

Moxibustion Ameliorates Synovial Inflammation in Rheumatoid Arthritis by Regulating the USP19/P62/NLRP3 Pathway

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Moxibustion Ameliorates Synovial Inflammation in Rheumatoid Arthritis by Regulating the USP19/P62/NLRP3 Pathway

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Abstract

Objective: This study aims to investigate the effects of moxibustion on the Ubiquitin-Specific Protease 19/P62/NOD-like receptor family, pyrin domain containing 3 (USP19/P62/NLRP3) pathway in RA mouse model, in order to explore its role and mechanisms in treating RA synovitis.

Methods: Male BALB/c mice were randomly divided into three groups: a control group (Ctrl), an RA model group (RA), and a moxibustion group (Mox), with 8 mice in each group. The RA model was established by intradermal injection at the base of the tail with bovine type II collagen emulsified in incomplete Freund's adjuvant. Mice in the Moxibustion group received moxibustion treatment at the bilateral acupoints of "Shenshu" (BL23) and "Zusanli" (ST36). Each acupoint was treated for 15 minutes per session. The treatment comprised 3 courses, with each course consisting of 7 daily sessions. Arthritis severity was assessed using the arthritis index (AI) score. Pathological changes in the ankle joints were examined by Hematoxylin and Eosin (H&E) staining. Immunofluorescence assay was used to detect the expression of proteins related to the USP19/P62/NLRP3 signaling pathway in synovial tissues. Quantitative real-time polymerase chain reaction (qRT-PCR) was employed to determine the expression levels of genes associated with the USP19/P62/NLRP3 signaling pathway in synovial tissues.

Results: After modeling, the mice exhibited marked redness and swelling of the feet, with a concomitant increase in the arthritis index score. Moxibustion treatment alleviated the symptoms of joint redness and swelling and reduced the arthritis index score. Meanwhile, HE staining revealed focal loss of synovial cells, loose arrangement of fibrous connective tissue, and inflammatory cell infiltration in the joints of RA model mice. These pathological changes were significantly ameliorated following moxibustion intervention. The study found that the expression of inflammatory cytokines and macrophage phenotypes was dysregulated in the RA model. Specifically, there was an up-regulation in

the expression of the pro-inflammatory cytokines IL-1 β and IL-18, as well as the pro-inflammatory macrophage marker CD38. Conversely, the expression of the anti-inflammatory cytokine Arg1, IL-4, and the anti-inflammatory macrophage marker CD163 was down-regulated. Moxibustion treatment effectively reversed these alterations. Furthermore, moxibustion was shown to modulate the expression of key components within the USP19/P62/NLRP3 pathway, including USP19, P62, LC3II, NLRP3, and IRF4, thereby alleviating synovial inflammation in RA.

Conclusion: Moxibustion treatment can ameliorate inflammatory injury in RA mice, regulate USP19 expression, further modulate autophagy levels and NLRP3 activity, thereby exerting a therapeutic effect on synovial inflammation in RA mice. The underlying mechanism may be mediated by the USP19/P62/NLRP3 pathway.

Key words: Rheumatoid arthritis; Synovitis; USP19; Autophagy; NLRP3

1.Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease primarily characterized by inflammatory arthritis, which typically involves the small joints of the hands and feet. The disease can result in significant disability and mortality [1]. Due to its complex pathogenesis and chronic, refractory nature, patients often require long-term medication to alleviate symptoms and clinical signs, imposing a substantial economic burden on both the patients and their families.

Autophagy is an intracellular lysosomal catabolic process that degrades the cell's own organelles and cytoplasm. It affects RA pathological changes by participating in synovial inflammation and plays a crucial role in coordinating inflammation [2]. In the early stage of RA progression, autophagy can enhance the protective effect on the body. However, with disease progression, excessive autophagy leads to reduced cell apoptosis, resulting in massive proliferation of synovial cells and exacerbation of the inflammatory response. Studies have found [3] increased expression of microtubule-associated protein 1A/1B-light chain 3 (LC3) and significantly decreased P62 levels in rat synovial tissue, indicating significant activation of the autophagic pathway in inflammatory cells. Furthermore, higher RA disease activity is associated with higher autophagy levels and LC3 expression [4]. Ubiquitin-specific peptidase 19 (USP19) is a newly discovered protease in recent years, belonging to the ubiquitin-proteasome family. Studies have revealed that ubiquitination modification is involved in the regulation of autophagic pathway, and the accurate coordination between the ubiquitination and autophagic systems can promptly clear these misfolded proteins [5]. To further understand how ubiquitination affects autophagy, researchers conducted a screen using small interfering RNAs targeting human deubiquitinase genes and found that USP19 can specifically participate in the regulation of Beclin-1 stability. Meanwhile, it regulates the K11-linked ubiquitination modification of

Beclin-1 through its deubiquitinase activity, identifying USP19 as a key regulatory factor involved in the autophagic process [6], which plays a significant role in combating inflammation and autoimmune diseases [7].

The NLRP3 inflammasome is a protein complex composed of NLRP3, Caspase-1, and ASC. As an important immune-inflammatory complex, it plays a key role in immune-inflammatory responses such as RA [8]. In RA, abnormal activation of the NLRP3 inflammasome is considered one of the important mechanisms driving synovial inflammation. Activated NLRP3 promotes the maturation and secretion of pro-inflammatory factors such as IL-1 β and IL-18 through interaction with Caspase-1, thereby exacerbating the inflammatory response [9]. Studies have shown [10, 11] that NLRP3 expression is significantly upregulated in the synovial tissue of RA patients and is closely associated with macrophage polarization and the secretion of inflammatory factors. Activation of NLRP3 not only promotes the polarization of M1-type macrophages but may also lead to the continuous exacerbation of inflammation by disrupting the cell membrane and releasing cytokines. Therefore, the NLRP3 inflammasome is regarded as a key regulatory factor in RA synovial inflammation, and its abnormal activation plays an important role in the pathogenesis of RA.

As an important part of traditional Chinese medicine (TCM), moxibustion is beneficial to qi strengthening and is able to warm the meridian. It can alleviate the symptoms of RA joint pain and swelling, reduce rheumatoid factor levels, inhibit inflammatory responses, and protect the joint synovium, playing an important role in the treatment of RA [12, 13]. Based on the favorable effects of acupuncture and moxibustion in regulating RA synovial inflammation, this experiment established an RA mouse model to observe the effects of moxibustion treatment on synovial inflammation, USP19 expression, autophagy levels, and NLRP3 activity in the RA model, to further investigate the mechanism of moxibustion in treating RA synovial inflammation.

