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Original paper

Impact of ligustrazine on chondrocyte apoptosis in knee osteoarthritis through FAK/PI3K/Akt pathway

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Abstract

Objective: To probe into the impact of ligustrazine on chondrocyte apoptosis in knee osteoarthritis (OA) and its possible mechanism.

Methods: Our team extracted the human primary chondrocytes to construct the IL-1β-induced chondrocyte damage model, detected the impact of ligustrazine on chondrocyte apoptosis through TUNEL and Western blot, applied Western blot to detect the impact of ligustrazine on matrix-degrading enzymes, matrix-associated proteins, as well as FAK/PI3K/Akt pathway, using ELISA detected the impact of ligustrazine on MCP-1, IL8, PEG2 and NO levels, applied FAK/PI3K/Akt pathway inhibitor PF573228 to inhibit FAK/PI3K/Akt pathway to detect whether the chondroprotective impact of ligustrazine was inhibited, constructed a New Zealand rabbit osteoarthritis model, applied HE staining to evaluate the impacts of ligustrazine and ligustrazine+PF573228 on cartilage histomorphology, and applied Western blot to detect the impacts of ligustrazine and ligustrazine+PF573228 on apoptotic proteins, matrix-degrading enzymes and matrix-related proteins in cartilage tissue.

Results: Ligustrazine signally inhibited IL-1β-induced chondrocyte apoptosis, reduced Bax, caspase-9, caspase-3, caspase-7, MMP3, MMP13, collagen X protein expression levels, and reduced MCP-1, IL8, PEG2, and MCP-1. NO contents, promoted collagen II and aggrecan protein expression. Additionally, ligustrazine activated the FAK/PI3K/Akt pathway, and applying PF573228 to inhibit the FAK/PI3K/Akt pathway abolished the chondroprotective impact of ligustrazine. At the in vivo level, ligustrazine activated the FAK/PI3K/Akt pathway to inhibit Bax, caspase-9, caspase-3, caspase-7, MMP3, MMP13, collagen X protein expression levels, promoted collagen II and aggrecan protein expression, and improved cartilage histomorphology.

Conclusion: Ligustrazine exerts chondroprotective impact through FAK/PI3K/Akt pathway

Keywords

Ligustrazine, FAK/PI3K/Akt pathway, knee osteoarthritis, chondrocyte apoptosis

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Introduction

Knee osteoarthritis (OA) is a universal clinical disease in orthopedics, also known as proliferative arthritis in the knee. It is a kind of osteoarthritis, which refers to the primary or secondary degeneration and structural disorder of the articular cartilage of the knee joint. Accompanied by subchondral hyperosteogeny and cartilage exfoliation, the joints are gradually destroyed and deformed, and finally a degenerative disease of knee joint dysfunction [1]. The main clinical manifestations are slow onset and development of joint pain, stiffness, tenderness, crepitus, swelling with limited functional activities. In severe cases, it is the key reason for joint dysfunction, even pain and disability in the elderly [2]. Every epidemiological survey implies that 240 people out of 100,000 suffer from this disease every year. The incidence of knee osteoarthritis is visually related to gender, age, BMI, occupation and other factors. Among them, the incidence of women is signally higher. For men, the elderly is at high risk of knee osteoarthritis. The prevalence rate can reach about 50% for people over 65, and about 80% for those over 75 [3].

At present, joint replacement clinically is still the main therapy for treating end-stage knee osteoarthritis, and there is still a lack of effective methods to prevent knee osteoarthritis from entering end-stage [4]. OA pathogenesis has not been fully studied. Among them, a large amount of evidence reveals that chondrocyte apoptosis acts pivotally in OA occurrence and development, and the number of chondrocyte apoptosis links closely with the severity of arthritis [5]. Chondrocytes are key in the structure of cartilage tissue. Mature chondrocytes are extremely weak in proliferation. Once chondrocytes enter the apoptotic process, they are almost impossible to regenerate. This will eventually result in declined extracellular matrix synthesis and enhanced degradation, thereby accelerating OA progression [6]. At present, it is generally believed that inhibiting chondrocyte apoptosis is available to delay OA progression.

Ligustrazine is the main active ingredient in the traditional Chinese medicine *Ligusticum chuanxiong Hort*. Ligustrazine injection has long been applied to treat ischemic stroke, coronary heart disease, diabetic nephropathy, and knee arthritis [7]. In the past few decades, a large number of research findings pointed out that ligustrazine has anti-inflammatory, -oxidant, -platelet, and -apoptotic as well as other pharmacological activities. In treating OA, ligustrazine has presented good clinical effect. First of all, studies have pointed out that ligustrazine is available to signally inhibit osteoarthritis-induced articular cartilage degradation. For example, the research by Liang [8] et al. pointed out that ligustrazine is available to reduce GAG degradation and MMP-3, MMP-13, COX-2, iNOS, type X collagen expressions, while increase TIMP-1 and type II collagen expressions. Additionally, other research findings indicate that ligustrazine can significantly inhibit OA-induced increase in oxidative stress, inhibit chondrocyte apoptosis, and reduce mitochondrial membrane potential [9]. But at present, the main mechanism of ligustrazine inhibiting chondrocyte apoptosis is still not clear. In this work, our team intend to probe into the main mechanism of ligustrazine inhibiting chondrocyte apoptosis, thus providing a basis for the clinical application of ligustrazine.

