

α -Chaconine Facilitates Chondrocyte Pyroptosis and Nerve Ingrowth to Aggravate Osteoarthritis Progression by Activating NF- κ B Signaling

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Background: With the rapid growth of the elderly population, the incidence of osteoarthritis (OA) increases annually, which has attracted extensive attention in public health. The roles of dietary intake in controlling joint disorders are perhaps one of the most frequently posed questions by OA patients, while the information about the interaction between dietary intake and OA based on scientific research is limited. α -Chaconine is the richest glycoalkaloid in eggplants such as potatoes. Previous evidence suggests that α -Chaconine is a toxic compound to nervous and digestive systems with potentially severe and fatal consequences for humans and farm animals, but its effect on OA development remains obscure.

Objective: To determine whether α -Chaconine deteriorates OA progression through sensory innervation and chondrocyte pyroptosis via regulating nuclear factor- κ B (NF- κ B) signaling, providing evidence for a possible linkage between α -Chaconine and OA progression.

Methods: We established a mouse OA model by destabilization of medial meniscus (DMM) surgery and then intra-articular injection of 20 or 100 μ M α -Chaconine into the OA mice for 8 and 12 weeks. The severity of OA progression was evaluated by histological staining and radiographic analyses. The expressions of matrix metabolic indicators, Col2, Mmp3, and Mmp13, as well as pyroptosis-related proteins, Nlrp3, Caspase-1, Gsdmd, IL-1 β , IL-18, were determined by immunohistochemistry. And the changes in sensory nerve ingrowth and activity of NF- κ B signaling were determined by immunofluorescence.

Results: We found that α -Chaconine could exacerbate mouse OA progression, resulting in subchondral sclerosis, osteophyte formation, and higher OARS scores. Specifically, α -Chaconine could augment cartilage matrix degradation and induce chondrocyte pyroptosis and nerve ingrowth. Mechanistical analysis revealed that α -Chaconine stimulated NF- κ B signaling by promoting I- κ B α phosphorylation and p65 nuclear translocation.

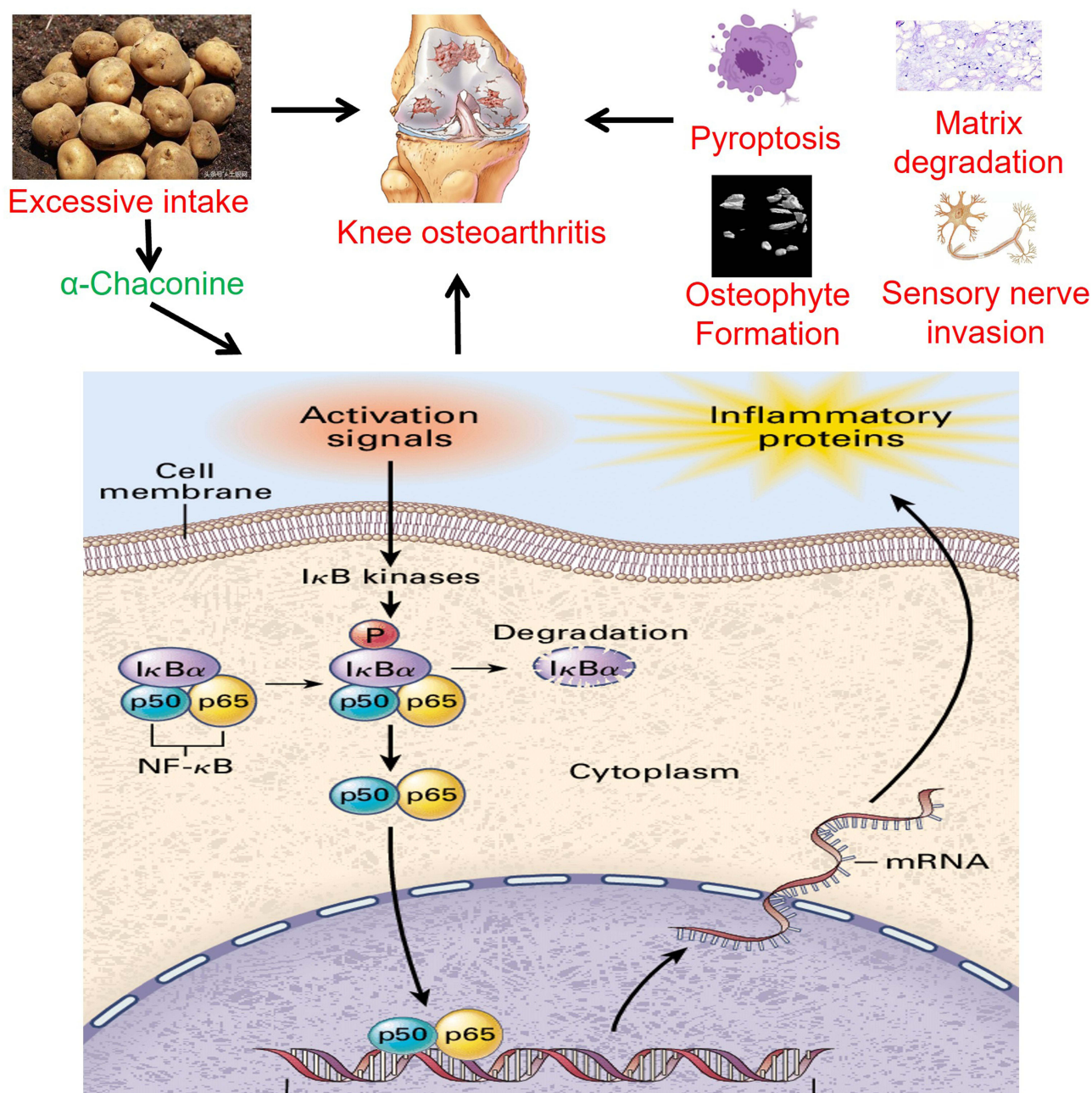
Conclusion: Collectively, our findings raise the possibility that α -Chaconine intake can boost chondrocyte pyroptosis and nerve ingrowth to potentiate OA progression by activating NF- κ B signaling.

Keywords: α -Chaconine, osteoarthritis, pyroptosis, nerve ingrowth, NF- κ B signaling

Introduction

Osteoarthritis (OA) is a major public health issue worldwide affecting 250 million people.^{1,2} Due to aging of the population, the prevalence of OA is continually increasing, leading to a great burden on health care system.³ Dramatic increase in the prevalence of OA in developed nations over the past half-century provides important clues as to which

Graphical Abstract



contributors are responsible for OA development, with obesity, metabolic syndrome, dietary changes, dysbiotic gut microbiome, and physical inactivity as the main conspicuous candidates,^{4,5} strongly implicating those vicious contributors of OA may be derived from dietary intake. Questions regarding the role of dietary intake in controlling OA are perhaps one of the most frequently posed ones by OA patients, while the information about the interaction between dietary intake and OA based on scientific research remains limited.⁶

Glycoalkaloids are present in many plants of the Solanaceae family (also known as nightshade plants), particularly in potatoes, and are considered part of the eggplant's defense system against insects and pests.⁷ α -Chaconine and α -solanine

account for 95% of total glycoalkaloids, and the content of α -Chaconine in potato is generally slightly higher than that of α -solanine (the ratio of α -chaconine to α -solanine ranges from 1.2 to 2.6).⁸ Increasing evidence suggests that glycoalkaloids have a variety of adverse as well as beneficial effects in cells, animals, and humans, which poses potential food safety concerns.⁹ A significant positive correlation has been documented between serum glycoalkaloid levels and normal dietary intake of potatoes by the individual concerned.⁷ And the accumulation of glycoalkaloids has been reported to be associated with OA-related swelling, pain and stiffness, whereas many OA patients do report symptom relief after the removal of potatoes from the diet.^{10–12} Moreover, high levels (3–6 mg/kg body weight) intake of glycoalkaloids can lead to acute intoxication, concomitant with vomiting, diarrhea, internal haemorrhage and abdominal pain followed by fever, weak pulse, in severe cases coma and death.^{13,14} Based on the above results, we hypothesize that α -Chaconine may be responsible for the aggravation of OA progression in patients with potato intake.