2 Materials and Methods

2.1 Animals

Male BALB/c mice aged 6-8 weeks, weighing 18-22g, were provided by Chengdu Dashuo Animal Technology Co., Ltd. [Specific Pathogen Free (SPF) grade, License No. SCXK (Chuan) 2020-030]. Before the experiment, the mice were acclimatized for one week. The mice were housed in an environment with a room temperature of 23 ± 1 °C, relative humidity of 50%-60%, regular ventilation, natural light-dark cycle, and free access to food and water. This experiment was approved by the Experimental Ethics Committee of Chengdu Hospital of Integrated Traditional Chinese and Western Medicine (Approval Number: 2023KT023).

2.2 Establishment of RA Model

Bovine type II collagen (CII) at a concentration of $2\text{mg} \cdot \text{mL}^{-1}$ was added dropwise to an equal volume of incomplete Freund's adjuvant and fully ground and mixed on ice to prepare a CII emulsion with a final concentration of $1\text{mg} \cdot \text{mL}^{-1}$. On the 1st day of the experiment, $50\mu\text{L}$ of the emulsion was injected into the base of the tail of the mice in the RA group and Mox group. 7 days later, a booster injection was given at the same dose using the same method. The Ctrl group was injected with the same dose of normal saline at the same time. After confirming successful modeling by arthritis index scoring, the experiment proceeded to the next step [14].

2.3 Moxibustion Methods

Treatment was initiated on the 14th day after modeling. Shenshu (BL23) is located 7 mm laterally below the spinous process of the second lumbar vertebra. Zusanli (ST36) is located below the knee joint and 6 mm beside the anterior tibial muscle. The rat's fur near the acupoints of BL23 and ST36 was shaved to be convenient for moxibustion. Moxibustion was applied to "BL23" and "ST36" acupoints for 15 minutes per acupoint daily with alternate use of left and right acupoints. One treatment course consisted of 7 consecutive daily sessions, and a total of 3 courses were administered. The treatment schedule is illustrated in Figure 1A and 1B.

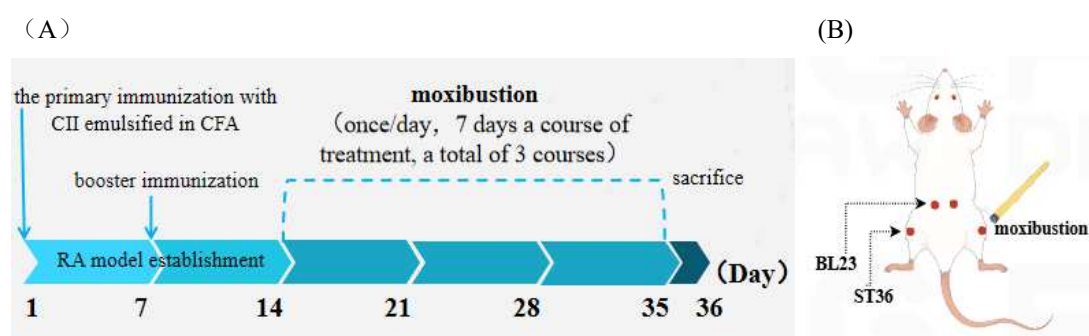


Figure 1(A) depicts the experimental flow chart. (B) represents the moxibustion diagram.

2.4 Arthritis index scoring

The arthritis index (AI) scoring method [14] was scored according to an established scale as follows: 0, no arthritis; 1, redness or mild swelling; 2, moderate swelling at the joint; 3, severe swelling; 4, severe swelling with an inability to bear weight. The total AI for each mouse was calculated as the sum of scores from all four paws, with higher scores indicating more severe arthritis. Scoring was recorded once before the initial immunization and once weekly after immunization, for a total of 6 times.

2.5 Histological Analysis

On the 36th day of the experiment, the mice were weighed and anesthetized by intraperitoneal injection of 1% sodium pentobarbital ($1\text{mL}/100\text{g}$) for in vivo sampling. The skin overlying the left ankle joint was incised, and the surrounding soft tissues were dissected. After trimming excess bone,

the joint was immersed and fixed in 4% paraformaldehyde solution. Subsequently, the samples underwent standard pathological processing, including dehydration, trimming, paraffin embedding, sectioning at 5 μ m thickness, HE staining, and coverslipping with mounting medium. Pathological changes of the mouse ankle joint were observed under a light microscope, and representative images were captured for documentation

2.6 Immunofluorescence Detection

After anesthesia, the surrounding soft tissues of the right ankle joint of the mice were dissected. Then the joint was opened, and the pale yellow synovial tissue was collected. The tissue samples were immediately fixed in 4% paraformaldehyde for 24 hours, followed by standard processing, including rinsing, decalcification in 10% EDTA solution, gradient ethanol dehydration, xylene transparency, and paraffin embedding for subsequent analysis. The fixed tissue paraffin blocks were taken out, sectioned at a thickness of 4 μ m, and antigen retrieval was performed after routine dewaxing and dehydration. The sections were washed 3 times in PBS solution, 5 minutes each time. Subsequently, goat serum was used for blocking at room temperature for 1 hour. After blocking, the sections were dried by flicking, and then incubated overnight at 4° C with primary antibodies (USP19 1:1000, P62 1:500, NLRP3 1:1000, LC3-II 1:100, IRF4 1:500). After washing 3 times with PBS, fluorescent secondary antibodies were added and incubated in the dark at room temperature for 50 minutes. Cell nuclei were counterstained with DAPI, and finally, the sections were mounted. Images were observed and captured under a fluorescence microscope, and fluorescence intensity was analyzed using Image-Pro Plus 6.0 software.

2.7 Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted using the Trizol method, and its concentration of RNA was measured. The RNA was then reverse-transcribed into cDNA, and qRT-PCR was performed with β -actin as the internal reference gene. The relative expression level of the target gene was calculated using the $2^{-\Delta\Delta CT}$ method. The primer sequences are shown in Table 1.

