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LINC01534 promotes the aberrant metabolic dysfunction and inflammation in IL-1β-simulated osteoarthritic chondrocytes by targeting miR-140-5p

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Abstract

Backgrounds: Long non-coding RNA 01534 (LINC01534) is highly expressed in the tissues of patients with osteoarthritis (OA). This study investigated the mechanism of LINC01534 on abnormal metabolic dysfunction and inflammation in OA chondro-cytes induced by IL-1β. Methods: The quantitative Real-Time PCR (qRT-PCR) was used to determine the expressions of LINC01534, aggrecan, collagen II and matrix metalloproteinase (MMPs) in OA cartilage tissue or OA chondrocyte model induced by IL-1β. The ex-pressions of aggrecan and collagen II in the chondrocyte were detected by Western Blot. The levels of TNF-α, IL-8, IL-6, MMP-13, MMP-9, MMP-3 and prostaglandin E2 (PGE2) in chondrocyte were determined by ELISA. Bioinformatics, dual luciferin gene reporting, RNA pulldown and Northern Blot were used to determine the interac-tion between LINC01534 and miR-140-5p. Results: The results showed that LINC01534 was up-regulated in both OA cartilage tissue and OA chondrocyte model. In addition, silencing LINC01534 significantly alleviated the inhibitory effect of IL-1β on expressions of aggrecan and collagen II in chondrocytes, and significantly down-regulated the expression of matrix metallopro-teinases in IL-1β-induced chondrocytes. Meanwhile, silencing LINC01534 also sig-nificantly inhibited the productions of pro-inflammatory factors NO, PGE2, TNF-α, IL-6 and IL-8 in the IL-1β-induced chondrocytes. Furthermore, miR-140-5p was con-firmed to be a direct target of LINC01534. More importantly, inhibition of miR-140-5p significantly reversed the inhibitory effect of silencing LINC01534 on inflammation and abnormal matrix degradation in the IL-1β-induced chondrocyte model of OA. Conclusion: Therefor, LINC01534 could promote the abnormal matrix degradation and inflammatory response of OA chondrocytes through the targeted binding of miR-140-5p.

Background

Osteoarthritis (OA) is a chronic inflammatory disease of the joints, its main pathological manifestations are the degeneration of articular cartilage tissue, the disorder of metabolism of chondrocyte extracellular matrix and inflammatory reaction, so it is also known as degenerative osteoarthritis disease (1). The pathogenesis of OA is related to a variety of factors, such as inflammation, obesity, age, gender, joint injury, etc., but the etiology and pathogenesis have not been fully understood (2, 3). Chondrocytes are the only cell type in articular cartilage (4). The aggrecan and collagen II synthesized and secreted by chondrocytes are the major components of the extracellular matrix of cartilage and play important roles in maintaining the structural and functional integrity of articular cartilage (5). In the pathogenesis of OA, chondrocytes have abnormal metabolic dysfunction, mainly manifested as inhibited synthesis of aggrecan and collagen II, and increased synthesis of matrix degrading enzyme matrix metalloproteinase (MMPs) (6). MMPs mainly degrade collagen fibers, of which MMP13 has the strongest ability to degrade type II collagen, while MMP-3 has a high lysis activity against polysaccharides (7). Therefore, MMPs can promote the degradation of OA cartilage extracellular matrix (8). In addition, the large release of inflammatory factors such as IL-1β, IL-6 and TNF-α not only damages chondrocytes, but also induces extracellular matrix degradation, which is also an important reason for promoting the development of OA (9, 10). IL-1β and TNF-α can promote the decomposition of aggrecan and collagen II and the synthesis of

MMPs, and play a synergistic role in the pathogenesis of OA (10). Furthermore, IL-1β stimulates chondrocytes to release NO, which enhances TNF-α-induced degradation of aggrecan (11). PGE2 is closely associated with inflammation and pain (12). Therefore, pro-inflammatory factors such as IL-1β, TNF-α, NO and PGE2 are involved in the pathogenesis of OA (13).

Studies have found that multiple long non-coding RNAs (lncRNAs) are involved in the pathogenesis of OA and can be potential targets for the treatment of OA (14). LINC01534 is a newly discovered lncRNA, which has been confirmed to be highly expressed in OA tissue (15). MicroRNA (miRNA) is a class of endogenous non-coding RNA molecules composed of 18-22 nucleotides, which is not only involved in tumorigenesis and development (16), but also closely related to the pathogenesis of OA (16). Studies have reported that miR-140-5p is abnormally expressed in bladder cancer, non-small cell lung cancer, gastric cancer, glioma and other tumor tissues (17, 18), and is also involved in the pathogenesis of rheumatoid arthritis and knee osteoarthritis (19). As a competitive endogenous RNA (ceRNA), lncRNA can bind to miRNA to inhibit the function of miRNA, thus affecting the occurrence and development of OA (20). However, the mechanism of LINC01534 and miR-140-5p in the progression of OA has not been reported. In this study, the effects of LINC01534 and miR-140-5p on abnormal metabolic dysfunction and inflammation in OA chondrocytes was explored by establishing the OA chondrocyte model induced by IL-1β, so as to provide a certain theoretical basis for the treatment of OA.

Methods

Tissues collection

25 patients (ages 32-59, 14 males and 11 females) with trauma or death were selected and normal cartilage tissue samples were obtained. In addition, 25 patients (aged 30-60 years, 15 males and 10 females) with OA were selected for endoscopic surgery to obtain OA cartilage tissue. All the tissue samples were frozen rapidly by liquid nitrogen, and then saved in the - 80 ℃ cryogenic refrigerator. Ethical approval was obtained from the Ethics Committee of The second Affiliated Hospital of Kunming Medical University. All patients or their families received written informed consent.

