Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Lentinan Inhibits AGE-Induced Inflammation and the Expression of Matrix-Degrading Enzymes in Human Chondrocytes

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Zhaozhen Zhang
Zhuqing Zha
Zhiwei Zhao
Wenjing Liu
Wuyin Li

Department of Bone Surgery, Luoyang Orthopedic Hospital of Henan Province (Henan Provincial Orthopedic Hospital), Zhengzhou City, Henan Province 450000, People's Republic of China

Background: Chondrocyte-mediated inflammation is an important pathological component of osteoarthritis (OA) development. There are currently no therapies that completely reverse the development of OA. Lentinan, a type of polysaccharide derived from Lentinus edodes, has been demonstrated to possess significant anti-viral, anti-cancer, and anti-inflammatory effects, and has been recently used in the treatment of several inflammatory diseases. However, little research has focused on the pharmacological effect of lentinan in human OA.

Materials and Methods: We evaluated the anti-inflammatory and anti-ROS effects of lentinan in SW1353 chondrocytes treated with AGEs using real-time polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and the nitro oxide-specific stain DAF-FM DA. The regulatory effects of lentinan on NF-κB and MAPK p38 signaling were investigated via promoter assay and Western blot analysis.

Results: We found that lentinan inhibits the production of pro-inflammatory cytokines, including IL-1β, TNF-α, IL-8 and the secretion of PGE_2 and NO, by reducing the expression of COX-2 and iNOS in AGE-challenged chondrocytes. Lentinan also reduces AGE-induced increased expression of matrix metalloproteinases-1, −3, and −13 (MMP-1, MMP-3, MMP-13). Furthermore, lentinan has a similar effect on a disintegrin and metalloproteinase with thrombospondin motifs-4 and −5 (ADAMTS-4, ADAMTS-5). Mechanistically, lentinan reduces the activation of NF-κB.

Conclusion: Our findings indicate that lentinan shows a protective effect against AGE-induced inflammatory response in chondrocytes. These findings suggest that lentinan is a promising agent for the treatment of OA that could be used as a dietary supplement for patients with OA.

Keywords: osteoarthritis, AGEs, lentinan, NF-κB, chondrocytes

Introduction

Osteoarthritis (OA) is a painful degenerative joint disease afflicting millions of people around the world. OA is characterized by inflammation, pain, swelling, physical disability, and changes in cartilage tissues throughout the body, including in the hips and knees. Previous studies have demonstrated that risk factors such as aging,[1] joint injury,[2] and obesity[3] play a vital role in the initiation and development of OA. However, there are no previous therapies that can completely impede the initiation and development of this disease. Advanced glycation end products (AGEs) result from the glycated endproducts of the maillard reaction (MR), which is a non-enzymatic reaction between sugars and proteins.[4] AGEs accumulate in cells and tissues through the innate process

Correspondence: Wuyin Li
Department of Bone Surgery, Luoyang Orthopedic Hospital of Henan Province (Henan Provincial Orthopedic Hospital), No. 100, Yongping Road, Zhengzhou City, Henan Province 450000, People's Republic of China
Tel +86-371-88820000
Email wuyinl128@163.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/DDDT.S243311

of non-enzymatic glycation as well as dietary intake. Research has demonstrated that the accumulation of AGEs is an age-related effect that occurs through the binding of AGEs to the receptor for AGEs (RAGE) in human chondrocytes.[5] The excessive formation of AGEs in chondrocytes plays a major role in the development of OA. It is well established that AGE stimulation increases the secretion of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-8, and upregulates the production of nitric oxide (NO) and prostaglandin E2 (PGE_2) by modulating the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).[6–9] In addition, AGEs promote the activation of p38 as well as the phosphorylation and degradation of IκBα, which leads to the activation of NF-κB signaling pathways, thereby inducing a cascade of pro-inflammatory cytokines and chemokines.[10] Furthermore, AGEs enhance the expression of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS).[11] These hydrolytic proteinases have a strong effect on the degradation of the cartilage extracellular matrix (ECM), which is mainly constituted by type Ⅱ collagen and aggrecan. Degradation of the ECM is a primary pathological characteristic of OA. The exploration of a novel agent with fewer side effects has become an important goal for the treatment of OA.

Natural molecules from food sources have been sought to treat OA. Chondroitin sulfate (CS) is an important proteoglycan that acts as a major component of the extracellular matrix in joints. CS isolated from animal cartilage has been shown to have potential treatment efficacy. Pharmacological studies have revealed its capacity to promote the synthesis of cartilage matrix while inhibiting the synthesis of proteolytic enzymes that degrade the cartilage matrix. Purified CS possesses anti-inflammatory effects in primary cultured chondrocytes.[12] Oral supplementation of CS has been recommended by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis and Musculoskeletal Diseases (ESCEO).[13] The biotechnological production of CS has been explored in the cultivation of Escherichia coli strains.[14] Hyaluronic acid is another main component of the cartilage ECM. Hyaluronic acid plays a central role in the maintenance of synovial fluid viscosity. Hyaluronic acid gel treatment reduces the production of key inflammatory cytokines including TNF-α, IL-6, and IL-8 in cultured human chondrocytes by suppressing the NF-κB pathway.[12,15] Pharmaceutical purified hyaluronic acid products show similar anti-inflammatory effects by countering the expression of TNF-α and IL-6 in human primary chondrocytes.[16] Dietary mushrooms are well known for their health benefits. Lentinan, a β-1,3-glucan, is a type of polysaccharide derived from Lentinus edodes.[17] Previous research has demonstrated that lentinan exerts important antiviral, anti-cancer, and anti-inflammatory effects.[18–21] As a biological response modifier (BRM), lentinan actualizes its effects by restoring and enhancing immune system responsiveness to stimulation and other physiologically active factors. These functions indicate that lentinan may have a protective effect on chondrocytes in OA. Although a large number of studies have reported the protective effects of lentinan in various diseases, few of them revealed whether lentinan possesses a beneficial effect on the progression and development of OA. In the present study, we investigated the pharmacological function of lentinan in AGE-challenged human chondrocytes to determine whether lentinan has a protective effect against the inflammatory response in human chondrocytes.

Materials and Methods

Cell Culture and Treatment

Lentinan was purchased from Kaifeng Pharmaceutical Co. (Henan, China)[22] as powder in a penicillin bottle. Lentinan was dissolved in PBS right before use. The chondrosarcoma SW1353 cells used in all experiments were purchased from ATCC. The cells were cultured in Leibovitz's L-15m medium (ATCC) containing 10% fetal bovine serum. For the AGE treatment experiments, SW1353 cells were seeded into 6-well plates, grown to full confluence, and then exposed to 100 μg/mL AGEs in the presence or absence of 250 and 500 μM lentinan[23,24] for 24 hours. To measure the levels of phosphorylated p38 and IκBα, cells underwent the same treatment for 2 hours.