Table 1 the primer sequences of qRT-PCR

Gene	Primer sequence	Length (bp)
USP19	F: 5'CAGCTGTACCAGAGGATCCA3'	120
	R: 5'GCTCCACTCAGGTAGTCCTT3'	
NLRP3	F: 5'GCTCTTACTGACTGGCATGAG3'	100
	R: 5'CGCAGCTCTAGGAGCATGTG3'	
IL-1 β	F: 5' AAGGAGAACCAAGCAACGACAAAA3'	150

	R: 5'TGGGGAACCTCTGCAGACTCAAAC 3'	
IL-4	F: 5'TGGTTCCATGCTTTCCTGGACTCCT3'	140
	R: 5'TTCCTGGGCCAAAGAGGAAGTGATT3'	
IL-18	F: 5'TGGTTCCATGCTTTCCTGGACTCCT3'	140
	R: 5'TTCCTGGGCCAAAGAGGAAGTGATT3'	
IRF4	F: 5'AGCCAGCTCATCCTGGACAT3 '	120
	R: 5'CTGGTTGTCCAGGACTGTCA 3'	
	F: 5'TCACCTGAGCTTTGATGTCG3 '	
Arg-1	R: 5' CTGAAAGGAGCCCTGTCTTG3'	100
	F: 5' GGCTGATTGAGTCCCTCTCCCAGAT3'	
P62	R: 5' CGGCGGGGGATGCTTTGAATACTGG3'	140
	F: 5' TCCTGGACAAGACCAAGTTTTT3'	
LC3-II	R: 5'CTGGGAGGCGTAGACCATATAGAG3 '	212
β -actin	F: 5'TGTCACCAACTGGGACGATA3'	165
	R: 5'GGGGTGTTGAAGGTCTCAA3'	

2.8 Statistical Analysis

Data were analyzed using SPSS 25.0. The Shapiro-Wilk (S-W) normality test was used to assess data distribution. For normally distributed data, one-way analysis of variance (One-way ANOVA) was used for comparisons among multiple groups. For non-normally distributed data, non-parametric tests were used for group comparisons. The arthritis index scores were compared among multiple groups using two-way repeated measures ANOVA. $P < 0.05$ was considered statistically significant.

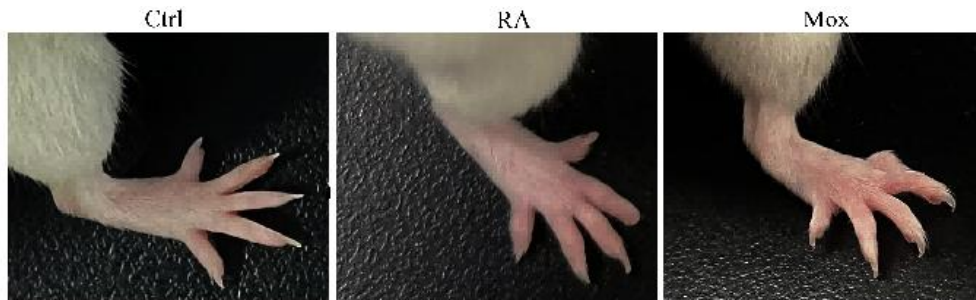
3. Results

3.1 Reduction of Arthritis Index Score by Moxibustion

Joint redness and swelling are the most direct manifestations of RA inflammation. After CII and

FCA injection, the joints exhibited redness, swelling, and an increased AI score. Following moxibustion treatment, joint redness and swelling symptoms were alleviated, and the AI score decreased accordingly, as shown in Figure 2A and 2B.

(A)



(B)

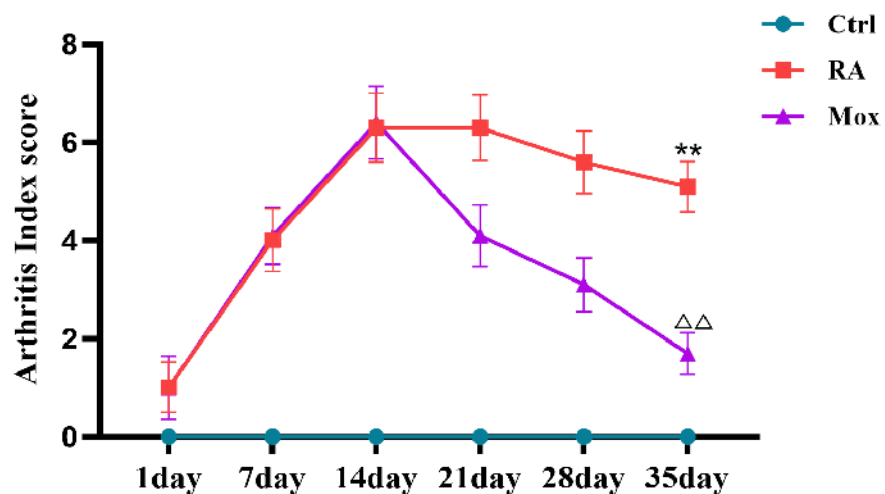


Figure 2 shows the effects of moxibustion on joint redness and swelling and arthritis index scores in mice. (A) represents the status of joint redness and swelling. (B) represents the arthritis index scores. Data were expressed as the mean $\pm$ SD ($n = 8$). Note: ** $P < 0.01$ versus the Ctrl group; $\triangle \triangle P < 0.01$ versus the RA group.

3.2 Alleviation of Joint Pathology by Moxibustion Treatment

Joint status directly reflects pathological changes. HE staining results showed that the RA group exhibited local loss of synovial cells, loosely arranged fibrous connective tissue, and inflammatory cell infiltration. After moxibustion treatment, the Mox group exhibited alleviated synovial cell loss, diminished inflammatory cell infiltration, and a more organized arrangement of fibrous connective tissue, as shown in Figure 3.