Persistent pain becomes a dominant clinical symptom for OA patients and a major driver of medical consultation and health service use.² OA is a whole-joint disease, involving all structural changes of joint tissues, including articular cartilage, subchondral bone, synovial tissue, meniscus, ligaments and infrapatellar fat pad, etc.^{15–17} An increasing body of evidence suggests that various pathogenesis associated with OA could worsen in combination, including cartilage degradation, subchondral bone sclerosis, nerve innervation, meniscal degeneration, synovial inflammation, inflammation and fibrosis of the infrapatellar fat pad.^{18–22} As OA disease advances, ongoing inflammatory stimulation and progressive damage to local tissues may affect the afferent processing of nociceptive signals from joints and surrounding tissues, resulting in pain sensitivity.^{23–25}

Pyroptosis is a type of proinflammatory programmed cell death triggered by inflammation involving the activation of nod-like receptor protein-3 (NLRP3)/Caspase-1, which cleave precursors of IL-1 β and IL-18 to promote their maturation and secretion.²⁶ Recent studies have shown that Nlrp3-mediated inflammasome contributes to chondrocyte pyroptosis and OA development through activation of nuclear factor- κ B (NF- κ B) signaling.²⁷ Besides, increasing evidence suggests that aberrant subchondral bone remodeling such as sensory nerve innervation contributes directly or indirectly to cartilage destruction, pain, and OA progression.^{20,28,29} By using biochemical and genetic approaches, Zhu et al reported that, in the context of OA progression, osteoclasts secrete axonal guidance molecules, Netrin-1, to induce calcitonin gene-related peptide-positive (Cgrp⁺) sensory nerve axonal growth in subchondral bone; and specific ablation of *Netrin-1* in osteoclasts reduces Cgrp⁺ sensory neurons in subchondral bone and alleviates OA pain behavior.¹⁸

NF- κ B family consists of five members: c-Rel, RelB, p52/p100, p50/p105, and RelA (p65).³⁰ Generally, p65 heterodimer is sequestered in cytoplasm in an inactive state complexed with inhibitor- κ B (I- κ B α). Once stimulated by inflammatory mediators or mechanical symbols, I- κ B kinase phosphorylates I- κ B α at Ser32 and Ser36 followed by proteasome-mediated degradation. It is widely acknowledged that NF- κ B signaling has been well affirmatory as the major calibrator of inflammatory responses in OA.^{30,31} Activation of NF- κ B signaling leads to upregulation of matrix metalloproteinases (Mmps) and decrease of Collagen 2 (Col2), eventually contributing to extracellular matrix degradation and triggering chondrocyte pyroptosis during OA onset and progression.³² Meanwhile, NF- κ B signaling has been documented to regulate osteoclast formation, differentiation, and functions in subchondral bone as well as Netrin-1 expression.³³ Beyond that, gene-profiling experiments performed in glycoalkaloid-treated intestinal epithelial cell-line Caco-2 suggest that NF- κ B signaling is significantly enriched by multiple targets induced by glycoalkaloid administration.³⁴ Accordingly, it is reasonable to speculate that NF- κ B signaling may mediate the roles of α -Chaconine on OA pathogenesis.

The present study aimed to explore the potential relationship between α -Chaconine treatment and OA development, based on destabilization of medial meniscus (DMM) surgery-induced OA model, thereby providing deep insights into the effects of α -Chaconine intake on human and animal health.

Materials and Methods

Chemicals and Reagents

α -Chaconine powder (99.67% of purity) was purchased from ChromaDex (Irvine, CA, USA). A voucher specimen (ASB-00003371-005) has been deposited at the First Affiliated Hospital of Zhejiang Chinese Medical University. Primary

antibodies against collagen 2 (Col2), matrix metalloproteinase 3 (Mmp3), matrix metalloproteinase 13 (Mmp13), Nlrp3, Caspase-1, Gasdermin D (Gsdmd), IL-1 β , IL-18, and phospho-i- κ B (p-i- κ B α) were purchased from Ruiying Biological Co. (Jiangsu, China). Primary antibodies against p65 and phospho-p65 (p-p65) were purchased from Cell Signaling Technology (Beverly, MA, USA). Gsdmd, Cgpr, and Netrin-1 were purchased from Abcam (Cambridge, MA, USA). Fluorescent secondary antibodies were purchased from Sungene Biotech Co. (Tianjin, China). Unless otherwise mentioned, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals and DMM-Induced OA Model

Eight-week-old male C57BL/6J mice (18–22 g) were provided by the animal experiment center of Zhejiang Chinese Medical University (Grade SPF, SCXK (Shanghai): 2017–0005). All mice were raised within a specific pathogen-free animal care facility and housed in a room at $23 \pm 2^\circ\text{C}$ with a 12 h-12 h light/dark cycle, and mice had *ad libitum* access to water and lab chow. All mouse procedures were approved by the Ethics Committee on Animal Experimentation of Zhejiang Chinese Medical University (ZSLL-2018-012).

Experimental Design of Animal Studies

All mice were allowed to acclimatize for 1-week before surgery. Forty mice were randomly and equally divided into four groups ($n = 10$ in each group): Sham group (Sham), OA model group (Vehicle), low-dose α -Chaconine group (OA mice treated with low-dose α -Chaconine), and high-dose α -Chaconine group (OA mice treated with high-dose α -Chaconine). The experimental mice were subjected to DMM surgery-induced OA model as we previously described.³⁵ Mice in the low- and high-dose α -Chaconine groups were intra-articularly injected with 6 μL of α -Chaconine (20 and 100 μM , respectively) per right knee joint twice a week, starting from day 10 post-DMM surgery, while mice in the Sham group and vehicle group received the same volume of saline. The microinjection was performed with a 10 μL microinjector (Hamilton Company, Reno, NV, USA) and 33#-gauge needles (Hamilton Company). All mice were anesthetized with 1% pentobarbital sodium and sacrificed at 8- and 12-week post-DMM surgery, and all knee joints were collected for further analyses (Figure 1).

Micro Computed Tomography (Micro-CT) Analysis

Before histologic processing, the knee joints were scanned using high-resolution micro-CT equipment (Skyscan1176, Bruker micro-CT N.V., Kontich, Belgium) at a voltage of 50 kV with a current of 500 μA and a resolution of 9 μm per pixel. Image reconstruction and quantitative morphologic analysis were achieved by using NRecon v1.6 and CTAn v1.15, respectively. Three-dimensional visualization images were reconstructed using 3D-creator software, CTVol v2.2. Coronal images of tibial subchondral bone were chosen to evaluate three-dimensional histomorphometric analyses. Bone

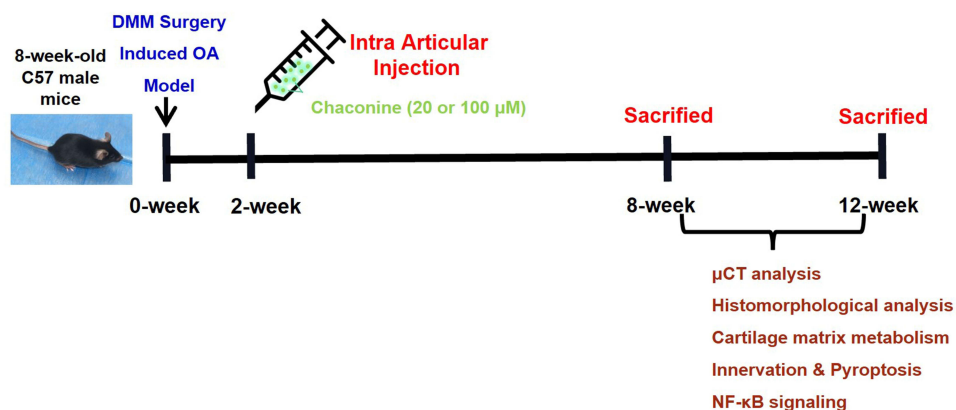


Figure 1 Schematic design of the project.

