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ORIGINAL RESEARCH

Aucubin Alleviates Intervertebral Disc Degeneration by Repressing NF-κB-NLRP3 Inflammasome Activation in Endplate Chondrocytes

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Background: Intervertebral disc degeneration (IDD) is a prevalent degenerative disease and often recognized as the primary cause of lower back pain (LBP). Aucubin (Au) is a natural compound with anti-inflammatory properties in various diseases. The present study aimed to confirm the therapeutic effect of Au on IDD and explore its potential mechanism in vivo and in vitro.

Methods: The process of IDD was simulated using the lumbar spine instability (LSI) model. In vivo, the therapeutic effect of Au on LSI-induced mice was evaluated by micro-CT and histomorphometry. Additionally, immunohistochemistry was applied to detect the cartilage metabolism and inflammasome activation in endplate. In vitro, the cytotoxicity of Au on ATDC5 cells was detected by Cell Counting Kit-8 (CCK-8), and the biological effects of Au were evaluated by Quantitative Real-time PCR (qRT-PCR) and Western blotting.

Results: Micro-CT analysis showed that Au administration significantly alleviated LSI-induced disc volume narrowing and endplate cartilage degeneration, which was further supported by Alcian Blue Hematoxylin/Orange G (ABH/OG) staining. Immunohistochemistry results verified that Au could increase the expression of Col2α1 and Aggrecan, reduce the expression of Mmp-13, and attenuate the degradation of the endplate extracellular matrix (ECM). Mechanistically, we found that Au treatment, both in vivo and in vitro, significantly inhibited NF-κB-NLRP3 inflammasome activation in chondrocytes as determined by the decreased expression of p-P65, NLRP3, and Caspase-1.

Discussion: Taken together, our findings have demonstrated for the first time that Au treatment ameliorated the degeneration of cartilage endplates in IDD may by inhibiting NF-κB-NLRP3 inflammasome activation in chondrocytes and provided a potential candidate for the treatment of IDD.

Keywords: Aucubin, cartilage endplate, intervertebral disc degeneration, NF-κB-NLRP3, inflammasome

Introduction

Low back pain (LBP) is a frequent disease that suffered people of all ages, and its prevalence increases with age.[1,2] Although the etiology of LBP remains largely unknown, intervertebral disc degeneration (IDD) is recognized as its major cause and target for treatment.[3] IDD is a very complex pathological process that involves multiple stress-induced tissue injuries, and is also strongly associated with age.[4] The prevalence of IDD is increasing due to the aging of the

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population.[5] The intervertebral disc (IVD) is one of the elements comprising the lumbar spine. It is located between two adjacent vertebrae and consists of a central nucleus pulposus (NP) surrounded by a fibrous ring and cartilaginous endplate (CEP) envelope.[6] Besides, it is capable of cushioning pressure from different directions and is prone to degradation accordingly.[7] The process of IDD consists of gradual structural changes accompanied by an anabolic-catabolic imbalance.[8] Cartilage extracellular matrix (ECM) degradation, immune response, apoptosis and pyroptosis of IVD cells are major molecular changes during IDD, ultimately causing alterations in the level of tissue.[9–11] There are multiple causative factors for IDD pathology, and the search for more effective interventions is particularly urgent.

The CEP is a layer of hydrated cartilage tissue dominated by chondrocytes.[12] Intervertebral cartilage cushions mechanical stress and has an important protective role for the spine. Degradation of ECM and calcification of intervertebral cartilage are the main features of IDD. However, Inflammatory factors including tumor necrosis factor alpha (TNF-α), and matrix metalloproteinases (Mmps) released by chondrocytes during IDD accelerate ECM degradation.[13] Pyroptosis is a type of programmed cell death, but excessive pyroptosis tends to cause cellular as well as ECM abnormalities. Highly active NF-κB signaling contributes to programmed chondrocyte death such as apoptosis and pyroptosis, which exacerbates CEP calcification.[14,15] The NLR pyrin domain containing 3 (NLRP3) inflammasome is a multiprotein complex composed of NLRP3 and the inflammatory protease Caspase-1, in addition, hyperactivation of NLRP3 inflammasome implicated in a wide of inflammatory disorders.[16,17] Besides, the NLRP3 is downstream of the NF-κB signaling pathway, and activation of the NLRP3 inflammasome leads to IDD by promoting pyroptosis in NP cell, and ECM degradation in IVD.[18,19] Therefore, endplate chondrocyte pyroptosis due to upregulation of NLRP3 by NF-κB activation may be an important causative factor for ECM degradation and cartilage calcification, and effective intervention in chondrocyte pyroptosis may be critical for treating IDD.

Aucubin (Au) is a natural compound that is widely found in Chinese herbs such as Cortex Eucommiae, Radix et Rhizoma Dioscoreae, Plantago Ovata, and many others. In past research has revealed that Au has potent anti-inflammatory effects.[20–22] In particular, Au inhibits NF-κB activity in chondrocytes and alleviates inflammatory matrix degradation in articular cartilage.[23,24] Furthermore, some previous researches have demonstrated Au could inhibit TNF-α and IL-1β induced nucleus pulposus cell ECM degradation in vitro.[25] These findings indicate that Au may have a protective role against IVD in pathological conditions.

In summary, we presumed that Au could modify inflammation-mediated chondrocyte pyroptosis and be a potential therapeutic approach for IDD. In the present study, we emphasized the therapeutic effects of Au on IDD and investigated the potential mechanisms in vivo and in vitro.

Materials and Methods

Mice

Ten-weeks-old male C57BL/6J mice were purchased from the Laboratory Animal Research Center of Zhejiang Chinese Medical University (Hangzhou, China). All experimental protocols for mice were approved by the Animal Experimentation Ethics Committee of Zhejiang Chinese Medical University and complied with the guidelines of the Care and Use of Laboratory Animals (LY23H270005).

