eIF5A downregulated by mechanical overloading delays chondrocyte senescence and osteoarthritis by regulating the CREBBP-mediated Notch pathway

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Aims

To examine how eukaryotic translation initiation factor 5A (eIF5A) regulates osteoarthritis (OA) during mechanical overload and the specific mechanism.

Methods

Histological experiments used human bone samples and C57BL/6J mice knee samples. All cell experiments were performed using mice primary chondrocytes. Messenger RNA (mRNA) sequencing was performed on chondrocytes treated with 20% cyclic tensile strain for 24 hours. Western blot (WB) and quantitative polymerase chain reaction were employed to detect relevant indicators of cartilage function in chondrocytes. We created the destabilization of the medial meniscus (DMM) model and the mechanical overload-induced OA model and injected with overexpressing eIF5A adenovirus (eIF5A-ADV). Cartilage degeneration was evaluated using Safranin O/Fast Green staining. Relative protein levels were ascertained by immunohistochemistry (IHC) and immunofluorescence (IF) staining.

Results

After OA initiation, eIF5A caused an upregulation of type II collagen (COL2) and a downregulation of matrix metalloproteinase 13 (MMP13), P16, and P21, which postponed the aggravation of OA. Further sequencing and experimental findings revealed that eIF5A knockdown accelerated the progression of OA by boosting the expression of histone acetyltransferase cyclic-adenosine monophosphate response element binding protein (CREB)-binding protein (CREBBP) to mediate activation of the Notch pathway.

Conclusion

Our findings identified a crucial functional mechanism for the onset of OA, and suggest that intra-articular eIF5A injections might be a useful therapeutic strategy for OA treatment.

Article focus

- Our study focused on the effects of mechanical stress and eukaryotic translation initiation factor 5A (eIF5A) on osteoarthritis (OA).
- We investigated the protective effect of eIF5A on chondrocytes in two different animal models.

Bone & Joint Research

Key messages

- Excessive mechanical stress can aggravate inflammation and senescence of chondrocytes.
- eIF5A is downregulated in OA chondrocytes induced by excessive mechanical stress, and affects intra-articular homeostasis mainly through cyclic-adenosine monophosphate response element binding protein (CREB)-binding protein (CREBBP) and Notch pathway.

Strengths and limitations

- The function of the eIF5A/CREBBP/Notch pathway axis in OA was revealed for the first time.
- We validated eIF5A in destabilization of the medial meniscus and the mechanical overload models.
- The specific mechanism between eIF5A and the Notch pathway remains to be investigated.

Introduction

Osteoarthritis (OA) is a chronic degenerative disease causing chronic pain and long-term disability in middle-aged and older people.[1-3] The pathogenesis of OA is related to many factors.[4] Degeneration of the cartilage extracellular matrix (ECM) is one of the hallmarks of OA, manifested by reduced type II collagen content.[5,6] Furthermore, a significant amount of matrix metalloproteinase (MMP), an essential enzyme for the degradation of type II collagen, is generated during the development of OA.[7,8] Articular cartilage breakdown is primarily caused by an imbalance resulting from a considerably greater increase in catabolic factors than anabolic factors.[9] The main pathological change in OA is the degeneration of cartilage. Therefore, it is particularly important to study chondrocyte senescence. Senescent cells exhibit positive senescence-associated β-galactosidase (SA-β Gal) staining and release factors related to secretory phenotypes associated with senescence.[10,11]

Appropriate mechanical loading may maintain the dynamic balance of the internal environment of the articular cartilage.[12,13] However, excessive mechanical loading is also an important risk factor for the development of OA.[14] Obesity, joint instability, and aberrant movement patterns can all contribute to excessive mechanical loading, which damages cartilage and alters the structural makeup of joints.[15] Two recent studies discovered that excessive mechanical loading leads to chondrocyte senescence and cartilage degeneration in mice, exacerbating the course of OA.[11,16]

Eukaryotic translation initiation factor 5A (eIF5A) is a highly evolutionarily conserved translation factor that is necessary for sustaining cell activity.[17] The primary function of eIF5A is to promote the elongation of messenger RNA (mRNA) encoding a specific peptide motif sequence.[18] It can also enhance translation termination by promoting the release of the peptide chain.[19,20] Nevertheless, it remains unclear how eIF5A and OA are related.

In this study, we tested the hypothesis that excessive mechanical stress affects eIF5A expression in chondrocytes. Additionally, we discovered that eIF5A regulates the Notch signalling pathway via cyclic-adenosine monophosphate (AMP) response element binding protein (CREB)-binding protein (CREBBP), which in turn affects chondrocyte function.

Methods

Human cartilage sample

The tibial plateau of five patients undergoing knee arthroplasty surgery (all with OA) was chosen. The OA group had substantial cartilage deterioration on the medial tibial plateau, whereas the control group had modest cartilage destruction on the lateral tibial plateau. After gaining informed consent, this study was carried out with approval from the Ethics Committee of the Third Affiliated Hospital of Southern Medical University. Histological analysis of the human cartilage sample is shown in the Results section.

Primary chondrocytes extract and culture

Primary chondrocytes from five-day-old mice were cultured in a complete medium containing Dulbecco's modified Eagle's medium F12 (DMEM-F12) (Gibco; Thermo Fisher Scientific, USA) with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin. To create an in vitro cellular OA model, chondrocytes were seeded in silicone films and cultured using a Flexell-5000 mechanical tensile system with 20% cyclic tensile stress at 0.5 Hz. The controls on the same plate were given no stress stimulation.

Cell transfection

Related small interfering RNA (siRNA) and overexpression plasmids (Tsingke Biotechnology, China) were used under the reagent instructions. We transferred siRNA/plasmids and a negative control (NC) into chondrocytes using lipofectamine 3000 (Thermo Fisher Scientific), changing the medium with a fresh one after eight hours. Following 48 hours of incubation in a cell incubator, the protein and RNA were retrieved for further analysis. The siRNA sequences used are listed in Supplementary Table i.

Total RNA extraction and qPCR analysis

Total RNA was extracted from the primary chondrocytes by TRIzol reagent (Takara Bio, Japan). Next, 1 mg of total RNA was purified with a genomic DNA remover, and reverse transcription was performed with 5 HiScrip II qRT SuperMix II (Vazyme Biotech, China). Amplification of total RNA using specific target gene primers and quantitative polymerase chain reaction (qPCR) was performed on LightCycler 96 (Roche, Switzerland). The primers used are listed in Supplementary Table ii.

Western blot analysis

The treated chondrocytes were lysed by radioimmunoprecipitation assay (RIPA) buffer (Jiangsu Beidase Institute of Biotechnology, China). The quantified proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene difluoride (PVDF) membrane (Beyotime Biotechnology, China). Blocking was performed with 5% skim milk in 50 mM Tris-buffered saline (TBS) (pH 7.4) containing 0.1% Tween-20 (TBST) buffer configuration, followed by overnight blot incubation at 4°C using specific primary antibodies. The next day, it was incubated with horseradish peroxidase-conjugated secondary antibodies (1:4,000) at room temperature and visualized in the chemiluminescence apparatus. The following primary antibodies were used for this Western blot (WB) analysis: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:10,000, HA721136; HUABIO, USA), eIF5A

(1:1,000, A2016; ABclonal, Germany), MMP13 (1:1,000, A11148; ABclonal), type II collagen (COL2) (1:1,000, A1560; ABclonal), P21 (1:1,000, ab109199; Abcam, UK), P16 (1:2,000, ab211542; Abcam), Notch intracellular domain (NICD) (1:1,000, 20687-1; Proteintech, USA), and CREBBP (1:1,000, A14237, ABclonal).

Senescence-associated β-galactosidase staining

We configured the dyeing working solution according to the SA-βGal (G1580; Solarbio, China) reagent instructions. The chondrocytes were treated with the fixation solution in the kit for 15 minutes at room temperature, then rinsed with phopshate-buffered saline and left exposed to the dyeing working solution overnight.

