

Effects of IL-1 β -induced ADAMTS-4/-5 and TAK1 on the degradation of cartilage aggrecan in the progression of human osteoarthritis

Qiqi Zhang

the people's hospital of bozhou city

Shenglin Xu

Henan Province Center for Disease Control and Prevention

Yinglei Fang

the people's hospital of Bozhou city

Changcheng Hua

Bozhou People's Hospital

Xiaohe Wang

Bozhou people's hospital

Yong Hu (✉ 975111530@qq.com)

<https://orcid.org/0000-0002-9922-9838>

Research article

Keywords: osteoarthritis, IL-1 β , ADAMTS-4/-5, TAK1, degradation

Posted Date: May 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-23866/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective

Interleukin-1 β (IL-1 β) is a potentially important cytokine involved in several pathological processes of osteoarthritis (OA). It is well known that a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4/-5) and transforming growth factor- β activated kinase 1 (TAK1) contribute to the degradation of human OA cartilage aggrecan. The purpose of this study was to investigate the mechanism by which IL-1 β induced the expression of ADAMTS-4/-5 and TAK1 in human OA cartilage aggrecan degradation.

Methods

The pathological changes of cartilage tissues and chondrocytes were observed by histomorphological analysis. OA chondrocytes were isolated from human articular cartilage tissues and stimulated with 10 ng/ml of IL-1 β at the per-designed times (24 h, 48 h, 72 h). Expression of ADAMTS-4/-5 and TAK1 in cartilage tissues and chondrocytes were measured using immunohistochemistry (IHC) and Western blot (WB).

Results

The level of IL-1 β in synovial fluid in the normal group was significantly lower than that in OA group. The expression of ADAMTS-4/-5 and TAK1 were simultaneously upregulated in human OA cartilage tissues, and ADAMTS-4 was more positively correlated with TAK1 than ADAMTS-5. Furthermore, the expression of ADAMTS-4/-5 and TAK1 were increased by stimulation with IL-1 β in human OA chondrocytes, but no positive correlation was found.

Conclusion

It is proposed that reducing the expression of ADAMTS-4/-5 in prevention of OA may involve the inhibition of TAK1 activity.

Background

With the rapid aging of the global population, more elderly people suffer from joint pain, deformity and dysfunction caused by osteoarthritis (OA) [1]. OA is a common orthopedic disease among the elderly and a chronic, inflammation-related progressive degenerative disease characterized by destruction of cartilage and aggrecan degradation of cartilage extracellular matrix (ECM), which is affected by many risk factors, such as gender, age, obesity, inflammation, and trauma [2, 3]. Although the pathogenesis of OA remains to be elucidated, it is speculated that the imbalance between anabolism and catabolism in cartilage ECM contributes to the progression of OA [1, 4]. Previous researchers have shown that collagens

and aggrecan are the key components of cartilage ECM [5]. Collagens can be degraded by collagenases or the matrix metalloproteinase (MMP) family, such as MMP1, MMP2, MMP3, MMP9, and MMP13 [6, 7]. The degradation of aggrecan is induced by aggrecanases, which belong to a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs), such as ADAMTS-1, ADAMTS-4, ADAMTS-5, ADAMTS-8, and ADAMTS-9 [7, 8, 9]. It has been reported that degradation and loss of aggrecan caused by ADAMTs was a key event in primary OA, while MMPs played an important role in late OA progression [10, 11]. While there are several reports in the literature indicating that both ADAMTS-4/-5 contribute to the pathogenesis of human OA, the question of which aggrecanase contributes more to cartilage aggrecan degradation remains somewhat controversial.

Numerous inflammatory cytokines, such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-17 (IL-17), and tumor necrosis factor- α (TNF- α) have been shown to play a pivotal role in the progression of cartilage aggrecan degradation in OA [12, 13]. Furthermore, these inflammatory cytokines suppress the expression of anabolic substances, such as aggrecan and type II collagen, as well as stimulate various catabolic enzymes, such as MMPs and ADAMTs through the cascade amplification of cell signal transduction pathways, which result in the development of OA [13, 14].

Transforming growth factor- β activated kinase 1 (TAK1) is a mitogen-activated protein kinase kinase kinase (MAP3K) family member, which can be activated by pro-inflammatory cytokines, such as IL-1, transforming growth factor (TGF), and TNF- α [15, 16]. As an important upstream kinase of MAPK and nuclear factor- κ B signaling pathways, TAK1 can regulate downstream transcription factors and then mediate the expression of cyclooxygenase 2 (COX2) and prostaglandin-e2 (PGE2) in chondrocytes, suggesting that TAK1 might participate in OA progression [15, 17]. However, there are fewer studies on the function of TAK1 in pathogenesis of OA progression.

Although previous research has contributed to the progression of OA pathogenesis, further clarification is needed. In the current study, we investigated the essential roles of IL-1 β , TAK1, ADAMTS-4/-5 in promoting cartilage aggrecan degradation in OA. To better understand the underlying pathogenesis mechanism, we also attempted to examine TAK1, ADAMTS-4/-5 expression induced by IL-1 β in cultured human OA chondrocytes. The aim of this study was to provide a novel experimental target for the prevention of OA and research the underlying mechanism.

Materials And Methods

Articular cartilage specimen collection

Normal human cartilage tissues were collected from 10 traumatic amputees (6 women and 4 men, mean age 24-40 years) who underwent above-the-knee amputations. OA cartilage tissues were obtained from 20 patients who underwent total knee arthroplasty. Diagnosis of the patients with OA (12 women and 8 men, mean age 52-76 years) was based on knee joint OA diagnostic criteria recommended by American Rheumatism Association Criteria. All cartilage tissues were collected anatomically from the femoral condyles and tibial plateaus, which were stored at -80 $^{\circ}$ in a liquid nitrogen refrigerator. All patients

provided informed consent for this research in accordance with the protocol approved by the institutional review board and ethics committee of the People's Hospital of Bozhou City and the First Affiliated Hospital of Anhui Medical University.

