Journal of Pain Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Effect of Acupotomy Modulation of NLRP3/ Caspase-1/GSDMD Pathway on Chondrocyte Pyroptosis in Knee Osteoarthritis

Yingjie Wang[1,2], Yuan Chen[1,2], Yao Yang[1,2], Yanling Zhou[1,2], Yonghui Yang[1], Junchen Zhu[1]

[1]Department of Orthopedics, The Second Affiliated Hospital of Anhui University of Chinese Medicine, Hefei, Anhui Province, People's Republic of China; [2]Graduate School, Anhui University of Traditional Chinese Medicine, Hefei, Anhui Province, People's Republic of China

Correspondence: Yonghui Yang; Junchen Zhu, Email 944588939@qq.com; 2006zhujc@163.com

Background: Knee osteoarthritis (KOA) is a prevalent degenerative joint disease characterized by progressive cartilage degradation and inflammation, often leading to pain and reduced mobility. Current therapeutic approaches, including pharmacological and surgical interventions, provide symptomatic relief but rarely address the underlying pathological mechanisms effectively. Acupotomy therapy, a minimally invasive technique integrating traditional Chinese medicine and modern surgical concepts, has shown promise in alleviating KOA symptoms. However, its mechanisms, particularly concerning chondrocyte pyroptosis, remain underexplored. This study investigates the role of acupotomy therapy in modulating chondrocyte pyroptosis via the NLRP3/Caspase-1/GSDMD signaling pathway in a rabbit KOA model.

Methods: Forty New Zealand rabbits were randomized into four groups: control, model, acupotomy, and drug-treated groups. A KOA model was induced via intra-articular injection of papain. Interventions included acupotomy therapy and oral celecoxib. Outcomes were assessed using behavioral scoring, micro-CT imaging, histological staining, serum inflammatory markers (ELISA), and the expression of NLRP3 inflammasome-associated proteins (qPCR and Western blot).

Results: Acupotomy therapy significantly improved behavioral scores and reduced knee joint space narrowing. Histological analyses revealed improved cartilage integrity and decreased inflammatory markers. Expression levels of NLRP3, Caspase-1, ASC, and GSDMD were downregulated in the acupotomy group compared to the model group.

Conclusion: This study highlights the therapeutic potential of acupotomy therapy in alleviating KOA symptoms and reducing chondrocyte pyroptosis by modulating the NLRP3 inflammasome pathway. These findings provide a mechanistic basis for its application in KOA treatment and a foundation for further clinical exploration.

Keywords: acupotomy, knee osteoarthritis, cartilage, cellular charring, NLRP3/Caspase-1/GSDMD pathway

Introduction

Knee osteoarthritis (KOA) is a prevalent degenerative joint disease characterized by the progressive degeneration and damage of knee cartilage, leading to pain, stiffness, and impaired mobility. It predominantly affects the elderly population, significantly reducing their quality of life and overall health.[1,2] With the global trend of population aging, the prevalence of KOA is expected to increase, posing a growing public health challenge and necessitating the development of effective therapeutic and management strategies.[3]

Current management approaches for KOA largely focus on conservative interventions, including the use of analgesics and anti-inflammatory drugs, while surgical options such as total knee arthroplasty are typically reserved for advanced cases.[4,5] However, these strategies have notable limitations. Pharmacological treatments often provide only temporary symptom relief and fail to address the underlying disease progression, whereas surgical interventions are associated with higher risks, extended recovery times, and significant financial burdens. These drawbacks highlight the urgent need for novel, safe, and effective therapies that target the root causes of KOA and promote cartilage repair.

Received: 7 May 2025
Accepted: 16 September 2025
Published: 23 September 2025

Acupotomy therapy, a technique rooted in traditional Chinese medicine, has been extensively applied in the treatment of joint disorders and has demonstrated promising clinical outcomes.[6] Previous studies have shown that acupotomy therapy can effectively alleviate pain and restore joint function in KOA patients.[7,8] Despite these encouraging results, the precise therapeutic mechanisms underlying its efficacy remain poorly understood, particularly in the context of cartilage repair and inflammation modulation.

The pathogenesis of KOA is complex, involving mechanical stress, biochemical factors, and inflammatory responses.[9,10] Recent research has identified neutrophil extracellular traps (NETs) and the NLRP3 inflammasome as key contributors to the inflammatory processes associated with KOA.[11,12] In chondrocytes, pyroptosis is primarily triggered by activation of the NLRP3 inflammasome, which recruits and activates Caspase-1, leading to the cleavage of gasdermin D (GSDMD) and the subsequent formation of membrane pores that execute cell death and amplify inflammation. This cascade not only exacerbates chondrocyte loss but also promotes the release of pro-inflammatory cytokines such as IL-1β and IL-18, creating a self-perpetuating inflammatory microenvironment that accelerates cartilage degradation.[13] Emerging evidence suggests that acupuncture-related interventions, including acupotomy, may exert anti-inflammatory effects partly through modulation of the NLRP3 inflammasome pathway. For example, clinical and preclinical studies have reported that acupuncture can downregulate NLRP3 activation, thereby reducing Caspase-1 activation and inhibiting GSDMD-mediated pyroptosis.[14,15] These findings provide a mechanistic rationale for investigating the effects of acupotomy on KOA through the NLRP3/Caspase-1/GSDMD pathway.

This study aims to investigate the therapeutic potential of acupotomy therapy in mitigating knee osteoarthritis through modulation of the NLRP3/Caspase-1/GSDMD pathway. Using a rabbit KOA model, we will explore the effects of acupotomy therapy on inflammation, cartilage repair, and functional recovery, providing experimental evidence to support its clinical application. By elucidating the underlying mechanisms, this research seeks to contribute to the development of innovative and effective treatments for KOA.

Materials and Methods

Animals and Grouping

Forty healthy 4-month-old New Zealand rabbits, weighing 2±0.2 kg, were purchased from Anhui Medical University Biological Co. Ltd. [Licence No. SYK (Anhui) 2019–011]. Due to the large number of rabbits required for this study, our institution's laboratory facilities were insufficient to accommodate the necessary number of rabbit cages. Therefore, the procurement and care of the rabbits were outsourced to the Anhui Provincial Public Health Institute, with Researcher Yuan Hui responsible for their breeding and care. The animals were housed individually in cages under drugsled conditions with a room temperature of 23±2°C and a humidity of 60±10%. The rabbits underwent a 7-day acclimatization period before being randomly assigned to four groups: control, model, acupotomy, and drugs, with 10 animals in each group. The experiment was approved by the Ethics Committee of Animal Management and Animal Welfare of Anhui Provincial Public Health Institute (2024LS001). All animal acupotomys during the study adhered to the guidelines outlined in the "Guiding Opinions on the Kind Acupotomy of Laboratory Animals".

