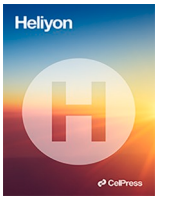
Heliyon 9 (2023) e17595

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



HTRA1 from OVX rat osteoclasts causes detrimental effects on endplate chondrocytes through NF-κB

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ARTICLE INFO

Keywords:
Osteoclast
Endplate cartilage
Estrogen deficiency
HTRA1
NF-κB

ABSTRACT

Endplate osteochondritis is considered one of the major causes of intervertebral disc degeneration (IVDD) and low back pain. Menopausal women have a higher rate of endplate cartilage degeneration than similarly aged men, but the related mechanisms are still unclear. Subchondral bone changes, mainly mediated by osteoblasts and osteoclasts, are considered an important reason for the degeneration of cartilage. This work explored the role of osteoclasts in endplate cartilage degeneration, as well as its underlying mechanisms. A rat ovariectomy (OVX) model was used to induce estrogen deficiency. Our experiments indicated that OVX significantly promoted osteoclastogenesis and anabolism and catabolism changes in endplate chondrocytes. OVX osteoclasts cause an imbalance between anabolism and catabolism in endplate chondrocytes, as shown by a decrease in anabolic markers such as Aggrecan and Collagen II, and an increase in catabolic markers such as a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) and matrix metalloproteinases (MMP13). Osteoclasts were also confirmed in this study to be able to secrete HtrA serine peptidase 1 (HTRA1), which resulted in increased catabolism in endplate chondrocytes through the NF-κB pathway under estrogen deficiency. This study demonstrated the involvement and mechanism of osteoclasts in the anabolism and catabolism changes of endplate cartilage under estrogen deficiency, and proposed a new strategy for the treatment of endplate osteochondritis and IVDD by targeting HTRA1.

1. Introduction

Endplate osteochondritis is a spinal condition that involves the degeneration of endplate cartilage, which can cause low back pain. Endplate osteochondritis is closely related to intervertebral disc degeneration (IVDD), and may even contribute to the initiation and development of IVDD [1,2]. Unfortunately, endplate osteochondritis has not received the same level of attention as IVDD. The pathophysiological mechanism underlying endplate osteochondritis is far from completely understood.

Menopause is an inevitable physiological stage for women. Menopause-related diseases are important clinical problems. The

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https://doi.org/10.1016/j.heliyon.2023.e17595
Received 2 March 2023; Received in revised form 19 June 2023; Accepted 21 June 2023
Available online 22 June 2023

prevalence of endplate cartilage degeneration in menopausal women is significantly higher than that in their male peers [3–5]. Previous studies have found that after ovariectomy (OVX), vertebral bone mass in rats is decreased, accompanied with a decrease in collagen II protein expression and calcification of the endplate cartilage [6,7]. Studies on knee articular osteoarthritis have found that subchondral bone changes are an important reason for the degeneration of articular cartilage in the early stage of estrogen deficiency [8,9]. Both endplate cartilage and articular cartilage belong to hyaline cartilage and form a functional complex with subchondral bone to bear stress. Considering the structural and functional similarities between endplate cartilage and articular cartilage, endplate osteochondritis may share some similar causes for cartilage degeneration with osteoarthritis. Endplate osteochondritis may have causes similar to that of osteoarthritis. There may be a causal relationship between endplate cartilage degeneration and adjacent bone loss after OVX.

Osteoclasts are bone-resorbing cells that play a key role in bone remodeling. Excessively activated osteoclasts under estrogen deficiency are an important cause of bone loss [10]. Studies have revealed that osteoclasts can promote hypertrophic differentiation and extracellular matrix degradation of knee articular chondrocytes by secreting exosomes [11,12]. Few studies have focused on the crosstalk between osteoclasts and chondrocytes in the vertebral endplate. Osteoclasts were confirmed to be able to secrete HtrA serine peptidase 1 (HTRA1) [13,14], which was found to be increased in the region of degraded knee osteoarthritic cartilage [15]. HTRA1 was first discovered in fibroblasts and is involved in some degenerative diseases such as osteoarthritis, IVDD, macular degeneration and cancer [16–18]. The present study assumed that osteoclasts under estrogen deficiency could lead to an imbalance between anabolism and catabolism in endplate chondrocytes, an important pathological characteristic of cartilage degeneration by secreting HTRA1. The elucidation of the involvement and mechanisms of osteoclasts in the anabolism and catabolism changes of endplate chondrocytes under estrogen deficiency could expedite the development of new and effective therapies for the treatment of menopausal endplate osteochondritis and IVDD.

2. Materials and methods

2.1. Primary cell sources

Primary cells were derived from OVX rats and Sham rats. The animal experiments were described in our previous study [19]. Six-month-old female Sprague-Dawley (SD) rats weighing 290 g–330 g were purchased from Peking University Health Science Center. The rats were raised 2–3 per cage under a 12 h light/dark cycle with controlled temperature (25 °C), humidity (49%–55%), and free access to food and water. The animal experiments were approved by the Animal Ethics Committee of Peking University (ethics approval number: LA2019209). Sham rats underwent bilateral laparotomy, and OVX rats underwent bilateral OVX operation. Nine weeks after surgery, rats were euthanized with carbon dioxide.

