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Original Article

Platelet-rich plasma combined with isometric quadriceps contraction regulates autophagy in chondrocytes via the PI3K/AKT/mTOR pathway to promote cartilage repair in knee osteoarthritis

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ABSTRACT

Background: This study investigated the molecular mechanism by which the combination of platelet-rich plasma (PRP) and isometric contraction of the quadriceps (ICQ) intervention regulates autophagy in chondrocytes to prevent and treat knee osteoarthritis (KOA).
Methods: Thirty Sprague-Dawley rats were divided into a control group (CG, n = 6) and a model group (n = 24). After one week, the model group was randomly divided into a joint intervention group (JIG), a rapamycin group (RAG), an MHY1485 group (MYG), and a model blank group (MBG), with JIG, RAG, and MYG receiving the same combined intervention.
Results: The trend of cartilage lesions in each group was CG < RAG < JIG < MYG < MBG. Compared with MBG and MYG, JIG and RAG showed downregulation of IL-1β, IL-6, IL-18, MMP-13, and TNF-α mRNA in the cartilage ($p < 0.01$); mTOR protein expression: compared with JIG, RAG showed downregulation, and MYG showed upregulation. Compared with RAG, MYG showed upregulation ($p < 0.01$); ATG5 protein expression: compared with RAG, MYG showed downregulation ($p < 0.01$); Beclin1, LC3-I, and ULK1 protein expression: compared with JIG, RAG showed upregulation, and MYG showed downregulation ($p < 0.01$). Compared with RAG, MYG showed downregulation ($p < 0.01$); P62 protein expression: compared with RAG, both MBG and RAG showed upregulation, and MYG showed downregulation ($p < 0.05$); LC3-II/LC3-I ratio: compared with JIG and RAG, the ratio in MYG was decreased ($p < 0.01$).
Conclusion: The combined intervention promotes autophagy in chondrocytes by inhibiting the PI3K/AKT/mTOR pathway, downregulating inflammatory factors and MMP-13 in the cartilage, upregulating autophagy markers, inhibiting matrix degradation, and promoting cartilage repair.

1. Introduction

Knee Osteoarthritis (KOA) is characterized by the degeneration of articular cartilage, osteophyte formation at the joint margins, subchondral bone proliferation, and narrowing of the joint space, representing a chronic degenerative disease [1–3]. KOA imposes a substantial economic burden on patients, families, and society [4,5]. Current treatment modalities include pharmacotherapy (non-steroidal anti-inflammatory drugs and chondroprotective agents) [6,7] and surgical interventions (arthroscopic debridement and total joint arthroplasty) [8,9]. However, pharmacological treatments cannot completely halt the pathological progression [10], and surgical options are costly [11]. Therefore, the search for effective strategies to intervene in KOA is a worthwhile area of research.

Our previous studies have completed three research projects on the intervention of platelet-rich plasma (PRP) in KOA, finding that PRP can promote chondrocyte proliferation and has reparative effects on cartilage in KOA animal models. Clinically, isometric

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contraction of the quadriceps femoris (ICQ) has been observed to significantly alleviate symptoms, improve joint function, and delay joint degeneration in KOA patients [12]. Further, using KOA animal models, we confirmed that combined interventions can reduce cartilage inflammatory responses, promote chondrocyte regeneration, and matrix tissue repair, with a synergistic effect compared to single interventions. Transmission electron microscopy revealed that PRP + ICQ combined intervention (combined intervention) resulted in the presence of primary lysosomes, autophagosomes, and autolysosomes in the cytoplasm of chondrocytes [13]. Subsequent RNA-seq sequencing, Gene Ontology (GO) enrichment, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis preliminarily identified the PI3K/AKT/mTOR molecular pathway as being significantly enriched. However, the mechanism of combined intervention in KOA remains unclear.

Previous studies have shown that the mTOR inhibitor Rapamycin (RAPA) activates autophagy in human chondrocytes in vitro and delays KOA expression [14]. In experimental mouse models, systemic administration of RAPA has been shown to reduce the severity of KOA [15]. Clinically, systemic administration of RAPA has side effects, including elevated serum cholesterol and triglycerides, anemia, proteinuria, rash, delayed wound healing, and diarrhea, leading to RAPA withdrawal [16]. Due to these side effects, intra-articular injection of RAPA may be more suitable for clinical application than systemic administration [17]. On the other hand, some scholars have chosen intra-articular injection of RAPA and found a positive impact on the recovery of joint cartilage in KOA rats, as overexpression of mTOR inhibits chondrocyte autophagy and accelerates cell apoptosis, exacerbating the degeneration of articular cartilage [18]. Intra-articular injection of the mTOR activator MHY1485 (which targets the ATP-binding domain of mTOR, inhibiting the fusion between autophagosomes and lysosomes, and inhibiting autophagy) in KOA rats was found to exacerbate KOA symptoms [18].

To more clearly explore whether combined intervention mediates KOA chondrocyte autophagy through the PI3K/AKT/mTOR pathway, this study continues our previous clinical [12] and animal [13] experiments, using our previous KOA animal model and intervention methods. We plan to inject mTOR inhibitors (RAPA) and activators (MHY1485) into the knee joint cavity of KOA rats to explore the mechanism by which combined intervention regulates autophagy in KOA. Clarifying the biological functions and mechanisms of combined intervention in KOA will provide a reference for clinical intervention in KOA. Research hypothesis: Combined intervention promotes chondrocyte autophagy by inhibiting the PI3K/AKT/mTOR pathway to intervene in KOA.

