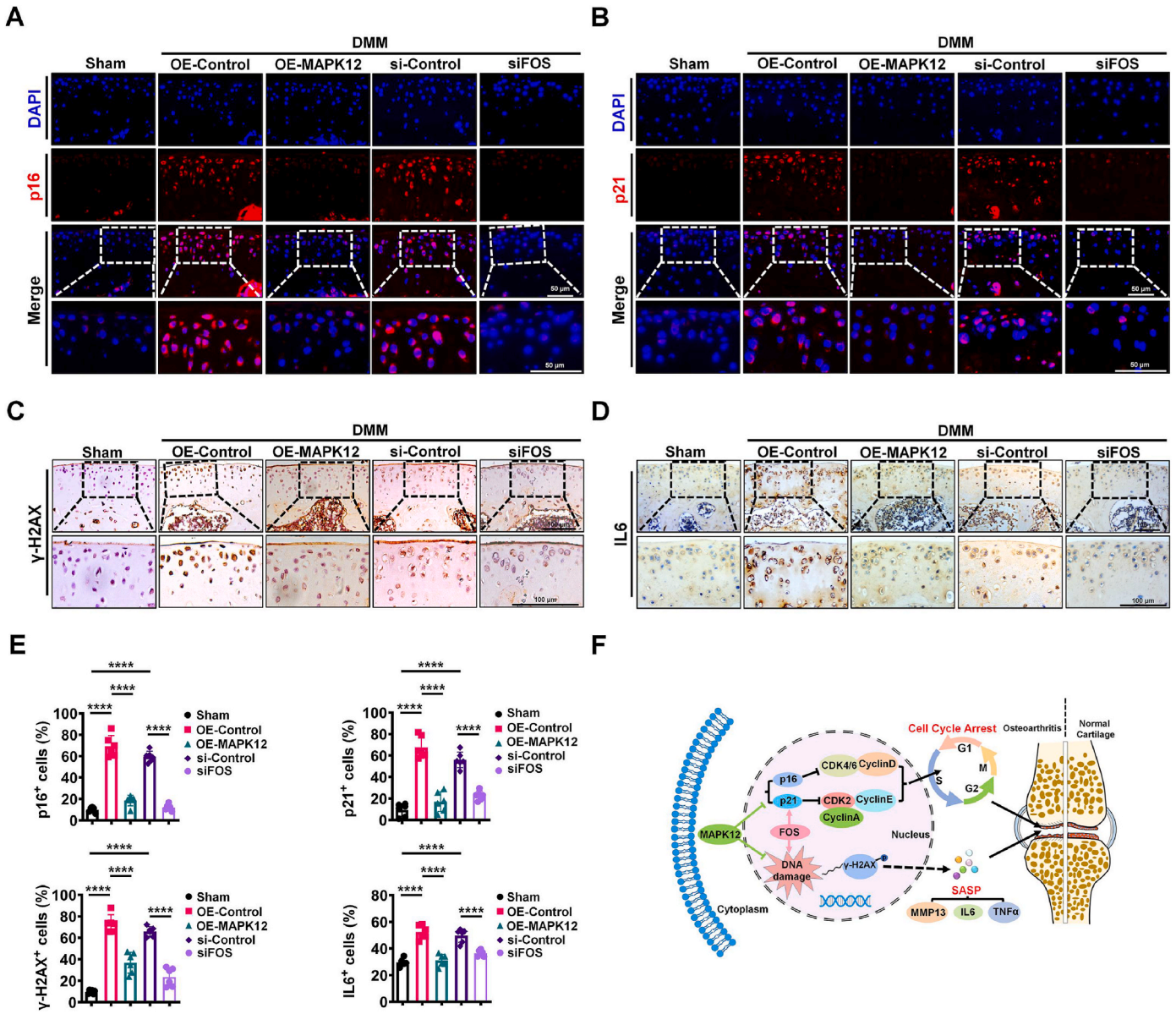

Figure 8. MAPK12 over-expression and FOS deficiency inhibit chondrocyte senescence in OA. (A) p16 protein levels were investigated in knee cartilage of mice by immunofluorescence. Scale bar = 50 μm. (B) p21 protein levels were investigated in knee cartilage of mice by immunofluorescence. Scale bar = 50 μm. (C) γ-H2AX protein levels were investigated in knee cartilage of mice by immunohistochemistry. Scale bar = 100 μm. (D) IL6 protein levels were investigated in knee cartilage of mice by immunohistochemistry. Scale bar = 100 μm. (E) Quantification of p16, p21, γ-H2AX and IL6 protein levels in knee cartilage of mice, n = 6. (F) Schematic illustration of the MAPK12 and FOS signaling pathways in OA by regulating cellular senescence. Statistical analysis was conducted using one-way ANOVA. *P* values were compared between OE-MAPK12 and OE-control group, between siFOS group and si-control group, between OE-control or si-control group and sham group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, ns, no significance.

over-expression or FOS deficiency through articular injection can effectively inhibit chondrocyte senescence, thus relieving the joint cartilage damage, meniscal injury, synovitis and joint pain of mice in OA. Although we detected downregulation of MAPK12 and upregulation of FOS expression in the joint cartilage tissue of 20-month-old aging mice, one limitation of this study was that DMM model was only conducted on 8-week-old mice, without investigating OA in aging mice. Given the increase in senescent chondrocytes with aging, future studies will further investigate the involvement of MAPK12 and FOS in spontaneous osteoarthritis induced by aging.