Real-Time Polymerase Chain Reaction (PCR)

RNA was extracted from 2–4 million SW1353 cells using a commercial RNA MiniPrep Purification Kit (Qiagen). Then, 1 μg RNA was used to synthesize cDNA using an RT-PCR One-step Kit (Bio-Rad, USA). Real-time PCR was performed using the SYBR-based real-time PCR method to measure the mRNA transcripts of the target genes and GAPDH (as a housekeeping control) using the Bio-Rad platform. The expression of each of the target genes was normalized to GAPDH and calculated using the $2^{-\Delta\Delta\ CT}$ method.

Enzyme-Linked Immunosorbent Assay (ELISA)

Roughly 1 × 10^4 SW1353 cells were used for ELISA. To measure the protein secretions of the target genes, the supernatants were collected from the culture medium by centrifugation at 1000 rpm for 10 min. Commercial ELISA kits for TNF-α, IL-1β, IL-8, MMP-1, MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 were purchased from R&D Systems and used in accordance with the manufacturer's instructions. The final values of the reaction results were extrapolated from the standard curve using a variable slope. The resulting data are presented as fold-changes using the non-treated group as a baseline.

Western Blot Analysis

After the indicated treatment, 4–6 million cells were lysed using RIPA buffer supplemented with protease inhibitor cocktail. Then, a total of 20 μg cell lysates was loaded onto 4–20% precasted polyacrylamide gel electrophoresis (PAGE) gel to separate the proteins according to size. The separated protein mix was then transferred onto polyvinylidene fluoride (PVDF) membranes and the corresponding protein levels were detected using their specific antibodies. The membranes were incubated overnight with primary antibodies at 4 °C, and then washed and incubated with HRP-conjugated secondary antibodies for 1 h. HRP substrate was added to detect the resulting protein signals which were visualized using Image J software.

4-Amino-5-Methylamino-2′,7′-Difluorofluorescein Diacetate (DAF-FM DA) Staining

The intracellular production of NO was measured by performing a cellular staining experiment using the cell-permeable fluorescent probe DAF-FM DA (Thermo-Fisher Scientific, USA). Briefly, 10,000 cells were grown to full confluence on a 96-well plate, and then incubated with 5 μM DAF-FM DA probe for 10 min. The resulting fluorescent signals were visualized using a fluorescence microscope, with excitation at 495 nm and emission detection at 515 nm.

Luciferase Activity of NF-κB

The NF-κB bind sites containing firefly luciferase vector were used to evaluate the transcriptional activity of NF-κB. Briefly, the cells were co-transfected with NF-κB promoter and a renilla luciferase promoter using Lipofectamine 3000 reagent from Invitrogen (cat # 11668027) in accordance with the manufacturer's instructions. After the indicated treatment, the total cell lysates were collected and the dual luciferase activity of renilla and firefly luciferase was measured. The relative luciferase activity was calculated by normalizing the firefly luciferase activity to that of renilla luciferase.

Statistical Analysis

All experimental data are presented as means ± S.E.M. in this study The statistical significance of differences was measured using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. A value of $P<0.05$ was considered statistically significant.

Results

Measuring the Production of Pro-Inflammatory Cytokines

Proinflammatory TNF-α, IL-8, and IL-1β are known to play a key role in the development of OA. Therefore, we tested the effects of lentinan on the production of these cytokines in AGE-induced chondrocytes. Firstly, we investigated the expression of TNF-α, as shown in Figure 1A. The mRNA level of TNF-α increased to 3.9-fold with AGE treatment alone and the secretion of TNF-α was upregulated to 792.3 from 123.5 pg/mL at baseline (Figure 1D). However, in the presence of the two doses of lentinan, AGE stimulation increased the mRNA level of TNF-α to 2.8- and 2.2-fold and the secretion of TNF-α to 568.2 and 432.5 pg/mL (Figure 1D), respectively. Secondly, we turned to the mRNA level and secretion of IL-8. When the two doses of lentinan were added, AGEs increased the mRNA level of IL-8 to 3.4- and 2.5-fold, compared with 4.6-fold upon AGE stimulation alone (Figure 1B). Furthermore, AGEs alone increased the secretion of IL-8 to 1544.3 pg/mL, but its secretion was decreased to 1192.6 and 878.6 pg/mL when exposed to the two doses of lentinan (Figure 1E). Similarly, the mRNA level of IL-1β was significantly suppressed to 2.6- and 1.9-fold by the two doses of lentinan from 3.6-fold with AGE treatment alone (Figure 1C), the secretion of IL-1β was reduced from 1099.5 to 781.5 pg/mL and 623.6 pg/mL by lentinan, respectively (Figure 1F).

The Expression of Matrix Metalloproteinases

To determine the impact of lentinan on the expression of MMPs, which play a major role in the degradation of type II collagen, the mRNA and protein levels of MMP-1, MMP-3, and MMP-13 were measured. As the results in Figure 2A

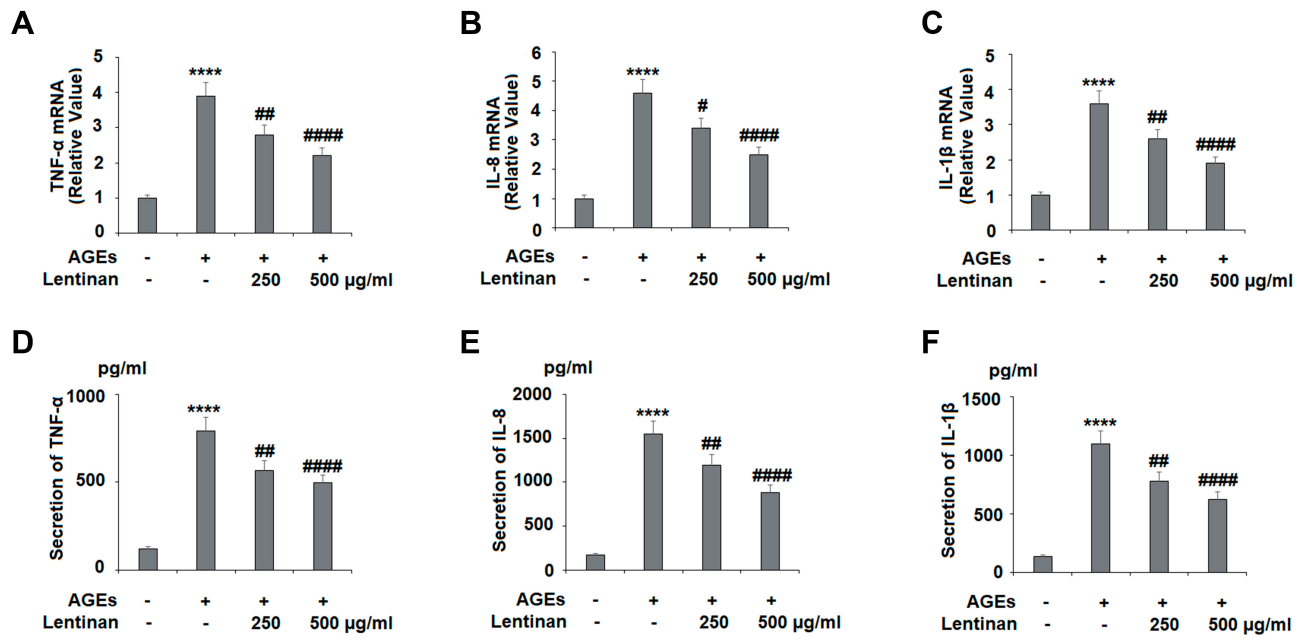

Figure 1 Lentinan reduced AGE-induced expression of TNF-α, IL-8, and IL-1β in human SW1353 chondrocytes. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 24 h. (**A**) mRNA of TNF-α; (**B**) mRNA of IL-8; (**C**) mRNA of IL-1β; (**D**) Secretion of TNF-α as measured by ELISA; (**E**) Secretion of IL-8 as measured by ELISA; (**F**) Secretion of IL-1β as measured by ELISA (****P<0.0001 vs vehicle group; #, ##, ####, P<0.05, 0.01, 0.0001 vs AGEs treatment group, n=4-5).

