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Experimental Study on PAA-mPEG-MSNs-miR129-3p Improving Rat Osteoarthritis Cartilage Degeneration by Upregulating Autophagy to Inhibit Inflammatory Response

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Abstract: Osteoarthritis (OA) is a chronic degenerative joint disease characterized by progressive cartilage degeneration and persistent inflammatory responses. This study aimed to investigate the therapeutic effects of intra-articular injection of a PAA-mPEG-MSNs-miR129-3p nanodelivery system on cartilage degeneration in a rat OA model and to explore its potential mechanisms. An OA model was established in rats by unilateral intra-articular injection of iodoacetic acid. Thirty rats were randomly divided into a normal group, model group, and PAA-mPEG-MSNs-miR129-3p treatment group (n = 10 per group). The treatment group received intra-articular injection of PAA-mPEG-MSNs-miR129-3p once weekly for four consecutive weeks, while the model group received an equivalent volume of blank carrier. After the intervention period, knee joint swelling and histological changes in cartilage tissue were evaluated using hematoxylin–eosin staining. The expression of the inflammation-related protein MMP-13 was detected by immunohistochemistry, and the mRNA expression levels of autophagy-related genes LC3B and Beclin-1 were analyzed using quantitative real-time PCR. Compared with the normal group, rats in the model group showed significant knee swelling, severe cartilage structural damage, markedly increased MMP-13 expression, and significantly decreased LC3B and Beclin-1 mRNA levels ($P < 0.05$). In contrast, treatment with PAA-mPEG-MSNs-miR129-3p significantly reduced joint swelling, improved cartilage histological structure, decreased MMP-13 expression, and increased the expression levels of LC3B and Beclin-1 ($P < 0.05$). These findings suggest that PAA-mPEG-MSNs-miR129-3p can alleviate cartilage degeneration and inflammatory responses in OA rats and enhance autophagy-related gene expression, indicating that its chondroprotective effects may be associated with restoration of autophagy activity and inhibition of inflammation-mediated cartilage degradation.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized primarily by pathological features such as articular cartilage degeneration, synovitis, and subchondral bone remodeling. Its development and progression are closely associated with multiple factors, including mechanical loading, extracellular matrix degradation, and inflammatory responses [1-3]. Recent studies indicate that inflammatory cytokines play a key role in the OA process, among which matrix metalloproteinase-13 (MMP-13) is one of the primary enzymes responsible for the degradation of type II collagen and the cartilage matrix, and is closely correlated with the extent of cartilage destruction [4]. Meanwhile, as a crucial mechanism for maintaining chondrocyte homeostasis, autophagy is often suppressed in OA. The expression of autophagy-related molecules, such as LC3B and Beclin-1, is downregulated, which is believed to potentially lead to the persistence of abnormal inflammatory responses and exacerbate cartilage degeneration [5,6]. Therefore, exploring OA treatment strategies from the dual regulatory perspective of inflammation and autophagy has become one of the current research hotspots.

As an important post-transcriptional regulatory factor, microRNA plays a significant biological role in the regulation of OA inflammation and the maintenance of cartilage homeostasis [7]. Our previous research found that miR129-3p is downregulated in OA cartilage tissues and chondrocytes, and its reduction is closely associated with impaired autophagy and enhanced inflammatory responses; conversely, upregulation of miR129-3p restores autophagic activity and suppresses the expression of inflammatory cytokines, thereby ameliorating chondrocyte injury. However, whether miR129-3p exerts similar protective effects at the whole-animal level, and whether its in vivo delivery method is safe and effective, remain to be further investigated.

The development of nanocarrier technology has provided new insights for nucleic acid drug delivery [8]. Based on previous work, this study utilized mesoporous silica nanoparticles (MSNs), methoxy polyethylene glycol (mPEG), and polyacrylic acid (PAA) to prepare a PPAA-mPEG-MSNs-miR129-3p nanodelivery system. An osteoarthritis (OA) rat model was established using the iodoacetic acid method, and interventions were performed via intra-articular injection of PAA-mPEG-MSNs-miR129-3p. The efficacy was systematically evaluated from aspects such as changes in cartilage histology, expression of the inflammatory factor MMP-13, and transcription levels of autophagy-related genes. This study aims to verify whether miR129-3p exerts chondroprotective effects in vivo by modulating autophagy and the inflammatory response, thereby providing experimental evidence for molecular targeted therapy of OA.