Primary chondrocyte culture

First, trypsin was used to digest the cartilage tissue for 10 min. Next, the cartilage was digested overnight in DMEM medium (10% FBS) using collagenase II. The digested chondrocytes were separated and centrifuged at 2000 g for 5 min. Chondrocytes were re-suspended using DMEM medium (10% FBS) and 100 μg/mL streptomycin and 100 U/mL penicillin were selectively added to the medium. Subsequent experiments selected chondrocytes between the first and third generation, and used 10 ng/mL IL-1β induction to establish the OA chondrocyte model.

Cell transfection

Si-LINC01534, miR-140-5p inhibitor (miR-140-5p-inh), miR-140-5p mimic, and their respective negative controls were purchased from Gene Pharma. Oligonucleotide or recombinant plasmids were transfected into chondrocytes using the Lipofectamine 2000 reagent.

The qRT-PCR assay

Total RNA was extracted from OA cartilage tissue or chondrocytes using Trizol reagent (Invitrogen), and was reversely transcribed into cDNA using Super Script II First Strand Synthesis System (Invitrogen). Then, SYBR Premix Ex TaqTM II kit (Takara) was used for qRT-PCR amplification of primers MMP-13, MMP-9, MMP-3, aggrecan, collagen II, miR-140-5p, LINC01534 (Sangon Biotech) and template cDNA. β-actin was used as the internal reference of mRNA and U6 as the internal reference of miRNA. The relative expression levels of each gene were calculated by $2^{-\triangle\triangle Ct}$ method.

Western Blot assay

Total proteins in cells were extracted using RIPA lysate and PMSF, and quantified using BCA protein concentration kit (Beyotime). Total protein was separated by 12% SDS-PAGE gel and transferred to cellulose acetate membrane. 5% skim milk was then used to seal the cellulose acetate membrane for 2 h. Next, the primary antibody was used to incubate the membrane under 4 ℃ for overnight, and then the membrane was incubated with a second antibody for 1 h at room temperature. GAPDH was used as the internal reference of the proteins. ECL reagent (Beyotime) and GelDocTM XR imaging system (Bio-Rad) were used to perform color imaging of the protein bands, and Quantity One software was used to analyze the grayscale value.

ELISA assay

The contents of IL-6, IL-8, TNF-α, PGE2, MMP-3, MMP-9 and MMP-13 in the cell supernatant were determined by ELISA kits (ebioscience), and the specific steps were strictly in accordance with the instructions of the kits.

Determination of NO

NO production in cell supernatant was determined using NO detection kit (NanJing JianCheng institute of biological engineering), and the specific steps were strictly in accordance with the instruction of the kit. After Griess reaction for 10 min, the absorbance value at 550 nm was determined, and the content of NO was calculated according to the standard nitrite curve.

Luciferase reporter gene assay

The mutant LINC01534 (LINC01534-mut) or wild-type LINC01534 (LINC01534-wt) was inserted into the pGL3 vector (Promega). The LINC01534-mut) or LINC01534-wt vectors were transfected into chondrocytes with miR-140-5p mimic or miR-NC using Lipofectamine 2000 reagent. The luciferase activity was measured after transfection for 48 h.

Pulldown assay

A pH 7.5 buffer (1 mM EDTA, 0.5 M NaCl, 20 mM Tris-HCl) was used to dissolve the biotinylated DNA probe complementary to LINC01534 RNA. The streptavidin-coupled beads were incubated with the DNA probe. RNA isolated by protease K digestion was determined using Northern Blot. The biomimetic miR-140-5p was transfected into the OA chondrocyte model and lytic the cells. Streptavidin-coupled beads were co-incubated with the lysis products of chondrocytes to form the biotin-miRNA-lncRNA complex. Binding RNA was extracted with Trizol reagent, and the enrichment of lncRNA LINC01534 was determined by qRT-PCR.

Northern Blot assay

Samples were separated with 15% polyacrylamide-urea gel and transferred to nylon membrane (Millipore) with positive charge. After UV irradiation, the nylon membrane was incubated with the 100 pmol 3-digoxigenin (DIG) labelled probes of miR-140-5p at 42 ℃ for the night. The extent of hybridization was determined using DIG light detection kit (Roche), and the specific steps were strictly in accordance with the instruction of the kit.

Statistical analysis

All data are expressed as mean standard ± deviation (SD). SPSS 19.0 software was used for data analysis, and t-test, ANOVA and Tukey's post-mortem were used for significance analysis. $P < 0.05$ indicated a significant difference.

Results

The mRNA expressions of LINC01534 in human OA cartilage tissues and OA chondrocyte model

The mRNA expression of LINC01534 in human OA cartilage tissue (n = 25) and normal cartilage tissue (n = 25) were determined by qRT-PCR. The results found that LINC01534 expression in OA cartilage tissue was remarkably higher than that in normal cartilage tissue (**Fig. 1A**, $P < 0.05$), indicating that LINC01534 was highly expressed in OA cartilage tissue. In addition, the mRNA expression of LINC01534 in primary chondrocytes was determined by qRT-PCR. The results revealed that the mRNA expression of LINC01534 in the OA chondrocyte model induced by IL-1β was significantly higher than that in the control group (**Fig. 1B**, $P < 0.05$), which suggested that LINC01534 was also highly expressed in the OA chondrocyte model.