Main Reagents and Equipment

RIPA cell lysate (P0013B), Trizol (15596018), paraformaldehyde (BL539A), PAGE gel procoagulant (T8090), TBST (Gooo-1L), PBS buffer (BL302), protein containment solution (PS108P), ECL ultrasensitive luminescence kit (BMU102-CN), and PAGE Gel Rapid Preparation Kit (PG211) were purchased from Beijing Solebaum Biotechnology Co., Ltd. Hematoxylin-Eosin Staining Kit, Fenghuang Solid Green Staining Solution (Anhui Tech Rabbit), RNA Rapid Extraction Kit (AC0205-B, Ciscojie Biotechnology Co., Ltd)., and ELISA Kit (EK0513-RB, Wuhan Doctoral Bioengineering Co., Ltd.) were also procured. Goat anti-mouse IgG and goat anti-rabbit IgG (Zhongsui Jinqiao), GAPDH antibody (60004-1-Ig), caspase-1 antibody (22915-1-AP), gephyrin D antibody (GSDMD, 20770-1-AP), NLRP3 antibody (19771-1-AP), and pyroptosis-associated speck-like protein (ASC) antibody (10500-1-AP) were purchased from Proteintech.

Instruments and equipment used included a small acupotomy (Hanzhang brand, 0.4 mm × 30 mm, former Beijing Huaxia Needle and Knife Medical Instrument Factory, batch number 20160038), a desktop freezer (Ningbo Xinzhi Biotechnology Co., Ltd)., a PCR instrument (Model: BIO RAD 622BR64428), an electrophoresis instrument (Model: EPS300), an electrophoresis

 https://doi.org/10.2147/JPR.S537826

tank (WIX-miniBLOT4), a horizontal shaking machine (Model: SLK-R3000-S), a micro high-speed freezing centrifuge (SCILOGEX CF1524R, USA), an embedding machine (Model: YB-7LF), and a slicer (Model: RM2235).

Model Establishment

The control group did not undergo modeling. The model, acupotomy, and drugs groups received intra-articular injections of a 4% papain solution mixed with an L-cysteine solution (0.03 mol/L) in a 2:1 ratio. The mixture was injected into the rabbits' left knee joints at a dose of 0.1 mL/kg on days 1, 4, and 7 over three consecutive weeks to induce the KOA model. Simultaneously, the left hind limb of each rabbit was immobilized using a softened resin bandage, maintaining the knee joint in a neutral position at 0° extension and the ankle joint dorsiflexed at 60°.[16] The success of the modeling was assessed using the Lequesne MG score, with a score of ≥6 indicating successful induction of the KOA model.

Intervention Treatment

The control group was kept normally without intervention. The model group, the acupotomy group and the drugs group were all constructed with osteoarthritis model of the knee by beating medicine combined with fixation. After successful modelling, the model group had no intervention, and the acupotomy group received acupotomy acupotomy once a week for 3 times. Acupotomy intervention: rabbits were fixed on the iron frame in supine position, nodules and adhesion lesion points at the quadriceps tendon and the medial and lateral collateral ligaments of the knee joints of the left hind limb were touched and identified, and four points were selected each time and marked with surgical markers. The skin was prepared and sterilised in accordance with the routine procedure, and the acupotomy was used to make a straight incision along the longitudinal axis of the lower limb in the direction of the parallel incision, with the tip of the acupotomy passing through the skin and soft tissues, and when a sense of toughness was felt under the acupotomy, three to four lifting and insertion incisions were performed on the striated nodules. After the operation was completed, the acupotomy was withdrawn and local pressure was applied to stop bleeding. The drugs group was given oral celecoxib (Celebrex, Pfizer Pharmaceuticals, National Drug Code: J20140072, 200 mg) at a dose of 18 mg/kg once a day for 3 weeks.

Lequesne MG Behavioural Score

The Modified Luckson Score MG Knee Index was used to look at localised pressure pain manifestations in the knee (0–3 points), joint mobility (0–3 points), joint swelling (0–2 points), and gait analysis (0–3 points). Higher total scores indicated more severe knee injury and poorer joint mobility function. The affected limbs of the rabbits were scored after modelling and the day after the end of the intervention.

ELISA Assay

After successful modelling and the end of the intervention, blood samples were collected from the marginal ear vein of rabbits in each group, and serum was obtained after centrifugation, and the concentrations of inflammatory factors TNF-α and IL-1β in serum were detected by ELISA kits, respectively. The specific operation was as follows: 50 µL of rabbit serum was added into a pre-coated 96-well plate and incubated for 60 min. After washing with washing buffer for 3 times, HRP-labelled antibody was added and incubated at 37°C for 60 min. After adding the termination solution, the OD value was measured at 450 nm, and the concentrations of TNF-α and IL-1β were calculated from the standard curve.

Hematoxylin-Eosin Staining and Safranin O-Fast Green Staining

Hematoxylin-eosin staining(HE) and Safranin O-Fast Green Staining were used to observe the cartilage tissue and cell morphology of rabbit knee joints: paraffin sections were dewaxed and hydrated according to the routine process and then subjected to hematoxylin immersion staining (5 min), routinely differentiated, blued, dehydrated and then immersed in eosin solution (3 s), dehydrated, transparent and sealed to obtain HE-stained slices of rabbit knee joints; Paraffin sections were stained with Weigert's solution for 5 minutes, followed by washing with distilled water. The sections were then differentiated using an acidic differentiation solution, washed again, and stained with Fast Green solution for 5–10 minutes. After rinsing with distilled water, the sections were washed with a weak acid solution to remove any residual Fast Green. The sections were then stained with Safranin O solution for 5–10 minutes. Following staining, the sections were dehydrated in the usual manner, cleared with xylene, and mounted with neutral balsam to create Safranin O-Fast Green stained sections of rabbit knee joints. The stained sections were then observed under a light microscope.