Cell isolation, culture, and stimulation with IL-1 β

Chondrocytes from human OA cartilage tissues were minced into small pieces less than 1 mm³ with the aseptic scalpel and washed 3 times with phosphate-buffered saline (PBS; Beyotime, Shanghai, China). After that, the cartilage pieces were digested at 37°C with 0.25% (w/v) trypsin-ethylenediaminetetraacetic acid (EDTA, Beyotime) solution for 30 min before incubating with 0.2% type α collagenase (Beyotime) in high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) at 37°C for 18-20 h. The cell suspension was filtered through 200- μ m meshes, centrifuged at 1,500 rpm for 10 min, and washed extensively with PBS (Beyotime). Subsequently, the isolated primary chondrocytes were resuspended in high-glucose DMEM (Gibco) containing 20% fetal bovine serum (FBS; Gibco), 100 U/ml of penicillin (Gibco), and 100 mg/ml of streptomycin (Gibco). Finally, cells were seeded in 100 cm² culture flasks and incubated at 37°C in an atmosphere of 5% CO₂. The medium was changed every other day. The isolated primary chondrocytes could be passaged after spreading 80-95% culture flasks in approximately 7 days.

Chondrocytes were passaged enzymatically, detaching with 0.25% (w/v) trypsin-EDTA solution (Beyotime), and cultured chondrocytes were split in a ratio of 1:3. The third-generation chondrocytes were used for the experiment and maintained for 24 h in high-glucose DMEM (Gibco) supplemented with 20% FBS (Gibco) and 1% penicillin (Gibco)/streptomycin (Gibco) at 37°C in an atmosphere of 5% CO₂.

Thereafter, chondrocytes in 6-well plates were washed with PBS (Beyotime) and treated with IL-1 β (10 ng/mL; Beyotime, Shanghai, China) in serum-free high-glucose DMEM (Gibco) for 24, 48, and 72 h. At the end of the stimulation period, the cell growth situation was observed by inverted microscope, while the cells were collected to analyze the expression of TAK1 and ADAMTS-4/-5 using Western blot assay. Cells in 96-well plates were stimulated in serum-free high-glucose DMEM (Gibco) with or without IL-1 β (10 ng/ml) for 0, 24, 48, or 72 h. At confluence, the effect of IL-1 β on the chondrocyte viability was detected by Cell Counting Kit-8 (CCK-8) assay.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of IL-1 β in the synovial fluid of normal and OA knee joints was collected in surgery and detected using commercial ELISA kits (Beyotime) according to the manufacturer's instructions. All the assays were performed in duplicate. The absorbance values were read at 450 nm.

Cell viability assay

The effect of IL-1 β on chondrocyte viability was measured by CCK-8 assay according to the manufacturer's instructions. Briefly, as indicated in the treatments with cells, 10 μ l CCK-8 solution

(Beyotime) was added to each well, and the cells in the 96-well plates were incubated at 37°C for 2 h. Absorbance at 450 nm (Leica Microsystems, Germany) was measured using a microplate reader (Bio-Rad, Hercules, CA, USA). The same conditions without IL-1 β were used as the control.

Immunohistochemistry assay

After cartilage tissues were collected from normal and OA patients, fixed with formalin, decalcified by 10% EDTA (Beyotime), conventionally dehydrated, embedded in paraffin, serial sliced into 3- 4- μ m slices, prepared for hematoxylin-eosin (HE) and immunohistochemical (IHC) slices. The operation steps of IHC were as follows: the cartilage tissue slices were baked in an oven at 65°C for 24 h, dewaxed in xylene, and dehydrated in a gradient concentration of ethanol. The cartilage tissues and chondrocytes sections were then washed in PBS (Beyotime) and incubated with proteinase K (Beyotime) and superoxide dismutase (SOD, Beyotime) for 30 min. Thereafter, endogenous peroxidases were blocked by 0.3% peroxide (Beyotime) for 20 min. Sections were incubated with dilution of primary antibodies (rabbit anti-TAK1, 1/200 dilution; rabbit anti-ADAMTS-4, 1/200 dilution; mouse anti-ADAMTS-5, 1/250 dilution; Abcam, Cambridge, MA, USA) overnight at 4°C. Next day, sections were incubated with a universal biotinylated secondary antibody (Beyotime) complex for 30 min and reaction was stopped by rinsing in PBS. DAB chromogenic reagent (Beyotime) was added for approximately 2 min, and sections were counterstained with Mayer's hematoxylin and Scott's blue with running tap water. Next, they were dehydrated with a gradient (Beyotime) concentration of ethanol, cleared with xylene, and mounted with a coverslip. The results were observed by inverted microscope (Olympus, Japan) and analyzed by ImagePro Plus version 6.0 (IPP 6.0).

Toluidine blue staining assay

The morphology and aggrecan degradation of cultured human OA chondrocytes were identified by toluidine blue staining as previously described. The slide glasses were taken out from 6-well plates and washed in PBS (Beyotime). Next, the slides were fixed with 4% paraformaldehyde (Beyotime) at room temperature for 30 min, stained with toluidine blue for 30 min, and rinsed rapidly in dehydrated alcohol. Finally, they were air-dried, dehydrated with a gradient concentration of ethanol, cleared with xylene, and mounted with cover slips. The stained cells were observed and captured using an inverted microscope (Olympus, Japan). The preparation of cartilage tissue slices was the same as that of IHC slices, and the remaining HE (Beyotime) staining steps are similar to toluidine blue staining.

Western blot assay

The total protein expression of TAK1 and ADAMTS-4/-5 were extracted from cartilage tissues and chondrocytes analyzed by western blot as previously described. Cartilage tissues (0.05 g), 499 μ l of radioimmunoprecipitation assay (RIPA, Beyotime) lysate and 1 μ l of benzenesulfonyl-fluoride (Beyotime) were put into a homogenizer and underwent adequate grinding and cracking for 30 min in the ice bath. The tissue lysates were collected in 2-ml centrifuge tubes. The stimulated chondrocytes were washed 3 times with ice-cold PBS, harvested by a scraper and incubated for 30 min with RIPA buffer (Beyotime).

The tissue lysates and cell lysates were centrifuged at 12,000 rpm for 10 min, and the supernatants were collected. The protein concentration was measured by a bicinchoninic acid (BCA) protein kit (Beyotime). Protein samples were heated at 95°C for 5 min with sample loading buffer, and 15 µl of protein was separated on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel (Beyotime). Cellular protein was transferred to polyvinylidene difluoride (PVDF, Beyotime) membranes by electroblotting and blocked with 5% nonfat milk in 0.1% Tris-Tween PBS (TPBS) for 1.5 h. The membranes were incubated with rabbit polyclonal antibodies for TAK1 (1/200 dilution; Abcam), ADAMTS-4 (1/200 dilution; Abcam), ADAMTS-5 (1/250 dilution; Abcam), and mouse mAb β-actin (1/500 dilution; Abcam) overnight at 4°C. Membranes were washed 3 times with TPBS and incubated with a goat-rabbit or goat-mouse immunoglobulin G-horseradish peroxidase (IgG-HRP)-conjugated secondary antibody (Abcam) at 1:10,000 dilution for 2 h. After washing, protein bands were detected using an enhanced chemiluminescence (ECL) kit (Beyotime) and visualized by autoradiography (Bioshine, Shanghai, China). Immunoblots were analyzed by densitometry using ImageJ software.