Animal model

All animal experiments were approved by the Southern Medical University Committee Animal Care and Use Committee (SMUL2021014) and followed the ARRIVE guidelines (36 mice in total). An ARRIVE checklist is included in the Supplementary Material. Animals were randomly divided into six groups, and eight-week-old male C57BL/6J mice were purchased and then maintained in a new experimental environment until ten to 12 weeks, followed by each of the two OA models. Before the destabilization of the medial meniscus model (DMM) surgery, we anaesthetized ten-week-old male C57BL/6J mice with sodium 1% pentobarbital (50 mg/kg weight) intraperitoneal injection. The connecting ligament of the medial meniscus and the tibia were cut open to destabilize the medial meniscus. Sham-operated mice only cut and closed the joint cavity. To achieve mechanical overload-induced OA, 60 cycles of 13.5 N axial compressive loads (twice per week) were applied to the knee joints of the anaesthetized mice. Sham-operated mice were subjected to a 0.5 N static axial compressive load on their knees using the same method.

Intra-articular adenovirus injection

We randomly allocated mice to three groups: sham surgery, injection with NC adenovirus, and injection with overexpressing eIF5A adenovirus (n = 6 per group). Adenovirus-mediated overexpression of eIF5A (ADV-eIF5A), sourced from Tsingke Biotechnology, was injected after surgery in both DMM and mechanical overload-induced OA models until four weeks later. Adenovirus injections of 5 nmol were given weekly, while the control group was given the same dose of NC.

Dynamic weightbearing test

In modelled mice (DMM and 13.5 N modelling groups), spontaneous pain was measured weekly using the static weightbearing weakness test (Bioseb, France). Measurement of pain is based on a previously published article.[21] Supplementary Figure c contains specific measurement schematics.

Histological analysis

Joint tissues were fixed with 4% Paraformaldehyde Fix Solution for 24 hours and decalcified in 0.5 M EDTA decalcified solution in a 37°C shaker for 14 days. After paraffin embedding, 4 μm thick serial mid-sagittal sections were continuously cut. Deparaffinization, rehydration, and Safranin O/Fast Green staining were performed. The Safranin O/Fast Green staining was scored according to the Osteoarthritis Research Society International (OARSI) grading system.[22]

Immunohistochemistry and immunofluorescence staining

Paraffin-sectioned specimens were processed as described in the previous paragraph. Antigen repair was performed with Tris-EDTA (TE9.0) at pH 9.0 for three hours at 65°C. To inactivate the endogenous peroxidase activity, a 3% concentration of hydrogen peroxide was added and reacted for ten minutes. Non-specific binding sites were blocked with 1% goat serum at room temperature for one hour and incubated with relevant primary antibodies overnight at 4°C. The next day, for immunohistochemistry (IHC), the sections were incubated with horseradish peroxidase-labelled secondary antibody (Jackson ImmunoResearch Laboratories, USA) for one hour at room temperature, then stained with 3,3-diaminobenzidine (DAB) and haematoxylin, dehydrated, and sealed. For immunofluorescence (IF), secondary antibodies were incubated with matched Alexa 594 dye (Life Technologies, Thermo Fisher Scientific) at room temperature for one hour, and then nuclei were labelled with 4,6-diamidino-2-phenylindole (DAPI). The primary antibodies used were: eIF5A (1:200, A2016; ABclonal), MMP13 (1:100, A11148; ABclonal), COL2 (1:1,000, A1560; ABclonal), P21 (1:1,000, ab109199; Abcam), P16 (1:200, ab211542; Abcam), NICD (1:200, 20687-1; Proteintech), and CREBBP (1:200, A14237; ABclonal).

Statistical analysis

All experiments were performed in duplicate or triplicate, with the data presented as the mean (SD). The data were analyzed with GraphPad Prism 9.0.0 (GraphPad Software, USA). An independent-samples *t*-test was used to analyze the two independent groups. For the comparison of the three datasets, one-way analysis of variance (ANOVA) was used. Statistical significance was set at $p < 0.05$.

Results

eIF5A is reduced in articular cartilage in OA patients and OA mice

To identify the potential mechanism of excessive mechanical stress with OA, we analyzed the mRNA expression profiles of control chondrocytes and cyclic tensile stretched chondrocytes. Differential expression analysis revealed that *eIF5A* was identified as the most upregulated gene (Figure 1a and Supplementary Figure aa). Next, we used 20% cyclic tensile strain to stretch mouse primary chondrocytes. Using qPCR and WB, it was found that the expression of eIF5A was significantly downregulated in overstressed chondrocytes. Additionally, stretched chondrocytes exhibited a decrease in the synthesis indicator COL2, an upregulation of the catabolic indicator MMP13, and upregulated P21 and P16, indicators of senescence (Figures 1b and 1c, Supplementary Figure ab).

To confirm the expression of eIF5A in the OA cartilage, we used OA cartilage from both humans and mice as material to perform eIF5A Safranin O/Fast Green staining and IHC staining. The results showed that compared with the NC group, the expression of eIF5A from OA patients was decreased (Figures 1d and 1f). Similarly, eIF5A expression was also downregulated with increasing cartilage damage in DMM-OA mice (Figures 1e and 1g).

