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Knockdown of KIAA1199 suppresses IL-1β-induced cartilage degradation and inflammatory responses in human chondrocytes through the Wnt/β-catenin signalling pathway

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ABSTRACT

The overproduction of proteolytic enzymes and dysregulation of extracellular matrix (ECM) metabolism have been shown to accelerate the degradation process of articular cartilage. The purpose of this study was to investigate the role of KIAA1199 and its association with the pathophysiology of osteoarthritis (OA). We found that the expression of KIAA1199 was significantly upregulated in OA cartilage compared with normal tissues. Serum levels of KIAA1199 were higher in OA patients than in non-OA patients. Furthermore, knockdown of KIAA1199 inhibited interleukin-1 beta (IL-1β)-induced ECM metabolic imbalance by regulating the expression of A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5; matrix metallopeptidase-13; aggrecan; and COL2A1. In addition, silencing of KIAA1199 significantly decreased the expression of inflammatory mediators such as prostaglandin E2, IL-6, and TNF-α. Mechanistic analyses further revealed that IL-1β-induced activation of the Wnt/β-catenin pathway was suppressed during KIAA1199 knockdown. Moreover, KIAA1199 expression was also upregulated in an in vivo rat OA model. Together, these results increase our understanding of the emerging role of KIAA1199 in the process of OA degeneration, and may lead to a novel molecular target to prevent cartilage degradation.

1. Introduction

Osteoarthritis (OA) is the most prevalent joint disorder and is a major cause of disability in the elderly [1]. The disease manifestations in the joint are well characterised, including progressive degradation of articular cartilage, synovial inflammation, subchondral bone remodelling, and osteophyte formation [2]. Although many factors are involved in the progression of OA, the degradation of articular cartilage is the major hallmark of OA [3]. The balance between anabolic and catabolic metabolisms in chondrocytes is critically important for matrix maintenance and cartilage function [4]. Previous studies have shown that several important proteases are involved in the pathogenesis of OA, including those of the matrix metalloproteinases (MMPs) and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) families [5]. Excessive production of ADAMTS-5 and MMP-13 is thought to be one of the factors responsible for this extracellular matrix (ECM) destruction [6].

Cytokines have been considered as important factors that initiate and accelerate the development of OA [7,8]. Interleukin-1 beta (IL-1β), a main proinflammatory cytokine, upregulates the expression of catabolic mediators and contributes to the exacerbation of cartilage erosion [9]. In addition, IL-1β potently suppresses the expression of cartilage-specific molecules, such as aggrecan and type II collagen, which are the main components of the ECM of cartilage [10]. IL-1β triggers a series of downstream signal pathways, including Wnt/β-catenin signalling, which is abnormally activated in OA chondrocytes [11]. Therefore, inhibition of IL-1β-induced catabolic metabolism and inflammatory responses may be a potential strategy to delay the progression of OA.

Cell migration-inducing and hyaluronan-binding protein (KIAA1199) was first described as an inner ear protein in which three point mutations were found to be associated with non-syndromic hearing loss [12]. Studies have suggested that KIAA1199 is associated with progression, metastases, and a poor prognosis in a variety of cancers [13,14]. A recent study reported that IL-1β markedly increased the expression of KIAA1199 and enhanced migration in pancreatic cancer [15]. Moreover, KIAA1199 is overexpressed by synovial

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Table 1
Primers of targeted genes.

	Forward	Reverse
KIAA1199	CCAACAACAACCTCATCAACTG	GCTCTGAATAACCTGGGGAGTA
ADAMTS-5	GACCGATGGCACTGAATGTAGGC	TCTCCTCCACATACTCCGCACTTG
MMP-13	GTCTTTCTTCGGCTTAGAGGTG	AGAGGAGTTACATCGGACCAAA
Collagen II	CAAGAACAGCATTGCCTATCTG	GATAACAGTCTTGCCCCACTTA
Aggrecan	GATCCTTACCGTAAAGCCCATC	CTCCAGTCTCATTCTCAACCTC
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC

fibroblasts and synovial tissues from OA and rheumatoid arthritis (RA) patients [16]. In addition, a recent study reported that KIAA1199 enhanced the proliferation and angiogenesis of synovial membrane and may be a potential diagnostic biomarker of RA-related angiogenesis [17]. However, the effects of KIAA1199 on the equilibrium between synthesis and breakdown of cartilage ECM and IL-1β stimulation have not been elucidated.

In the present study, we investigated the potential effects of KIAA1199 in human chondrocytes and the progression of OA. Notably, KIAA1199 was highly upregulated in OA cartilage compared with normal tissues. In addition, knockdown of KIAA1199 protected chondrocytes from IL-1β-induced injury by regulating the expression of catabolic enzymes and production of proinflammatory cytokines via the Wnt/β-catenin signalling pathway. The results of this study extend our understanding of the molecular mechanism of cartilage metabolism, and suggest that KIAA1199 may be a potential molecular target in the treatment and prevention of OA.

2. Materials and methods

2.1. Cartilage samples

This study adhered to the tenets of the Declaration of Helsinki. All experiments were approved by the Ethical Committee of the Second Affiliated Hospital of Zhejiang University. Human cartilage was obtained from donors undergoing hip replacement due to osteoarthritis (OA patients) or femoral neck fracture (non-OA patients) with informed consent in accordance with the requirements of the local ethics committee. OA was diagnosed in accordance with the American College of Rheumatology criteria [18]. The main symptoms of these patients were hip pain and difficulty moving. At the time of surgery, hip OA patients had symptomatic disease requiring medical treatment, and none had received intra-articular steroid injections within 3 months prior to surgery. Patients with femoral neck fracture presented no complaint of hip pain, with no OA radiographic changes, and were considered as non-OA patients.