Statistical analysis

All experimental data in this study are expressed as the means ± deviation (SD) and analyzed by SPSS 13.0 software. Student's *t*-test and one-way analysis of variance (ANOVA) were used to analyze the statistical significance. $p < .05$ (*) was considered a statistically significant difference.

Results

ELISA results

The concentration level of IL-1β in synovial fluid of normal and OA knee joints were detected using ELISA. As shown in Fig. 1, IL-1β in synovial fluid in the normal group was significantly lower than in the OA group ($p < .01$).

HE staining assay

HE staining was used to observe the morphology and aggrecan degradation of cartilage tissues. As shown in (Fig. 2A), in the normal group, the surface of cartilage tissue was smooth or showed microscopic change, and the chondrocytes were located in the cartilage lacunae, and arranged regularly layer by layer. In the OA group, the surface was irregular, with collagen fibrosis and fissure, the staining of the sub-cartilage matrix was inhomogeneous, and the degenerative chondrocytes were arranged in clusters.

IHC analysis of expression of ADAMTS-4/-5 and TAK1 in cartilage tissues

The expression of ADAMTS-4/-5 and TAK1 in normal and OA cartilage tissues were analyzed by IHC. As shown in Fig. 2B, ADAMTS-4-positive chondrocytes were localized in superficial and transitional zones in the OA group, while no or negligible staining was observed in the normal group. The location of ADAMTS-

5-positive chondrocytes were similar to ADAMTS-4. Both ADAMTS-4/-5 expression in the normal group were significantly lower than in the OA group ($p < .01$) (Fig. 2C and D). TAK1-positive chondrocytes, which were stained with brownish-yellow granules in normal group, were similar in the OA group, but the amount and the staining intensity was decreased. The expression of TAK1 in normal group was significantly lower than in the OA group ($p < .01$) (Fig 2E). The expression of ADAMTS-4/-5 and TAK1 were consistent with western blot analysis results (Fig 2F).

Phenotypic determination of primary human OA chondrocytes

The morphology of primary human OA chondrocytes is shown in Fig. 3A. The phenotype was determined by toluidine blue staining and IHC of aggrecan staining, as shown in Fig. 3B and 3C.

Effect of IL-1 β on the morphology and aggrecan degradation of cultured OA chondrocytes

The change of morphology and aggrecan degradation in cultured OA chondrocytes were obvious at the premeditated times. As shown in Fig. 3D, the adherent primary human chondrocytes grew faster. Then, the chondrocyte edges protruded and pseudopodium extended, presenting as multipolar or stellate. A high density of chondrocytes was arranged closely, presenting a cobblestone-like appearance, and they secreted a large amount of aggrecan. The chondrocyte gap was found to expand, stretching out more pseudopodium for cellular junction and aggrecan degradation after stimulation with or without IL-1 β at 10 ng/ml for 0, 24, 48, and 72 h by an inverted microscope.

Effect of IL-1 β on chondrocyte viability

The effect of 10 ng/ml IL-1 β on chondrocyte viability was detected by CCK-8 assay. As shown in Fig. 3E, CCK-8 assays revealed that 10 ng/ml IL-1 β had no significant cytotoxic effect on human OA chondrocytes after incubation at a concentration of 10 ng/ml for 24, 48, and 72 h ($p > .05$).

Effect of IL-1 β on the expression of ADAMTS-4/-5 and TAK1 in cultured human OA chondrocytes

Expression of ADAMTS-4/-5 and TAK1 induced by 10 ng/ml of IL-1 β in cultured human OA chondrocytes were analyzed by Western blot. As shown in Fig. 3F, the result showed that 10 ng/ml of IL-1 β stimulation significantly induced the expression of ADAMTS-4 and TAK1, while ADAMTS-5 protein was slightly increased in response to the stimulation of IL-1 β .

The respective correlation between ADAMTS-4/-5 and TAK1

The above findings showed that the expression of ADAMTS-4/-5 and TAK1 were simultaneously upregulated in the OA group compared to the normal group (Fig. 2C-E). Furthermore, we observed that the respective correlation between ADAMTS-4/-5 and TAK1 were analyzed by Spearman's rank correlation analyses, and ADAMTS-4 was also more positively correlated with TAK1 than ADAMTS-5 ($p < .05$) (Fig. 4A-B). Herein, results showed that expression of ADAMTS-4/-5 and TAK1 were induced by stimulation

with IL-1 β in human OA chondrocytes, but interestingly, the respectively positive correlation was not found between them.

Discussion

With the rapid aging of global population, the incidence rate of OA has increased in recent years, and the morbidity is > 10%. [4, 18]. OA is a chronic progressive joint disease, characterized by arthrosynovitis, degeneration and destruction of cartilage, subchondral bone sclerosis, and osteophyte formation [19]. In recent years, several studies have improved our understanding of the heterogeneous and complicated pathogenesis of OA development and provided potential targets for clinical therapy of OA, but the precise etiological mechanism of OA remains to be elucidated. Aggrecan and hyaluronan fill the interstices of collagen meshwork, which together with diaspora chondrocytes constitute the articular cartilage [9, 20]. The proteoglycan aggrecan and type II collagen are the two major components of articular cartilage extracellular matrix [5, 21]. The subsequent destruction of articular cartilage, degradation of cartilage extracellular matrix and progressive loss of joint function are induced by the imbalance between anabolic and catabolic cartilage extracellular matrix, which is a pathological feature in the progression of OA [22]. Proteoglycan aggrecan plays an important role in compression resistance and elasticity of articular cartilage extracellular matrix, which makes articular cartilage adjust to joint biomechanical movement [21, 23]. Thus, the loss of proteoglycan aggrecan is considered to be a crucial event in the pathogenesis of OA [24].