2.2. Isolation of rat primary BMMs and endplate chondrocytes

For isolation of bone marrow-derived macrophages (BMMs), the femurs from Sham and OVX rats were collected, and both ends were cut off with a rongeur under sterile conditions. The marrow cavity was rinsed with a sterile syringe containing phosphate buffer. The rinsed liquid was then centrifuged at 800 rpm for 5 min, and the cells were resuspended in α-MEM medium (HyClone, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, USA), 1% penicillin/streptomycin (HyClone, USA) and 30 ng/mL M-CSF (Peprotech, USA). Three days later, mature rat primary BMMs were obtained.

For chondrocyte isolation, endplate cartilage from the spinal segments of Sham rats was detached, cut into small pieces, and then digested with 0.2% type II collagenase (Solarbio, China) at 37 °C for 4–6 h. The endplate chondrocytes were washed and resuspended in DMEM (HyClone, USA) containing 10% fetal bovine serum (Thermo Fisher, USA) and 1% penicillin/streptomycin (HyClone, USA) in a cell incubator under 5% CO_2 at 37 °C. Every 2–3 days, the culture medium was replaced, and cells were passaged after reaching 70–80% confluence. All experiments were performed using passage 1 cells.

2.3. Osteoclast differentiation and identification

For osteoclast differentiation, BMMs were treated with 30 ng/mL M-CSF (Peprotech, USA) and 50 ng/mL RANKL (Peprotech, USA) for 6 days. For TRAP staining, cells were fixed with 4% paraformaldehyde at room temperature for 20 min, and then stained with a TRAP staining kit (Solarbio, China) according to the manufacturer's instructions.

2.4. Treatment with osteoclast-conditioned medium

Osteoclast-conditioned medium (OC-CM), which contained secreted proteins from osteoclasts in OVX and Sham rats, was used to treat Sham endplate chondrocytes to explore the mechanism stimulating changes in Sham endplate chondrocytes. The supernatants incubated with osteoclasts from OVX or Sham rats for 2 days were harvested and centrifuged at a speed of 1000 g for 5 min. The supernatants were diluted at a ratio of 1:1 with fresh culture medium and added to chondrocytes for 3 days. A blank control group using pure medium was applied in the study. Pure medium refers to regular culture medium for endplate chondrocytes that was not collected from supernatant from Sham or OVX osteoclasts.

2.5. Treatment with rHTRA1

Recombinant rat HTRA1 (rHTRA1, CUSABIO, China) was used to treat endplate chondrocytes. Different concentrations of 0, 5, 10 and 20 ng/mL were applied to investigate dosage effects. The intervention lasted for 3 days.

2.6. Treatment with NF-κB antagonist and agonist

The NF-κB agonist Betulinic acid (MCE, USA) and antagonist Sulfasalazine (MCE) were used to test the role of NF-κB in changes in endplate chondrocytes due to HTRA1. The treatments were performed at the concentration of 10 μM for both Betulinic acid and Sulfasalazine for 3 days.

2.7. CCK-8 assay

Cells were inoculated into 96-well plates at a density of 4×10^3. Then, 100 μL complete culture medium containing 10% CCK-8 solution (Meilunbio, China) was added to each well. After incubating in a 37 °C incubator for 1 h, the absorbance at 450 nm was detected using a microplate tester (Molecular Devices, USA).

2.8. RT-qPCR

Total RNA was extracted using TRIzol (Thermo Fisher Scientific, USA). The concentration and purity of RNA were measured by a spectrophotometer (Thermo Fisher Scientific, USA). 1 μg RNA was used for reverse transcription, and the final reaction volume was 20 μL. ABI QuantStudio5 (Thermo Fisher Scientific, USA) was used for real-time reverse transcription polymerase chain reaction (RT-PCR). The relative transcription levels were standardized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are listed in Table 1.

2.9. ELISA

Culture medium of osteoclasts from the Sham group and OVX group was collected and centrifuged at 1500 rpm for 10 min. An ELISA kit (Meimian, China) was used to detect the expression level of HTRA1 in the supernatant according to the manufacturers' instructions.

2.10. Western blot

Cells were lysed with RIPA buffer (Applygen, China) on ice and the concentration of protein was determined by a BCA kit (Beyotime, China) following the manufacturers' protocol. Nuclear proteins were extracted using a nuclear and cytoplasmic protein separation kit (Beyotime, China) according to the manufacturer's instructions. After being denatured with an equal volume of sample buffer (Bio-Rad, USA) at 100 °C for 5 min, protein samples were separated on 10% SDS–PAGE gels (Beyotime, China) and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membranes were blocked with 5% skim milk powder for 1 h and incubated with primary antibodies at 4 °C overnight. The membranes were washed with TBST, and incubated with HRP-conjugated secondary antibodies for 2 h. An ECL kit (Boster, China) was used to visualize the immune complexes, and the protein levels were analyzed with ImageJ software (NIH, USA). All the antibodies used in this study are listed in Table 2.