Abbreviations: DMM Surgery, destabilization of medial meniscus surgery; OA Model, osteoarthritis model; μCT analysis, micro computed tomography analysis.

volume/total volume (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N, 1/mm) on subchondral bone medial compartment were calculated using X-Tek computational algorithms from 50 consecutive images.

Histology Staining

After fixation in 4% paraformaldehyde for 48 h, the knee joints were decalcified with 14% EDTA solution for 21 days, dehydrated by alcohol, embedded in paraffin, and sectioned at 4 μ m. The sections were stained with Safranin O/Fast green. Cartilage destruction in the medial tibial plateau of the joint was scored by two blinded pathologists using the OARSI system, as previously described.³⁶ Osteophyte formation was evaluated semi-quantitatively using osteophyte formation scores, including osteophyte volume and osteophyte maturity.³⁷

Immunohistochemistry (IHC) and Immunofluorescence (IF) Analyses

IHC analysis was performed on 4 μ m-thick sections using SP Link Detection Kits (ZSGB-BIO, Beijing, China), according to the manufacturer's instructions. Briefly, sections were blocked with PBS containing 10% normal goat serum for 30 min, and subsequently incubated with primary antibodies, Col2 (diluted 1:1000), Mmp3 (diluted 1:100), Mmp13 (diluted 1:100), Nlrp3 (diluted 1:100), Caspase-1 (diluted 1:100), Gsdmd (diluted 1:100), IL-1 β (diluted 1:100), IL-18 (diluted 1:100) at 4 °C overnight, respectively. The polymer-HRP labeled secondary antibody was added for 30 min the following day. The immunohistochemical reaction was visualized by incubation with 0.05% diaminobenzidine (DAB). Positive staining appeared in brown. Images were captured with a microscope (Carl Zeiss, Göttingen, Germany). For IF assay, sections were incubated with primary antibodies, including Cgrp (diluted 1:200), Netrin-1 (diluted 1:200), p-i-kb α (diluted 1:200), p65 (diluted 1:200) at 4°C overnight, then treated with fluorescent-conjugated secondary-antibody (Sungene Biotech, Tianjin, China) for 30 min in the dark. After DAPI counterstaining, the images were captured with a fluorescence microscope (Carl Zeiss, Göttingen, Germany). The number of positive cells was quantified in a blinded manner with Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA) as we previously described.³⁸

Statistical Analysis

All numerical data were presented as mean \pm SD. One-way analysis of variance (ANOVA) and *post-hoc* Tukey's test were performed using GraphPad Prism 8 software (GraphPad Software Inc., La Jolla, CA). A *P* value of less than 0.05 was statistically significant.

Results

α -Chaconine Deteriorates OA Progression in DMM Surgery-Induced OA Mice

OA is an age-related degenerative joint disease characterized by progressive articular cartilage destruction, osteophyte formation, and subchondral bone sclerosis et al.³⁹ To investigate the effects of α -Chaconine on OA progression, mice that underwent DMM surgery were intra-articularly injected with two optimized concentrations of α -Chaconine (20 and 100 μ M, concentrations used in the previous in vitro and in vivo studies of α -Chaconine^{40–43}) (Figure 1). The morphological changes of knee joints were evaluated by Safranin O/Fast green staining, and we found that articular cartilage erosion was obviously observed in OA mice, and high-dose α -Chaconine could exacerbate the articular cartilage damage (Figure 2A). The mouse OA progression was further assessed by the OARSI score system, and we found that high-dose α -Chaconine treatment aggravated increased OARSI scores in DMM surgery-treated mice (Figure 2B). Moreover, the radiographic changes of knee joints were examined using micro-CT analysis. We found that high-dose α -Chaconine could significantly increase osteophyte maturity but have no effect on osteophyte volume (Figure 2C–E). Interestingly and intriguingly, compared with the OA mice, low-dose α -Chaconine could reduce increased BV/TV and Tb.Th in subchondral bone 8- and 12-week post-surgery, while high-dose α -Chaconine further enhanced the above indexes (Figure 2F and G). In addition, high-dose α -Chaconine increased Tb.N in subchondral bone 8-week post-surgery, whereas low-dose α -Chaconine reduced Tb.N 12-week post-surgery (Figure 2H). Our findings suggest that only high-dose α -Chaconine may aggravate OA progression in DMM surgery-induced murine OA model.