2. Materials and methods

This study was approved by the Ethics Committee of Chengdu Sport University (Approval No.202213). Female Sprague-Dawley rats (SCXK (Chuan) 2020-030, SPFSD, 2 months old, weighing 180–220 g) were purchased from Chengdu Dashuo Laboratory Animal Co., Ltd. and were raised strictly according to the standards for animal experiments.

2.1. Grouping and intervention methods subsequently

After a 7-day acclimatization period, 30 rats were randomly divided into the Control Group (CG, n = 6) and the Model Group (n = 24). One week after the modeling, the Model Group rats were randomly divided into the Joint Intervention Group, Rapamycin Group (RAG), MHY1485 Group (MYG), and Model Blank Group (MBG), all of which underwent 4 weeks of PRP + ICQ intervention. On this basis, RAG received intra-articular injection of the mTOR inhibitor Rapamycin, and MYG received intra-articular injection of the mTOR activator MHY1485. The injections were administered for 4 weeks, at a frequency of 2 times per week, with a dosage of 0.3 mg (50 μL) per knee, at the site 1 mm lateral to the medial side of the patellar ligament of the rat knee [13]. The normal group received an injection of sterile saline (50 μL). MBG and CG were normally fed without additional intervention. After 4 weeks, the rats were euthanized, and the knee joint cartilage was subjected to pathological, RT-PCR, and Western Blot testing (Fig. 1-A).

2.2. Knee joint inflammation modeling

Using our previously established method [13], rats were anesthetized by intraperitoneal injection of pentobarbital sodium (2 % concentration, 0.2 mL/100 g). With the knee joint in a flexed position, under ultrasound guidance, the knee joint cavity of the hind limbs was injected with sodium iodoacetate (50 μL, 40 mg/mL) (CAS No. 305-53-3; MDL: MFCD00002686, 270.00 units/mg) [18], with the injection point being 1 mm lateral to the medial side of the patellar ligament of the rat knee. The normal group received an injection of sterile saline (50 μL).

2.3. Platelet-rich plasma preparation

An additional 5 female Sprague-Dawley rats, 2 months old, were anesthetized by intraperitoneal injection of pentobarbital sodium (0.3 %, 0.1 mL/100 g). Approximately 6 mL of blood was collected from the abdominal aorta. A 1 mL sample of whole blood was used for platelet counting, and the remaining blood was transferred into a tube containing ACD anticoagulant at a ratio of 1:10 and mixed well. PRP was prepared using a double centrifugation method [19], and a centrifuge (Xiangyi, Hunan, China, Model: CHT 210R) was used for the second centrifugation (First: 200 g, 10 min; Second: 200 g, 10 min). The resulting PRP had a platelet concentration approximately 6 times the baseline concentration [20,21]. The PRP was injected twice (the first injection was one week after modeling, and the second injection was 2 weeks apart), with 0.1 mL of PRP injected into each knee joint cavity of the rats.

2.4. Electrical stimulation to simulate isometric quadriceps contraction

Referring to our previous intervention method [13], the current intensity was selected to be 0.5–2 mA (0.5 mA in the first week, increasing by 0.5 mA every week, up to 2 mA in the fourth week), with a frequency of 1 Hz. The stimulation lasted for 15 min per session, 5 times per week, for a total of 4 weeks.

2.5. Cartilage examination

The knee joint cartilage of the rats was taken for pathological observation with HE staining and electron microscopy, and the expression of proteins such as PI3K, AKT, mTOR, ATG5, Beclin1, LC3 (LC3-I, LC3-II, and the ratio of LC3-II/LC3-I), P62, and ULK1 in the cartilage was detected by Western Blot (primers and antibodies are shown in Table 1 and Table 2).

2.6. Statistical analysis

All measured data were processed for mean ± standard deviation using SPSS 20.0. Pathological scores and cartilage inflammatory factors, as well as Western Blot data, were measured once. Data were first tested for normality and homogeneity of variance

