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RESEARCH ARTICLE

SIRT1 Inhibits Bovine Cartilage ECM Degradation and Apoptosis by Activating Nrf2/HO-1 and Inhibiting NF-κB Pathways

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ABSTRACT

This study explored the potential mechanism of SIRT1 in bovine osteoarthritis (OA). *In vivo* experiments evaluated the severity of damage to bovine tarsal cartilage tissue through pathological staining and Mankin scoring. When compared with healthy bovines, cartilage in OA-affected bovines displayed ECM degradation and increased Mankin scores ($P<0.01$). In the cartilage of OA-affected cattle, the protein expression of inflammatory factors (TNF-α, IL-1β, COX-2) and ECM degradation markers (MMP-3, MMP-13) was increased ($P<0.05$), the expression levels of key pathway proteins (including SIRT1, nuclear Nrf2 and HO-1) were decreased ($P<0.05$), and the expression of nuclear p65 and p-IκBα was upregulated ($P<0.05$). *In vitro* studies, we developed a bovine primary chondrocyte inflammation model using 10ng/mL IL-1β. IL-1β treatment significantly upregulated the expression levels of ECM degradation markers in chondrocytes ($P<0.01$). Transmission electron microscopy revealed mitochondrial ultrastructural damage, and flow cytometry indicated an apoptosis rate of 38.1%. ML334 treatment significantly enhanced the levels of nuclear Nrf2, HO-1, and Bcl-2 ($P<0.05$), while simultaneously decreasing nuclear p65, BAX, Cleaved caspase-3, and ECM degradation markers ($P<0.05$). JSH-23 treatment successfully downregulated nuclear p65 and ECM degradation marker levels ($P<0.05$). Treatment with the SIRT1 agonist SRT2104 not only increased the level of SIRT1, nuclear Nrf2, and HO-1 ($P<0.05$), but also effectively attenuated the expression of nuclear p65 ($P<0.05$). This experiment strongly supports the claim that SIRT1 effectively protects bovine cartilage ECM from degradation and apoptosis through a dual mechanism of action (activation of the Nrf2/HO-1 pathway and inhibition of the NF-κB pathway).

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INTRODUCTION

Lameness in dairy cows is undoubtedly one of the most prevalent and devastating health challenges in the global dairy farming industry (Urban-Chmiel *et al*., 2024). It has been reported that in the past 30 years, among nearly 4,000 dairy herds, the average proportion of dairy cows with a lameness level of 3 or above (grades 1-5) was 5.1-45%, and in some individual herds, the highest percentage could reach 88% (Thomsen *et al*., 2023). It not only seriously affects animal welfare but can also lead to decreased milk production and culling, thereby reducing the overall performance of dairy cows. A high incidence (85%, 23/27) of grossly visible degenerative arthritic lesions was observed in culled dairy bulls upon post-mortem examination (Heinola *et al.,* 2013).

In bovines over 5 years old, OA is one of the common causes of lameness, clinically manifested as joint swelling and slowly progressive lameness (AKGÜN *et al.,* 2025). Articular cartilage is mainly composed of chondrocytes, ECM, and non-collagen proteins, which maintain cartilage health and normal joint function. As OA progresses, the production of proteoglycans and collagen fibers in cartilage ECM decreases, leading to cartilage degeneration (Xian *et al.,* 2024; Hao *et al.,* 2025). Our earlier work (Ma *et al.,* 2022) revealed that rat osteoarthritis is characterized by

suppressed expression of HO-1 and Nrf2, accompanied by elevated levels of reactive oxygen species (ROS) and inflammatory factors. Notably, elevated ROS further activated nuclear NF-κB, leading to subsequent cartilage degradation. Given that SIRT1 is a multifunctional deacetylase capable of broadly regulating the activity of multiple transcription factors, including NF-κB and Nrf2 (Yang *et al.,* 2022), it is an important candidate molecule linking oxidative stress and inflammatory responses. Through these actions, SIRT1 can regulate chondrocytes' various physiological functions (such as apoptosis, autophagy, inflammation, and oxidative stress) (Dvir-Ginzberg and Steinmeyer 2013; Liao *et al.,* 2024; Ren *et al.,* 2024). Furthermore, numerous studies demonstrated that SIRT1 regulates multiple physiological processes and disease pathogenesis in bovines, including embryonic development, bovine fertility, mastitis, and inflammatory responses (Idrees *et al.,* 2022; Fan, *et al.,* 2023; Zhang *et al.,* 2024; Worku and Verma, 2024). However, its regulatory role in OA-affected bovine remains unclear.

Although the SIRT1, NF-κB, and Nrf2 molecular pathways have been extensively investigated in rodents (Widowati *et al.,* 2022; Meng *et al.,* 2024), chondrocytes from different species may differ in their response sensitivity to inflammatory factors (e.g., IL-1β), gene regulatory efficiency, and the activity of key pathway proteins. This study innovatively deciphers the regulatory network of SIRT1, NF-κB, and Nrf2 in OA-affected bovine, validating their efficacy in bovine chondrocyte physiology. This study aims to verify the following hypothesis: SIRT1 alleviates the destruction of bovine cartilage ECM and apoptosis by coordinating the Nrf2/HO-1 (activation) and NF-κB (inhibition) dual pathway mechanisms, thereby slowing the pathological progression of bovines with OA.