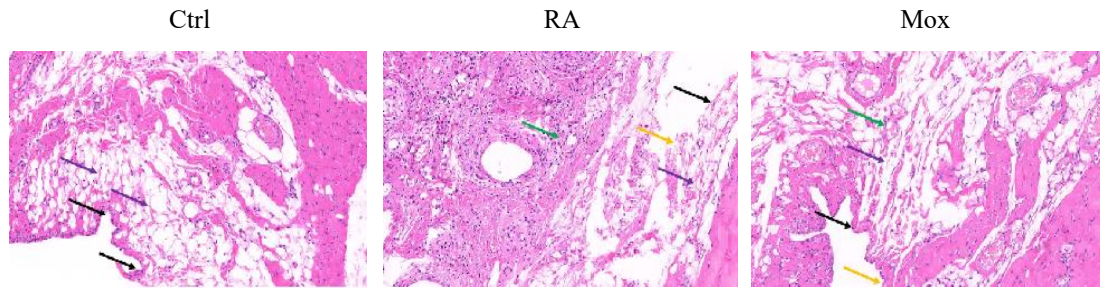
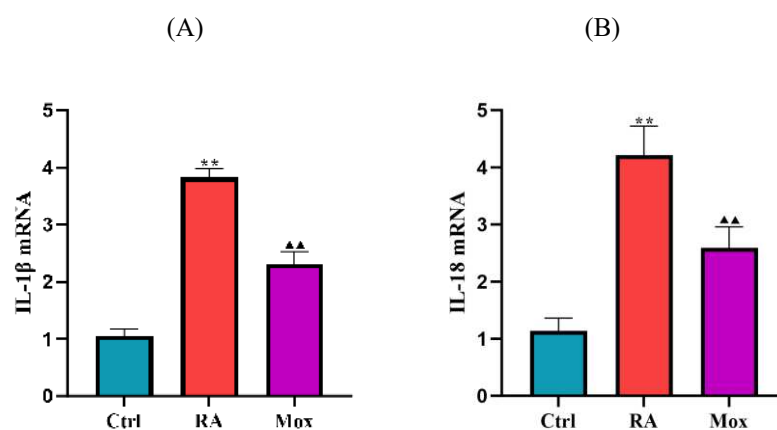


Figure 3 shows the effect of moxibustion on joint pathology in RA mice. The pathological changes of the joint are shown (magnification, $\times 400$; scale bar, 20 μm). Note: Synovial cells (black arrow); absence of synovial cells (yellow arrow); loose arrangement of fibrous connective tissue (purple arrow); inflammatory cell infiltration (green arrow).

3.3 Alleviation of RA Inflammation by Moxibustion Treatment

Inflammatory cytokines, as the main mediators sustaining synovial inflammation in RA, contribute to RA progression in various forms. The expression of pro-inflammatory cytokine is increased in RA, which collectively promote the recruitment of leukocytes in the joints. This subsequently induces angiogenesis, promotes inflammatory cell infiltration and synovial cell proliferation, and can effectively stimulate the release of other pro-inflammatory mediators, thereby promoting synovial inflammation. The study found that after injection of CII, the expressions of IL-1 β (Fig.4A) and IL-18 (Fig.4B) were increased, while the expressions of Arg1 (Fig.4C) and IL-4 (Fig.4D) were decreased. After moxibustion intervention, this situation changed, that is, the expressions of IL-1 β and IL-18 were decreased, and the expressions of Arg1 and IL-4 were increased, as shown in Figure 4A, 4B, 4C and 4D.



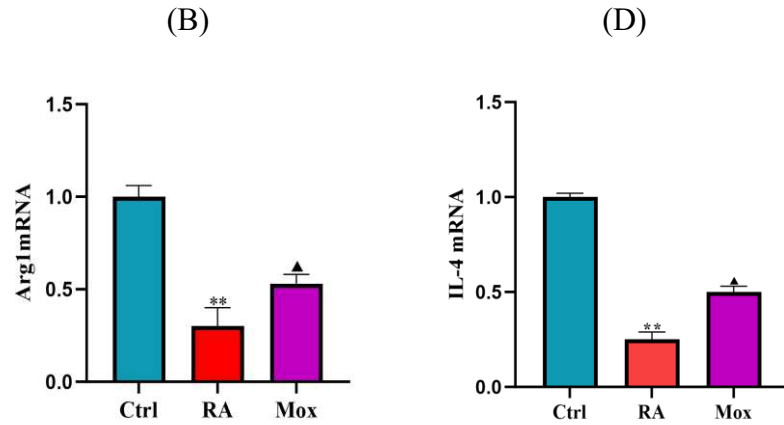
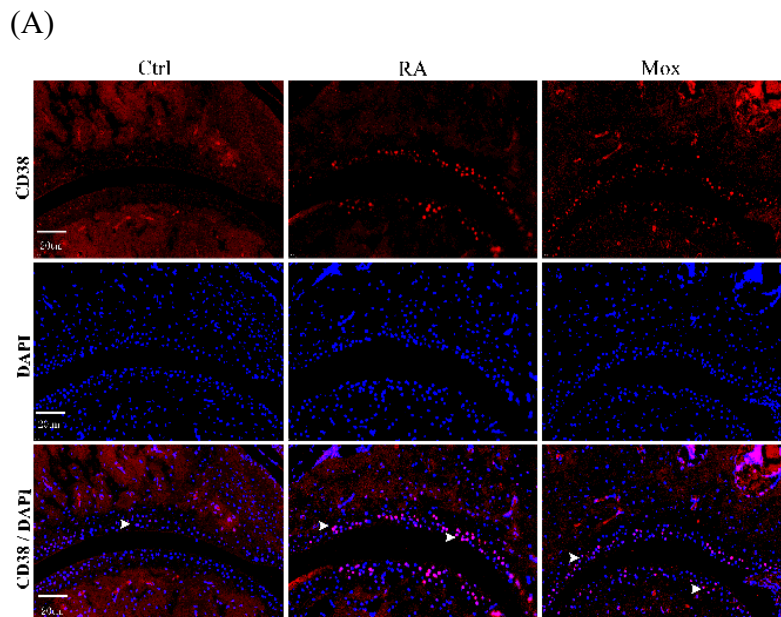


Figure 4 shows the effect of moxibustion on alleviating the inflammatory response in RA mice. The levels of the inflammatory cytokines IL-1 β (A), IL-18 (B), Arg1(C) and IL-4 (D) were measured by ELISA. Data were expressed as the mean $\pm$ SD ($n = 8$). Note: ** $P < 0.01$ versus the Ctrl group; ▲ $P < 0.05$ versus the RA group; ▲ $P < 0.01$ versus the RA group.