In vivo Studies

Aucubin (Aucubin, MUST BIO-TECHNOLOGY CAS: 479-98-1, purity ≥ 98.0%, ChengDu, China), an iridoid glycoside from natural plants, has antioxidative and anti-inflammatory bioactivities (Figure 1A). We employed lumbar spine instability (LSI) surgery to establish a mouse model of IDD.[26–28] As reported in previous studies,[29] mice were anesthetized with sodium pentobarbital, and the supraspinous ligament, the interspinous ligament, and the spinous processes between the lumbar 3 (L_3) to lumbar 5 (L_5) were excised with a machete. Meanwhile, mice subjected to sham surgery group were only resected for paraspinal muscle tissue. Finally, antibiotics were used to suture the incision to prevent infection. Mice in the Au group were injected intraperitoneally with Au (10 mg/kg/day) for 8 weeks. Equal amounts of normal saline as Vehicle were administered to mice in the sham-operated and model groups. Groups of mice

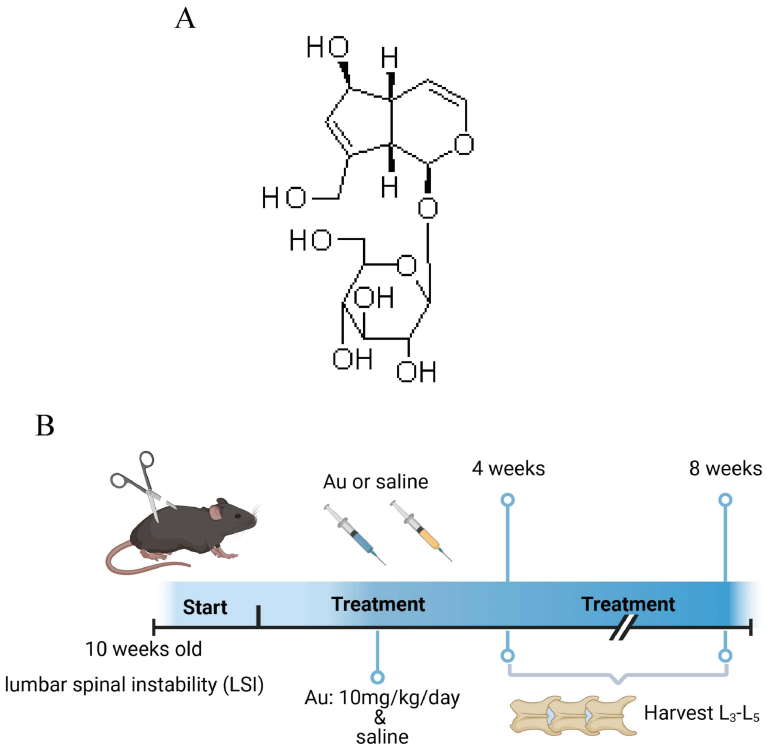

Figure 1 Experimental model diagram. (**A**) Au chemical structure formula. (**B**) LSI Surgery and Au Treatment Timeline.

were euthanized at 4 and 8 weeks after surgery. (Figure 1B). Six mice were used in each group (Sham, LSI+Vehicle, LSI+Au).

Micro-CT Analyses

All isolated lumbar spine samples were scanned by a high-resolution MIcro-CT scanner (SkyScan, 1176) with scanning parameters set at 45 kV, 500 μA, and 780 ms exposure time. Successive vertebrae from L_3-L_5 were scanned and three-dimensional images were generated by visualization software (CTVolx, v3.0, SkyScan). Five contiguous coronary images of the regions of interest were used for three-dimensional reconstruction. The invisible space between L_4 and L_5 was defined as the ROI for the analysis of the IVD.

Histological Analyses

About histological staining, vertebral samples were fixed in 4% paraformaldehyde (PFA) for 3 days. After fixation, samples were decalcified in 14% Ethylene Diamine Tetra acetic Acid (EDTA) solution (pH = 7.2) for 14 days. Subsequently, samples were embedded in paraffin and sectioned at 3 μm in thickness. The slices after dewaxing and rehydrating were stained with Alcian Blue Hematoxylin/ Orange G (ABH/OG) to assess histopathology and endplate scoring.

Immunohistochemical Staining

For immunohistochemistry, the slices after dewaxing and rehydrating were antigen restoration in 60 °C with 0.01 M sodium citrate for 4 h. After that, the slices were incubated with 0.3% Triton X-100 for 10 min at room temperature and then blocked with an endogenous peroxidase blocker (ZSGB-BIO, PV-6001, Beijing, China) for 15 min. The slices were then treated with primary antibody overnight at 4 °C, treated for reaction enhancer 15 min and then with a homologous secondary antibody for 20 min the next day. Positive expression highlighted by DAB (ZSGB-BIO, ZLI-9018, Beijing) staining. These are the primary antibodies used in this study: Collagen II (Abcam, ab34712), MMP-

13 (Abcam, ab39012), Aggrecan (Abcam, ab216965), p-P65 (Arigo, ARG51518), TNF-α (Arigo, ARG56080), NLRP3 (HuaBio, ET1610-93), Casapase-1 (HuaBio, ET1608-69).

Cell Culture and Viability Assay

In vitro, the ATDC5 chondrocyte cell line (RIKEN Cell Bank, Tsukuba, Japan) was used and cultured in Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) (Gibco, USA) medium supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco, USA). Cells were cultured at 37 °C with 5% CO_2. The cytotoxic effect of Aucubin on chondrocytes was evaluated using Cell Counting Kit-8 (CCK-8, Bioss, Beijing, China). ATDC5 cells were seeded in 96-well plates at a density of 8000 cells per well, and the cells were treated with different doses (0, 1, 5, 10, 20, 50, 100, 200 μM) of Au for 12 and 24 h after adhesion. Finally, the absorption of each well was analyzed at 450 nm using a microwell plate reader.

Quantitative RT-PCR

The total RNA of ATDC5 cells was extracted with TRIzol reagent after the cells were treated with 10 ng/mL TNF-α and different doses of Au. The cells were then subjected to Real-time polymerase chain reaction (qRT-PCR) using Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Q712-02). Quantitative analysis was performed using QuantStudio™ Real-Time PCR software. The β-actin was used as a quantitative analysis reference gene. Table 1 lists the primer sequences for the target genes used in this study.

Western Blot Analysis

Cell lysates were prepared by adding phosphatase and protease inhibitors (Roche Diagnostics, Basel, Switzerland) to the RIPA lysis buffer. All Cells added to the lysate were lysed on ice for more than 30 minutes. Total proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto 0.22 μm NC membranes (MilliporeSigma, Burlington, MA, USA). The target proteins were quantified with β-actin (Proteintech, HRP-60008) before detection. Membranes with 5% skimmed milk (Beyotime, P0216) were blocked for 1 h at room temperature (RT = 25 °C) and then incubated with primary antibodies. Next day, the membranes were washed with Tris-buffered saline (TBS)-0.1% Tween 20 (TBST) and subsequently incubated with a horseradish peroxidase-conjugated secondary goat anti-rabbit IgG antibody for 1 hour at room temperature. After washing in TBST, protein immunoreactivity was detected using an ECL Reagent (4A Biotech, Beijing, China).