2. Materials and Methods

2.1 Animals and Grouping

Thirty healthy male Sprague-Dawley (SD) rats, weighing 220–250 g, were provided by the Experimental Animal Center of our institution and housed in specific pathogen-free (SPF) animal facilities. The room temperature, humidity, and light conditions were kept constant, and the rats were allowed free access to food and water. After one week of acclimatization, the rats were randomly divided into three groups using a random number table method: the Normal group, the Model group, and the PAA-mPEG-MSNs-miR129-3p Treatment group, with 10 rats in each group. All experimental procedures were performed in accordance with the ethical guidelines for experimental animals and were approved by the Animal Ethics Committee.

2.2 Induction of Osteoarthritis

The OA model was established via intra-articular injection of monosodium iodoacetate (MIA) into the knee joint. After anesthetizing and fixing the rats, the joint capsule was accessed by puncture adjacent to the right patellar ligament, and a specific dose of MIA solution was injected; the normal control group received an equivalent volume of physiological saline. Following model induction, some rats gradually exhibited manifestations such as limping, reduced weight-bearing capacity, and knee joint swelling, indicating successful model establishment.

2.3 Preparation and Administration of the Nanodelivery System

The PAA-mPEG-MSNs-miR129-3p nanodelivery system was prepared by our research group according to established methods: Firstly, mesoporous silica nanoparticles (MSNs) were synthesized. Subsequently, the MSNs were modified with mPEG and surface-functionalized with polyacrylic acid (PAA). On this basis, mimic-miR129-3p was loaded to form the PAA-mPEG-MSNs-miR129-3p complex for subsequent use.

Starting one week after model induction, rats in the treatment group received a weekly intra-articular injection of this complex into the affected knee joint for 4 consecutive weeks; the model group received an equivalent volume of blank carrier, and the normal group received no treatment. The general condition and joint changes of the animals were recorded.The physical properties of the nanodelivery system are shown in Figure 1.

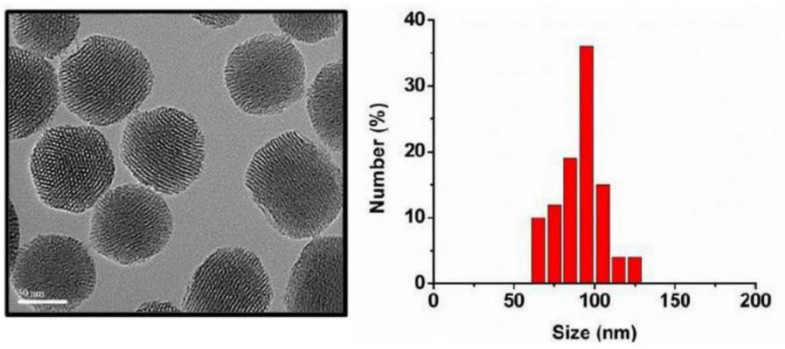

Figure 1: Nanodelivery System

2.4 Histological Evaluation of Knee Joints

At 4 weeks, rats from all groups were euthanized. Right knee joint specimens were harvested, and the surrounding soft tissues were removed. The specimens were then fixed in 4% polyformaldehyde, followed by routine decalcification, dehydration, paraffin embedding, and sectioning. Hematoxylin-Eosin (HE) staining was performed. Histological changes, including the morphology of the articular cartilage surface, cell arrangement, tidemark structure, and integrity of the extracellular matrix, were observed under an optical microscope.

2.5 Immunohistochemical Detection of MMP-13 Expression

The expression level of the cartilage tissue inflammation-related protein MMP-13 was detected using immunohistochemistry. After deparaffinization and hydration, the paraffin sections underwent antigen retrieval and blocking, followed by incubation with the primary anti-MMP-13 antibody overnight at 4 ℃. Subsequently, the secondary antibody was applied, and DAB was used

for color development. The sections were counterstained with hematoxylin, dehydrated, and mounted. Positive signals were observed as brownish-yellow granular distributions. Semi-quantitative analysis was performed by measuring the mean optical density (IOD) using image analysis software.

2.6 Detection of Autophagy-Related Gene mRNA Expression

To evaluate changes in autophagy levels, knee joint cartilage tissues from each group were rapidly frozen and ground in liquid nitrogen. Total RNA was extracted using the TRIzol method and reverse transcribed into cDNA. The mRNA expression levels of LC3B and Beclin-1 were detected by real-time quantitative PCR (qPCR), with GAPDH used as the internal reference gene. Relative expression levels were calculated using the 2^{-DeltaDelta Ct} method. All samples were analyzed in triplicate.

2.7 Statistical Analysis

Analysis was performed using SPSS 26.0 statistical software. Measurement data were expressed as bar{x} pm s. Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA), and pairwise comparisons were performed using the LSD-t test. A P-value < 0.05 was considered statistically significant.

