miR-200a/b relieves IL-1β-induced cell injury in knee articular chondrocytes ex-vivo by targeting fucosyltransferase 4 (FUT4)

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Research

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Abstract

Background: Osteoarthritis (OA) is a common subtype of arthritis with prevalence increase with age, and is characterized by the degeneration of articular cartilage. Chondrocytes play curial role in the formation of the articular cartilage. This work aimed to figure out the effect of miR-200a/b in chondrocytes of OA, as well as the underlying molecular mechanism.

Methods: Cell viability, apoptosis, pro-inflammatory factors secretion, and matrix degradation were detected with cell counting kit-8 (CCK-8), flow cytometry, enzyme-linked immunosorbent assay (ELISA), and western blotting, separately. Expression of miR-200a/b and fucosyltransferase 4 (FUT4) was measured by RT-qPCR (RNA level) and western blot (protein level). The relationship between miR-200a/b and FUT4 was verified by dual-luciferase assay and RNA immunoprecipitation (RIP) assay.

Results: Interleukin 1β (IL-1β) induced OA cell model in primary chondrocytes ex-vivo, as evidenced by cell viability inhibition, apoptosis rate promotion, and IL-6 and tumor necrosis factor α (TNF-α) resection enhancement, as well as Collegen 2a1 (Col2a1) and Aggrecan expression inhibition. Expression of miR-200a/b was downregulated in knee articular cartilage of OA patients and IL-1β-induced primary chondrocytes. miR-200a/b overexpression decreased IL-1β-induced cell injuries, which was further blocked by FUT4 upregulation. Mechanically, FUT4 was negatively regulated by miR-200a/b via target binding.

Conclusion: miR-200a/b could alleviate IL-1β-induced chondrocyte injuries via targeting its downstream gene FUT4, suggesting that miR-200a/b-FUT4 axis might be a potential candidate to the treatment of OA.

Background

Osteoarthritis (OA) is classic age-related arthritis characterized by serious pain, limited movement, and crepitus [1]. The prevalence and severity of OA increase with age, thus OA has been becoming one common thereat in the old populations [2]. Synovium and articular cartilage degradation injury and a variable degree of inflammation are the mainly pathological changes of OA [3]. Articular chondrocyte is the only cell type in articular cartilage, therefore plays the pivotal role in the fate of articular cartilage [4]. The aberrant functions of chondrocytes result in the imbalance of extracellular matrix (ECM) synthesis [5], which lead to degradation of the articular cartilage tissue. Therefore, chondrocytes have been becoming as the preferred cells in OA researches, and apoptosis of chondrocytes is suggested to contribute to the pathogenesis of OA [6]. Although chondrocytes comprise 2–3% of total cartilage volume [4], it is necessary to study the cellular functions of chondrocytes for a better understanding of the pathogenesis of OA.

It has been widely suggested that the aetiology of OA was attributed to the deregulation of gene expression in chondrocytes [7]. microRNAs (miRNAs) belong to small non-coding RNAs that are essentially endogenous transcripts. Data indicated that miRNAs are direct regulators for about one third of the genes via target binding [8]. Recently, miRNAs has also been shown to be involved in maintaining

cartilage homeostasis in OA, as well as inflammatory response during development [9]. For example, several miRNAs have been demonstrated to participate in OA, such as miRNA (miR)-320, miR-21 and miR-140 [10–12]. The miR-200 family is mainly characterized as tumor suppressor [13], and miR-200a-3p (miR-200a) and miR-200b-3p (miR-200b) have been preliminarily identified in OA [14, 15]. However, the potential role of miR-200a/b in OA development remains to be widely studied.

The fucosyltransferase 4 (FUT4) is one member of FUTs family that are key enzymes in the surface of cells and take part in multiple biological processes, including inflammation and cancer progression [16, 17]. In OA, FUT4 has been reported to be complicated in the progression of OA [18]. Interleukin 1beta (IL-1β) is a pro-inflammatory cytokine that contributes to the onset and progression of OA [19, 20]. Therefore, we aimed to investigate the role of miR-200a/b in IL-1β-induced OA model in primary chondrocytes ex-vivo. The mechanism study was also performed to explore the targeted functional protein of miR-200a/b in modulating knee articular chondrocyte progression including cell progression, inflammation, and matrix degradation.

Materials And Methods

Patients and tissue specimens

With the written informed consents obtained from each patient, a total of 60 knee cartilage tissues were collected from 2016 to 2018 in the Baoji Traditional Chinese Medicine Hospital, including 30 OA specimens from OA patients (mean age: 42.5 years old) and 30 normal specimens from emergency traumatic amputated patients (mean age: 34.6 years old). All patients with arthritis, including rheumatoid arthritis (RA), OA and septic arthritis, were excluded in control group. The samples were put into liquid nitrogen at once and stored for cryopreservation. This study was approved by the ethic committee of the Baoji Traditional Chinese Medicine Hospital.

Cell culture

Under aseptic conditions, the cartilage tissues were removed of fibrous connective tissues, and cut into small pieces. After washing with sterile phosphate-buffered saline (PBS), cartilage tissues were digested with 0.25% trypsin (Invitrogen, Carlsbad, CA, USA) for 30 min, and then with 0.2% collagenase Type II (Col2; Millipore Corp., Billerica, MA, USA) in Dulbecco's modified Eagle's medium (DMEM; Gibco, Life Technologies, Carlsbad, CA, USA) for 10 h at 37 ^{o}C. Subsequently, these cells were filtered with 40 mm filter, and then centrifuged and washed at least three times, last re-suspended in growth culture media supplemented with DMEM (Gibco), 10% fetal bovine serum (Gibco) and 100 U/ml penicillin and 100 μg/ml streptomycin solution (Invitrogen). The cells were cultured in humidified sterile air with 5% (v/v) CO_2 at 37 ^{o}C, and the first passage chondrocytes were obtained for 10 days. The chondrocyte cells were treated with 0.25% trypsin (Invitrogen) for cell passage.