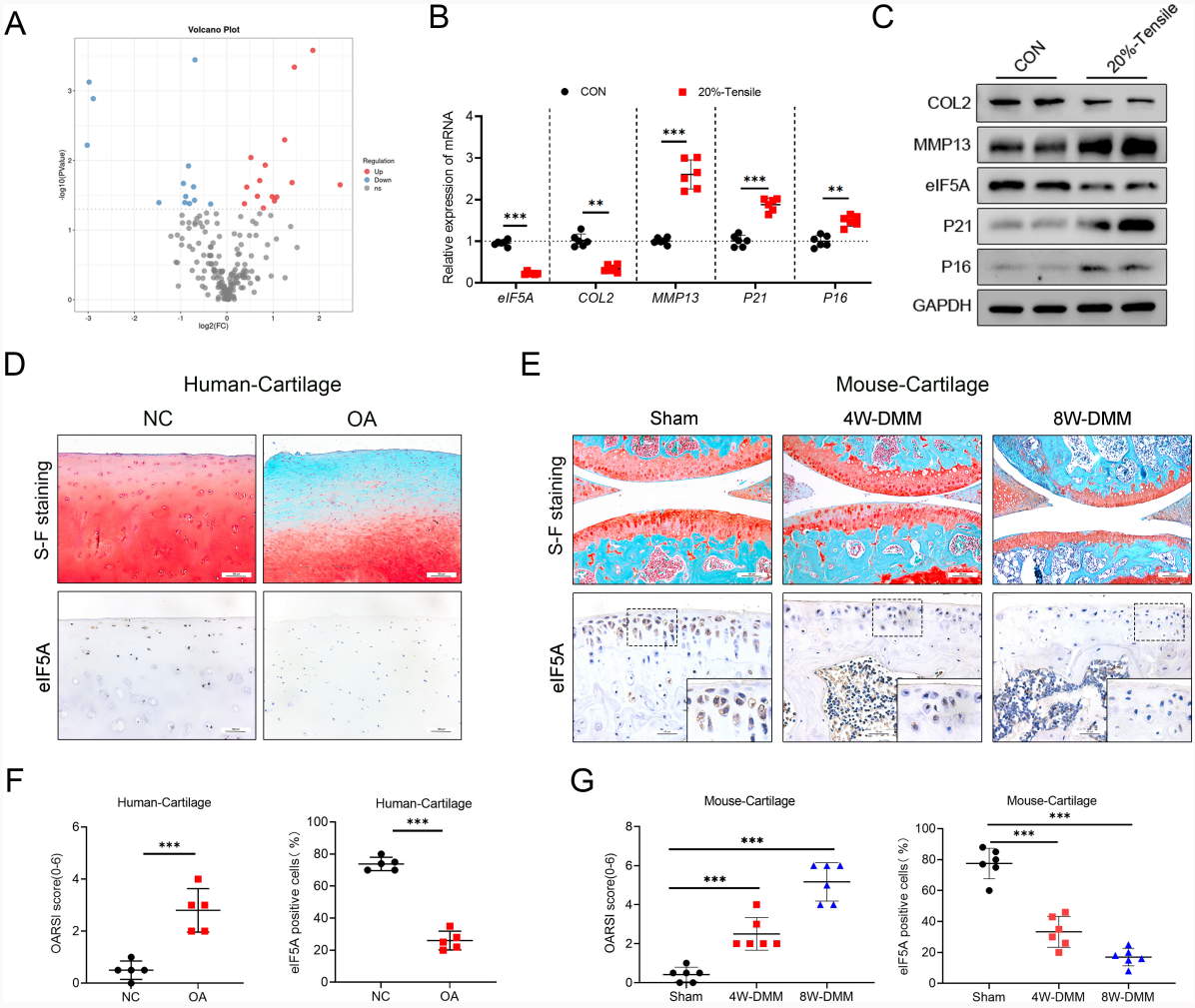

Fig. 1
Eukaryotic translation initiation factor 5A (eIF5A) is reduced in articular cartilage in osteoarthritis (OA) patients and OA mice. a) Volcano plots of differentially expressed genes after stretching. b) Quantitative polymerase chain reaction (qPCR) analysis of eIF5A, type II collagen (COL2), matrix metalloproteinase 13 (MMP13), P21, and P16 in primary chondrocytes treated with 20% cyclic tensile strain for 24 hours; n = 6 per group. c) Western blot (WB) analysis of eIF5A, COL2, and MMP13 in primary chondrocytes treated with 20% cyclic tensile strain for 24 hours. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the loading control. d) Safranin O/Fast Green staining (upper) and immunohistochemistry (IHC) staining of eIF5A (lower) of human knee cartilage from the medial (OA) and lateral (controls) tibial plateau of OA patients. Scale bar: 100 µm. e) Safranin O/Fast Green staining (upper) and IHC of eIF5A (lower) of control group (CON) and destabilization of the medial meniscus (DMM) knee cartilage of C57 mouse. Knee tissue was obtained at four and eight weeks (4W and 8W) after the DMM procedure. Scale bars: 100 µm, 50 µm. f) Osteoarthritis Research Society International (OARSI) grades for human articular cartilage of the medial and lateral tibial plateau in Fig. 1c and quantification of eIF5A in each group; n = 6 per group. g) OARSI grades for the joints described in Fig. 1e and quantification of eIF5A in each group; n = 6 per group. An independent-samples *t*-test and one-way analysis of variance were used for statistical analyses (*p < 0.05, **p < 0.01, ***p < 0.001). FC, fold change; NC, negative control; ns, not significant.

Knockdown/overexpression of *eIF5A* can aggravate or rescue the injury of chondrocytes in OA

To further determine the effects of eIF5A on chondrocytes and OA, eIF5A was silenced using siRNA (Supplementary Figure ac). Therefore, the two most effective knockdown siRNAs were selected for further investigation. The results of qPCR and WB showed that knockdown of eIF5A resulted in downregulation of COL2 expression and upregulation of MMP13, P16, and P21 expression (Figures 2a and 2c). We also used plasmids to overexpress the level of eIF5A in chondrocytes, as determined by qPCR (Supplementary Figure ad). Subsequently, we used 20% cyclic tensile strain to stimulate chondrocytes to induce OA and overexpress eIF5A in the culture environment. qPCR results revealed that after 20% cyclic tensile strain stimulations, eIF5A decreased in primary chondrocytes, as did the expression of COL2. However, the level of MMP13, P16, and P21 increased. Interestingly, the chondrocyte damage caused by 20% cyclic tensile strain can be rescued after OE-eIF5A (Figures 2b and 2d).

Cell IF staining of eIF5A and SA-βGal showed that the degree of chondrocyte senescence was slightly aggravated after eIF5A knockout. Similarly, eIF5A overexpression could rescue SA-βGal staining enhanced by 20%-tensile in primary chondrocytes (Figures 2a to 2h). This result indicates that eIF5A may play a role in delaying chondrocyte ageing.

eIF5A can inhibit cartilage degeneration and delay OA progression in DMM-OA mice

In order to determine the delaying effect of eIF5A on OA, we established a mouse model of DMM and injected adenovirus weekly (Figure 3a). Measuring the mice's pain weekly, we observed that mice showed knee pain after DMM, but the

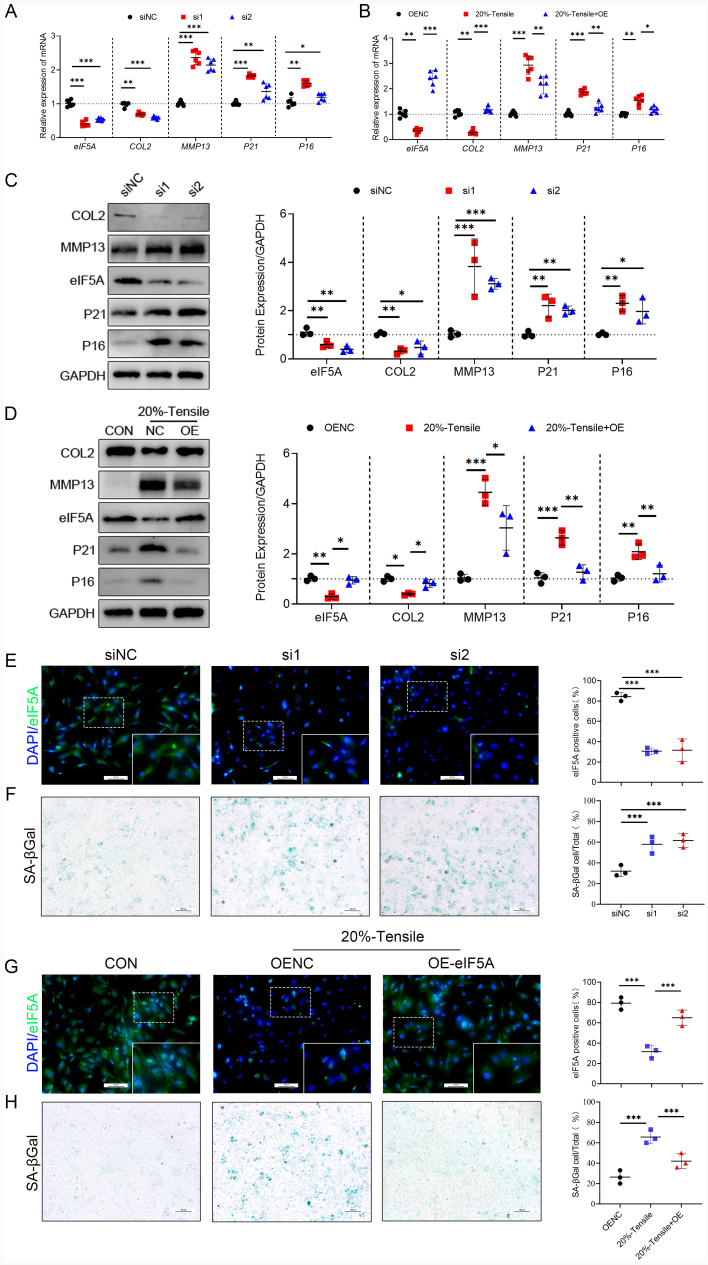

Fig. 2

Knockdown/overexpression of eukaryotic translation initiation factor 5A (eIF5A) can aggravate or rescue the injury of chondrocytes in osteoarthritis (OA). a) Quantitative polymerase chain reaction (qPCR) analysis of *eIF5A*, type II collagen (*COL2*), martrix metalloproteinase 13 (*MMP13*), *P21*, and *P16* in primary chondrocytes transfected with si-eIF5A; n = 6 per group. b) qPCR analysis of *eIF5A, COL2, MMP13, P21*, and *P16* in mouse primary chondrocytes treated with or without OE-eIF5A under 20% cyclic tensile strain; n = 6 per group. c) Western blot (WB) analysis and quantification of eIF5A, COL2, MMP13, P21, and P16 in si-*eIF5A*-transfected primary chondrocytes; n = 3 per group. d) WB analysis and quantification of eIF5A, COL2, MMP13, P21, and P16 in mouse primary chondrocytes treated with or without OE-eIF5A at 20% cyclic tensile strain; n = 3 per group. e) and f) Immunofluorescence (IF) and senescence-associated β-galactosidase (SA-βGal) staining of eIF5A after transfection of primary chondrocytes with si-*eIF5A* and corresponding quantitative plots; n = 3 per group. g) and h) IF and SA-βGal staining of primary mouse chondrocytes treated with or without OE-eIF5A at 20% cyclic tensile strain and corresponding quantification plots; n = 3 per group. An independent-samples *t*-test and one-way analysis of variance were used for statistical analyses (*p < 0.05, **p < 0.01, ***p < 0.001). CON, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; ns, not significant; OE, over-expression.