2.11. Statistical analysis

Every experiment was independently repeated three times, and the data are presented as the means ± SDs. GraphPad Prism 8.0.1 (GraphPad, USA) was utilized for statistical analysis. Student's t-test was used to assess the statistically significant difference between two groups. For three or more groups, one-way analysis of variance (ANOVA) with Tukey's correction was used. For each analysis, a p value < 0.05 was considered statistically significant.

Table 1
Primer sequences for real-time PCR.

Gene	Forward	Reverse
GAPDH	GCAAGTTCAACGGCACAG	GCCAGTAGACTCCACGACA
Nfatc1	TGCTCCTCCTCCTGCTGCTC	CGTCTTCCACCTCCACGTCG
Ctsk	TCCTCAACAGTGCAAGCGAA	CCAGCGTCTATCAGCACAGA
Aggrecan	CCACTGGAGAGGACTGCGTAG	GGTCTGTGCAAGTGATTCGAG
Col2a1	CTCAAGTCGCTGAACAACCA	GTCTCCGCTCTTCCACTCTG
Adamts5	CACGACCCTCAAGAACTTTTGC	TCACATGAATGATGCCCACATAA
Mmp13	CAAGATGTGGAGTGCCTGATGTGG	GCGTGTGCCAGAAGACCAGAAG
HtrA1	TTATCGCTGATGTGGTGGAG	AATGAATCCTGACCCACTCG

Table 2
The antibodies used in this study.

Antibodies	Source	Catalog number
Rabbit anti-Collagen II	Abcam	ab34712
Rabbit anti-HTRA1	Immunoway	YN2041
Rabbit anti-β-actin	Proteintech	20536-1-AP
Mouse anti-Aggrecan	Abcam	ab3773
Rabbit anti-ADAMTS5	Abcam	ab41037
Rabbit anti-MMP13	Abcam	ab39012
Rabbit anti-p65	CST	8242
Alexa Fluor 488-labeled Goat Anti-Rabbit IgG (H + L)	Beyotime	A0423
HRP-labeled Goat Anti-Rabbit IgG (H + L)	Beyotime	A0208
HRP-labeled Goat Anti-Mouse IgG (H + L)	Beyotime	A0216

3. Results

3.1. Enhanced osteoclastogenesis and osteoclast-secreted HTRA1 after OVX

Osteoclast formation was confirmed through TRAP staining. Osteoclastogenesis through BMMs from OVX rats was significantly greater than that from Sham rats (Fig. 1A). The expression of the osteoclastogenesis-related genes *Nfatc1* and *Ctsk* was significantly higher in OVX osteoclasts than in Sham osteoclasts (Fig. 1B). Compared with BMMs from Sham rats, BMMs from OVX rats showed significantly higher cell viability (Fig. 1C). ELISA analysis indicated a significant increase in the release of HTRA1 from osteoclasts in OVX rats (Fig. 1D). Moreover, increased HTRA1 expression at the protein and mRNA levels was observed in OVX osteoclasts (Fig. 1E and F).

3.2. Changes in endplate chondrocytes after OVX

The viability of OVX endplate chondrocytes was significantly lower than that of Sham endplate chondrocytes (Fig. 2A). The expression of the anabolism-related markers, *Col2a1* and *Aggrecan,* were significantly decreased in OVX endplate chondrocytes compared with Sham endplate chondrocytes (Fig. 2B). The expression of catabolism-related markers, a disintegrin and metalloproteinase with thrombospondin motifs 5 (*Adamts5*) and matrix metalloproteinases (*Mmp13*), were significantly increased in OVX endplate chondrocytes (Fig. 2B). Similar trends were observed at the protein level (Fig. 2C).