2.2. Chondrocyte isolation

Cartilage was washed with phosphate-buffered saline three times and dissected into pieces followed by digestion with 0.1% collagenase II for 13 h in Dulbecco's modified Eagle's medium (DMEM). The released chondrocytes were seeded onto 6-well plates (2×10^5 cells/well) in DMEM containing 10% foetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Confluent chondrocytes (approximately 80–90%) were serum-starved overnight, and then were used in experiments.

2.3. Cell treatment and small interfering RNA transfection

KIAA1199 expression in chondrocytes was silenced by transfection of small interfering RNA (siRNA, Biomics, Nantong, China). Chondrocytes were seeded at 2.0×10^5 cells/well in 6-well plates and transfected with siRNAs specific to KIAA1199 (si-KIAA1199; 50 nM) or with negative control siRNA (si-NC; 50 nM) as a negative control using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Then, the cells were incubated with IL-1β (5 ng/mL) for 24 h. Quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting were conducted to confirm the effects of siRNA transfection.

2.4. RNA isolation and RT-PCR

Total RNA was isolated from cartilage/chondrocytes using TRIzol (Invitrogen, Carlsbad, CA, USA), dissolved in diethylpyrocarbonate-treated water, and stored at −80 °C prior to use. Total RNA (1 μg) was reverse-transcribed using the PrimeScript-RT reagent kit (TaKaRa Biotechnology, Kusatsu, Japan) by using a 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA), and the reverse transcriptase reaction product was analyzed by real-time quantitative polymerase chain reaction (RT-PCR) using the SYBR Premix Ex TaqTM (TaKaRa Biotechnology, Kusatsu, Japan), as detailed in the manufacturer's guidelines. All experiments were performed in triplicate and mRNA levels were standardised to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers used in the analysis are listed as Table 1. Data were calculated using the $2^{\hat{}(-\triangle\triangle CT)}$ method [19].

2.5. Enzyme-linked immunosorbent assay

Blood samples were obtained from patients undergoing hip replacement surgery, and serum was obtained by centrifugation (1000 rpm) for 10 min at 4 °C and stored at −80 °C. Serum levels of KIAA1199 (Cusabio, Houston, TX, USA; detection range:1 ng/mL-24 ng/mL; sensitivity:0.4 ng/mL) and production of prostaglandin E2 (PGE_2; MyBioSource, San Diego, CA, USA; detection range:7.8 pg/mL-500 pg/mL; sensitivity:1 pg/mL), IL-6(R&D Systems, Minneapolis, MN, USA; detection range:3.1 pg/mL-300 pg/mL; sensitivity:0.7 pg/mL), and tumour necrosis factor alpha (R&D Systems, Minneapolis, MN, USA; detection range:15.6 pg/mL-1000 pg/mL; sensitivity:6.23 pg/mL TNF-α) secreted by human chondrocytes were measured using enzyme-linked immunosorbent assay kits.

2.6. Treatment of transfected chondrocytes with DKK-1 and LiCl

To explore the association between KIAA1199 and Wnt signal pathway, an activator (LiCl) and an inhibitor (Dickkopf-1) of the Wnt signal pathway were applied. Human chondrocytes transfected with siRNA-KIAA1199 or siRNA-NC were pre-treated with Dickkopf-1 (DKK-1; 100 ng/mL) or LiCl (10 mM) for 1 h, followed by IL-1β (10 ng/mL) for 24 h. At the indicated times, cells were harvested for western blotting.

2.7. Western blotting

Protein lysates were extracted from chondrocytes with cell lysis buffer, loaded, and resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (10% resolving gel with a 5% stacking gel), and subsequently transferred to a polyvinylidene difluoride membrane (Millipore, Burlington, MA, USA). The membrane was blocked with 5% nonfat milk and probed with diluted primary

antibodies against aggrecan, type II collagen, MMP-13, ADAMTS-5, β-catenin, or GAPDH at 4 °C overnight and incubated with corresponding horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Reactive protein bands were detected using enhanced chemiluminescence and visualised using the Bio-Rad system (Bio-Rad, Hercules, CA, USA).

2.8. Establishment of a rat OA model

Twenty-four male Sprague Dawley rats weighing 180–200 g were obtained from the Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China). Rats were randomly divided into two groups, a sham-operated group (control group) and osteoarthritis group (OA group). The OA group rats were subjected to surgically induced OA by destabilisation of the medial meniscus (DMM) as described previously [20]. Four rats of each group were sacrificed at 4 weeks, 6 weeks, and 8 weeks after surgery. Cartilage from femoral condyles was isolated for detection of mRNA and protein expression, as well as for histological evaluation and immunohistochemistry. All animal procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China).

2.9. Histological evaluation and immunohistochemical analysis

Femoral condyles were fixed in 10% neutral buffered formalin, then decalcified with 10% EDTA, dehydrated, and then embedded in paraffin. The paraffin blocks were cut into 5-μm sections, which were stained with Safranin O/Fast Green for proteoglycan detection. To further evaluate the expression of KIAA1199, immunohistochemistry was performed using paraffin-embedded tissue. Sections were incubated with a primary antibody to KIAA1199 for 1 h at room temperature. Immunoperoxidase detection was performed with EnVision (Nashville, TN, USA) anti-rabbit antibodies (Dakocytomation EnVision + system labelled polymer-horseradish peroxidase anti-rabbit antibodies) for 30 min. Sections were then developed using 3,3′-diaminobenzidine for 10 min, and counterstained using Harris haematoxylin.

2.10. Statistical analysis

All data are expressed as the mean ± standard deviation. Statistically significant differences were calculated with Student's *t*-test using GraphPad Prism software (GraphPad, San Diego, CA, USA). A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. KIAA1199 is upregulated in human OA cartilage tissue

To assess the role of KIAA1199 in the progression of OA, we first detected the expression of KIAA1199 in human OA and normal articular cartilage tissues. Significantly higher mRNA and protein expressions of KIAA1199 in clinical samples were found in OA cartilage compared with non-OA cartilage tissues (Fig. 1(1–2)). To further confirm these results, we examined the expression of KIAA1199 in cartilage using immunohistochemical analysis. KIAA1199 was highly upregulated in OA cartilage tissue (Fig. 1(3)), which was consistent with the PCR and western blot results. In addition, in our serum samples, the level of KIAA1199 in OA patients was higher compared with that in non-OA patients, indicating that KIAA1199 may have played a role in the progression of OA (Fig. 1(1)).