Collectively, MAPK12 can block cell cycle arrest of G0/G1 and S phases through p16/CDK4, 6/CyclinD and p21/CDK2/CyclinA/E axis respectively, as well as inhibit DNA damage and subsequent SASP including MMP13, IL6, TNFα, thus leading to the protection of chondrocyte against OA. However, FOS can promote p16 and p21 mediated irreversible cell cycle arrest, induce DNA damage response and SASP, thereby leading to senescence of chondrocyte, eventually aggravating OA (Fig. 8F). Therefore, MAPK12 and FOS might be potential therapeutic targets in OA.

5. Conclusions

In summary, we screened out 4 hub DEGs from two public datasets of OA and senescence by bioinformatics analysis. Then MAPK12 and FOS were preliminarily identified by *in vitro* or *in vivo* experiments and OA patient tissues, which might serve as novel senescence-related biomarkers and in OA and potential therapeutic targets in regulating the occurrence and development of OA. In addition, further studies are

needed to explore the depth mechanism associated with senescence of MAPK12 and FOS in OA.

Ethics approval

Approval was granted by the Ethics Committee of Chongqing Medical University (March 23, 2021).

Author contributions

Conceptualization: FJG, NNG. Data curation: NNG, MLX and LD. Formal analysis: NNG, MLX and LD. Investigation: BK, KWL and YLY. Methodology: ZXB, MTF and YMP. Validation: NNG, MLX, and LD. Writing-original draft: NNG. Writing-review & editing: FJG, NNG. Final approval of the article: FJG. All authors contributed to the article and approved the submitted version.

Declaration of competing interest

The authors declare that the paper was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81871769, 82272550), Postdoctoral Initial Foundation of Chongqing Medical University (No. R1079), Postdoctoral Research Foundation of China (No. 2021M700632), Chongqing Postdoctoral Science Foundation (No. 2020-379-46), CQMU Program for Youth Innovation in Future Medicine (W0146).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jot.2024.06.008.