IL-1β stimulation

For OA cell model in primary chondrocytes, all experiments were depend on 1–3 passage cells exposed with IL-1β (Sigma-Aldrich, St. Louis, MO, USA). IL-1β was dissolved in ultrapure water to a storage concentration of 10 mg/ml according to the instruction. For IL-1β stimulation, chondrocytes were incubated in serum-free growth culture media containing IL-1β in a series of concentrations (0, 5, 10 and 20 ng/ml) for 24 h.

Cell counting kit-8 (CCK-8) assay

The viability of cells was determined according to CCK-8 (Dojindo Laboratories, Kumamoto, Japan) manufactures. In brief, the chondrocytes and transfected chondrocytes were treated with 0, 5, 10 and 20 ng/ml of IL-1β for 24 h. IL-1β-induced chondrocytes were seeded onto 96-well plate (Corning, NY, USA) at a density of 1×10^4 cells/well for 24 h. Cells were cultured with 20 μl CCK-8 solution (5 g/l) in PBS for another 2 h, and the optical density was measured at 450 nm using a microplate reader. Three independent wells were performed in each group.

Apoptosis assay

IL-1β-induced chondrocytes were seeded onto 6-well plate (Corning) with 1×10^5 cells per well for 24 h and analyzed by Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (Beyotime, Shanghai, China) on flow cytometry. Apoptotic cells were labelled complying with the protocol. In short, the adherent were digested with 0.25% trypsin (Invitrogen) without EDTA, and harvested. Then, the cells were washed with ice PBS for twice, and re-suspended in 500 μl PBS. Next, 100 μl of cells in each group was stained in the binding buffer containing 5 μl FITC-Annexin V and 5 μl PI for 30 min at 25 ^{o}C in the dark. Fluorescence of labeled cells was analyzed on cytoFLEX LX flow cytometer (Beckman-Counter Electronics, Jiangsu, China) using CytExpert software.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was conducted to measure the concentration of IL-6 and TNF-α released. Chondrocytes and transfected chondrocytes were seeded onto 24-well plates (Corning) for 24 h. After IL-1β stimulation, the culture supernatant was collected for estimation of inflammatory factors in line with the manufacturer's instruction. The ELISA kits including human IL-6 kit (ab46027), and human TNF-α kit (ab46087) were purchased from Abcam (Cambrige, UK).

Real-time Quantitative PCR (RT-qPCR)

Total RNA from tissues and primary chondrocytes was isolated using TRIzol reagent (Life Technologies) following the protocol. Reverse transcription kit (Abcam) and/or Bestar QPCR RT Kit (DBI Bioscience, Germany) were utilized to reverse transcribe into cDNA depending on 300 ng of total RNA sample. The amplification of cDNA was performed using SYBR Premix Ex Taq Master Mix (Invitrogen) and/or Bestar SybrGreen qPCR MasterMix (DBI Bioscience) relying on special primers. The expression of miR-200a/b and FUT4 mRNA was analyzed on ABI PRISM 7900 Real-time PCR System (Applied Biosystems, Foster City, CA), and calculated according to the comparative threshold cycle value ($2^{-\Delta\Delta Ct}$) method, compared with internal control GAPDH (for mRNA) or U6 small nuclear RNA (U6, for miRNA). All primers were

synthesized by Ribobio (Guangzhou, China), including miR-200a: 5′-TAACACTGTCTGGTAACGATGT-3′ (sense) and 5′-CATCTTACCGGACAGTGCTGGA-3′ (antisense); miR-200b: 5′-GCTGCTGAATTCCATCTAATTTCCAAAAG-3′ (sense) and 5′-TATTATGGATCCGCCCCCAGGGCAATGGG-3′ (antisense); FUT4: 5′-CCGGCGAAGTTATCAAGGGTT-3′ (sense) and 5′-AAAGGAACAACTTTCCCCGA-3′ (antisense); U6: 5′-ACCCTGAGAAATACCCTCACAT-3′ (sense) and 5′-GACGACTGAGCCCCTGATG-3′ (antisense); GAPDH: 5′-GAAGATGGTGATGGGATTTC-3′ (sense) and 5′-GAAGGTGAAGGTCGGAGT-3′ (antisense). The reactions were performed in quadruplicate for each sample.

Cell transfection

The primary chondrocytes were seeded onto 6-well plate (Corning) at density of 5×10^4/well prior to transfection for 24 h. For overexpression, the coding domain sequence (CDS) of FUT4 (NM_002033.3) was cloned into the multiple cloning site (MCS) of the pcDNA3.1 vector (Invitrogen); miR-200a mimic, miR-200b mimic and the negative control miR-NC mimic were purchased from GenePharma (Shanghai, China). Cell transfection with oligonucleotides (30 nM) or plasmids (2 μg) into primary chondrocytes was performed by lipofectamine 3000 reagent (Invitrogen) according to the manufacturer's instructions. After transfection for 36 h, the cells were collected for functional analysis.

Dual-luciferase reporter assay

The putative target prediction of miR-200a/b and FUT4 was performed through DIANA Tools software. The potential binding sites of miR-200a/b on wild type of FUT4 3′UTR (FUT4-WT) were cloned by PCR method into plasmid pGL4-luciferase report vector (Promega, Madison, WI, USA), as well as the mutant of FUT4 3′UTR (FUT4-MUT). Cells were plated onto 24-well plate (Corning) at 1×10^4 cells/well, followed by co-transfection with 20 ng of FUT4-WT/MUT and 20 nM of either miR-200a/NC mimic or miR-200b/NC mimic in 293T cells for 48 h. All transfection groups were carried out in triplicate. Cells were collected to measure the relative luciferase activity using the dual-luciferase reporter assay system (Promega) in line with the manufacturer's information.

RNA immunoprecipitation (RIP)

RIP was performed with chondrocyte extract after the primary chondrocytes were transfected with miR-200a mimic, miR-200b mimic or miR-NC mimic for 36 h. Magna RIP™ RNA-binding protein immunoprecipitation kit (Millipore) was chosen to obtain RIP-Ago2 and RIP-IgG through incubation with anti-Ago2 (ab32381, 1:25) or anti-IgG (ab2410, 1:100) at 4 °C for 16 h. Then, the enriched expression of FUT4 mRNA in the RIP samples was detected using RT-qPCR.