3.2. Knockdown of KIAA1199 inhibits IL-1β induced inflammatory responses in human OA chondrocytes

Because the expression of KIAA1199 was upregulated in osteoarthritic chondrocytes, we speculated that it may play a crucial role in OA development. The effects of KIAA1199 siRNA were detected using qRT-PCR and western blotting. The results showed that expression of KIAA1199 was significantly downregulated in osteoarthritic chondrocytes transfected with si-KIAA1199, when compared with cells transfected with si-NC, both at the mRNA and protein levels (Fig. 2a, b). In addition, we determined the expression of proinflammatory cytokines, which greatly influenced the pathogenesis of osteoarthritis. Moreover, silencing of KIAA1199 alleviated IL-1β-induced inflammation and subsequently led to a significant reduction of PGE2, IL-6, and TNF-α levels (Fig. 2c).

3.3. Silencing of KIAA1199 alleviates IL-1β-induced injury in human OA chondrocytes

After confirmation of the knockdown of KIAA1199, we further evaluated the effect of KIAA1199 on IL-1β-induced extracellular matrix degradation in OA chondrocytes. Briefly, the transfected chondrocytes were incubated with IL-1β (10 ng/mL) for 24 h. Both the mRNA and protein expressions of aggrecan and COL2A1 were significantly decreased in IL-1β-stimulated human OA chondrocytes. However, knockdown of KIAA1199 significantly increased the expression of aggrecan and COL2A1 (Fig. 3). Next, we detected the effects of KIAA1199 on ADAMTS-5 and MMP-13 expression. Silencing of KIAA1199 strikingly inhibited IL-1β-induced upregulations of ADAMTS-5 and MMP-13 (Fig. 3). Collectively, these findings indicated that silencing of KIAA1199 resulted a chondro-protective effect by normalising the ECM matrix metabolic imbalance in IL-1β-induced OA chondrocytes.

3.4. Silencing of KIAA1199 attenuates IL-1β-induced Wnt/β-catenin signalling in human OA chondrocytes

To further investigate the mechanism of KIAA1199 involved in IL-1β-induced cartilage degradation, we characterised some signal pathways that were related to cartilage metabolism. Knockdown of KIAA1199 significantly decreased IL-1β-induced β-catenin expression in human OA chondrocytes (Fig. 4a). In addition, we characterised the expression of cyclin D1, a main downstream target gene involved in the Wnt/β-catenin signal pathway, and found that expression of cyclin D1 was decreased after knockdown of KIAA1199 in IL-1β-induced chondrocytes. To confirm that the chondro-protective effect of KIAA1199 interference was related to the Wnt/β-catenin signalling pathway, we added an agonist (LiCl) and antagonist (DKK-1), and found that expression of β-catenin was upregulated by stimulation with LiCl and downregulated by stimulation with DKK-1 in chondrocytes transfected with KIAA1199. The combination of DKK-1 and knockdown of KIAA1199 led to significantly lower expressions of catabolic mediators such as MMP-13 and ADAMTS-5 (Fig. 4b). Based on these findings, we speculated that KIAA1199 was associated with IL-1β-induced cartilage degradation and was, at least in part, attributed to its inhibition of the Wnt/β-catenin signal pathway.

3.5. Gross morphological assessment and histological observation of cartilage

To further evaluate the effect of KIAA1199 on OA development in vivo, a surgically induced rat OA model was developed by destabilisation of the DMM. First, histological analysis of cartilage matrix loss was performed using Safranin O staining. In the control sham group, cartilage from the femoral condyles was smooth with no defects or osteophytes. However, rats in the OA group exhibited varying degrees of cartilage loss compared with the control group (Fig. 5.1). Afterwards, we evaluated the expression of KIAA1199 in articular cartilage by immunochemical staining, which showed that KIAA1199 was rarely expressed in normal cartilage, while a striking increase of KIAA1199 expression was found in the OA model, and was upregulated with the development of cartilage degradation (Fig. 5.2). KIAA1199 expression