pain was partially relieved by adenovirus injection (Figure 3b). The mice were euthanized after four weeks, and the knees were fixed and stained with Safranin O/Fast Green. The results show that articular cartilage injury in the exogenous adenovirus injection group was obviously less than in the OA control group (Figures 3c and 3i). Compared with the OA control group, eIF5A and COL2 were elevated (Figures 3d, 3e, 3j, and 3k). Simultaneously, the IF results indicate that MMP13 expression was downregulated (Figures 3f and 3l). In addition, the senescence-related hallmarks P16 and P21 were significantly downregulated after eIF5A injections (Figures 3g, 3h, 3m, and 3n).

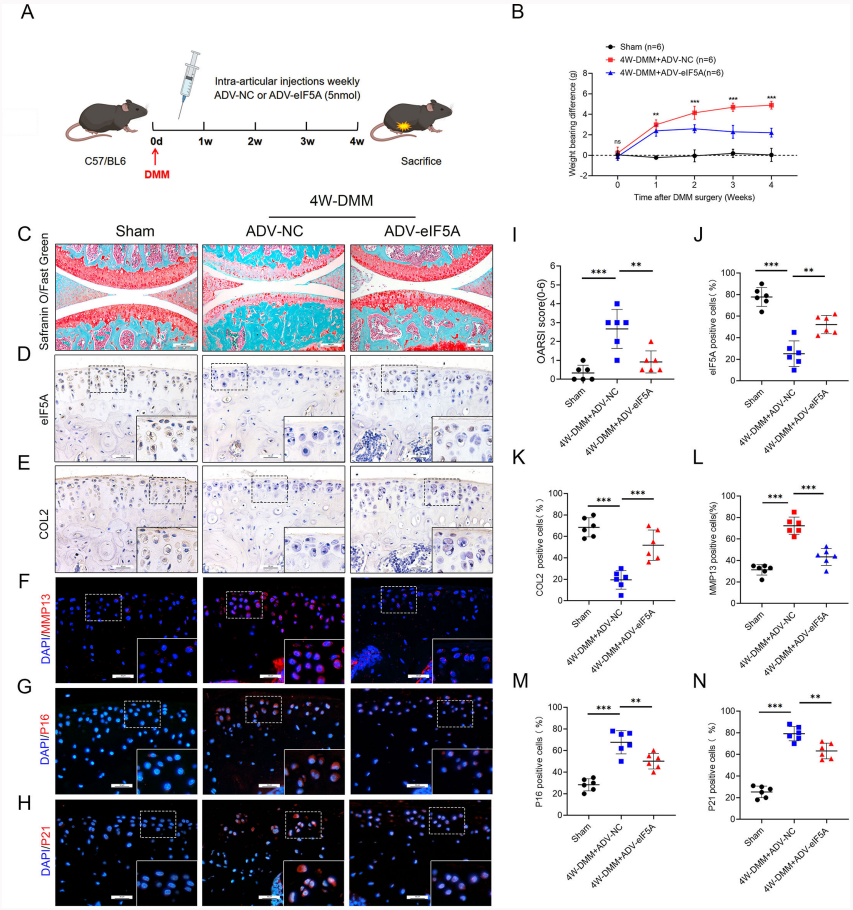

Fig. 3
Eukaryotic translation initiation factor 5A (eIF5A) can inhibit cartilage degeneration and delay osteoarthritis (OA) progression in destabilization of the medial meniscus (DMM)-OA mice. a) Protocol for intra-articular injection of ADV-NC or ADV-eIF5A in medial meniscus instability (DMM) mice. b) Analysis of weekly weightbearing asymmetry in mice. c) Safranin O/Fast Green staining of knee cartilage from control, DMM, and post-DMM eIF5A adenovirus-injected mice. Scale bar: 100 μm. d) and e) Immunohistochemistry (IHC) staining of eIF5A and type II collagen (COL2) in knee cartilage of control, DMM, and post-DMM eIF5A adenovirus-injected mice. Scale bar: 50 μm. f) to h) Immunofluorescence (IF) staining of matrix metalloproteinase (MMP13), P16, and P21 in knee cartilage of control, DMM, and post-DMM eIF5A adenovirus-injected mice. Scale bar: 50 μm. i) to n) Quantitative analysis of the Osteoarthritis Research Society International (OARSI) scoring scale in mice in Fig. 3a and the number of positive particles in Fig. 3d to 3h of articular cartilage; n = 6 per group. One-way analysis of variance was used for statistical analyses (*p < 0.05, **p < 0.01, ***p < 0.001). 4W, four weeks; ADV, adenovirus; NC, negative control; ns, not significant.

Intra-articular injection of eIF5A partially relieved the progression of 13.5 N overload-induced OA

A previous study showed that the articular cartilage of mouse knee joints experienced proteoglycan loss and impairment after being subjected to multiple load fragments at a peak load of 13.5 N.[11] Thus, we developed a model of excessive mechanical loading of 13.5 N. eIF5A adenovirus was injected weekly for four weeks (Figure 4a). Pain was measured weekly, and we found that after mechanical overloading, mice also showed knee pain that was partially relieved by adenovirus injection (Figure 4b). Remarkably, mice with OA induced by 13.5 N overload also experienced cartilage degeneration and joint fibrillation. The expression of eIF5A and COL2 was downregulated, while MMP13 and senescence markers P16 and P21 were upregulated (Figures 4c to 4n). Similarly, the eIF5A adenovirus could reverse this alteration. The above experimental results demonstrate the benign role of eIF5A in experimental OA.

eIF5A suppresses OA by inhibiting the Notch pathways

Next, we sought to determine which molecules and signalling pathways are responsible for chondrocyte degeneration after *eIF5A* knockdown. We analyzed transcript genes and pathways after treating mouse primary chondrocytes with si-eIF5A. The data demonstrated that the Notch pathway was considerably active following si-eIF5A (Figure 5a). Subsequently, we verified gene expression changes in the Notch pathway-related genes by qPCR and found the expression changes in *CREBBP* to be most associated with *eIF5A* (Figure 5b). To confirm the association between downstream molecules and OA, we performed immunohistochemical staining of OA cartilage for Notch pathway markers NICD and CREBBP. Interestingly, NICD and CREBBP expression was significantly upregulated after DMM. Nevertheless, in OA cartilage injected with eIF5A-ADV, NICD and CREBBP expression started to decline (Figures 5c and 5d). Moreover, through qPCR we discovered that overexpressing eIF5A in chondrocytes might somewhat mitigate the stretch-induced elevation of NICD and CREBBP (Figures 5f and 5g).

