

Positive feedback loop of lncRNA FAM201A/miR-146a-5p/POU2F1 regulates IL-1 β -induced chondrocyte injury *in vitro*

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Abstract. Numerous studies have previously demonstrated that long non-coding RNAs (lncRNAs) serve an important regulatory role in osteoarthritis (OA). In particular, the lncRNA family with sequence similarity 201 member A (FAM201A) was previously found to be downregulated in necrotic femoral head samples. However, the role of FAM201A in IL-1 β -induced chondrocyte injury remains unclear. It was hypothesized that FAM201A may exert a protective effect on IL-1 β -induced chondrocyte injury in OA by sponging microRNAs (miRNAs/miRs). The purpose of the present study was to explore the role and molecular mechanism of FAM201A in IL-1 β -induced chondrocyte injury. A model of OA was established by stimulation C-28/I2 cell with IL-1 β *in vitro*. The expression levels of FAM201A following IL-1 β -induced chondrocyte injury were detected via reverse transcription-quantitative PCR. Luciferase reporter assay was used to assess the possible associations among FAM201A, miR-146a-5p and POU class 2 homeobox 1 (POU2F1). Chromatin immunoprecipitation assay was performed to analyze the interaction between POU2F1 and miR-146a-5p. ELISA, TUNEL and western blotting were performed to measure the level of inflammation, lactate dehydrogenase release, apoptosis and the expression of apoptosis-related proteins (Bcl-2, Bax, cleaved caspase 3 and cleaved caspase 9), respectively. The expression levels of FAM201A were found to be downregulated following IL-1 β -induced chondrocyte injury. Overexpression of FAM201A exerted a protective effect against IL-1 β -induced chondrocyte injury. In addition, FAM201A could upregulate the expression levels of POU2F1

by sponging miR-146a-5p. Further experiments revealed that POU2F1 could bind to the promoter region of FAM201A and subsequently regulate the expression levels of POU2F1, indicating a role for the FAM201A/miR-146a-5p/POU2F1 positive feedback loop in IL-1 β -induced chondrocyte injury. The present study revealed the protective effects of the FAM201A/miR-146a-5p/POU2F1 positive feedback loop on IL-1 β -induced chondrocyte injury and provided a potential therapeutic target for OA.

Introduction

Osteoarthritis (OA) is one of the main causes of disability among the middle-aged and elderly population (1). Its main pathological characteristics include the gradual loss of articular cartilage and formation of osteophytes, with symptoms including joint pain, deformity and loss of joint function, leading to a decline in the quality of life of patients (2). According to the latest demographic data, the prevalence of knee OA in China ranges from 1.3-11.1% (2). By contrast, for individuals aged ≥ 60 years the prevalence of symptomatic knee OA is 19.4% (3). IL-1 β is frequently used to establish the OA model *in vitro* (4). At present, treatment methods for OA include surgery, physiotherapy and drug therapy (5,6). However, they can only relieve the symptoms of arthritis instead of effectively curing the condition in addition to being limited by the occurrence of adverse reactions (7). Therefore, it is necessary to develop novel effective treatment methods to prevent the occurrence of OA.

Long non-coding RNAs (lncRNAs) are a type of RNA transcripts that do not encode proteins and are typically >200 nucleotides in length (8). lncRNAs have been reported to be involved in cartilage destruction during OA pathogenesis (9). The mechanism underlying the role of lncRNA in OA, especially through its reported epigenetic regulatory effects, has become a topic of intense research over the past decade (10). A previous study demonstrated that expression levels of the lncRNA family with sequence similarity 201 member A (FAM201A) are decreased in necrotic femoral head samples (11). Whereas, FAM201A upregulation has been observed to promote the migration

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and invasion of cancer cells (10,12,13). However, the role of FAM10A in OA remains poorly understood. A previous study reported that FAM201A could inhibit the expression of microRNA (miR)-146a-5p, which is increased in knee OA (14). Furthermore, it has been reported that miR-146a expression is upregulated in IL-1 β -treated chondrocytes (15). Smad4 can be knocked down to weaken the effects of the TGF- β signaling pathway to increase apoptosis, whilst promoting the development of OA (16). Inhibition of Smad4 can also promote the expression of proinflammatory factors and MMP13 (17). Through ENCORI database analysis, it was previously found that miR-146a-5p can target POU class 2 homeobox 1 (POU2F1). A previous study has shown that POU2F1 expression is downregulated in the articular cartilage tissues of patients with OA (18). In addition, inflammation can lead to the downregulation of POU2F expression in the liver (18). Therefore, it was hypothetically speculated in the present study that the lncRNA FAM201A/miR-146a-5p/POU2F1 system can regulate IL-1 β -induced chondrocyte apoptosis, inflammatory response and extracellular matrix degradation, thereby aggravating OA injury.

Materials and methods

Bioinformatics analysis. ENCORI database (<https://starbase.sysu.edu.cn/>) was used to predict the binding sites between FAM201A and miR-146a-5p as well as miR-146a-5p and POU2F1. The site of interaction between POU2F1 and FAM201A promoter was predicted by JASPAR database (<https://jaspar.genereg.net/>).

Cell culture and treatment. The human C-28/I2 chondrocytes were obtained from BeNa Culture Collection (Beijing Beina Chunglian Institute of Biotechnology). The cells were cultured in DMEM/F12 medium (Hyclone; Cytiva) containing 10% FBS (Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin in a 5% CO₂ incubator at 37°C. Cells in logarithmic phase were used for subsequent experiments. Cells in model group were stimulated with IL-1 β (Sigma-Aldrich; Merck KGaA) (10 ng/ml) at 37°C for 24 h (19). Untreated cells were considered as control.

Cell transfection. Small interfering RNAs (siRNAs) against POU2F1 (siRNA-POU2F1), siRNA negative control (siRNA-NC), miR-146-5p mimic, miR-146-5p inhibitor and inhibitor/mimic NC were synthesized by Shanghai GenePharma Co., Ltd. Full length of FAM201A and POU2F1 was inserted into pcDNA3.1 plasmid (Abcam) to construct overexpression (Ov)-FAM201A and Ov-POU2F1. An empty pcDNA3.1 plasmid was used as the negative control (Ov-NC). Cells were seeded into 6-well plates at a density of 3x10⁵ cells/well and cultured for 24 h at 37°C. In accordance with the instructions, these aforementioned indicated plasmids or RNA sequences (2–4 μ g/ml) were transfected into the cells using Lipofectamine[®] 2000 (Thermo Fisher Scientific, Inc.) at 37°C for 48 h. Transfection efficiency was detected using reverse transcription-quantitative PCR (RT-qPCR) at 48 h after transfection. The sequences used were as follows: siRNA-POU2F1 sense, 5'-GGUGUUUGUAGACUAUUAU ATT-3' and antisense, 5'-UAUAUAGUCUACAAACACCTT-3';

siRNA-NC sense, 5'-UUCUCCGAACGUGUCACGUTT-3' and antisense, 5'-ACGUGACACGUUCGGAGAATT-3'; miR-146a-5p mimic sense, 5'-UGAGAACUGAAUCCAUG GGUU-3' and antisense, 5'-AACCCAUGGAAUUCAGUUC UCA-3'; NC mimic sense, 5'-UUUGUACUACACAAAAGUA CUG-3' and antisense, 5'-CAGUACUUUUGUGUAGUAC AAA-3'; miR-146a-5p inhibitor, 5'-AACCCAUGGAAUUCAG UUCUCA-3'; and NC inhibitor, 5'-CAGUACUUUUGUGUAG UACAAA-3'.