Real-Time Fluorescence Quantitative PCR

The total RNA was extracted by Trizol method after weighing 100 mg of cartilage tissue and cutting it into pieces. cDNA was reverse transcribed from the total RNA using a reverse transcription kit and used as a template for PCR amplification. GAPDH was used as an internal reference gene, and the relative expression of each group of samples was calculated using the $2^{-\Delta\Delta}$Ct method. The primer sequences are shown in Table 1.

Western Blot

NLRP3 inflammatory vesicle-associated protein expression in cartilage tissues was detected by Western blot. About 100mg of cartilage tissue samples were weighed and tissue lysis was performed by adding 600μL of RIPA lysate to extract the total protein. After loading, electrophoresis and protein transfer of the protein samples, the samples were closed at room temperature for 2 hours on a shaker using Western Closure Solution with 5% skimmed milk powder. Primary antibody was added for overnight incubation at 4°C. The secondary antibody was added and incubated the following day. Protein signals were detected using an ECL luminescence kit and the grey values of the film strips were quantified using Image-J software.

Statistical Analysis

All data analyses were performed using R software (version 4.0.1; https://www.r-project.org/) and GraphPad Prism (version 9.0). Data were expressed as mean ± standard deviation. Comparisons between groups were conducted using the *t*-test, while paired t-tests were employed for within-group pre-post comparisons. Statistical significance was set at $P < 0.05$.

Results

Comparison of Lequesne MG Behavioural Scores in Rabbits in Each Group

The Lequesne MG behavioral scores, an established indicator of knee joint mobility, demonstrated significant differences among the experimental groups. KOA model rabbits exhibited markedly higher scores compared to the control group ($P < 0.05$), indicating substantial impairment in knee joint function. Following intervention, the scores of KOA model rabbits treated with Drugs and acupotomy were significantly lower than those of the model group ($P < 0.05$), reflecting partial restoration of knee joint mobility. However, these scores remained higher than those of the control group. Notably, acupotomy treatment demonstrated a superior effect in improving knee joint function compared to Drugs, suggesting its potential as an effective therapeutic modality for KOA (Table 2).

Serum Inflammation Levels in Rabbits in Each Group

ELISA results revealed significantly elevated levels of inflammatory factors TNF-α and IL-1β in the serum of KOA model rabbits compared to the control group ($P < 0.05$). Both acupotomy and Drugs interventions significantly reduced these levels compared to the model group ($P < 0.05$), indicating effective mitigation of systemic inflammation. However, the levels of inflammatory factors in the intervention groups remained higher than those in the control group ($P < 0.05$).

Table 1 Target Genes and Primer Sequences for qRT-PCR

Gene	Upstream Primer (5′→3′)	Downstream Primer (3′→5′)	Amplication Length (bp)
GAPDH	AGACACGATGGTGAAGGTCG	TGCCGTGGGTGGAATCATAC	164
NLRP3	GTGTCAAGACCACAGCTCCA	GTAATTGGGACCATCGGCCT	147
Caspase-1	GCCTGGTCTTGTGATGTGGA	TGATAGCACTCTTGGCTTCGT	159
ASC	GCAACTGCGAGAAGGCTATG	GTGAGCTCCAAGCCATACGA	247
GSDMD	CGATCTCATTCCGGTGGACAG	GGTTTCCAGAACCTTGAGCTTG	269

Table 2 Lequesne MG Scores of Rabbits in Each Group (n=9)

Groups	Control	Model	Drugs	Acupotomy
Pre-intervention	0.00±0.00	8.00±0.71	7.78±0.67	8.11±0.78
Post-intervention	0.00±0.00	7.56±0.73	4.56±0.53[a]	2.56±1.13[b,c]
t-value		1.079	14.500	11.045
P-value		0.312	0.000	0.000

Notes: comparisons with the model group. [a]t= 10.028, P=0.000; [b]t=11.163, P=0.000. Comparisons with the Drugs group. [c]t= 4.811, P=0.000.

Table 3 Serum Inflammatory Factor Levels in Rabbits in Each Group (n=9)

Groups	TNF-α (pg/mL)		IL-1β (pg/mL)	
	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention
Control	151.63±15.16	147.75±27.14	128.14±21.44	141.34±20.55
Model	350.28±32.84[b]	326.72±55.12[b]	300.11±11.35[b]	308.35±13.20[b]
Drugs	343.95±21.86[b]	232.20±41.92[abc]	292.78±32.10[b]	200.17±33.47[abc]
Acupotomy	357.90±28.47[b]	228.39±26.32[abc]	301.11±19.60[b]	202.89±22.31[abc]

Note: Within-group comparison, [a]P<0.05; comparison with Control group [b]P<0.05; comparison with model group [c]P<0.05.

Notably, there was no statistically significant difference in the reduction of inflammatory levels between the acupotomy and Drugs groups ($P > 0.05$), suggesting comparable anti-inflammatory effects (Table 3).

CT Examination of the Knee Joints of Rabbits in Each Group

To establish the KOA model, rabbits underwent intra-articular injection of papain mixed with L-cysteine, followed by immobilization of the hind limb with a softened resin bandage. This procedure induced significant cartilage degeneration and joint damage, as confirmed by gross morphological observations, which revealed severe cartilage surface erosion and irregularity (Figure 1A and B). Following successful modeling, acupotomy treatment was performed to release adhesions and nodules in the knee joint, aiming to restore structural integrity and improve mobility (Figure 1C). CT imaging demonstrated substantial structural differences among the experimental groups (Figure 1D). In the KOA model group, joint spaces were significantly narrowed, and osteophyte formation was pronounced compared to the control group ($P < 0.05$). Acupotomy treatment led to notable improvements, including restored joint space and reduced osteophyte formation, as evidenced by smoother joint surfaces and more uniform joint spaces in the CT images. In contrast, the Drugs group showed moderate improvements with some residual irregularities. These results underscore the therapeutic efficacy of acupotomy in mitigating structural damage and restoring joint function in KOA.