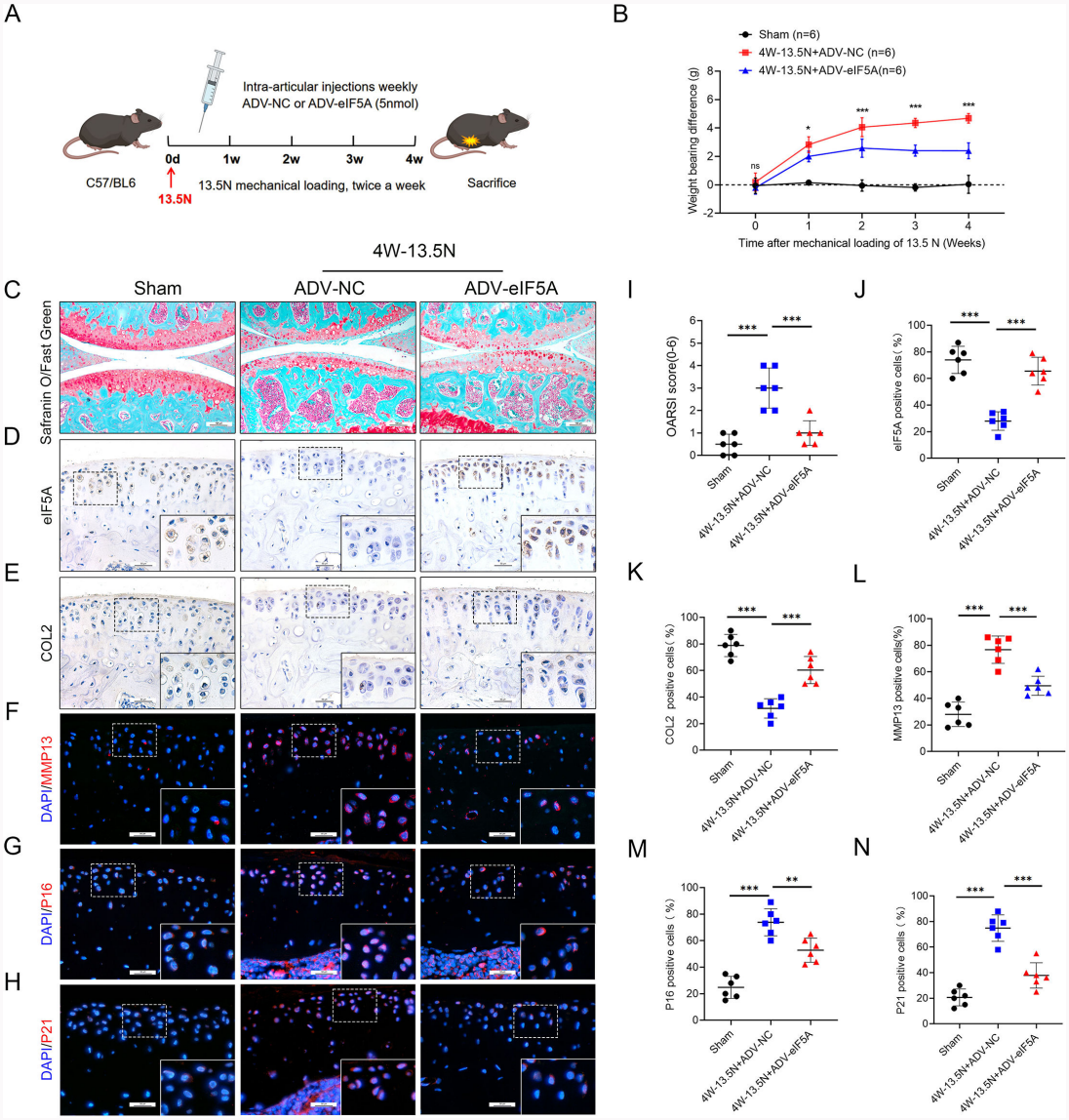

Fig. 4

Intra-articular injection of eukaryotic translation initiation factor 5A (eIF5A) partially relieved the progression of 13.5 N overload-induced osteoarthritis (OA). a) Protocol for intra-articular injection of ADV-NC or ADV-eIF5A in mice after stimulation with an excessive mechanical load of 13.5 N (13.5 N model). b) Analysis of weekly weightbearing asymmetry in mice. c) Safranin O/Fast Green staining of knee cartilage from control, 13.5 N, and post-13.5 N eIF5A adenovirus injected mice. Scale bar: 100 μm. d) and e) Immunohistochemistry (IHC) staining of eIF5A and type II collagen (COL2) in knee cartilage of control, 13.5 N group, and post-13.5 N eIF5A adenovirus-injected mice. Scale bar: 50 μm. f) to h) Immunofluorescence (IF) staining for matrix metalloproteinase (MMP13), P16, and P21 in knee cartilage of control, 13.5 N group, and post-13.5 N eIF5A adenovirus-injected mice. Scale bar: 50 μm. i) to n) Quantitative analysis of the Osteoarthritis Research Society International (OARSI) scoring scale in mice in Fig. 4a and the number of positive particles in Fig. 4d to 4h of articular cartilage; n = 6 per group. One-way analysis of variance was used for statistical analyses (*p < 0.05, **p < 0.01, ***p < 0.001). ADV, adenovirus; NC, negative control; ns, not significant.

eIF5A delays OA through inhibiting CREBBP expression

To further substantiate the connection between eIF5A and downstream CREBBP, we designed and validated siRNA and overexpression plasmids for CREBBP (Supplementary Figures ba and bb). We demonstrated that following 20% stretched chondrocytes, there was a significant rise in NICD and CREBBP, a decrease in the expression of COL2, and an upregulation of the expression of MMP13, P16, and P21. The tensile that caused chondrocyte injury could be rescued by si-CREBBP (Figures 6a and 6c). In the interim, we also verified the therapeutic effect of eIF5A on CREBBP. First, we used the CREBBP overexpression plasmid to stimulate primary chondrocytes and applied eIF5A overexpression to the group at the same time. Unexpectedly, chondrocytes with CREBBP overexpression showed a phenotype similar to that of stretch, with a notable rise in NICD and CREBBP expression. Overexpression of eIF5A concurrently mitigated the impact caused by CREBBP (Figures 6b and 6d). In addition, we also performed SA-βGal staining, which strongly verified CREBBP involvement in chondrocyte ageing (Figures 6e to 6h).

Discussion

This study demonstrates that eIF5A plays a critical role in mediating chondrocyte damage and ageing in OA development. We observed that eIF5A could postpone OA caused by high mechanical stress. This impact was probably brought