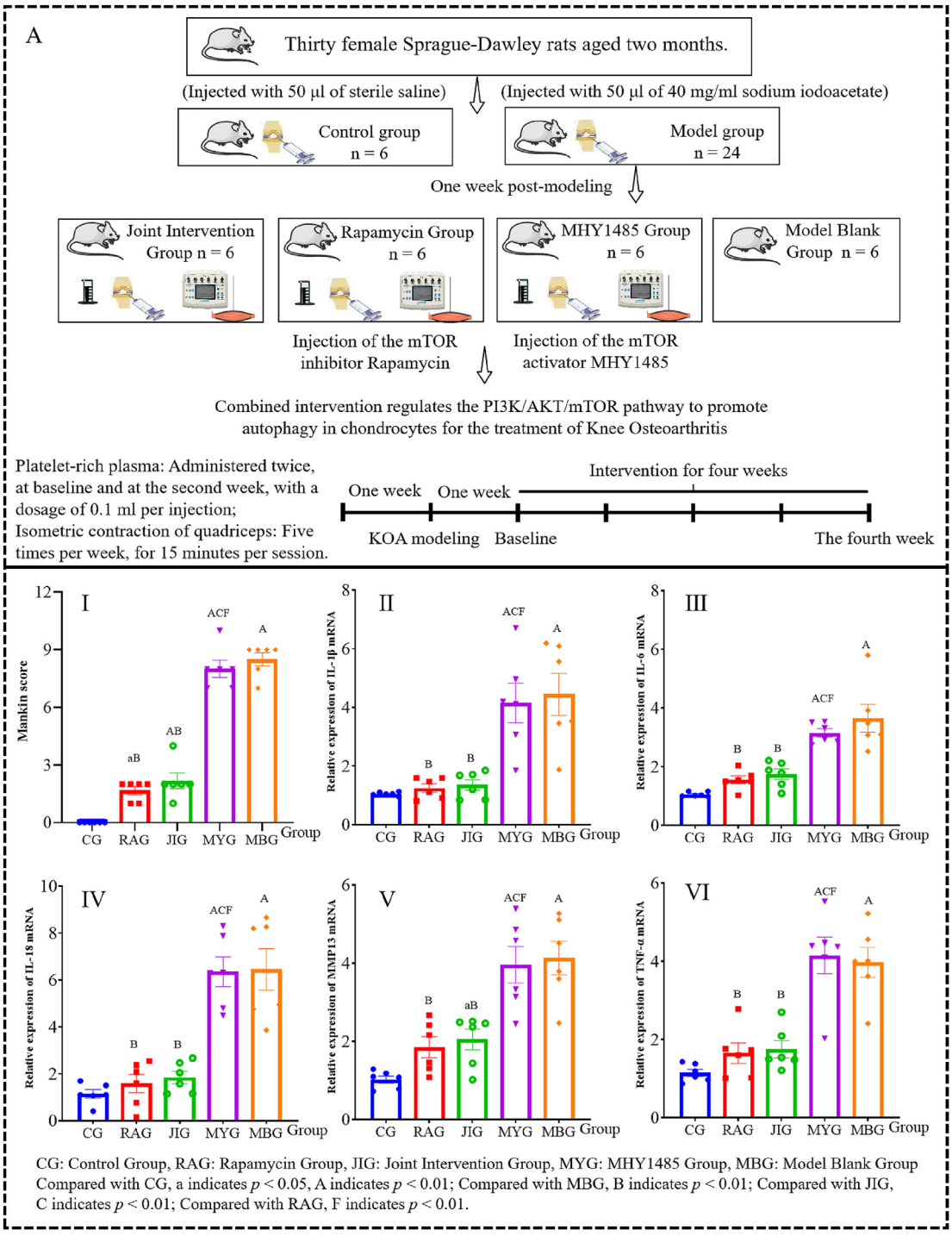

Fig. 1. Experimental grouping and intervention process (A) and Mankin scoring and relative expression of inflammatory cytokine mRNA in cartilage tissue (I-VI).

using the Shapiro-Wilk test and Levene's tests. If the data met the criteria for normal distribution and homogeneity of variance, one-way ANOVA was used, followed by post-hoc comparison of differences between groups. If the data did not meet the criteria for normal distribution or homogeneity of variance, the Kruskal-Wallis H test was used to compare differences between groups. To ensure the overall Type I error rate for each ANOVA was no greater than 0.05, post-hoc comparisons were adjusted using the Bonferroni method [22,23]. The level of significance was set at $\alpha = 0.05$.

3. Results

Cartilage Pathological Examination HE staining revealed severe cartilage damage in the MBG and MYG, while the JIG and RAG showed more intact cartilage structures. Transmission electron microscopy observed changes in the number of autophagic lysosomes and lysosomes in the cytoplasm of chondrocytes, this study noted that chondrocytes in the MBG exhibited significant morphological alterations, including an increase in cell volume and the formation of cytoplasmic protrusions, which are closely

Table 1
Experimental antibody information.

Name	Manufacturer	Antibody Type	Specification
β-actin Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	AC026
AKT Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	ET1609-47
ATG5 Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	ET1611-38
Becline1 Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	HA721216
LC3 Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	ET1701-65
mTOR Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	ET1608-5
PI3K Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	ET1609-70
P62 Antibody	Aiboke biological (Wuhan)	Rabbit Cloned Antibody	ab211342
ULK1 Antibody	Merck Life Science (USA)	Rabbit Cloned Antibody	ET1704-63
IgG (H + L)	Merck Life Science (USA)	Goat Anti-Rabbit	AS014

Table 2
Primers and base sequences.