Effect of LINC01534 on the metabolic dysfunction of chondrocytes induced by IL-1β

The chondrocytes were transfected with LINC01534 siRNA to investigate the effect of LINC01534 on IL-1β-induced chondrocyte metabolic dysfunction. The results showed that LINC01534 expression was inhibited in primary chondrocytes transfected with si-LINC01534 (**Fig. 2B**, $P < 0.05$), which indicated that si-LINC01534 successfully silenced the expression of INC01534 in the cells. In addition, the mRNA expressions of the main components of the chondrocyte extracellular matrix (aggrecan and collagen II) in

the chondrocytes of the IL-1β group was remarkably lower than that of the control group, suggesting that IL-1β inhibited the expression of the main components of the chondrocyte extracellular matrix. The mRNA expressions of aggrecan and collagen II in chondrocytes of the IL-1β + si-LINC01534 group were significantly higher than those in the IL-1β group (**Fig. 2B**, $P < 0.05$), indicating that silencing LINC01534 alleviated the inhibitory effect of IL-1β on the major components of the extracellular matrix. At the same time, we also used Western Blot to determine the protein expressions of aggrecan and collagen II, and the results showed the same expression trend as the results of qRT-PCR (**Fig. 2D and 2E**, $P < 0.05$). Moreover, the mRNA expression levels of MMP-13, MMP-9 and MMP-3 in chondrocytes were determined by qRT-PCR. The results showed that IL-1β remarkably up-regulated the mRNA expressions of MMP-13, MMP-9 and MMP-3 in chondrocytes ($P < 0.05$), while silencing LINC01534 significantly inhibited the mRNA expressions of MMP-3, MMP-9 and MMP-13 in chondrocytes induced by IL-1β (**Fig. 2C**, $P < 0.05$). Furthermore, the contents of MMP-13, MMP-9 and MMP-3 in the supernatant of chondrocytes were determined by ELISA. It was found that silencing LINC01534 also significantly inhibited the protein secretion of MMP-3, MMP-9 and MMP-13 in IL-1β-induced chondrocytes (**Figs. 2F-2H**, $P < 0.05$). The above results suggested that silencing LINC01534 could remarkably alleviate the abnormal metabolism dysfunction of IL-1β-induced chondrocytes, suggesting that LINC01534 could promote the degradation of chondrocyte extracellular matrix.

Effect of LINC01534 on inflammatory responses of chondrocytes induced by IL-1β

The secretions of inflammatory factors in chondrocyte supernatant were measured to investigate the role of LINC01534 in the inflammatory response of chondrocytes induced by IL-1β. The results showed that the levels of PGE2, NO, IL-6, IL-8 and TNF-α in the chondrocyte supernatant in the IL-1β group were significantly higher than those in the control group (**Fig. 3A-E**, $P < 0.05$), indicating that IL-1β induced chondrocyte inflammation. In addition, silencing LINC01534 remarkably reduced the contents of PGE2, NO, IL-6, IL-8 and TNF-α in the chondrocyte supernatant induced by IL-1β, which indicated that silencing LINC01534 significantly suppressed the chondrocyte inflammatory response induced by IL-1β.

Identification of miR-140-5p as a direct target of LINC01534

First, we transfected si-LINC01534 or siNC into the OA chondrocyte model to determine the expression of miR-140-5p. The results showed that silencing LINC01534 significantly upregulated the expression of miR-140-5p in the OA chondrocyte model (**Fig. 4A**, $P < 0.05$). In addition, we transfected the recombinant LINC01534 plasmid or pNC into the OA chondrocyte model to determine the expression of LINC01534 and miR-140-5p. The results showed that the recombinant plasmid LINC01534 significantly up-regulated the expression of LINC01534 in the OA chondrocyte model (**Fig. 4B**, $P < 0.05$), and remarkably inhibited the expression of miR-140-5p (**Fig. 4C**, $P < 0.05$), which indicated that LINC01534 negatively regulated the expression of miR-140-5p in the OA chondrocyte model. In order to further explore the interaction between LINC01534 and miR-140-5p, bioinformatics was used to find that miR-140-5p might contain the binding site of LINC01534 (**Fig. 4D**). In addition, after co-transfection with miR-140-5p, the luciferase activity of cells in the LINC01534-wt group was significantly lower than that in the LINC01534-mut group (**Fig. 4E**, *P*

< 0.05). Furthermore, the results of pulldown and Northern Blot analysis showed that the Bio-LINC01534 probe could pulldown miR-140-5p (**Fig. 4F**). Moreover, the results of pulldown and qRT-PCR showed that LINR01534 was remarkably enriched after Bio-miR-140-5p-wt transfection ($P < 0.05$), while Bio-miR-140-5p-mut transfection showed no such effect (**Fig. 4G**). These results indicated that miR-140-5p was the direct target of LINC01534 in the OA chondrocyte model.