RT-qPCR. Total RNA was extracted from cells using TRIzol[®] reagent (Thermo Fisher Scientific, Inc.). RNA was synthesized into cDNA using a reverse transcription kit (Promega Corporation) according to the manufacturer's protocol. The qPCR reaction was performed using SYBR Green Supermix (Applied Biosystems; Thermo Fisher Scientific, Inc.) in the ABI 7500 PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec (22 cycles). The relative expression levels of target genes were calculated using the 2^{- $\Delta\Delta$ C_q} method (20) and normalized to those of the housekeeping genes GAPDH or U6. The sequences used were as follows: FAM201A forward, 5'-CTCGGGACCTCTAGC CAGT-3' and reverse, 5'-TGTGGGCAGATGTGGTTTCC-3'; miR-146a-5p forward, 5'-ACACTCCAGCTGGGTGAGAAC TGAATTCCA-3' and reverse, 5'-TGGTGTCTGTGGAGTCG-3'; POU2F1 forward, 5'-GTAGTACCTTCTTTCCCCACCC-3' and reverse, 5'-TTGGTTTGTGTGCCTGTGTTG-3'; GAPDH forward, 5'-AATGGGCAGCCGTTAGGAAA-3' and reverse, 5'-GCGCCCAATACGACCAAATC-3'; and U6 forward, 5'-CTCGCTTCGGCAGCACAC-3' and reverse, 5'-AACGCTTC ACGAATTTGCGT-3'.

TUNEL assay. Apoptosis was detected using the TUNEL Apoptosis Assay kit (cat. no. C1088; Beyotime Institute of Biotechnology). Briefly, cells were washed with PBS and fixed with 4% paraformaldehyde at room temperature for 20 min. Subsequently, cells were treated with 0.1% Triton X-100 for 10 min, and then incubated with TUNEL reagent at 37°C in the dark for 60 min. The nuclei were stained with 5 μ g/ml DAPI at room temperature for 5 min. The morphological changes of apoptotic cells were observed under a fluorescence microscope in three fields of view (Olympus FV500; Olympus Corporation). Bright green fluorescence was considered to indicate apoptotic cells.

ELISA. According to the manufacturer's protocols, corresponding ELISA kits (Nanjing Jiancheng Bioengineering Institute) were used to analyze the levels of IL-6 (IL-6 Assay Kit, cat. no. H007-1-1), TNF- α (TNF- α Assay Kit, cat. no. H052-1) and IL-1 β (IL-1 β Assay Kit, cat. no. H002) in the cell culture supernatant. The samples in each group were analyzed using an Automatic Microplate Reader (Syngene).

Luciferase reporter assay. The C-28/I2 cells were inoculated into 96-well plates at a density of 2x10⁴ cells per well. When the cell confluence reached 60%, the cells were co-transfected with the reporter construct with the *Renilla* luciferase expression vector pRL-TK (Promega Corporation), encoding either the wild-type (WT) POU2F1 or FAM201A 3'-untranslated region

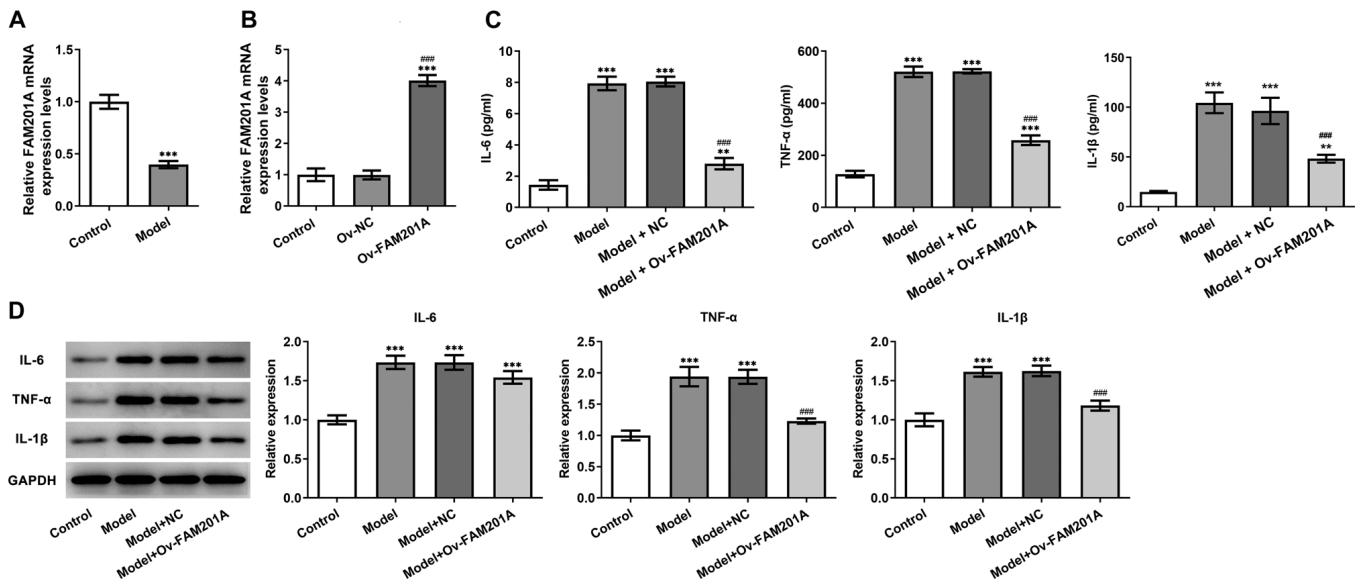


Figure 1. Overexpression of FAM201A attenuates the inflammatory response in IL-1 β -induced C-28/I2 cells. (A) RT-qPCR assay was performed to measure the expression of FAM201A in IL-1 β -treated C-28/I2 cells. (B) Efficiency of FAM201 overexpression was examined by RT-qPCR. (C) ELISA was performed to measure the inflammatory response in IL-1 β -treated C-28/I2 cells. (D) Western blot assay was performed to measure the inflammatory response in IL-1 β -treated C-28/I2 cells. ** P <0.01 and *** P <0.001 vs. Normal or Control; *** P <0.001 vs. Ov-NC or Model. RT-qPCR, reverse transcription-quantitative PCR; FAM201A, family with sequence similarity 201 member A; Ov, overexpression; NC, negative control.

(3'-UTR) or the mutant (MUT) POU2F1 or FAM201A 3'-UTR plasmid generated by a Phusion Site-directed Mutagenesis kit (Thermo Fisher Scientific, Inc.; cat. no. F541) and miR-146-5p mimics or miR-NC using Lipofectamine[®] 2000 reagent. At 48 h after transfection, the relative luciferase intensity of each group was detected using a Dual-Luciferase assay kit (Promega Corporation) by comparison with *Renilla* luciferase activity.

Chromatin immunoprecipitation (ChIP) assay. The ChIP assay was performed using a ChIP assay kit (Pierce[™] Agarose ChIP kit; Thermo Fisher Scientific, Inc.; cat. no. 26156). Briefly, the chromatin fragments derived from C-28/I2 cells that extracted by the lysis buffer included in the ChIP kit (Thermo Fisher Scientific, Inc.) were immunoprecipitated with 10 μ g antibody against FAM201A (cat. no. ab184572; Abcam) or 5 μ g mouse IgG (cat. no. sc-2025; Santa Cruz Biotechnology, Inc.) at 4°C overnight. All antibodies were mixed with the dilution buffer and 250 μ g magnetic beads (Thermo Fisher Scientific, Inc.) followed by incubation for 1 h at 4°C. DNA fragments and these antibodies were incubated at 4°C overnight. The next day, products of immunoprecipitation were treated with the ChIP elution buffer and centrifuged at 6,000 \times g for 1 min. Subsequently, precipitated DNA samples were detected via RT-qPCR.