Methods

Primary chondrocyte culture

The isolation and culture of human-derived chondrocytes are as described above [10]. Here is a brief description in the following. In accordance with the approved guidelines, our team processed normal human articular cartilage tissues, and later prepared the digested chondrocytes with 0.2% collagenase II (Sigma) in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA). After filtration and centrifugation, our team washed them with sterile phosphate buffer saline (PBS), and the cultured the extracted chondrocytes in DMEM containing 10% fetal bovine serum (FBS, Invitrogen) at 37°C, 5% CO2 humidified incubator. We changed the medium every two days, and obtained the first generation of human-derived primary chondrocytes after two weeks.

Western blot

Our team lysed the cartilage or chondrocyte tissue was with RIPA lysis buffer containing protease inhibitors, and then centrifuged it in a low-temperature high-speed centrifuge at 14000×g for 30 min at 4°C, and taken the supernate. We applied the BCA experiment to determine the protein content of the sample and adjusted it to the same concentration with RIPA lysis buffer. After protein denaturation, our team 20μg sample loading each, electrophoresed in 8-12% SDS-polyacrylamide gel (Bio-Rad, USA), and later transferred to PVDF membrane. After blocking with 5% skim milk for 1 hour, our team incubated the primary antibody overnight at 4°C, later incubated the PVSF membrane with the peroxidase-bound secondary antibody for 2 hours, performed the color reaction through ECL, and quantified the protein through Image J software.

TUNEL

In this work, our team applied the TUNEL to detect chondrocyte apoptosis in situ, spread the primary chondrocytes in a 6-well plate, washed with PBS, fixed with 4% paraformaldehyde for 20 minutes, blocked with 5% BSA for 1 hour,

and then treated with the TUNEL reaction mixture for 1 hour (Roche), finally examined the apoptotic cells under microscopes (LSM 510; Zeiss: Jena, Germany), and calculated the percentage of TUENL-positive cells (apoptosis).

ELISA to detect IL8 and MCP-1 level

After the drug treatment, our team collected the supernate in cell culture solution, and detected IL-8 and MCP-1 contents in cell supernate via ELISA, collected the cells and applied BCA to detect the total cell protein contents, and expressed IL-8 and MCP-1 content as the ratio of protein content (ng/g).

Detection of NO and PGE2 contents in cell supernate

After the drug treatment, our team collected the supernate in cell culture solution, and detected NO and PGE2 contents in cell supernate via ELISA, and applied nitrate reductase to determine the NO content at 550nm.

Animal feeding

In this work, our team purchased 80 male SD clean-grade New Zealand rabbits from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (production license number: SCXK (Beijing) 2018-004, animal qualification number: No.1100112011035947), kept all experimental animals in a clean-grade animal room, with breeding temperature at 20-26°C (daily temperature difference ≤ 4°C), and 40-70% relative humidity, artificial lighting, alternating light and shade for 12 hours.

Osteoarthritis model [11]

In total 80 clean-grade New Zealand rabbits qualified for quarantine, our team selected 20 at random as normal control group, and the remaining animals were modeled. After fasting overnight before surgery and weighing, our team injected intraperitoneally 2% sodium pentobarbital (60 mg/kg) into rabbits for anesthesia. After anesthesia, made rabbits lie supine and fixed them, performed skin preparation skin on the right knee joint, and measured it. Later our team routinely disinfected the scope of surgery, spread surgical towels, applied a scalpel to make a longitudinal incision on the inside of the knee, separated the subcutaneous tissue to find the white patellar tendon, and cut the joint capsule along the inner side, dislocated the lateral patella (toward the outside of the knee), bent the knee joint to find the anterior cruciate ligament, cut and exposed the inner meniscus with ophthalmic scissors, put the meniscus back into the joint cavity, straightened the knee joint, reset the patella, and finally closed the wound with suture layer by layer. Through the anterior drawer test our team verified the operation findings. Each animal was injected intramuscularly with 80,000 units of penicillin for 3 consecutive days. If an infected animal was found, the number of injections could be elevated. They were returned to the cage after surgery. Treadmill exercise (20 m/min) was started on the second day after the operation, for 6 consecutive days per week, one day off, each time 30 min for 3 consecutive weeks. Our team screened the successful animals molding, and after random grouping, administered the animals in each group in accordance with the dosage, concentration and volume in the following table. The specific dosage was in the following(Table 1)

Table 1. Test design for dosage

	Groups	Dosage (mg/kg)	Volume (ml/each)	The number of animals
1	Normal control group	0	2	20
2	Model control group	0	2	20
3	Ligustrazine group	20mg/kg	2	20
4	PF573228+ ligustrazine group	10mg/kg + 20mg/kg	2	20

HE staining

Our team sacrificed the rabbits to be tested, separated cartilage tissues, fixed with them 4% paraformaldehyde/PBS (pH 7.4) for 24 hours, later embedded in paraffin to prepare 20 μm paraffin sections, applied HE staining kit (Biyuntian Biological Technology Co., Ltd.) for staining in strict accordance with the instructions.