Effects of miR-140-5p on abnormal metabolic dysfunction and inflammatory responses in the OA chondrocyte model mediated by LINC01534

The miR-140-5p inhibitor was used to investigate the effect of miR-140-5p on abnormal metabolic dysfunction and inflammatory response inhibited by silenced LINC01534 in the OA chondrocyte model. The qRT-PCR results showed that the miR-140-5p inhibitor significantly inhibited the mRNA expression of miR-140-5p in the OA chondrocyte model (**Fig. 5A**, $P < 0.05$). Additionally, miR-140-5p inhibitor significantly down-regulated the mRNA expressions of aggrecan and collagen II promoted by silenced LINC01534 in the OA chondrocyte model. (**Fig. 5B**, $P < 0.05$). Furthermore, miR-140-5p inhibitor also remarkably up-regulated the mRNA and protein expressions of MMP-3, MMP-9 and MMP-13 inhibited by silenced LINC01534 in the OA chondrocyte model (**Figs. 5C-E**, $P < 0.05$). Moreover, miR-140-5p inhibitor also significantly increased the contents of PGE2, NO, IL-6, IL-8, and TNF-α decreased by silenced LINC01534 in the OA chondrocyte model (**Figs. 5F-I**, $P < 0.05$). The above results suggested that LLINC01534 could promote abnormal metabolic dysfunction and inflammatory responses in the IL-1β-induced chondrocyte model of OA by targeting miR-140-5p.

Discussion

OA is a degenerative osteoarthritis disease characterized by reduction of chondrocytes and degeneration of articular cartilage tissue (21). Studies have confirmed that a variety of long non-coding RNAs (lnc RNAs) are related to the pathogenesis of OA (22). LINC01534 is a newly discovered LNC RNA, which has been confirmed to be highly expressed in OA tissue (15). However, the specific mechanism of LINC01534 in the pathogenesis of OA remains unclear. In this study, the mRNA expression of LINC01534 in human OA cartilage tissue (n = 25) and normal cartilage tissue (n = 25) was determined by qRT-PCR. The results found that LINC01534 was highly expressed in OA cartilage tissue, which was consistent with the results reported in the literature (15). Therefore, the results of this study suggested that LINC01534 might be involved in the pathogenesis of OA.

The immortalized chondrosarcoma cell line and the primary chondrocytes derived from the patient are often used for the *in vitro* study of OA, and the primary chondrocytes can better reflect the intracellular changes during the development of OA (23). Currently, IL-1β is commonly used as an inducer *in vitro* experiments to simulate the inflammatory environment of OA (24). In the present study, IL-1β induction was used to establish the OA chondrocyte model, and qRT-PCR results showed that LINC01534 was also highly expressed in the OA chondrocyte model. Studies have found that lncRNA can be used as a ceRNA to bind miRNA, thereby inhibiting the function of miRNA and affecting the pathogenesis of OA (25). MiR-

140-5p is not only abnormally expressed in various tumor tissues, but also involved in the pathogenesis of rheumatoid arthritis and knee osteoarthritis (26-29). However, the interaction mechanism between LINC01534 and miR-140-5p in the development of OA is still unclear. This study found that LINC01534 negatively regulated the expression of miR-140-5p in the OA chondrocyte model. In order to further explore the interaction between LINC01534 and miR-140-5p, bioinformatics was used to find that miR-140-5p might contain the binding site of LINC01534. In addition, the direct interaction between LINC01534 and miR-140-5p was confirmed by luciferase activity determination and RNA pulldown assay. The results of this study suggested that miR-140-5p was a direct target of LINC01534 in the OA chondrocyte model.

In the process of the development of OA, abnormal metabolic dysfunction occurs in chondrocytes, and the synthesis of aggrecan and collagen II, the main components of chondrocyte extracellular matrix, are inhibited, while the synthesis of MMPs is increased, thus promoting the abnormal degradation of chondrocyte extracellular matrix (30-32). In addition, the large release of inflammatory factors such as IL-1β, IL-6, NO, PGE2 and TNF-α not only damages chondrocytes, but also induces extracellular matrix degradation, which is also an important reason for promoting the development of OA (33-35). In this study, it was found that silencing LINC01534 alleviated the inhibitory effect of IL-1β on the major components of chondrocytes, and significantly inhibited the synthesis of MMP-3, MMP-9 and MMP-13, while miR-140-5p inhibitor remarkably reversed this effect. Furthermore, this study also found that silencing LINC01534 significantly reduced the contents of PGE2, NO, IL-6, IL-8, and TNF-α in the chondrocyte supernatant induced by IL-1β, while miR-140-5p inhibitor showed the opposite effect. Zhao *et al.* found that lncRNA PVT1 inhibited the expression of aggrecan and collagen II in IL-1β-induced OA chondrocytes by targeting miR-149, up-regulated the secretions of MMPs, TNF-α, IL-8, IL-6, NO, and PGE2, and ultimately aggravated chondrocyte catabolism and inflammation (36). Therefore, the results of this study suggested that LLINC01534 could promote abnormal metabolic dysfunction and inflammatory responses in the IL-1β-induced chondrocyte model of OA by targeting miR-140-5p.

Conclusions

In conclusion, the present study assessed the molecular mechanism of LINC01534 on abnormal metabolic dysfunction and inflammation in OA chondrocytes induced by IL-1β. These results confirmed that LINC01534 could promote the abnormal matrix degradation and inflammatory response of OA chondrocytes through the targeted binding of miR-140-5p, which was expected to be a potential target for the treatment of OA.

Abbreviations

Long non-coding RNA 01534 (LINC01534); osteoarthritis (OA); quantitative Real-Time PCR (qRT-PCR); matrix metalloproteinase (MMPs); prostaglandin E2 (PGE2); MicroRNA (miRNA); competitive endogenous RNA (ceRNA); 3-digoxigenin (DIG).

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of The second Affiliated Hospital of Kunming Medical University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.Written informed consent was obtained from all individual participants included in this study.