Western blot

The total protein in IL-1β-treated chondrocytes was extracted by RIPA lysis buffer (Beyotime), and the concentration was determined using BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA). 20 μg of proteins were separated on 8–12% SDS-PAGE and transferred onto PVDF membrane (Millipore). After blocking with 5% nonfat milk, membranes carrying proteins were incubated with primary antibodies and HRP-conjugated secondary antibodies. β-actin was used as an internal standard to normalize protein level.

The bands were visualized by ECL reagent (Millipore) according to the standard protocol. The primary antibodies were provided from Abcam and as follows: anti-FUT4 (ab181461, 1:500), anti-Collegen IIA1 (Col2a1; ab188570, 1:5000), anti-Aggrecan (ab52141, 1:100), and anti-β-actin (ab8227, 1:1000).

Statistical analysis

Data were presented as mean ± standard deviation (SD) from three independent experiments, and analyzed on Graphpad Prism 6.0 (GraphPad Software Inc., La Jolla, USA). Two tailed Student's t test was used to calculate statistical significance between two groups test, and one-way analysis of variance was for multiple groups. Result with P value < 0.05 was considered to be statistically significant.

Results

Expression of miR-200a/b was downregulated in OA cartilage tissues

Then, RT-qPCR analysis was used to measure miR-200a/b expression in OA, and we found that relative miR-200a level was lower in this cohort of OA patients than control (Fig. 1A). Similar results were obtained about miR-200b expression status in the same OA patients (Fig. 1B). These data suggested a potential role of miR-200a/b in OA.

The cell injury induced by IL-1β in primary chondrocytes

Therefore, we established and verified the cell model of OA in chondrocytes. The primary chondrocytes were isolated and cultivated with 5, 10 and 20 ng/ml of IL-1β for 12 h ex-vivo. Then cell injury was measured. CCK-8 assay showed that 10 and 20 ng/ml of IL-1β exposure significantly induced a 38% and 57% cell viability inhibition, respectively (Fig. 2A); flow cytometry determined that apoptosis rate was distinctively increased in response to IL-1β from 5.7–16.9% (10 ng/ml) and to 25.6% (20 ng/ml) (Fig. 2B and 2C). Released pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were dramatically upregulated when the primary chondrocytes were exposed with 10 and 20 ng/ml of IL-1β (Fig. 2D and 2E). In addition, western blotting was utilized to assess cartilage matrix degradation, and the decrease of Col2a1 and Aggrecan indicated the degradation of chondrocyte matrix in response to 10–20 ng/ml of IL-1β (Fig. 2F). Then, expression of miR-200a/b in IL-1β-induced cell injury in this cell model of OA was clarified. RT-qPCR analysis revealed that relative miR-200a and miR-200b levels were consistently downregulated in IL-1β-treated primary chondrocytes compared with control group (without IL-1β exposure) (Fig. 2G and 2H). Taken together, IL-1β could induce inflammatory injury and matrix degradation in chondrocytes in a certain of dose-dependent manner.

FUT4 expression was directly modulated by miR-200a/b in chondrocytes

Recently, FUT4 had been reported to be aberrantly upregulated in OA cartilage tissues [18], we hypothesized FUT4 played an important role in knee articular chondrocytes. As predicted by DIANA Tools (http://DianaTools/microT_CDS), FUT4 3'UTR was predicted to be a potential target of both miR-200a and miR-200b (Fig. 3A and 3B). Subsequently, this potential target binding relationship was further identified. With co-transfection, the relative luciferase activity of FUT4-WT was significantly reduced in 293T cells transfected miR-200a mimic or miR-200b mimic, compared with miR-NC mimic transfection (Fig. 3C and 3D); whereas miR-200a/b mimic had no effect on the luciferase activity of FUT4-MUT. Moreover, the enrichment level of FUT4 was up to 4.6-fold in RIP-Ago2 derived from miR-200a mimic-transfected primary chondrocytes (Fig. 3E); moreover, that was 6.4-fold enrichment of FUT4 in miR-200b-transfected chondrocytes (Fig. 3F). These results indicated a target binding relationship between miR-200a/b and FUT4.

Next, the expression of FUT4 in IL-1β-induced knee OA model was figured out. Expression of FUT4 both on mRNA level and protein level was gradually upregulated by IL-1β in primary chondrocytes (Fig. 4A and 4B). What's more, exogenous administration of miR-200a or miR-200b could dramatically reduce FUT4 protein expression (Fig. 4C and 4E), and knockdown of miR-200a or miR-200b via anti-miRNAs transfection exerted the opposite effect (Fig. 4D and 4F). These results showed FUT4 was negatively modulated by miR-200a/b in IL-1β-induced primary chondrocytes via target binding.

miR-200a/b protected primary chondrocytes against IL-1β-induced cell injury via downregulating FUT4

Functionally, the roles of miR-200a and miR-200b in IL-1β-induced primary chondrocyte injury were explored, as well as the contribution of FUT4 in this OA cell model. IL-1β-induced chondrocytes were forcedly restored miR-200a expression via miRNA mimic transfection, as accompanied with FUT4 lower expression (Fig. 5A); meanwhile, pcDNA-FUT4 vector administration rescued FUT4 expression in miR-200a-overexpressed chondrocytes under IL-1β insult. Cell viability was promoted, whereas apoptosis rate was diminished by miR-200a mimic in IL-1β-induced primary chondrocytes, which were partially overturned in the presence of pcDNA-FUT4 (Fig. 5B and 5C). The highly expressed IL-1β, IL-6 and TNF-α in IL-1β-induced primary chondrocytes were attenuated by miR-200b restoration, and this effect was further blocked by FUT4 upregulation (Fig. 5D and 5E). On the contrary, the low expression of Col2a1 and Aggrecan was enhanced in chondrocytes under IL-1β stress when miR-200a was upregulated, and this promotion was counteracted by FUT4 (Fig. 5F). Notably, introduction of pcDNA-FUT4 vector could not only abolish the inhibitory effect of miR-200b upregulation on FUT4 expression (Fig. 6A), but also the suppressive role of miR-200b overexpression in IL-1β-induced cell viability inhibition, apoptosis, inflammation response, and matrix degradation (Fig. 6B-6F). Collectively, these data suggested that miR-200a/b could attenuate IL-1β-induced cell injury in primary chondrocytes, and this protective effect relied on directly inhibiting its downstream target gene FUT4.