Statistical Analysis

In the present study, GraphPad Prism 8.2 software (San Diego, CA) was used for all statistical analyses. Data were presented as mean ± standard deviation (S.D.). Comparisons between the two groups above were statistically analyzed by

Table 1 The primer sequences for the target genes.

Gene	Primer Sequence (5'→3')
Aggrecan Forward	CAGTGCGATGCAGGCTGGCT
Aggrecan Reverse	CCTCCGGCACTCGTTGGCTG
Mmp-13 Forward	TTTGAGAACACGGGGAAGA
Mmp-13 Reverse	ACTTTGTTGCCAATTCCAGG
Col2α1 Forward	TGGTCCTCTGGGCATCTCAGGC
Col2α1 Reverse	GGTGAACCTGCTGTTGCCCTCA
IL-1β Forward	GCAACTGTTCCTGAACTCAACT
IL-1β Reverse	ATCTTTTGGGGTCCGTCAACT
TNF-α Forward	CCCTCACACTCAGATCATCTTCT
TNF-α Reverse	GCTACGACGTGGGCTACAG
β-actin Forward	GGAGATTACTGCCCTGGCTCCTA
β-actin Reverse	GACTCATCGTACTCCTGCTTGCTG

one-way analysis of variance (ANOVA), and a p value of less than 0.05 was considered a statistically significant difference.

Results

Au Attenuated of LSI-Induced Disc Degeneration

The intervertebral disc serves as an essential tissue for cushioning stress and is extremely resilient. However, degenerative discs undergo hardening that makes it difficult to return to the original level after prolonged exposure to mechanical forces transmitted from the vertebral. Therefore, we induced IDD using LSI and investigated the disc height and disc volume using Micro-CT assays. Sagittal plane images showed that the LSI procedure decreased the disc height between L_4 and L_5, in particular the posterior region. However, the loss of height can be partially mitigated by the treatment of Au (Figure 2A–D). Moreover, in an attempt to further observe the volume changes of the intervertebral disc, we performed a 3-dimensional reconstruction of the L_4-L_5 intervertebral disc and endplates, the red area represents IVD (Figure 2E and F). Similarly, Au ameliorated the LSI-induced disc volume narrowing (Figure 2G and H). This evidence indicated that Au administration was effective in alleviating LSI-induced IDD.

Au Suppressed Calcification and Degeneration of Cartilage Endplates in LSI-Induced Mice

Based on the Micro-CT results, Au improved disc degeneration and effectively slowed the progression of IDD. The cartilaginous endplate is an important source of nutrient supply to IVD. Aiming to further explore the histologic effects of Au on IDD, we explored the effects of Au on CEP using ABH/OG staining, which was able to distinguish cartilaginous and calcified tissues clearly. In tissue sections, samples from the Au-treated group showed reduced cartilage matrix loss and suppressed endplate calcification at 4 weeks (Figure 3A). At 8 weeks, samples from the mice received Au treatment had significantly lower areas of calcification compared to vehicle-treated mice (Figure 3B). Additionally, the cartilage endplate score was much lower in the Au treatment group than that in the LSI surgical induction group after both 4 and 8 weeks (Figure 3C and D). Consistently, and it was clear that the calcified endplate areas at both 4 and 8 weeks were significantly decreased following Au treatment (Figure 3E and F). From the analysis of the histological examination, it was evident that Au treatment could alleviate endplate calcification and degeneration.

Au Alleviated LSI-Induced Metabolic Disturbances in Endplate Cartilage Matrix

Since the homeostasis of cartilage is determined by the balance of anabolism as well as catabolism, we then examined the expression of Col2α1 and Aggrecan as well as Mmp-13 on endplates by immunohistochemical tests (Figure 4A–C). We found that the expression of Col2α1 and Aggrecan were down-regulated in response to LSI and were rescued with Au intervention (Figure 4D and E). In contrast, the expression of Mmp-13 was increased after modeling and inhibited by Au treatment (Figure 4F). Collectively, these results provided sufficient evidence that Au treatment has maintained cartilage metabolism in LSI-induced mice.

Au Ameliorated IDD by Inhibiting NF-κB Signaling Activity

The series of immune responses induced by inflammation is an important reason for exacerbating the process of IDD. Besides, in a previous report, NF-κB signaling was activated in endplate chondrocytes during IDD pathology. We further explore whether Au ameliorated endplate degeneration by inhibiting NF-κB signaling activity in vivo. As expected, the expression of p-P65, a key activator protein for NF-κB signaling, was markedly downregulated in endplate chondrocytes in the Au-treated group (Figure 5A and C). We found that Au diminished the elevation of TNF-α expression in endplate chondrocytes induced by LSI surgery (Figure 5B and D). These data suggested Au has the potential to alleviate IDD by inhibiting NF-κB signaling activity and attenuating endplate cartilage ECM degradation.