HE and Safranin O-Fast Green Staining of Rabbit Knee Joints in Each Group

Histological analysis revealed significant differences in cartilage morphology across the experimental groups. The control group exhibited a well-preserved cartilage structure with evenly distributed chondrocytes, clear nuclei, and normal morphology. In contrast, the model group showed substantial cartilage degradation, characterized by a reduced number of chondrocytes, scattered arrangement, wrinkled or ruptured nuclei, and thinning of the cartilage layer. Both acupotomy and Drugs treatments improved cartilage structure, as evidenced by smoother cartilage surfaces, clearer structural layers, and an increased number of chondrocytes with distinct nuclei. Notably, the acupotomy group demonstrated more pronounced improvements compared to the Drugs group (Figure 2).

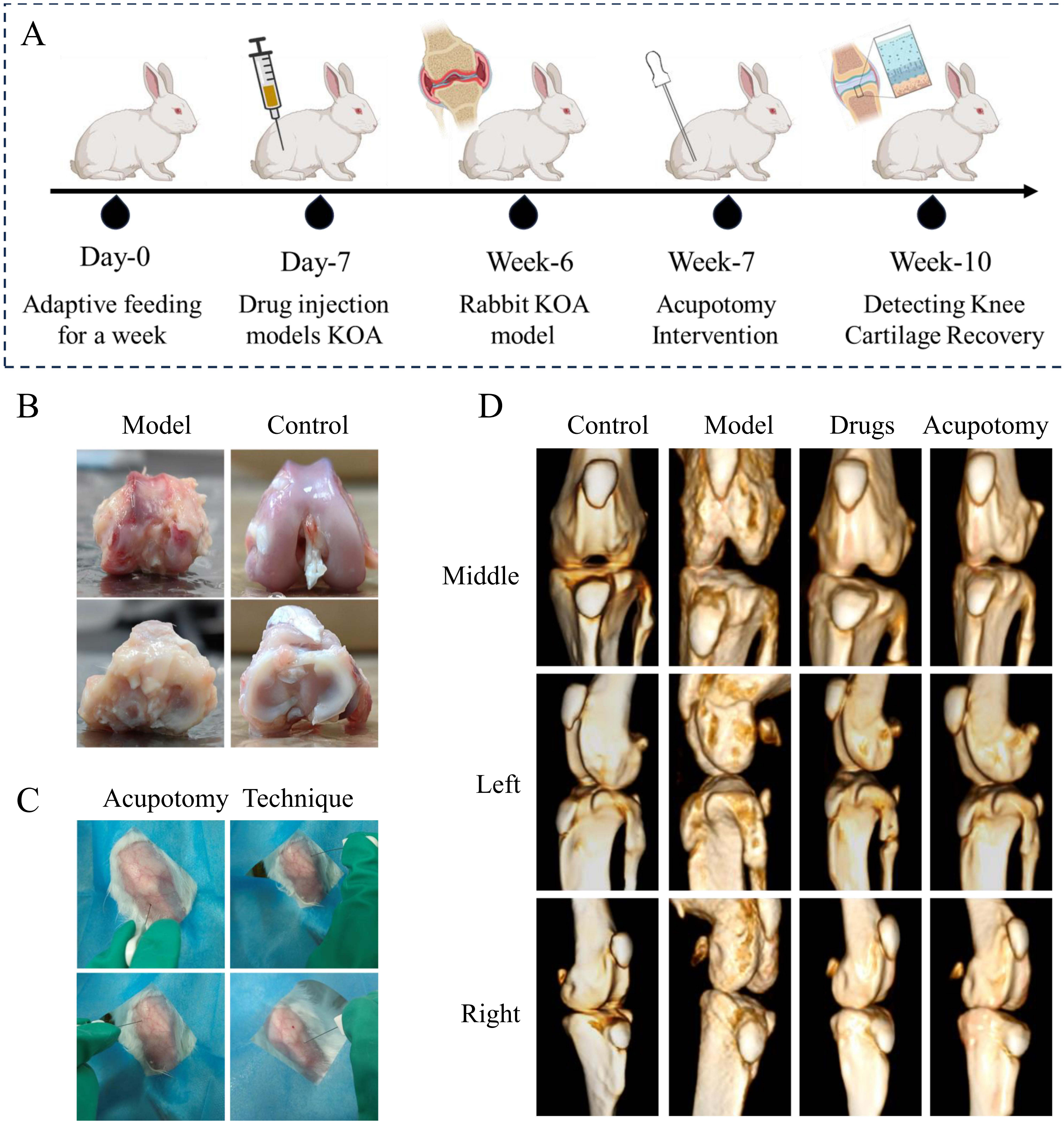

Figure 1 (**A**) Flowchart of animal experiment. (**B**) Gross observation of structural changes in rabbit knee joints after successful modeling. (**C**) Photographic depiction of the acupotomy procedure performed on rabbit knee joints. (**D**) CT images of rabbit knee joints in different experimental groups (Control, Model, Acupotomy, Drug), illustrating differences in joint space width and osteophyte formation.

Expression Levels of NLRP3 Inflammatory Vesicle-Associated mRNA in Cartilage Tissues of Rabbit Knee Joints in Various Groups

Quantitative PCR analysis revealed significant upregulation of NLRP3, GSDMD, caspase-1, IL-1β and ASC mRNA expression in the cartilage tissues of the KOA model group compared to the control group ($P < 0.05$). Both acupotomy and Drugs treatments significantly downregulated the expression of these inflammasome-related genes compared to the model group ($P < 0.05$), with the acupotomy group showing a more pronounced reduction. These findings suggest that acupotomy exerts a stronger regulatory effect on inflammasome activation at the transcriptional level (Figure 3).