show, the mRNA levels of MMP-1, MMP-3, and MMP-13 significantly increased to 3.7-, 3.3-, and 4.7-fold by AGE treatment alone, respectively. However, 250 μg/mL lentinan reduced the mRNA levels of these three factors to 2.6-, 2.4-, and 3.2-fold, respectively. Furthermore, 500 μg/mL lentinan significantly reduced them to 1.5-, 1.6-, and 1.9-fold. Congruously, as shown in Figure 2B, the results of ELISA analysis show that AGE treatment increased the protein levels of MMP-1, MMP-3, and MMP-13 from 125.7, 93.7, and 143.6 pg/mL to 493.4, 552.3, and 666.9 pg/mL. Meanwhile, the two doses of lentinan reduced the protein level of MMP-1 to 325.6 and 228.7 pg/mL, MMP-3 to 381.2, 299.5 pg/mL, and MMP-13 to 481.2, 333.5 pg/mL, respectively. Taken together, these findings imply that lentinan has a strong inhibitory effect on the expression of MMPs.

Expression Changes of ADAMTS-4 and ADAMTS-5

As ADAMTS plays a major role in aggrecan degeneration, we investigated the effect of lentinan on the expression of ADAMTS-4 and ADAMTS-5. The results in Figure 3A show that the mRNA levels of ADAMTS-4 and

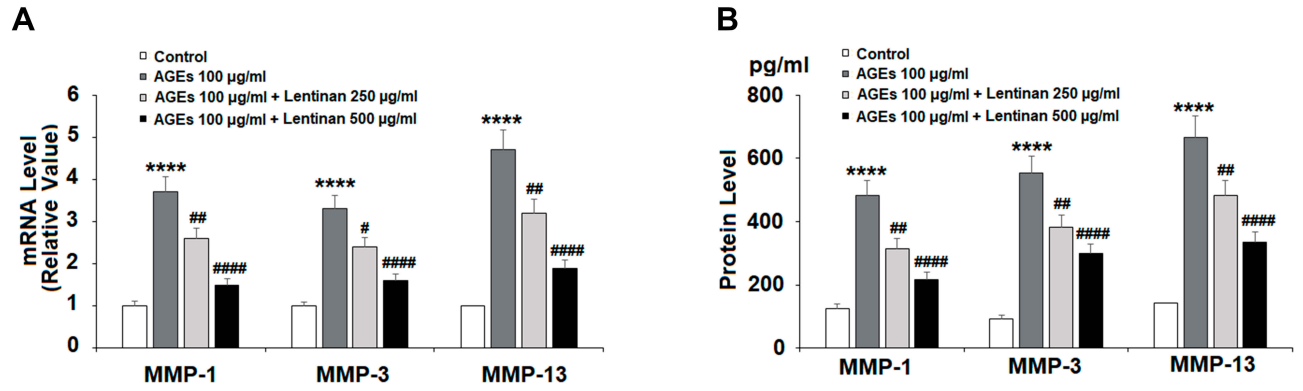

Figure 2 Lentinan suppressed AGE-induced expression of MMP-1, MMP-3, and MMP-13 in human SW1353 chondrocytes. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 24 h. (**A**) mRNA levels of MMP-1, MMP-3, and MMP-13; (**B**) Protein levels of MMP-1, MMP-3, and MMP-13 as measured by ELISA (****, P<0.0001 vs vehicle group; #, ##, ####, P<0.05, 0.01, 0.0001 vs AGEs treatment group, n=3-4).

ADAMTS-5 were significantly enhanced to 3.7-and 3.4-fold by exposure to AGEs alone, while 250 μg/mL lentinan showed an inhibitive effect on these two enzymes, reducing their mRNA expression to 2.6- and 2.2-fold, and 500 μg/mL lentinan further reduced them to 1.8- and 1.6-fold. Congruously, AGE treatment enhanced the protein levels of ADAMTS-4 and ADAMTS-5 to 582.4 and 668.9 pg/mL, while 250 μg/mL lentinan was able to decrease them to 391.3 and 492.1 pg/mL, respectively. In addition, 500 μg/mL lentinan further decreased the protein levels of ADAMTS-4 and ADAMTS-5 to 278.5 and 335.6 pg/mL, as shown in Figure 3B. Our data reveal that lentinan suppresses the expression of ADAMTS-4 and ADAMTS-5 at both the mRNA and protein levels.

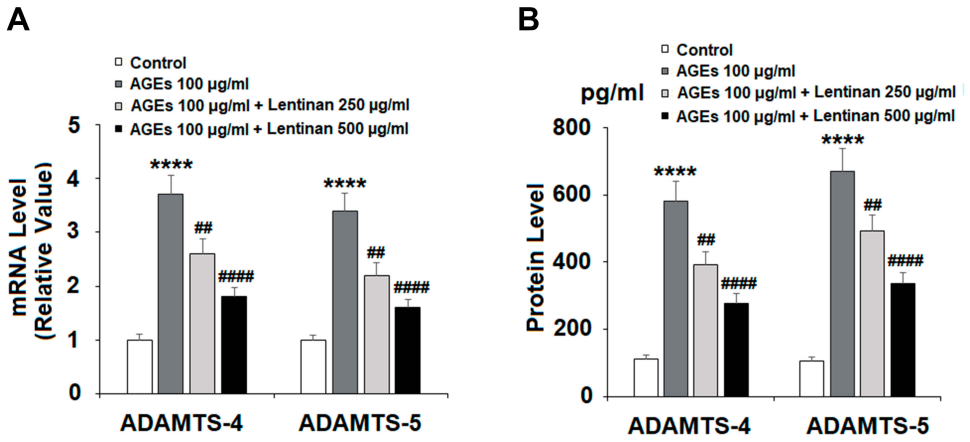

Figure 3 Lentinan inhibited AGE-induced expression of ADAMTS-4 and ADAMTS-5 in human SW1353 chondrocytes. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 24 h. (**A**) mRNA of ADAMTS-4 and ADAMTS-5; (**B**) Protein of ADAMTS-4 and ADAMTS-5 as measured by ELISA (****, P<0.0001 vs vehicle group; ##, #####, P<0.01, 0.0001 vs AGEs treatment group, n=3-4).