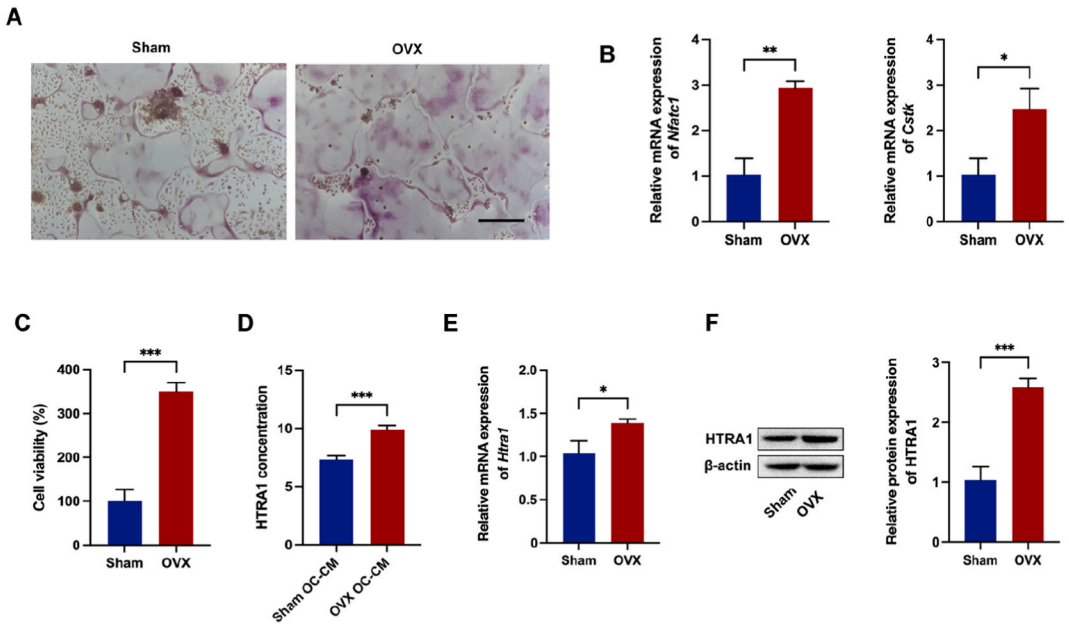

Fig. 1. Promoted osteoclastogenesis and HTRA1 release due to estrogen deficiency. (A) TRAP staining of BMMs on day 6. (B) The mRNA expression of Nfatc1 and Ctsk in BMMs on day 6. (C) CCK-8 assay of BMMs at 72 h. (D) ELISA of HTRA1 concentration in OVX OC-CM and Sham OC-CM. (E) The mRNA expression of HTRA1 in BMMs after treatment with M-CSF (30 ng/mL) and RANKL (50 ng/mL) for 6 days. (F) The protein expression of HTRA1 in BMMs after treatment with M-CSF (30 ng/mL) and RANKL (50 ng/mL) for 6 days. Refer to the Supplementary Fig. 1 for full scans of blots. Error bars represent the mean ± SD, n = 3. *p < 0.05, **p < 0.01, ***p < 0.001. Scale = 100 μm. BMMs = bone marrow-derived macrophages.

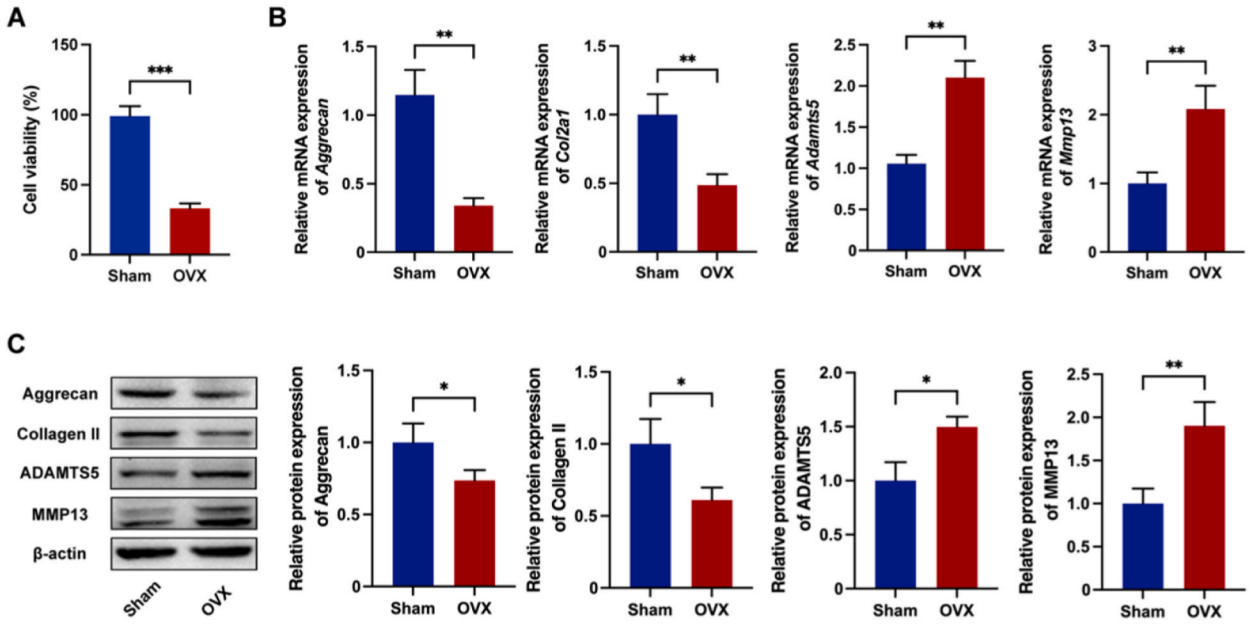

Fig. 2. Pathologic changes in endplate chondrocytes induced by estrogen deficiency. (A) CCK-8 assay of endplate chondrocytes at 72 h. (B) The mRNA expression of *Aggrecan, Col2a1, Adamts5* and *Mmp13* in endplate chondrocytes. (C) The protein expression of Aggrecan, Collagen II, ADAMTS5 and MMP13 in endplate chondrocytes. Refer to the Supplementary Fig. 1 for full scans of blots. Error bars represent the mean ± SD, n = 3. *p < 0.05, **p < 0.01, ***p < 0.001.