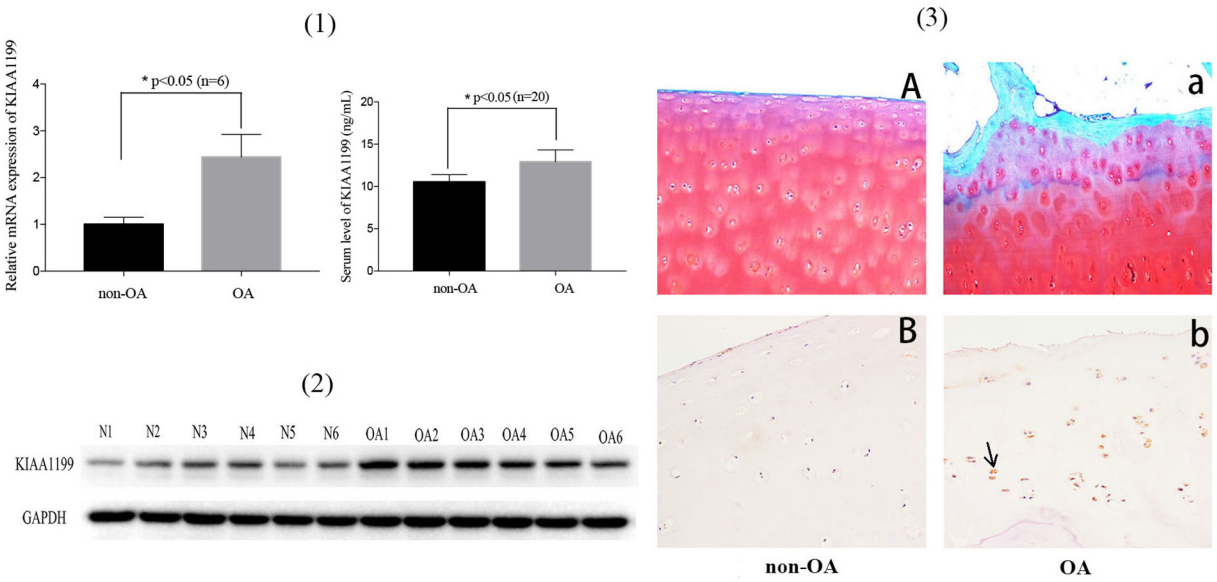

Fig. 1. Expression of KIAA1199 in cartilage and serum level of KIAA1199 analysis.
(1). qRT-PCR analysis of KIAA1199 mRNA expression in OA and normal cartilage tissues. Relative mRNA expression was normalized to GAPDH. Serum levels of KIAA1199 were measured by Enzyme Linked Immunosorbent Assay (ELISA). Value represents Mean ± S.D. *P < 0.05, OA cartilage group vs. non-OA cartilage group. (2). Western blot analysis of KIAA1199 protein expression in OA and normal cartilage. (3). Safranin O staining and immunohistochemistry of human articular cartilage. OA cartilage showed loss of Safranin O staining, uneven surface and higher expression of KIAA1199 (3a, 3b), arrow shows the positive staining of KIAA1199 in chondrocytes.

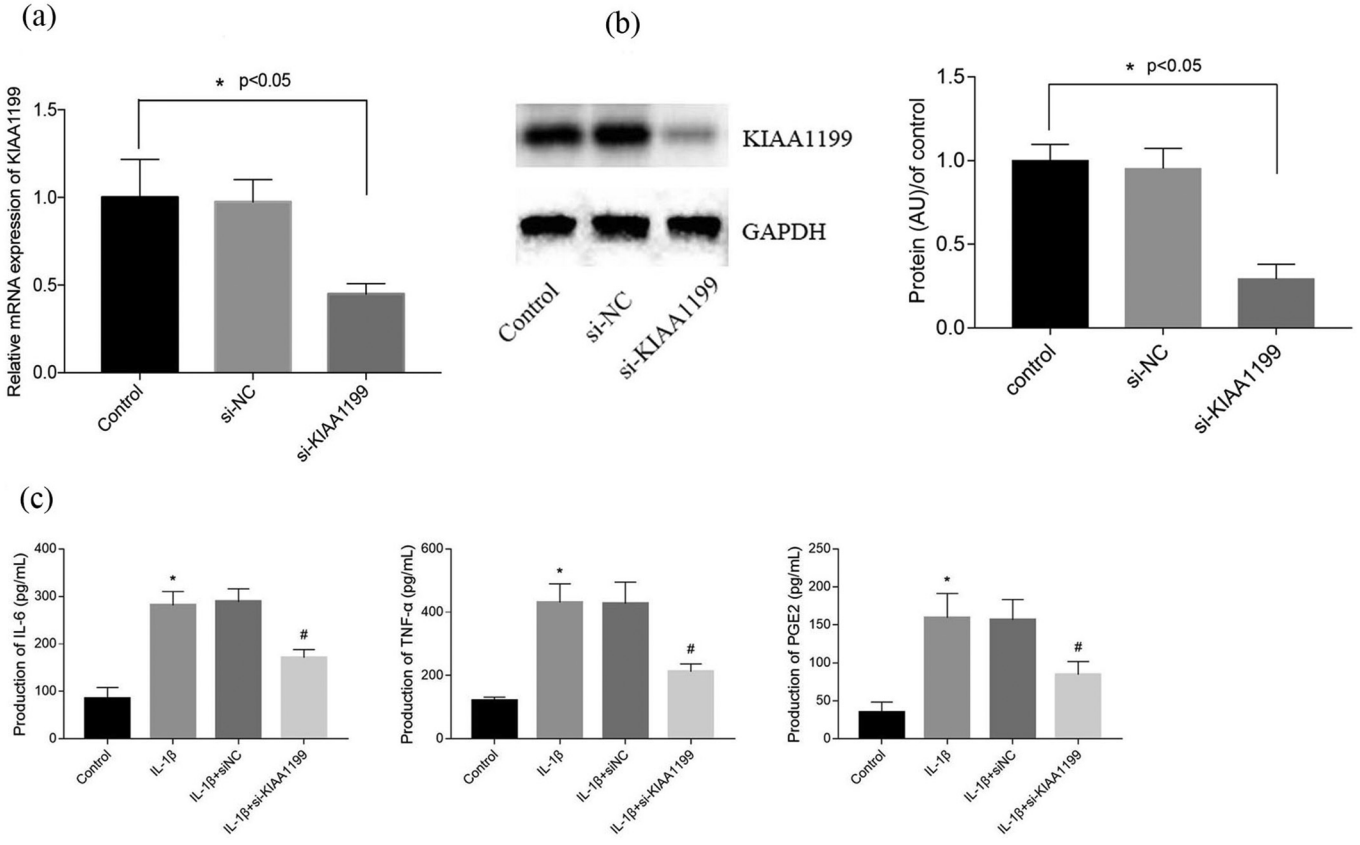

Fig. 2. Effects of KIAA1199 on inflammatory response in IL-1β stimulated human OA chondrocytes.
(a, b). qRT-PCR and western blot analyses were used to evaluate the efficiency of KIAA1199 knockdown. Densitometry was performed to analyze the ratio of the density between the KIAA1199 protein and GAPDH (*P < 0.05 compared with control group). (c). Human OA chondrocytes transfected with si-KIAA1199 or si-NC were treated with IL-1β for 24 h and the cultural supernatant was collected for Enzyme Linked Immunosorbent Assay (ELISA). The levels of IL-6, TNF-α and PGE2 were detected using ELISA. Value represents Mean ± S.D. *P < 0.05 vs. control group, #P < 0.05 vs. IL-1β treated group.