References

[1] Kang D, Lee J, Jung J, Carlson BA, Chang MJ, Chang CB, et al. Selenophosphate synthetase 1 deficiency exacerbates osteoarthritis by dysregulating redox homeostasis. Nat Commun 2022;13(1):779.
[2] Cuollo L, Antonangeli F, Santoni A, Soriani A. The senescence-associated secretory phenotype (sasp) in the challenging future of cancer therapy and age-related diseases. Biology 2020;9(12).
[3] Katz JN, Arant KR, Loeser RF. Diagnosis and treatment of hip and knee osteoarthritis: a review. JAMA 2021;325(6):568–78.
[4] Thorup AS, Strachan D, Caxaria S, Poulet B, Thomas BL, Eldridge SE, et al. Ror2 blockade as a therapy for osteoarthritis. Sci Transl Med 2020;12(561).
[5] Wang KD, Ding X, Jiang N, Zeng C, Wu J, Cai XY, et al. Digoxin targets low density lipoprotein receptor-related protein 4 and protects against osteoarthritis. Ann Rheum Dis 2022;81(4):544–55.
[6] Zhang Y, Li S, Jin P, Shang T, Sun R, Lu L, et al. Dual functions of microrna-17 in maintaining cartilage homeostasis and protection against osteoarthritis. Nat Commun 2022;13(1):2447.
[7] Wiley CD, Campisi J. The metabolic roots of senescence: mechanisms and opportunities for intervention. Nat Metab 2021;3(10):1290–301.
[8] Chen X, Gong W, Shao X, Shi T, Zhang L, Dong J, et al. Mettl3-mediated m(6)a modification of atg7 regulates autophagy-gata4 axis to promote cellular senescence and osteoarthritis progression. Ann Rheum Dis 2022;81(1):87–99.
[9] Kowald A, Passos JF, Kirkwood TBL. On the evolution of cellular senescence. Aging Cell 2020;19(12):e13270.
[10] Conaghan PG, Cook AD, Hamilton JA, Tak PP. Therapeutic options for targeting inflammatory osteoarthritis pain. Nat Rev Rheumatol 2019;15(6):355–63.
[11] Latourte A, Cherifi C, Maillet J, Ea HK, Bouaziz W, Funck-Brentano T, et al. Systemic inhibition of il-6/stat3 signalling protects against experimental osteoarthritis. Ann Rheum Dis 2017;76(4):748–55.
[12] Wang T, He C. Pro-inflammatory cytokines: the link between obesity and osteoarthritis. Cytokine Growth Factor Rev 2018;44:38–50.
[13] Liao CR, Wang SN, Zhu SY, Wang YQ, Li ZZ, Liu ZY, et al. Advanced oxidation protein products increase tnf-alpha and il-1beta expression in chondrocytes via nadph oxidase 4 and accelerate cartilage degeneration in osteoarthritis progression. Redox Biol 2020;28:101306.
[14] Ji ML, Jiang H, Wu F, Geng R, Ya LK, Lin YC, et al. Precise targeting of mir-141/200c cluster in chondrocytes attenuates osteoarthritis development. Ann Rheum Dis 2021;80(3):356–66.
[15] Broeren MG, de Vries M, Bennink MB, van Lent PL, van der Kraan PM, Koenders MI, et al. Functional tissue analysis reveals successful cryopreservation of human osteoarthritic synovium. PLoS One 2016;11(11):e0167076.
[16] Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, et al. Cellular senescence: defining a path forward. Cell 2019;179(4):813–27.
[17] Su C, Sun Q, Liu S, Wang H, Feng L, Cao Y. Targeting p38gamma to inhibit human colorectal cancer cell progression. Biochem Biophys Res Commun 2019;517(1):172–9.
[18] Wang F, Qi XM, Wertz R, Mortensen M, Hagen C, Evans J, et al. P38 gamma mapk is essential for aerobic glycolysis and pancreatic tumorigenesis. Cancer Res 2020;80(16):3251–64.
[19] Shi C, Cheng WN, Wang Y, Li DZ, Zhou LN, Zhu YC, et al. P38gamma overexpression promotes osteosarcoma cell progression. Aging (Albany NY) 2020;12(18):18384–95.
[20] Zhang SP, Teo KYW, Chuah SJ, Lai RC, Lim SK, Toh WS. Msc exosomes alleviate temporomandibular joint osteoarthritis by attenuating inflammation and restoring matrix homeostasis. Biomaterials 2019;200:35–47.
[21] Cao Y, Tang S, Nie X, Zhou Z, Ruan G, Han W, et al. Decreased mir-214-3p activates nf-kappab pathway and aggravates osteoarthritis progression. EBioMedicine 2021;65:103283.