Western blot analysis. Total RNA was extracted from cells using RIPA buffer (cat. no. P0013B; Beyotime Institute of Biotechnology). The protein concentration was detected using a bicinchoninic acid protein quantitative kit (cat. no. P0012; Beyotime Institute of Biotechnology). A total of 20 μ g protein was separated by 10% SDS-PAGE and subsequently transferred onto PVDF membranes. These membranes were blocked with 5% skimmed milk at room temperature for 30 min and incubated with primary antibodies (1:1,000) against POU2F1

(cat. no. ab178869; Abcam), IL-6 (cat. no. ab233706; Abcam), TNF- α (cat. no. ab183218; Abcam), IL-1 β (cat. no. ab254360; Abcam), MMP1 (cat. no. ab137332; Abcam), MMP13 (cat. no. ab39012; Abcam), collagen II (cat. no. ab34712; Abcam), Aggrecan (cat. no. ab3778; Abcam), Bcl-2 (cat. no. ab182858; Abcam), Bax (cat. no. ab182733; Abcam), caspase 3 (cat. no. ab32150; Abcam), cleaved caspase 3 (cat. no. ab2302; Abcam), caspase 9 (cat. no. ab65608; Abcam), cleaved caspase 9 (cat. no. ab2324; Abcam) and GAPDH (cat. no. ab9485; Abcam) overnight at 4°C. Subsequently, the membranes were incubated with the corresponding HRP-conjugated Goat Anti-Rabbit IgG H&L secondary antibody (cat. no. ab205718; Abcam) at 37°C for 2 h at 1:10,000. An ECL Plus kit (cat. no. P0018; Beyotime Institute of Biotechnology) was used to visualize the protein bands. The densitometry analysis was performed using ImageJ software (version 1.8.0; National Institutes of Health).

Statistical analysis. All data were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc.). All experiments in this study were repeated three times. The measurement data are presented as the mean \pm standard deviation. Unpaired Student's t-test was used to compare two groups of data. Comparisons among multiple groups were performed using one-way ANOVA followed by Tukey's post hoc test. P <0.05 was considered to indicate a statistically significant difference.

Results

FAM201A expression is downregulated by IL-1 β treatment in C-28/I2 cells. To simulate OA *in vitro*, C-28/I2 cells were stimulated with 10 ng/ml IL-1 β for 24 h, then FAM201A expression was detected by RT-qPCR assay, results revealed that FAM201A expression in C-28/I2 cells was significantly decreased in the model group compared with that in the normal C-28/I2 cells (Fig. 1A).

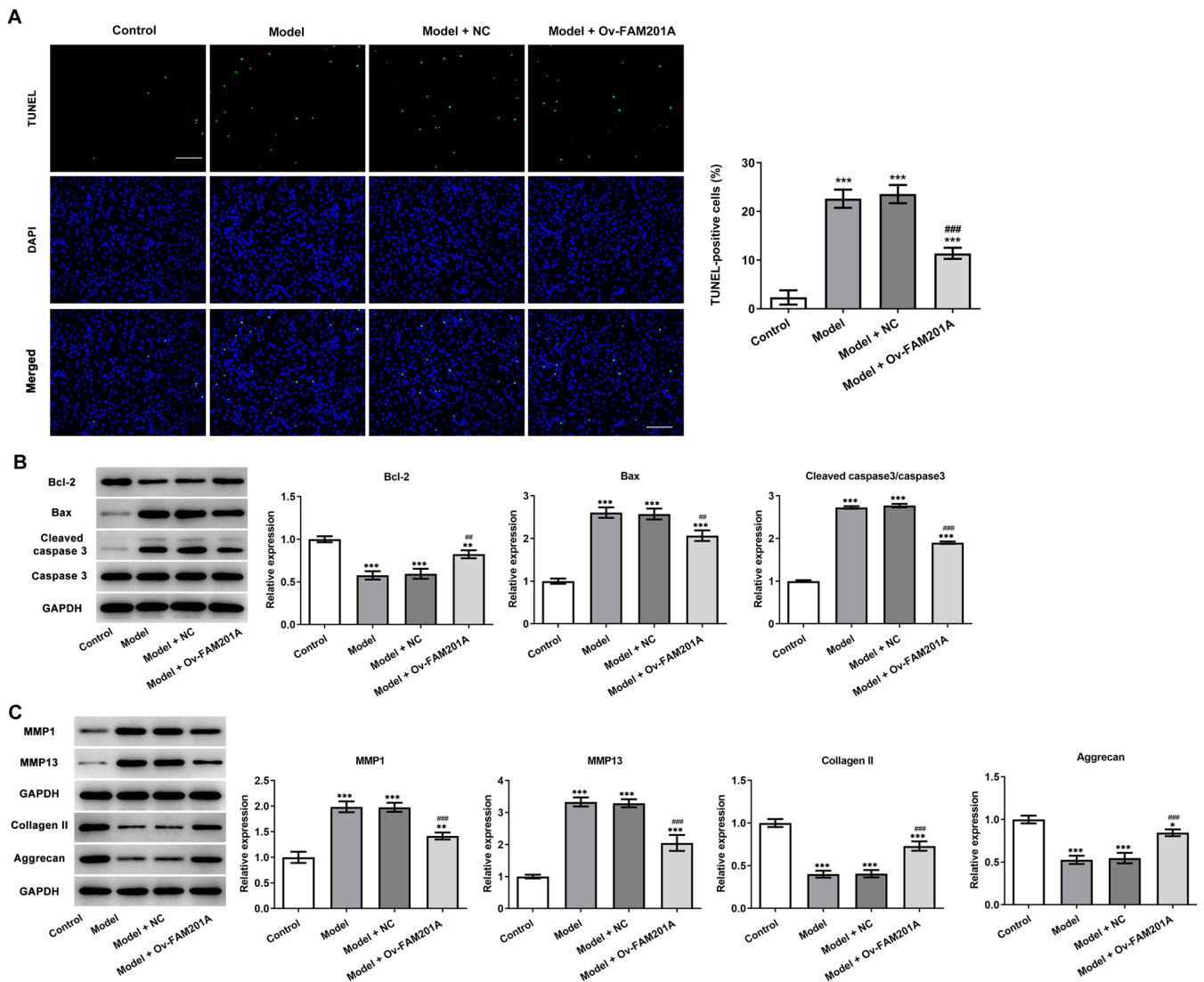


Figure 2. Overexpression of FAM201A attenuates cell apoptosis and matrix degradation by IL-1 β -treated C-28/I2 cells. (A) Effect of FAM201A overexpression on cell apoptosis was detected using a TUNEL assay (scale bar, 50 μ m). (B) Effect of FAM201A overexpression on the expression of apoptosis-related proteins was detected via western blotting. (C) Effect of FAM201A on the expression of matrix degradation-related proteins was detected via western blotting. * P <0.05, ** P <0.01 and *** P <0.001 vs. Control; ## P <0.01 and ### P <0.001 vs. Model. FAM201A, family with sequence similarity 201 member A; Ov, overexpression; NC, negative control.