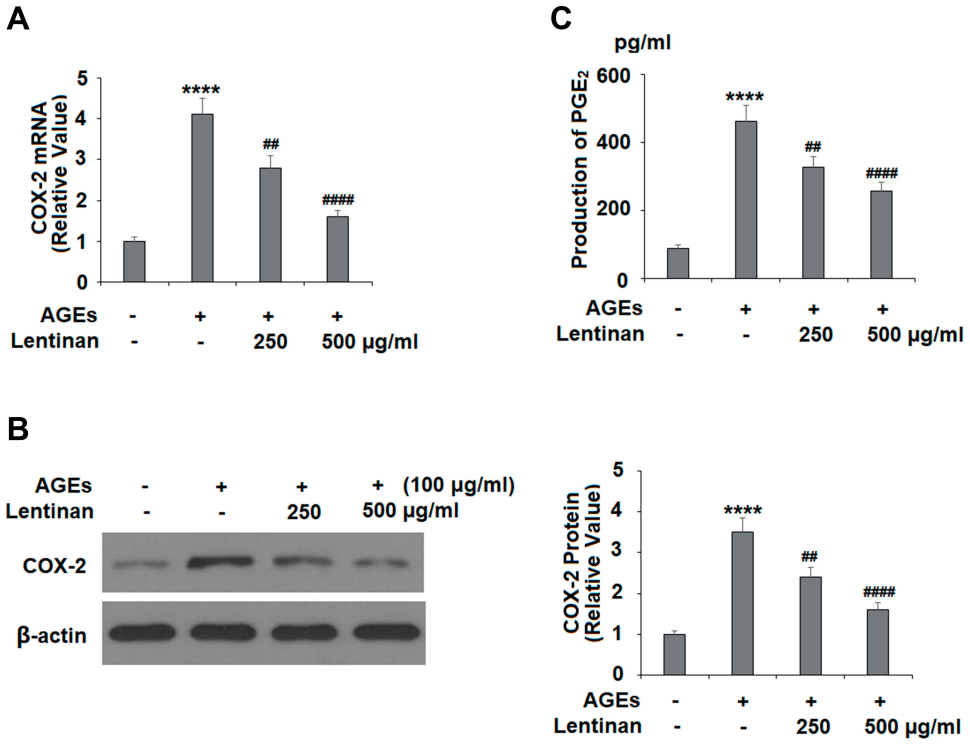

Figure 4 Lentinan prevented AGE-induced COX-2 expression and PGE_2 generation. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 24 h. (**A**) mRNA of COX-2; (**B**) Protein of COX-2 as measured by Western blot analysis; (**C**) Production of PGE_2 (****, P<0.0001 vs vehicle group; ##, #####, P<0.01, 0.0001 vs AGEs treatment group, n=4-5).

Expression Changes of COX-2 and PGE_2

The results in Figure 4A shows that lentinan reduced the mRNA level of COX-2 to 2.8- and 1.6-fold, compared with 4.1-fold via exposure to AGEs alone, in a concentration-dependent manner. In addition, the results of Western blot analysis in Figure 4B reveal that the two doses of lentinan significantly decreased the protein levels of COX-2 to 2.4- and 1.6-fold, compared to 3.5-fold with AGE treatment alone. For the production of PGE_2, the baseline of the protein level of PGE_2 was 88.8 pg/mL, and the addition of AGEs enhanced it to 462.3 pg/mL. However, two doses of lentinan were able to reduce the protein level of PGE_2 to 325.8 and 256.7 pg/mL, respectively (Figure 4C).

Expression of iNOS and Production of NO

In order to determine the effect of lentinan on the expression of iNOS and the generation of NO, Western blot analysis and DAF-FM staining approaches were employed. The results in Figure 5A show that stimulation with AGEs upregulated the mRNA level of iNOS to 3.1-fold from baseline, while 250 μg/mL lentinan reduced it to 2.7-fold, and 500 μg/mL lentinan further reduced the mRNA level of iNOS to 2.1-fold. As for the protein level of iNOS, the data in Figure 5B reveal that the two doses of lentinan decreased the iNOS protein level to 2.1- and 1.3-fold, respectively, as compared to the exposure to AGEs alone. The results of DAF-FM staining in Figure 5C show that the two doses of lentinan significantly downregulated the protein level of NO to 2.4- and 1.6-fold, respectively, as opposed to the increase to 3.4-fold induced by AGE treatment alone.

Activation of P38

In order to test whether lentinan affects the activation of the p38/MAPK pathway, the phosphorylation of p38 (p-p38) was tested. As shown in Figure 6, the level of p-p38 was significantly increased to 3.8-fold upon exposure to AGEs alone, while the two doses of lentinan reduced the level of p-p38 to 2.6-and 1.5-fold, respectively. These results reveal that lentinan has a potent inhibitory effect on the activation of p38.

Phosphorylation and Degradation of IκBα

It is well documented that the phosphorylation and degradation of IκBα is a trigger for the activation of the NF-κB signaling pathway. Our data in Figure 7 show that AGEs increased the phosphorylated level of IκBα (p-IκBα) to 3.2-fold, while the two doses of lentinan reduced the level of p-IκBα to 2.1- and 1.2-fold, respectively. On the contrary, AGE treatment suppressed the total level of IκBα to 34% of that of baseline. However, 250 μg/mL lentinan significantly increased the total level of IκBα to 69% of that of base line, while 500 μg/mL lentinan recovered 98% IκBα level.

The Effects of Lentinan on AGE-Induced NF-κB Activation

As the phosphorylation and degradation of IκBα is a hallmark of NF-κB activity, we measured the nuclear translocation and luciferase activity of NF-κB promoter. The results in Figure 8A reveal that the two doses of lentinan reduced the nuclear translocation of NF-κB to 2.7- and 1.7-fold, respectively, as compared with 3.8-fold upon exposure to AGEs alone. The luciferase activity of