Consent for publication

Not applicable.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WW and SXH: analyzed and interpreted the patient data, experiments work and manuscript writing. ZHW: literature research, research design and manuscript editing. JJD, DX, YXL, LRR, NNK and JL: literature research, clinical research and data anlysis. All authors read and approved the final manuscript.

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Figures

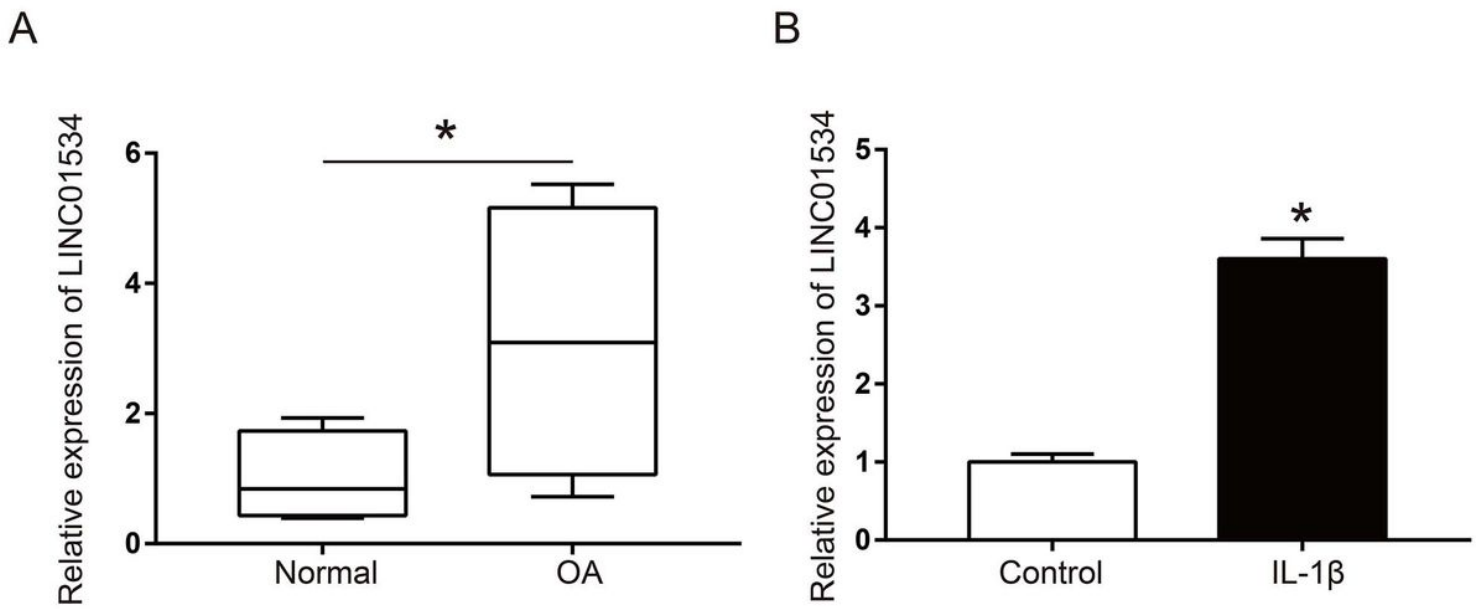


Figure 1

The mRNA expressions of LINC01534 in human OA cartilage tissues and OA chondrocyte model. (A) Detection of the mRNA expression of LINC01534 in human OA cartilage (n = 25) and normal cartilage (n = 25) by qRT-PCR. (B) Detection of the mRNA expression of LINC01534 in primary chondrocytes by qRT-PCR. *P < 0.05.