MATERIALS AND METHODS

Experimental animals: Tarsal and carpal joints of 6 bovines (4.2±0.4 years old) were obtained from a slaughterhouse. Clinical examination and pathological staining results showed that 3 bovines had early OA, and the other 3 were healthy. All chondrocytes used in the experiment were extracted from the tarsal or carpal joints. All experimental procedures in this study were reviewed and formally approved by the Animal Ethics Committee of Northeast Agricultural University (NEAUEC2024 03 48).

Experimental grouping: Six bovines were used as the experimental animal model and divided into a control group and an OA group.

Lameness scoring system: The gait of each group of bovines was assessed using lameness rating scale (Sprecher *et al.,*1997).

Cartilage collection and storage: Cartilage tissue collected from the tarsal joints of bovine was decalcified, embedded, sectioned, and stained with Safranin O-Fix Green and Hematoxylin-Eosin (H&E).

Isolation and culture of bovine articular chondrocytes: Cartilage tissue was digested with 0.25% trypsin solution (Gibco, USA) and 0.2% type II collagenase solution (Gibco). Cells were collected by centrifugation (1000rpm, 5min) and seeded in complete culture medium for incubation in a cell culture incubator.

Chondrocyte identification: Chondrocytes were fixed, washed, stained with toluidine blue (Solarbio, China), and then observed under a microscope (n=3 per group).

Immunofluorescence: Cells were seeded into confocal culture dishes (n=6 per group), and then fixed, washed, permeabilized, and incubated with antibodies. 200μL DAPI dye (1:1000, Beyotime, Cat. No.:C1002) was added. Observation and recording were performed using a fluorescence microscope (Leica).

Bovine chondrocyte growth curve assay: Bovine chondrocytes (1×10^5 cells/well) were seeded into cell culture plates in hexaplicate. Day 0 was designated as seeding. On days 0, 1, 3, 5, 7, 9, and 11, cell counting was carried out and chondrocyte growth curves were plotted (n=6 per group).

CCK8 assay: The IL-1β treatment group (MedChemExpress, USA) was prepared with five concentration gradients: 0, 2.5, 5, 10, and 20ng/mL. Each group was prepared in hexaplicate (n=6 per group). After 24h of culture, CCK8 solution (APExBIO, USA) was added. The absorbance was measured at 450nm.

Bovine chondrocyte inflammation model: Passaged cell in the logarithmic growth phase were maintained in culture until they reached 70-80% confluency. Culture medium (serum-free) containing different concentrations of IL-1β was introduced, followed by culturing it in an incubator (37°C, 5% CO_2) for 24h.

Transmission Electron Microscopy: Chondrocytes (n=6 per group) were fixed with 2.5% glutaraldehyde (4°C, 24h), followed by stepwise dehydration (ethanol), washing (PBS), embedding (resin), sectioning, and staining (2% aqueous uranyl acetate,1h). Samples were observed using an H-7650 electron microscope (Hitachi, Japan).

Apoptosis Detection: The apoptosis rate of bovine chondrocytes was detected using apoptosis detection kit (Abbkine, China), with each group repeated 6 times (n=6).

Western blot: The approach was consistent with that of previous studies (Ma *et al.*, 2022). PVDF membranes (n=3) were processed using enhanced chemiluminescence (Abbkine, China). Protein signal images were acquired using an imaging system (Tannon, China). In the experiment, antibodies TNF-α, IL-1β and COX-2 were purchased from Wanleibio, China. Antibodies MMP-13, SIRT1, Nrf2, HO-1, p65, p-IκBα, BAX, Bcl-2 and Lamin B1 were purchased from Affinity Biosciences, China. Antibodies GAPDH and Cleaved Caspase-3 were purchased from Abclonal, China.

Statistical Methods: All experimental data, derived from three independent replicates, are summarized as mean ± SD. We utilized SPSS 22.0 for all computational

procedures. Initial assessments for normal distribution and equal variance were performed using Shapiro-Wilk and Levene's tests. Subsequently, a one-way ANOVA followed by Tukey's post-hoc analysis was applied to identify significant differences, which were defined by a $P<0.05$.

RESULTS

Clinical symptoms and lameness score

Histopathological characteristics of articular cartilage in bovines with OA: Fig. 1A shows that the lameness score of bovines with OA was greater than that of healthy bovines ($P<0.01$). Fig. 1B shows representative histopathological images of the tarsal joints of each group. In the Control group, the cartilage structure was intact; the cartilage surface appeared smooth; chondrocytes were evenly distributed, and under Safranin O staining, collagen fibers exhibited a regular blue-purple reticular pattern (Fig. 1B, yellow arrow), thereby preserving the mechanical stability of the cartilage tissue. In the OA group, slight wrinkles and irregularities were observed on the cartilage surface, the blue color of the cell nuclei became sparse, chondrocytes were lost, the number decreased, the arrangement was disordered, and cells were missing in some areas. The staining intensity of the cartilage ECM was diminished; the red color faded and became uneven, indicating the substantial loss of acidic proteoglycan in the ECM during OA. In areas with more severe lesions, the cartilage lacuna was abnormal in morphology, part of the cartilage lacuna was enlarged, and the number of chondrocytes in it decreased, and vacuolation and pyknosis occurred (Fig. 1B, yellow box). The collagen fiber structure was more obviously exposed, showing a broken and disordered state (Fig. 1B, yellow arrow), and clustering of chondrocytes near the subchondral bone area was also seen. The Mankin score of the OA group was greater than that of the control group ($P<0.01$) (Fig. 1C), indicating that the articular cartilage tissue of the OA group bovines was severely damaged.