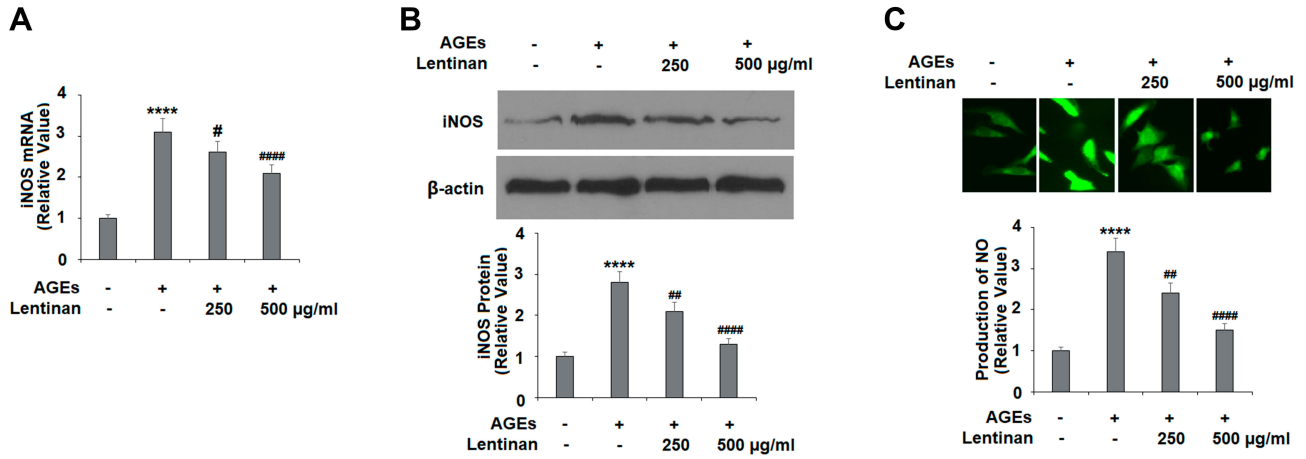

Figure 5 Lentinan inhibited AGE-induced iNOS expression and production of NO. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 24 h. (**A**) mRNA of iNOS; (**B**) Protein of iNOS as measured by Western blot analysis; (**C**) Production of NO as measured by DAF-FM staining (****, P<0.0001 vs vehicle group; #, ##, ####, P<0.05, 0.01, 0.0001 vs AGEs treatment group, n=4-5).

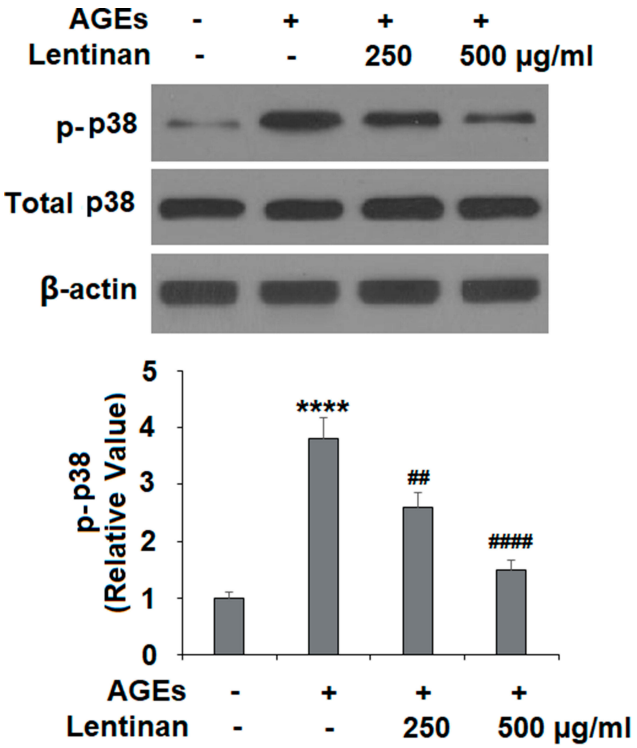

Figure 6 Lentinan inhibited AGE-induced activation of p38. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 2 h. Phosphorylated and total levels of p38 were measured (****, P<0.0001 vs vehicle group; ##, ####, P<0.01, 0.0001 vs AGEs treatment group, n=4-5).

NF-κB was induced to 123.5-fold by AGEs, which was ameliorated to 78.2- and 57.3-fold by lentinan in a dose-dependent manner, respectively (Figure 8B).

Discussion

OA is a degenerative joint disease afflicting millions of people worldwide, primarily those over the age of 65.[25] As cartilage degradation is a hallmark of the inflammatory process of OA, the inhibition of inflammatory factors that promote cartilage degradation has been shown to be an effective target for the treatment of OA. Although a large number of studies have proposed treatments for this disease, a safe and therapeutic strategy is still urgently needed. Lentinan, a polysaccharide derived from Lentinus edodes, has shown protective properties in inflammatory skin diseases,[26] inflammatory bowel disease,[27] and different cancers.[28] In the present study, we employed lentinan to antagonize inflammation induced by AGEs in human chondrocytes. This is the first time the effect of lentinan in AGE-challenged chondrocytes has been measured. The results show that lentinan significantly suppresses the inflammatory response in chondrocytes.

When binding with RAGE, AGEs accumulate in the joints. The resulting excessive levels of AGEs stimulate chondrocytes to release various pro-inflammatory cytokines and chemokines such as IL-1β, TNF-α, and IL-8, which play a critical role in the progression of OA.[29–31] IL-1β and TNF-α have been demonstrated to upregulate the expression of ADAMTS and MMPs, especially MMP-1, MMP-3, and MMP-13, in chondrocytes.[31,32] IL-8 plays a vital role in OA by attracting neutrophils and monocytes to promote an inflammatory response in chondrocytes.[33,34] COX-2 and iNOS are two primary regulatory enzymes involved in various inflammatory responses that work by modulating the production of PGE_2 and NO, which are responsible for the development of inflammation. Furthermore, studies have reported that PGE_2 and NO contribute to articular inflammation and destruction by increasing MMP activity and production.[35,36] Lentinan, as a biological response modifier, has been proven to be safe and effective as a treatment and adjunctive therapy for inflammatory diseases.[23,24] In our study, lentinan treatment significantly inhibited the expression of pro-inflammatory cytokines and chemokines, including IL-1β, TNF-α, IL-8, PGE_2, and NO. These results reveal that lentinan exerts anti-inflammatory effects in AGE-induced chondrocytes. In addition, as overexpression of pro-inflammatory cytokines and chemokines exists in various

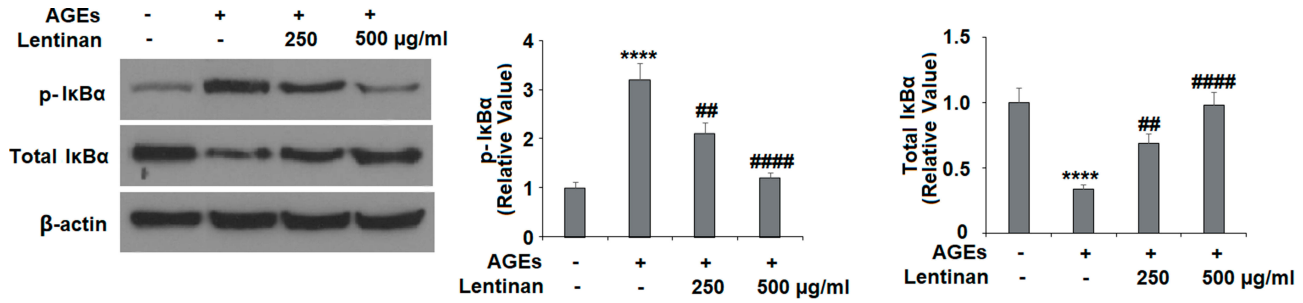

Figure 7 Lentinan inhibited AGE-induced phosphorylation and degradation of IκBα. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 2 h. Phosphorylated and total levels of IκBα were measured by Western blot analysis (****, P<0.0001 vs vehicle group; ##, ####, P<0.01, 0.0001 vs AGEs treatment group, n=4-5).