3.3. Changes in endplate chondrocytes with OC-CM treatment

To explore whether osteoclasts were involved in the process of endplate cartilage degeneration after estrogen decline, Sham endplate chondrocytes were treated with OC-CM or pure medium. The expression of *Col2a1* and *Aggrecan* was significantly decreased and the expression of *Adamts5* and *Mmp13* was significantly increased in endplate chondrocytes after treatment with Sham OC-CM, compared with pure medium. Compared with Sham OC-CM, OVX OC-CM treatment further decreased the expression of *Col2a1* and *Aggrecan*, and enhanced the expression of *Adamts5* and *Mmp13* in endplate chondrocytes (Fig. 3).

3.4. The effects of HTRA1 on endplate chondrocytes

Sham endplate chondrocytes were treated with HTRA1 recombinant protein (rHTRA1), and the expression of anabolism- and catabolism-related markers was detected by real-time PCR and Western blot analysis. The results showed that rHTRA1 decreased the expression of Collagen II and Aggrecan, and increased the expression of ADAMTS5 and MMP13 in a dose-dependent manner (Fig. 4A and B).

3.5. Involvement of NF-κB in the changes in endplate chondrocytes mediated by HTRA1

NF-κB has been shown to be a key regulator of chondrocyte catabolism, promoting the expression of matrix-degrading enzymes that contribute to cartilage destruction. Increased levels of nuclear p65, an indicator of NF-κB pathway activation, were observed in endplate chondrocytes after OVX OC-CM treatment (Fig. 5A). Compared with Sham OC-CM treatment, OVX OC-CM treatment enhanced the expression of Adamts5 in endplate chondrocytes (Fig. 5B). Intervention with rHTRA1 and Betulinic acid, an agonist of NF-κB pathways, also enhanced the expression of Adamts5 in endplate chondrocytes. Treatment with Sulfasalazine, an antagonist of NF-κB pathways, retarded the increases in Adamts5 induced by OVX OC-CM (Fig. 5B).

4. Discussion

Metabolism of both bone tissue and degeneration of the endplate cartilage can be changed by estrogen withdrawal. Whether abnormal bone remodeling due to estrogen deficiency can affect endplate cartilage degeneration deserves investigation. The answer to the question might shed light on the diagnosis and treatment of endplate osteochondritis due to menopause. The present study examined the alterations in osteoclasts and endplate chondrocytes in OVX rats, determined the influence of osteoclasts from OVX rats on endplate chondrocytes, and explored the possible mechanisms.

The cell viability of BMMs increased significantly in OVX rats, and estrogen deficiency promoted the differentiation of osteoclast precursors into osteoclasts. These results demonstrated that estrogen deficiency promoted osteoclastogenesis. Other studies have also