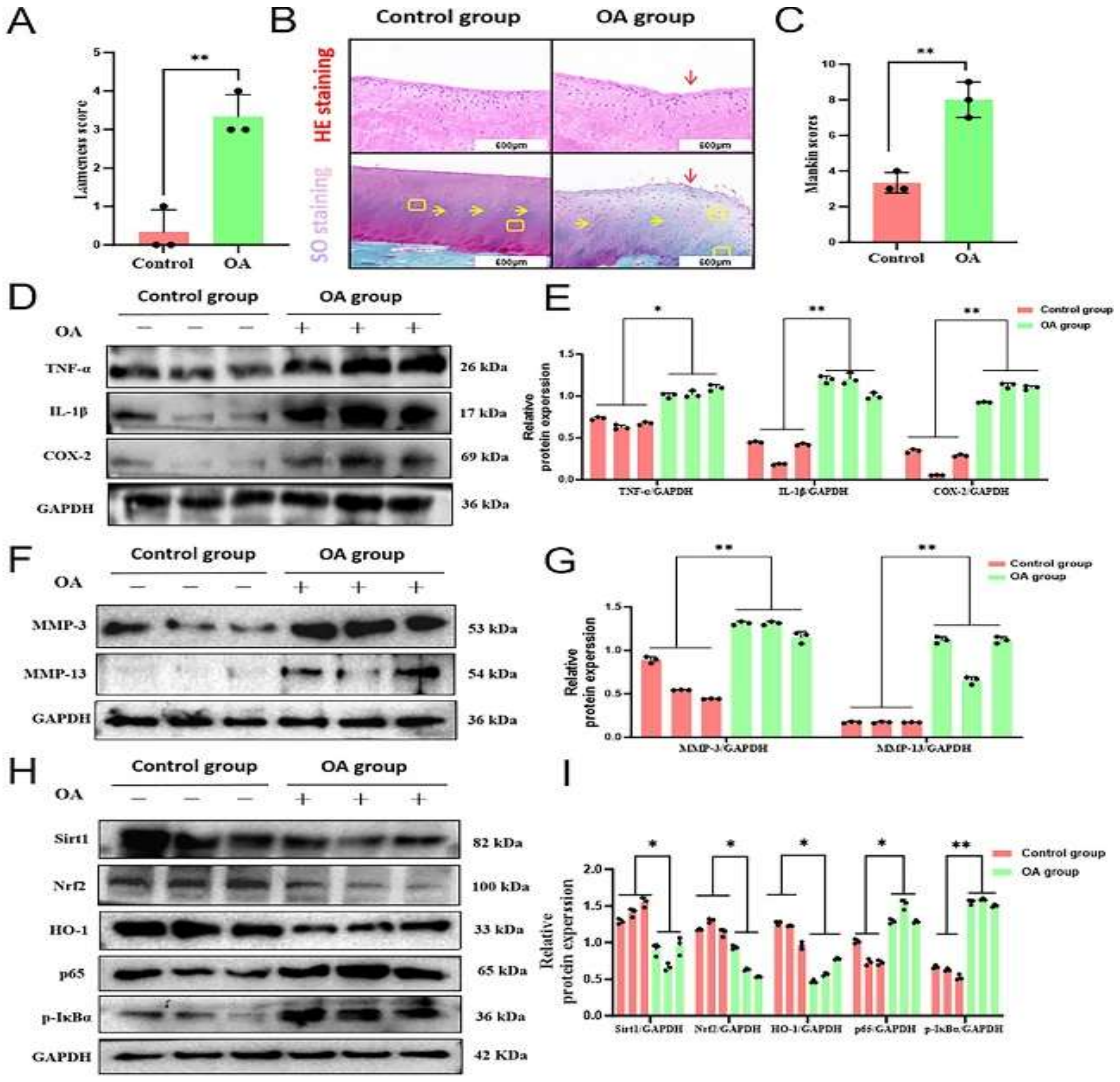

Fig. 1: Pathological changes, ECM degradation, and alterations of key pathway proteins in the cartilage of a bovine with OA. (A) The lameness score of bovines in the OA group was significantly increased ($P<0.05$). (B, C) HE staining and safranin O staining of OA bovine cartilage indicated cartilage damage, with an elevated Mankin score ($P<0.05$). (D, I) Western Blot analysis showed that the levels of inflammatory and ECM degradation markers were increased in OA cartilage, while the expression of SIRT1, Nrf2, and HO-1 was decreased, and the expression of p65 and p-IκBα was increased ($P<0.05$). *$P<0.05$, **$P<0.01$.

Changes in inflammatory and ECM degradation markers in cartilage of OA bovines: Western blot analysis (Fig. 1D-G) showed significantly elevated levels of TNF-α ($P<0.05$), IL-1β, COX-2, MMP-3, and MMP-13 ($P<0.01$) proteins in the cartilage tissue of lame bovines. These differences in protein expression levels molecularly demonstrate the presence of inflammation (TNF-α, IL-1β, COX-2) and ECM degradation (MMP-3, MMP-13) in the affected joints of lame bovines, thus molecularly validating that the lameness in this study was due to OA.