Primer Name	Upstream	Downstream
β-actin	gggaaatcgtgcgtgacatt	gcggcagtggccatctc
IL-1β	aatctcacagcagcatctcgacaag	tccacgggcaagacataggtagc
IL-6	acttccagccagttgccttcttg	tggtctgttgtgggtggtatcctc
IL-18	cgaccgaacagccaacgaatcc	gtcacagccagtcctcttacttcac
MMP-13	cagccctatcccttgatgccattac	gggtgcagacgccagaagaatc
TNF-α	caccacgctctttctgtctactgaac	tgggctacgggcttgtcactc
LC3	ccgtcctggacaagaccaagttccttt	acactcaccatgctgtgcccattca
ULK1	tacacagcaagggcatcattcacc	cgggcaaatccaaagtcagcaatc
Beclin1	tcaagatcctggaccgagtgacc	ctcctctcctgagttagcctcttcc
ATG5	acggagagaagaagagccaggtg	tgctgatgtgaaggaagttgtctgg

associated with the progression of cartilage degeneration. In contrast, chondrocytes in the JIG and the RAG displayed more regular and healthy morphologies, suggesting that the intervention measures may have played a positive role in maintaining the normal shape and function of chondrocytes. Furthermore, we observed an increase in the number of autophagic vesicles and autolysosomes in the chondrocytes of the intervention groups compared to the model group, which may be related to the activation of the autophagy process, thereby aiding in the repair of chondrocytes and the maintenance of the matrix. With a trend of pathological changes as CG < RAG < JIG < MYG < MBG (Fig. 2). Compared with MBG and MYG, the Mankin scores of JIG and RAG were significantly reduced to varying degrees ($p < 0.01$) (Fig. 1, I).

Expression of Cartilage Inflammatory Factors and MMP-13 mRNA The mRNA expression of inflammatory factors in the five groups (Fig. 1, II-VI). Shapiro-Wilk test showed that the data met the normal distribution. Levene's test found that IL-1β ($F_{(4, 25)} = 0.997$), IL-6 ($F_{(4, 25)} = 0.490$), IL-18 ($F_{(4, 25)} = 1.201$), MMP-13 ($F_{(4, 25)} = 1.304$), and TNF-α ($F_{(4, 25)} = 0.949$) had homogeneous variances ($p > 0.05$). ANOVA results showed differences among groups for IL-1β ($F_{(4, 25)} = 14.070$), IL-6 ($F_{(4, 25)} = 21.172$), IL-18 ($F_{(4, 25)} = 25.222$), MMP-13 ($F_{(4, 25)} = 16.960$), and TNF-α ($F_{(4, 25)} = 20.047$) ($p < 0.001$). Further post-hoc comparisons were made to determine differences among groups. The mRNA expression of IL-1β, IL-6, IL-18, MMP-13, and TNF-α showed a consistent trend, with downregulation in JIG and RAG compared to MBG and MYG ($p < 0.01$).

Autophagy and Pathway Protein Expression in Knee Cartilage Relative protein expression in the cartilage of the five groups (Fig. 3). Shapiro-Wilk test showed that the data met the normal distribution. Levene's test found that PI3K ($F_{(4, 25)} = 0.915$), AKT ($F_{(4, 25)} = 0.924$), mTOR ($F_{(4, 25)} = 1.394$), ATG5 ($F_{(4, 25)} = 1.700$), Beclin1 ($F_{(4, 25)} = 1.133$), LC3-I ($F_{(4, 25)} = 0.534$), LC3-II ($F_{(4, 25)} = 1.547$), LC3-I/LC3-II ($F_{(4, 25)} = 1.734$), P62 ($F_{(4, 25)} = 1.184$), and ULK1 ($F_{(4, 25)} = 1.116$) had homogeneous variances ($p > 0.05$). ANOVA showed differences among groups for mTOR ($F_{(4, 25)} = 94.492$), ATG5 ($F_{(4, 25)} = 12.467$), Beclin1 ($F_{(4, 25)} = 171.079$), LC3-II ($F_{(4, 25)} = 248.526$), LC3-I/LC3-II ($F_{(4, 25)} = 51.422$), P62 ($F_{(4, 25)} = 4.378$), and ULK1 ($F_{(4, 25)} = 46.448$) ($p < 0.01$); the relative expression of PI3K, AKT, and LC3-I proteins is shown in Fig. 3 (I, II, and VI). ANOVA showed no statistically significant differences among groups ($p > 0.05$).

mTOR Protein Expression: Compared with MBG, RAG and JIG were downregulated ($p < 0.01$). Compared with JIG, RAG was downregulated and MYG was upregulated ($p < 0.01$). Compared with RAG, MYG was upregulated ($p < 0.01$); ATG5 Protein Expression: Compared with MBG, RAG, JIG, and MYG were upregulated ($p < 0.01$). Compared with RAG, MYG was downregulated ($p < 0.05$); Beclin1, LC3-I, and ULK1 Protein Expression: Compared with MBG, RAG, JIG, and MYG were upregulated ($p < 0.01$). Compared with JIG, RAG was upregulated and MYG was downregulated ($p < 0.05$). Compared with RAG, MYG was downregulated ($p < 0.01$); LC3-II/LC3-I Ratio: Compared with MBG, the ratio in RAG, JIG, and MYG was increased ($p < 0.01$). Compared with JIG, MYG's ratio was decreased ($p < 0.01$). Compared with RAG, MYG's ratio was decreased ($p < 0.01$); P62 Protein Expression: Compared with RAG, MBG and RAG were upregulated and MYG was downregulated ($p < 0.05$).

4. Discussion

The objective of this study was to verify whether the combined intervention mediated by the PI3K/AKT/mTOR pathway could regulate autophagy in KOA chondrocytes, thereby affecting the symptoms of KOA in rats. Our hypothesis was confirmed that the combined intervention could promote autophagy in chondrocytes and intervene in KOA by inhibiting the PI3K/AKT/mTOR pathway.