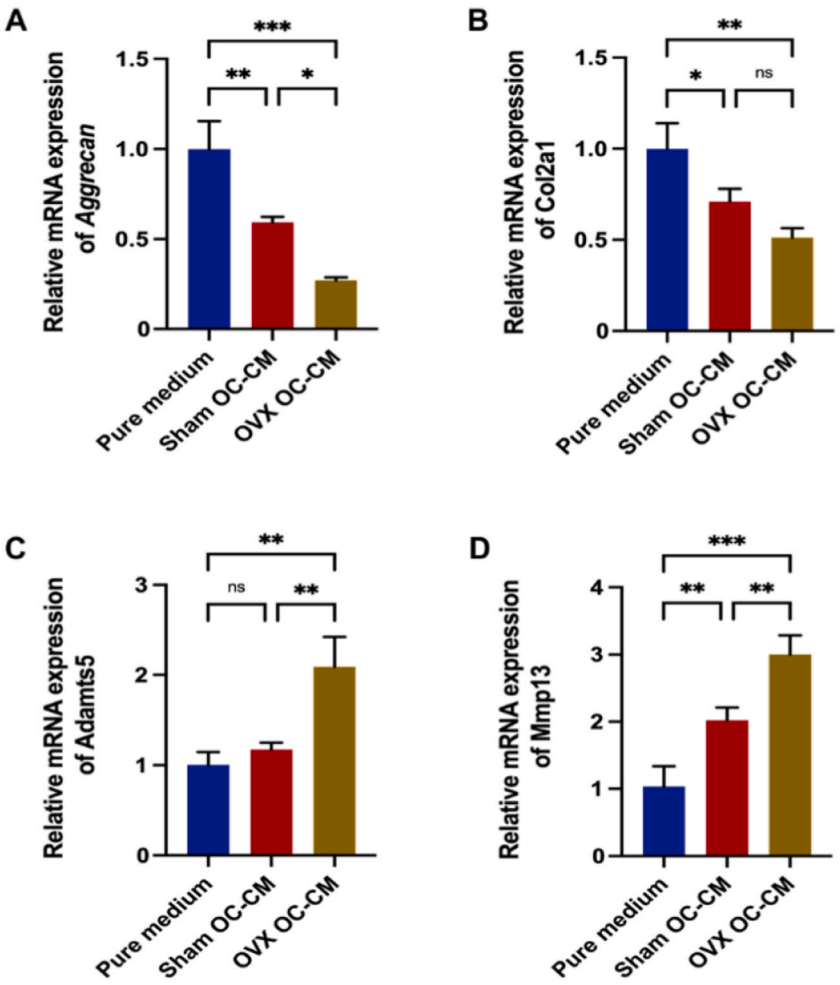

Fig. 3. OVX rat osteoclasts contribute to changes in endplate chondrocytes. The mRNA expression of (A) *Aggrecan*, (B) *Col2a1*, (C) *Adamts5* and (D) *Mmp13* in endplate chondrocytes under different conditioned media. Error bars represent the mean ± SD, n = 3. *p < 0.05, **p < 0.01, ***p < 0.001, ns = 'not significant'. OC-CM = osteoclast-conditioned medium.

reported that estrogen can negatively regulate osteoclast differentiation [20,21]. This study demonstrated increased mRNA expression of *Nfatc1* and *Ctsk* in OVX osteoclasts. *Nfatc1* is specific to osteoclasts, and *Ctsk* is expressed by both osteoclastic and nonosteoclastic skeletal cells [22]. The expression of *Ctsk* and *Nfatc1* has been reported to increase significantly with osteoclast differentiation, and both are used to evaluate osteoclastogenesis [23]. The abnormally increased activities of osteoclasts might be the critical sources by which bone tissue affects endplate cartilage.

Cartilage degeneration is attributed to the imbalance between anabolism and catabolism of chondrocytes. During cartilage degeneration, the expression of extracellular matrix (ECM) molecules, such as type II collagen and sulfated proteoglycans, the essential components of chondrocyte anabolism, is suppressed. Meanwhile, the expression of catabolism-related factors are increased, such as ADAMTs and MMPs family [24–26]. This is the first study to reveal the metabolic changes in endplate chondrocytes after OVX and provide the evidence that estrogen deficiency can promote endplate cartilage degeneration at the cellular level. The results confirmed that endplate chondrocytes in OVX rats experienced increased catabolism and decreased anabolism. Similar effects of estrogen deficiency were also observed in articular cartilage and intervertebral discs in OVX rats/mice [27–29]. Those studies demonstrated that estrogen deficiency can lead to pathological changes in articular chondrocytes through the AMPK/Foxo3a pathway and pathological changes in nucleus pulposus cells through the Wnt/β-catenin pathways [27–29]. Thus, the estrogen level has similar effects on chondrocytes at different locations and different types of cells through different pathways.

The interaction between osteoclasts and endplate chondrocytes may be one of the main causes for the degeneration of endplate cartilage under estrogen deficiency. To our knowledge, there have been no studies investigating the effects of osteoclasts on endplate chondrocytes. In articular cartilage, osteoclasts can interact with chondrocytes within the bone-cartilage unit [30]. A reduced trabecular bone mass that is correlated with defects in chondrogenesis and chondrocyte differentiation was observed in mice lacking Beclin1, an autophagy-related protein [31]. The present study observed increased viability of osteoclasts and decreased viability of endplate chondrocytes, implying that activated osteoclasts might be harmful to endplate chondrocytes. Treatment with OVX OC-CM