Changes of key proteins in SIRT1, Nrf2/HO-1 and NF-κB pathways in cartilage of bovines with OA: Western blot analyses (Fig. 1H and I) demonstrated that, in comparison to the control group, the expression levels of Nrf2 and HO-1, critical proteins in the Nrf2/HO-1 pathway, were notably reduced in OA-affected bovines ($P<0.05$), whereas the levels of p65 ($P<0.05$) and p-IκBα ($P<0.01$), critical proteins in the NF-κB pathway, were elevated. Notably, the protein level of SIRT1 was significantly reduced ($P<0.05$). This indicates that the Nrf2/HO-1 pathway is suppressed and the NF-κB pathway is activated in bovines with OA, and that SIRT1 participates in the onset and progression of OA.

Observation and identification of chondrocyte morphology in bovine: The isolation and culture process of primary chondrocytes in bovine is shown in Fig. 2A. Bovine chondrocytes were spindle-shaped and exhibited a healthy polygonal morphology typical of primary chondrocytes (Fig. 2B). Toluidine blue staining found that the nuclei of chondrocytes were stained dark, the cytoplasm was light, and the proteoglycans in the cartilage ECM were also stained with toluidine blue, confirming that they were chondrocytes (Fig. 2C). We further detected the level of Type II collagen marker protein in bovine second-generation chondrocytes by immunofluorescence. The results showed specific green fluorescence with blue fluorescence was visible in the nuclei (Fig. 2D).

Chondrocyte growth curve: The growth curve of bovine P2 chondrocytes was drawn. According to the cell counting results, the cell proliferation rate increased continuously after 1 day of culturing and reached a peak at 7 days of culture (Fig. 2E). Therefore, the second-generation bovine chondrocytes were selected for subsequent experiments.