3.4 Regulation of the Expression of Different Macrophage Phenotypes by Moxibustion

Macrophage infiltration is a characteristic feature of the RA pathological process. By secreting various inflammatory factors and matrix metalloproteinases, macrophages coordinate inflammation and hyperplasia, which is crucial for the synovial inflammatory process. In RA, resting macrophages can polarize from one phenotype to another upon inflammatory stimulation. The study found that in the RA group, the expression of the pro-inflammatory macrophage phenotype marker CD38 (Fig.5A) increased, while the expression of the anti-inflammatory macrophage phenotype marker CD163 (Fig.5B) decreased. After moxibustion treatment, CD38 expression decreased, and CD163 expression increased, as shown in Figure 5A and 5B



(B)

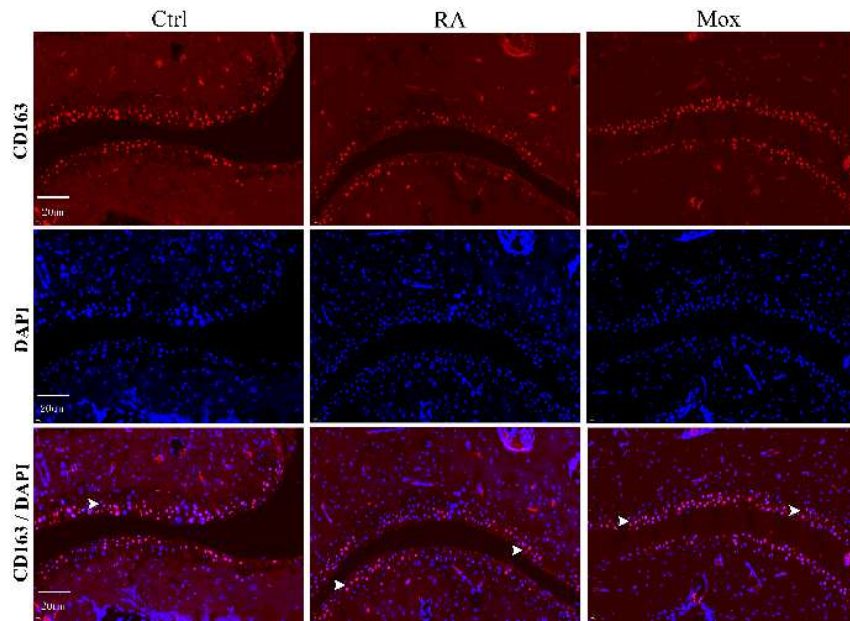
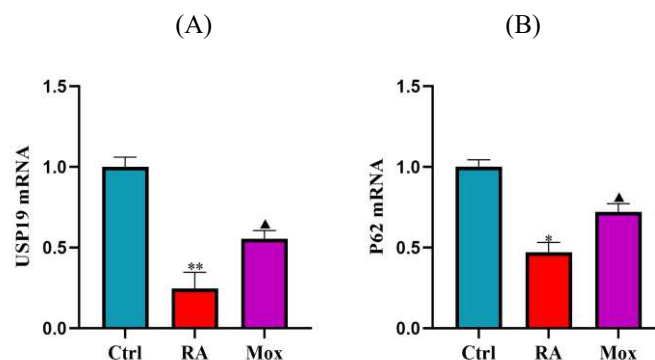


Figure 5 shows the effect of moxibustion on the expression of different macrophage phenotypes. Moxibustion inhibited M1-polarized Mφs and advocated M2-polarized Mφs. Immunofluorescence was performed to examine the expression of different macrophages markers (CD38 (A) and CD163 (B)) in synovium (magnification, $\times 400$; scale bar, 20 μm). Note: arrows represent the positive expression.

3.5 Effect of Moxibustion on the Expression of Genes in the USP19/P62/NLRP3 Pathway

The study found that in the RA group, the expression levels of USP19mRNA (Fig.6A) and P62mRNA (Fig.6B) decreased, while the expression levels of NLRP3mRNA (Fig.6C), IRF4mRNA (Fig.6D), and LC3-II mRNA (Fig.6E) increased. After moxibustion intervention, the expression levels of USP19mRNA and P62mRNA increased, while the expression levels of NLRP3mRNA, IRF4mRNA, and LC3-II mRNA decreased, as shown in Figure 6A to 6E.



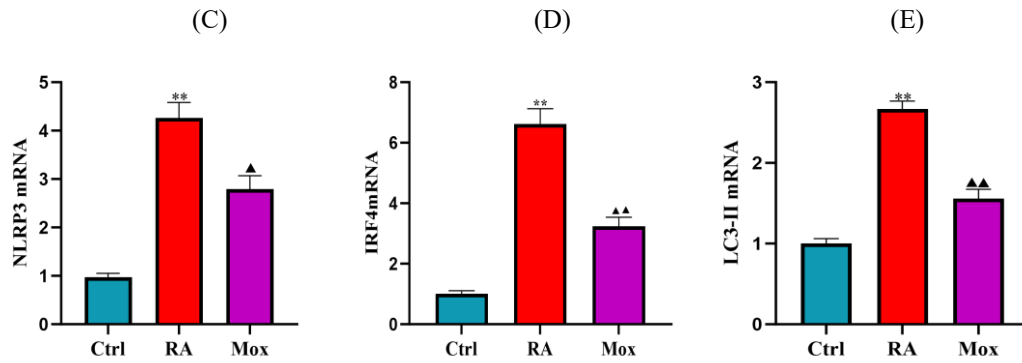
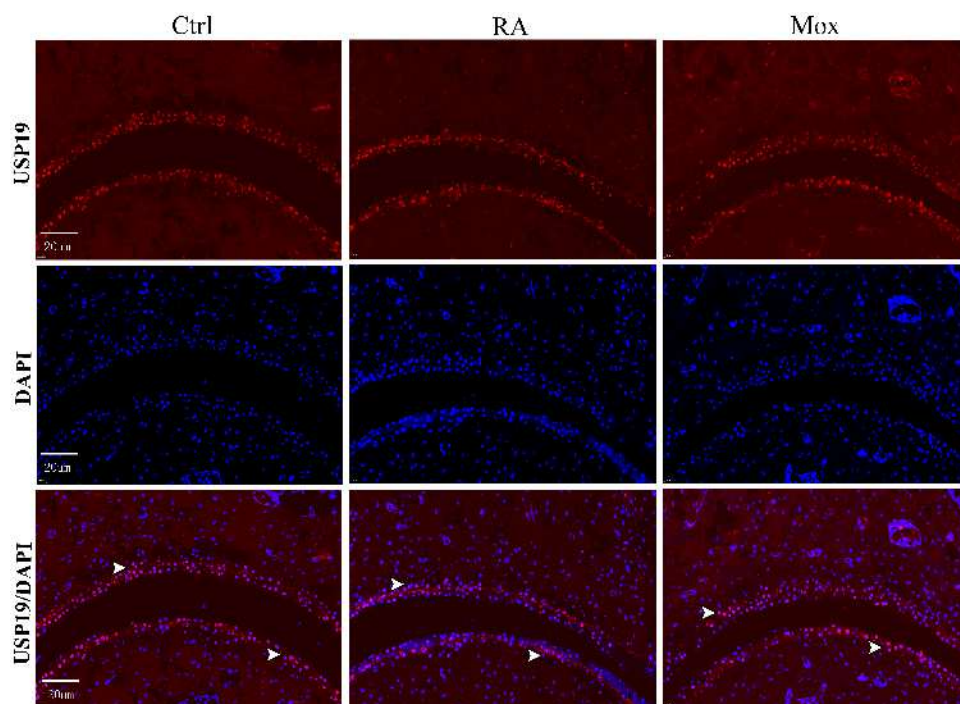