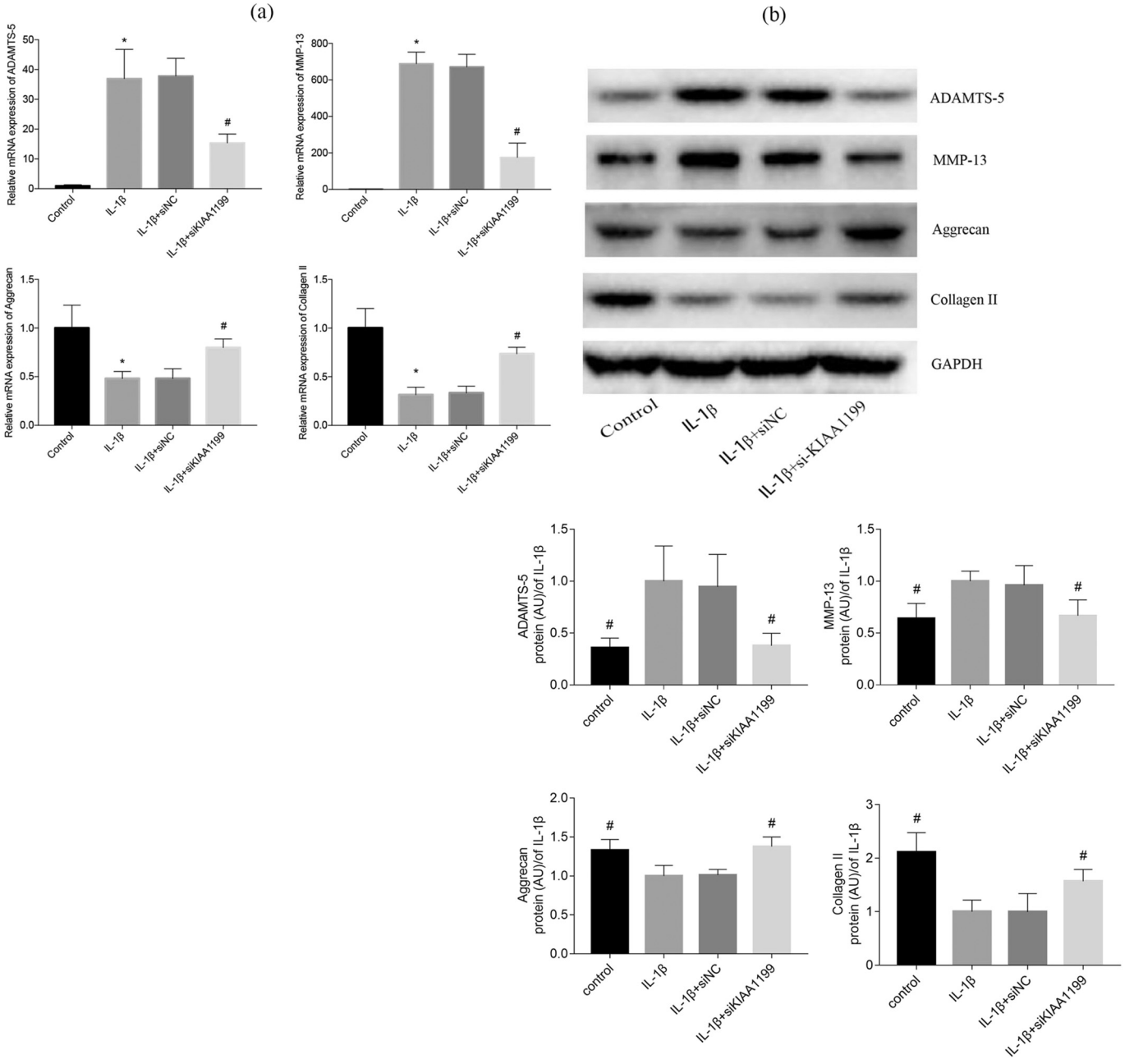

Fig. 3. Effects of KIAA1199 knockdown on IL-1β stimulated human OA chondrocytes.
Human OA chondrocytes transfected with si-KIAA1199 or si-NC were treated with IL-1β for 24 h and the cells was collected for qRT-PCR and western blotting analysis. (a). The relative mRNA expression of ADAMTS-5, MMP-13, aggrecan and collagen II was detected by qRT-PCR. Value represents Mean ± S.D. *P < 0.05 vs. control group, #P < 0.05 vs. IL-1β treated group. (b). Protein levels of ADAMTS-5, MMP-13, aggrecan and collagen II were assayed by western blotting. Densitometry was performed to analyze the ratio of the density between the protein and GAPDH (#P < 0.05 vs. IL-1β treated group).

was consistent with cartilage loss. Together, these results suggested that KIAA1199 was involved in the progression of cartilage degeneration.

3.6. Expression of KIAA1199 in the OA model

In OA cartilage, the relative mRNA and protein expression levels of KIAA1199 were significantly upregulated compared with the control group (Fig. 6a, b). In addition, KIAA1199 expression was gradually upregulated with the progression of OA.

4. Discussion

To identify new biomarkers for the improvement of diagnosis and targeted therapies, a better understanding of the biology and molecular profiles of OA is needed. The present study aimed to characterise the potential role of KIAA1199, a protein recently reported to be involved in hyaluronan depolymerisation, in the progression of OA. The results showed that knockdown of KIAA1199 exerted chondro-protective effects on OA. In addition, we analyzed KIAA1199-related signalling pathways to further understand its mechanism in OA chondrocytes.