Statistical experiment method

Our team applied descriptive analysis for general status and other information, quantified indicators, and calculated by group mean ± standard deviation ($\bar{X} \pm SD$). We performed the comparison between the two groups through t test, and $p<0.05$ was considered statistically significant. In the comparison between multiple groups, our team analyzed the data of various indicators based on the following procedures:

Our team applied Levene's to test the homogeneity of variance. If there was no statistical significance ($P>0.05$), applied one-way analysis of variance (ANOVA) for statistical analysis. If ANOVA was statistically significant ($P\leq0.05$), applied LSD for a comprehensive comparison. If the variance was not uniform ($P\leq0.05$), our team performed Dunnett's T3 test for statistical analysis and applied SPSS 23.0 for the above statistical operations.

Results

IL-1β induced chondrocyte apoptosis and increased metabolic activity in a dose-dependent manner

The pathological process of osteoarthritis is manifested as chondrocyte apoptosis, increased matrix degrading-enzyme expression, enhanced extracellular matrix (ECM) degradation, and increased inflammatory factor expression in articular cartilage tissue. A large number of studies have revealed that exposure of chondrocytes to the pro-inflammatory cytokine IL-1β is available to induce pathological phenotypes similar to that in the articular chondrocytes during OA progression, including cell apoptosis and increased decomposition activity. Therefore, our team applied 0, 3, 10, 15 μg/ml IL-1β to treat chondrocytes. Consistent with literature reports, IL-1β increased apoptosis-related proteins expression levels, including caspase-9, caspase-3, and caspase-7 in a dose-dependent manner (P<0.05) (Figure 1A).

Additionally, IL-1β was up-regulated in a dose-dependent manner by which it promoted matrix degrading-enzyme expression levels, like MMP3, MMP13, and collagen X (P<0.05), while down-regulated collagen II, and aggrecan protein expression levels (P<0.05) (Figure 1B). Our subsequent research findings pointed out that IL-1β increased MCP-1, IL-8, NO and PGE2 contents in a dose-dependent manner (P<0.05) (Figure 1C), which indicates that IL-1β induces chondrocyte apoptosis and catabolism activity in a dose-dependent manner.

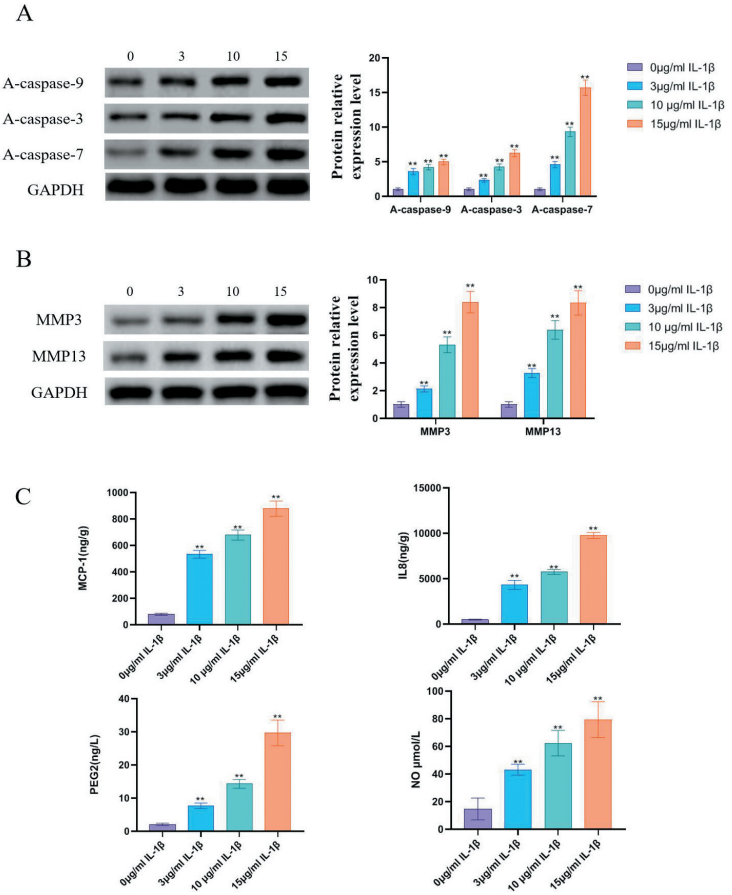

Figure 1. IL-1β induced chondrocyte apoptosis and enhanced its metabolic activity in a dose-dependent manner. Figure 1A Western blot to detect the impact of IL-1β on apoptotic protein content such as caspase-9, caspase-3, and caspase-7 in chondrocytes (n=3). Figure 1B Western blot to detect the impact of IL-1β on chondrocyte matrix degrading enzymes like MMP3, MMP13 as well as extracellular matrix collagen II, aggrecan and collagen X expression levels (n=3). Figure 1C Impact of IL-1β on inflammation-related factor content like MCP-1, IL-8, NO and PGE2. ** represented the control group V.S. IL-1β model group P<0.05;