Previous studies have reported the degradation of proteoglycan aggrecan mediated by aggrecanase in the initial phase of OA articular cartilage destruction [9, 25]. Aggrecanases belong to the ADAMTS family and play a crucial role in aggrecan degradation, which occurs in the aggrecanase-specific site (Glu373-Ala374) existing in the interglobular domain (IGD) region [26]. While both ADAMTS-4 and ADAMTS-5 are the most efficient aggrecanases related to human OA joint disease, the question of which one contributes more to the degradation of human articular cartilage remains controversial [10, 27, 28]. Previous results revealed that ADAMTS-4 plays a significant role in aggrecan catabolism by using immunoprecipitation with anti-ADAMTS-4 and anti-ADAMTS-5 antibodies in bovine and porcine chondrocytes in vitro [29, 30]. Recent mouse knockout research results have shown that ADAMTS-5 but not ADAMTS-4 plays an essential role in proteoglycan degradation [31]. The cartilage damage of human OA was found to be induced by the role of both ADAMTS-4/-5 using siRNA in vitro [32]. Several reports have suggested that ADAMTS-4 might be the principal aggrecanase in human OA cartilage aggrecan degradation [33].

In this study, we demonstrated that ADAMTS-4-positive chondrocytes were localized in superficial and transitional zones in the OA group, while no or negligible staining was observed in the normal group. The location of ADAMTS-5-positive chondrocytes were similar to ADAMTS-4, and both ADAMTS-4/-5 expression in the normal group were significantly lower than in the OA group. Western blot results showed that expression of ADAMTS-4/-5 were consistent with IHC results. Our findings are consistent with previous studies showing that both ADAMTS-4/-5 are the major aggrecanases to attenuate the degradation of aggrecan in human OA cartilage tissues [32, 34]. In the present study, we deduced that

ADAMTS-4 degrades more proteoglycan aggrecans than ADAMTS-5 in human OA cartilage tissue, while ADAMTS-5 is primarily responsible for the physiologic equilibrium of proteoglycans in normal cartilage and cooperative with ADAMTS-4 cleaving more proteoglycan aggrecan in OA cartilage tissues. This finding is consistent with some studies in human OA cartilage tissue [32–35].

Because IL-1 β can stimulate the secretion of inflammatory cytokines (such as IL-6, TNF- α), promote associated catabolic factors, such as COX-2, nitric oxide (NO), induce nitric oxide synthase (iNOS) and PGE₂, and increase expression of matrix-degrading proteinases (such as MMPs and ADAMTS), it is considered to be the key inducer and amplifier mediating the initiation and progression of OA cartilage degradation [6, 14, 36]. Our findings showed that IL-1 β in synovial fluid in the normal group was significantly lower than in the OA group. Our study showed that cultured OA chondrocytes gradually became spindle-shaped with less abundant cytoplasm, reduced refractivity, expanded intercellular space, aggrecan degradation after stimulation with IL-1 β . The regulation of IL-1 β on the expressions of ADAMTS-4/-5 in human OA cartilage chondrocytes remain somewhat controversial. In this study, stimulation with 10 ng/ml of IL-1 β stimulation the expression of ADAMTS-4/-5 in human OA cartilage chondrocytes. With the extension of incubation time with IL-1 β , the expression of ADAMTS-4 was increased, and ADAMTS-5 was slightly upregulated. The reasons may be that the expression of ADAMTS-4 was inducible and sensitive to IL-1 β stimulation, while the expression of ADAMTS-5 was constitutive and insensitive to the induction of IL-1 β . These results were consistent with previous studies on the effect of IL-1 β on the expression of ADAMTS-4/-5 associated with individual differences and enzyme activity in human OA chondrocytes [37, 38]. This result suggested that both ADAMTS-4/-5 contributed to the degradation of cartilage aggrecan in IL-1 β -treated human OA chondrocytes, while ADAMTS-4 seemed more sensitive to IL-1 β in vitro. This finding was consistent with a previous study in which ADAMTS-4 seemed to play a more significant role than ADAMTS-5 in aggrecan catabolism and degradation of human OA arthritis cartilage [34].

TAK1 is a key regulator in upstream MAPKs and NF- κ B signaling pathways induced by transduction cascades of inflammatory cytokines (such as IL-1, TGF- β , and TNF- α), which is involved in immune activation, catabolic factor generation, pain-related molecule expression, matrix-degrading enzyme expression, and cell apoptosis [16, 39, 40]. It has been reported that inhibition of TAK1 through siRNA could reduce the expression of MMP13, MMP1, and TNF- α induced by IL-1 β [41]. The expression of COX-2, MMP-3, MMP-13, and ADAMTS-4 were reduced by injecting TAK1 inhibition in injured porcine cartilage [42]. In the present study, western blot confirmed that expression of TAK1 in the OA group was significantly higher than in the normal group in human cartilage tissues. Our results showed that IL-1 β stimulation induced the expression of TAK1 in human OA chondrocytes, which is coincident to a previous study that showed that TAK1 is responsive to many proinflammatory cytokines [41, 43]. The ADAMTS-4/-5 expression were upregulated and positively correlated with TAK1 in OA cartilage tissue, further indicating that suppressing TAK1 might reduce ADAMTS-4/-5 expression, causing the aggrecan degradation of cartilage tissues in the initial and progression of OA. Herein, we observed that the expression of ADAMTS-4/-5 and TAK1 were upregulated in cultured human OA chondrocytes. The expression of ADAMTS-4/-5 and TAK1 were not positively correlated, although stimulation with IL-1 β

induced an increase in their expression. The reason for this may be that the amount of ADAMTS-4/-5 expression was slightly upregulated and hard to detect by western blot. However, their important role in cartilage aggrecan degeneration are undeniable, and previous studies have confirmed that ADAMTS-5 is constitutively expressed for the physiological equilibrium of aggrecan in normal cartilage and conjunction with ADAMTS-4 in the degradation of aggrecan, which may make aggrecan more impressible to cleavage by ADAMTS-4 [32]. Recently, a study indicated that the differently increased amounts of ADAMTS-4/-5 mediated by IL-1 β may be associated with individual differences, and ADAMTS-4 is primarily responsible for the cytokine-induced cartilage aggrecan degeneration [34, 44]. This finding is consistent with the results of some in vitro studies about human OA chondrocytes [7, 23, 45]. Thus, these data indicated that active TAK1 might participate in IL-1 β -induced ADAMTS-4/-5 expression in the degeneration and progression of OA cartilage aggrecan. It is useful to reduce TAK1 activity in the retardant development of pathogenesis in OA.