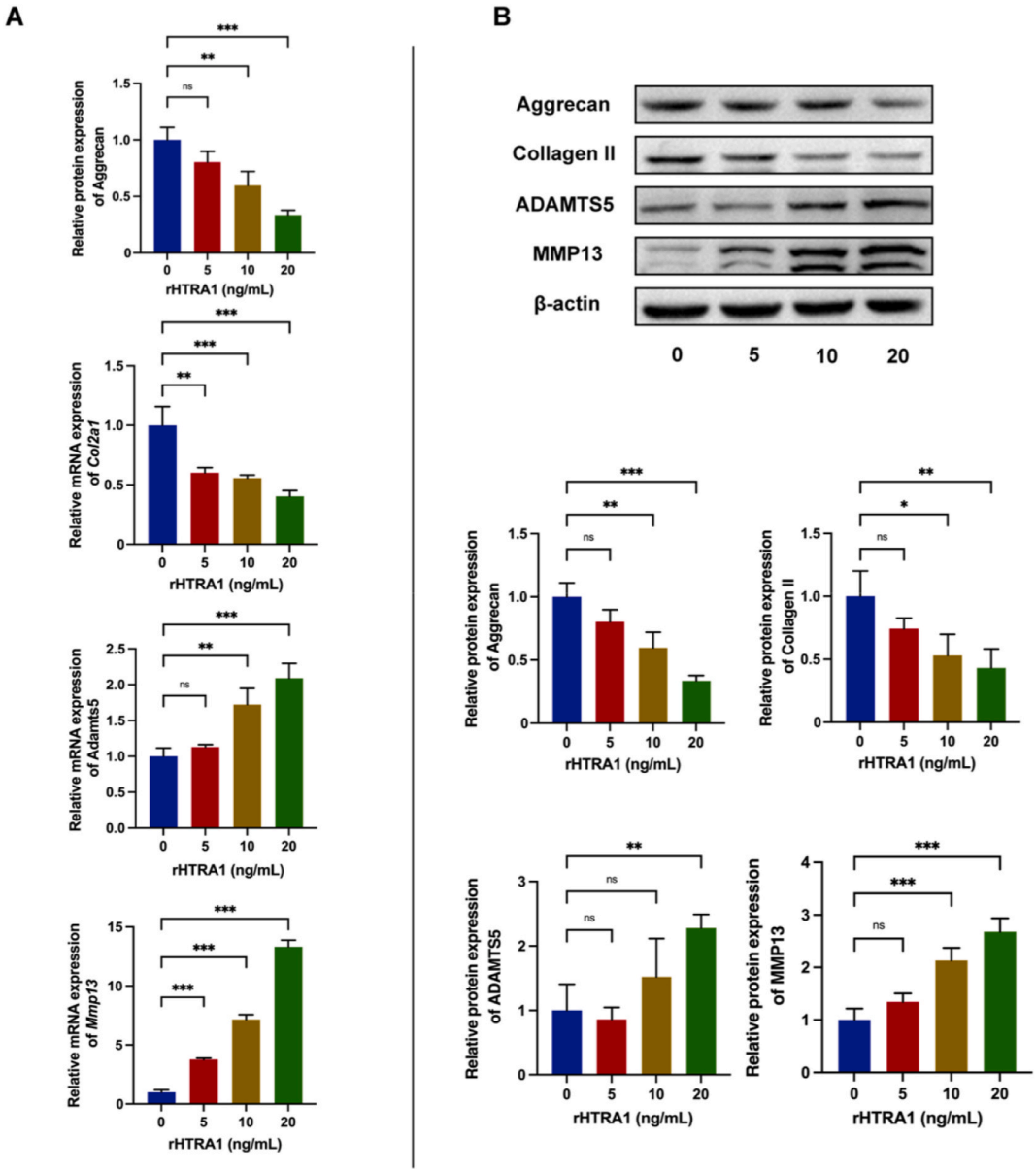

Fig. 4. The role of HTRA1 in changes in endplate chondrocytes. (A) The mRNA expression of *Aggrecan, Col2a1, Adamts5* and *Mmp13* in endplate chondrocytes after treatment with different doses of rHTRA1 (0, 5, 10 or 20 ng/mL). (B) The protein expression of Aggrecan, Collagen II, ADAMTS5 and MMP13 in endplate chondrocytes after treatment with different doses of rHTRA1 (0, 5, 10 or 20 ng/mL). Refer to the Supplementary Fig. 1 for full scans of blots. Error bars represent the mean ± SD, n = 3. *p < 0.05, **p < 0.01, ***p < 0.001, ns = 'not significant'. BMMs = bone marrow-derived macrophages. rHTRA1 = HTRA1 recombinant protein.

could stimulate inflammatory changes in endplate chondrocytes, including decreased anabolism and increased catabolism. This study chose OC-CM treatment, rather than a transwell co-culture system. The transwell system is ideal for investigating interaction between two types of cells in one system, while conditioned-medium treatment is able to highlight the effects and mechanisms of the conditioned cells (osteoclasts) on the target cells (endplate chondrocytes).

The relevant mechanisms remain unknown. There are multiple ways for osteoclasts to influence endplate chondrocytes. Osteoclasts can secrete exosomes which include different types of miRNAs to act on chondrocytes to induce chondrocyte hypertrophy and differentiation [11]. Mature osteoclasts can also release growth factors and other complexes to regulate the metabolic activities of articular chondrocytes during bone tissue dissolution [32,33]. As mentioned before, HTRA1 attracted our attention because HTRA1 can be secreted by osteoclasts [13,14], and its expression increases in degraded osteoarthritic knee cartilage [15]. Based on the results from ELISA of the osteoclast supernatant, the present study observed increased HTRA1 secretion in the osteoclasts after estrogen withdrawal. Similar results were observed in the mRNA and protein levels of HTRA1 in osteoclasts. HTRA1 is a serine protease that is