Overexpression of FAM201A attenuates the inflammatory response, apoptosis and matrix degradation of IL-1 β -treated C-28/I2 cells. The physiological function of FAM201A in IL-1 β -treated C-28/I2 cells was next investigated. As shown in Fig. 1B, the successful overexpression of FAM201A in C-28/I2 cells following transfection with the Ov-FAM201A plasmid was confirmed via RT-qPCR. Subsequently, ELISA results showed that the secretion levels of IL-6, TNF- α and IL-1 β were significantly decreased in the model + Ov-FAM201A group compared with those in the model group (Fig. 1C). Results from western blotting showed that overexpression of FAM201A reduced the inflammatory response in the model group compared with the model group (Fig. 1D). TUNEL assay (Fig. 2A) and western blotting (Fig. 2B) results showed that IL-1 β -induced C-28/I2 cell apoptosis was significantly suppressed in the model + Ov-FAM201A group compared with the model group, while the expression levels of apoptosis-related proteins were also significantly reduced

and anti-apoptosis protein Bcl-2 was increased. These findings suggested that the overexpression of FAM201A could attenuate the apoptosis of IL-1 β -treated C-28/I2 cells. The expression levels of proteins associated with extracellular matrix degradation and components of the extracellular matrix were subsequently detected via western blotting. MMP1 and MMP13 expression levels were significantly decreased, while collagen II and Aggrecan expression levels were significantly increased in the model + Ov-FAM201A group compared with the model group (Fig. 2C).

FAM201A directly targets miR-146a-5p. To further study the mechanism underlying the function of FAM201A in IL-1 β -treated C-28/I2 cells, miR-146a-5p was predicted to be a target of FAM201A through the ENCORI database (Fig. 3A). The expression of miR-146a-5p was found to be significantly decreased in the model + Ov-FAM201A group compared with the model group (Fig. 3B).

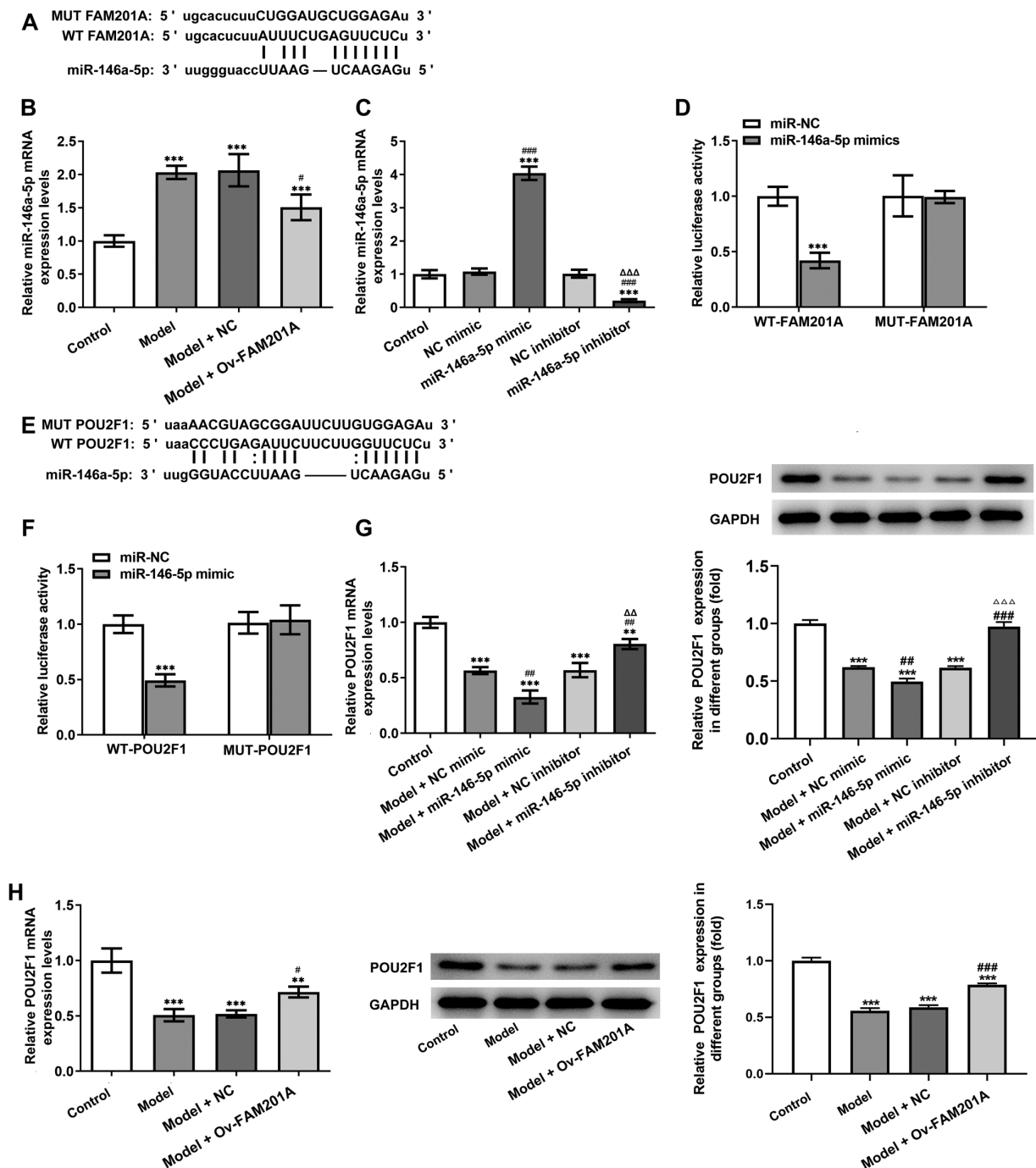


Figure 3. FAM201A directly targets miR-146a-5p. (A) miR-146a-5p was predicted to be a target gene of FAM201A through the ENCORI database. (B) RT-qPCR was performed to measure the expression levels of miR-146a-5p in IL-1 β -treated C-28/I2 cells. ***P<0.001 vs. Control; #P<0.05 vs. Model. (C) Expression levels of miR-146a-5p after transfection with the miR-146a-5p mimic and miR-146a-5p inhibitor was measured by RT-qPCR. ***P<0.001 vs. Control; ###P<0.001 vs. NC mimic; $\Delta\Delta\Delta$ P<0.001 vs. NC inhibitor. (D) Potential relationship between miR-146a-5p and FAM201A was detected by dual-luciferase reporter assay. ***P<0.001 vs. miR-NC. (E) Downstream target genes of miR-146a-5p were predicted using the ENCORI database. (F) Relationship between miR-146a-5p and POU2F1 was detected using the dual-luciferase reporter assay. ***P<0.001 vs. miR-NC. (G) The expression levels of POU2F1 after transfection with the miR-146a-5p mimic or miR-146a-5p inhibitor was measured by RT-qPCR. **P<0.01 and ***P<0.001 vs. Control; ##P<0.01 and ###P<0.001 vs. Model + NC mimic; $\Delta\Delta$ P<0.01 and $\Delta\Delta\Delta$ P<0.001 vs. Model + NC inhibitor. (H) RT-qPCR was performed to measure the expression of POU2F1 in IL-1 β -treated C-28/I2 cells. **P<0.01 and ***P<0.001 vs. Control; #P<0.05 and ###P<0.001 vs. Model. miR, microRNA; FAM201A, family with sequence similarity 201 member A; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; POU2F1, POU class 2 homeobox 1; WT, wild-type; MUT, mutant; Ov, overexpression.