Figure 6 shows the expression of genes related to USP19/P62/NLRP3 pathway. The expression level of mRNA was examined in synovial tissue by qRT-PCR. (A) represents the expression of USP19mRNA. (B), (C), (D) and (E) respectively represent the expression of P62mRNA, NLRP3mRNA, IRF4mRNA and LC3-II mRNA. Data were expressed as the mean $\pm$ SD ($n = 8$). Note: ** $P < 0.01$ versus the Ctrl group; ▲ $P < 0.05$ versus the RA group; ▲▲ $P < 0.01$ versus the RA group

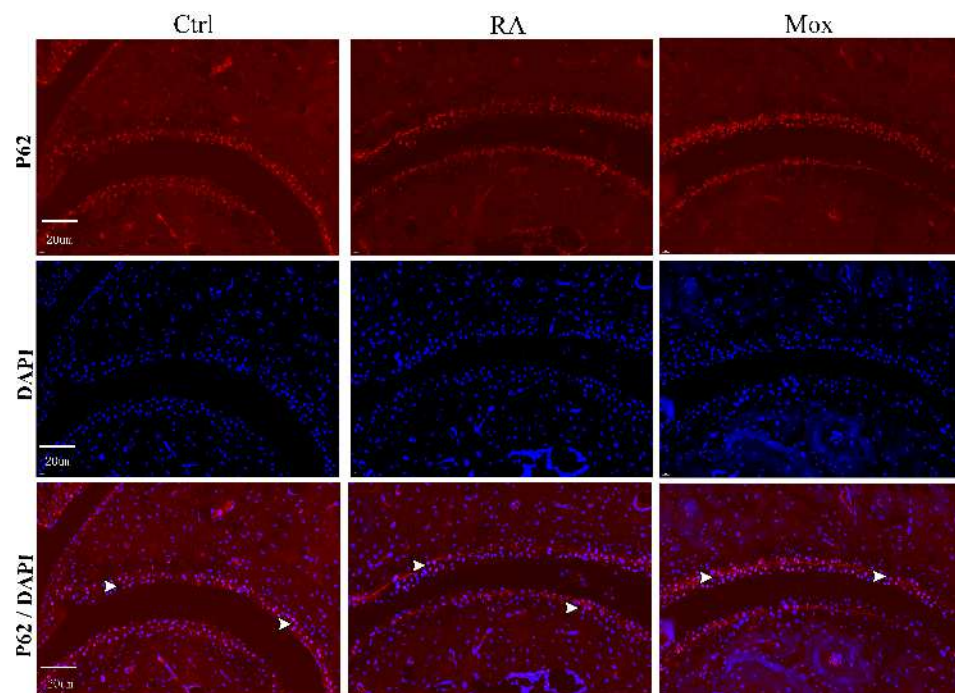
3.6 The Effect of Moxibustion on the Expression of Proteins Related to the USP19/P62/NLRP3 Pathway

The synovium is a target of RA invasion, and persistent synovial inflammation is a critical factor in RA disease progression. Immunofluorescence detection showed that the expressions of USP19 (Fig.7A) and P62 (Fig.7B) proteins in the synovium of the RA group were decreased, while the expressions of NLRP3 (Fig.7C), IRF4 (Fig.7D), and LC3-II (Fig.7E) proteins were increased. After moxibustion intervention, the expressions of USP19 and P62 proteins were increased, while the expressions of NLRP3, IRF4, and LC3-II proteins were decreased. The expression of synovial proteins was consistent with gene expression. After moxibustion treatment, autophagy and NLRP3 activity were inhibited, as shown in Figure 7.

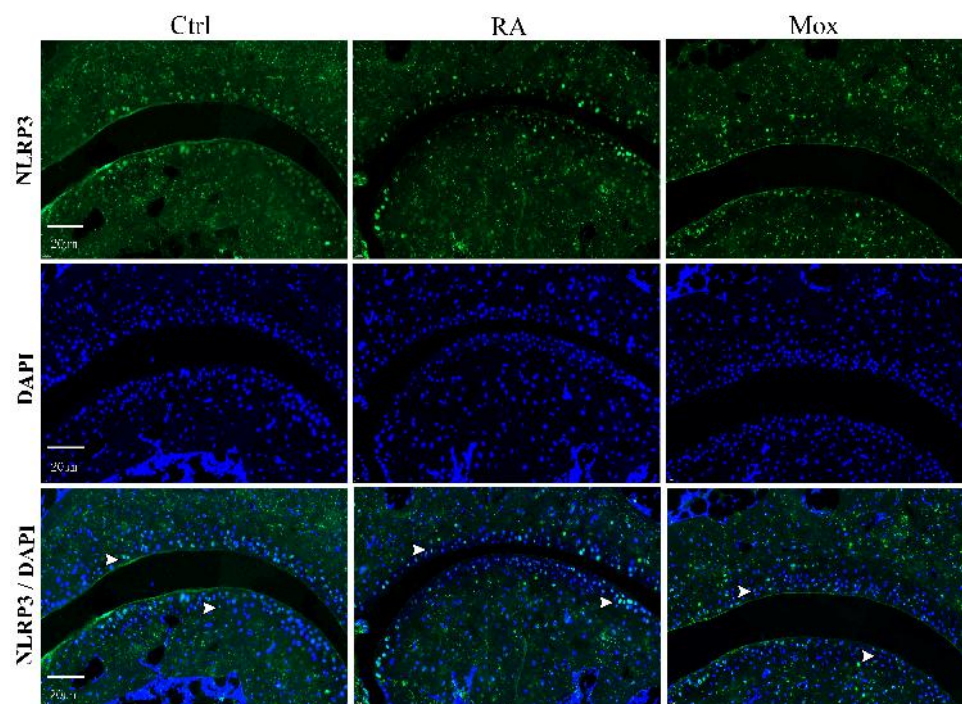
(A)