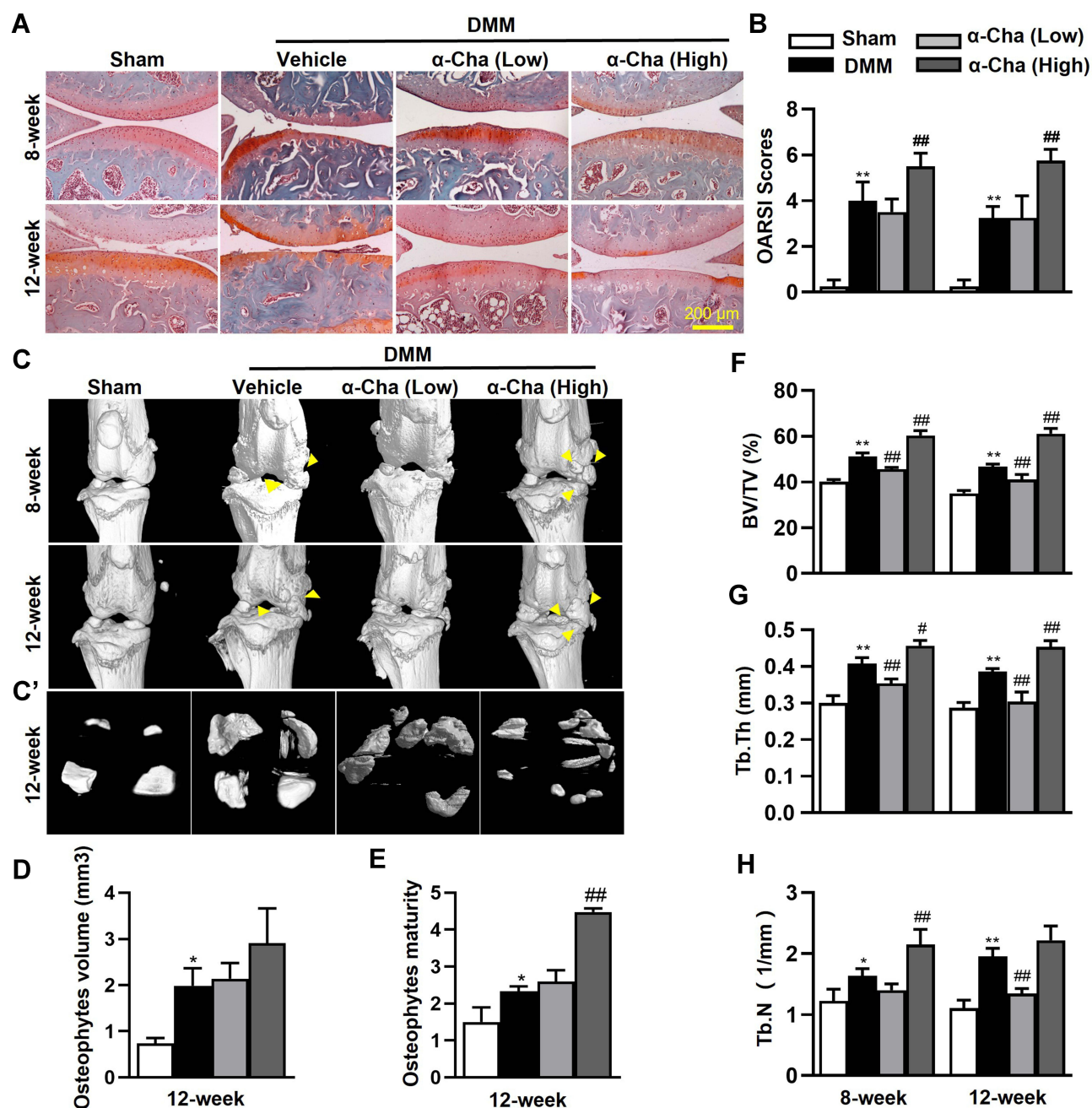


Figure 2 α -Chaconine (α -Cha) deteriorates OA progression. (A) Safranin O/Fast green staining of cartilage. (B) OARSI scores for cartilage structure damage in (A). (C-C') Representative three-dimensional images of knee joints. Three-dimensional reconstructions of knee joints in (C) and osteophytes (C') were used to visualize calcification of meniscus and osteophyte formation 8- and 12-week after DMM surgery. Yellow triangles indicated osteophytes. (D-H) Statistical results of bone morphological parameters in (C). Data were expressed as the mean \pm SD. * $P < 0.05$, ** $P < 0.01$ vs Sham group, # $P < 0.05$, ### $P < 0.01$ vs Vehicle group.

Abbreviations: α -Cha (Low), low-dose α -Chaconine group; α -Cha (High), high-dose α -Chaconine group; BV/TV, bone volume/total volume; Tb.Th, trabecular thickness; Tb.N, trabecular number.

α -Chaconine Accelerates Articular Cartilage Matrix Degradation in OA Mice

Stable matrix content is crucial for maintaining the physiological function of knee joint.⁴⁴ To explore the effects of α -Chaconine on matrix metabolism of OA mice, we determined Col2, Mmp13, and Mmp3 expressions in articular cartilage by IHC analysis. The IHC results showed that, compared with Sham mice, lower Col2 and higher Mmp3 and Mmp13 expressions were observed in OA mice; and high-dose α -Chaconine could further exacerbate these alterations.

Unexpectedly, low-dose α -Chaconine may partially attenuate Mmp13 expression in OA mice (Figure 3A–F). The above results suggest that high-dose α -Chaconine could accelerate matrix degradation in articular cartilage of OA mice.

α -Chaconine Promotes Articular Chondrocytes Pyroptosis in OA Mice

Given the widely accepted role of IL-1 β in OA pathology and the maturation of IL-1 β by inflammasomes, the molecular mechanism for nod-like receptor protein-3 (NLRP3) inflammasome-mediated pyroptosis during OA progression has gained extensive attention.^{35,38} To identify whether α -Chaconine affects articular chondrocyte pyroptosis, we analyzed the expressions of key pyroptosis-related proteins, Nlrp3, Caspase-1, Gsdmd, IL-1 β , and IL-18, in articular cartilage by IHC staining. Consistent with our previous findings,^{35,38} the expression levels of Nlrp3, Caspase-1, Gsdmd, IL-1 β , and IL-18 were significantly increased in OA mice, and high-doses α -Chaconine treatment could further accelerate their increases. Unexpectedly, low-doses α -Chaconine treatment could downregulate these increases to a similar level to the Sham group, except Gsdmd (Figure 4A–J). The above findings indicate that α -Chaconine exposure aggravates chondrocyte pyroptosis-mediated inflammation.

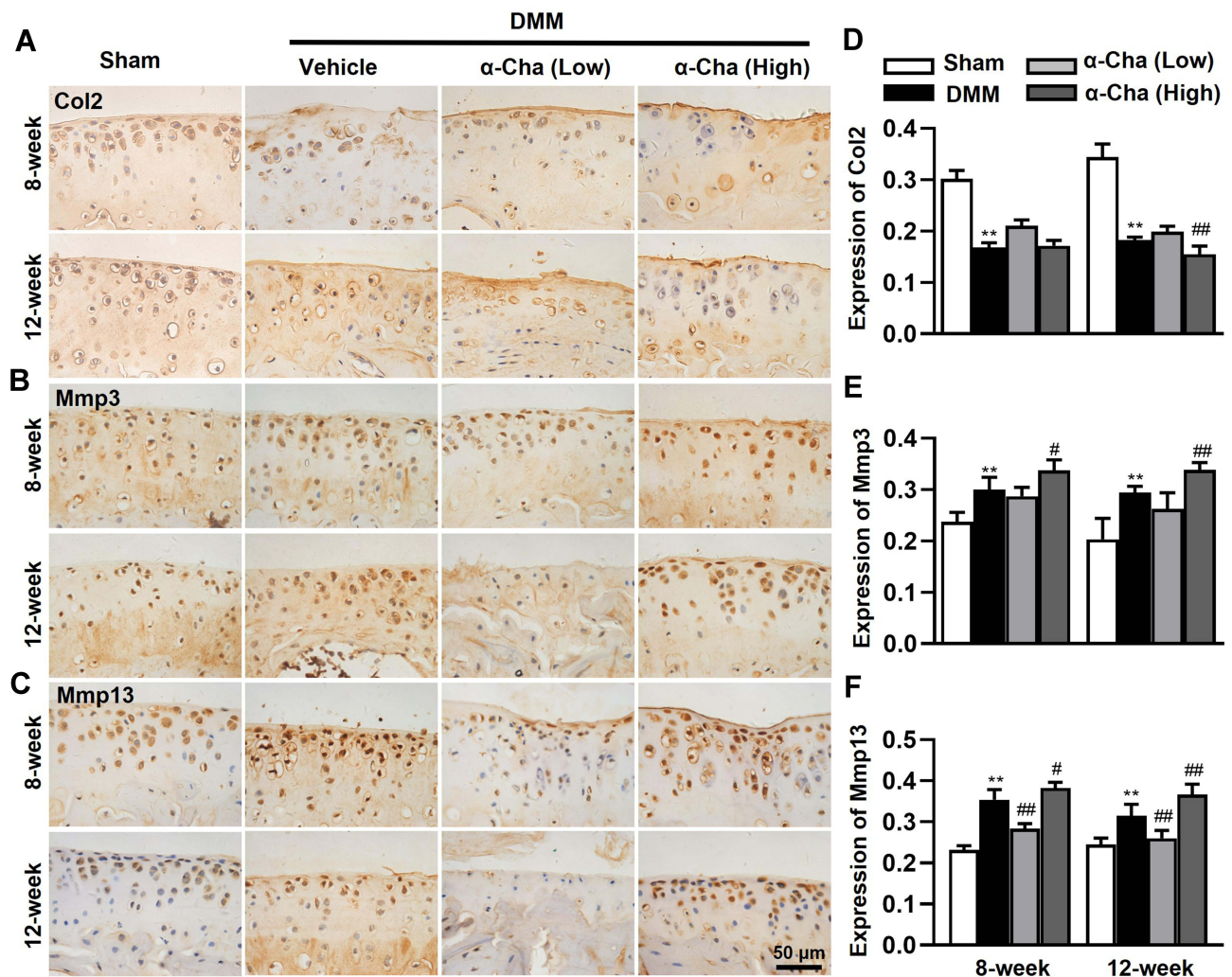


Figure 3 α -Chaconine accelerates articular cartilage matrix degradation. (A–C) Immunohistochemistry results of Col2, Mmp3, and Mmp13 proteins. (D–F) The ratios of immunoreactive positive cells of Col2, Mmp3, and Mmp13 in (A–C). Data were expressed as the mean \pm SD. ** P < 0.01 vs Sham group, # P < 0.05, ### P < 0.01 vs Vehicle group.