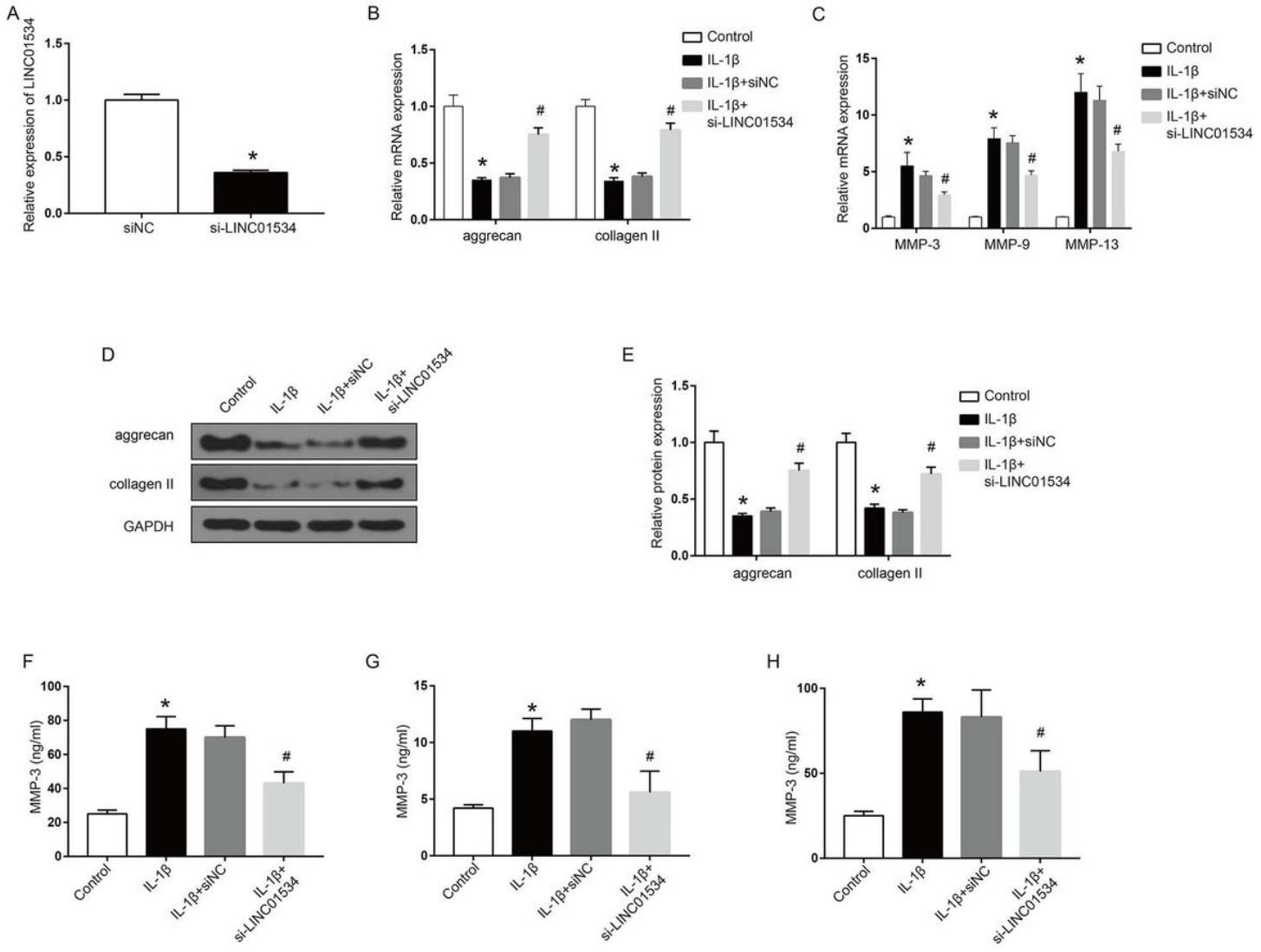


Figure 2

Effect of LINC01534 on the metabolic dysfunction of chondrocytes induced by IL-1β. (A) Detection of the expression of LINC01534 in chondrocytes transfected with si-LINC01534 or siNC by qRT-PCR. (B) Detection of the mRNA expressions of ag-grecan and collagen II in chondrocytes by qRT-PCR. (C) Detection of the mRNA ex-pressions of MMP-3, MMP-9 and MMP-13 in chondrocytes by qRT-PCR. (D, E) De-tection of the protein expressions of MMP-3, MMP-9 and MMP-13 in chondrocytes by Western Blot. (F-G) Detection of the contents of MMP-3, MMP-9 and MMP-13 in chondrocyte supernatant by ELISA. *P < 0.05.