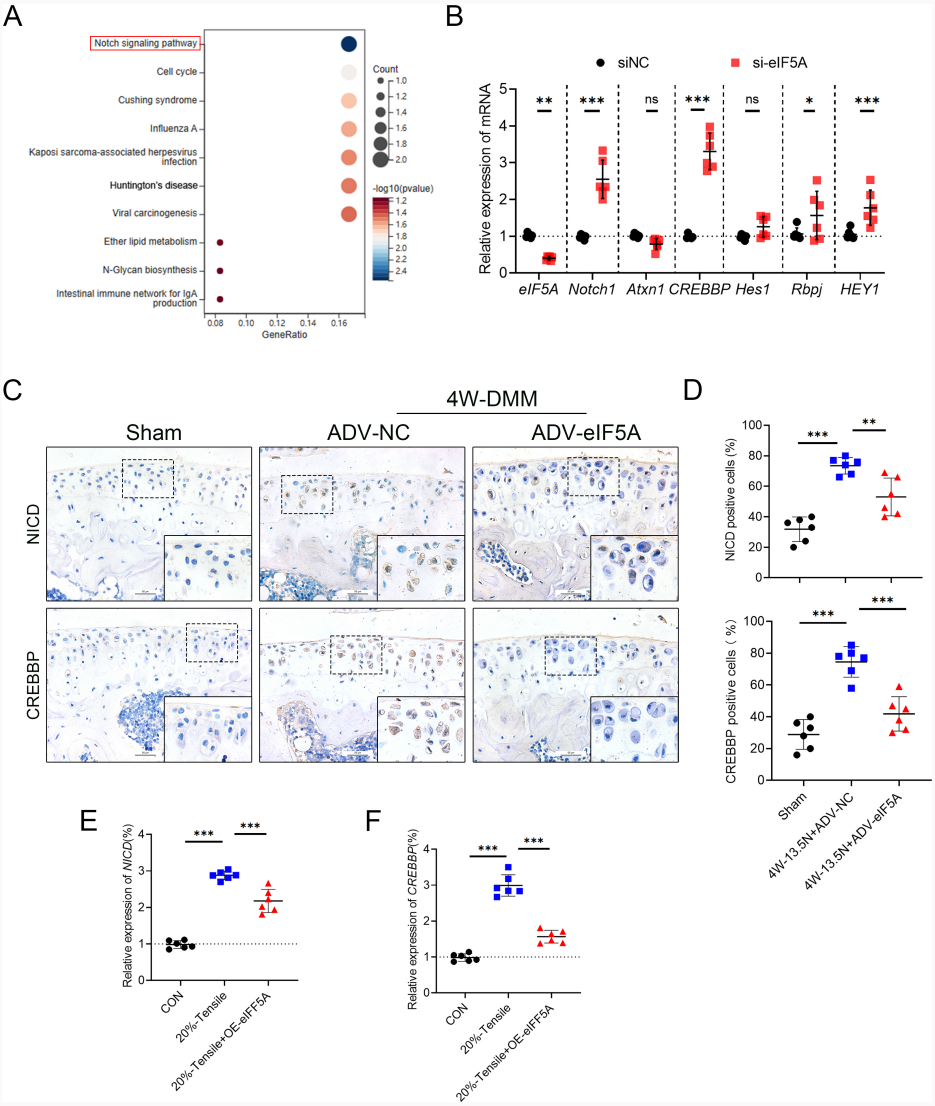

Fig. 5

Notch pathway activation was most pronounced as eukaryotic translation initiation factor 5A (eIF5A) was downregulated. a) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differential genes in chondrocytes after si-eIF5A. b) Quantitative polymerase chain reaction (qPCR) analysis of key molecules of the Notch pathway in chondrocytes after si-eIF5A; n = 6 per group. c) and d) Immunohistochemistry (IHC) staining of Notch intracellular domain (NICD) and cyclic-AMP response element binding protein (CREB)-binding protein (CREBBP) in knee cartilage of control, destabilization of the medial meniscus (DMM), and post-DMM eIF5A adenovirus-injected mice, and quantification of positive particles; n = 6 per group. e) and f) qPCR analysis of NICD and CREBBP in primary mouse chondrocytes treated with or without OE-eIF5A under 20% cyclic tensile strain; n = 6 per group. An independent-samples *t*-test and one-way analysis of variance were used for statistical analyses (*p < 0.05, **p < 0.01, ***p < 0.001). CON, control; IgA, immunoglobulin A; ns, not significant.

about by inhibiting the Notch signalling pathway and downregulating CREBBP (Figure 7). Our findings investigated the function of the eIF5A/CREBBP/Notch pathway axis in OA, suggesting that eIF5A overexpression might be a useful therapeutic strategy for OA.

Recent evidence has revealed that mechanical overloading greatly contributes to OA and cartilage deterioration.[23,24] In a new study, Zhu et al[16] discovered that chondrocyte catabolism and ageing were exacerbated by 0.5 Hz, 20% cyclic tensile strain stimulation for 24 hours. Alternatively, continuous stimulation of C57 mice with 13.5 N of mechanical stress exacerbated the loss of cartilage proteoglycan. These results imply that excessive mechanical stress induces cartilage senility and catabolism. eIF5A is one of the most substantially downregulated genes from our chondrocyte sequencing results following 20% cyclic tensile strain stimulation.

eIF5A, a highly conserved protein that is expressed in humans and many other eukaryotes, is essential for eukaryotic cell proliferation.[25,26] Numerous data point to the connection between eIF5A and cellular anabolism as well as the prevention of senescence.[27,28] Nevertheless, the relationship between eIF5A and chondrocyte degeneration and OA has not been reported. In this study, we created both in vitro and in vivo mechanical OA models to confirm the expression of eIF5A. Furthermore, we discovered that cartilage destruction was successfully reversed in both the DMM and mechanical OA models by intra-articular injection of eIF5A-ADV.

The Notch signalling pathway is critically linked to articular cartilage function and ageing.[29] Research has demonstrated that chondrocytes with elevated expression of

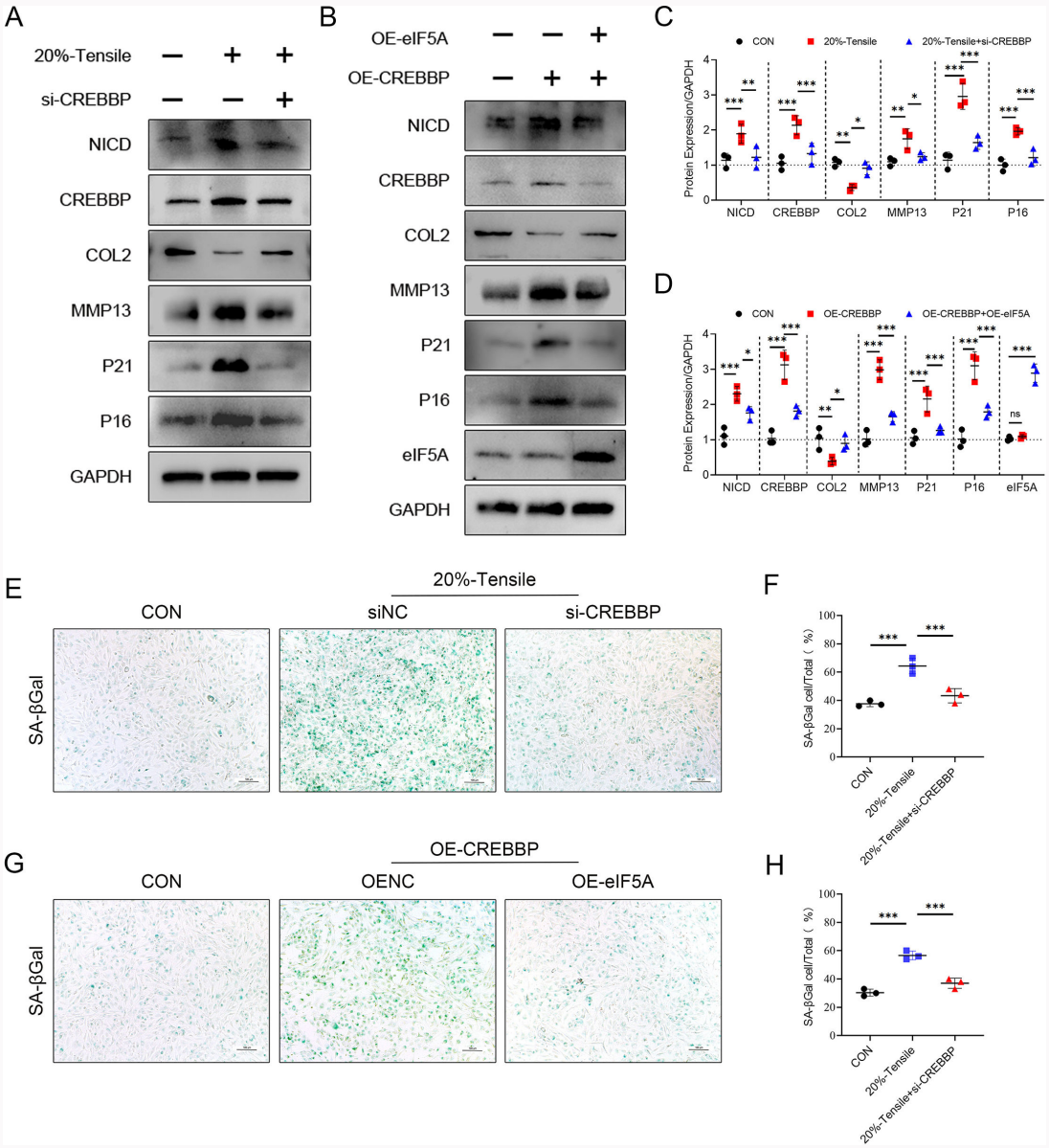

Fig. 6

Eukaryotic translation initiation factor 5A (eIF5A) delays osteoarthritis (OA) through inhibiting cyclic-adenosine monophosphate (AMP) response element binding protein (CREB)-binding protein (CREBBP) expression. a) Western blot (WB) analysis of Notch intracellular domain (NICD), CREBBP, type II collagen (COL2), matrix metalloproteinase (MMP13), P21, and P16 in primary mouse chondrocytes treated with or without si-CREBBP at 20% cyclic tensile strain. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the loading control. b) The WB analysis of NICD, CREBBP, COL2, MMP13, P21, P16, and eIF5A in mouse primary chondrocytes treated with or without OE-eIF5A after OE-CREBBP. GAPDH was used as the loading control. c) and d) Quantification of the expression of each indicator in Fig. 6a and 6b; n = 3 per group. e) and f) Senescence-associated β-galactosidase (SA-βGal) staining of primary mouse chondrocytes treated with or without si-CREBBP at 20% elongation strain loading and corresponding quantification plots; n = 3 per group. g) and h) SA-βGal staining of primary mouse chondrocytes treated with or without OE-eIF5A after OE-CREBBP and corresponding quantification plots; n = 3 per group. One-way analysis of variance was used for statistical analyses (*p < 0.05, **p < 0.01, ***p < 0.001). CON, control; ns, not significant.