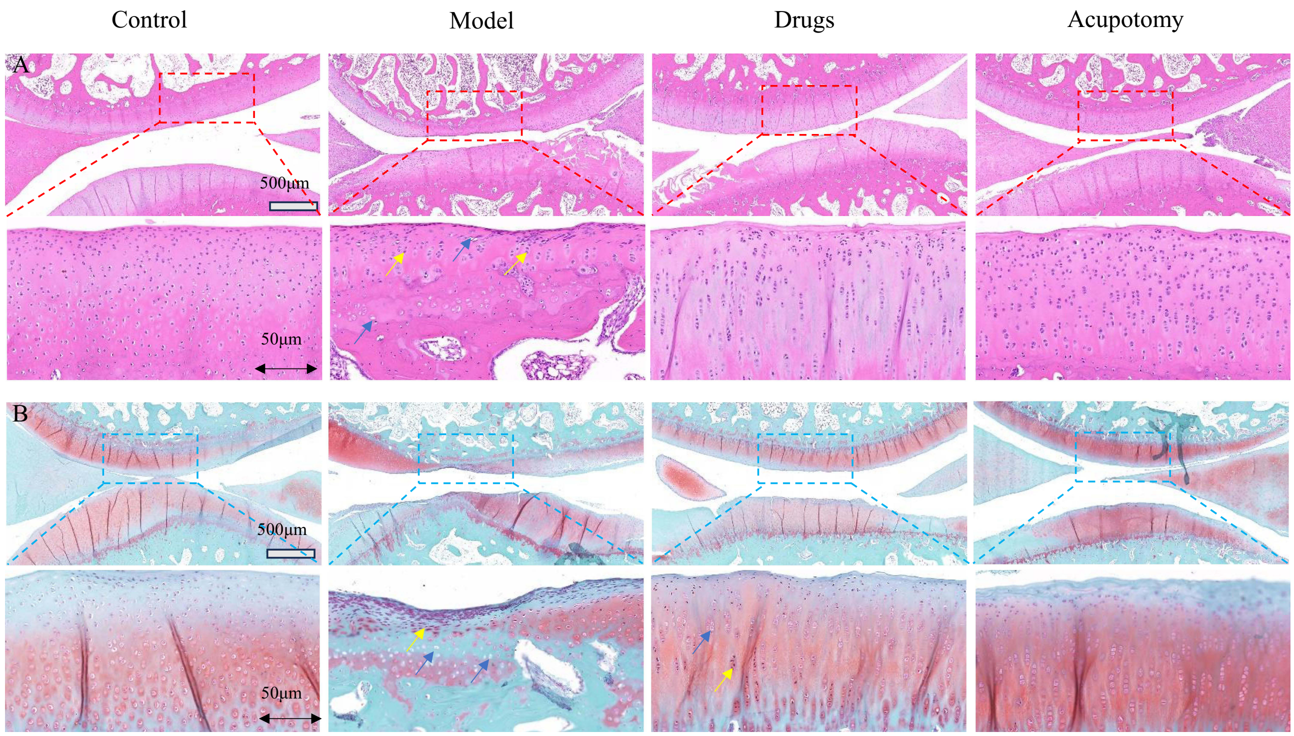

Figure 2 Histopathological analysis of rabbit knee joints. (**A**) Hematoxylin and Eosin (HE) staining of rabbit knee joints. Yellow arrows indicate deeply stained nuclei appearing dark blue-purple, while blue arrows highlight swollen cells with crumpled, broken, or absent nuclei. (**B**) Safranin O-Fast Green Staining of rabbit knee joints. Similar cellular changes are observed, with yellow arrows marking darkly stained nuclei and blue arrows pointing to cellular swelling and nuclear degradation.

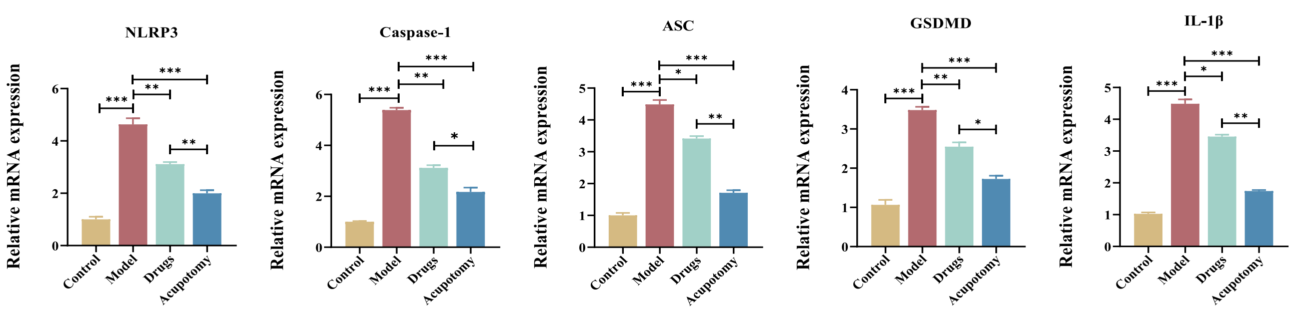

Figure 3 Expression of mRNA related to the NLRP3 inflammasome. (Comparisons with the blank group, n=3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are presented as mean ± standard deviation).
Abbreviations: NLRP3, NOD-like receptor protein 3 antibody; caspase-1, cysteine aspartate protease 1; ASC, apoptosis-associated speck-like protein; GSDMD, gephyrin D.

Expression of NLRP3 Inflammatory Vesicle-Associated Protein in Rabbit Cartilage Tissues of Various Groups

Western blot analysis demonstrated elevated expression of NLRP3, GSDMD, caspase-1, IL-1β and ASC proteins in the cartilage tissues of the KOA model group compared to the control group ($P < 0.05$). Intervention with acupotomy or Drugs significantly reduced the expression levels of these proteins compared to the model group ($P < 0.05$). Consistent with mRNA findings, the acupotomy group exhibited a more substantial reduction in protein expression compared to the Drugs group. These results highlight the potential of acupotomy to modulate inflammasome activity and mitigate cartilage damage in KOA (Figure 4A and B).