4.1. Combined intervention promotes cartilage repair and reduces expression of inflammatory factors

In this study, mTOR inhibitors and activators were injected into the knee joint cavity of KOA model rats. After 4 weeks of combined intervention, HE staining revealed that the additional injection of the mTOR activator MHY1485 in the model rats resulted in severe cartilage damage, while the cartilage damage was less severe with only the combined intervention. In contrast, the additional injection of the mTOR inhibitor resulted in more intact cartilage structure. Consistent conclusions were also drawn from Mankin scores and transmission electron microscopy results, which showed changes in the number of autophagic lysosomes and lysosomes in the cytoplasm of chondrocytes, correlating with the morphological changes observed in HE staining.

Chondrocyte apoptosis is an important manifestation of KOA, which is related to the destruction of cartilage and the extracellular matrix. Transmission electron microscopy confirmed the presence of cellular morphological changes in KOA cartilage samples, such as

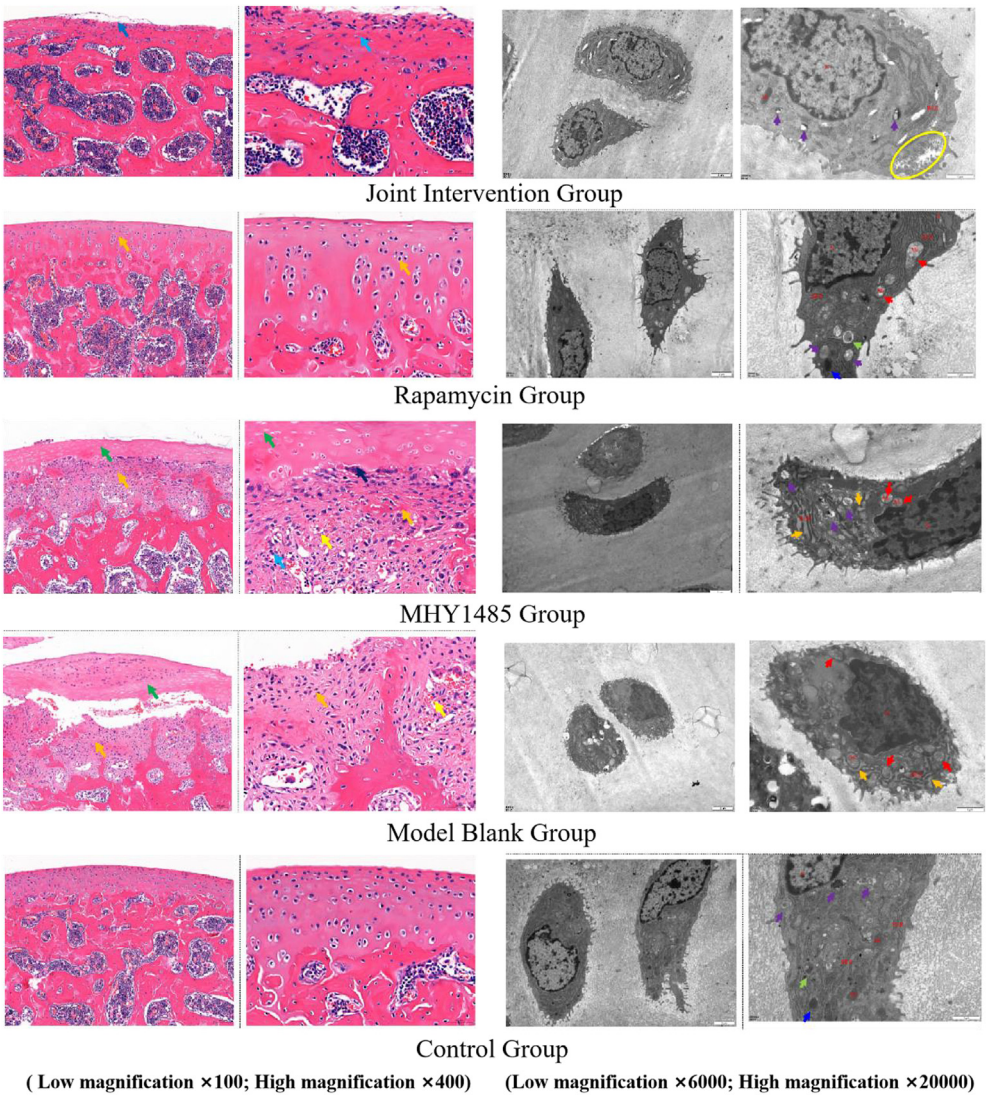

Fig. 2. Representative HE staining and transmission electron microscopy images from one sample per group.
Note: **HE staining:** damage to the cartilage layer (↑), proliferation of fibrous tissue (↑), increased osteoclasts (↑), formation of new capillaries (↑), hemorrhage (↑), reduction in the number of trabeculae (↑); **Transmission electron microscopy:** expansion of the rough endoplasmic reticulum (↑), primary lysosomes (↑), autophagic lysosomes (↑), lipid droplets (↑), mitochondrial swelling (↑), ribosome loss (○).