Subsequently, miR-146a-5p mimic and miR-146a-5p inhibitor were successfully used to modify the expression of miR-146a-5p in C-28/I2 cells (Fig. 3C). Results of the dual-luciferase reporter assays showed that compared with that in the miR-NC group, co-transfection with the miR-16-5p mimic significantly decreased the luciferase activity of the

WT-FAM201A plasmid, but not that of the MUT-FAM201A plasmid (Fig. 3D). Therefore, these findings suggested that FAM201A could negatively target miR-16-5p.

miR-146a-5p directly targets POU2F1. The downstream target genes of miR-146a-5p were subsequently predicted by using

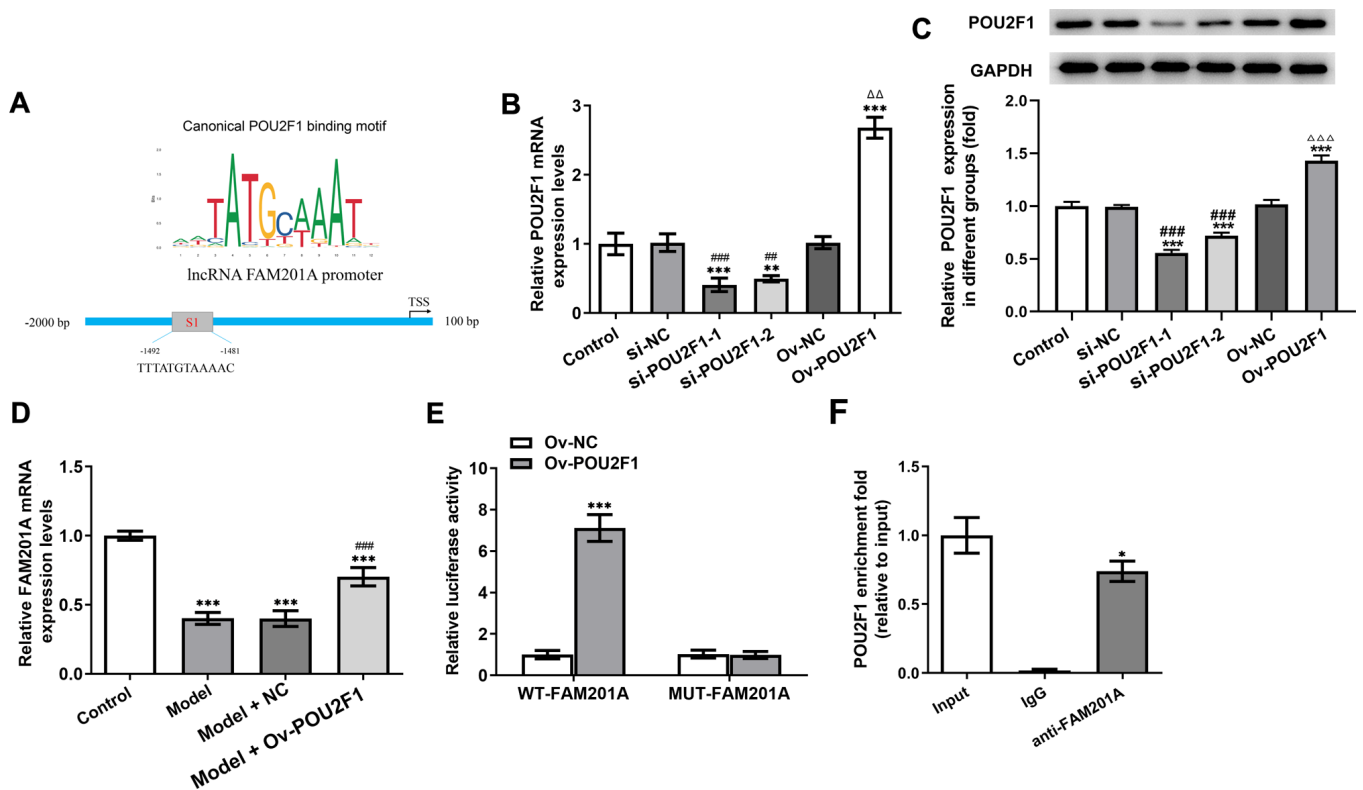


Figure 4. POU2F1 and FAM201A promoters combine to form a positive feedback loop. (A) JASPAR analysis predicted a POU2F1 binding site on the FAM201A promoter. (B and C) Expression of POU2F1 after transfection with siRNA-POU2F1 or Ov-POU2F1 was measured via RT-qPCR. ** $P < 0.01$ and *** $P < 0.001$ vs. Control; ## $P < 0.01$ and ### $P < 0.001$ vs. si-NC; $\Delta\Delta P < 0.01$ and $\Delta\Delta\Delta P < 0.001$ vs. Ov-NC. (D) Expression of FAM201A was measured via RT-qPCR. *** $P < 0.001$ vs. Control; ## $P < 0.001$ vs. Model. (E) Relationship between POU2F1 and FAM201A was detected using a dual-luciferase reporter assay. *** $P < 0.001$ vs. Ov-NC. (F) Chromatin immunoprecipitation assay coupled with qPCR was used to verify POU2F1 binding to the promoter of FAM201A. * $P < 0.05$ vs. IgG. POU2F1, POU class 2 homeobox 1; FAM201A, family with sequence similarity 201 member A; si, small interfering RNA; RT-qPCR, reverse transcription-quantitative PCR; Ov, overexpression; NC, negative control; WT, wild-type; MUT, mutant.

the ENCORI database. As shown in Fig. 3E, POU2F1 was predicted to be a target gene of miR-146a-5p. Results of the dual-luciferase reporter assays showed that compared with that in the miR-NC group, miR-146a-5p mimic significantly decreased the luciferase activity of the WT-POU2F1 plasmid, but not that of the MUT-POU2F1 plasmid (Fig. 3F). In addition, the expression of POU2F1 was significantly inhibited in the model + miR-146a-5p mimic group compared with the model + NC mimic group, whereas the expression of POU2F1 was significantly increased in the model + miR-146a-5p inhibitor group compared with the model + NC inhibitor group (Fig. 3G). The expression levels of POU2F1 after FAM201A overexpression was then detected by RT-qPCR. As shown in Fig. 3H, expression of POU2F1 was significantly increased in the model + Ov-FAM201A group compared with the model + NC group.

POU2F1 and FAM201A promoters combine to form a positive feedback loop. It was predicted using the JASPAR database that there may be a site of interaction (S1) between POU2F1 and FAM201A (Fig. 4A). Therefore, the relationships among FAM201A, miR-146a-5p and POU2F1 were studied further. The expression of POU2F1 in cells was manipulated by transfection with either siRNA-POU2F1 or pcDNA-POU2F1. As shown in Fig. 4B and C, compared with that in the control group, the expression of POU2F1 in the group transfected

with shRNA-POU2F1-1 was the lowest, whereas that in the pcDNA-POU2F1 group was the highest. In addition, the expression of FAM201A was significantly promoted in the model + Ov-POU2F1 group compared with the model group (Fig. 4D). Results of the dual-luciferase reporter assays showed that compared with that in the Ov-NC group, overexpression of POU2F1 significantly enhanced the luciferase activity of the WT-FAM201A plasmid, but not that of the MUT-FAM201A plasmid (Fig. 4E). Results of the ChIP assay revealed the enrichment of POU2F1 on the promoter of FAM201A (Fig. 4F).