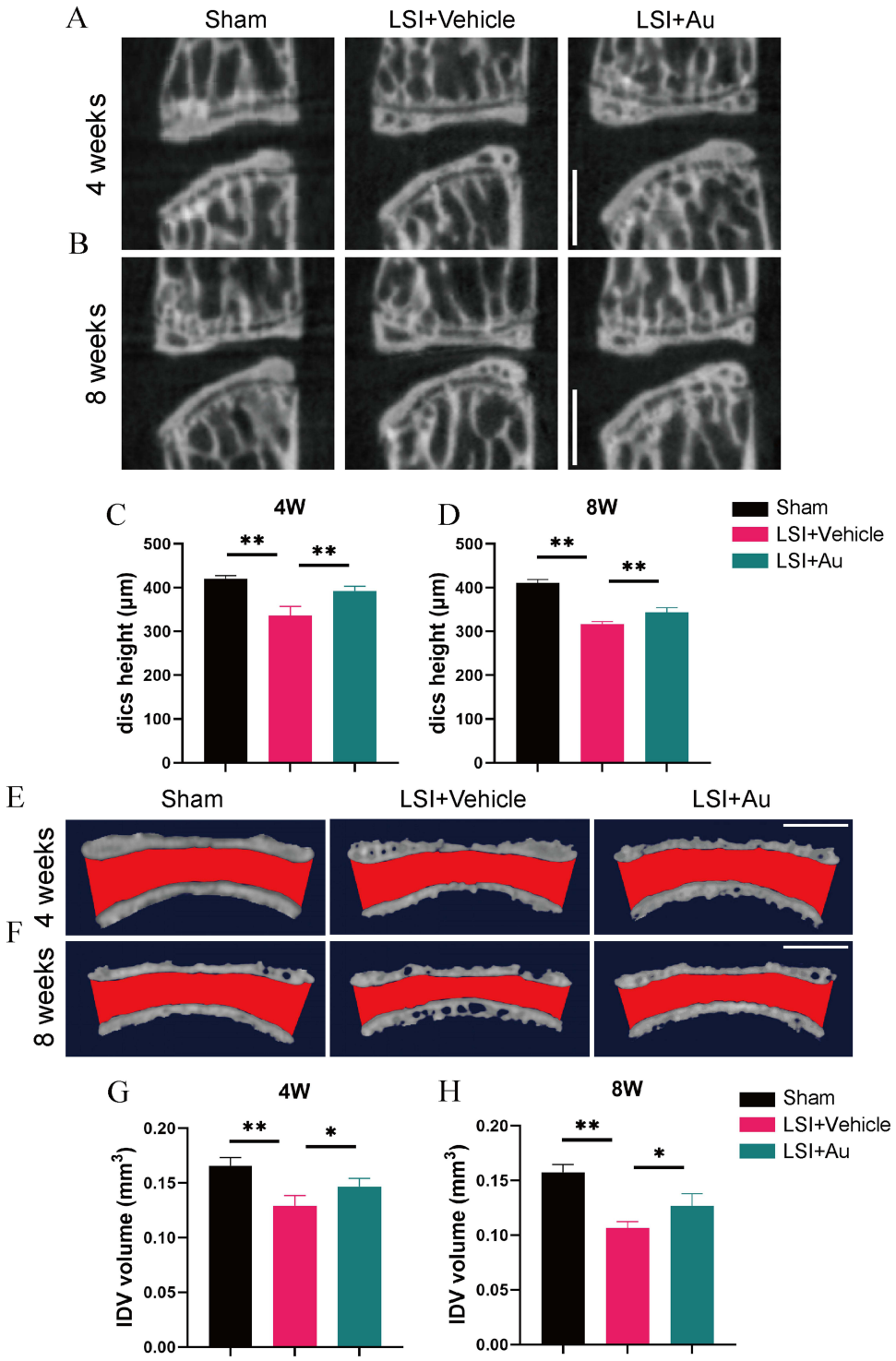

Figure 2 Au mitigated IDD in LSI mice. (**A** and **B**) Representative micro-CT images of midsagittal plane at L_4-L_5. Scale bar = 500 μm. (**C** and **D**) Quantitative analysis of disc height at L_{4-5}. (**E** and **F**) Representative 3D-reconstruction images of L4–5 endplate with coronal plane. Red area indicated IVD space. Scale bar = 500 μm. (**G** and **H**) Quantitative analysis of the IVD volume of ROI. The dose of Au was 10 mg/kg/day. Data were presented as means ± S.D. *$p < 0.05$, **$p < 0.01$; $n \geq 4$ in each group.

Au Rescued TNF-α-Induced Chondrocyte Catabolism and NF-κB Signaling Activation in vitro

Given that Au attenuated endplate chondrocyte degeneration in vivo, we used ATDC5 cells in culture to investigate the protective effect of Au against TNF-α-induced chondrocyte inflammation in vitro.[30] CCK-8 assay showed that treatment of