Discussions

The therapies for OA patients were mainly to relieve the pain and inflammation [21]. Researches had shown that the important pathological feature of OA contained articular cartilage degeneration, which was largely attributed to apoptosis of chondrocytes [22]. At present, the medications were often accompanied with severe side effects. Therefore, studies of the basically molecular mechanism of chondrocytes on cytological level might contribute to a better understanding and novel therapy of OA [23]. In this study, we used the primary chondrocytes in knee articular and investigated the effect of miR-200a/b on IL-1β-induced chondrocyte injury. The results showed that IL-1β, as OA cell model stimulation, could evoke cell viability inhibition, apoptosis, inflammation and matrix degradation in primary chondrocytes ex-vivo, and miR-200a/b was downregulated in OA knee cartilage and IL-1β-induced chondrocytes. Functionally, restoration of miR-200a/b significantly alleviated IL-1β-induced knee articular chondrocyte cell injury through negatively regulating its downstream target gene FUT4.

It was generally believed that miR-200 family, consisting of five miRNAs miR-141/200a/200b/200c/429 served pivotal role in cancer incidence [13]. Very recently, several members of miR-200 family had been declared to participate in OA progression. For example, miR-141 was associated with pathological destruction of cartilage through regulating cell migration and cell spreading of OA fibroblast-like synoviocytes, apoptosis of chondrocytes, and lipid metabolism [15, 24, 25]. Besides, serum miR-200c together with miR-100 and miR-1826 was proposed to be potential diagnostic biomarkers for knee OA [26]. Few studies had been published to clarify the potential function of miR-200a and miR-200b in OA pathogenesis. For instance, Wang et al. [27] discovered that forkhead box C1 positively modulate OA synovial fibroblast proliferation and pro-inflammatory cytokine production partially via regulated by miR-200a. In terms of miR-200b,Wu et al. [14] demonstrated that miR-200b was downregulated in OA chondrocytes and could serve as a repair factor for OA cartilage, because its upregulation significantly suppressed MMPs levels and promoted Col2 levels via declining DNMT3A expression. Luckily, we managed to verify the expression patter of miR-200a/b in knee cartilage of OA patients and IL-1β-induced OA chondrocytes model ex-vivo, which supported the findings of previous researches [14, 27]. What's more, FUT4 as a novel, common downstream target gene of miR-200a/b was came to light. Here, we performed functional experiments to detect the chondrocyte cell viability, apoptosis rate, production of pro-inflammation factors (IL-6 and TNF-α), and expression of ECM proteins (Col2a1 and Aggrecan).

FUTs were involved in signal transduction, inflammatory, tumor progression, and metastasis. For example, FUTs mediated the multidrug resistance of human hepatocellular carcinoma [16]. As for arthritis, accumulating evidences implied that FUTs had controlled the development of RA and juvenile idiopathic arthritis [28, 29]. For example, FUT1 and FUT7 were upregulated in RA synovial fibroblast cell and synovial fluid, respectively [28, 29]. FUT1 and FUT2 mediated angiogenesis and cell adhesion in RA [29, 30]. However, FUTs in OA had not been widely studied. Hu et al. [18] revealed the expression profile of FUTs, and mRNA expression of FUT1, FUT2, FUT3, and FUT4 were remarkably upregulated in healthy and OA human cartilage tissues, while FUT7 mRNA expression was downregulated. Furthermore, Li et al. [31] appointed that most of FUTs were higher in RA compared to OA synovial tissues, including FUT1, FUT2, FUT7 and FUT4. According to our findings, FUT4 expression both on mRNA level and protein level were dramatically increased in IL-1β-induced OA cell model ex-vivo, and overexpression of FUT4 attenuated

miR-200a/b-induced effects on the reduction of apoptosis and secretions of IL-6 and TNF-α. At the same time, more researches should be launched to explore which signaling pathway(s) underlies miR-200a/b/FUT4 axis-modulated chondrocytes progression, such as NF-κB [18] and MAPK/ERK [14] pathway.

Conclusion

In summary, our study demonstrates that miR-200a/b overexpression rescued cell viability, but alleviated apoptosis, inflammatory response and matrix degradation in IL-1β-treated primary chondrocytes; and high-expression of FUT4 blocked the protective effect of miR-200a/b in knee articular chondrocyte injury ex-vivo. This work suggested miR-200a/b-FUT4 axis as a potential candidate to improve chondrocyte viability in knee OA [32], even though further evidence should be obtained to verify the above conclusion in vivo and the underlying signaling pathway need more investigations to be fully disclosed.

Declarations

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Availability of data and materials

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

Authors' contribution:

All authors made substantial contribution to conception and design, acquisition of the data, or analysis and interpretation of the data; take part in drafting the article or revising it critically for important intellectual content; gave final approval of the revision to be published; and agree to be accountable for all aspect of the work.

Ethics approval and consent to participate

The present study was approved by the ethical review committee of Baoji Traditional Chinese Medicine Hospital

Consent for publication

All authors consent this work for publication

Conflicts of interest

The authors have no conflicts of interest to declare.