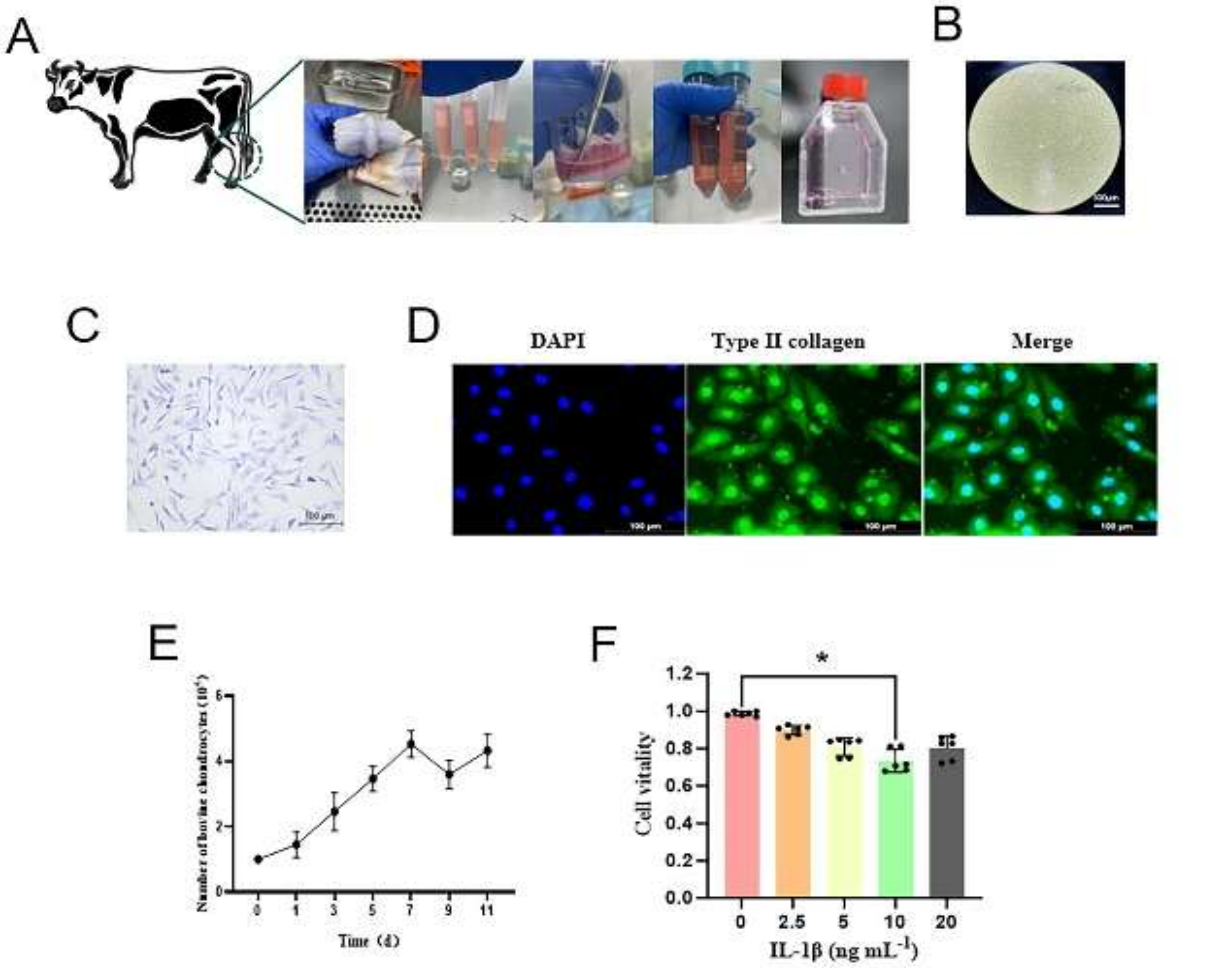

Fig. 2: Isolation, culture, and identification of bovine chondrocytes. (A) Flow chart of primary chondrocyte isolation and culture. (B) Observation of the morphological structure of bovine chondrocytes under a microscope. (C) Toluidine blue staining of chondrocytes. (D) Immunofluorescence detection of Type II collagen marker protein levels in second-generation bovine chondrocytes. (E) Within 7 days of culture of second-generation bovine chondrocytes, the number of bovine chondrocytes showed an increasing trend. (F) CCK-8 method detected that 10ng/mL of IL-1β concentrations significantly reduced the activity of chondrocytes after 24 hours of treatment. *$P<0.05$, **$P<0.01$.

Screening of IL-1β dosage: Among the IL-1β concentrations of 2.5, 5, 10, and 20ng/mL, chondrocyte activity showed a significant decrease at 10ng/mL after 24h of treatment (P<0.05) (Fig. 2F). Therefore, 10ng/mL IL-1β was used as the induction dose for the *in vitro* inflammatory model of bovine chondrocytes.

IL-1β accelerates chondrocyte ECM degradation, mitochondrial damage and apoptosis: To evaluate the impacts of IL-1β on bovine chondrocyte ECM breakdown and mitochondrial function, we measured the protein abundances of MMP-3 and MMP-13 in IL-1β-exposed chondrocytes via Western blotting and immunofluorescence assays (Fig. 3A and B). Our findings indicated that, in comparison to the control group, the protein levels of MMP-3 and MMP-13 in IL-1β-exposed chondrocytes were markedly elevated (P<0.01), indicating that IL-1β can promote the breakdown of chondrocyte ECM. The nuclear morphology of bovine chondrocytes in the control group was basically normal, the bovine chondrocyte membrane was smooth (yellow dotted line), the cytoplasm contained abundant mitochondria, and the mitochondrial cristae were parallel and intact (red box). In the IL-1β group, the bovine chondrocyte membrane was tortuous (yellow dotted line), the mitochondria in the cytoplasm were swollen, the mitochondrial matrix became rarefaction, vacuolization occurred, the double membrane was damaged and the cristae were broken, lysosomes appeared, and the mitochondria were damaged (Fig. 3C). We observed mitochondrial apoptosis directly using transmission electron microscopy. We additionally investigated the abundances of critical apoptosis-associated proteins in bovine chondrocytes. Western blotting data (Fig. 3D) demonstrated that, in comparison with the control group, the abundances of Bax and Cleaved Caspase-3 proteins were notably elevated in the IL-1β group (P<0.01), whereas the abundance of Bcl-2 protein was markedly reduced (P<0.05). These findings, from mitochondrial morphology to molecular level, validated that IL-1β can induce mitochondrial apoptosis in bovine chondrocytes.