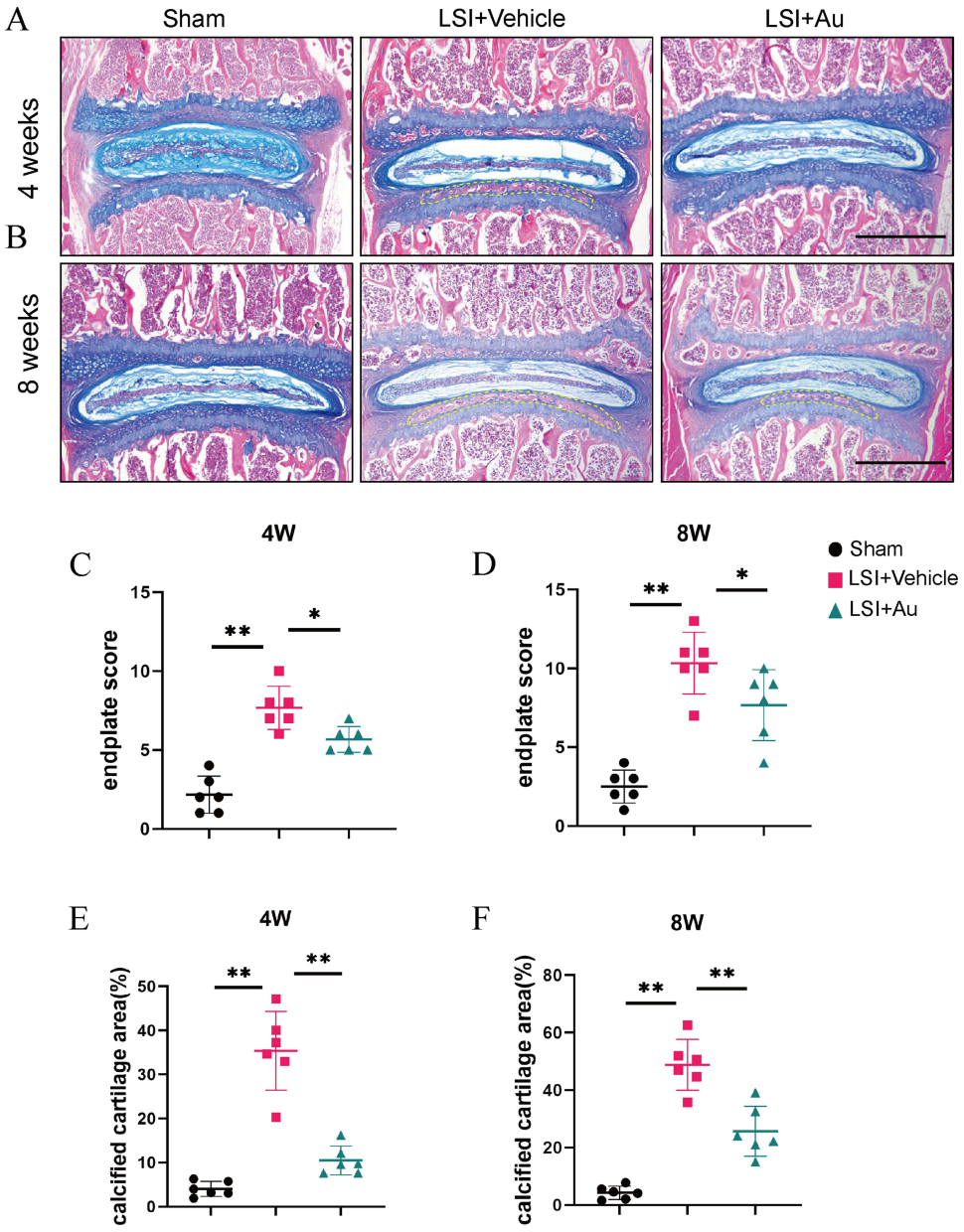

Figure 3 Au attenuated cartilaginous endplate calcification in LSI mice. (**A** and **B**) Alcian Blue Hematoxylin/Orange G staining of cranial $L_{4\text{-}5}$ endplate at 4 weeks and 8 weeks after operations. Yellow dashed boxes indicated regions shown as calcification. Scale bar = 500 μm. (**C** and **D**) Quantitative analysis of $L_{4\text{-}5}$ endplate score at 4 and 8 weeks post-operation. (**E** and **F**) Quantitative analysis of $L_{4\text{-}5}$ endplate calcified cartilage area at 4 and 8 weeks post-operation. The dose of Au was 10 mg/kg/day. Data were presented as means ± S.D. *$p < 0.05$; **$p < 0.01$; n = 6 in each group.

Au at doses ranging from 1 to 200 μM for 12 and 24 hours had no effect on the viability of ATDC5 cells (Figure 6A and B). Therefore, we chose the centered three doses as our main intervention doses. In qRT-PCR assay, Au promoted the mRNA expression of *Aggrecan* and *Colα1* in ATDC5 cells after TNF-α treatment, in addition to decreasing the TNF-α-induced expression of *Mmp-13* (Figure 6C–E), which were highly in keeping with the immunohistochemistry results. Also, the expressions of *IL-1β* and *TNF-α* were both inhibited by Au (Figure 6F and G). Furthermore, we verified the protein expressions by Western blotting experiments (Figure 6H). Consistently, Au rescued TNF-α-induced decreased levels of Col2α1 and inhibited the Mmp-13 expression as well as P65 phosphorylation (Figure 6I and J). All of these results indicated that Au protected chondrocytes from catabolism and inflammation, at least in part, by repressing NF-κB signaling.

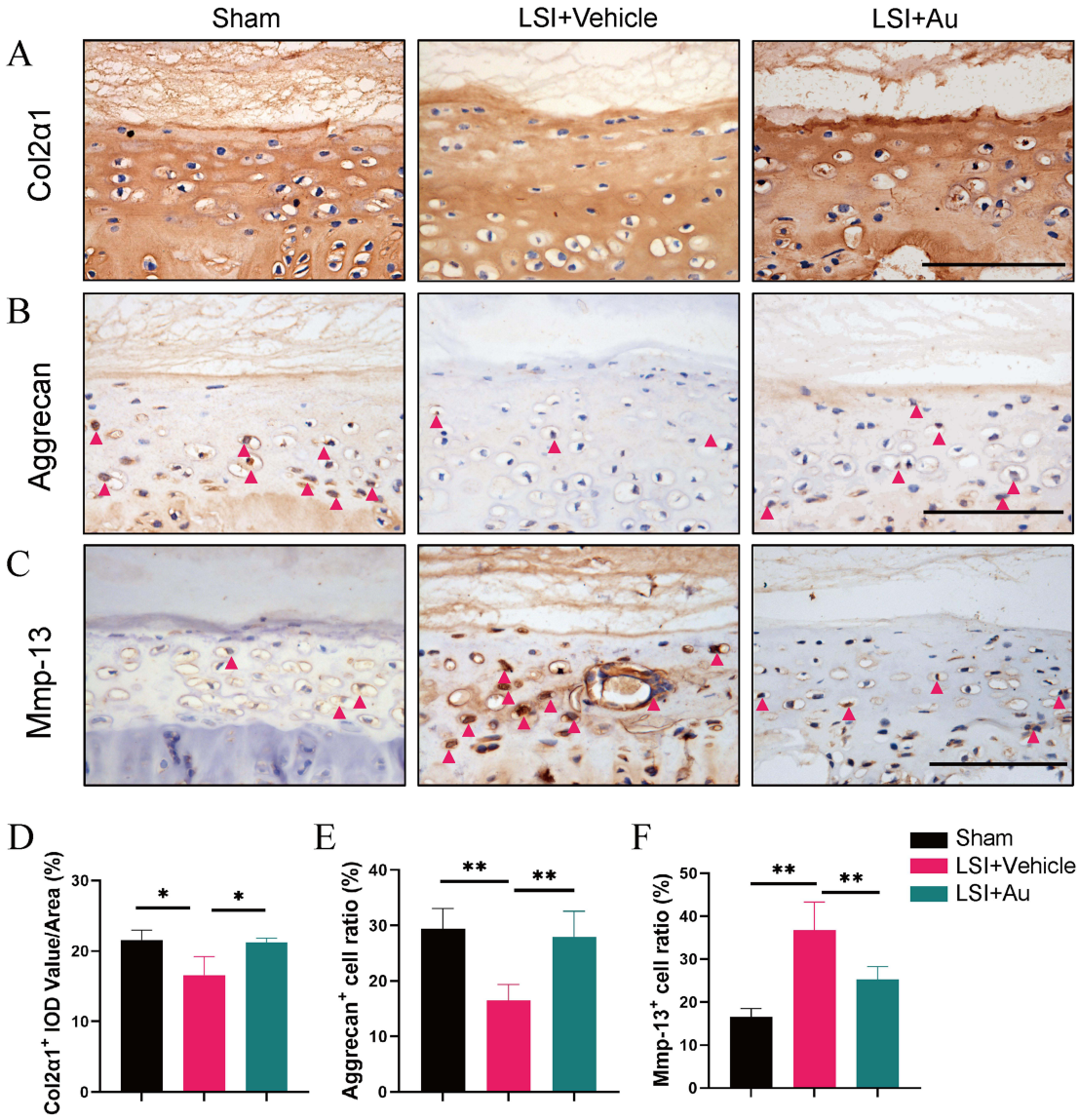

Figure 4 The effect of CGA on LSI-induced anabolism and catabolism in cartilage endplate. (**A**) The representative images of immumohistochemical staining and quantifications of Col2α1 in the caudal $L_{4\text{-}5}$ endplate at 4 weeks. (**B**) The representative immumohistochemical images for Aggrecan and the ratio of positive cells in the whole caudal $L_{4\text{-}5}$ endplate at 4 weeks. Red arrows indicated positive cells. Scale bar = 100 μm. (**C**) Immunostaining images and quantificational evaluation of Mmp-13 expression in caudal $L_{4\text{-}5}$ endplate at 4 weeks post operation. Red arrows indicated positive cells. Scale bar = 100 μm. (**D**–**F**) Quantitative analyses of the rate of positive cells and average IOD value. The dose of Au was 10 mg/kg/day. Data were presented as means ± S.D. *p < 0.05; **p < 0.01; n ≥ 3 in each group.