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Figures

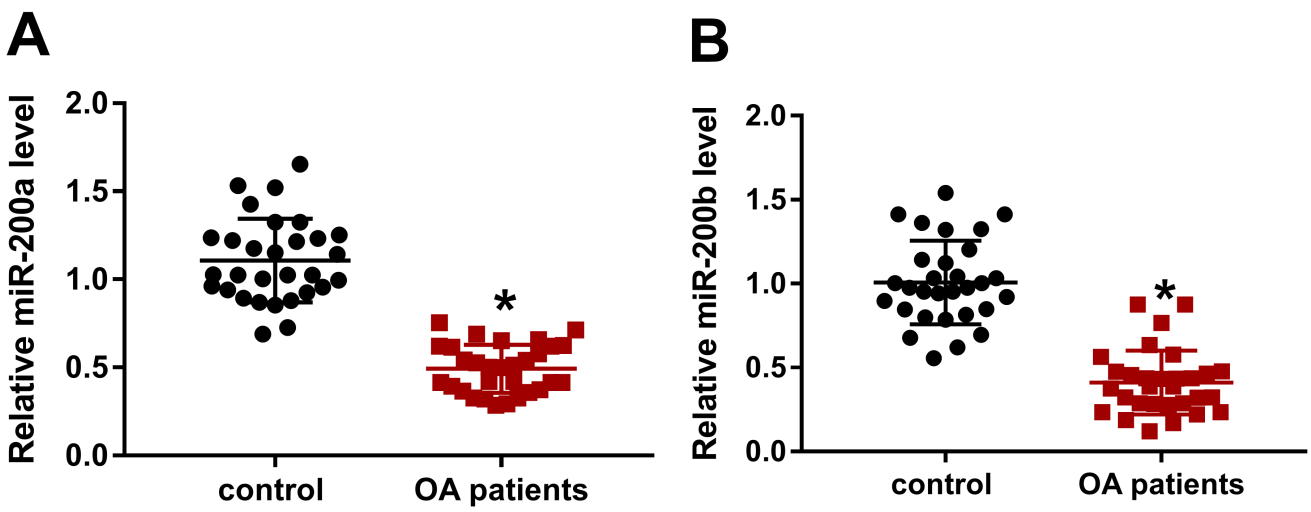

Figure 1

The expression of miR-200a/b in OA patients and IL-1β-induced primary chondrocytes. RT-qPCR detected the expression level of (A) miR-200a and (B) miR-200b in cartilage tissues from OA patients (n=30) and control patients (n=30). * $P < 0.05$.

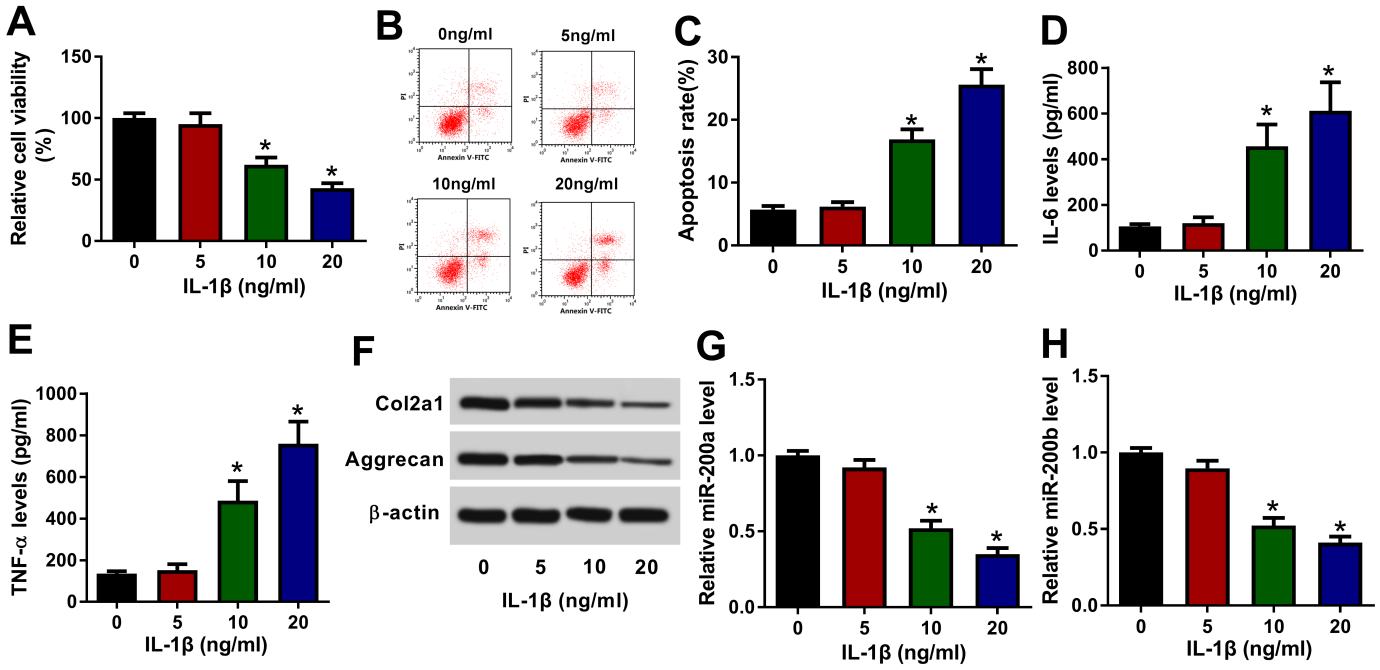

Figure 2

The cell injury induced by IL-1β in primary chondrocytes derived from knee articular cartilage tissues. Primary chondrocytes were exposed to 0, 5, 10 and 20 ng/ml of IL-1β for 12 h. (A) Cell viability (%) was measured by CCK-8. (B and C) Apoptosis rate (%) was recorded by flow cytometry. (D and E) Expression of IL-6 and TNF-α was evaluated by ELISA. (F) Western blotting detected Collegen 2a1 (Col2a1) and Aggrecan protein expression. (G and H) RT-qPCR detected the expression level of miR-200a and miR-200b in IL-1β-induced chondrocytes. All experiments were compared with control group (without IL-1β exposure). * $P < 0.05$.