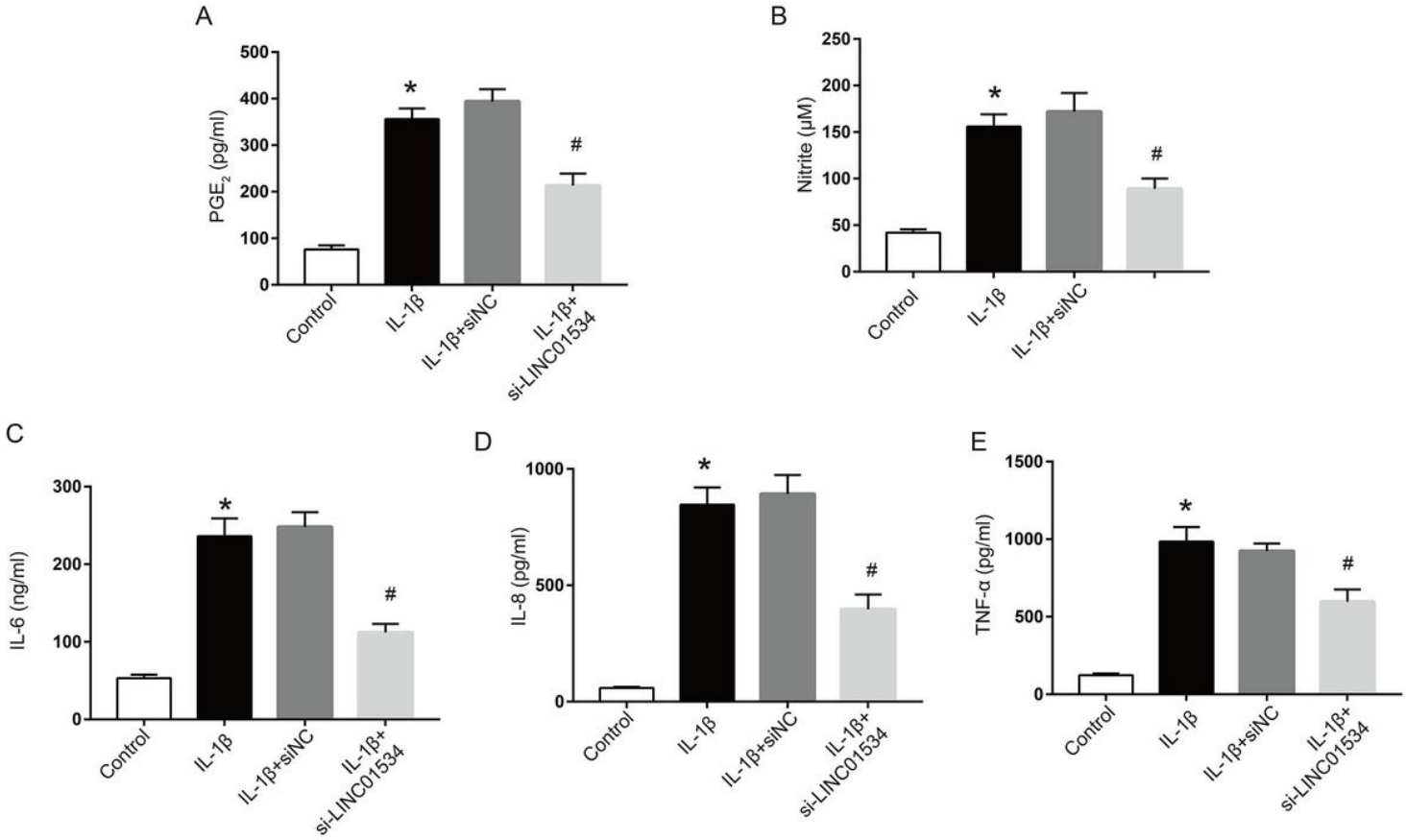


Figure 3

Effect of LINC01534 on inflammatory responses of chondrocytes induced by IL-1β. (A-E) Determination of PGE2 (A), NO (B), IL-6 (C), IL-8 (D) and TNF-α (E) in chondrocytes. *P < 0.05, #P < 0.05.

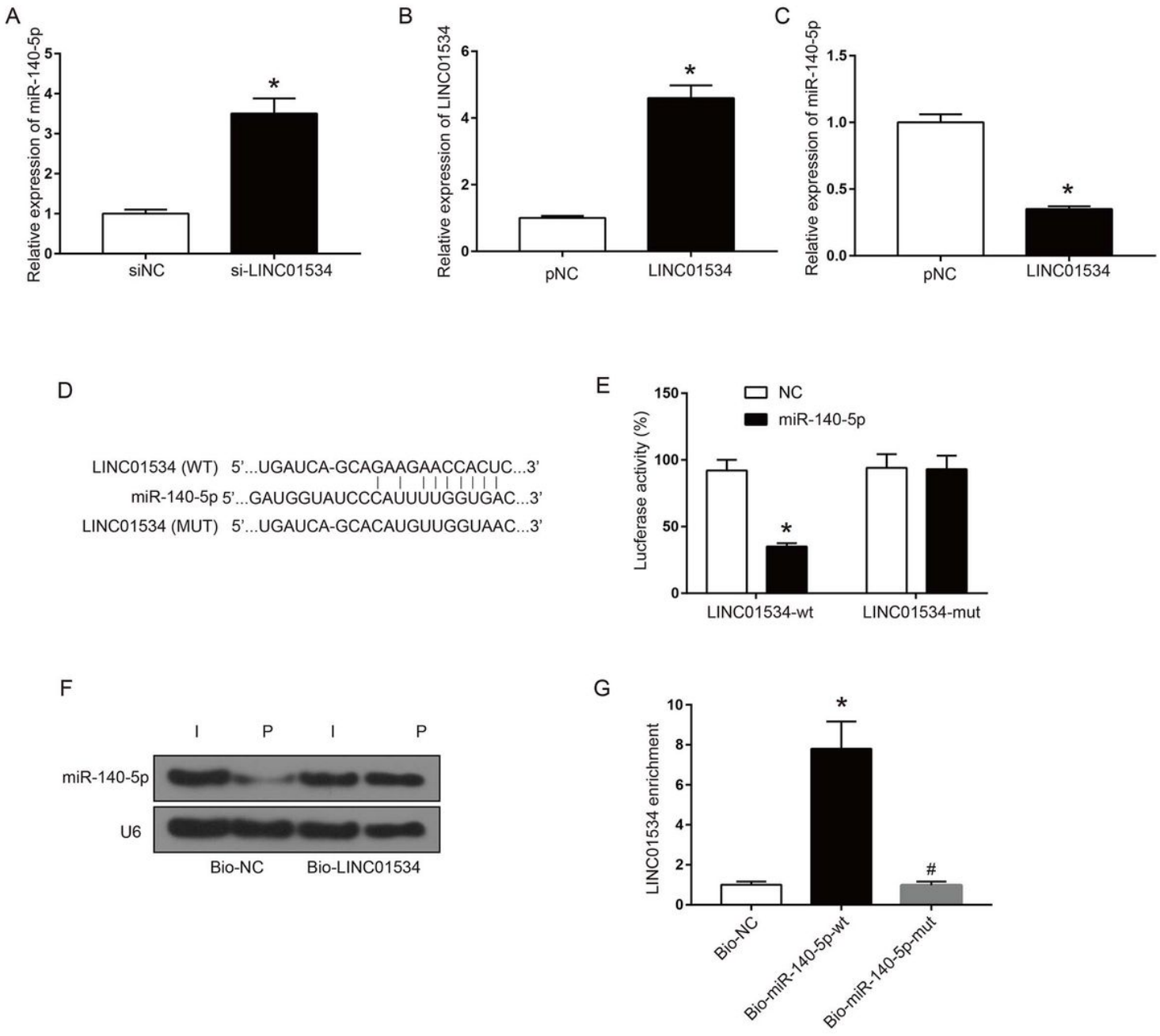


Figure 4

Identification of miR-140-5p as a direct target of LINC01534. (A) Determination of the expression of miR-140-5p in si-LINC01534 or siNC-transfected OA chondro-cyte model by qRT-PCR. (B, C) Determination of the expression of LINC01534 or miR-140-5p in OA chondrocyte model transfected with recombinant LINC01534 plas-mid or pNC by qRT-PCR. (D) Schematic diagram of binding sites of miR-140-5p and LINC01534. (E) Determination of the luciferase activity in OA chondrocytes transfect-ed with LINC01534-wt or LINC01534-mut and miR-140-5p or miR-NC. (F) Deter-mination of Bio-linc01534 probe binding to miR-140-5p by Pulldown and Northern blot analysis. (G) Determination of the enrichment effect of Bio-miR-140-5p-wt or Bio-miR-140-5p-mut on LINR01534 by Pulldown and qRT-PCR. *P < 0.05, #P < 0.05.