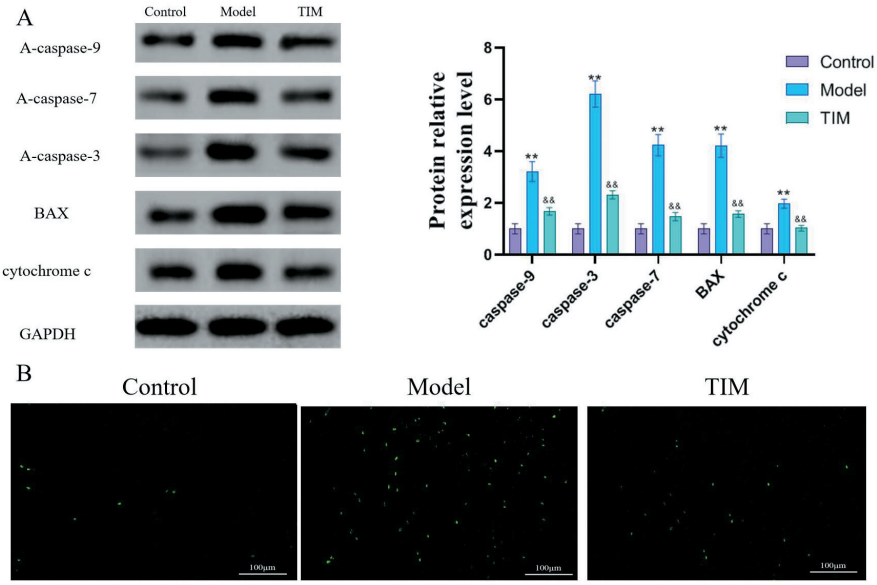

Figure 2. Ligustrazine inhibited IL-1β-induced chondrocytes apoptosis. Figure 2A Western blot test to detect the impact of ligustrazine on caspase-9, caspase-3, caspase -7, Bax, cytochrome c protein and other apoptotic protein levels (n=3). Figure 2B TUNEL to detect the impact of ligustrazine on chondrocyte apoptosis level induced by IL-1β (n=3). ** represented the control group V.S. IL-1β model group $P<0.05$; && represented the IL-1β model group V.S. Ligustrazine group $P<0.05$.

Ligustrazine inhibited IL-1β-induced chondrocyte apoptosis

Since articular cartilage completely relies on chondrocyte to maintain the extracellular matrix, damage to chondrocyte function and viability will result in articular cartilage failure. The degree of cartilage damage is certainly related to chondrocyte apoptosis. Chondrocyte apoptosis and matrix loss may form a positive feedback loop that promotes each other.

In this work, our team applied TUNEL and Western blot to probe into the impact of ligustrazine on IL-1β-induced chondrocyte apoptosis level. Our research pointed out that in comparison with those in the IL-1β model group, ligustrazine could signally inhibit IL-1β-induced increase in caspase-9, caspase-3, caspase-7, Bax, cytochrome c protein expressions ($P<0.05$) (Figure 2A). TUNEL findings pointed out that in comparison with those in the IL-1β model group, ligustrazine could visually inhibit the IL-1β-induced increase in the number of apoptotic cells ($P<0.05$) (Figure 2B). Our research findings indicate that ligustrazine is available to visually inhibit IL-1β-induced chondrocyte apoptosis.

Ligustrazine inhibited IL-1β-induced increase in inflammation-related factors and metabolic activity

Aiming to further detect the impact of ligustrazine on IL-1β-induced increase in inflammation-related factor levels and enhanced metabolic activity, our team applied Western blot to detect the impact of ligustrazine on MMP3 and MMP13, as well as collagen II, aggrecan, and collagen X protein expression levels. The experimental findings pointed out that in comparison with those in the IL-1β model group, ligustrazine could signally inhibit IL-1β-induced increase in MMP3, MMP13, collagen X protein expressions ($P<0.05$). Additionally, in comparison with those in the IL-1β model group, ligustrazine could visually increase collagen II and aggrecan protein expressions inhibited by IL-1β (Figure 3A).

Our subsequent research findings pointed out that ligustrazine could signally inhibit the IL-1β-induced increase in MCP-1, IL-8, NO and PGE2 levels ($P<0.05$) (Figure 3B). The research findings indicate that ligustrazine inhibited IL-1β-induced increase in inflammation-related factor level and the metabolic activity.

Ligustrazine activated FAK/PI3K/Akt pathways

Aiming to probe into the relative mechanism of ligustrazine inhibiting chondrocyte apoptosis, increase in matrix decomposition protein expression, and inflammation-related factor expression. In this research, our team probed into the influence of ligustrazine on the FAK/PI3K/Akt pathway. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that accelerates cytoskeleton dynamics to regulate cell