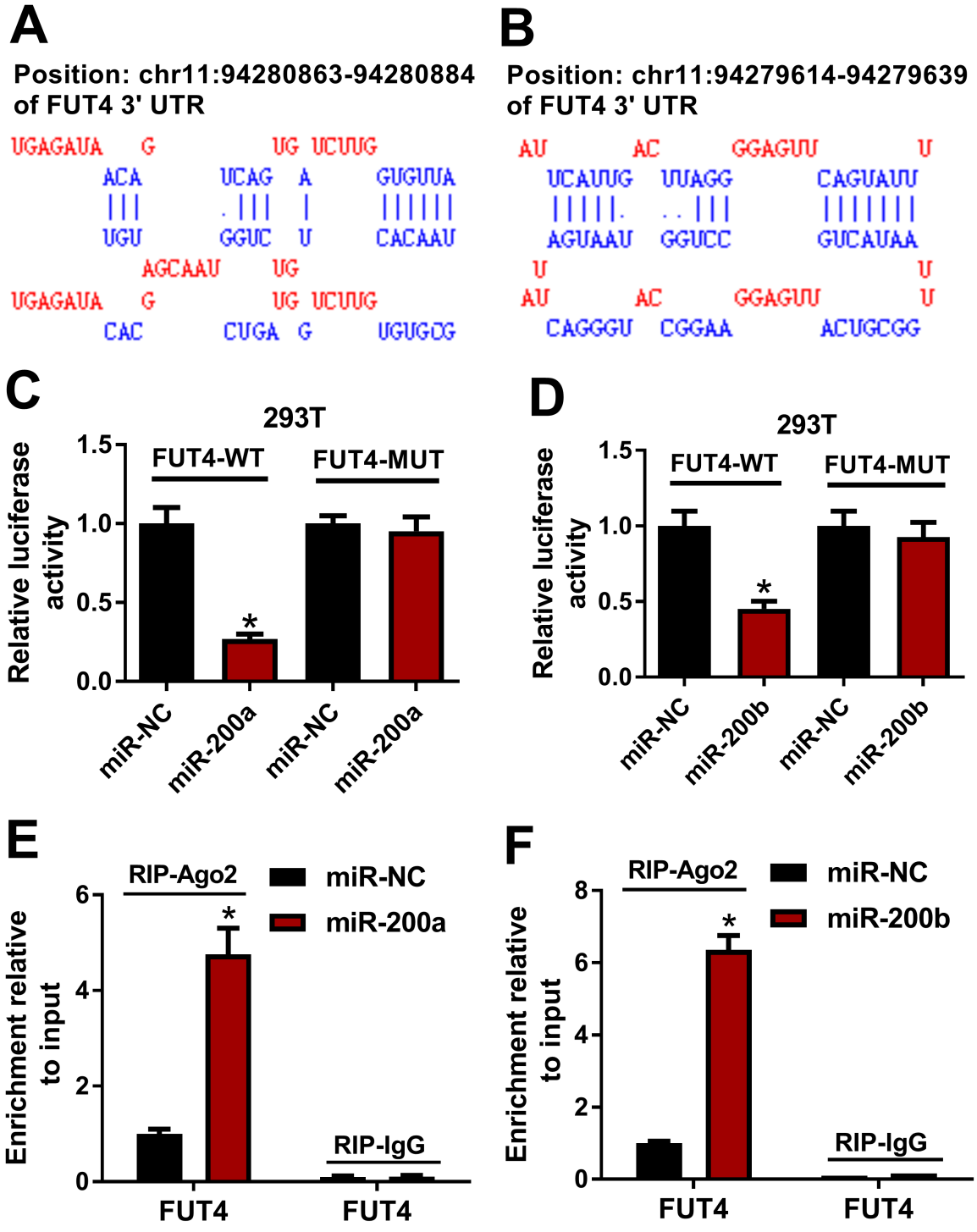

Figure 3

The relationship between miR-200a/b and FUT4. (A and B) Predicted binding sites of miR-200a and miR-200b on FUT4 3'UTR were presented. FUT4-WT, the wide type of FUT4 3' UTR fragment; FUT4-MUT, the mutant of FUT4-WT. (C and D) The dual-luciferase assay confirmed the relative luciferase activity of the wide type and mutant of FUT4 3' UTR fragment (FUT4-WT/MUT) in HEK 293T (293T) cells transfected with miR-200a mimic (miR-200a), miR-200b mimic (miR-200b) or miR-NC mimic (miR-NC). (E and F) RT-

qPCR detected the enrichment of FUT4 in Ago2-mediated RNA immunoprecipitation (RIP-Ago2) and RIP-IgG derived from primary chondrocytes transfected with miR-200a, miR-200b or miR-NC. * P < 0.05.