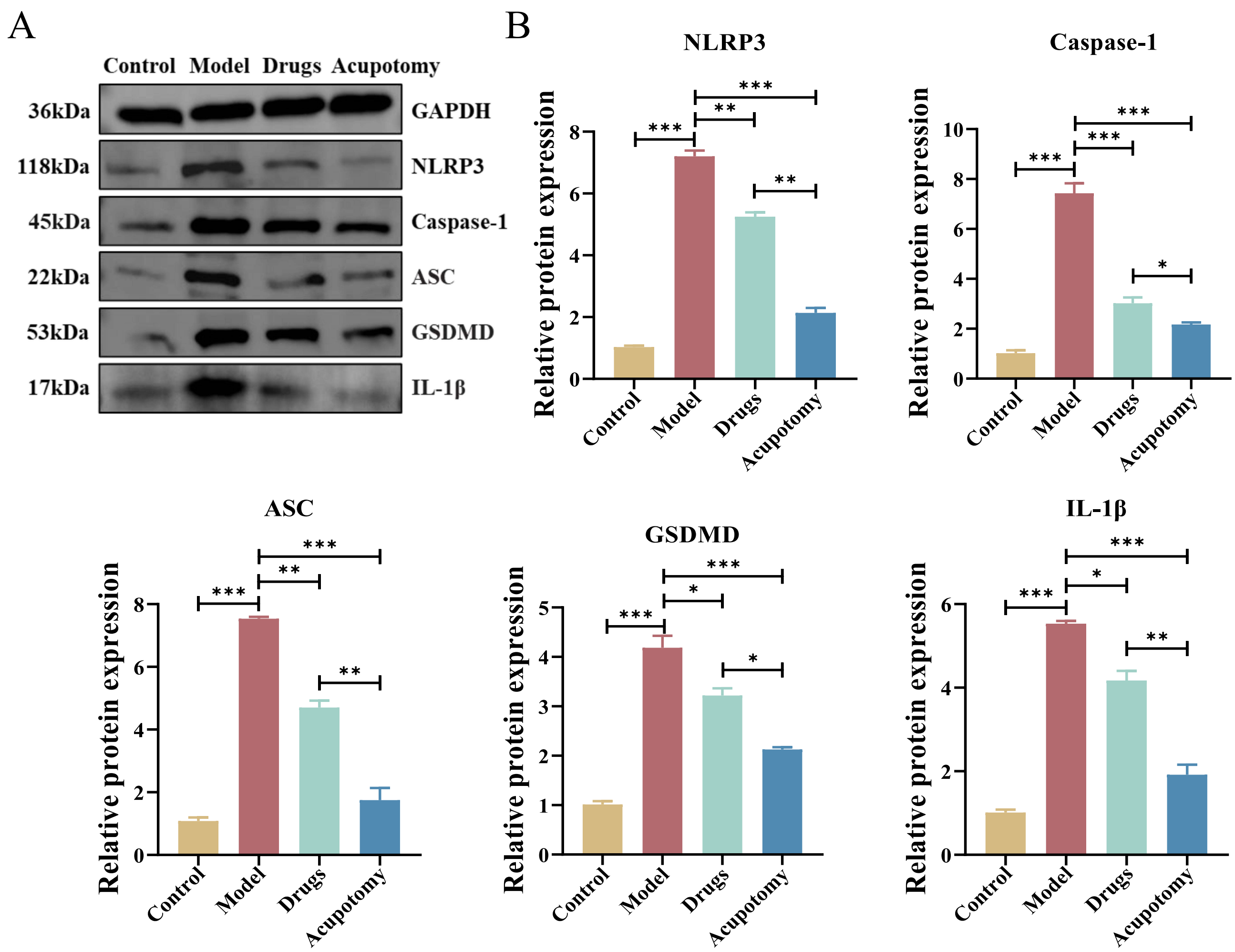

Figure 4 Protein expression levels of NLRP3 inflammasome-related components. (**A**) Western blot analysis of protein bands, (**B**) Quantification of protein expression. (Comparisons with the blank group, n=3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are presented as mean ± standard deviation).

Abbreviations: NLRP3, NOD-like receptor protein 3 antibody; caspase-1, cysteine aspartate protease 1; ASC, apoptosis-associated speck-like protein; GSDMD, desmoplakin D.

Discussion

In Traditional Chinese Medicine (TCM), knee osteoarthritis (KOA) is classified under the category of "Bi Syndrome", specifically manifesting as "knee Bi" or "tendon Bi". TCM views knee pain not merely as a localized symptom but as an external manifestation of systemic dysfunction, involving internal organ imbalance and impaired circulation of qi and blood.[17] The " Huangdi Neijing Suwen - Bi Syndrome" states: "When wind, cold, and dampness combine, they lead to Bi Syndrome", emphasizing that the invasion of pathogenic factors, such as wind, cold, and dampness, along with trauma or overuse, results in qi and blood stagnation, causing pain described as "pain due to obstruction". Additionally, the "Suwen - Theory of Ancient Heavenly Truth" mentions: "By the ages of seven or eight, liver qi declines, tendons become immobile, essence decreases, kidney storage fails, and the body becomes frail". This suggests that aging and weakness, leading to liver and kidney insufficiency, cause the deterioration of sinews and bones, another mechanism of knee pain described as "pain due to insufficiency". These two pathomechanisms—"pain due to obstruction" and "pain due to insufficiency"—are linked to the formation of adhesions, scarring, and striated nodules in the soft tissues surrounding the knee joint.[18,19]

Acupotomy therapy is an acupotomy method that combines the principles of traditional Chinese acupuncture with modern Western anatomy. Through its unique dual action of needles and blades, it effectively loosens and separates adhesions and scar tissue, thereby alleviating pain caused by adhesions.[20] Additionally, this therapy promotes blood supply to the surrounding tissues of the affected area, enhances blood circulation, and accelerates the metabolism of inflammatory substances.[21] This helps to restore the mechanical balance of the tissues around the joints and improve knee joint function. This acupotomy not only targets the lesions directly but also aids in the overall recovery of the patient by improving local blood supply and metabolic conditions.[22] It exemplifies the integration of the holistic perspective and localized acupotomy wisdom in traditional Chinese medicine. Therefore, further investigation into the underlying mechanisms of acupotomy therapy is crucial for advancing its application in the clinical acupotomy of KOA.