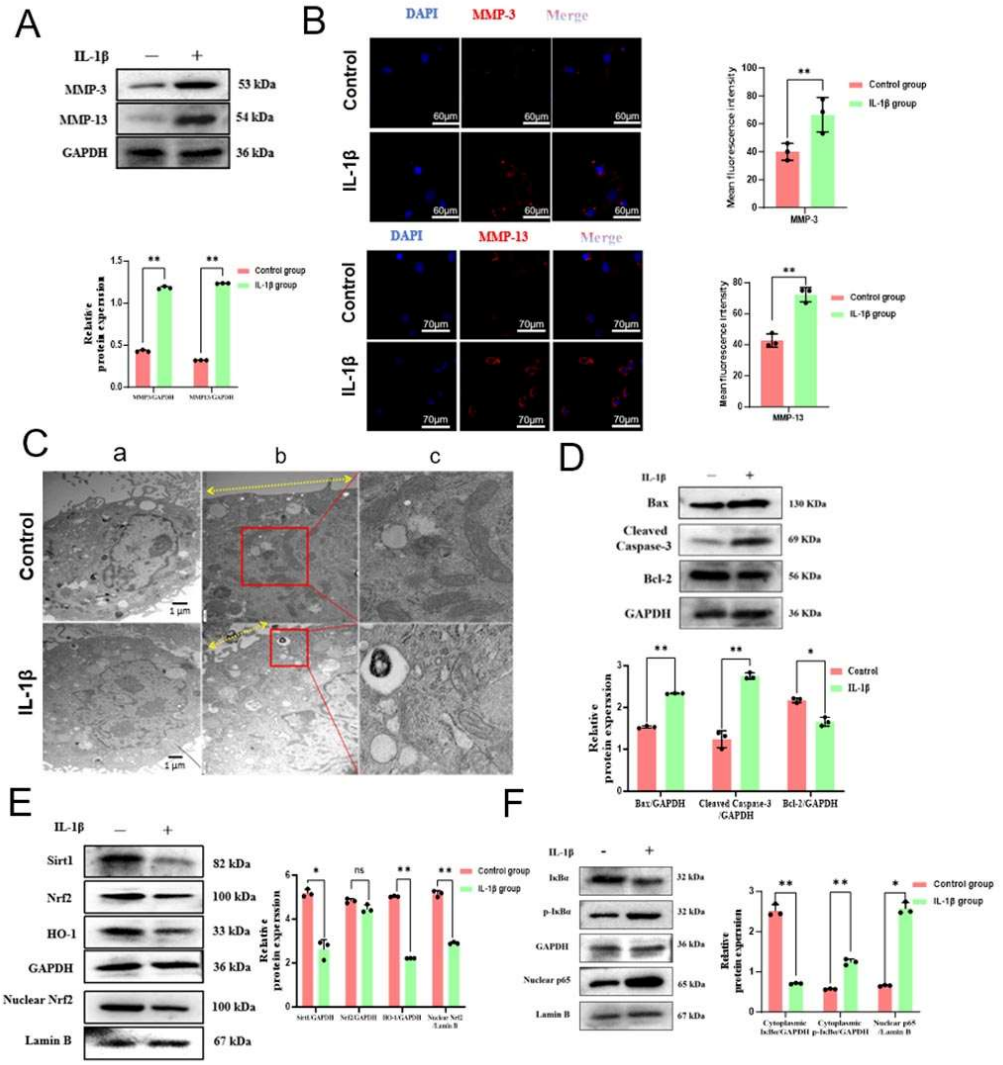

Fig. 3: Changes in SIRT1, Nrf2/HO-1, and NF-κB pathway-related proteins in bovine chondrocytes. (A, B) Western blot and immunofluorescence showed increased levels of ECM degradation markers (P<0.01). (C) Transmission electron microscopy showed chondrocyte morphology and mitochondrial damage. (D-F) Western blot showed increased apoptosis-related levels, with significant changes in key proteins of the SIRT1, Nrf2/HO-1, and NF-κB pathways (P<0.05). *P<0.05, **P<0.01.

IL-1β regulates chondrocyte dual pathways via SIRT1: To investigate the activity of the Nrf2/HO-1 and NF-κB pathways in the IL-1β group, we employed Western blotting to measure the protein abundances of critical proteins in these two pathways in bovine chondrocytes following IL-1β intervention. Our findings indicated (Fig. 3E and F) that, in comparison to the control group, the protein levels of SIRT1, HO-1, nuclear Nrf2, and IκBα in chondrocytes of the IL-1β group were markedly reduced (P<0.05), while the protein levels of p-IκBα and nuclear p65 were notably elevated (P<0.05). This suggests that 10ng/mL IL-1β suppressed the SIRT1 protein and Nrf2/HO-1 pathways in chondrocytes, while simultaneously stimulating the NF-κB pathway, thus facilitating degenerative changes in cartilage.

SIRT1 inhibits bovine cartilage ECM degradation and apoptosis through a dual pathway: To further verify the regulatory roles of the Nrf2/HO-1 and NF-κB pathways, we intervened in bovine chondrocytes with the Nrf2/HO-1 pathway agonist ML334 (100μM; MedChemExpress) and the NF-κB inhibitor JSH-23 (20μM; MedChemExpress). The results showed that the levels of nuclear Nrf2, HO-1, and Bcl-2 proteins were significantly decreased in the IL-1β group (P<0.05), while the levels of nuclear p65, MMP-3, MMP-13, BAX, and Cleaved Caspase-3 proteins were significantly increased (P<0.05), and ML334 significantly reversed these changes (Fig. 4A and D). The levels of nuclear p65, MMP-3, and MMP-13 proteins were significantly increased in the IL-1β group (P<0.05), and JSH-23 significantly reversed this result (Fig. 4G and H). Furthermore, immunofluorescence analysis revealed reduced Nrf2 nuclear translocation and increased p65 nuclear translocation in the IL-1β group compared to the control group. However, the addition of ML334 increased Nrf2 nuclear translocation while decreasing p65 nuclear translocation (Fig. 4C and F).