Overexpression of FAM201A upregulates POU2F1 expression and attenuates the inflammatory response, apoptosis and matrix degradation in IL-1 β -treated C-28/I2 cells. As shown in Fig. 5A and B, western blotting and ELISA demonstrated that the expression levels of inflammatory-related proteins, including IL-6, TNF- α and IL-1 β , were upregulated in the model + Ov-FAM201A + si-POU2F1 group compared with the model + Ov-FAM201A + si-NC. As shown in Fig. 6A, TUNEL revealed that POU2F1 knockdown partially reversed the inhibitory effect of FAM201A overexpression on the apoptosis of IL-1 β -treated C-28/I2 cells. Consistently, western blotting also demonstrated that the changes in the expression levels of Bcl-2, Bax and cleaved caspase 3/caspase 3 observed in the model + Ov-FAM201A + si-NC were markedly blocked

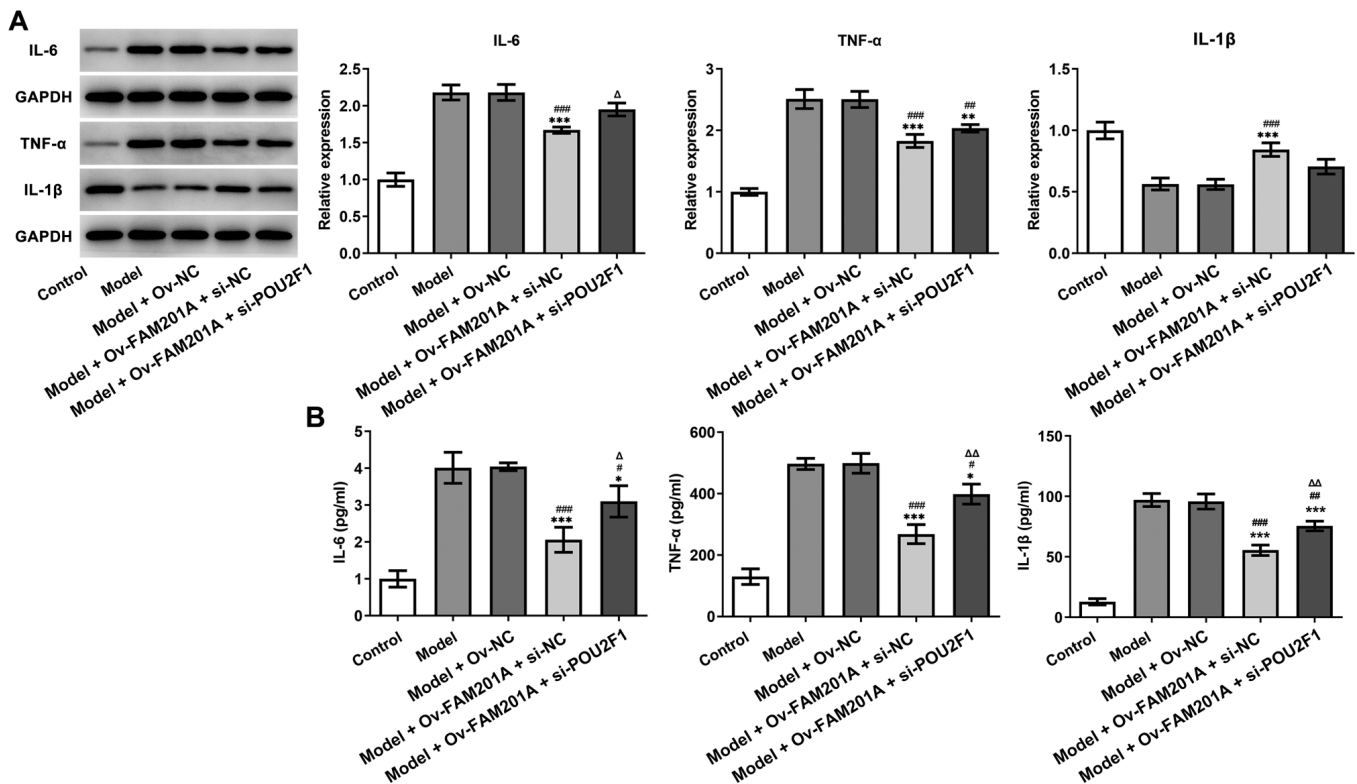


Figure 5. Overexpression of FAM201A upregulates POU2F1 expression and attenuates the inflammatory response in IL-1 β -treated C-28/I2 cells. (A) Western blotting was performed to detect the inflammatory response in IL-1 β -treated C-28/I2 cells. (B) ELISA was performed to detect the inflammatory response in IL-1 β -treated C-28/I2 cells. * P <0.05, ** P <0.01 and *** P <0.001 vs. Control; $^{\Delta}$ P <0.05, $^{\Delta\Delta}$ P <0.01 and $^{\Delta\Delta\Delta}$ P <0.001 vs. Model; $^{\Delta}$ P <0.05 and $^{\Delta\Delta}$ P <0.01 vs. Model + Ov-FAM201A + si-NC. POU2F1, POU class 2 homeobox 1; FAM201A, family with sequence similarity 201 member A; si, small interfering RNA; Ov, overexpression; NC, negative control.

in the model + Ov-FAM201A + si-POU2F1 group (Fig. 6B). Furthermore, the expression of extracellular matrix-related proteins, including MMP1, MMP13, collagen II and Aggrecan, was measured by western blotting. The results showed that POU2F1 knockdown partially reversed the effects of FAM201A overexpression on these proteins expression in IL-1 β -treated C-28/I2 cells (Fig. 6C).

Discussion

OA is one of the most common joint diseases and is becoming a public health concern worldwide (1). At present, the treatment strategy for OA is mainly focused on relieving the symptoms (5). However, the underlying mechanisms of OA development remain to be fully elucidated, forcing the majority of patients with advanced OA to eventually require joint replacement surgery. Therefore, studies on OA have focused on investigating the epigenetic regulation of its pathogenesis and the discovery of potential therapeutic targets, with non-coding RNAs, including lncRNAs and miRNAs, of interest (6,21). Previous studies have reported that lncRNAs serve a key role in the development of bone and cartilage tissues (9,22). This suggests that lncRNAs may regulate the balance between anabolic and catabolic processes in the articular cartilage, which could be utilized for the diagnosis and treatment of OA. Furthermore, recent studies have shown that lncRNAs can be used as personalized therapeutic biomarkers to prevent, halt or even reverse the progression of OA (23-26). These lncRNAs

may be used to investigate the underlying molecular mechanism involved in cartilage damage and may be beneficial for the development of novel therapies for OA. Huang *et al* (11) recently found that FAM201A expression was associated with the occurrence of femoral head necrosis. However, to the best of our knowledge, no relevant studies on the potential effects of lncRNA FAM201A on OA currently exists. In the present study, it was found that the expression of the lncRNA FAM201A was decreased in IL-1 β -treated chondrocytes. In addition, after exploring the function of FAM201A in these chondrocytes, overexpression of FAM201A alleviated the inflammatory damage caused by IL-1 β , suggesting the protective role of FAM201A against OA.