Previous studies suggested that KIAA1199 was overexpressed and was correlated with malignant behaviour in a variety of cancers [21,22]. Recently, KIAA1199 was found to promote migration and invasion through the enhancement of the Wnt/β-catenin signalling pathway and the MMP-mediated epithelial-to-mesenchymal transition progression in gastric cancer cells [23]. Furthermore, a study reported

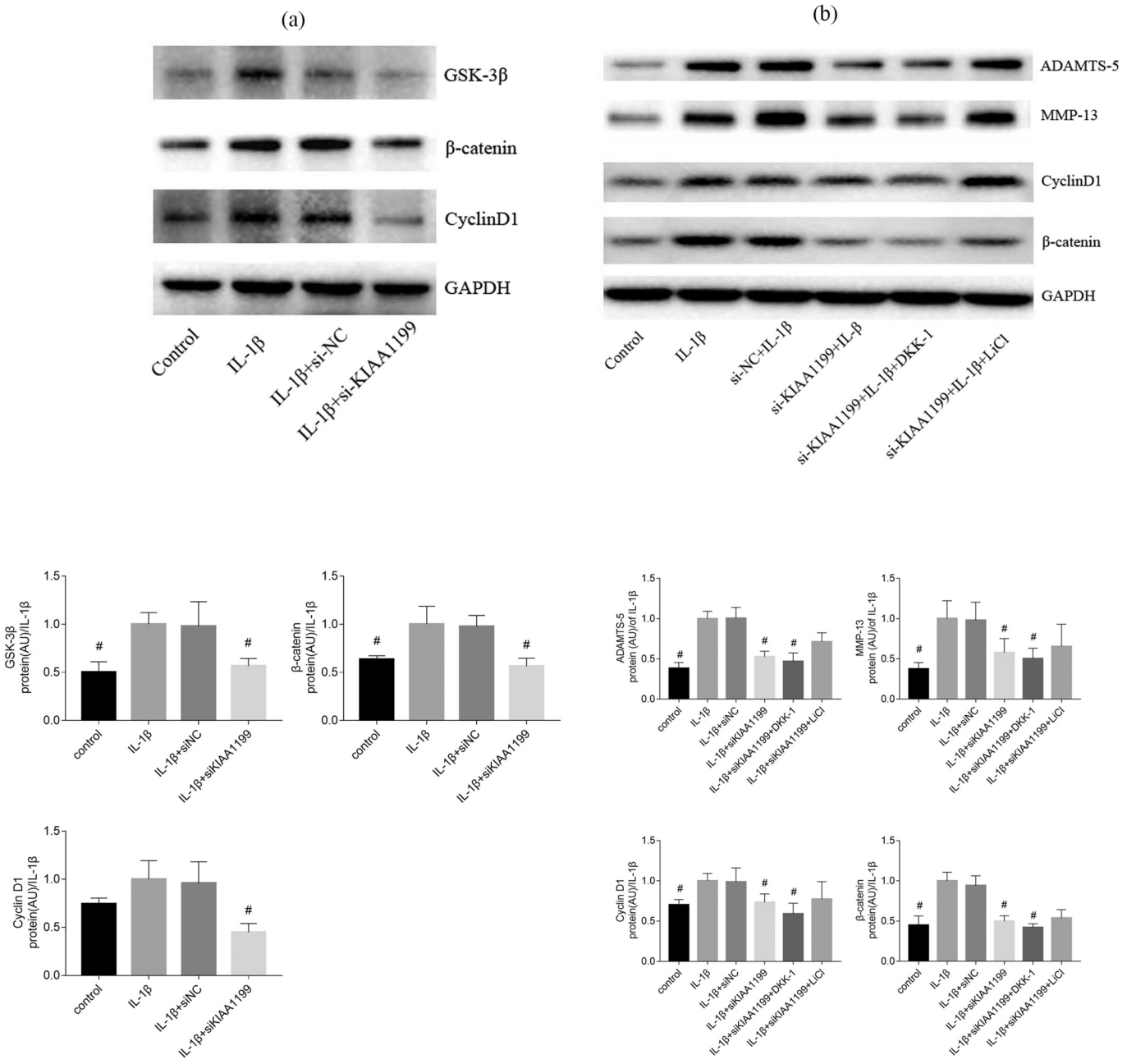

Fig. 4. Knockdown of KIAA1199 attenuated IL-1β activated Wnt/β-catenin signalling pathway in OA chondrocytes.
(a). Human OA chondrocytes transfected with si-KIAA1199 or si-NC were treated with IL-1β for 24 h and the cells were collected for protein analysis. The protein expression of GSK3β, β-catenin and cyclinD1was detected by western blotting analysis. Densitometry was performed to analyze the ratio of the density between the protein and GAPDH ($^{\#}P < 0.05$ vs. IL-1β treated group). (b). Human chondrocytes transfected with si-KIAA1199 or si-NC were treated with DKK-1 (100 ng/mL) or LiCl (10 mM) for 1 h followed by IL-1β (10 ng/mL) for 24 h. At the indicated time, cells were harvested for western blotting analysis. Densitometry was performed to analyze the ratio of the density between the protein and GAPDH ($^{\#}P < 0.05$ vs. IL-1β treated group).

that KIAA1199 was a newly identified protein, which had the ability to degrade hyaluronan, suggesting a possible linkage between KIAA1199 expression, hyaluronan degradation, and cancer progression [14]. In the present study, we found that KIAA1199 was overexpressed at both the mRNA and protein levels in OA cartilage in contrast to normal tissue, which was consistent with a previous study [24]. Moreover, the serum level of KIAA1199 was upregulated in OA patients compared with those without OA. To reconfirm the involvement of KIAA1199 in the progression of OA, we established an OA animal model in vivo. Using this model, KIAA1199 was gradually upregulated with the development of OA, indicating that KIAA1199 was related to cartilage degradation during OA. However, the exact mechanisms by which KIAA1199 accelerates OA progression remain unknown.