Conclusion

In this study, we confirmed that ADAMTS-4/-5 and TAK1 played a vital role in OA cartilage aggrecan degradation and cartilage destruction. ADAMTS-4 may contribute to more cartilage aggrecan degradation than ADAMTS-5. Taken together, it is speculated that inhibiting TAK1 activity, which is the upstream predominant transducer of signaling pathways in OA-relevant inflammatory cytokines, could be useful to reduce the cartilage aggrecan degradation in pathophysiology of OA through reducing of ADAMTS-4/-5 expression induced by IL-1 β . In summary, we provide a novel experimental basis for studying the pathogenesis of OA. Of course, our experiment is flawed, and further research is needed on the effect of cartilage aggrecan degradation in the pathogenesis of OA by inhibiting TAK1 activity.

Abbreviations

OA: Osteoarthritis; IL-1: Interleukin-1 β ; ADAMTS-4/5: A disintegrin and metalloproteinase with thrombospondin motifs-4/5; TAK1: Transforming growth factor- β activated kinase 1; IHC: Immunohistochemistry; WB: Western blot; PBS: Phosphate-buffered saline; EDTA: Ethylenediaminetetraacetic acid; DMEM: Dulbecco's modified Eagle's medium; ELISA: Enzyme-linked immunosorbent assay; HE: Hematoxylin-eosin; ECM: Extracellular matrix; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; MMP: Matrix metalloproteinase; ADAMTs: A disintegrin and metalloproteinase with thrombospondin motifs; IL-6: Interleukin transforming growth factor -6; IL-17: Interleukin-17; TNF- α : Tumor necrosis factor- α ; MAP3K: Mitogen-activated protein kinase kinase kinase; TGF: Transforming growth factor; COX2: Cyclooxygenase 2; PGE2: prostaglandin-e2; NO: Nitric oxide; iNOS: Induce nitric oxide synthase;

Declarations

Acknowledgements

Not applicable.

Authors' contributions

QQZ conceived and designed the study. QQZ and SLX performed all the experiments. YLF collected clinical tissue samples and experimental data. CCH analyzed and interpreted the data. YH supervised this study. All the authors read and approved the final manuscript.

Funding

This study was supported by the Natural Science Foundation project of Anhui Province (1708085MH215).

Availability of data and materials

All data of this study are available to author for scientific research.

Ethics approval and consent to participate

All patients were provided informed consent for this research in accordance with the protocol approved by the institutional review board and ethics committee of the People's Hospital of Bozhou City and the First Affiliated Hospital of Anhui Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

¹Department of Orthopaedics, The People's Hospital of Bozhou City, 616# Du Zhong Road, Bozhou, 236800, Anhui, China

²Department of Orthopaedics, The First Affiliated Hospital of Anhui Medical University, 218# Ji Xi Road, Hefei, 230032, Anhui, China

References

1. Lane NE, Shidara K. Osteoarthritis year in review 2016: clinical. *Osteoarthritis Cartilage*. 2017;25:209–15.
2. Mobasher A. An update on the pathophysiology of osteoarthritis. *Ann Phys Rehabil Med*. 2016;59:333–9.

3. Greene MA. Aging-related inflammation in osteoarthritis. *Osteoarthritis Cartilage*. 2015;23:1966–71.
4. Wang P, Mao Z, Pan Q, et al. Histone deacetylase-4 and histone deacetylase-8 regulate interleukin-1 β -induced cartilage catabolic degradation through MAPK/JNK and ERK pathways. *Int J Mol Med*. 2018;41:2117–27.
5. Olivotto E, Otero M, Marcu KB. Pathophysiology of osteoarthritis: canonical NF- κ B/IKK β -dependent and kinase-independent effects of IKK α in cartilage degradation and chondrocyte differentiation. *RMD Open*. 2015;1:e000061.
6. Ding QH, Ye CY, Chen EM, et al. Emodin ameliorates cartilage degradation in osteoarthritis by inhibiting NF- κ B and Wnt/ β -catenin signaling in-vitro and in-vivo. *Int Immunopharmacol*. 2018;61:222–30.
7. da Silva MA, Yamada N, Clarke NM. Cellular and Epigenetic Features of a Young Healthy and a Young Osteoarthritic Cartilage Compared with Aged Control and OA Cartilage. *J Orthop Res*. 2009;27:593–601.
8. Yaykasli KO, Hatipoglu OF, Yaykasli E, et al. Leptin induces ADAMTS-4, ADAMTS-5, and ADAMTS-9 genes expression by mitogen-activated.
9. protein kinases. and NF- κ B signaling pathways in human chondrocytes. *Cell Biol Int*. 2015;39:104–12.
10. Verma P. ADAMTS-4 and ADAMTS-5: key enzymes in osteoarthritis. *J Cell Biochem*. 2011;112:3507–14.
11. Yang B, Ni J, Long H, et al. IL-1 β -induced miR-34a up-regulation.
12. inhibits Cyr61. to modulate osteoarthritis chondrocyte proliferation through ADAMTS-4⁺. *J Cell Biochem*. 2018;119:7959–70.
13. Jabłońska-Trypuć A, Matejczyk M. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. *J Enzyme Inhib Med Chem*. 2016;31:177–83.
14. Tsuchida AI, Beekhuizen M. et al. Cytokine profiles in the joint depend on pathology, but are different between synovial fluid, cartilage tissue and cultured chondrocytes. *Arthritis Res Ther*. 2014;16:441., 't Hart MC.
15. Chan CM, Macdonald CD, Litherland GJ, et al. Cytokine-induced MMP13 expression in human chondrocytes is dependent on activating Transcription factor 3 (ATF3) regulation. *J Biol Chem*. 2017;292:1625–36.
16. Lee SA, Moon SM, Han SH, et al. The Effect of the Prethanol Extract of *Trifolium pratense* Leaves on Interleukin-1 β -Induced Cartilage Matrix Degradation in Primary Rat Chondrocytes. *Cell Tissues Organs*. 2018;206:95–105.
17. Sai K, Morioka S, Takaesu G, et al. Inhibition of TAK1 (MAP3K7) upregulates unfolded protein response and prevents high-fat diet-induced obesity. *FASEB J*. 2014;28:765.4.