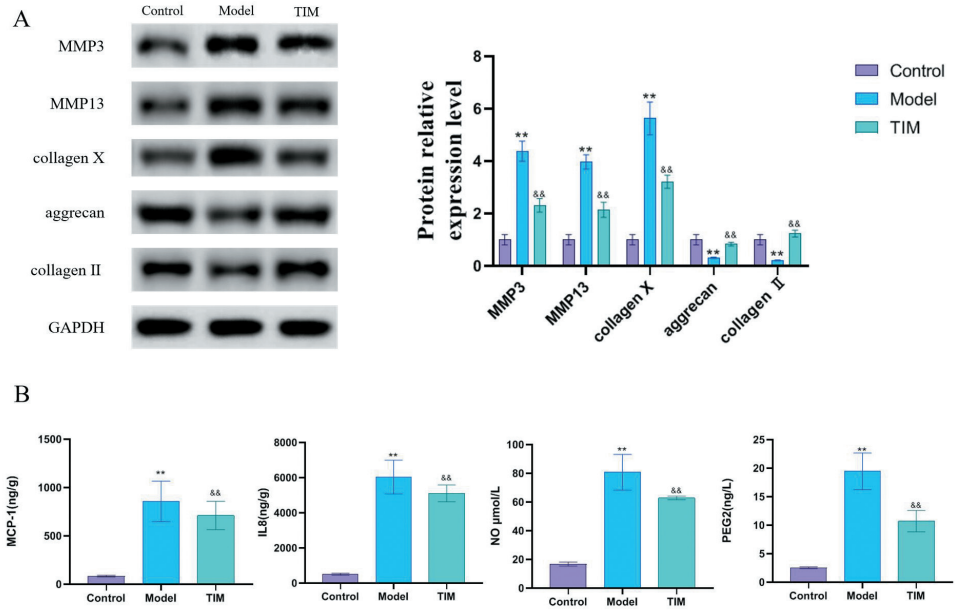

Figure 3. Ligustrazine inhibited IL-1β-induced increase in inflammation-related factor levels and enhanced metabolic activity. Figure 3A Western blot to detect the impact of ligustrazine on IL-1β-induced MMP3, MMP13, collagen II, aggrecan, and collagen X protein expression levels (n=3). Figure 3B Impact of ligustrazine on inflammation-related factor level including MCP-1, IL-8, NO and PGE2. **represented the control group V.S. IL-1β model group $P<0.05$; && represented the IL-1β model group V.S. Ligustrazine group $P<0.05$.

movement. A large number of studies have pointed out that the FAK/PI3K/Akt pathway acts pivotally in the pathological process of osteoarthritis. In this work, our experimental findings pointed out that in comparison with the IL-1β model group, ligustrazine could signally promote p-FAK/FAK, p-PI3K/PI3K and p-Akt/Akt protein expression levels ($P<0.05$) (Figure 4). Our findings pointed out that ligustrazine could activate the FAK/PI3K/Akt pathway, and its protective effect on chondrocytes may be related to the activation of the FAK/PI3K/Akt pathway.

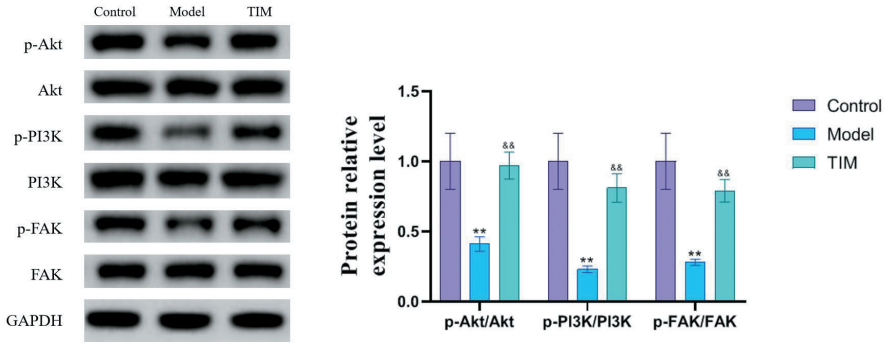

Figure 4. Ligustrazine activated the FAK/PI3K/Akt pathway. Western blot to detect the impact of ligustrazine on p-Akt/Akt, p-PI3K/PI3K and p-FAK/FAK expression levels in chondrocytes (n=3). ** represented the control group V.S. IL-1β model group $P<0.05$; && represented the IL-1β model group V.S. Ligustrazine group $P<0.05$.