NICD upregulate MMP13 levels while exacerbating cellular senescence.[30,31] CREBBP is one of the activating molecules of the Notch pathway.[32,33] Some researchers discovered that the CREBBP/EP300 mutation caused histone H3 lysine 27 acetylation (H3K27) to become deacetylated in clinical patients with diffuse large B cell lymphoma (DLBCL), hence enhancing gene transcription and the Notch signalling pathway.[34] In the present work, after knocking out eIF5A in primary mouse chondrocytes, we observed that the expression level of CREBBP was significantly elevated. CREBBP is most likely to be crucial in establishing a connection between eIF5A and the Notch signalling pathway.[35,36] We next demonstrated that overexpression of CREBBP promoted chondrocyte degeneration and senescence and that subsequent overexpression of eIF5A slowed this process. Conversely, the knockdown of CREBBP reduced the activation of NICD signalling in OA, thereby alleviating OA. These findings imply that CREBBP may activate the Notch pathway, which in turn may worsen the pathophysiology of OA.

However, this study has some limitations. We did not construct transgenic knockout mice to confirm whether a primary decrease in eIF5A would result in OA. Alternatively,

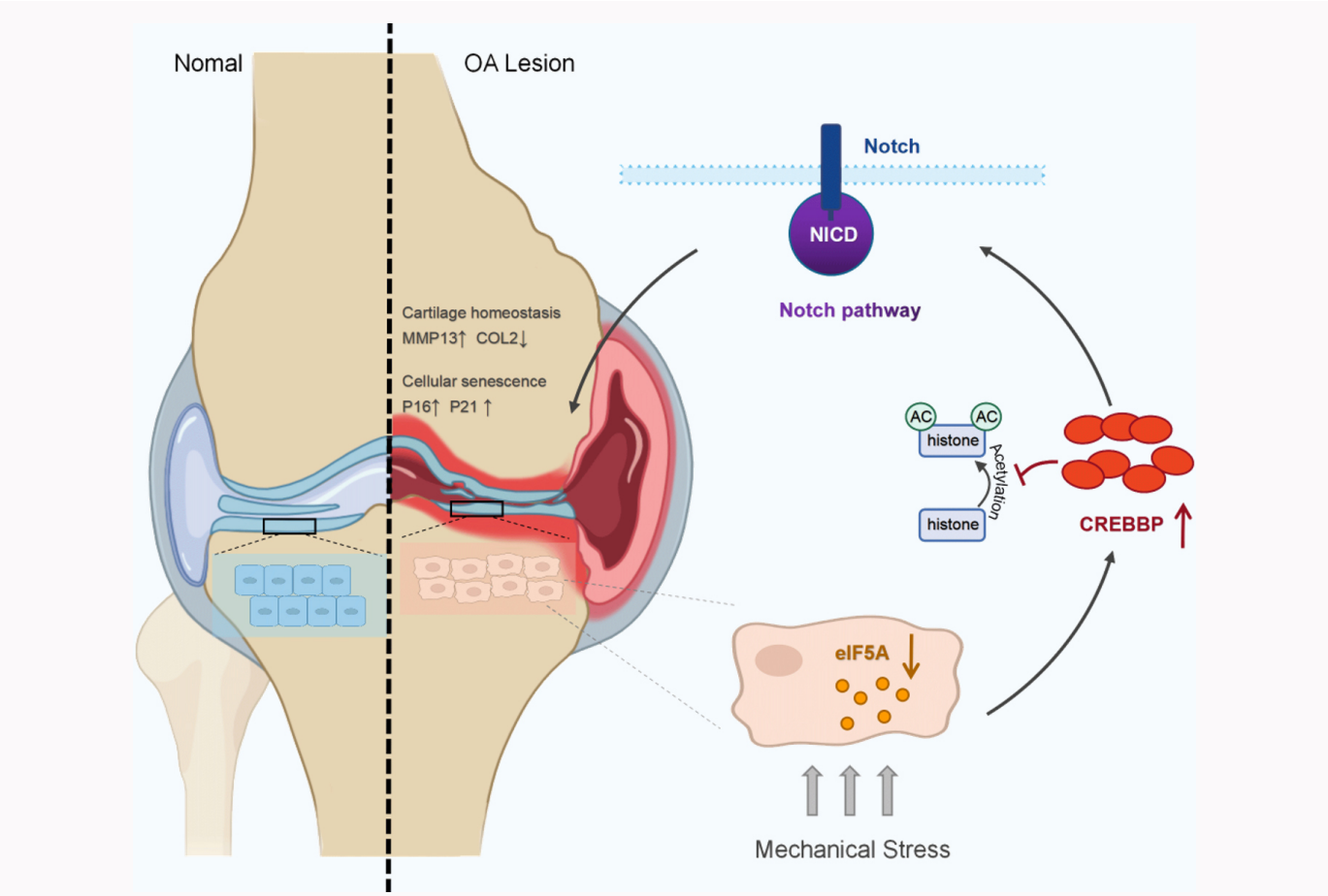

Fig. 7
Model of the specific mechanisms by which eukaryotic translation initiation factor 5A (eIF5A) regulates chondrocyte homeostasis under mechanical loading. Excessive mechanical loading leads to downregulation of eIF5A expression in chondrocytes and affects the Notch pathway via cyclic-adenosine monophosphate (AMP) response element binding protein (CREB)-binding protein (CREBBP), thereby promoting chondrocyte catabolism and senescence and accelerating osteoarthritis (OA) progression. AC, acetylation; NICD, Notch intracellular domain.

the role of eIF5A injection in the joint cavity in relieving joint pain also deserves further exploration. Although CREBBP induction of histone deacetylation to activate Notch signalling has been widely accepted in recent years, and we have found it to be a downstream molecule of eIF5A affecting OA, the precise mechanism of action between eIF5A and CREBBP remains to be investigated.

In conclusion, our study revealed a substantial reduction in eIF5A in OA caused by excessive mechanical stress. Conversely, eIF5A partially reversed OA lesions by delaying cartilage catabolism and chondrocyte ageing. Moreover, sequencing and experimental outcomes verified that eIF5A suppressed Notch signalling by interacting with CREBBP. These findings point to a possible role for eIF5A in the development of OA, suggesting that intra-articular targeted eIF5A injections could be a useful strategy for treating the disease.

Supplementary material

Western blot strips, and all of the primers used for quantitative polymerase chain reaction. An ARRIVE checklist is also included to show that the ARRIVE guidelines were adhered to in this study.