Abbreviations: α -Cha (Low), low-dose α -Chaconine group; α -Cha (High), high-dose α -Chaconine group; Col2, collagen 2; Mmp3, matrix metalloproteinase 3; Mmp13, matrix metalloproteinase 13.

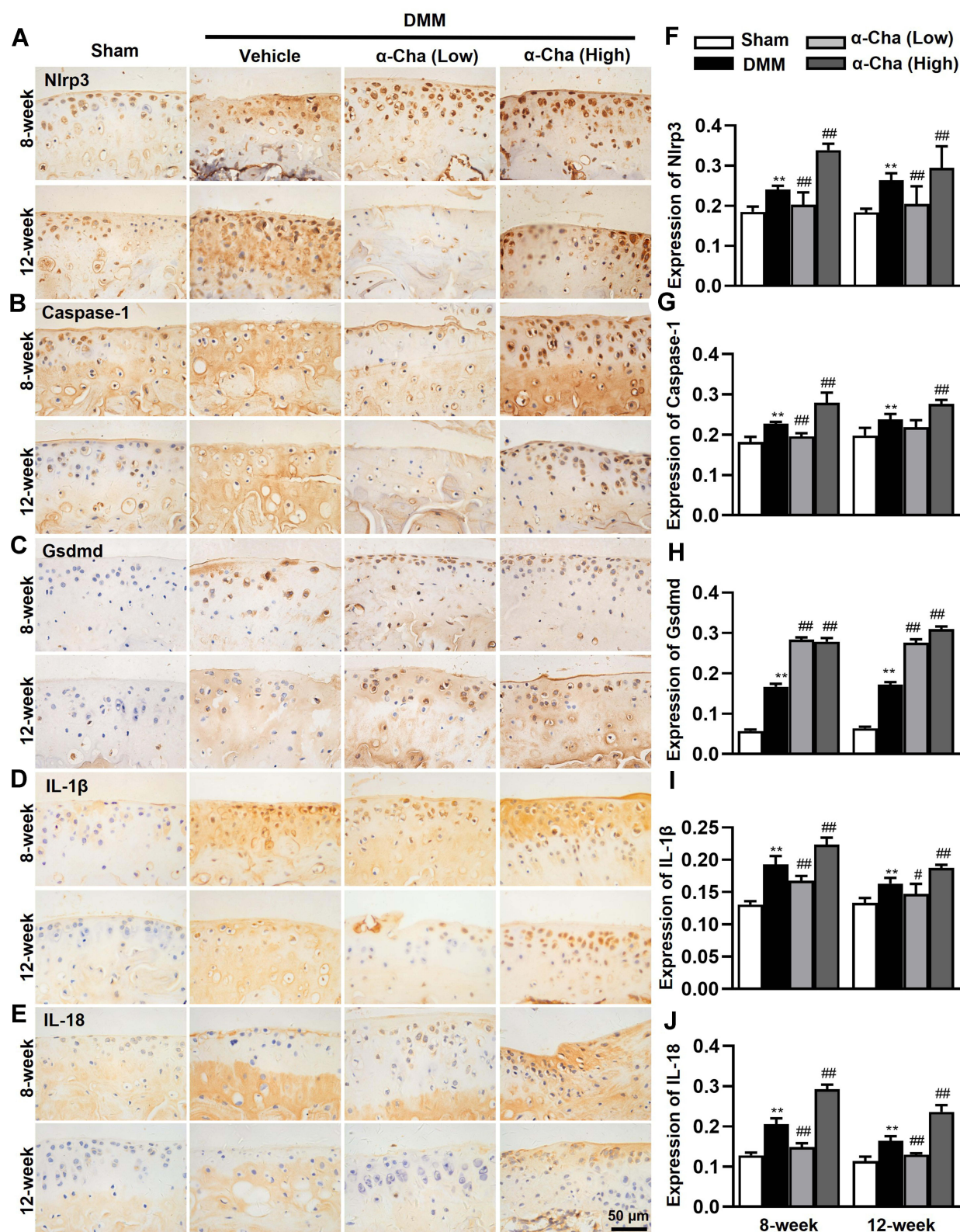


Figure 4 α -Chaonine promotes chondrocyte pyroptosis. (A–E) Immunohistochemistry staining results of Nlrp3, Caspase-1, Gsdmd, IL-1 β , and IL-18 proteins in articular cartilage 8- and 12-week post-DMM surgery. (F–J) The ratios of immunoreactive positive cells of Nlrp3, Caspase-1, Gsdmd, IL-1 β , and IL-18 in (A–E). Data were expressed as the mean \pm SD. ** P < 0.01 vs Sham group, # P < 0.05, ## P < 0.01 vs Vehicle group.

Abbreviations: α -Cha (Low), low-dose α -Chaonine group; α -Cha (High), high-dose α -Chaonine group; Nlrp3, nod-like receptor protein-3; Gsdmd, Gasdermin D.

α -Chaconine Facilitates Nerve Ingrowth in OA Mice

A study carried out in the mouse OA model has demonstrated that secretion of Netrin-1 by osteoclasts is strongly associated with the recruitment of Cgrp⁺ sensory nerve innervation in subchondral bone and hypersensitivity to pain.¹⁸ To evaluate whether α -Chaconine regulates sensory nerve ingrowth in subchondral bone, we examined expression changes in its specific marker, Cgrp, as well as Netrin-1 using IF analysis. Consistent with previous results,^{20,45} DMM surgery resulted in a significantly higher number of Cgrp⁺ neurons in cartilage and subchondral bone of OA mice (Figure 5A, C and E). Notably, α -Chaconine administration substantially elevated Cgrp⁺ nerve cell ratio in subchondral bone of OA mice in a dose-dependent manner (Figure 5E). Similarly, an enhanced expression of Netrin-1 was found in cartilage and subchondral bone of DMM surgery-treated mice, and α -Chaconine-treated mice had a higher expression of Netrin-1 than OA mice (Figure 5B, D and F). Based on the above results, we speculated that α -Chaconine could stimulate