KOA is characterized by structural changes throughout the knee joint, including progressive degeneration of articular cartilage, subchondral bone sclerosis, osteophyte formation, inflammation, abnormal angiogenesis, and inward growth of subchondral bone nerves.[1,23] The pathogenesis of KOA is complex and involves various factors, including cartilage degradation, subchondral bone remodeling, ectopic bone formation, joint capsule thickening, and synovitis.[24] Inflammatory response plays a key role in the pathological process of KOA. Emerging evidence suggests that pro-inflammatory cytokines and inflammatory responses lead to overproduction of catabolic enzymes,[25] further exacerbating the metabolic imbalance of chondrocytes during the progression of osteoarthritis. Numerous studies have highlighted the role of IL-1βin the development of OA and the involvement of NLRP3-mediated inflammatory vesicles in the promotion of various articular compartments has attracted much attention. Pyroptosis is a pro-inflammatory programmed cell death mechanism.[26,27] It is characterised by cell swelling and rupture, cell membrane cleavage, release of cytoplasmic contents into extracellular compartments and chromosomal DNA breaks.[28] A common activation of pyroptosis is mediated by the NLRP3 inflammasome, which consists of NLRP3, pyroptosis-associated speck-like protein (ASC) containing the cystatin recruitment domain, and Caspase-1.[29] In this activation pathway, NLRP3 is activated in response to intra- and extra-cellular stimuli, which in turn leads to the recruitment of ASC, binding to Caspase-1, and the formation of NLRP3 inflammasome. This activated NLRP3 inflammatory vesicles further activate Caspase-1, causing it to cleave interleukin 1β (IL-1β) precursors, promoting their maturation and secretion.[30] In addition, activated Caspase-1 releases its active N-terminal structural domain by cleaving GSDMD proteins, which translocates to the cytoplasmic membrane and promotes cell swelling and enlarges the pores in the cell membrane, which ultimately leads to the rupture of the cell membrane and the release of IL-1β and IL-18.[31] Large amounts of IL-1β are released extracellularly, which in turn damages knee chondrocytes and promotes the process of KOA.NLRP3-mediated chondrocyte pyroptosis has been accepted as a form of cell death during the pathogenesis of KOA.[32]

The results of this experiment indicated that acupotomy therapy alleviates nodules and adhesions around the joint, reduces abnormal traction of quadriceps muscle fibers as well as the patella, and regulates stress distribution around the joint. This therapy can significantly improve knee joint function and reduce pain. After acupotomy, the number of osteophytes on the surface of the rabbit knee joint decreased; the cartilage layer was relatively smooth and well-structured, with an increased number of chondrocytes arranged in an orderly manner, and serum inflammatory factor levels also decreased. The expression levels of NLRP3, caspase-1, ASC, and GSDMD mRNA and proteins in cartilage tissue were significantly downregulated, indicating that the mechanical cutting and loosening effects of the acupotomy on striated nodular lesions convert mechanical energy into thermal energy, which accelerates blood circulation and the metabolism of inflammatory substances, reduces local knee joint pain, and downregulates the expression of NLRP3 inflammasome-related genes to inhibit chondrocyte pyroptosis, thereby achieving effective therapeutic outcomes.

Conclusion

In summary, acupotomy release therapy significantly improved motor symptoms and behavioral abilities in KOA model rabbits by downregulating the expression of inflammasome-associated proteins, attenuating the inflammatory response, and reducing chondrocyte pathological damage. Acupotomy therapy can repair cartilage damage by mediating chondrocyte pyroptosis and reducing the release of inflammatory mediators in the knee joint. This finding provides a new theoretical basis and therapeutic approach for treating KOA with acupotomy therapy. Given the complex pathological mechanisms of KOA, future studies should explore combining acupotomy therapy with other therapeutic strategies to achieve a more comprehensive and in-depth understanding of KOA acupotomy.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request. All data will be made available upon request.

Author Contributions

All authors played an important role in the conception of the article, research design, data collection, analysis, interpretation, writing, revision, and critical review, thus ensuring the accuracy and reliability of the research results. Each author reviewed the final published manuscript and agreed to submit it to the Journal of Pain Research. Authors accept reviews and are responsible for all aspects of the research.

Funding

Supported by the National Natural Science Foundation of China (No.81774436), Major Project of Scientific Research of Anhui Provincial Department of Education (2022AH040074), Yangtze River Delta Famous Chinese Medicine Workshop (Zhan Hongsheng's Workshop) [Secret of the Development of Traditional Chinese Medicine in Anhui (2023) No.16].

Disclosure

The authors report no conflicts of interest in this work.