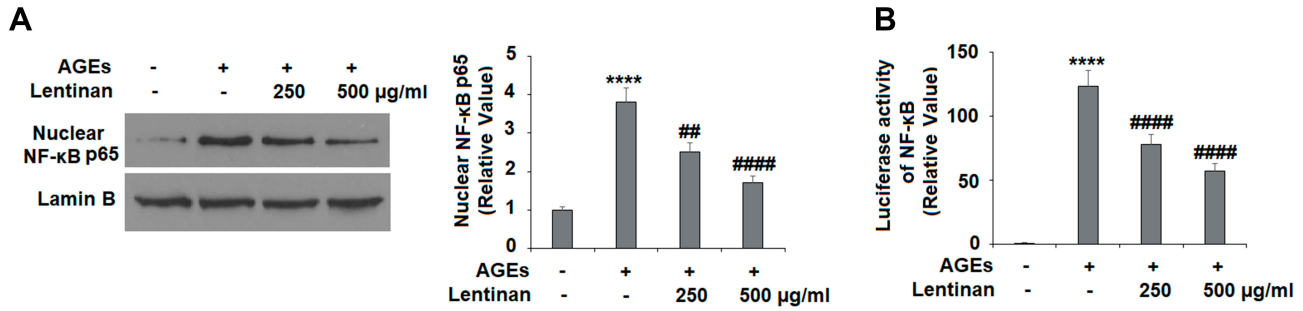

Figure 8 Lentinan inhibited AGE-induced activation of NF-κB. Cells were stimulated with 100 μg/mL AGEs with or without lentinan (250 and 500 μg/mL) for 2 h. (**A**) Nuclear translocation of NF-κB; (**B**) Luciferase activity of NF-κB (****, P<0.0001 vs vehicle group; ##, #####, P<0.01, 0.0001 vs AGEs treatment group, n=4-5).

inflammatory diseases and promotes their progression and development, lentinan has the potential for applications in other related fields.

MMPs and ADAMTS are the primary hydrolytic proteinases involved in the destruction of the cartilage matrix in AGE-induced chondrocytes.[37,38] Research has found that high expression of MMPs and ADAMTS, including MMP-1, MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5, in chondrocytes induces the destruction of the cartilage matrix. On the other hand, the degraded products further promote the deterioration of the cartilage matrix, forming a vicious circle that accelerates cartilage matrix breakdown.[39] Multiple studies have revealed that the production of MMPs and ADAMTS is modulated by the expression of pro-inflammatory cytokines and chemokines.[35,36] In our study, AGE treatment significantly increased the production of MMPs and ADAMTS in chondrocytes. However, a disadvantage of this experiment is not being able to differentiate whether AGE treatment enhances the production of MMPs and ADAMTS directly or whether this occurs as an indirect result of the increase in the expression of pro-inflammatory cytokines. Regarding this, we speculate that lentinan might suppress the expression of MMPs and ADAMTS in a direct or indirect way. More research is required to demonstrate the inhibitory effect of lentinan on the expression of MMPs and ADAMTS. Our study suggests that lentinan has the potential to maintain the integrity of the cartilage matrix.

The NF-κB signaling pathway plays a vital role in the progression and development of inflammatory diseases. In OA, the NF-κB pathway has been proven to modulate the inflammatory response, production of cytokines, and degradation of the cartilage matrix.[40] Under normal conditions, NF-κB is maintained in its inactive state in the cytoplasm by IκBα, a type of inhibitory protein. However, AGE stimulation leads to the activation of the p38/MAPK pathway and the phosphorylation of IκBα, resulting in nuclear translocation of p65 and the activation of NF-κB.[41] When activated, NF-κB upregulates the production of various of cytokines and chemokines such as IL-1β, TNF-α, PGE_2, and NO. These factors not only contribute to the development of the inflammatory response in OA chondrocytes, but also leads to the expression of MMPs and ADAMTS, which are responsible for the breakdown of the cartilage matrix. Our research provides evidence that lentinan inhibits the activation of the p38/MAPK pathway, thereby preventing IκBα from undergoing phosphorylation and degradation. Therefore, lentinan treatment modulates the activation of the NF-κB signaling pathway in a dose-dependent manner.

In this study, we reported that 250–500 μg/mL of lentinan displays a protective effect against AGE- induced inflammation and the expression of matrix-degrading enzymes in human SW1353 chondrocytes. Consistently, 250–500 μg/mL lentinan has been shown to suppress inflammasome activation in bone marrow-derived macrophages.[23] Additionally, 500 μg/mL lentinan has displayed an anti-inflammatory capacity by inhibiting the expression of TNFR1 and IL-8 in Caco-2 cells.[24] Interestingly, several studies have used varied doses of chondroitin sulfate (CS) in cultured chondrocytes. For example, 10–25 μg/mL CS is shown to be responsive for extracellular matrix metabolism.[42,43] At high doses, 200 μg/mL CS has been shown to suppress cytokine-induced chondrocyte activation.[44,45] Based on these facts, we recognize that both lentinan and CS could be effective at doses of 100–200 μg/mL in vitro. Importantly, previous studies have shown that CS treatment could promote the synthesis of ECM[42] and exhibit anti-inflammatory effects in cultured chondrocytes.[46,47] CS also suppresses NF-κB accumulation in the nucleus.[48] We showed that lentinan suppresses AGE-induced expression of inflammatory cytokines, matrix metalloproteinases (MMPs), and the activation of NF-κB in chondrocytes. Therefore, we speculate that lentinan might have a comparable beneficial effect to CS. Based on the published studies on CS, it is

typically administered orally at doses ranging from 800 to 1200 mg/day. Previous work has shown that at 2 hour following oral administration, plasma levels of CS can reach 0.6–10 μg/mL.[49] However, other studies have administered lentinan via intraperitoneal injection. Compared to CS (molecular weight: 10–40 kDa), 500 kDa lentinan is a much larger polysaccharide, which makes it difficult to pass through the semipermeable synovial membrane. Thus, the oral administration of lentinan could be long-term goal for investigation.

There are several limitations to the current study. Firstly, we investigated the effects of lentinan in human SW1353 chondrosarcoma cells but not in human primary chondrocytes. Investigation of primary chondrocytes is limited by a lack of a sufficient number of chondrocytes from operative procedures and the fact that after isolation, the biological and functional characteristics of primary chondrocytes differ considerably between donors and preparations [51]. Therefore, human SW1353 cells are widely used as a substitute for primary chondrocytes [52]. It should be noted that SW1353 cells exhibit only a limited chondrocytic phenotype in line with previous reports showing an epithelial phenotype of SW1353 cells after long-term culture [53]. Secondly, the pathological mechanisms of osteoarthritis are complicated. In addition to AGEs, there are a variety of toxins such as IL-1β that are associated with the progression and development of OA. Therefore, future in vivo studies using ideal animal models are necessary to clarify the beneficial effects of lentinan in OA.