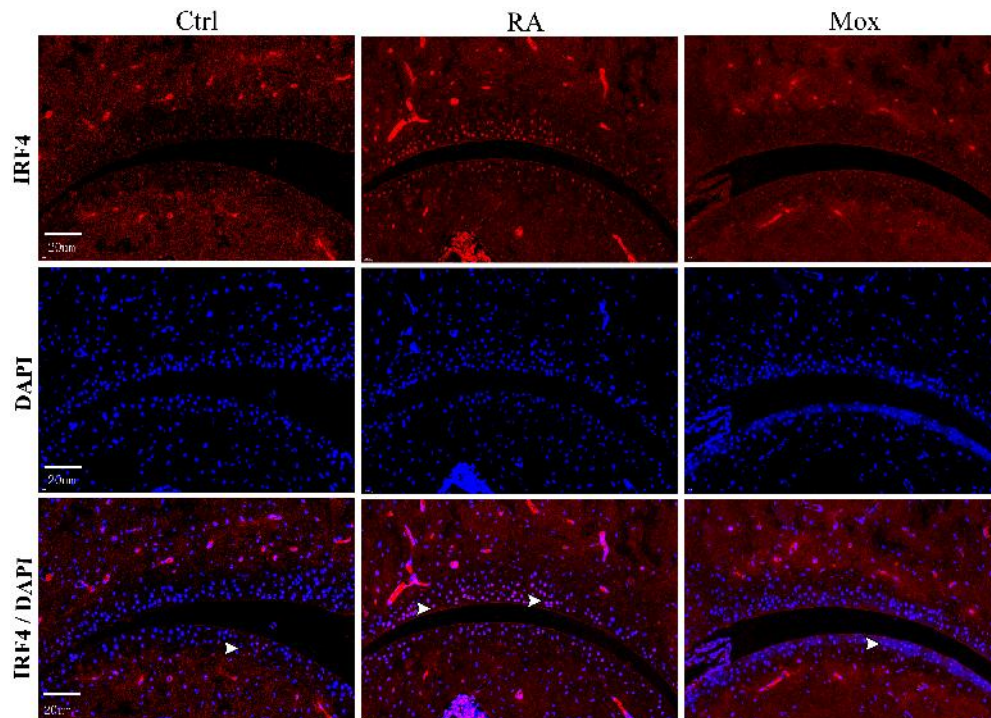
(B)



(C)



(D)



(E)

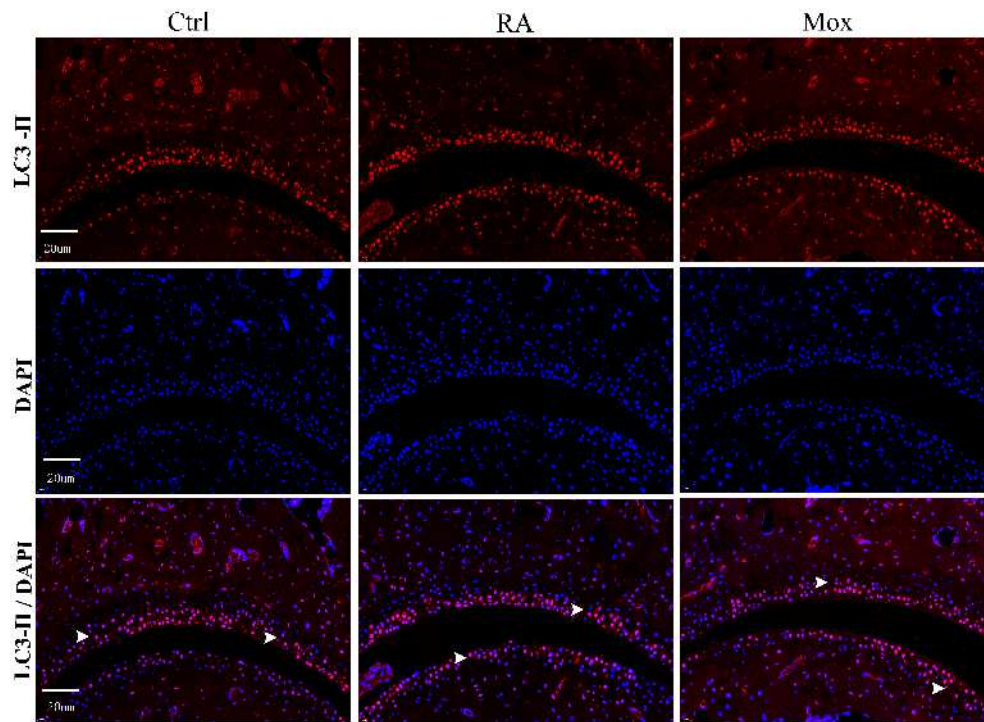


Figure 7 shows the effect of moxibustion on the expression of USP19/P62/NLRP3 pathway related proteins. Immunofluorescence was used to examine the expression of USP19 (A). (B), (C), (D) and (E) respectively represent the expression of P62, NLRP3, IRF4 and LC3-II in synovial tissue (magnification, $\times 400$; scale bar, 20 μm). Note: arrows represent the positive expression.

4 Discussion

Moxibustion is an important part of traditional Chinese medicine, which has the effects of strengthening the body resistance to eliminate pathogenic factors and warming meridians to promote circulation. It can effectively alleviate the clinical symptoms of pain, swelling, and stiffness in RA [12, 13]. Studies have shown that acupuncture at the “Zusanli” (ST36) acupoint contributes to the regulation of central neurotransmitters and the restoration of the dynamic balance of T cells, thereby regulating the immune function of the body [15-16]. Modern studies have also confirmed that moxibustion at the “Shenshu” (BL23) acupoint can regulate the function of the hypothalamic-pituitary-adrenal axis in mice, promote the secretion of anti-inflammatory factors such as IL-4 and IL-10, and significantly inhibit synovial inflammatory responses [17-18]. The results of this study show that moxibustion can effectively ameliorate the joint pathology of RA mice, regulate the expression of inflammatory factors, and alleviate synovial inflammation.