Au Protected Endplate Chondrocyte Against NLRP3 Inflammasome Activation

Active NF-κB signaling tends to activate a number of downstream inflammatory factors thereby exacerbating the inflammatory response. NLRP3 as an inflammasomes downstream of NF-κB signaling has been shown to cite as a key protein in the initiation of cellular pyroptosis. Meanwhile, from recent studies, we focused on the effects of endplate chondrocyte pyroptosis on IDD.[31] Moreover, the accumulated NLRP3 inflammasome activates the protease Caspase-1 to induce pyroptosis, which is a key component of cellular pyroptosis.[32] Therefore, we evaluated the expression of NLRP3 and Caspase-1 on the endplates with immunohistochemical staining (Figure 7A and B). The results showed that NLRP3 and Caspase-1 expression in the Au treatment group was lower than that in the LSI surgery group (Figure 7C and D). To verify the reliability of this mechanism in vitro, we intervened ATDC5 cells with the same dose as in previous data and confirmed the protein expressions by Western blotting (Figure 7E). These data showed that Au treatment substantially attenuated the

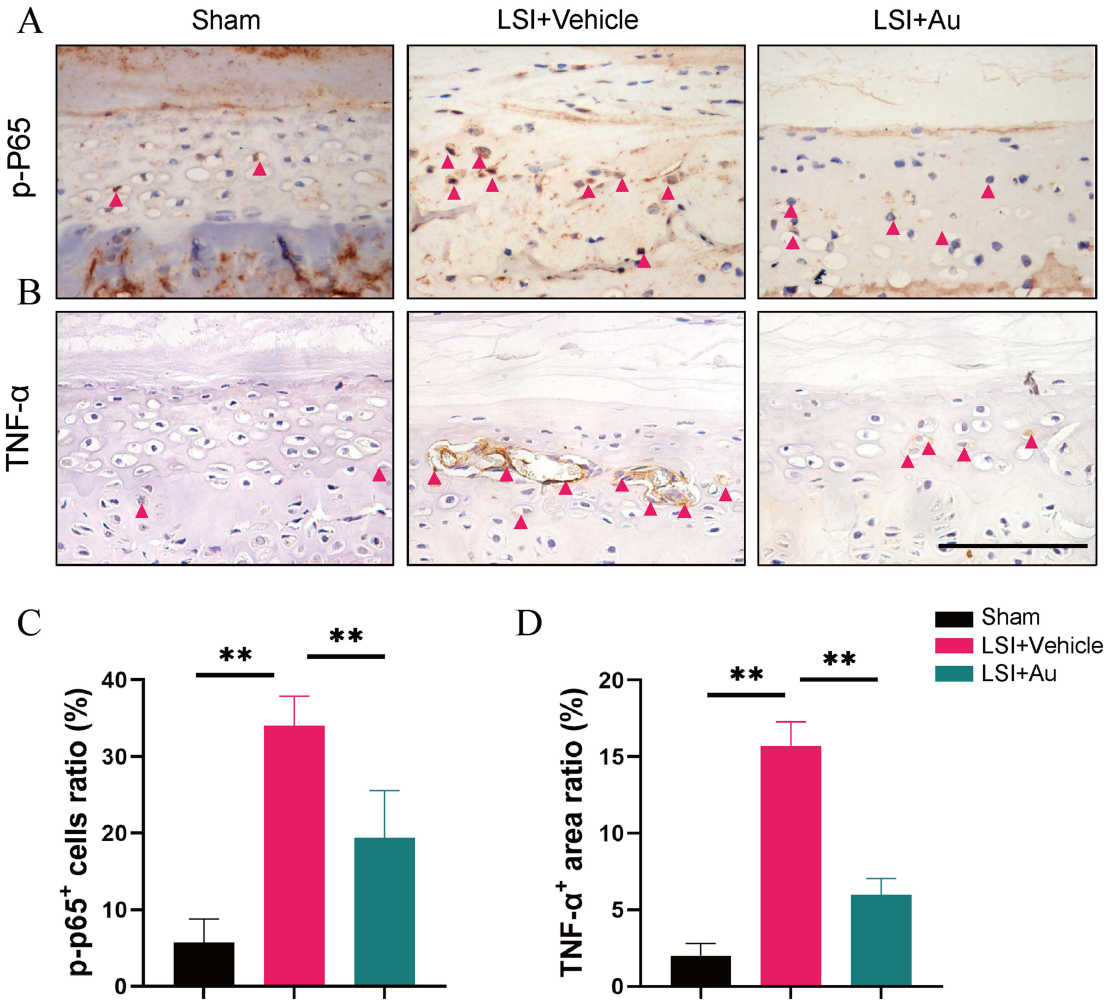

Figure 5 Au repressed LSI-induced activation of NF-κB signaling in caudal L4-5 endplate. (**A** and **B**) Representative immunohistochemical images of P-p65 and TNF-α in the caudal L_{4-5} endplate at 4 weeks. Red arrows indicated positive expressions. Scale bar = 100 μm. (**C** and **D**) Quantitative analyses of the percent of phosphorylated-p65 positive cells and TNF-α positive area in the endplates. The dose of Au was 10 mg/kg/day. Data were presented as means ± S.D. $^{**}p < 0.01$; n ≥ 4 in each group.

TNF-α-induced elevation of NLRP3 and Capase1 expression (Figure 7F and G). Taken together, we have demonstrated that Au alleviated IDD by suppression the NF-κB-NLRP3 inflammasome activation in endplate chondrocyte.

Discussion

The prevalence of LBP is at the forefront of the morbidity of chronic diseases.[33] LBP can lead to severe, lifelong disability, with the attendant high cost of treatment.[34,35] Moreover, the etiology of LBP is multifactorial, and disc degeneration is undoubtedly a crucial factor. Although many treatments have been applied to IDD, treatments that are more cost-effective and specific to the source of the pain need to be investigated.[36] Here, our data demonstrated, for the first time, Au suppresses ECM degradation and retards endplate calcification of by suppressing the NF-κB-NLRP3 inflammasome activation. The data from this study suggest that Au is a potential drug for the therapy of IDD.