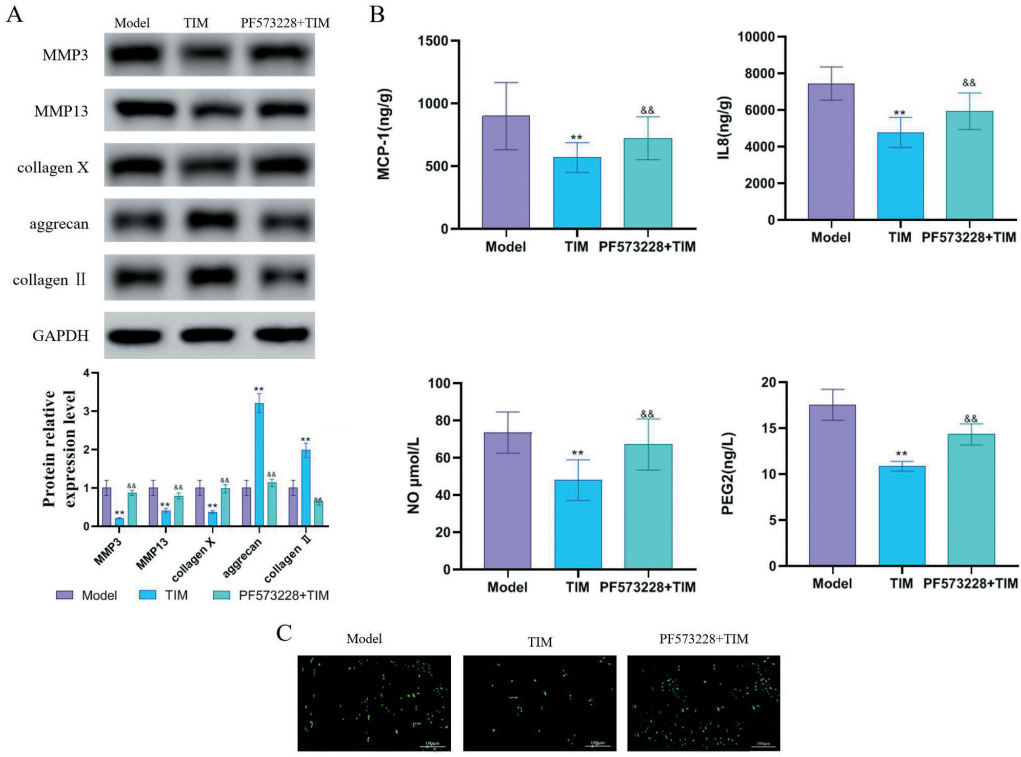

Figure 5. Inhibiting FAK/PI3K/Akt pathway abolished the chondroprotective effect of ligustrazine. Figure 5A Western blot to detect the impact of inhibiting FAK/PI3K/Akt signaling pathway on the ability of ligustrazine to inhibit matrix degradation (n=3). Figure 5B TUNEL to detect the impact of inhibiting FAK/PI3K/Akt signaling pathway on the anti-apoptotic effect of ligustrazine (n=3). Figure 5C Impact of inhibiting FAK/PI3K/Akt signaling pathway on the anti-inflammatory effect of ligustrazine (n=3). ** represented the ligustrazine group V.S. IL-1β model group $P<0.05$; && represented the ligustrazine group V.S.PF573228 + ligustrazine group $P<0.05$.

Inhibiting FAK/PI3K/Akt pathway abolished the chondroprotective effect of ligustrazine

In this work, with aim to further probe into whether the protective effect of ligustrazine on chondrocytes was achieved by activating the FAK/PI3K/Akt pathway, our team applied PF573228 to inhibit the FAK/PI3K/Akt signaling pathway in chondrocytes. PF573228 is a widely used FAK signaling pathway inhibitor.

The TUNEL findings indicated that in comparison with the ligustrazine group, the amount of apoptosis in the PF573228+ligustrazine group elevated signally ($P<0.05$) (Figure 5A). As shown in Figure 5B, in comparison with the ligustrazine group, MMP3, MMP13, and collagen X expression levels in the PF573228+ligustrazine group elevated ($P<0.05$), while collagen II and aggrecan expression levels decreased signally ($P<0.05$). As shown in Figure 5C, in comparison with the ligustrazine group, MCP-1, IL-8, NO and PGE2 contents in the cells in the PF573228+ligustrazine group elevated significantly ($P<0.05$). Our findings revealed that ligustrazine could activate the FAK/PI3K/Akt pathway to inhibit IL-1β-induced chondrocyte apoptosis, elevate matrix degradation activity, as well as inflammatory factor expression.

Ligustrazine improved OA-induced cartilage damage through activating FAK/PI3K/Akt pathway

Based on Western blot findings, ligustrazine could activate the FAK/PI3K/Akt pathway. In comparison with the model group, the p-Akt/Akt, p-PI3K/PI3K and p-FAK/FAK protein expression levels elevated signally in the cartilage tissue in the ligustrazine treatment group ($P<0.05$), while those in the PF573228+ligustrazine group were visually decreased ($P<0.05$) (Figure 6A).

Aiming to further probe into the protective mechanism of ligustrazine on rabbit cartilage tissue damage at the *in vivo* level, our team constructed a knee osteoarthritis model with