References

1. **He C-P**, **Chen C**, **Jiang X-C**, **et al**. The role of AGEs in pathogenesis of cartilage destruction in osteoarthritis. *Bone Joint Res*. 2022;11(5):292–300.
2. **Lv G**, **Wang B**, **Li L**, **et al**. Exosomes from dysfunctional chondrocytes affect osteoarthritis in sprague-dawley rats through FTO-dependent regulation of PIK3R5 mRNA stability. *Bone Joint Res*. 2022;11(9):652–668.
3. **Mo H**, **Wang Z**, **He Z**, **et al**. Decreased Peli1 expression attenuates osteoarthritis by protecting chondrocytes and inhibiting M1-polarization of macrophages. *Bone Joint Res*. 2023;12(2):121–132.
4. **Zhou Y**, **Li J**, **Xu F**, **Ji E**, **Wang C**, **Pan Z**. Long noncoding RNA H19 alleviates inflammation in osteoarthritis through interactions between TP53, IL-38, and IL-36 receptor. *Bone Joint Res*. 2022;11(8):594–607.
5. **Chen M-F**, **Hu C-C**, **Hsu Y-H**, **et al**. The role of EDIL3 in maintaining cartilage extracellular matrix and inhibiting osteoarthritis development. *Bone Joint Res*. 2023;12(12):734–746.
6. **Luobu Z**, **Wang L**, **Jiang D**, **Liao T**, **Luobu C**, **Qunpei L**. CircSCAPER contributes to IL-1β-induced osteoarthritis in vitro via miR-140-3p/EZH2 axis. *Bone Joint Res*. 2022;11(2):61–72.
7. **Wang M**, **Tan G**, **Jiang H**, **et al**. Molecular crosstalk between articular cartilage, meniscus, synovium, and subchondral bone in osteoarthritis. *Bone Joint Res*. 2022;11(12):862–872.
8. **Piñeiro-Ramil M**, **Sanjurjo-Rodríguez C**, **Rodríguez-Fernández S**, **et al**. Generation of human immortalized chondrocytes from osteoarthritic and healthy cartilage: a new tool for cartilage pathophysiology studies. *Bone Joint Res*. 2023;12(1):46–57.
9. **Bolduc JA**, **Collins JA**, **Loeser RF**. Reactive oxygen species, aging and articular cartilage homeostasis. *FREE Radic Biol Med*. 2019;132:73–82.

10. **Coryell PR**, **Diekman BO**, **Loeser RF**. Mechanisms and therapeutic implications of cellular senescence in osteoarthritis. *Nat Rev Rheumatol*. 2021;17(1):47–57.
11. **Zhang H**, **Shao Y**, **Yao Z**, **et al**. Mechanical overloading promotes chondrocyte senescence and osteoarthritis development through downregulating FBXW7. *Ann Rheum Dis*. 2022;81(5):676–686.
12. **Boocock M**, **McNair P**, **Cicuttini F**, **Stuart A**, **Sinclair T**. The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. *Osteoarthritis Cartilage*. 2009;17(7):883–890.
13. **Van Ginckel A**, **Hall M**, **Dobson F**, **Calders P**. Effects of long-term exercise therapy on knee joint structure in people with knee osteoarthritis: a systematic review and meta-analysis. *Semin Arthritis Rheum*. 2019;48(6):941–949.
14. **Li B**, **Ding T**, **Chen H**, **et al**. *CircStrn3* targeting *microRNA-9-5p* is involved in the regulation of cartilage degeneration and subchondral bone remodelling in osteoarthritis. *Bone Joint Res*. 2023;12(1):33–45.
15. **Lu R**, **Wang Y-G**, **Qu Y**, **et al**. Dihydrocaffeic acid improves IL-1β-induced inflammation and cartilage degradation via inhibiting NF-κB and MAPK signalling pathways. *Bone Joint Res*. 2023;12(4):259–273.
16. **Zhu J**, **Liu L**, **Lin R**, **et al**. RPL35 downregulated by mechanical overloading promotes chondrocyte senescence and osteoarthritis development via hedgehog-Gli1 signaling. *J Orthop Translat*. 2024;45:226–235.
17. **Barba-Aliaga M**, **Alepuz P**. Role of eIF5A in mitochondrial function. *Int J Mol Sci*. 2022;23(3):1284.
18. **Puleston DJ**, **Buck MD**, **Klein Geltink RI**, **et al**. Polyamines and eIF5A hypusination modulate mitochondrial respiration and macrophage activation. *Cell Metab*. 2019;30(2):352–363.
19. **Gonzalez-Menendez P**, **Phadke I**, **Olive ME**, **et al**. Arginine metabolism regulates human erythroid differentiation through hypusination of eIF5A. *Blood*. 2023;141(20):2520–2536.
20. **Vu VV**, **Emerson JP**, **Martinho M**, **et al**. Human deoxyhypusine hydroxylase, an enzyme involved in regulating cell growth, activates O2 with a nonheme diiron center. *Proc Natl Acad Sci USA*. 2009;106(35):14814–14819.
21. **Ko C-Y**, **Lin Y-Y**, **Achudhan D**, **et al**. Omentin-1 ameliorates the progress of osteoarthritis by promoting IL-4-dependent anti-inflammatory responses and M2 macrophage polarization. *Int J Biol Sci*. 2023;19(16):5275–5289.
22. **Glasson SS**, **Chambers MG**, **Van Den Berg WB**, **Little CB**. The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage*. 2010;18:S17–S23.
23. **Kong K**, **Jin M**, **Zhao C**, **et al**. Mechanical overloading leads to chondrocyte degeneration and senescence via Zmpste24-mediated nuclear membrane instability. *i Sci*. 2023;26(11):108119.
24. **Wang S**, **Li W**, **Zhang P**, **et al**. Mechanical overloading induces GPX4-regulated chondrocyte ferroptosis in osteoarthritis via Piezo1 channel facilitated calcium influx. *J Adv Res*. 2022;41:63–75.
25. **Park MH**, **Wolff EC**. Hypusine, a polyamine-derived amino acid critical for eukaryotic translation. *J Biol Chem*. 2018;293(48):18710–18718.
26. **Schuller AP**, **Wu CC-C**, **Dever TE**, **Buskirk AR**, **Green R**. eIF5A functions globally in translation elongation and termination. *Mol Cell*. 2017;66(2):194–205.
27. **Melis N**, **Rubera I**, **Cougnon M**, **et al**. Targeting eIF5A hypusination prevents anoxic cell death through mitochondrial silencing and improves kidney transplant outcome. *J Am Soc Nephrol*. 2017;28(3):811–822.
28. **Zhang H**, **Alsaleh G**, **Feltham J**, **et al**. Polyamines control eIF5A hypusination, TFEB translation, and autophagy to reverse B cell senescence. *Mol Cell*. 2019;76(1):110–125.
29. **Sassi N**, **Laadhar L**, **Driss M**, **Kallel-Sellami M**, **Sellami S**, **Makni S**. The role of the Notch pathway in healthy and osteoarthritic articular cartilage: from experimental models to ex vivo studies. *Arthritis Res Ther*. 2011;13(2):208.
30. **Hosaka Y**, **Saito T**, **Sugita S**, **et al**. Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. *Proc Natl Acad Sci USA*. 2013;110(5):1875–1880.
31. **Cao H**, **Yang P**, **Liu J**, **et al**. MYL3 protects chondrocytes from senescence by inhibiting clathrin-mediated endocytosis and activating of Notch signaling. *Nat Commun*. 2023;14(1):6190.
32. **Dutto I**, **Scalera C**, **Prosperi E**. CREBBP and p300 lysine acetyl transferases in the DNA damage response. *Cell Mol Life Sci*. 2018;75(8):1325–1338.
33. **Yi F**, **Cai C**, **Ruan B**, **et al**. Regulation of RB1CC1/FIP200 stability and autophagy function by CREBBP-mediated acetylation in an intrinsically disordered region. *Autophagy*. 2023;19(6):1662–1677.
34. **Huang Y-H**, **Cai K**, **Xu P-P**, **et al**. CREBBP/EP300 mutations promoted tumor progression in diffuse large B-cell lymphoma through altering tumor-associated macrophage polarization via FBXW7-NOTCH-CCL2/CSF1 axis. *Signal Transduct Target Ther*. 2021;6(1):10.
35. **Imam S**, **Dar P**, **Aziz SW**, **et al**. Immune cell plasticity allows for resetting of phenotype from effector to regulator with combined inhibition of Notch/eIF5A pathways. *Front Cell Dev Biol*. 2021;9:777805.
36. **Ishfaq M**, **Maeta K**, **Maeda S**, **Natsume T**, **Ito A**, **Yoshida M**. Acetylation regulates subcellular localization of eukaryotic translation initiation factor 5A (eIF5A). *FEBS Lett*. 2012;586(19):3236–3241.

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Data sharing

The data that support the findings for this study are available to other researchers from the corresponding author upon reasonable request.

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Ethical review statement

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