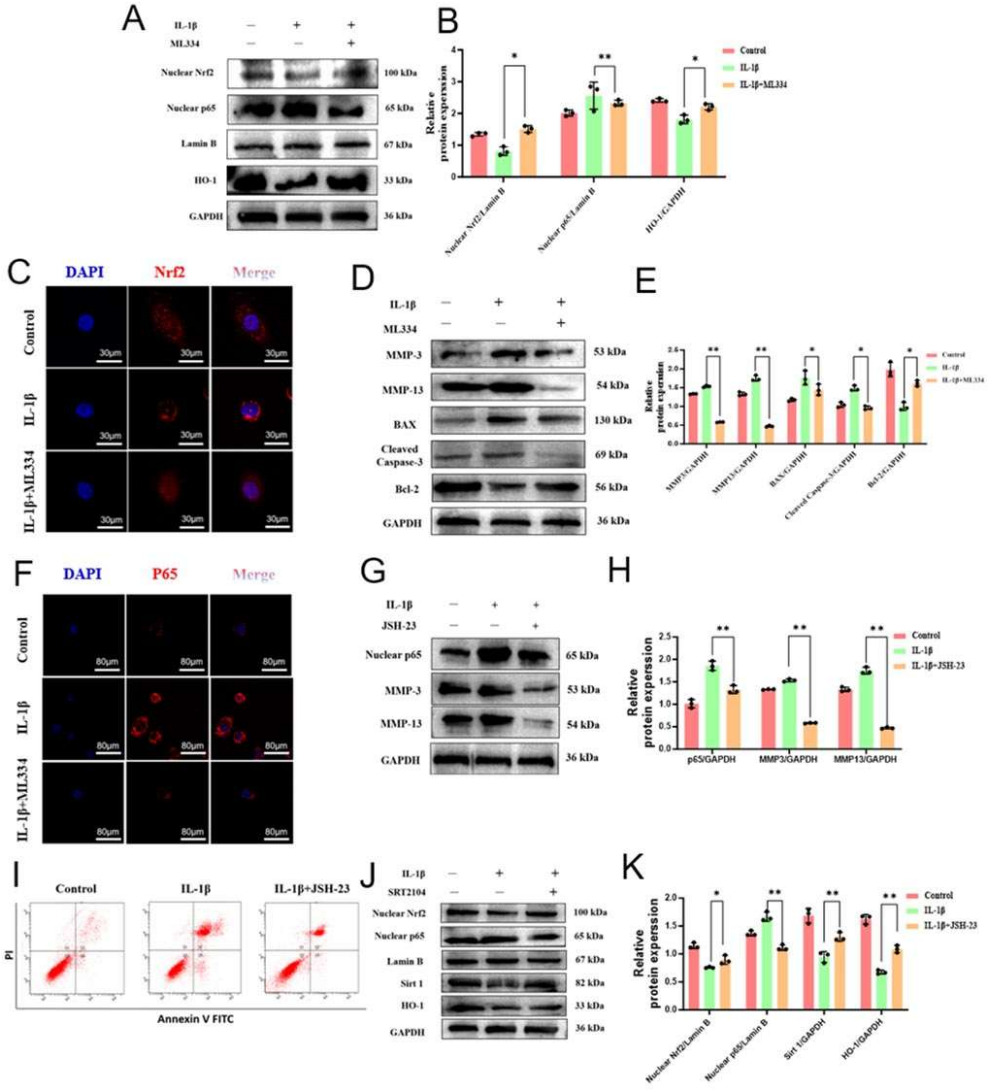

Fig. 4: SIRT1 activates Nrf2/HO-1 and inhibits the NF-κB pathway, thereby inhibiting ECM degradation and apoptosis in bovine chondrocytes. (A-F) After ML334 intervention, significant changes were observed in ECM degradation markers, key proteins of the Nrf2/HO-1 and NF-κB pathways, and apoptosis levels (P<0.05). (E-I) After JSH23 intervention, significant changes were observed in ECM degradation markers, key proteins of the NF-κB pathway, and apoptosis levels (P<0.05). (J, K) After SRT2104 intervention, significant changes were observed in key proteins of the SIRT1, Nrf2/HO-1, and NF-κB pathways (P<0.05), *P<0.05, **P<0.01.

In addition, the apoptosis rate was 7.9% in the control group, increased to 38.1% in the IL-1β group, and decreased by 18.2% after JSH-23 intervention compared to the IL-1β group (Fig. 4I). To clarify whether the regulation of the Nrf2/HO-1 and NF-κB pathways in chondrocytes induced by IL-1β is related to SIRT1, we intervened in chondrocytes with the SIRT1 agonist SRT2104 (20μM, MedChemExpress) to observe the levels of key proteins in the pathway. The results showed that the levels of nuclear Nrf2, SIRT1, and HO-1 proteins were significantly decreased in the IL-1β group ($P<0.05$), while the levels of nuclear p65 protein were significantly increased. SRT2104 was able to significantly reverse these changes (Fig. 4J and K).

DISCUSSION

In this study, healthy bovines and those with cartilage degeneration were screened by lameness scores and pathological tests. Further, by finding that inflammatory and cartilage metabolism markers were significantly upregulated in OA affected bovines' cartilage, we found that this may be related to the SIRT1-mediated dual pathway. To further verify its potential regulatory mechanism, we used IL-1β to establish an *in vitro* inflammatory model of bovine chondrocytes and a target pathway blocking/activation model. The experimental findings demonstrated that SIRT1 can suppress chondrocyte apoptosis and ECM breakdown by stimulating the Nrf2/HO-1 pathway and suppressing the NF-κB signaling cascade.