In the context of this study, we used a mouse model of LSI that is similar in imaging and histopathologic examination to the human manifestations of IDD.[26,27] We successfully reproduced the phenotype of IDD in mice using LSI surgery. For example, compared with the Sham group, lumbar spine Micro-CT at 4 and 8 weeks postoperatively showed the IVD height narrowing, and 3D reconstruction demonstrated a reduction in the IVD volume. In parallel, ABH/OG staining also revealed a distinct endplate cartilage calcification layer, these phenotypes are in accordance with prior findings.[37,38] Of note, all of these manifestations of IDD partially reversed with Au treatment. Previous studies have found that endplate

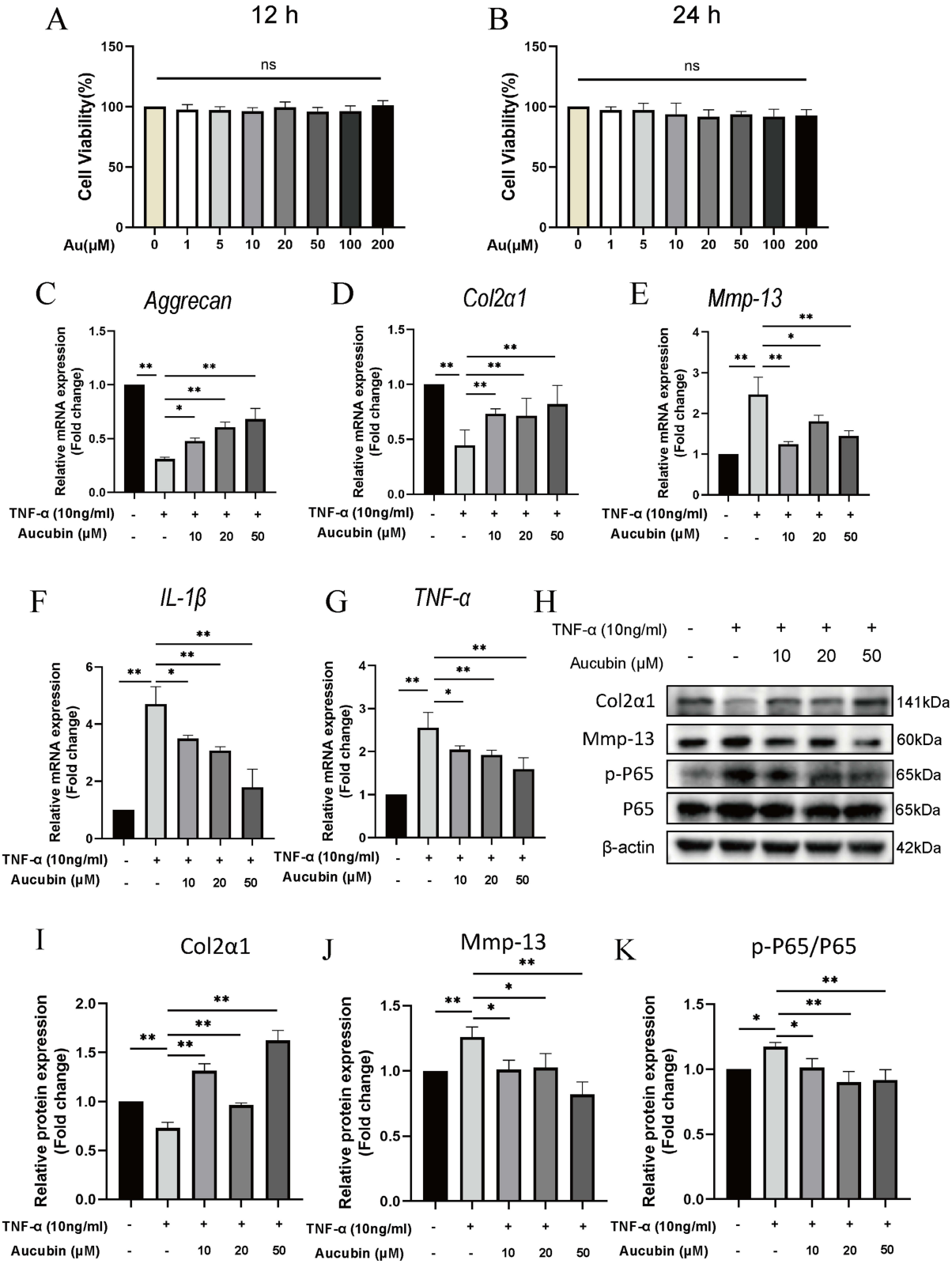

Figure 6 The effect of Au on TNF-α-induced chondrocytes in vitro. (**A** and **B**) Cell viability of ATDC5 cell cultured with various concentrations of Au for 12 h and 24 h. (**C**–**G**) The gene expression of TNF-α-induced ATDC5 cell treated with various concentrations of Au for 24 h. (**H**) The protein expression of TNF-α-induced ATDC5 cell treated with various concentrations of Au for 24 h. (**I**–**K**) Quantitative analyses of the relative protein expression. Data were presented as means ± S.D. *$p < 0.05$; **$p < 0.01$, ns, significant difference, as compared to group treated with TNF-α only (the second column), n ≥ 3 in each group.