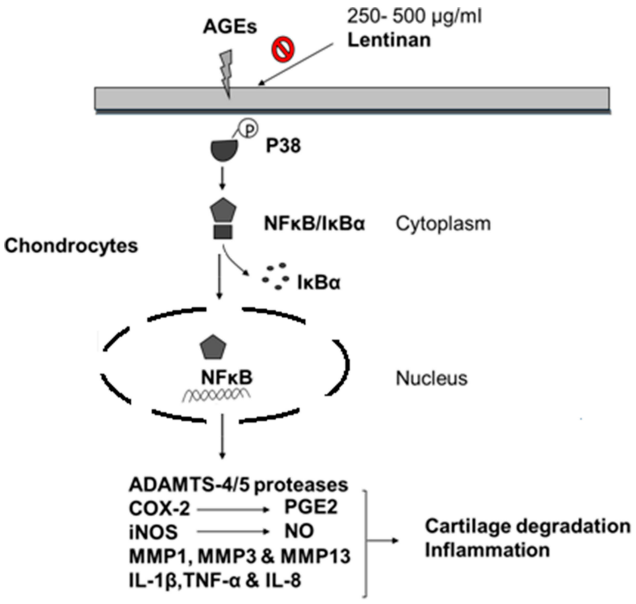

Figure 9 A representative schematic of the molecular mechanisms.

In conclusion, this study demonstrates the protective effects of lentinan in AGE-induced chondrocytes by inhibiting the production of pro-inflammatory cytokines and chemokines such as IL-1β, TNF-α, IL-8, PGE_2, and NO, suppressing the expression of regulatory enzymes including COX-2 and iNOS, and downregulating the expression of hydrolytic enzymes via the modulation of NF-κB signaling by suppressing the phosphorylation of p38 and the phosphorylation and degradation of IκBα (Figure 9). These findings suggest that lentinan may be effective in the treatment of chondrocyte-based inflammation in OA.

Ethics Statement

The chondrosarcoma SW1353 cells used in all experiments were purchased from ATCC. All experiments were approved by the ethics committee of Henan Provincial Orthopedic Hospital.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Rahmati M, Nalesso G, Mobasheri A, Mozafari M. Aging and osteoarthritis: central role of the extracellular matrix. *Ageing Res Rev*. 2017;40:20–30. doi:10.1016/j.arr.2017.07.004
2. Gelber AC, Hochberg MC, Mead LA, Wang NY, Wigley FM, Klag MJ. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. *Ann Intern Med*. 2000;133(5):321–328. doi:10.7326/0003-4819-133-5-200009050-00007
3. Berenbaum F, Eymard F, Houard X. Osteoarthritis, inflammation and obesity. *Curr Opin Rheumatol*. 2013;25(1):114–118. doi:10.1097/BOR.0b013e32835a9414
4. Maillard LC. Action des acides amines sur les sucres: formation des melanoidines par voie methodique. *Compt Rend*. 1912;154:66–68.
5. Loeser RF, Yammani RR, Carlson CS, et al. Articular chondrocytes express the receptor for advanced glycation end products: potential role in osteoarthritis. *Arthritis Rheum*. 2005;52(8):2376–2385. doi:10.1002/art.21199
6. Gao F, Zhang S. Salicin inhibits AGE-induced degradation of type II collagen and aggrecan in human SW1353 chondrocytes: therapeutic potential in osteoarthritis. *Artif Cells Nanomed Biotechnol*. 2019;47(1):1043–1049. doi:10.1080/21691401.2019.1591427
7. Takahashi N, Harada M, Azhary JMK, et al. Accumulation of advanced glycation end products in follicles is associated with poor oocyte developmental competence. *Mol Hum Reprod*. 2019;25(11):684–694. doi:10.1093/molehr/gaz050
8. Sun W, Gao Y, Ding Y, et al. Catalpol ameliorates advanced glycation end product-induced dysfunction of glomerular endothelial cells via regulating nitric oxide synthesis by inducible nitric oxide synthase and endothelial nitric oxide synthase. *IUBMB Life*. 2019;71(9):1268–1283. doi:10.1002/iub.2032
9. Matsumoto T, Kojima M, Takayanagi K, Katome T, Taguchi K. Amplification of the COX/TXS/TP receptor pathway enhances uridine diphosphate-induced contraction by advanced glycation end products in rat carotid arteries. *Pflugers Arch*. 2019;471(11–12):1505–1517. doi:10.1007/s00424-019-02330-y