18. Ninomiya-Tsuji J, Kishimoto K, Hiyama A, et al. The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature*. 1999;398:252–6.
19. Klatt AR, Klinger G, Paul-Klausch B, et al. TAK1 mediates the collagen-II-dependent induction of the COX-2 gene and PGE2 release in primary human chondrocytes. *Connect Tissue Res*. 2010;51:452–8.
20. Guillemain F, Rat AC, Mazieres B, et al. Prevalence of symptomatic hip and knee osteoarthritis: a two-phase population-based survey. *Osteoarthritis Cartilage*. 2011;19:1314–22.
21. Li H, Peng Y, Wang X, et al. Astragaloside inhibits IL-1 β -induced inflammatory response in human osteoarthritis chondrocytes and ameliorates the progression of osteoarthritis in mice. *Immunopharmacol Immunotoxicol*. 2019;41:497–503.
22. Huang K. Aggrecanase and aggrecan degradation in osteoarthritis: a review. *J Int Med Res*. 2008;36:1149–60.
23. Tang Q, Zheng G, Feng Z, et al. Wogonoside inhibits IL-1 β induced catabolism and hypertrophy in mouse chondrocyte and ameliorates murine osteoarthritis. *Oncotarget*. 2017;8:61440–56.
24. Zhong HM, Ding QH, Chen WP, et al. Vorinostat, a HDAC inhibitor, showed anti-osteoarthritic activities through inhibition of iNOS and MMP expression, p38 and ERK phosphorylation and blocking NF- κ B nuclear translocation. *Int Immunopharmacol*. 2013;17:329–35.
25. Caterson B, Flannery CR, Hughes CE, et al. Mechanisms involved in cartilage proteoglycan catabolism. *Matrix Biol*. 2000;19:333–44.
26. Sandy JD. Proteolytic cleavage of normal and osteoarthritic cartilage and matrix [J]. Oxford: Oxford University Press; 2003. pp82–92.
27. Dancevic CM. Current and emerging therapeutic strategies for preventing inflammation and aggrecanase-mediated cartilage destruction in arthritis. *Arthritis Res Ther*. 2014;16:429.
28. Durham TB, Klimkowski VJ, Rito CJ, et al. Identification of potent and selective hydantoin inhibitors of aggrecanase-1 and aggrecanase-2 that are efficacious in both chemical and surgical models of osteoarthritis. *J Med Chem*. 2014;57:10476–85.
29. Apte SS. Anti-ADAMTS5 monoclonal antibodies: implications for aggrecanase inhibition in osteoarthritis. *Biochem J*. 2016;473:e1–4.
30. Bayliss MT, Hutton S, Hayward J, et al. Distribution of aggrecanase (ADAMts 4/5) cleavage products in normal and osteoarthritic human articular cartilage: the influence of age, topography and zone of tissue. *Osteoarthritis Cartilage*. 2001;9:553–60.
31. Tortorella MD, Malfait AM, Deccico C, et al. The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. *Osteoarthritis Cartilage*. 2001;9:539–52.
32. Powell AJ, Little CB. Low molecular weight isoforms of the aggrecanases are responsible for the cytokine-induced proteolysis of aggrecan in a porcine chondrocyte culture system. *Arthritis Rheum*. 2007;56:3010–9.
33. Stanton H, Rogerson FM, East CJ, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature*. 2005;434:648–52.

34. Song RH, Tortorella MD, Malfait AM, et al. Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum.* 2007;56:575–85.
35. Naito S, Shiomi T, Okada A, et al. Expression of ADAMTS4 (aggrecanase-1) in human osteoarthritic cartilage. *Pathol Int.* 2007;57:703–11.
36. Zhang E, Yan X, Zhang M, et al. Aggrecanases in the human synovial fluid at different stages of osteoarthritis. *Clin Rheumatol.* 2013;32:797–803.
37. Haneda M, Hayashi S, Matsumoto T, et al. Depletion of aquaporin 1 decreased ADAMTS-4 expression in human chondrocytes. *Mol Med Rep.* 2018;17:4874–82.
38. Kapoor M, Martel-Pelletier J, Lajeunesse D, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Rheumatol.* 2011;7:33–42.
39. Imada K, Oka H, Kawasaki D, et al. Anti-arthritic action mechanisms of natural chondroitin sulfate in human articular chondrocytes and synovial fibroblasts. *Biol Pharm Bull.* 2010;33:410–4.
40. Hu P, Chen W, Bao J, et al. Cordycepin modulates inflammatory and catabolic gene expression in interleukin-1beta-induced human chondrocytes from advanced-stage osteoarthritis: an in vitro study. *Int J Clin Exp Pathol.* 2014;7:6575–84.
41. Cheng J, Hu X, Dai L, et al. Inhibition of transforming growth factor β -activated kinase 1 prevents inflammation-related cartilage degradation in osteoarthritis. *Sci Rep.* 2016;6:34497.
42. Uchida K, Takano S, Matsumoto T, et al. Transforming growth factor activating kinase 1 regulates extracellular matrix degrading enzymes and pain-related molecule expression following tumor necrosis factor- α stimulation of synovial cells: an in vitro study. *BMC Musculoskelet Disord.* 2017;18:283.
43. Klatt AR, Klinger G, Neumüller O, et al. TAK1 downregulation reduces IL-1beta induced expression of MMP13, MMP1 and TNF-alpha. *Biomed Pharmacother.* 2006;60:55–61.
44. Ismail HM, Didangelos A, Vincent TL, et al. Rapid activation of transforming growth factor beta-activated Kinase 1 in chondrocytes by phosphorylation and K63-linked polyubiquitination upon injury to animal articular cartilage. *Arthritis Rheumatol.* 2017;69:565–75.
45. Ismail HM, Yamamoto K, Vincent TL, et al. Interleukin-1 acts via the JNK-2 signaling pathway to induce Aggrecan degradation by human Chondrocytes. *Arthritis Rheumatol.* 2015;67:1826–36.
46. Adler N, Schoeniger A. Polyunsaturated fatty acids influence inflammatory markers in a cellular model for canine osteoarthritis. *J Anim Physiol Anim Nutr (Berl).* 2018;102:e623–32.
47. Kamm JL, Nixon AJ. Cytokine and catabolic enzyme expression in synovium, synovial fluid and articular cartilage of naturally osteoarthritic equine carpi. *Equine Vet J.* 2010;42:693–9.

Figures

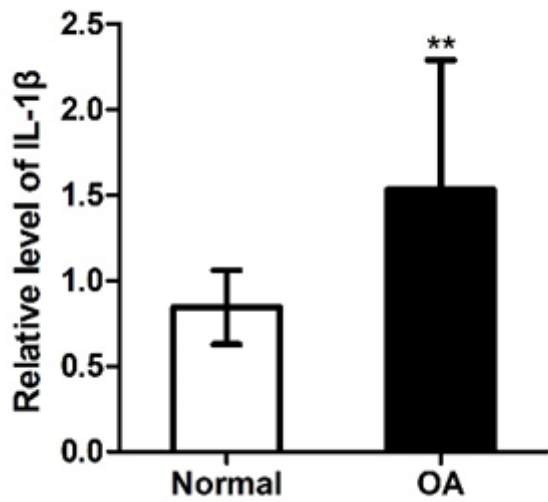


Figure 1

Comparison of the concentration levels of IL-1 β in synovial fluid of normal and OA knee joint using ELISA. IL-1 β level is represented as optical density (OD) values at 450nm. **P < 0.01 compared to normal group.