USP19 belongs to the ubiquitin-proteasome family, is localized on the endoplasmic reticulum membrane, and plays a key role in the endoplasmic reticulum-associated degradation (ERAD) pathway

[19]. The ubiquitin-proteasome system is involved in the regulation of mitochondrial function [20]. USP19 promotes mitophagy by removing the K11-linked ubiquitin chain that stabilizes Beclin1. Studies have shown that knockdown of USP19 reduces the level of FUN14 domain-containing protein 1 (FUND C1), suggesting that USP19 may be involved in the mitophagy process by regulating the expression of FUND C1 in BEAS-2B cells treated with cystathionase (CSE) [21]. As a target of the unfolded protein response, USP19 is involved in physiological and pathological processes including inflammation, autophagy, and DNA damage repair. A growing number of studies have shown that USP19 plays a key role in immune-inflammatory regulation, modulates various intracellular signals and immune responses, and exerts a significant role in combating inflammation and autoimmune diseases [22]. USP19 promotes autophagy by stabilizing Beclin-1-mediated clearance of reactive oxygen species (ROS), inhibits the activation of the NLRP3 inflammasome, and produces anti-inflammatory effects [23]. Researchers used CRISPR/Cas9 technology to generate gene knockout mice and found that in mice with peritonitis, the deletion of USP19 reduced the proportion of type 2 macrophages (M2). Meanwhile, siRNA-mediated silencing of USP19 specifically enhanced the activation of the NLRP3 inflammasome and upregulated the secretion of interleukin-1 β (IL-1 β), thereby exacerbating peritoneal inflammation in mice [23]. The results of this study show that the expression of USP19 in the synovial tissue of RA mice is decreased. Moxibustion can up-regulate the expression of USP19, inhibit the activity of the NLRP3 inflammasome, and reduce the level of autophagy, which is consistent with the above studies.

Autophagy is an important catabolic process in organisms, which exerts a cytoprotective effect by degrading intracellular pathogens, damaged organelles, and protein aggregates [24]. The activation of the autophagic mechanism in mammals is achieved through mammalian target of rapamycin complex 1 (mTORC1), and upstream signal transduction is mediated by enzymes such as phosphatidylinositol 3-kinase (PI3K), which stimulate the autophagic process [25]. Autophagy-related protein 1 regulated by Beclin-1 or autophagy and Beclin 1 regulator 1 (AMBRA1) acts as a positive regulator of autophagy, which induces Beclin-1-related autophagy, thereby transmitting the protein through the action of the class III PI3K complex. Beclin-1 is associated with triggered autophagic activity, which is affected by its binding to the B-cell lymphoma-2 (Bcl-2) gene, which inhibits the autophagic process [26]. Impairment of the autophagic mechanism or increased autophagic level can have negative effects on cells [27]. Studies have found that autophagy is activated in RA mice, and the high expression of autophagy-related proteins Beclin-1, Atg5, Atg12, and LC3B exacerbates joint synovial hyperplasia and inflammatory infiltration [28]. Autophagy has a dual role in rheumatoid arthritis fibroblast-like synoviocytes (RASf). It can promote endoplasmic reticulum stress-induced cell death and inhibit proteasome inhibition-induced apoptosis [29]. Autophagy also exacerbates joint inflammation by

altering the secretion of pro-inflammatory cytokines [30]. Specifically, autophagy can promote the secretion of IL-1 β . Similarly, autophagy regulates the interferon- γ (IFN- γ) signaling pathway and cellular inflammation by limiting the production of ROS and regulating interleukin-23 (IL-23) and transforming growth factor- β (TGF- β). In RA, the level of autophagy is positively correlated with disease activity, and inhibiting autophagy can alleviate the symptoms of RA [4]. Researchers evaluated the effect of the autophagy inhibitor 3-Methyladenine (3-MA) in collagen-induced arthritis (CIA) mice and found that 3-MA significantly reduced the level of inflammatory cytokines, inhibited oxidative levels and bone-resorbing cytokines, and significantly inhibited the abnormal autophagy level of the ankle joint in CIA mice, indicating that 3-MA protects against bone destruction in CIA-induced mouse arthritis through anti-inflammatory effects and autophagy inhibition [31]. This study found that moxibustion treatment regulates the expression of autophagy-related proteins P62 and LC3II, inhibits autophagy levels, and alleviates synovial inflammation in RA mouse, which is consistent with the above studies.

The NLRP3 inflammasome is an important part of the immune-inflammatory response, a protein complex composed of NLRP3, Caspase-1, and apoptosis-associated speck-like protein containing a CARD (ASC) [32]. When NLRP3 is stimulated by abnormal signals, NLRP3 assembles with ASC and Caspase-1 to form a heterodimer, initiating various downstream inflammatory signaling pathways. Studies have shown that activated Caspase-1 cleaves Gasdermin D protein, leading to the destruction and lysis of the cell membrane, promoting the polarization of macrophages to the pro-inflammatory M1 type, and causing the release of a large number of activated inflammatory factors in the cell, thereby leading to the continuous deterioration and expansion of the inflammatory response [33]. NLRP3 acts as a trigger for synovial inflammation. Clinical and animal studies have shown that NLRP3 expression is significantly up-regulated in RA [34, 35]. Researchers analyzed RA patients in the active phase and found that the mRNA and protein expressions of NLRP3, Caspase-1, and ASC, like IL-1 β and IL-18, are significantly higher than those in normal people, and the peripheral blood mononuclear cells (PBMC) in their serum secrete more IL-1 β than normal people under the stimulation of NLRP3 [36]. Researchers used *in vivo* blocking and found that inhibiting the activity of NLRP3 converts macrophages from the M1 phenotype to the M2 phenotype and alleviates the inflammatory response [37]. Meanwhile, after treatment with punicalagin, CIA mice downregulate the expression of NLRP3 and Caspase-1 to reduce pyroptosis, thereby preventing inflammatory cell death caused by the release of IL-1 β and IL-18, resulting in a significant alleviation in RA inflammatory pathological responses [38]. The results of this study show that the expression of NLRP3 in the synovial tissue of RA mice is upregulated, and after moxibustion treatment, the activity of the NLRP3 inflammasome is significantly inhibited, the inflammatory factors IL-1 β and IL-18 are decreased, and the phenotypic polarization of

macrophages is corrected, thereby significantly alleviating RA synovial inflammation.

In conclusion, moxibustion treatment can regulate USP19 expression in RA models, reduce autophagy levels, inhibit NLRP3 inflammasome activation, regulate the expression of inflammatory factors and macrophage polarization phenotypes, thereby alleviating RA synovial inflammation. USP19 plays an important role in the regulation of autophagy and NLRP3 activity in RA models, but the specific mechanism remains to be further studied.

5 Data availability

Data supporting the results of this study are available from the corresponding authors. The all original data supporting the findings of this study were supplied by Yu-mei Zhong under license and cannot be made freely available. Requests for access to these data should be made to Yu-mei Zhong, zhongyumei1205@163.com.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication

The authors declare consent for publication.

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