nuclear volume loss, apoptotic bodies, cellular surface blebbing, and even changes in nuclear shape [24]. Further testing of IL-1β, IL-6, IL-18, MMP-13, and TNF-α mRNA expression among groups showed consistent conclusions. Compared with the single combined intervention, the additional injection of the mTOR inhibitor downregulated these factors to varying degrees, while the injection of the mTOR activator upregulated them to varying degrees. Previous studies have shown that the overexpression of inflammatory factors and MMP-13 in articular cartilage is conducive to the occurrence and development of KOA [25]. KOA produces pro-inflammatory mediators, including IL-6, IL-1β, and TNF-α [26]. Among them, IL-1β, TNF-α, IL-6, IL-18, and MMP-13 in the joint space are one of the causes of KOA cartilage degradation [27]. In experiments with KOA chondrocytes, it has also been confirmed that after ozone intervention, the expression levels of IL-6, TNF-α, and MMP-13 mRNA in KOA chondrocytes decreased, and transmission electron microscopy observed an increase in autophagosomes in chondrocytes [28]. Therefore, downregulating the expression levels of these factors and enhancing autophagy may be a sign of alleviating KOA symptoms.

Rapamycin is an effective immunomodulator that can activate autophagy in KOA [29]. In KOA mouse models, the injection of rapamycin into the knee joint cavity was found to downregulate mTOR expression, activate autophagy markers such as LC3 in chondrocytes, and reduce the degree of cartilage lesions [16,30,31]. Li et al. [17]. injected rapamycin and the mTOR activator MHY1485 into the knee joint cavity of KOA rats, indicating that mTOR inhibition induces autophagy, reduces cell apoptosis, and cartilage loss. Conversely, the injection of the mTOR activator had the opposite effect. The study [17] is consistent with the conclusions of this study, and differs from previous studies [16,17,30,31] in that this study added a combined intervention, indicating that the injection of the mTOR inhibitor further increased the effect of KOA intervention, while the injection of MHY1485 to some extent inhibited the beneficial effects of the combined intervention.