Recently, the competing endogenous RNA network pathway has been widely reported to be the classical mechanism of action mediated by lncRNAs (27), which exerts its influence on biological processes by regulating the expression of miRNAs. A study previously conducted by Hu *et al* (26) showed that HOX transcript antisense RNA promoted the development of OA by regulating the miR-17-5p/fucosyltransferase 2/ β -catenin axis. In addition, the lncRNA plasmacytoma variant translocation 1 mediates inflammatory injury by targeting miR-27b-3p/TNF receptor-associated factor 3 in chondrocytes (28). The current study subsequently investigated the possible target miRNA of FAM201A, and the results demonstrated the interaction between FAM201A and miR-146a-5p. Of note, miR-146a-5p has been reported to be involved in IL-1 β -induced chondrocyte injury and OA

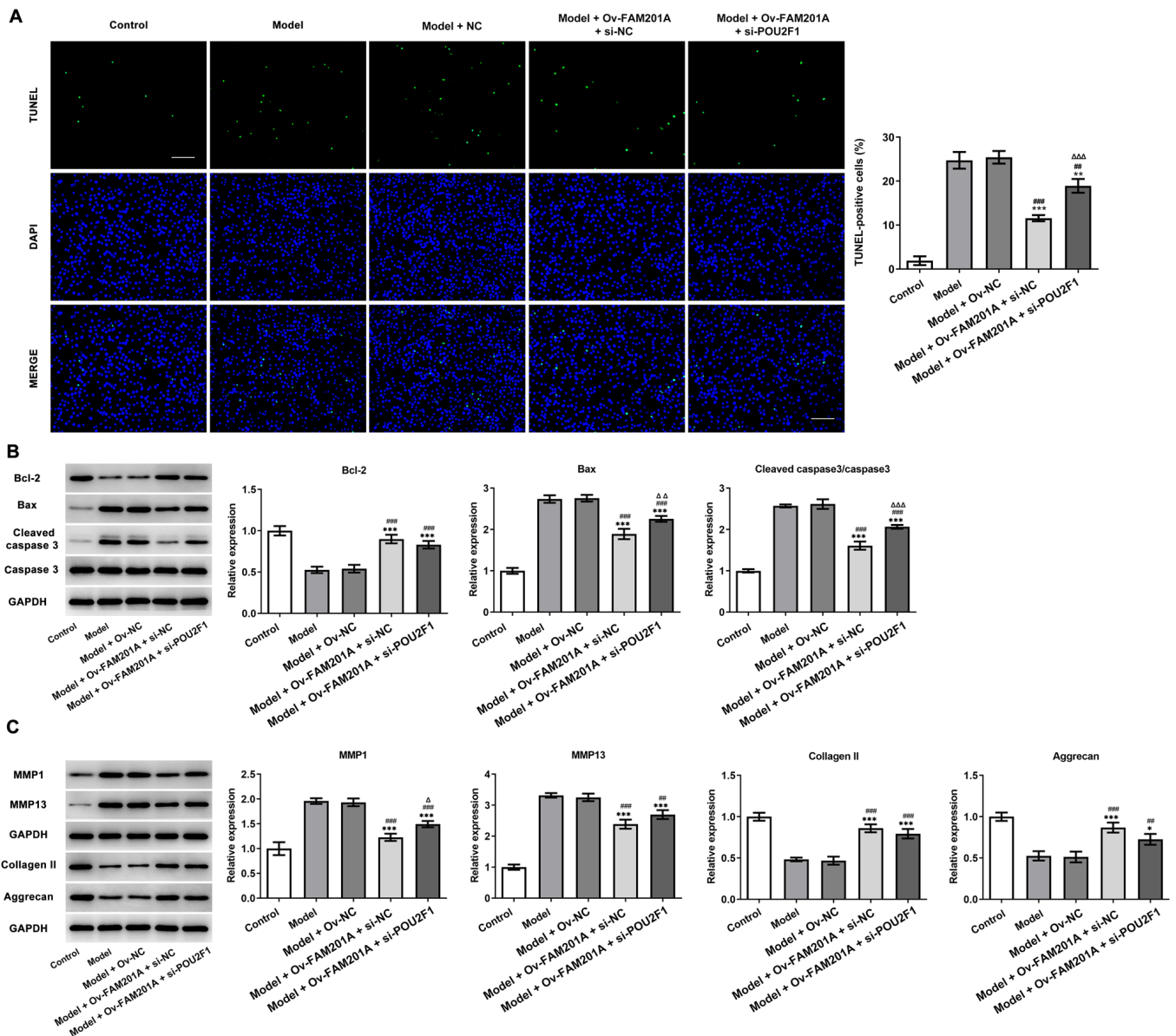


Figure 6. Silencing of POU2F1 reverses the effect of FAM201A overexpression on apoptosis and matrix degradation in IL- β -treated C-28/I2 cells. (A) Effects of POU2F1 silencing on the apoptosis of Ov-FAM201A C-28/I2 cells were detected via a TUNEL assay (scale bar, 50 μ m). (B) Effects of POU2F1 silencing on the expression of apoptosis-related proteins in Ov-FAM201A C-28/I2 cells were detected via western blotting. (C) Effects of POU2F1 silencing on the expression of matrix degradation-related proteins in Ov-FAM201A C-28/I2 cells were detected via western blotting. * P <0.05, ** P <0.01 and *** P <0.001 vs. Control; ## P <0.01 and ### P <0.001 vs. Model; ΔP <0.05, $\Delta\Delta P$ <0.01 and $\Delta\Delta\Delta P$ <0.001 vs. Model + Ov-FAM201A + si-NC. POU2F1, POU class 2 homeobox 1; FAM201A, family with sequence similarity 201 member A; si, small interfering RNA; Ov, overexpression; NC, negative control.

through multiple mechanisms (29-31). Further experiments in the present study demonstrated that miR-146a-5p inhibited the expression of FAM201A and overexpression of miR-146a-5p reversed the protective effects of FAM201A on IL- β -treated chondrocytes. These data revealed that FAM201A may exhibit its protective role against OA via targeting miR-146a-5p.

The current study next demonstrated that POU2F1 was a downstream target gene of miR-146a-5p, and POU2F1 was subsequently found to bind to the promoter of FAM201A. In a previous study, the positive feedback loop LINC00511/miR-150-5p/SP1 was reported to modulate chondrocyte apoptosis and proliferation in OA (32). The existence of the FAM201A/miR-146a-5p/POU2F1 positive feedback loop that regulates OA progression was therefore hypothesized. In accordance with this hypothesis, results showed that silencing of

POU2F1 markedly blocked the effects of FAM201A overexpression on IL- β -induced chondrocytes inflammation and apoptosis. Thus, these results implied that lncRNA FAM201A could directly regulate the expression of POU2F1 by binding to miR-146a-5p to exert protective effects against inflammation in injured chondrocytes. However, the potential downstream mechanisms that are regulated by the FAM201A/miR-146a-5p/POU2F1 axis during OA has not been covered in this study. miR-146a-5p has been reported to promote IL- β -induced chondrocyte apoptosis via NF- κ B signaling (15). NF- κ B signaling can be activated in response to IL- β stimulation, and has been widely accepted to contribute to OA upon activation (33,34). Therefore, future research may explore the involvement of NF- κ B signaling in FAM201A/miR-146a-5p/POU2F1 axis-mediated chondrocyte injury.