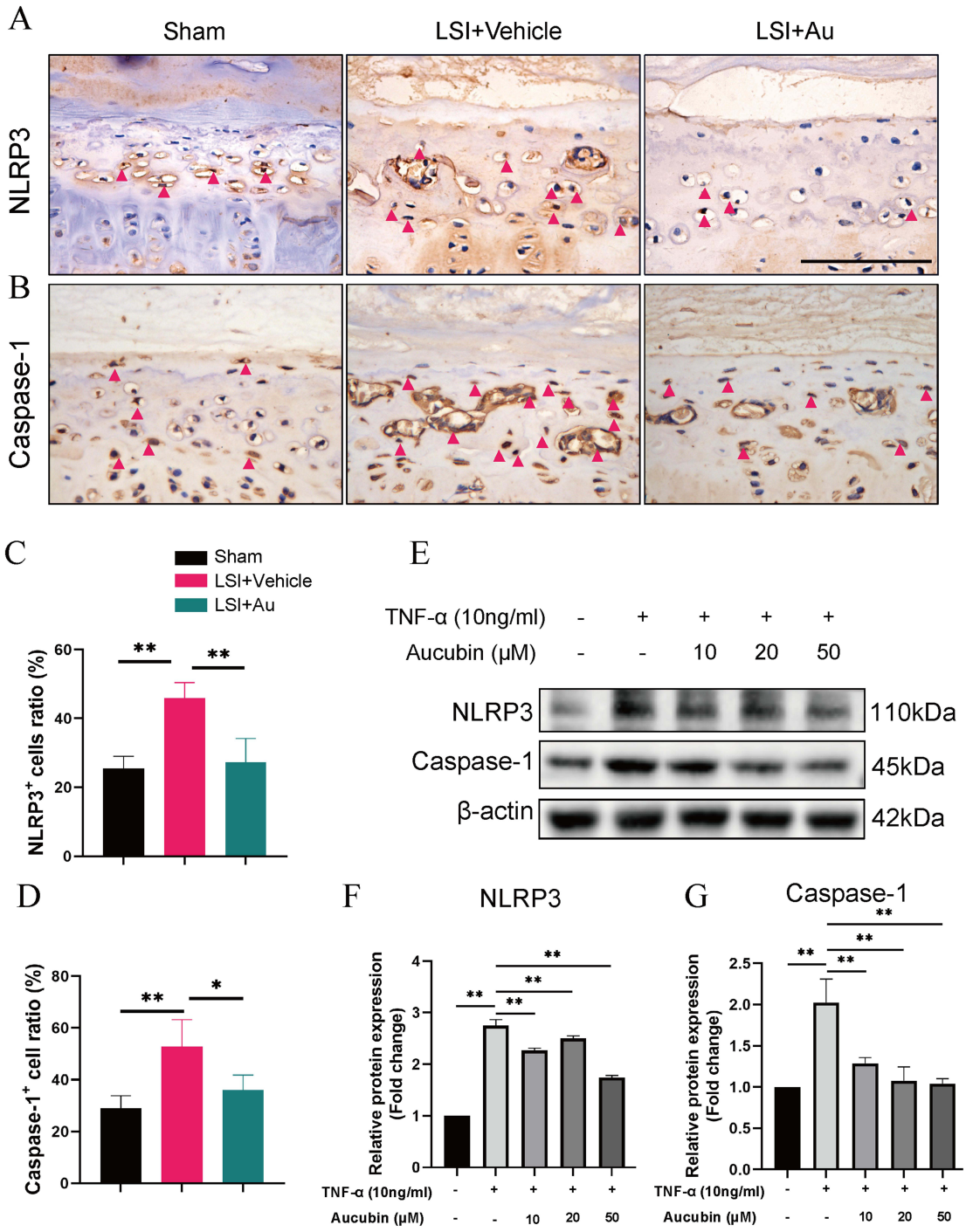

Figure 7 Au inhibits chondrocyte pyroptosis in vivo and in vitro. (**A** and **B**) Representative immunohistochemical images of NLRP3 and Caspase-1 in the caudal $L_{4\text{-}5}$ endplate at 4 weeks. Red arrows indicated positive expressions. Scale bar = 100 μm. (**C** and **D**) Quantitative analyses of the percent of NLRP3 and Caspase-1 positive cells in the endplates. The dose of Au was 10 mg/kg. (**E**) The protein expression of TNF-α-induced ATDC5 cell treated with various concentrations of Au for 24 h. (**F** and **G**) Quantitative analyses of the relative protein expression. Data were presented as means ± S.D. *p < 0.05; **p < 0.01; as compared to group treated with Vehicle in vivo or TNF-α in vitro (the second column), n ≥ 3 in each group.

cartilage is the sole source of nutrients for IVD and its inflammation exacerbates IVD degeneration.[39–41] This seems to be further evidence that Au mitigated IDD by relieving inflammation and degradation of CEP.

Recently, increasing research focuses on endplate cartilage degeneration.[42] The IVD is avascular tissue, and the CEP adjacent to it is its only source of nutrients.[43] In addition to the destruction of the nucleus pulposus, which is the main

structure of the IVD, recent, studies have shown that degeneration of the fibrous ring and cartilage endplates is also a major factor in the development of IDD.[44] Degeneration of the vertebral endplates is manifested early by calcification of the cartilage, a change that is also considered to be the initial pathologic manifestation.[45] However, previous studies have shown that TNF-α can induce the expression of transcription factors associated with osteogenesis in CEPs, which is similar to our findings.[12] TNF-α may be a main cause of endplate cartilage calcification. In the present study, ABH/OG staining revealed significant areas of calcification in the L_4-L_5 inferior endplate at 4 and 8 weeks after surgery. Immunohistochemical results suggest decreased matrix synthesis and increased matrix degradation. In addition, since matrix metabolism is closely related to cartilage calcification.[46] Therefore, we supposed that Au could slow the progression of IDD by restoring the metabolic homeostasis of endplate cartilage.

NF-κB signaling is involved in IDD pathophysiology through a variety of roles, including the promotion of gene transcription and protein translation involved in inflammation and catabolism.[47–49] It has been widely reported that Au could suppress NF-κB signaling to modulate the inflammatory response.[50,51] From our findings on IDD, we suggest that disruption of chondrocyte pyroptosis and matrix metabolic homeostasis is caused by aberrant activation of the NF-κB signaling. NLRP3/Caspase-1 axis activation plays an important role in neuroinflammation and neuronal injury in various neurological diseases.[52,53] Previous studies have identified the effect of pyroptosis in nucleus pulposus cells during IDD,[14,54,55] and recently, Caspase-1 and NLRP3 expression has been reported to be upregulated in CEP of patients with degenerative disease.[56] Consistently, as both in vivo and in vitro studies presented in the current study, Au inhibited the phosphorylation of P65 and reduced the activity of NF-κB signaling, thereby decreased the expression of NLRP3 and Caspase-1, suggested that Au reduced chondrocyte pyroptosis by decreasing the activation of NF-κB signaling. Besides, the expressions of Col2α1 and Aggrecan were restored in the Au-treated group, while the expression of Mmp-13 and TNF-α and IL-1β was decreased. Thus, Au may protect CEP degeneration by reducing pyroptosis and alleviating severe inflammation. Although the anti-inflammatory effects of Au have been extensively studied in many other diseases, including neuronal injury,[57] myocardial damage,[58] brain injury[59] and osteoarthritis,[24] our study demonstrates, for the first time, that Au attenuates the inflammatory response and pyroptosis of endplate chondrocytes in IDD.

Inevitably, this study still has some limitations. Due to the limitations of the experimental facilities and other objective conditions, we were unable to determine the effect of Au on behavioral assessments such as gait analysis and spontaneous activity in this study. In addition, our findings do not completely exclude the possibility that Au may mitigate IDD by attenuating chondrocyte pyroptosis through pathways other than NF-κB signaling.

Conclusion

In summary, our study shows for the first time that Au could alleviate LSI-induced cartilage degeneration as well as IDD by inhibiting NF-κB signaling activity and attenuating endplate chondrocyte pyroptosis and suggested its potential pharmacological effects on IDD.

Data Sharing Statement

The data used to provide support for the results of this study can be obtained from the corresponding authors.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare that they have no conflict of interest.

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