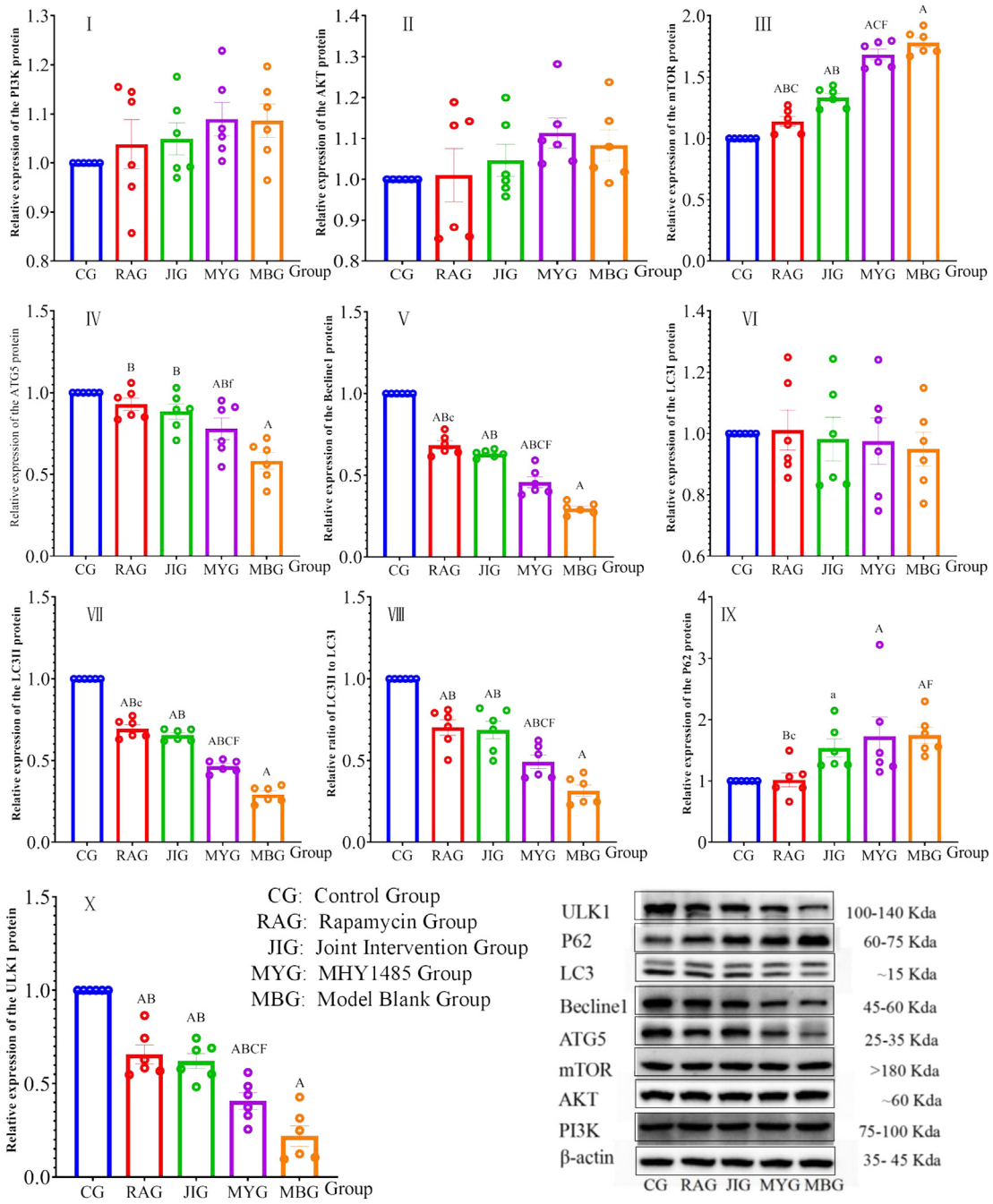

Fig. 3. Different groups have different protein expressions.
Note: Taking the relative value of CG as 1, the other groups are calculated proportionally. Compared with CG, a indicates $p < 0.05$, and A indicates $p < 0.01$; compared with MBG, b indicates $p < 0.05$, and B indicates $p < 0.01$; compared with JIG, c indicates $p < 0.05$, and C indicates $p < 0.01$; compared with RAG, f indicates $p < 0.05$, and F indicates $p < 0.01$.

4.2. Combined intervention promotes autophagy in knee cartilage cells

The results of the autophagy-promoting protein (ATG5, Beclin1, LC3, and ULK1) tests showed that compared with the simple combined intervention, the additional injection of the mTOR inhibitor resulted in upregulation of the expression of ATG5, Beclin1, LC3, and ULK1 to varying degrees. On the contrary, the additional injection of the mTOR activator resulted in downregulation of the expression of the above autophagy proteins to varying degrees. The results of the P62 (inhibits autophagy) test found that compared with the simple combined intervention, the additional injection of the mTOR inhibitor rapamycin resulted in downregulation of P62 expression. In contrast, the additional injection of the mTOR activator resulted in upregulation of P62 expression.

ATG5 plays an important role in the formation of autophagosomes [32], participating in the extension of the phagocytic cell membrane in autophagosomes [33]. Beclin1, phosphorylated by ULK1, serves as an overall scaffold of the PI3K complex, promoting the localization of autophagy proteins to autophagosomes [34]. When the PI3K complex combines with different regulatory proteins, it selectively participates in different processes of autophagy.

In addition, LC3 includes interconvertible LC3-I and LC3-II, which are involved in the formation of the autophagosome membrane [35]. Both LC3 and LC3-II are used as molecular markers for autophagy. The degree of autophagy is directly proportional to the expression of LC3-II, and the size of the LC3-II/LC3-I ratio can also reflect the level of autophagy [36]. At the same time, P62 is also a widely studied autophagy substrate [37]. During the formation of autophagosomes, P62 can selectively wrap into autophagosomes and then be degraded by proteases in autolysosomes, and the expression of P62 protein is negatively correlated with autophagy activity [38]. ULK1 is a protein necessary for the formation of autophagosomes, and the activation of ULK1 kinase induces autophagy [39].

Autophagy dysfunction is related to cartilage degeneration, and inducing autophagy can alleviate cartilage degeneration to a certain extent. Low expression of autophagy-related proteins such as ULK1, LC3, and Beclin1 has been found in the cartilage of KOA patients [40]. In a rat temporomandibular joint arthritis model, transmission electron microscopy observed an increase in autophagosomes in chondrocytes with early degenerated joint cartilage. With the decline of mTOR activity, the transcription and expression of LC3-II and Beclin-1 also showed an increasing trend [41]. Carames et al. [42]. found that in KOA mice with less joint cartilage lesions, the expression of ULK1, Beclin-1, and LC3-II in the middle and deep layers was downregulated, indicating that the reduction of autophagy marker expression is related to an increase in chondrocyte apoptosis. Similar conclusions have also been drawn from human experiments. Autophagy can maintain the stable state of normal adult joint cartilage cells, and Beclin-1, ATG5, and LC3 are highly expressed in the superficial layer of cartilage cells [43]. It may inhibit the expression of inflammatory and catabolic factors related to the pathogenesis of KOA by activating autophagy proteins (Beclin1, ATG5, ATG7, and LC3, etc.) [44]. This study to some extent verified previous studies [40–44], indicating that the combined intervention promotes autophagy in knee cartilage cells.

4.3. Combined intervention inhibits the PI3K/AKT/mTOR pathway to promote autophagy in chondrocytes and intervene in knee osteoarthritis

This study showed that compared with the MBG, the expression of mTOR in the RAG and JIG was downregulated to varying degrees. Compared with the JIG, the RAG was downregulated, and the MYG was upregulated. Compared with the RAG, the MYG was upregulated. An early study supported the conclusion of this study, which showed that the degree of cartilage degeneration in the rapamycin intervention group was significantly reduced compared with the control group. Rapamycin inhibits the phosphorylation of ribosomal protein S6 (a target of mTOR) and the activation of LC3, alleviating the symptoms of KOA by activating autophagy [15].