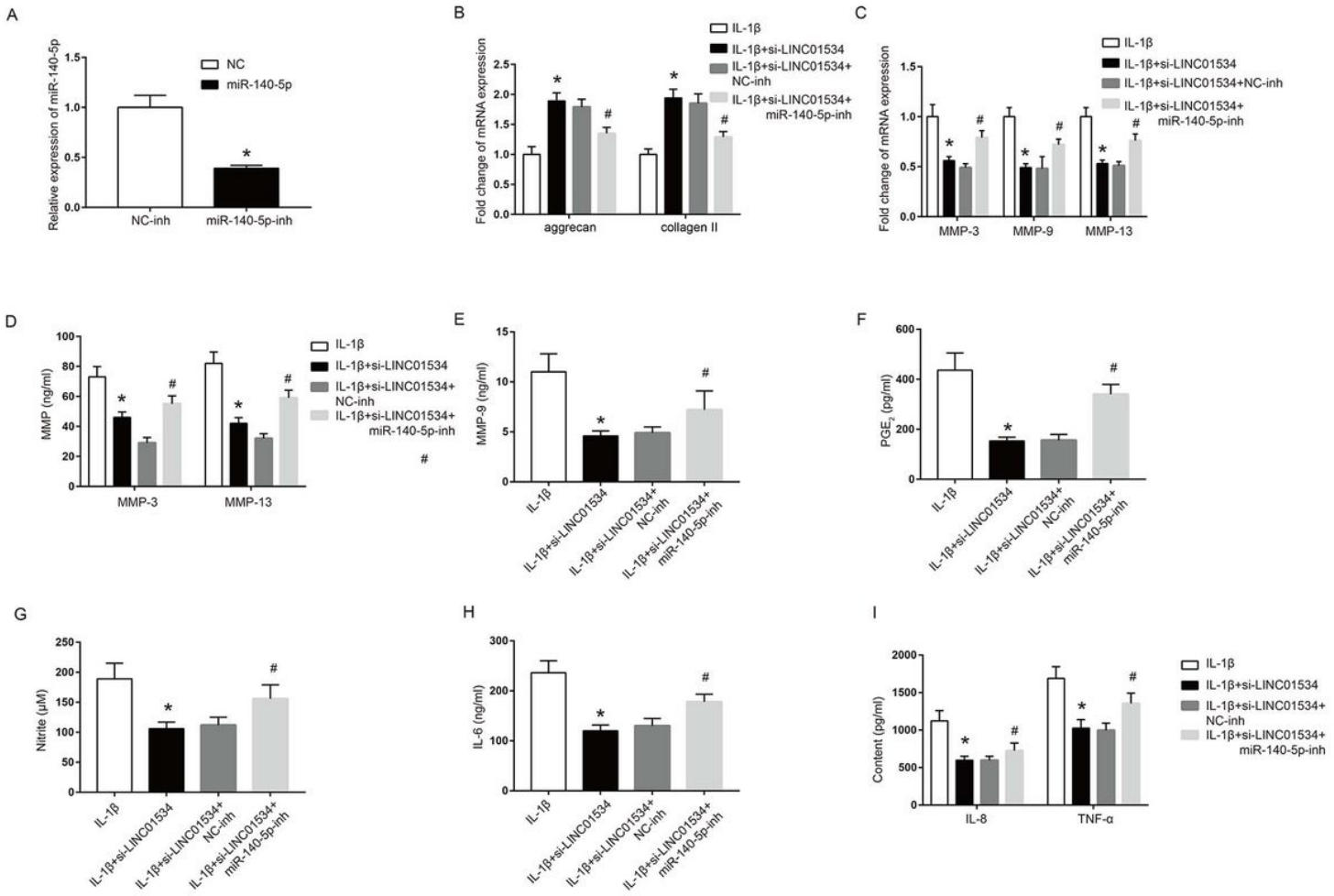


Figure 5

Effects of miR-140-5p on abnormal metabolic dysfunction and inflammatory responses in the OA chondrocyte model mediated by LINC01534. (A) Effect of miR-140-5p inhibitor on the mRNA expression of miR-140-5p in chondrocytes. (B) Effect of miR-140-5p inhibitor on the mRNA expressions of aggrecan and collagen II in chondrocytes. (C) The effect of miR-140-5p inhibitor on the mRNA expressions of MMP-3, MMP-9 and MMP-13 in chondrocytes. (D) Effect of miR-140-5p inhibitor on MMP-3 and MMP-13 contents in the supernatant of chondrocytes. (E) Effect of miR-140-5p inhibitor on MMP-9 content in the supernatant of chondrocytes. (F-I) Ef-fect of miR-140-5p inhibitor on the contents of PGE2 (F), NO (G), IL-6 (H), IL-8 and TNF-α (I) in in the supernatant of chondrocytes. *P < 0.05, #P < 0.05.