3. Results

3.1 PAA-mPEG-MSNs-miR X 41 alleviates rat knee joint swelling and improves cartilage histological structure

Compared with the normal group, the knee joints of rats in the model group showed obvious swelling and restricted movement. After sample collection, the articular cartilage surface was observed to be rough and detached, the tide mark structure was blurred, and there was disordered arrangement of chondrocytes accompanied by fading of cartilage matrix staining, indicating degenerative changes. Compared with the model group, the degree of knee joint swelling in the PAA-mPEG-MSNs-miR129-3p treatment group was significantly reduced. HE staining showed that the cartilage surface was relatively smooth, the tide mark structure was relatively clear, the arrangement of chondrocytes was more regular, and the matrix staining was significantly improved. This suggests that this treatment can effectively alleviate the structural destruction of OA cartilage and improve degenerative changes. See Figure 2.

3.2 PAA-mPEG-MSNs-miR129-3p reduces the expression level of MMP-13 in cartilage tissue

Immunohistochemical analysis results showed that the expression level of MMP-13 in the cartilage tissue of the normal group was relatively low, with only a small amount of brownish-yellow positive particles observed. In contrast, the expression of MMP-13 in the model group was significantly enhanced, diffusely distributed around the chondrocytes and in the extracellular matrix, with a significantly increased mean optical density value ($P < 0.05$). Compared with the model group, the positive expression of MMP-13 in the treatment group was significantly reduced; the range and intensity of positive staining were both markedly decreased, and the mean optical density value was lower ($P < 0.05$). These results suggest that PAA-mPEG-MSNs-miR129-3p can effectively inhibit the excessive expression of inflammation-related proteins in OA cartilage tissue. See Figure 2.

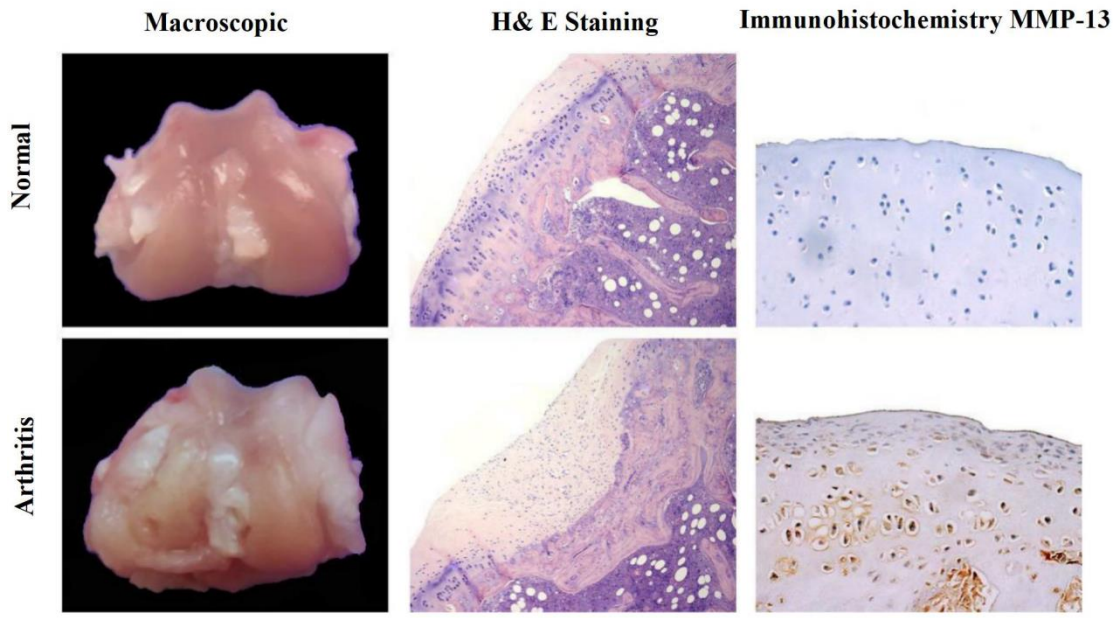

Figure 2: H&E and immunohistochemistry (IHC) staining.

3.3 PAA-mPEG-MSNs-miR129-3p Upregulates Autophagy-Related Gene Expression in Cartilage Tissue

Real-time quantitative PCR results demonstrated that, compared with the normal group, the mRNA expression levels of LC3B and Beclin-1 in the cartilage tissue of rats in the model group were significantly decreased ($P < 0.05$). In comparison with the model group, the expression of both markers was significantly elevated in the treatment group ($P < 0.05$), with levels basically restored to near-normal (Table 1). This suggests that PAA-mPEG-MSNs-miR129-3p can enhance the expression of autophagy-related genes in OA cartilage tissue.