The PI3K/AKT/mTOR pathway is involved in the development of KOA. The activation of mTOR can inhibit autophagy. Chondrocyte apoptosis in joint cartilage is related to the upregulation of mTOR and the downregulation of ATG5, Beclin-1, and LC3-II [45,46]. Specific knockout of the mTOR gene in mice resulted in increased autophagy levels in chondrocytes and a reduction in the number of chondrocyte apoptosis compared with normal mice [47]. The regulation of mTOR is affected by various factors, among which both types I and III PI3K are involved in the regulation of autophagy, activating the downstream Akt phosphorylation, positively regulating mTOR, and inhibiting autophagy. On the contrary, when the downstream Akt is reduced, it induces autophagy. In addition, type III PI3K can directly combine with Beclin-1 to form a complex and promote autophagy [48]. An important function of the PI3K/AKT/mTOR signaling pathway is to regulate autophagy. Inhibiting the transmission of this signaling pathway is an important protective agent against the pathophysiology of KOA [49]. Inflammation occurs in the joint cartilage of KOA model rats, which inhibits the proliferation of chondrocytes and reduces autophagy, while inhibiting this signaling pathway increases autophagy in joint cartilage cells and reduces the inflammatory response [50]. Similar conclusions were also found in human experiments, in which autophagy markers (LC3, Beclin1, and p62) were detected in the cartilage of KOA patients and traumatic amputees, and the expression of related proteins in this signaling pathway was tested, finding that activating this molecular signaling pathway inhibits the proliferation and autophagy of chondrocytes [51]. In vitro studies have shown that the injection of rapamycin (10 μm) can activate autophagy and reduce chondrocyte apoptosis [52]. This study found that the combined intervention has a similar intervention effect to rapamycin, indicating that it may promote autophagy in chondrocytes by inhibiting the PI3K/AKT/mTOR pathway.

Rapamycin inhibits mTOR, which triggers a negative feedback loop, inducing the activation of Akt signaling [53]. In adult joint cartilage, the PI3K/Akt signaling pathway promotes matrix synthesis and the survival of chondrocytes. The activation of Akt in human joint cartilage cells significantly increases the synthesis of proteoglycans and the expression of type II collagen [54]. It is worth noting that this study found that although there were different trends in the expression of PI3K and AKT proteins among groups (upregulation trends in MBG and MYG), the differences were not statistically significant. The insufficiency of autophagy in the cartilage tissue of KOA model rats may be due to the overactivation of the PI3K/AKT/mTOR signaling pathway. This is consistent with previous studies [55], which found that the signaling pathway was overactivated in the cartilage of KOA model rats, manifested by the upregulation of PI3K, AKT, and mTOR proteins. However, some studies hold different opinions, and as the symptoms of KOA are alleviated, the differences in the expression of PI3K and AKT proteins among groups are statistically significant [56,57], which may be related to the intervention time not being long enough. Previous studies generally lasted 5–10 weeks [56,57], while this study lasted for 4 weeks, and further research is needed to extend the intervention time.

The reasons for the effects of the combined intervention on KOA are as follows: PRP contains a high concentration of platelets, which can release various growth factors after activation, promoting cartilage repair [58]. The cytokines in PRP can regulate the joint environment by reducing the production of pro-inflammatory mediators, reducing joint inflammation and pain [59]. ICQ enhances the strength of the quadriceps, improves joint stability, reduces joint pain and stiffness [60,61], and accelerates blood circulation in the knee joint area, promoting the recovery of the knee joint [62]. The combined intervention uses PRP to provide bioactive factors to promote tissue repair and uses ICQ to promote local blood circulation, enhance muscle strength, improve joint stability, and reduce inflammatory responses.

There are limitations to this study: First, the effective components of platelet-rich plasma and the optimal intensity of isometric contraction of the quadriceps need further clarification; second, Local injection of Rapamycin offers the advantage of reduced systemic side effects and the ability to achieve high concentrations directly at the joint site, which is particularly suitable for joint diseases as it can increase the drug concentration within the joint cavity while minimizing potential impacts on other organs. However, local injection also has its limitations, such as potential uneven drug distribution or the need for frequent injections to maintain effective concentrations. Future research can further explore the optimal dosage and frequency of local injections, as well as their combination with other treatment methods, to

maximize therapeutic effects and minimize potential side effects. And this study only used mTOR inhibitors and activators for in vivo verification and did not delve into the molecular biological mechanism of the combined intervention in KOA from the cellular level, and these issues are expected to be resolved in subsequent studies.

5. Conclusion

The combined intervention of platelet-rich plasma and isometric contraction of the quadriceps, by inhibiting the PI3K/AKT/mTOR pathway, promotes autophagy in chondrocytes, downregulates the expression of inflammatory factors such as IL-1β, IL-6, IL-18, and TNF-α, as well as MMP-13, upregulates the expression of autophagy markers such as ATG5, Beclin1, LC3, and ULK1, inhibits matrix degradation, and promotes cartilage repair.

Compliance with ethical standards

All animals were cared for in accordance with the Chengdu Sport University policy for animal welfare, which complies in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and the 1964 Declaration of Helsinki and its later amendments. This study was approved by the Ethics Committee of Chengdu Sport University (Approval No.202213).

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Author contributions

LC, SWC, YJT and BXH: designing this study, writing initial draft and revision, revising language and content, supervision, project administration, and funding acquisition. SWC: making figure and table. LC, YJT and BXH: rechecking the manuscript and putting forward suggestions for amendment. All authors contributed to the article and approved the submitted version.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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