[22] Schonthaler HB, Guinea-Viniegra J, Wagner EF. Targeting inflammation by modulating the jun/ap-1 pathway. Ann Rheum Dis 2011;70(Suppl 1):i109–12.
[23] Grabiec AM, Korchynskyi O, Tak PP, Reedquist KA. Histone deacetylase inhibitors suppress rheumatoid arthritis fibroblast-like synoviocyte and macrophage il-6 production by accelerating mrna decay. Ann Rheum Dis 2012;71(3):424–31.
[24] Safiri S, Kolahi AA, Smith E, Hill C, Bettampadi D, Mansournia MA, et al. Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the global burden of disease study 2017. Ann Rheum Dis 2020;79(6):819–28.
[25] Zheng L, Zhang Z, Sheng P, Mobasheri A. The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. Ageing Res Rev 2021;66:101249.
[26] Xie JW, Wang Y, Lu LY, Liu L, Yu XJ, Pei FX. Cellular senescence in knee osteoarthritis: molecular mechanisms and therapeutic implications. Ageing Res Rev 2021;70.
[27] Gu Y, Yan R, Wang Y, Zeng Y, Yao Q. High trb3 expression induces chondrocyte autophagy and senescence in osteoarthritis cartilage. Aging (Albany NY) 2022;14(13):5366–75.
[28] Lotz MK, Carames B. Autophagy and cartilage homeostasis mechanisms in joint health, aging and oa. Nat Rev Rheumatol 2011;7(10):579–87.
[29] Gao SG, Zeng C, Li LJ, Luo W, Zhang FJ, Tian J, et al. Correlation between senescence-associated beta-galactosidase expression in articular cartilage and disease severity of patients with knee osteoarthritis. International Journal of Rheumatic Diseases 2016;19(3):226–32.
[30] Richard D, Liu Z, Cao J, Kiapour AM, Willen J, Yarlagadda S, et al. Evolutionary selection and constraint on human knee chondrocyte regulation impacts osteoarthritis risk. Cell 2020;181(2):362–381 e28.
[31] Philipot D, Guerit D, Platano D, Chuchana P, Olivotto E, Espinoza F, et al. P16ink4a and its regulator mir-24 link senescence and chondrocyte terminal differentiation-associated matrix remodeling in osteoarthritis. Arthritis Res Ther 2014;16(1):R58.
[32] Xie J, Huang Z, Yu X, Zhou L, Pei F. Clinical implications of macrophage dysfunction in the development of osteoarthritis of the knee. Cytokine Growth Factor Rev 2019;46:36–44.
[33] Diekman BO, Sessions GA, Collins JA, Knecht AK, Strum SL, Mitin NK, et al. Expression of p16(ink) (4a) is a biomarker of chondrocyte aging but does not cause osteoarthritis. Aging Cell 2018;17(4):e12771.
[34] Xu WT, Liu R, Dai Y, Hong SC, Dong HK, Wang H. The role of p38 gamma in cancer: from review to outlook. Int J Biol Sci 2021;17(14):4036–46.
[35] Zheng ST, Yang CC, Liu T, Liu Q, Dai F, Sheyhidin I, et al. Clinicopathological significance of p38 beta, p38 gamma, and p38 delta and its biological roles in esophageal squamous cell carcinoma. Tumor Biol 2016;37(6):7255–66.
[36] Freund A, Patil CK, Campisi J. P38mapk is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 2011;30(8):1536–48.
[37] Bent EH, Gilbert LA, Hemann MT. A senescence secretory switch mediated by pi3k/akt/mtor activation controls chemoprotective endothelial secretory responses. Gene Dev 2016;30(16):1811–21.
[38] Long DL, Loeser RF. P38gamma mitogen-activated protein kinase suppresses chondrocyte production of mmp-13 in response to catabolic stimulation. Osteoarthritis Cartilage 2010;18(9):1203–10.
[39] Motomura H, Seki S, Shiozawa S, Aikawa Y, Nogami M, Kimura T. A selective c-fos/ap-1 inhibitor prevents cartilage destruction and subsequent osteophyte formation. Biochem Biophys Res Commun 2018;497(2):756–61.
[40] Ji QB, Xu XJ, Zhang Q, Kang L, Xu YM, Zhang K, et al. The il-1 beta/ap-1/mir-30a/adamts-5 axis regulates cartilage matrix degradation in human osteoarthritis. Journal of Molecular Medicine-Jmm 2016;94(7):771–85.