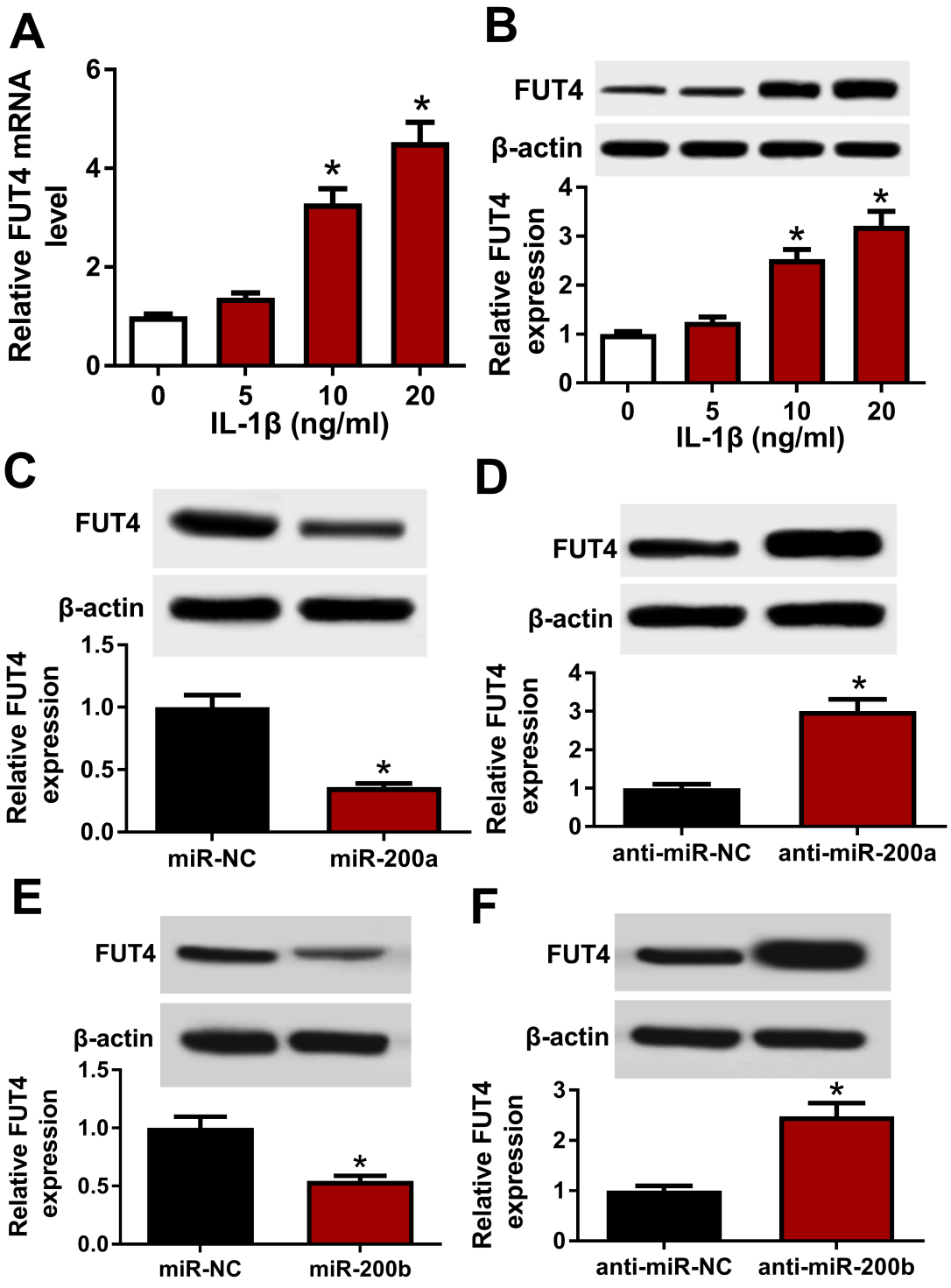

Figure 4

The expression of FUT4 in primary chondrocytes. (A) Relative FUT4 mRNA level and (B) protein level in IL-1β-induced primary chondrocytes were detected by RT-qPCR and western blotting, respectively. (C-F) Western blotting determined relative FUT4 protein expression in primary chondrocytes transfected with

miR-200a, anti-miRNA of miR-200a (anti-miR-200a), miR-200b, anti-miR-200b, miR-NC, or anti-miR-NC. * P < 0.05.