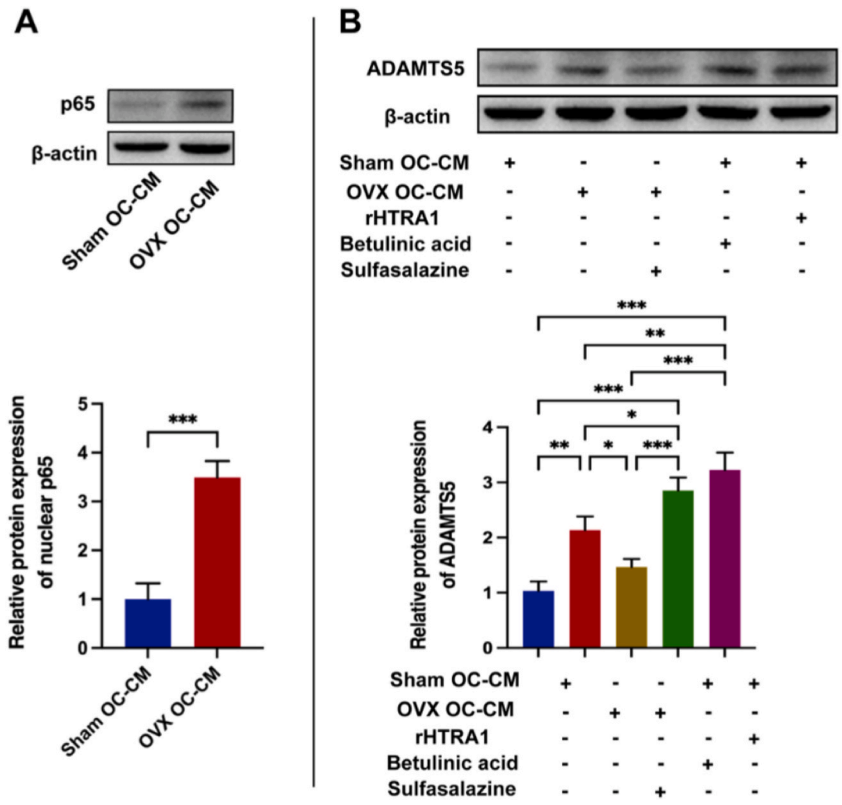

Fig. 5. The involvement of NF-κB in changes in endplate chondrocytes mediated by HTRA1. (A) The levels of nuclear p65 in endplate chondrocytes after treatment with OVX OC-CM or SHAM OC-CM. (B) The protein expression of ADAMTS5 in endplate chondrocytes after treatment with SHAM OC-CM or OVX OC-CM in the presence of rHTRA1, Betulinic acid or Sulfasalazine. Refer to the Supplementary Fig. 1 for full scans of blots. Error bars represent the mean ± SD, n = 3. *p < 0.05, **p < 0.01, ***p < 0.001. OC-CM = osteoclast conditioned medium. rHTRA1 = HTRA1 recombinant protein.

involved in a variety of biological processes [34–36]. During RANKL-induced osteoclast formation, the HTRA1 concentration is enhanced in a time-dependent manner [14], which might explain the HTRA1 increase after OVX.

Since no study has reported the effects of HTRA1 on endplate chondrocytes, we designed in vitro experiments using rHTRA1 treatment. Even a relatively low dose of rHTRA1 was detrimental to endplate chondrocytes, with increased ADAMTS5/MMP13 and decreased Aggrecan/Collagen II expression at both the mRNA and protein levels. In knee joints, HTRA1 was reported to stimulate MMPs and destroy the extracellular matrix of articular cartilage in early osteoarthritis [37]. The detrimental roles of HTRA1 were also observed in articular chondrocytes and nucleus pulposus cells [38–40]. Therefore, HTRA1 might be treated as an inflammatory marker of degeneration in articular cartilage, nucleus pulposus and endplate cartilage.

The involvement of NF-κB during the regulation of HTRA1 was investigated in this study. NF-κB can promote the expression of osteoarthritis-related proinflammatory and destructive mediators [41,42]. Studies in the literature have demonstrated that activation of NF-κB can stimulate the decomposition and inhibit the synthesis of extracellular matrix in endplate cartilage [43,44]. The activation of NF-κB might directly or indirectly target MMP13, leading to endplate cartilage degeneration [44]. Moreover, NF-κB can induce chondrocyte catabolism by regulating MMP3, MMP13 and ADAMTS5 [45,46]. HTRA1 upregulated the expression of MMP13, ADAMTS5 and the NF-κB axis in endplate chondrocytes. Our results also showed that increased NF-κB can induce chondrocyte catabolism by regulating MMP13 and ADAMTS5 [45,46]. Intervention of Sham endplate chondrocytes with the agonist and inhibitor of NF-κB verified the role of NF-κB during HTRA1 regulation in endplate chondrocytes.

Several limitations are worth mentioning. First, although this study focused on the influence of OVX osteoclasts on endplate chondrocytes, the two types of cells actually interacted with each other. The effects of chondrocytes with endplate osteochondritis on osteoclasts also deserve future investigation. Second, the present study emphasized the effects of OVX osteoclasts on endplate chondrocytes, and only provided primary results about the mechanism. More comprehensive experimental design is required to better reveal and confirm the mechanism. In addition, this study is an in vitro study at large. All the results might not be the same as those in vivo condition.

5. Conclusions

Estrogen deficiency can promote osteoclastogenesis. Changes in osteoclasts under estrogen deficiency contribute to pathological changes in endplate chondrocytes. Increased secretion of HTRA1 from OVX osteoclasts plays an important role by regulating NF-κB. Osteoclasts might be a potential target for the protection of endplate chondrocytes under estrogen deficiency.

Author contribution statement

Longting Chen, Yiming Zhong, Shang Sun: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Zihuan Yang, Haofeng Hong, Da Zou: Performed the experiments; Wrote the paper.
Chunli Song: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Weishi Li: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Huijie Leng: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Dr. Huijie Leng was supported by National Natural Science Foundation of China {12172011, 11872076}.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17595.

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