In conclusion, the results from the present study demonstrated that the positive feedback loop of FAM201A/miR-146a-5p/POU2F1 could regulate IL-1 β -induced inflammation and apoptosis in chondrocytes. This study may provide novel targets for the therapeutic treatment of OA.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Jin L and KG conceived and designed the study. KG, WW, Jie L and DX performed the experiments. YL, MB and LL analyzed and interpreted the data. Jin L and KG drafted the manuscript and revised it for critically important intellectual content. Jin L and KG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Mandl LA: Osteoarthritis year in review 2018: Clinical. *Osteoarthritis Cartilage* 27: 359-364, 2019.
- Alonso B, Bravo B, Mediavilla L, Gortazar AR, Forriol F, Vaquero J and Guisasaola MC: Osteoarthritis-related biomarkers profile in chronic anterior cruciate ligament injured knee. *Knee* 27: 51-60, 2020.
- Shi D, Dai J, Xu Z, Chen D and Jiang Q: Update on basic and clinical aspects of osteoarthritis. *Ann Transl Med* 3: 142, 2015.
- Nasi S, Ea HK, So A and Busso N: Revisiting the Role of Interleukin-1 Pathway in Osteoarthritis: Interleukin-1 α and -1 β , and NLRP3 Inflammasome Are Not Involved in the Pathological Features of the Murine Meniscectomy Model of Osteoarthritis. *Front Pharmacol* 8: 282, 2017.
- Cheng W, Gan D, Hu Y, Zheng Z, Zeng Q, Li L, Wang X, Zhang Y, Xu Z, Qin L, *et al*: The effect and mechanism of QufengZhitong capsule for the treatment of osteoarthritis in a rat model. *J Orthop Translat* 28: 65-73, 2021.
- He M, Zhong X, Li Z, Shen K and Zeng W: Progress in the treatment of knee osteoarthritis with high tibial osteotomy: A systematic review. *Syst Rev* 10: 56, 2021.
- Glyn-Jones S, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H and Carr AJ: Osteoarthritis. *Lancet* 386: 376-387, 2015.
- Ou F, Su K, Sun J, Liao W, Yao Y, Zheng Y and Zhang Z: The lncRNA ZBED3-AS1 induces chondrogenesis of human synovial fluid mesenchymal stem cells. *Biochem Biophys Res Commun* 487: 457-463, 2017.
- Jiang SD, Lu J, Deng ZH, Li YS and Lei GH: Long noncoding RNAs in osteoarthritis. *Joint Bone Spine* 84: 553-556, 2017.
- He W, Qiao ZX and Ma B: Long noncoding RNA FAM201A mediates the metastasis of lung squamous cell cancer via regulating ABCE1 expression. *Eur Rev Med Pharmacol Sci* 23: 10343-10353, 2019.
- Huang G, Zhao G, Xia J, Wei Y, Chen F, Chen J and Shi J: FGF2 and FAM201A affect the development of osteonecrosis of the femoral head after femoral neck fracture. *Gene* 652: 39-47, 2018.
- Jia H, Wu D, Zhang Z and Li S: TCF3-activated FAM201A enhances cell proliferation and invasion via miR-186-5p/TNKS1BP1 axis in triple-negative breast cancer. *Bioorg Chem* 104: 104301, 2020.
- Huang J, Yu Q, Zhou Y, Chu Y, Jiang F and Wang Q: FAM201A knockdown inhibits proliferation and invasion of lung adenocarcinoma cells by regulating miR-7515/GLO1 axis. *J Cell Physiol* 236: 5620-5632, 2021.
- Rousseau JC, Millet M, Croset M, Sornay-Rendu E, Borel O and Chapurlat R: Association of circulating microRNAs with prevalent and incident knee osteoarthritis in women: The OFELY study. *Arthritis Res Ther* 22: 2, 2020.
- Shao J, Ding Z, Peng J, Zhou R, Li L, Qian Q and Chen Y: miR-146a-5p promotes IL-1 β -induced chondrocyte apoptosis through the TRAF6-mediated NF- κ B pathway. *Inflamm Res* 69: 619-630, 2020.
- Li J, Huang J, Dai L, Yu D, Chen Q, Zhang X and Dai K: miR-146a, an IL-1 β responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. *Arthritis Res Ther* 14: R75, 2012.
- Zhang X, Wang C, Zhao J, Xu J, Geng Y, Dai L, Huang Y, Fu SC, Dai K and Zhang X: miR-146a facilitates osteoarthritis by regulating cartilage homeostasis via targeting Camk2d and Ppp3r2. *Cell Death Dis* 8: e2734, 2017.
- Tian H: Detection of differentially expressed genes involved in osteoarthritis pathology. *J Orthop Surg Res* 13: 49, 2018.
- Wang Q, Xu X, Kang Z, Zhang Z and Li Y: Paeonol prevents IL-1 β -induced inflammatory response and degradation of type II collagen in human primary chondrocytes. *Artif Cells Nanomed Biotechnol* 47: 2139-2145, 2019.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
- Schaukowitch K and Kim TK: Emerging epigenetic mechanisms of long non-coding RNAs. *Neuroscience* 264: 25-38, 2014.
- Sun H, Peng G, Ning X, Wang J, Yang H and Deng J: Emerging roles of long noncoding RNA in chondrogenesis, osteogenesis, and osteoarthritis. *Am J Transl Res* 11: 16-30, 2019.
- Cen X, Huang XQ, Sun WT, Liu Q and Liu J: Long noncoding RNAs: A new regulatory code in osteoarthritis. *Am J Transl Res* 9: 4747-4755, 2017.
- Wang Z, Hao J and Chen D: Long Noncoding RNA Nuclear Enriched Abundant Transcript 1 (NEAT1) Regulates Proliferation, Apoptosis, and Inflammation of Chondrocytes via the miR-181a/Glycerol-3-Phosphate Dehydrogenase 1-Like (GPD1L) Axis. *Med Sci Monit* 25: 8084-8094, 2019.
- Xu J and Xu Y: The lncRNA MEG3 downregulation leads to osteoarthritis progression via miR-16/SMAD7 axis. *Cell Biosci* 7: 69, 2017.
- Hu J, Wang Z, Shan Y, Pan Y, Ma J and Jia L: Long non-coding RNA HOTAIR promotes osteoarthritis progression via miR-17-5p/FUT2/ β -catenin axis. *Cell Death Dis* 9: 711, 2018.
- Ju C, Liu R, Zhang YW, Zhang Y, Zhou R, Sun J, Lv XB and Zhang Z: Mesenchymal stem cell-associated lncRNA in osteogenic differentiation. *Biomed Pharmacother* 115: 108912, 2019.
- Lu X, Yu Y, Yin F, Yang C, Li B, Lin J and Yu H: Knockdown of PVT1 inhibits IL-1 β -induced injury in chondrocytes by regulating miR-27b-3p/TRAF3 axis. *Int Immunopharmacol* 79: 106052, 2020.

29. Meng Q, Liang C, Hua J, Zhang B, Liu J, Zhang Y, Wei M, Yu X, Xu J and Shi S: A miR-146a-5p/TRAF6/NF- κ B p65 axis regulates pancreatic cancer chemoresistance: Functional validation and clinical significance. *Theranostics* 10: 3967-3979, 2020.
30. Zhao G and Gu W: Effects of miR-146a-5p on chondrocyte interleukin-1 β -induced inflammation and apoptosis involving thioredoxin interacting protein regulation. *J Int Med Res* 48: 300060520969550, 2020.
31. Zhang H, Zheng W, Li D and Zheng J: miR-146a-5p Promotes Chondrocyte Apoptosis and Inhibits Autophagy of Osteoarthritis by Targeting NUMB. *Cartilage*: Jul 27, 2012 (Epub ahead of print). doi: 10.1177/19476035211023550.
32. Zhang Y, Dong Q and Sun X: Positive Feedback Loop LINC00511/miR-150-5p/SP1 Modulates Chondrocyte Apoptosis and Proliferation in Osteoarthritis. *DNA Cell Biol* 39: 1506-1512, 2020.
33. Jimi E, Fei H and Nakatomi C: NF- κ B Signaling Regulates Physiological and Pathological Chondrogenesis. *Int J Mol Sci* 20: 6275, 2019.
34. Rigoglou S and Papavassiliou AG: The NF- κ B signalling pathway in osteoarthritis. *Int J Biochem Cell Biol* 45: 2580-2584, 2013.



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