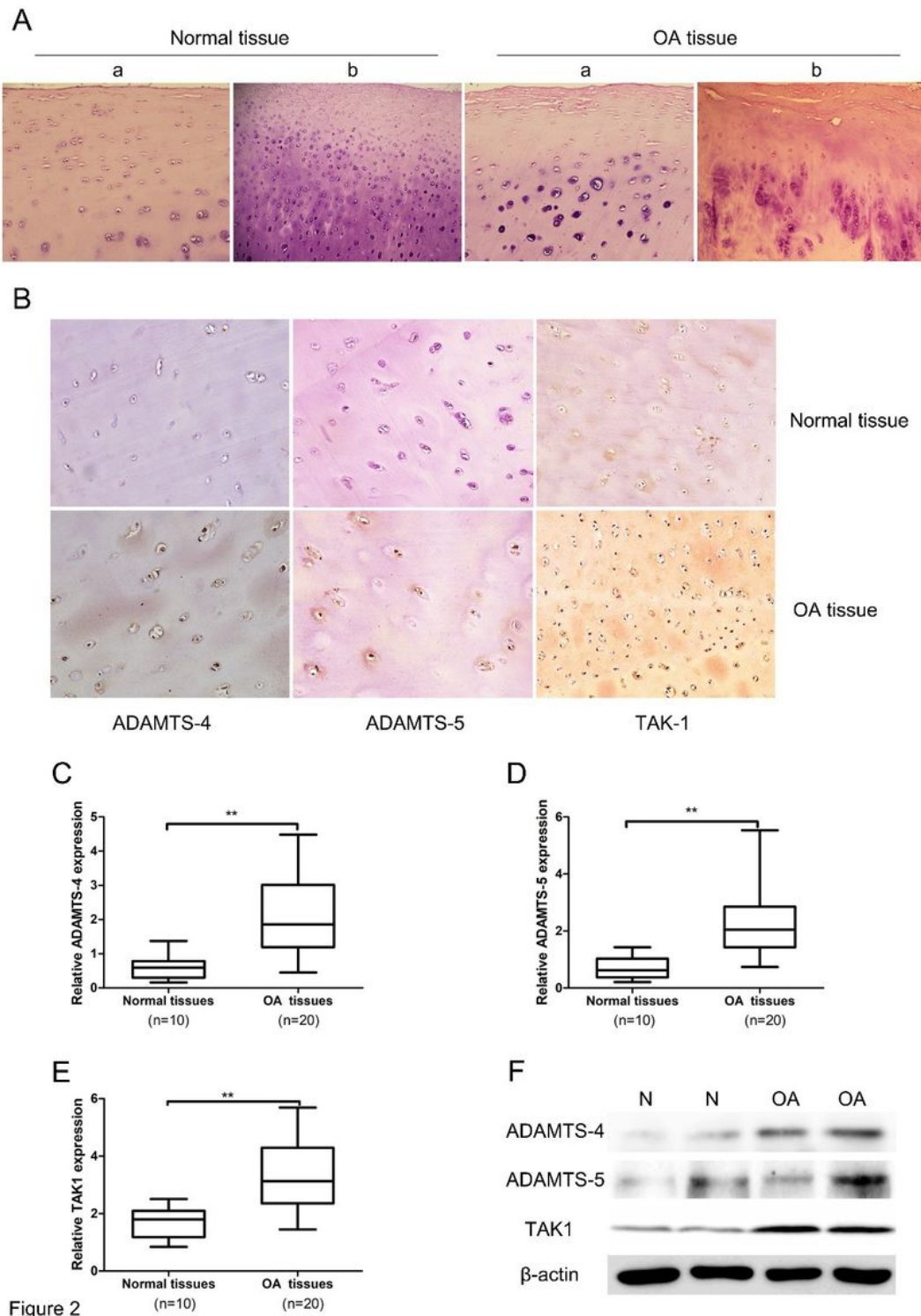


Figure 2

Figure 2

The expression of ADAMTS-4/-5 and TAK1 in normal and OA cartilage tissues. (A) The morphology of normal and OA cartilage tissues was revealed using HE staining (a, 200x; b, 100x). (B) ADAMTS-4/-5 and TAK1 content in normal and OA cartilage tissues were evaluated using IHC assays (200x). (C), (D) and (E) The expression of ADAMTS-4/-5 and TAK1 in normal and OA cartilage tissues were determined by IHC

using Image-Pro Plus Version 6.0 (IPP 6.0) system. ** P < 0.01 (F) The protein levels of ADAMTS-4/-5 and TAK1 in normal and OA cartilage tissues were determined using Western blot assays.