Many cytokines, such as IL-1β, IL-6, and TNF-α, have been implicated in synovial inflammation and cartilage degradation in the progression of OA [25–27]. IL-1β increases the production of a variety of proinflammatory mediators, including PGE2, MMPs, and ADAMTS to promote the degradation of cartilage [28,29]. Accumulated evidence suggests that inhibition of inflammation by IL-1β may be an effective therapeutic target for symptom relief and structural changes in OA [30]. Therefore, we investigated the anti-inflammatory effect of KIAA1199 knockdown in IL-1β-induced chondrocytes. As shown in the present study, inhibition of KIAA1199 significantly attenuated IL-1β-induced PGE2 production, which was characterised by numerous

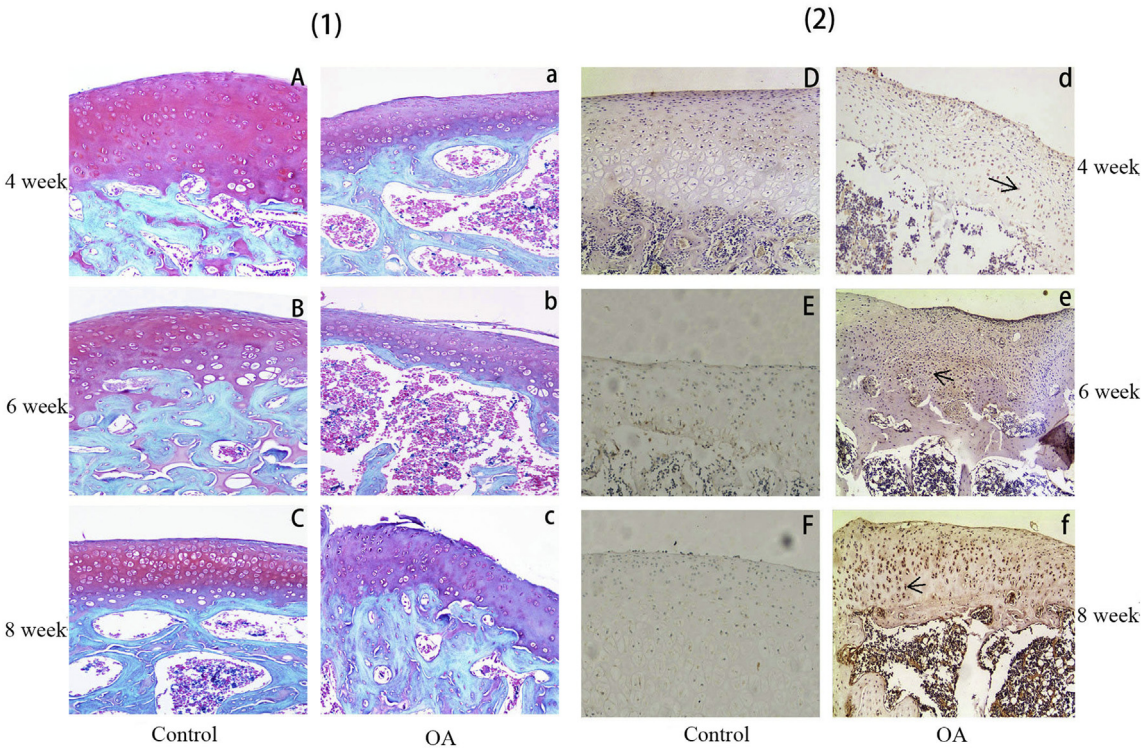

Fig. 5. Safranin O staining, immunohistochemistry of articular cartilage in OA model.
Cartilage obtained from sham group showed no reduction of Safranin O staining, smooth articular surface and slight expression of KIAA1199 (1A–1C, 2D–2F). Cartilage retrieved from OA group showed loss of Safranin O staining, uneven surface and higher expression of KIAA1199 (1a–1c, 2d–2f). The arrow shows the positive staining of KIAA1199 in chondrocytes. Original magnification ×200.

pathological effects, such as inhibiting chondrocytes proliferation and ECM synthesis, in the pathogenesis of OA. In addition, silencing of KIAA1199 decreased the production of TNF-α and IL-6 in IL-1β-stimulated human OA chondrocytes. However, IL-1β could not directly upregulate KIAA1199 expression in human chondrocytes. Collectively, these findings indicated that inhibition of KIAA1199 had an anti-inflammatory effect in OA tissues.

MMPs, a family of proteolytic enzymes, are mainly involved in the breakdown of the ECM in OA [31]. Increased expression of MMPs is closely related to OA progression. Among these MMPs, MMP-13 is considered to play a crucial role in the progression of OA by degrading the ECM, such as the degradation of type II collagen, which is the main component of ECM [32]. Hence, inhibition of MMP-13 expression may confer chondro-protection, which would be of therapeutic value in the treatment of OA [33]. Aggrecanases belong to the ADAMTS family, whose members are characterised by disintegrin and metalloproteases with thrombospondin motifs. Studies have reported that ADAMTS-4 and ADAMTS-5 are the most efficient aggrecanases related to joint disease, but ADAMTS-5 is more active than ADAMTS-4, and rather than ADAMTS-4, the deletion of ADAMTS-5 protects against OA by decreasing aggrecan degradation [34,35]. Importantly, we showed that knockdown of KIAA1199 exerted an anti-catabolic effect by downregulating the expression of MMP-13 and ADAMTS-5, as well as upregulating type II collagen and aggrecan in IL-1β-stimulated OA chondrocytes. These findings imply that silencing of KIAA1199 may serve as a new target for prevention of IL-1β-induced cartilage degradation.