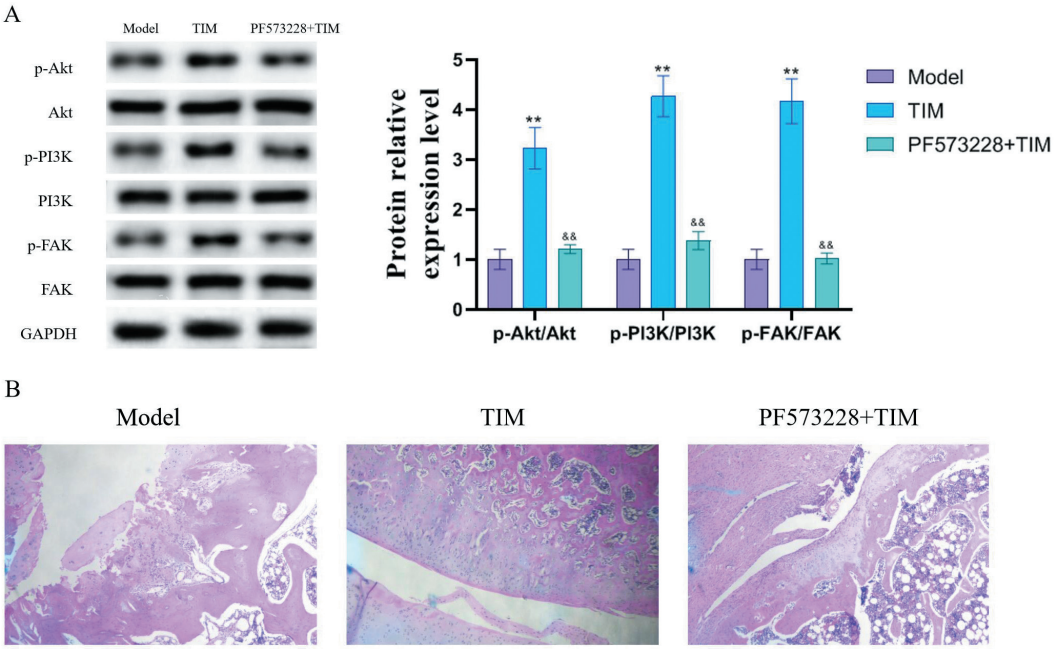

Figure 6. Ligustrazine improved cartilage damage induced by osteoarthritis through activating FAK/PI3K/Akt pathway. Figure 6A Western blot to detect the impacts of PF573228+Ligustrazine and Ligustrazine on FAK/PI3K/Akt signaling pathway (n=3). Figure 6B HE staining to detect the impact of inhibiting FAK/PI3K/Akt signaling pathway on the protective effect of ligustrazine on cartilage tissue morphology (n=3). ** represented the ligustrazine group V.S. PF573228+ligustrazine group $P<0.05$; && represented the ligustrazine group V.S. model group $P<0.05$.

meniscus tear of the right knee joint to investigate the protection mechanism of ligustrazine on rabbit cartilage tissue damage. The HE staining findings were shown in Figure 6B. The experimental findings implied that the cartilage surface of the model group had fallen off, the boundaries of each layer disappeared, and chondrocytes decreased. The morphology of articular cartilage in the ligustrazine group was signally improved, while that in the PF573228+ligustrazine group was visually worse.

Ligustrazine inhibited apoptosis induced by osteoarthritis model and enhanced metabolic activity

In this work, our team applied Western blot to determine the impact of ligustrazine and PF573228+ligustrazine on apoptotic protein level in cartilage tissue. Our work pointed out that in comparison with the model group, ligustrazine could visually inhibit the increase in caspase-9, caspase-3, caspase-7, Bax, and cytochrome c protein expression levels ($P<0.05$). Compared with the ligustrazine group, PF573228+Ligustrazine signally elevated caspase-9, caspase-3, caspase-7, Bax, and cytochrome c protein expression levels (Figure 7A).

Subsequently, Western blot findings pointed out that in comparison with the model group, ligustrazine could signally inhibit MMP3, MMP13, and collagen X expression levels ($P<0.05$), and up-regulate collagen II and aggrecan expression levels ($P<0.05$). In comparison with the ligustrazine group, PF573228+ligustrazine observably elevated MMP3, MMP13, and collagen X expression levels ($P<0.05$), while inhibited collagen II and aggrecan expression levels ($P<0.05$) (Figure 7B).

Discussion

Knee osteoarthritis, among the most universal chronic joint diseases, features a high incidence among the elderly worldwide and is the main cause of disability. Knee osteoarthritis affects approximately 60% men and 70% women over 65. Due to aging population, the rising rate of obesity, and the lack of clear treatments to prevent the progression of this disease, its harm is still increasing [12]. In the past few decades, treatment strategies for knee osteoarthritis have been greatly enriched, like non-drug, drug and surgical treatments [13]. However, none of these treatments is available to completely reverse the cartilage destruction in OA progression.

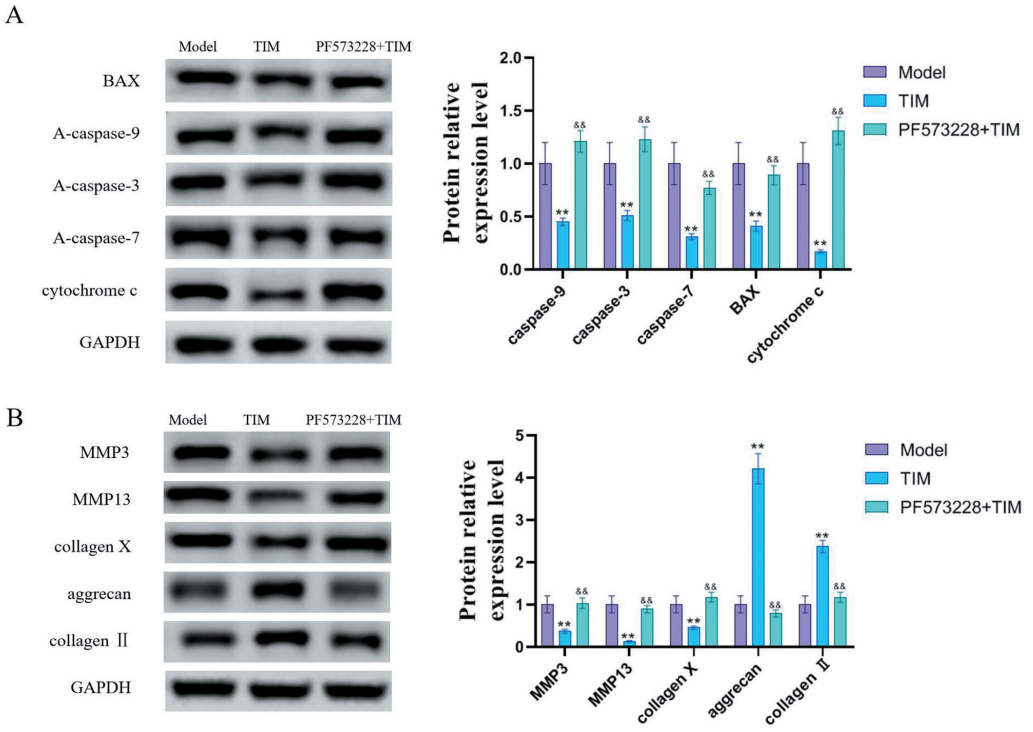

Figure 7. Ligustrazine inhibited cell apoptosis induced by osteoarthritis model and enhanced metabolic activity. Figure 7A Western blot to detect the impact of PF573228+ligustrazine on apoptosis-related protein expression (n=3). Figure 7B Western blot to detect the impact of PF573228+ligustrazine on MMP3, MMP13, collagen X, collagen II, and aggrecan expression levels (n=3). ** represented the ligustrazine group V.S. PF573228+ligustrazine group $P<0.05$; && represented the ligustrazine group V.S. the model group $P<0.05$.