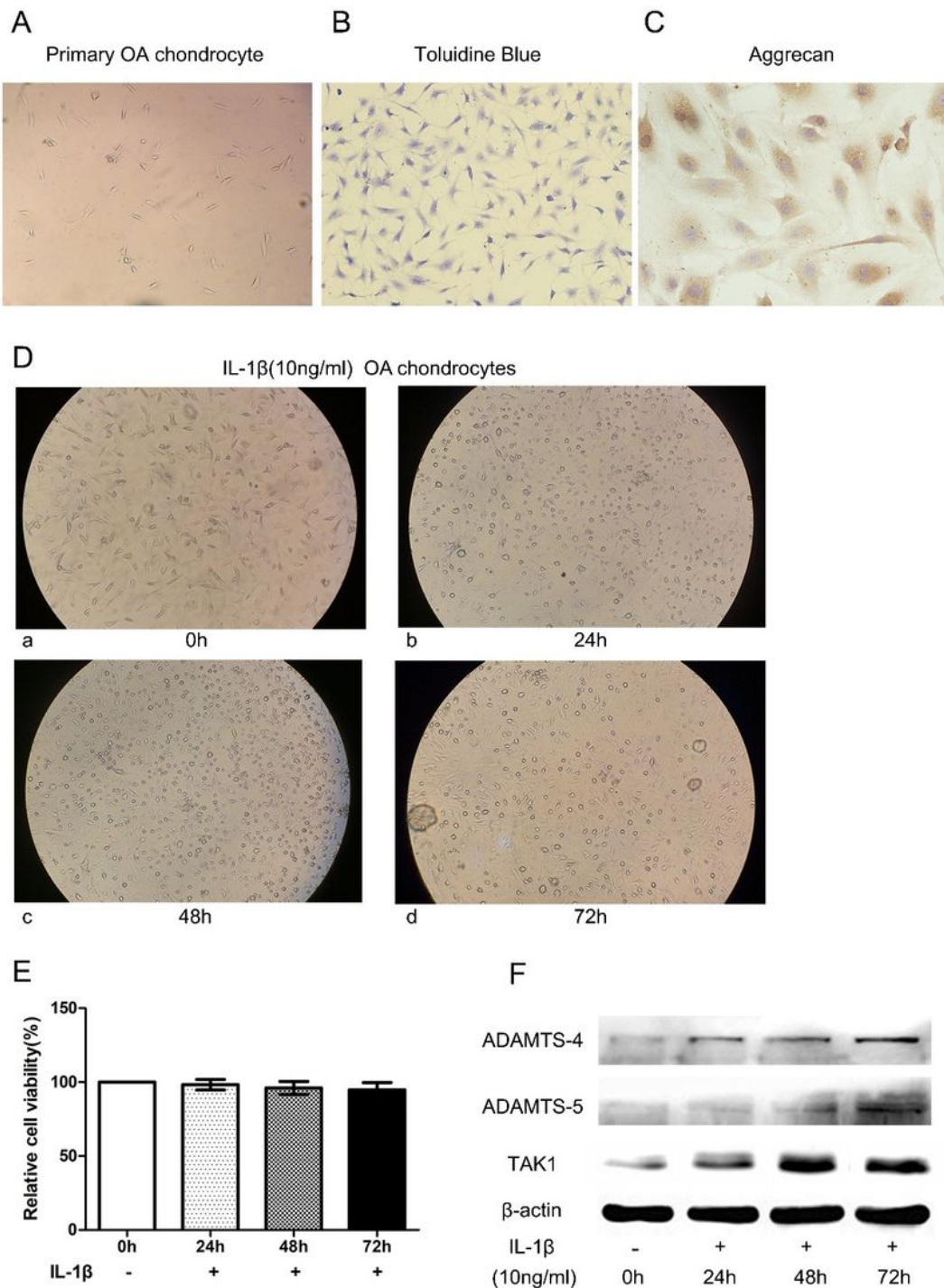


Figure 3

Figure 3

Effect of IL-1 β on the expression of ADAMTS-4/-5 and TAK1 in cultured human OA chondrocytes. (A) The morphology of primary human OA chondrocytes (100x). (B) Toluidine blue stain was performed to stain the phenotype in OA chondrocytes (200x). (C) Immunohistochemical (IHC) was used to identify

chondrocytes using aggrecan antibody (400x). (D) The human OA chondrocytes were cultured with or without IL-1 β (10ng/ml) for 0h, 24h, 48h and 72h. The morphology and aggrecan degradation of chondrocyte images were observed using an inverted microscope (100x). (E) Chondrocytes were cultured with or without 10ng/ml IL-1 β for 0h, 24h, 48h and 72h followed by CCK-8 assay analysis. (F) The protein levels of ADAMTS-4/-5 and TAK1 in cultured human OA chondrocytes with or without 10ng/ml IL-1 β for 0h, 24h, 48h and 72h were determined using Western blot assays.

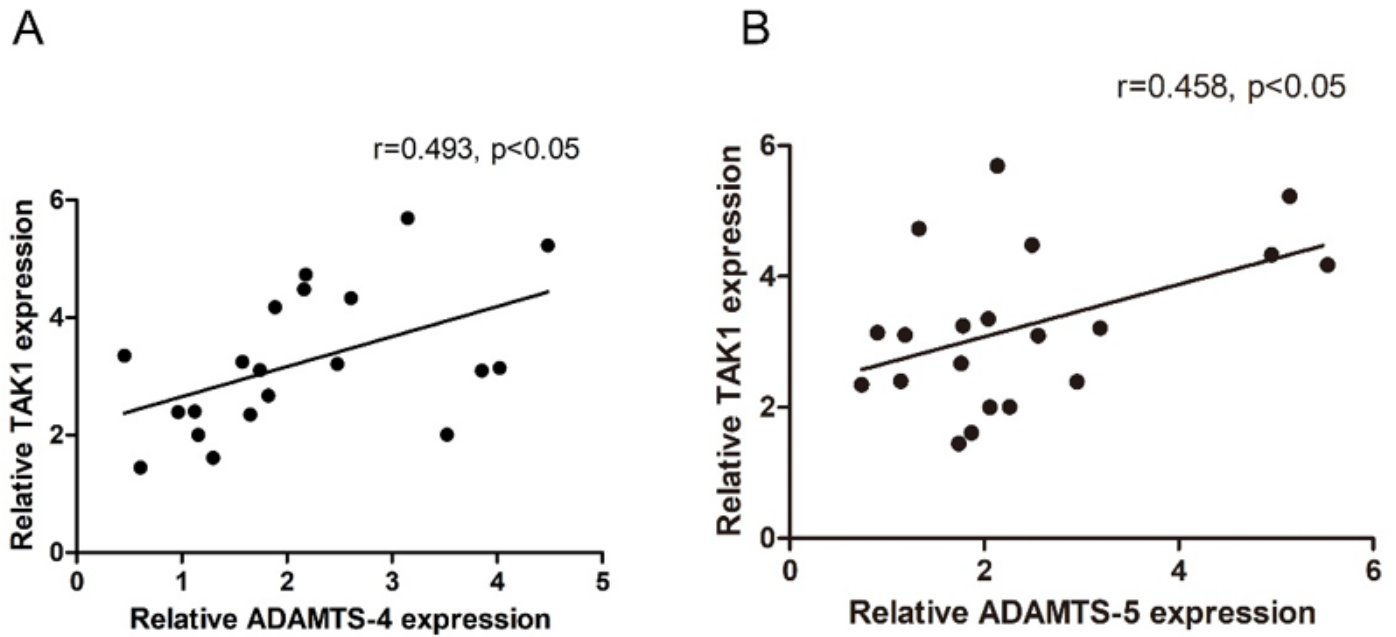


Figure 4

Figure 4

The respective correlation between ADAMTS-4/-5 and TAK1. The respective correlation between ADAMTS-4/-5 and TAK1 in OA cartilage tissues using Spearman's rank correlation analyzes.