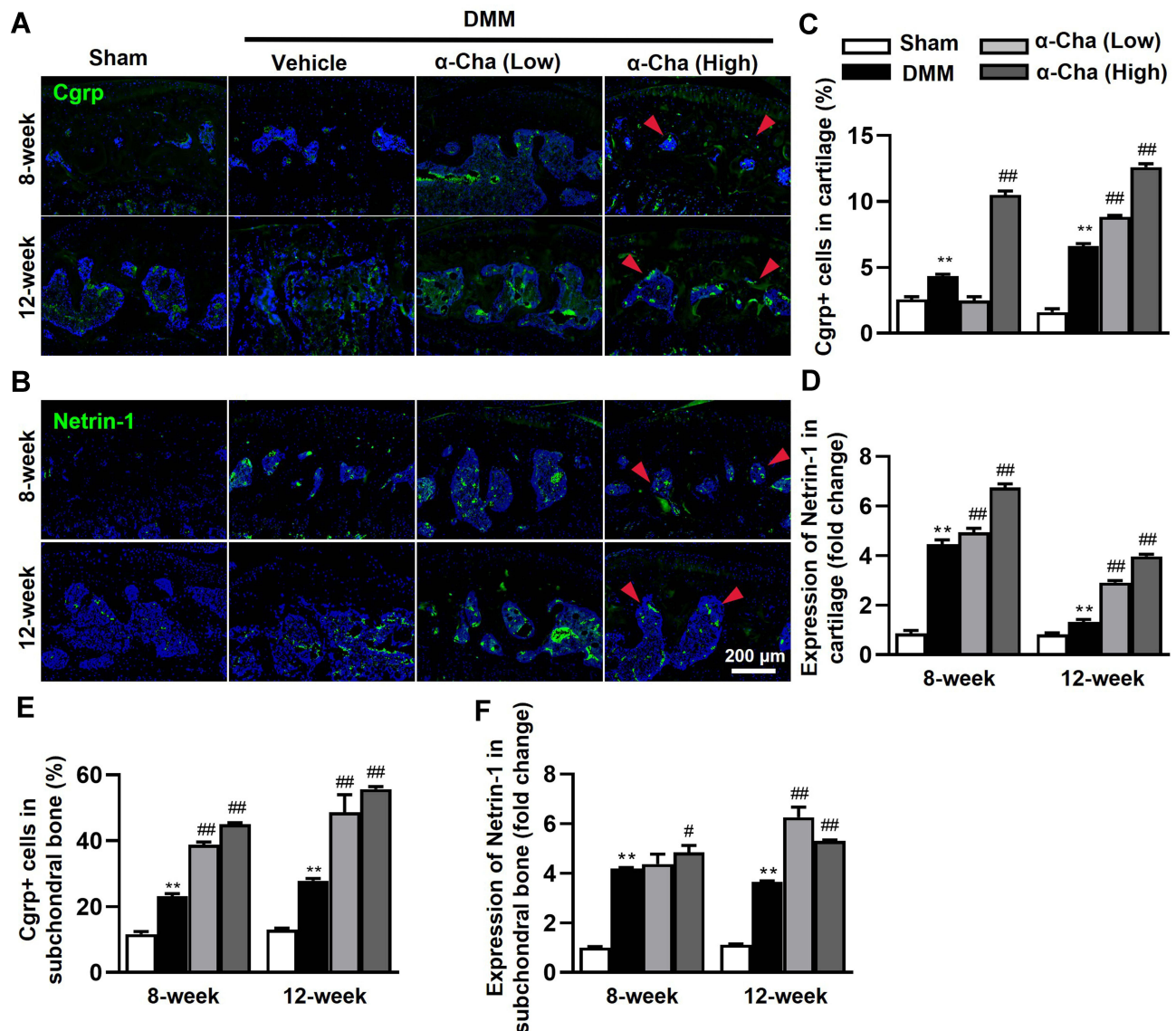


Figure 5 α -Chaconine recruits sensory nerve ingrowth in subchondral bone. (A and B) Immunofluorescence staining of Cgrp and Netrin-1 in subchondral bone 8- and 12-Week post-surgery. (C and D) Quantification of Cgrp-positive cells and positive staining of Netrin-1 in cartilage. (E and F) Quantification of Cgrp-positive cells and positive staining of Netrin-1 in subchondral bone. Red arrows indicated Cgrp-positive neurons and positive expression of Netrin-1. Data were expressed as the mean \pm SD. ** P < 0.05, *** P < 0.01 vs Sham group, # P < 0.05, ## P < 0.01 vs Vehicle group.

Abbreviations: α -Cha (Low), low-dose α -Chaconine group; α -Cha (High), high-dose α -Chaconine group; Cgrp, calcitonin gene-related peptide-positive.

the sensitivity of OA-related pain, which may partly be due to the elevated secretion of Netrin-1 and its subsequent promotion of Cgrp⁺ sensory neuron recruitment.

α -Chaconine Further Activates NF- κ B Signaling in OA Mice

Subsequently, to clarify the molecular mechanism by which α -Chaconine-induced chondrocyte pyroptosis and sensory innervation, we examined NF- κ B signaling activity by analyzing the expression of p65 as well as the phosphorylation status of I- κ B α , p-i- κ B α . As shown in Figure 6A and B, we found that OA mice displayed a significant increase in nuclear translocation of p65 and p-i- κ B α levels in articular cartilage and subchondral bone throughout OA modeling, while it was noteworthy that α -Chaconine dose-dependently augmented p-i- κ B α and p65 levels (Figure 6A–F). Taken together, these results suggest that α -Chaconine might strengthen chondrocyte pyroptosis and sensory nerve ingrowth to exacerbate OA development by activating NF- κ B activity (Figure 7).