References

1. Zheng X, Qiu J, Gao N, et al. Paroxetine attenuates chondrocyte pyroptosis and inhibits osteoclast formation by inhibiting NF-κB pathway activation to delay osteoarthritis progression. *Drug Des Devel Ther*. 2023;17:2383–2399. doi:10.2147/dddt.S417598
2. Liew JW, King LK, Mahmoudian A, et al. A scoping review of how early-stage knee osteoarthritis has been defined. *Osteoarthritis Cartilage*. 2023;31(9):1234–1241. doi:10.1016/j.joca.2023.04.015
3. Dong Y, Yan Y, Zhou J, Zhou Q, Wei H. Evidence on risk factors for knee osteoarthritis in middle-older aged: a systematic review and meta analysis. *J Orthopaedic Surg Res*. 2023;18(1):634. doi:10.1186/s13018-023-04089-6
4. Arden NK, Perry TA, Bannuru RR, et al. Non-surgical management of knee osteoarthritis: comparison of ESCEO and OARSI 2019 guidelines. *Nat Rev Rheumatol*. 2021;17(1):59–66. doi:10.1038/s41584-020-00523-9
5. Xu W, Xiao Y, Zhao M, et al. Effective treatment of knee osteoarthritis using a nano-enabled drug acupuncture technology in mice. *Adv Sci*. 2023;10(28):e2302586. doi:10.1002/advs.202302586
6. Chen C, Liu D, Guo SX, et al. Clinical effects of ultrasound-guided acupotomy in knee osteoarthritis treatment. *J Visualized Exp*. 2024;(206). doi:10.3791/66587
7. Wang C, Zhu JC, Zheng ZW, et al. [Effects of acupotomy on partial movement gait and serum tumor necrosis factor-α, interleukin-1β in patients with knee osteoarthritis]. *Zhongguo gu shang*. 2022;35(9):848–852. doi:10.12200/j.issn.1003-0034.2022.09.009
8. Zhu J, Zheng Z, Liu Y, et al. The effects of small-needle-knife therapy on pain and mobility from knee osteoarthritis: a pilot randomized-controlled study. *Clin Rehabil*. 2020;34(12):1497–1505. doi:10.1177/0269215520938852
9. Lu M, Meng DH, She ZY, et al. Promotion and mechanism of acupotomy on chondrocyte autophagy in knee osteoarthritis rabbits. *Chin J Integr Med*. 2024;30(9):809–817. doi:10.1007/s11655-024-3759-8
10. Xilin C, Yan G, Juan LU, et al. Acupotomy ameliorates subchondral bone absorption and mechanical properties in rabbits with knee osteoarthritis by regulating bone morphogenetic protein 2-Smad1 pathway. *J Tradi Chin Med*. 2023;43(4):734–743. doi:10.19852/j.cnki.jtcm.20230404.001
11. Ge W, Zhang X, Wang Q, et al. Dicoumarol attenuates NLRP3 inflammasome activation to inhibit inflammation and fibrosis in knee osteoarthritis. *Mol Med Rep*. 2024;29(6). doi:10.3892/mmr.2024.13224
12. An F, Sun B, Liu Y, et al. Advances in understanding effects of miRNAs on apoptosis, autophagy, and pyroptosis in knee osteoarthritis. *Mol Genet Genomic*. 2023;298(6):1261–1278. doi:10.1007/s00438-023-02077-3
13. Tang H, Gong X, Dai J, et al. The IRF1/GBP5 axis promotes osteoarthritis progression by activating chondrocyte pyroptosis. *J Orthop Transl*. 2024;44:47–59. doi:10.1016/j.jot.2023.11.005
14. Zhao LR, Xing RL, Wang PM, et al. NLRP1 and NLRP3 inflammasomes mediate LPS/ATP-induced pyroptosis in knee osteoarthritis. *Mol Med Rep*. 2018;17:5463–5469. doi:10.3892/mmr.2018.8520
15. Zhang W, Zhang L, Yang S, et al. Electroacupuncture ameliorates knee osteoarthritis in rats via inhibiting NLRP3 inflammasome and reducing pyroptosis. *Molecular Pain*. 2023;19:1–12. doi:10.1177/17448069221147792
16. Xing L, Chen X, Guo C, et al. Electroacupuncture exerts chondroprotective effect in knee osteoarthritis of rabbits through the mitophagy pathway. *Journal of Pain Research*. 2023;16:2871–2882. doi:10.2147/jpr.S416242
17. Weiwei M, Mei D, Juan L, et al. Electroacupuncture improves articular microcirculation and attenuates cartilage hypoxia in a male rabbit model of knee osteoarthritis. *J Trad Complementary Med*. 2024;14(4):414–423. doi:10.1016/j.jtcme.2024.01.002
18. Lu MY, Yang YH, Wu X, et al. [Effect of needle-knife on chondrocyte apoptosis of knee joint in rabbits with knee osteoarthritis based on CircSERPINE2-miR-1271-5P-ERG axis]. *Zhongguo Zhen Jiu*. 2023;43(4):447–453. doi:10.13703/j.0255-2930.20220411-k0001
19. Liu J, Zeng WQ, Lin QX, et al. [Acupotomy relieves pain and improves motor function by regulating autophagy and apoptosis of cartilage in rabbits with knee osteoarthritis apoptosis]. *Zhen ci yan jiu*. 2022;47(12):1080–1087. doi:10.13702/j.1000-0607.20211184
20. LongFei X, Yan G, XiLin C, et al. Application of acupotomy in a knee osteoarthritis model in rabbit. *J Visualized Exp*. 2023;(200). doi:10.3791/65584

21. Luxue Q, Changqing G, Ruili Z, et al. Acupotomy inhibits aberrant formation of subchondral bone through regulating osteoprotegerin/receptor activator of nuclear factor-κB ligand pathway in rabbits with knee osteoarthritis induced by modified Videman method. *J Trad Chin Med*. 2022;42(3):389–399. doi:10.19852/j.cnki.jtcm.2022.03.006
22. Wenting Z, Changqing G, Mei DU, et al. Acupotomy alleviates knee osteoarthritis in rabbit by regulating chondrocyte mitophagy Pink1-Parkin pathway. *J Trad Chin Med*. 2024;44(3):468–477. doi:10.19852/j.cnki.jtcm.20240402.001
23. Tao T, Shi MP, Tan BY, et al. Evaluation of traditional Chinese exercise for knee osteoarthritis (KOA): an overview of systematic reviews. *Syst Rev*. 2024;13(1):187. doi:10.1186/s13643-024-02606-0
24. Zhou J, Wu J, Fu F, et al. α-Solanine attenuates chondrocyte pyroptosis to improve osteoarthritis via suppressing NF-κB pathway. *J Cell Mol Med*. 2024;28(4):e18132. doi:10.1111/jcmm.18132
25. Hu H, Yang B, Li Y, Zhang S, Li Z. Blocking of the P2X7 receptor inhibits the activation of the MMP-13 and NF-κB pathways in the cartilage tissue of rats with osteoarthritis. *IntJ Mol Med*. 2016;38(6):1922–1932. doi:10.3892/ijmm.2016.2770
26. Hu J, Zhou J, Wu J, et al. Loganin ameliorates cartilage degeneration and osteoarthritis development in an osteoarthritis mouse model through inhibition of NF-κB activity and pyroptosis in chondrocytes. *J Ethnopharmacol*. 2020;247:112261. doi:10.1016/j.jep.2019.112261
27. Zhang Z, Fu F, Bian Y, et al. α-Chaconine facilitates chondrocyte pyroptosis and nerve ingrowth to aggravate osteoarthritis progression by activating NF-κB signaling. *J Inflamm Res*. 2022;15:5873–5888. doi:10.2147/jir.S382675
28. Rao Z, Zhu Y, Yang P, et al. Pyroptosis in inflammatory diseases and cancer. *Theranostics*. 2022;12(9):4310–4329. doi:10.7150/thno.71086
29. Huang Y, Xu W, Zhou R. NLRP3 inflammasome activation and cell death. *Cell Mol Immunol*. 2021;18(9):2114–2127. doi:10.1038/s41423-021-00740-6
30. Fu J, Wu H. Structural mechanisms of NLRP3 inflammasome assembly and activation. *Ann Rev Immunol*. 2023;41:301–316. doi:10.1146/annurev-immunol-081022-021207
31. Liu W, Liu A, Li X, et al. Dual-engineered cartilage-targeting extracellular vesicles derived from mesenchymal stem cells enhance osteoarthritis treatment via miR-223/NLRP3/pyroptosis axis: toward a precision therapy. *Bioact Mater*. 2023;30:169–183. doi:10.1016/j.bioactmat.2023.06.012
32. Tong L, Yu H, Huang X, et al. Current understanding of osteoarthritis pathogenesis and relevant new approaches. *Bone Res*. 2022;10(1):60. doi:10.1038/s41413-022-00226-9