Therefore, there is still an urgent need for new drugs for knee osteoarthritis. Ligustrazine is the main active ingredient in the traditional Chinese medicine *Ligusticum chuanxiong Hort*. Recently, ligustrazine has also been confirmed as a potential drug candidate for treating knee osteoarthritis [14]. Although clinical evidence reveals that ligustrazine is available to signally improve knee osteoarthritis, the relevant molecular mechanism is still not clear.

In this work, our team studied the impact of ligustrazine on knee osteoarthritis and IL-1β-induced chondrocyte damage *in vitro* and *in vivo*. Our research findings indicate that ligustrazine could reduce IL-1β-induced chondrocyte apoptosis through activating the FAK/PI3K/Akt pathway *in vitro* and *in vivo*, inhibit the apoptotic proteins Bax, caspase-9, caspase-3, caspase-7 protein expression level, as well as matrix-degrading enzymes MMP3 and MMP13 expression levels, and reduce collagen X (index of chondrocyte hypertrophy) protein expression level, and promote matrix proteins collagen II and aggrecan expressions. Additionally, ligustrazine could inhibit inflammatory factors MCP-1 and IL8 contents in the supernate of primary chondrocytes and reduce PEG2 and NO contents through activating the FAK/PI3K/Akt pathway *in vivo*. In the pathological mechanism of knee osteoarthritis, NO and PGE2 had the impact of inhibiting matrix synthesis [15]. In conclusion, our research results implied that ligustrazine could inhibit chondrocyte apoptosis, inhibit cartilage mechanism degradation, promote cartilage matrix synthesis, and inhibit inflammatory factor expression through activating the FAK/PI3K/Akt pathway.

In vitro and *in vivo*, ligustrazine can inhibit chondrocyte apoptosis, cartilage mechanism degradation, and promote cartilage matrix synthesis, which is in line with our research findings. Studies by Ju and Xu [16] pointed out that, ligustrazine inhibits chondrocyte apoptosis *ex vivo* and *in vivo*, protects cartilage tissue morphology, and inhibits cartilage mechanism degradation. As the only resident cells in cartilage tissue, chondrocytes adhere to the adjacent extracellular matrix to survive. This phenomenon is called anchorage dependence [17]. A large number of research findings pointed out that the destruction of proteoglycan and col-

lagen network in the cartilage matrix in knee osteoarthritis is the main pathological feature of cartilage degeneration. Therefore, the possible reason for chondrocyte apoptosis is under that pathological environment of knee osteoarthritis, the anchorage of chondrocytes to the extracellular matrix is destroyed [18]. For example, the research findings by Pulai [19] et al. pointed out that α5β1 integrin provides the binding between the extracellular matrix and the chondrocyte skeleton, and blockingα5β1 integrin knockout *in vitro* is available to signally promote chondrocyte apoptosis. Since articular cartilage completely relies on chondrocyte to maintain the extracellular matrix, damage to chondrocyte function and viability will result in articular cartilage failure. Therefore, chondrocyte apoptosis will further promote cartilage degeneration. Our research results thus pointed out that one of the mechanisms of ligustrazine in OA treatment may be that ligustrazine is available to inhibit matrix degradation and chondrocyte apoptosis induced by OA pathological conditions.

Subsequently, our work also pointed out that ligustrazine activates the FAK/PI3K/Akt pathway to inhibit chondrocyte apoptosis, cartilage mechanism degradation, while promote cartilage matrix synthesis. FAK is a key molecule in the signal transduction pathway mediated by integrin, and FAK phosphorylation is available to further activate the downstream PI3K/Akt pathway [20]. PI3K/Akt acts pivotally in the pathological process of OA, and is closely related to chondrocyte growth, differentiation, apoptosis and matrix remodeling [21]. For example, Tao [22] et al. pointed out that miR-34a can promote articular chondrocyte proliferation and inhibit its apoptosis through activating the PI3K/Akt pathway. Additionally, Fan [23] et al. pointed out that miR-155 is available to inhibit cartilage matrix degradation and promote cartilage matrix synthesis through activating PI3K/Akt. Consistent with our findings, Yang [24] et al. pointed out that ligustrazine can protect against myocardial injury induced by acute myocardial ischemia through activating the PI3K/Akt pathway. In summary, *in vitro* and *in vivo* ligustrazine is available to reduce IL-1β-induced chondrocyte apoptosis, inhibit cartilage mechanism degradation, and promote cartilage matrix synthesis through activating the FAK/PI3K/Akt pathway.

Venue of the research

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Declaration of Conflicting Interests

The authors declare that they have no competing interests.

Authors' contributions

GS designed the research study. JY performed the research. PR provided help and advice on the experiments. JY and XY analyzed the data. GS and XY wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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