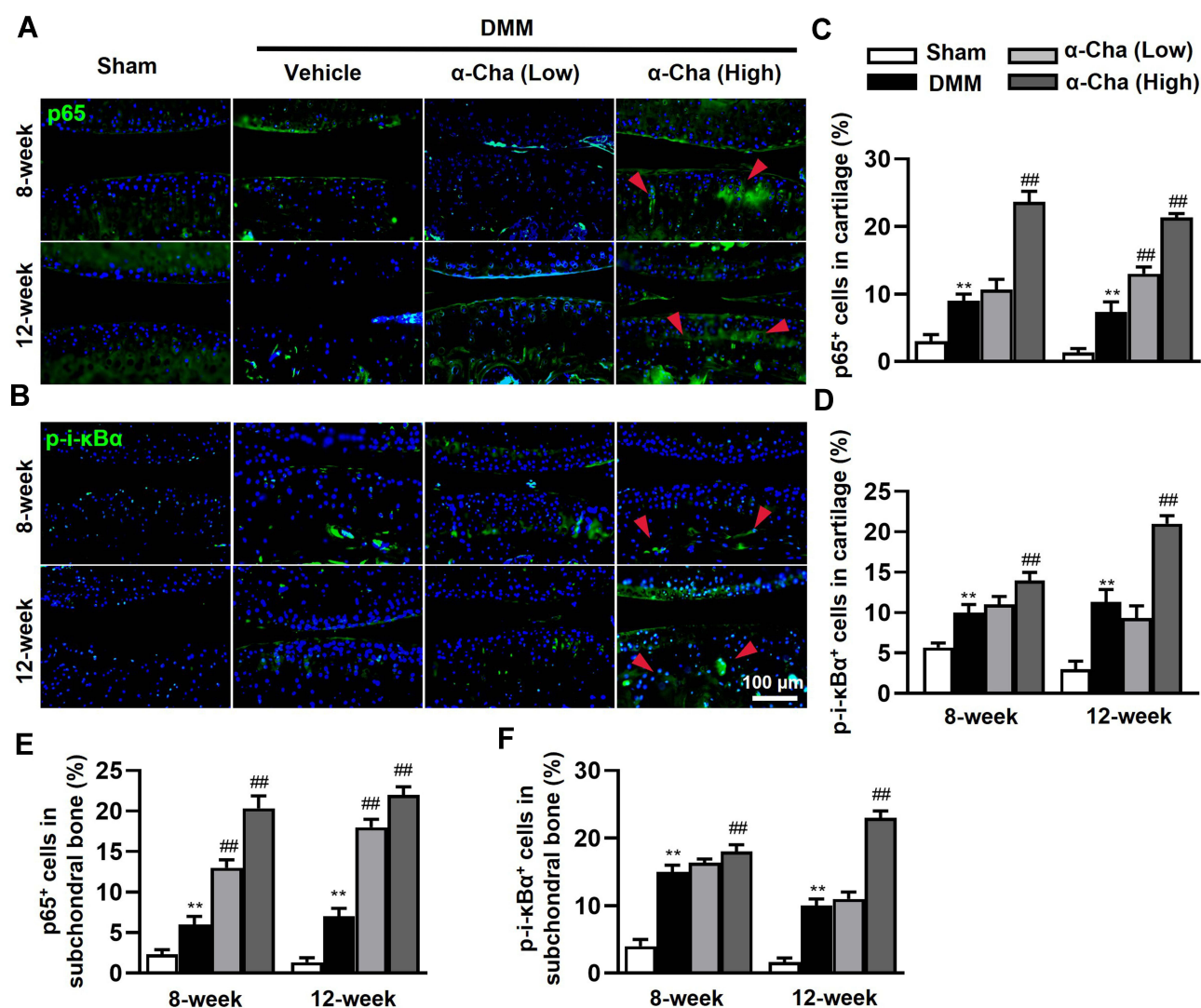


Figure 6 α -Chaconine activates NF- κ B signaling pathway of knee joint. (A and B) Immunofluorescence staining of p65 and p-i- κ B α both in articular cartilage and subchondral bone 8 and 12-week post-surgery. (C and D) Quantification of p65 and p-i- κ B α positive cells in cartilage. (E and F) Quantification of p65 and p-i- κ B α positive cells in subchondral bone. Red arrows indicate the positive expression of p65 and p-i- κ B α . Data are expressed as the mean \pm SD. ** P < 0.01 vs Sham group, ### P < 0.01 vs Vehicle group.

Abbreviations: α -Cha (Low), low-dose α -Chaconine group; α -Cha (High), high-dose α -Chaconine group; p-i- κ B α , phospho-i- κ B.

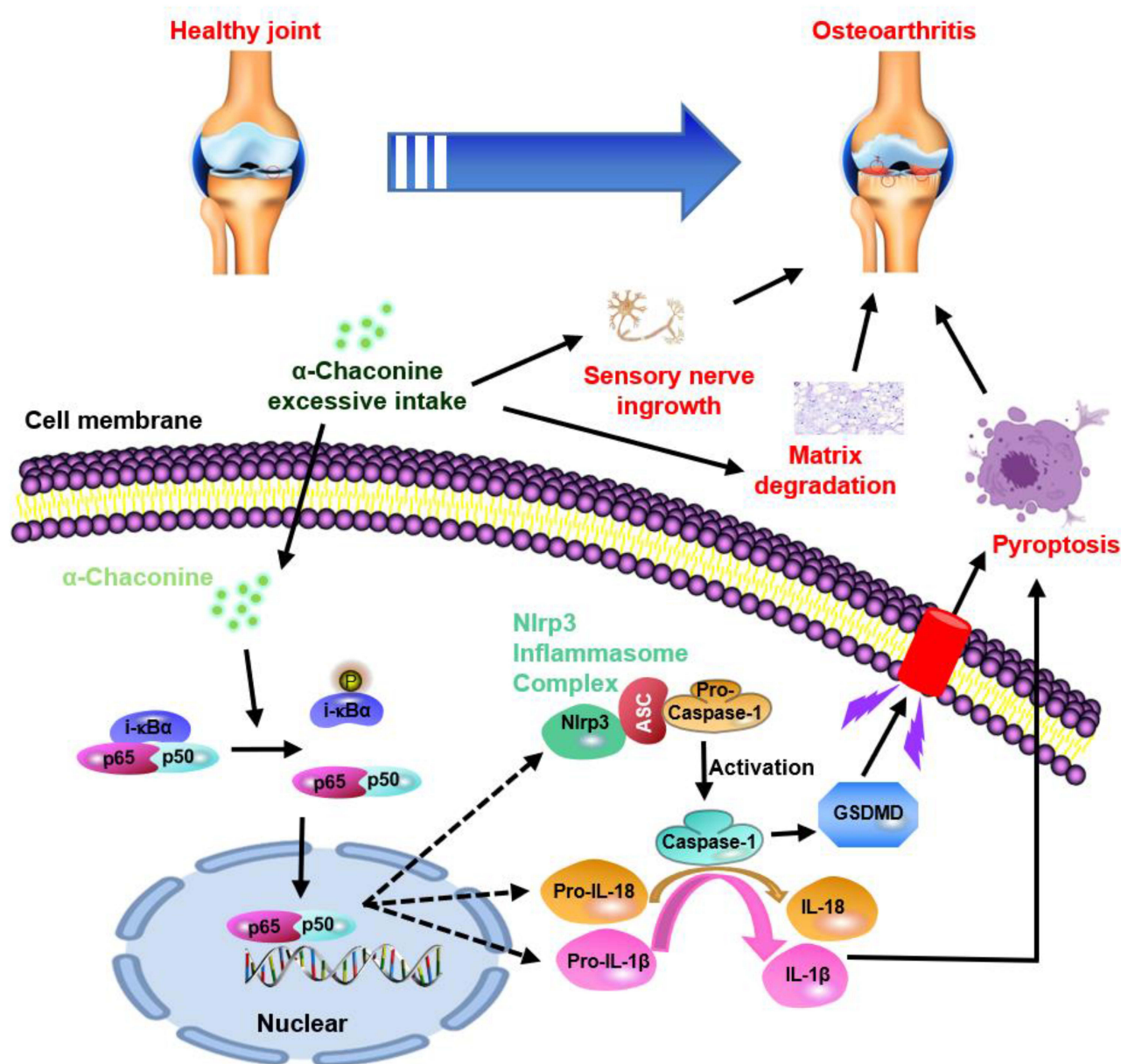


Figure 7 A schematic diagram illustrating the role of α -Chaconine in exacerbating OA progression.

Discussion

Overwhelming evidence from studies in animal models and clinical patients confirms that high-level glycoalkaloids may have toxic effects on the health of humans and animals.^{7,9,13,14} Unfortunately, no data are available on the association between glycoalkaloid exposure and OA progression. In the current study, we aimed to investigate the effects of α -Chaconine on the progression of mouse OA model induced by DMM surgery. We found that α -Chaconine could impair articular cartilage and contribute to matrix degradation, osteophyte formation, chondrocyte pyroptosis, as well as sensory nerve ingrowth. Subsequent mechanistic analysis revealed that α -Chaconine could significantly activate NF- κ B signaling activity, which acts as an upstream activator of chondrocyte dysfunction and pyroptosis, and sensory nerve innervation in subchondral bone. To the best of our knowledge, our findings provide the first in vivo evidence supporting excessive α -Chaconine exposure aggravates OA development.

As one of the most common degenerative diseases encountered clinically, OA is the primary cause of mobility impairment in the old due to pain and is also an extensive cause of disability worldwide.⁴⁶ As the primary symptom of

this disease, joint pain is arising from any part dysfunction of a joint, such as inflammatory-mediated damage to articular chondrocytes, bones, ligaments, tendons, and the progressive degradation of chondrocyte extracellular matrix.⁴⁷ It has been well established that the increased expressions of matrix-degrading enzymes, such as Mmp3 and Mmp13, are the reason contributing to the symptoms and progression of OA.⁴⁸ Moreover, failure to correctly maintain cartilage homeostasis leads to softer, less elastic, and pitted cartilage, concomitant with the formation of osteophytes, or bone spurs.⁴⁹ In our research, α -Chaconine promoted osteophyte formation and exacerbated cartilage degradation with higher Mmp3 and Mmp13 but lower Col2, indicating that α -Chaconine is detrimental to OA progression.