The Nrf2 and NF-κB pathways are key regulatory hubs in the development of bovine inflammatory diseases. Multiple studies have shown that these pathways play important roles in mediating oxidative stress and inflammation: During the development of crack lesions in bovine tissue, PI3K/Akt can activate the NF-κB pathway, promoting the development of local tissue inflammation (Zhai *et al.,* 2025). The melatonin-dependent Nrf2 pathway confers bovine oocytes' resistance to β-hydroxybutyrate-induced oxidative stress damage (Zhang *et al.,* 2025). Chlorogenic acid alleviates heat stress-induced damage to bovine mammary epithelial cells by simultaneously upregulating Nrf2 and inhibiting NF-κB-mediated NLRP3 inflammasome activation (Huang *et al*. 2025). Aucubin has been found to have bidirectional regulatory capacity in a bovine endometrial cell inflammation model, reducing both NF-κB p65 and IκB phosphorylation while simultaneously activating the Keap1/Nrf2 pathway (Gao *et al.,*2021). This evidence strongly supports strategies for intervening in and treating bovine inflammatory diseases by targeting the Nrf2/NF-κB dual pathway. To understand the progression mechanisms of OA, many researchers have explored the key roles of chondrocyte apoptosis and ECM degradation. In this pathological process, the Nrf2/HO-1 and NF-κB pathways, due to their central regulatory role, have become a major focus of research in this field (Hodgkinson *et al.,* 2022; Du *et al.,* 2024; Li *et al.,* 2024). In a bovine chondrocyte model, studies have found that hyaluronic acid treatment induces Akt phosphorylation in bovine chondrocytes, whereas inhibiting Akt activity using siRNA prevents hyaluronic acid-mediated Nrf2 accumulation demonstrating that hyaluronic acid regulates Nrf2 by activating Akt to reduce ROS production (Onodera *et al.,* 2015). In a bovine nasal cartilage explant model, sulforaphane was shown to inhibit matrix-degrading proteases and cartilage destruction via NF-κB (Davidson *et al.,* 2013). Researchers have found that asiaticoside can both activate Nrf2/HO-1 signal transduction to enhance the antioxidant and anti-apoptotic effects of chondrocytes and inhibit NF-κB activity to eliminate OA inflammation by interfering with p65 DNA binding (Luo *et al.,* 2022). Li also observed that these two pathways synergistically regulate each other, jointly participating in oxidative stress, inflammatory response, and chondrocyte ECM regulation during OA (Li *et al.,* 2024). Although existing studies suggest that either the Nrf2 or NF-κB pathway can regulate the progression of OA, the crosstalk mechanism between these two pathways has not been demonstrated in bovines with OA, and *in vivo* validation in bovine remains lacking. We found that abnormal expression of key pathway proteins (including SIRT1, Nrf2, and HO-1) was first observed in bovines with OA. To clarify the specific mechanism of action, bovine chondrocytes were induced with IL-1β; this treatment suppressed the Nrf2/HO-1 pathway while activating the NF-κB pathway. Intervention with ML334 resulted in only minimal nuclear translocation of p65, accompanied by reduced downstream ECM metabolism and apoptosis. This indicates that in bovine chondrocytes, Nrf2 can inhibit the NF-κB pathway and exert targeted regulation of p65 nuclear translocation. Additionally, intervention with JSH-23 led to decreased expression of the ECM metabolism markers compared to IL-1β treatment alone, demonstrating that NF-κB can directly regulate ECM metabolism in bovine chondrocytes.

The SIRT1 pathway and oxidative stress response play critical roles in activating inflammatory responses in bovine. SIRT1 has been reported as a target of melatonin in improving bovine endometrial epithelial cell damage (Li *et al.,* 2024). Studies have also found that salidroside improves inflammatory injury in bovine hepatocytes by activating the AMPK/SIRT1 pathway (Li *et al.,* 2025). In acute inflammatory states, the NF-κB pathway amplifies the inflammatory response by activating glycolysis. Conversely, SIRT1 inhibits OA by deacetylation of the p65 subunit in the NF-κB complex, thereby inhibiting NF-κB activity and enhancing oxidative metabolism (Kauppinen *et al.,* 2013). Several studies have shown that there is an important connection between SIRT1 and Nrf2. SIRT1 can enhance of Nrf2, activate HO-1, and prevent oxidative damage. Furthermore, recent studies have found that SIRT1, as a key upstream regulatory molecule, can simultaneously regulate NF-κB and Nrf2. In a cisplatin-induced kidney injury model, decreased SIRT1 protein expression led to increased NF-κB acetylation, promoting its entry into the cell nucleus and amplifying renal inflammation, while Nrf2 and the expression of its downstream proteins. However, this phenomenon could be reversed under the action of theaflavins (Alanazi *et al.,*2025). To investigate how SIRT1 alleviates ECM degradation and apoptosis in bovine degenerative arthritis through the NF-κB and Nrf2/HO-1 cascade, we treated with the SIRT1 agonist SRT2104 in an IL-1β-induced inflammatory model of bovine chondrocytes to verify the relationship among the SIRT1, Nrf2/HO-1 and NF-κB pathways. We found that IL-1β-induced alterations in the

nuclear levels of key proteins (e.g., Nrf2 and p65) were reversed. This indicates that SIRT1 can target and regulate a dual pathway to resist ECM degradation and chondrocyte apoptosis in bovine OA patients.

Conclusions: In summary, SIRT1 inhibits ECM degradation and apoptosis in chondrocytes by activating the Nrf2/HO-1 pathway and inhibiting the NF-κB pathway.

Authors contribution: Tianwen Ma and Chengwei Wei conceptualized and designed the study and secured funding; Zongsheng Qiu conducted the research and collected the data; Tiantian Gao, Xiaoyan Guo, Jingjing Qi, Hui Bai, and Zhiheng Zhang analyzed and interpreted the data; and Yanan Li, Yuhui Ma, Jingjing Qi, and Jiantao Zhang visualized the data.

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Data availability: The data that support the findings of this study are available in the Materials and Methods, Results, and/or Supplemental Material of this article.

Ethics approval and consent to participate: All experimental procedures in this study were reviewed and formally approved by the Animal Ethics Committee of Northeast Agricultural University (NEAUEC2024 03 48).

Competing interests: The authors declare that they have no competing interests.

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