The Wnt/β-catenin signalling pathway participates in a series of cellular events, including cell differentiation, proliferation, migration, and cartilage homeostasis [36]. Many studies have shown the important role of Wnt/β-catenin signalling in the pathogenesis of OA [37,38]. Our previous study also showed that β-catenin was upregulated in an OA animal model [39]. In the present study, we investigated the relationship between KIAA1199 and Wnt/β-catenin signalling. Knockdown of KIAA1199 decreased the expression of β-catenin and Wnt/β-catenin-targeted genes, including those of cyclin D1, MMP-13, and ADAMTS-5. To characterise the role of Wnt/β-catenin signalling in KIAA1199-knockdown-mediated protection in OA chondrocytes, we activated Wnt/β-catenin signalling by stimulation with an agonist (LiCl), which significantly decreased the chondro-protective effects of KIAA1199 silencing. In contrast to the control group, chondrocytes treated with the agonist showed significantly higher expressions of MMP-13 and ADAMTS-5. In addition, we inactivated Wnt/β-catenin signalling by stimulation with DKK-1, indicating that the protective effects of KIAA1199 knockdown were enhanced. These data suggested that suppression of KIAA1199 resulted in anti-inflammatory and chondro-protective effects in IL-1β-induced chondrocytes, at least in part, via inhibiting the Wnt/β-catenin signalling pathway, indicating that KIAA1199 knockdown may prevent cartilage degeneration and inflammatory responses in patients with OA.

5. Conclusion

Expression of KIAA1199 was highly upregulated in osteoarthritic cartilage. KIAA1199 knockdown exerted anti-catabolic and anti-inflammatory effects on IL-1β-induced human chondrocytes via regulation of the Wnt/β-catenin signalling pathway. Therefore, pharmacological inhibition of KIAA1199 may represent a promising therapeutic target for OA.

Declaration of Competing Interest

The authors declared no conflict of interest.

Acknowledgements

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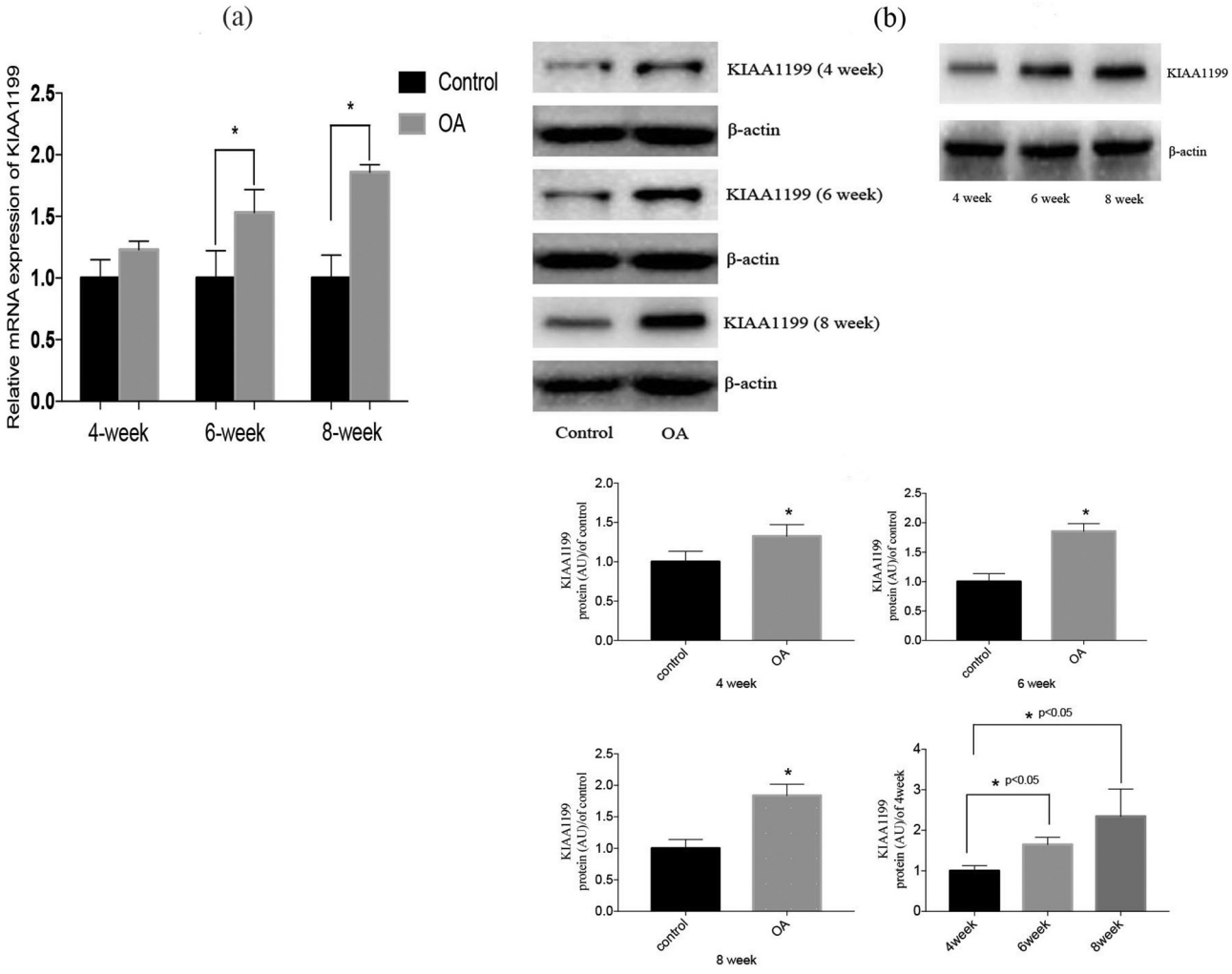

Fig. 6. Expression of KIAA1199 in OA animal model.
Cartilage retrieved from OA group showed higher expression of KIAA1199 by using qRT-PCR and Western blotting analysis. (a). Relative mRNA expression of KIAA1199 in articular cartilage was analyzed by qRT-PCR. *P < 0.05 vs. control group. (b). Protein expression of KIAA1199 in cartilage was detected by western blotting. Densitometry was performed to analyze the ratio of the density between the protein and GAPDH (*P < 0.05 compared with control group).

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