Pyroptosis is morphologically and mechanistically distinct from other forms of cell death, characterized by Nlrp3- and Caspase-1-mediated inflammasome, triggering the release of pyrogenic cytokines IL-1 β and IL-18.^{50,51} This process can induce cell rupture and inflammatory response, facilitating the pathogenesis of many chronic inflammatory diseases.^{52–54} Substantial evidence from our and others' studies suggests that chondrocyte pyroptosis contributes to the pathology of OA, leading to cartilage degeneration, formation of osteochondral fissures and osteophytes, and increase in nociceptive input in joint tissue.^{35,38,55} In agreement with these studies, we found that α -Chaconine remarkably increased the expressions of IL-1 β and IL-18 proteins via activating pyroptosis, suggesting α -Chaconine could cause a state of hyperalgesia and OA pain.

Knee pain not only needs outbreaks of inflammatory factors induced by pyroptosis but also needs the participation of nociceptive neurons in the transmission of pain.^{20,27} A previous clinical study has reported that aberrant remodeling of subchondral bone results in sensory nerve innervation, thereby aggravating pain in OA patients.⁵⁶ Further mechanism analysis demonstrated that netrin-1 secreted by osteoclasts during aberrant subchondral bone remodeling could induce Cgrp⁺ sensory innervation and exaggerate the sensation of pain,¹⁸ while reducing Netrin-1 secretion could lower Cgrp⁺ sensory neurons in the subchondral bone and significantly relieve OA-related pain.^{18–20} Consistently, the present study identified that α -Chaconine could significantly intensify the expression of Netrin-1 and the ratio of Cgrp⁺ neurons in OA mice. Based on the above results, we speculate that α -Chaconine may contribute to the expansion of nociceptive input by eliciting chondrocytes pyroptosis and sensory innervation. To verify whether α -Chaconine aggravates the development of human OA disease and the corresponding pain sensitivity, a clinical trial (eg, NCT00080171 registered in ClinicalTrials.gov) needs to be adopted to identify the correlation between serum α -Chaconine levels with pain sensitivity and OA progression in clinical OA patients.

It has been well established that NF- κ B signaling is involved in OA pathophysiology through various effects, including upregulation of inflammatory and catabolism-related proteins.⁵⁷ Our recent findings on degenerative joint diseases have shown that aberrant activation of NF- κ B pathway leads to chondrocyte pyroptosis and disruption of matrix metabolic homeostasis.^{35,38,58–60} Emerging evidence suggests that the activity of NF- κ B signaling is strongly correlated with the expression of pain-related neuropeptides such as Netrin-1, Cgrp, contributing to neuropathic pain.^{33,61} All these findings suggest that NF- κ B signaling cascade is a known regulator of pyroptosis and nerve ingrowth. Consistently, in our study presented here, α -Chaconine activated NF- κ B signaling both in articular chondrocytes and subchondral bone marrow, suggesting that α -Chaconine may induce chondrocyte pyroptosis and sensory innervation by activation of NF- κ B signaling.

Potatoes serve as major, inexpensive low-fat food sources providing energy (starch), high-quality protein, fiber, and vitamins, which are consumed massively worldwide.⁶² They do, however, also produce glycoalkaloids that at appropriate levels may be toxic to bacteria, fungi, viruses, and insects.⁶³ In high amounts, these compounds can also produce toxic effects in humans. Acute toxicity experiments have identified that glycoalkaloids could cause rapid damage to plasma membrane, leading to the lethal disorder of metabolism,⁶⁴ which mainly results from acetylcholinesterase inhibition and cell membrane disruption that affect the digestive and other organs.^{40,65} Toxicities induced in other species include hepatotoxicity in mice,⁶⁶ increased hepatic ornithine decarboxylase activity in rats,⁶⁷ craniofacial malformations in hamsters,⁶⁸ and anatomical developmental toxicities in frog embryos.⁶⁹ Likewise, our consequences also confirmed that α -Chaconine treatment is detrimental to OA progression by strengthening matrix metabolic degradation, potentiating chondrocyte pyroptosis and nerve ingrowth. Overall, our data indicated that long-time exposure to α -Chaconine may be harmful to OA development.

Besides, several limitations were noted in the present study. 1) Different from normal intragastric ingestion of α -Chaconine, in this study, we locally injected α -Chaconine into the joint cavity to focus on the direct pathogenic roles of

α -Chaconine on OA joint tissues. Different administration routes of α -Chaconine (eg, gavage, intravenous injection, and intraperitoneal injection) are needed to further validate the findings in this study. In addition, it needs to be clarified whether α -Chaconine has a long-term cumulative toxic effect on knee function and phenotypic characteristics of normal mice without receiving OA modeling. 2) As α -Chaconine is more toxic than α -solanine, the present study focused on the deleterious effects of α -Chaconine on OA progress. However, except for α -Chaconine, is there anything else in potatoes (eg, α -solanine, which is concomitantly present in the potato and may have a similar or lower toxicity and teratogenicity) alone or together with α -Chaconine synergistically exacerbate OA progression remains uncertain, further work will be required to address this point. 3) To further confirm the effects of α -Chaconine on OA pain, it is necessary to comprehensively evaluate the joint function and behavior phenotypes of OA mice using motor coordination analysis, mechanical analysis, and thermal hyperalgesia analyses in the future. In addition, we focused only on the pathological changes of cartilage and subchondral bone, without evaluating other parts of the joint tissue (eg, meniscus and infrapatellar fat) involved in OA and OA pain. The implementation of all these aspects will unprecedentedly enhance our understanding of the multiple and overlapping aspects of glycoalkaloids in diet, which will benefit consumers of potatoes.

Conclusions

In summary, we found that high-dose α -Chaconine contributes to cartilage degradation, bone structure destruction, chondrocyte pyroptosis, and aberrant nerve ingrowth via activation of NF- κ B signaling, thereby exacerbating OA progression. Our results provide new evidence and insights into the potential deleterious roles of α -Chaconine or eggplant high intake on OA development.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Zhiguo Zhang: Conceptualization, Methodology, Investigation, Visualization, Writing-original draft, Writing-review & editing. Fangda Fu: Methodology, Investigation, Investigation, Formal analysis, Writing-review & editing. Yishan Bian: Methodology, Investigation, Writing-review & editing. Huihao Zhang: Methodology, Investigation, Writing-review & editing. Sai Yao: Methodology, Investigation, Writing-review & editing. Chengcong Zhou: Methodology, Investigation, Visualization, Writing-review & editing. Yuying Ge: Methodology, Investigation, Writing-review & editing. Yuying Chen: Investigation, Writing-review & editing. Huan Luo: Investigation, Writing-review & editing. Weifeng Ji: Conceptualization, Investigation, Writing-review & editing. Kun Tian: Conceptualization, Investigation, Writing-review & editing. Ming Yue: Conceptualization, Investigation, Writing-review & editing. Weibin Du: Conceptualization, Investigation, Writing-review & editing. Hongting Jin:

Conceptualization, Investigation, Writing-review & editing. Peijian Tong: Conceptualization, Writing-review & editing. Chengliang Wu: Conceptualization, Methodology, Formal analysis, Writing-original draft, Writing-review & editing. Hongfeng Ruan: Conceptualization, Methodology, Formal analysis, Writing-original draft, Writing-review & editing.

Disclosure

The authors report no conflicts of interest in this work.

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