10. Nonaka K, Bando M, Sakamoto E, et al. 6-shogaol inhibits advanced glycation end-products-induced IL-6 and ICAM-1 expression by regulating oxidative responses in human gingival fibroblasts. *Molecules*. 2019;24(20):E3705. doi:10.3390/molecules24203705
11. Tong C, Liang H, Liu X, et al. The protective effects of exenatide against AGEs-induced articular matrix degradation in human primary chondrocytes. *Am J Transl Res*. 2019;11(4):2081–2089.
12. Stellavato A, Restaino OF, Vassallo V, et al. Comparative analyses of pharmaceuticals or food supplements containing chondroitin sulfate: are their bioactivities equivalent? *Adv Ther*. 2019;36(11):3221–3237. doi:10.1007/s12325-019-01064-8
13. Restaino OF, Finamore R, Stellavato A, et al. European chondroitin sulfate and glucosamine food supplements: a systematic quality and quantity assessment compared to pharmaceuticals. *Carbohydr Polym*. 2019;15(222):114984. doi:10.1016/j.carbpol.2019.114984
14. Restaino OF, Di Lauro I, Di Nuzzo R, De Rosa M, Schiraldi C. New insight into chondroitin and heparosan-like capsular polysaccharide synthesis by profiling of the nucleotide sugar precursors. *Biosci Rep*. 2017;37(1):1. doi:10.1042/BSR20160548
15. Stellavato A, Vassallo V, La Gatta A, et al. Novel hybrid gels made of high and low molecular weight hyaluronic acid induce proliferation and reduce inflammation in an osteoarthritis in vitro model based on human synoviocytes and chondrocytes. *Biomed Res Int*. 2019;23 (2019):4328219.
16. Stellavato A, De Novellis F, Reale S, De Rosa M, Schiraldi C. Hybrid complexes of high and low molecular weight: evaluation using an in vitro model of osteoarthritis. *J Biol Regul Homeost Agents*. 2016; Oct-Dec;30(4 Suppl 1):7–16.
17. Mizuno T. Development of anti-tumor polysaccharides from mushroom fungi, Food Foods Ingredients J. *Jpn*. 1996;167:69–85.
18. Ren G, Xu L, Lu T, Yin J. Structural characterization and anti-viral activity of lentinan from Lentinus edodes mycelia against infectious hematopoietic necrosis virus. *Int J Biol Macromol*. 2018;115:1202–1210. doi:10.1016/j.ijbiomac.2018.04.132
19. Shirasaka T, Nakano K, Takechi T, et al. Anti-tumor effect of 1 M tegafur-0.4 M 5-chloro-2,4- dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. *Cancer Res*. 1996;56:2602–2606.
20. Ooi VEC, Liu F. Immunomodulation and anti-cancer activity of polysaccharide–protein complexes. *Curr Med Chem*. 2000;7:715–729. doi:10.2174/0929867003374705
21. Liu Y, Zhao J, Zhao Y, et al. Therapeutic effects of lentinan on inflammatory bowel disease and colitis-associated cancer. *J Cell Mol Med*. 2019;23(2):750–760.
22. Liu Y, Zhao J, Zhao Y, et al. Therapeutic effects of lentinan on inflammatory bowel disease and colitis-associated cancer. *J Cell Mol Med*. 2019;23(2):750–760.
23. Ahn H, Jeon E, Kim JC, et al. Lentinan from shiitake selectively attenuates AIM2 and non-canonical inflammasome activation while inducing pro-inflammatory cytokine production. *Sci Rep*. 2017;7 (1):1314. doi:10.1038/s41598-017-01462-4
24. Nishitani Y, Zhang L, Yoshida M, et al. Intestinal anti-inflammatory activity of lentinan: influence on IL-8 and TNFR1 expression in intestinal epithelial cells. *PLoS One*. 2013;8(4):e62441. doi:10.1371/journal.pone.0062441
25. Dillon CF, Rasch EK, Qiuping G, Hirsch R. Prevalence of knee osteoarthritis in the United States: arthritis data from the third national health and nutrition examination survey 1991–1994. J Rheumatol. 2006. 33(11):2271–2279.
26. Zi Y, Jiang B, He C, Liu L. Lentinan inhibits oxidative stress and inflammatory cytokine production induced by benzo(a)pyrene in human keratinocytes. *J Cosmet Dermatol*. 2019;28.
27. Liu Y, Zhao J, Zhao Y, et al. Therapeutic effects of lentinan on inflammatory bowel disease and colitis-associated cancer. *J Cell Mol Med*. 2019;23(2):750–760.
28. Zhang M, Zhang Y, Zhang L, Tian Q. Mushroom polysaccharide lentinan for treating different types of cancers: A review of 12 years clinical studies in China. *Prog Mol Biol Transl Sci*. 2019;163:297–328.
29. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. *Nat Rev Rheumatol*. 2015;11(1):35–44. doi:10.1038/nrrheum.2014.162
30. Kleine SA, Gogal RM, George C, Thaliath M, Budsberg SC. Elevated synovial fluid concentration of monocyte chemoattractant Protein-1 and Interleukin-8 in dogs with osteoarthritis of the stifle. *Vet Comp Orthop Traumatol*. 2019;22.
31. Jia T, Qiao J, Guan D, Chen T. Anti-inflammatory effects of licochalcone A on IL-1β-stimulated human osteoarthritis chondrocytes. *Inflammation*. 2017;40(6):1894–1902. doi:10.1007/s10753-017-0630-5
32. Tortorella MD, Malfait AM, Deccico C, Arner E. The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. *Osteoarthritis Cartilage*. 2001;9:539–552. doi:10.1053/joca.2001.0427
33. Devitt BM, Queally JM, Vioreanu M, et al. Cobalt ions induce chemokine secretion in a variety of systemic cell lines. *Acta Orthop*. 2010;81(6):756–764. doi:10.3109/17453674.2010.537806
34. Lotz M, Terkeltaub R, Villiger PM. Cartilage and joint inflammation. Regulation of IL-8 expression by human articular chondrocytes. *J Immunol*. 1992;148:466–473.
35. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):33–42. doi:10.1038/nrrheum.2010.196
36. Sun FF, Hu PF, Xiong Y, Bao JP, Qian J, Wu LD. Tricetin Protects rat chondrocytes against IL-1β-induced inflammation and apoptosis. *Oxid Med Cell Longev*. 2019;2019:4695381. doi:10.1155/2019/4695381
37. Kobayashi M, et al. Role of interleukin-1 and tumor necrosis factor α in matrix degradation of human osteoarthritic cartilage. *Arthritis Rheum*. 2005;52:128–135. doi:10.1002/art.20776
38. Nagase H, Itoh Y, Binner S. Interaction of alpha 2-macroglobulin with matrix metalloproteinases and its use for identification of their active forms. *Ann N Y Acad Sci*. 1994;732:294–302. doi:10.1111/j.1749-6632.1994.tb24744.x
39. Roman-Blas JA, Jimenez SA. NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthr Cartil*. 2006;14 (9):839–848. doi:10.1016/j.joca.2006.04.008
40. Mohetaer M, Li G, Wang Y, et al. Protective effects of gemigliptin against type II collagen degradation in human chondrocytes. *Biomed Pharmacother*. 2018;104:590–594. doi:10.1016/j.biopha.2018.04.018
41. Mengshol JA, Vincenti MP, Coon CI, et al. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-jun N-terminal kinase, and nuclear factor jB: differential regulation of collagenase 1 and collagenase 3. *Arthritis & Rheumatol*. 2000;43:801–811. doi:10.1002/1529-0131(200004)43:4<801::AID-ANR10>3.0.CO;2-4
42. Wang L, Wang J, Almqvist KF, Veys EM, Verbruggen G. Influence of polysulphated polysaccharides and hydrocortisone on the extracellular matrix metabolism of human articular chondrocytes in vitro. *Clin Exp Rheumatol*. 2002;20(5):669–676.
43. Lippiello L. Osteoarthritis and Cartilage, Glucosamine and chondroitin sulfate: biological response modifiers of chondrocytes under simulated conditions of joint stress. 2003;11(5):335–342.
44. Calamia V, Ruiz-Romero C, Rocha B, et al. Pharmacoproteomic study of the effects of chondroitin and glucosamine sulfate on human articular chondrocytes. *Arthritis Res Ther*. 2010;12(4):R138. doi:10.1186/ar3077
45. Jomphe C, Gabriac M, Hale TM, et al. Chondroitin sulfate inhibits the nuclear translocation of nuclear factor-kappaB in interleukin-1beta-stimulated chondrocytes. *Basic Clin Pharmacol Toxicol*. 2008;102(1):59–65.

46. Calamia V, Lourido L, Fernández-Puente P, et al. Secretome analysis of chondroitin sulfate-treated chondrocytes reveals anti-angiogenic, anti-inflammatory and anti-catabolic properties. *Arthritis Res Ther*. 2012;14(5):R202. doi:10.1186/ar4040
47. Stellavato A, Tirino V, de Novellis F, et al. Biotechnological chondroitin a novel glycosamminoglycan with remarkable biological function on human primary chondrocytes. *J Cell Biochem*. 2016;117(9):2158–2169. doi:10.1002/jcb.25556
48. Jomphe CJ, Gabriac M, Hale TM, et al. Chondroitin sulfate inhibits the nuclear translocation of nuclear factor-kappaB in interleukin-lbeta-stimulated chondrocytes. *Basic Clin Pharmacol Toxicol*. 2008;102:59–65. doi:10.1111/j.1742-7843.2007.00158.x
49. Volpi N. Oral bioavailability of chondroitin sulfate (Condrosulf) and its constituents in healthy male volunteers. *Osteoarthritis Cartilage*. 2002;10:768–777. doi:10.1053/joca.2002.0824