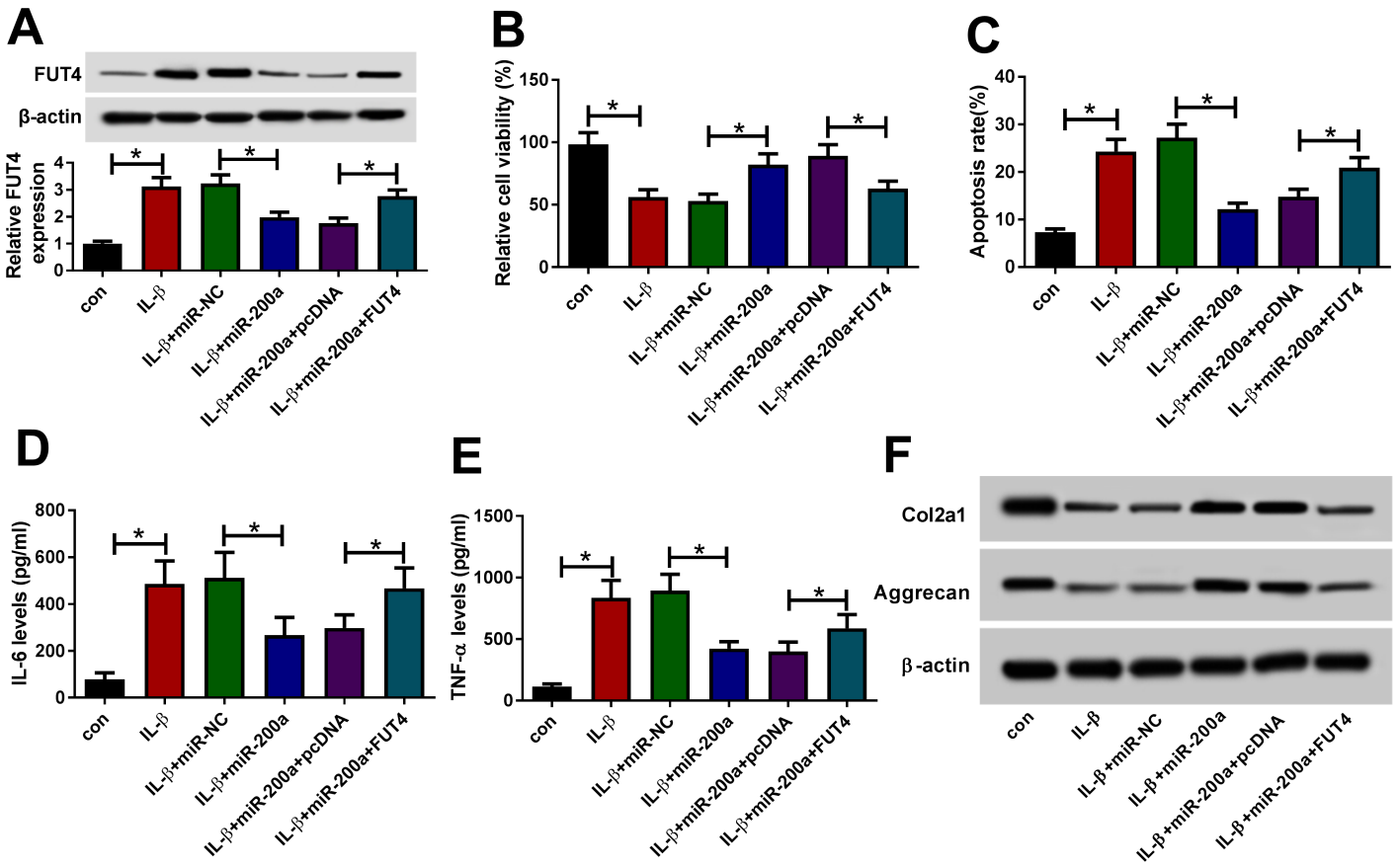

Figure 5

The role of miR-200a and FUT4 in IL-1β-induced primary chondrocytes. Chondrocytes were transfected with miR-200a or miR-NC, and co-transfected of miR-200b and pcDNA-FUT4 vector (FUT4) or the empty vector (pcDNA). After IL-1β (20 ng/ml) treatment for 12 h, (A) western blotting measured relative FUT4 protein expression, (B) CCK-8 assay tested relative cell viability (%), (C) flow cytometry detected apoptosis rate(%), (D and E) ELISA evaluated expression of IL-1β, IL-6 and TNF-α, (F) western blotting measured Col2a1 and Aggrecan levels. * P < 0.05.

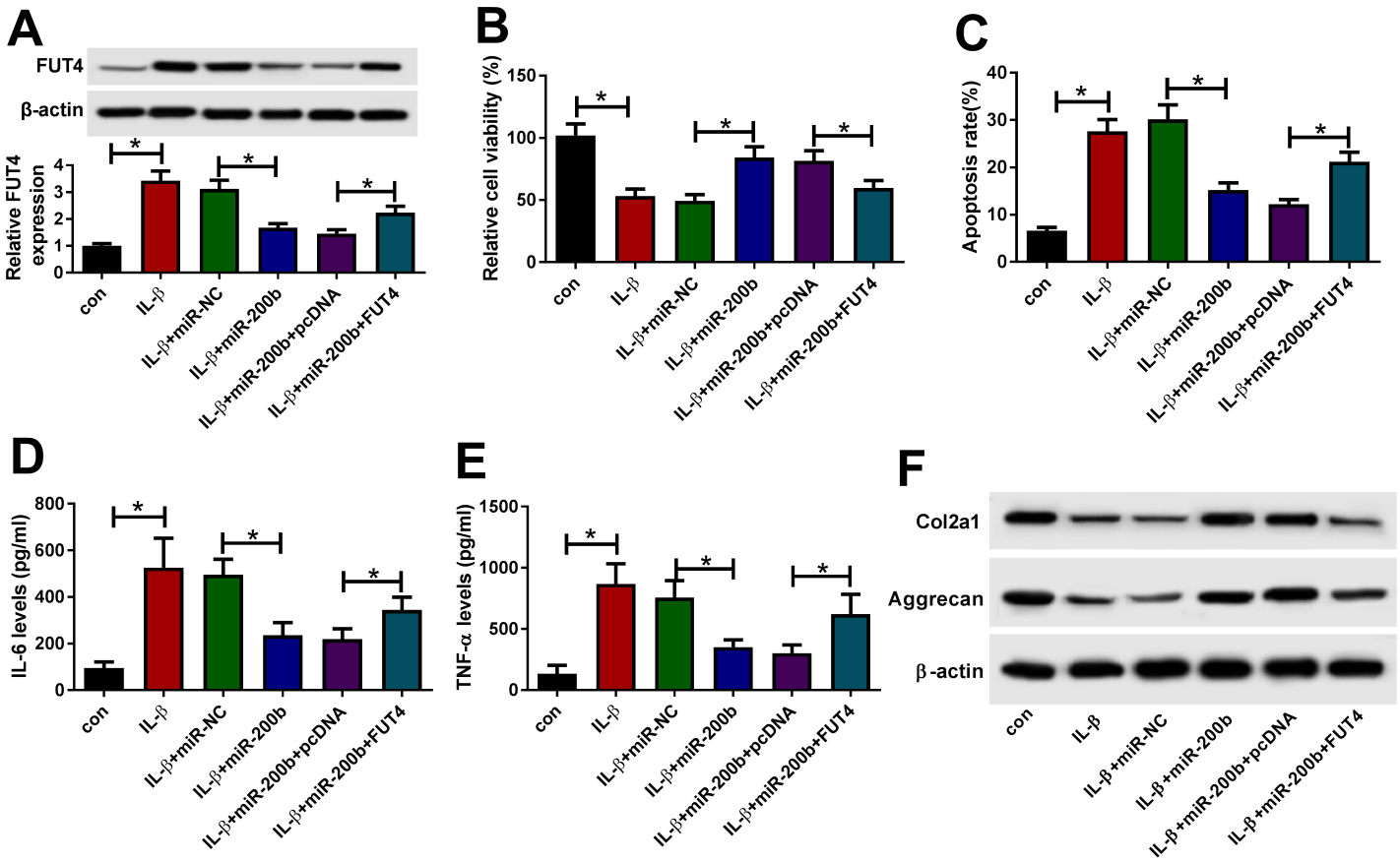

Figure 6

The role of miR-200b and FUT4 in IL-1β-induced primary chondrocytes. Chondrocytes were transfected with miR-200b or miR-NC, and co-transfected of miR-200b and FUT4 or pcDNA. After IL-1β (20 ng/ml) treatment for 12 h, (A) western blotting measured relative FUT4 protein expression, (B) CCK-8 assay tested relative cell viability (%), (C) flow cytometry detected apoptosis rate(%), (D and E) ELISA evaluated expression of IL-1β, IL-6 and TNF-α, (F) western blotting measured Col2a1 and Aggrecan levels. * $P < 0.05$.