Table 1. Comparison of LC3B and Beclin-1 mRNA Relative Expression Levels in Knee Articular Cartilage Tissues among Different Groups($\bar{x}\pm s$)

Group	n	LC3B	Beclin-1
Control Group	10	1.00 ±0.18	1.00 ±0.16
Model Group	10	0.42 ±0.11*	0.39 ±0.10*
Treatment Group	10	0.87 ±0.15#	0.82 ±0.14#

4. Discussion

Osteoarthritis (OA) is a chronic degenerative joint disease caused by the interaction of multiple factors. Its core pathological changes include chondrocyte dysfunction, increased extracellular matrix degradation, and persistent low-grade inflammation[9]. Among these, inflammatory cytokines and matrix-degrading enzymes are considered to play key roles in the progression of OA. In particular, MMP-13 serves as the primary enzyme for degrading type II collagen, and its abnormal elevation is regarded as a critical molecular basis for cartilage destruction[10–12]. Meanwhile, extensive research in recent years has revealed that autophagy plays an important role in maintaining chondrocyte homeostasis and stress adaptation. A decline in autophagy levels may lead to persistent inflammation, increased cell apoptosis, and imbalance in matrix homeostasis, thereby driving the progression of OA [13–15]. Results from the animal experiments in this study showed that

the expression of MMP-13 was significantly upregulated in the cartilage tissue of the OA model rats, while the expression levels of the autophagy-related genes LC3B and Beclin-1 were markedly downregulated. These findings are consistent with previous research results, suggesting that autophagy function in cartilage tissue is suppressed during OA progression, accompanied by enhanced inflammation-mediated cartilage matrix degradation.

miR129-3p is downregulated in OA cartilage tissue and chondrocytes; upregulation of miR129-3p enhances autophagy and suppresses the expression of inflammatory factors[16,17]. This study further confirms in an in vivo animal model that following intra-articular injection of PAA-mPEG-MSNs-miR129-3p, the structural destruction of rat knee articular cartilage is significantly alleviated, MMP-13 expression is downregulated, while LC3B and Beclin-1 mRNA expression levels are restored to near-normal levels. This indicates that miR129-3p can similarly modulate autophagy and inflammation processes in vivo, thereby exerting a chondroprotective effect. Integrating the results of prior and current research, it can be inferred that the upregulation of miR129-3p may activate the autophagy pathway, reduce inflammation-mediated matrix degradation reactions, and interrupt the vicious cycle during OA progression, highlighting its potential value as a therapeutic target.

It is worth noting that the PAA-mPEG-MSNs nanodelivery system adopted in this study possesses excellent biocompatibility and local delivery advantages. It enables the stable release of miRNA within the joint cavity, avoiding systemic exposure and rapid clearance, thereby providing a feasible delivery strategy for the application of nucleic acid drugs in OA. Compared with Simple miRNA administration, nanocarriers can enhance its stability and local concentration, thereby augmenting the therapeutic efficacy [18]. The results of this study also support this view at the animal level. However, the long-term metabolism, safety, and immune response of the nanodelivery system in the body still require further systematic evaluation, which is also one of the important directions for future research.

Of course, this study also has certain limitations. First, the sample size is relatively limited, and the research scale needs to be expanded to improve the reliability of the conclusions. Second, this study only detected the expression of autophagy-related genes and did not further explore the upstream signaling pathways and downstream effector molecules; the molecular mechanism by which miR129-3p regulates the autophagy-inflammation network remains to be elucidated in depth. Furthermore, this experiment was a study on animal models, and whether its results can be fully extrapolated to the clinic still requires support from more evidence.

In summary, the present study demonstrates that PAA-mPEG-MSNs-miR129-3p can ameliorate OAcartilage degeneration in rat joints, suppress the expression of MMP-13, and upregulate the levels of autophagy-related genes. Its chondroprotective effect may be associated with the restoration of autophagic function and the mitigation of inflammation-mediated extracellular matrix degradation. This study provides in vivo experimental evidence supporting miR129-3p as a potential molecular therapeutic target for OA, while also highlighting that nanodelivery systems may serve as a crucial carrier modality for future OA nucleic acid therapeutics, warranting further investigation and translational application.

5. Conclusions

In conclusion, this study demonstrates that intra-articular administration of the PAA-mPEG-MSNs-miR129-3p nanodelivery system can effectively alleviate cartilage degeneration in a rat osteoarthritis model. The treatment significantly reduced joint swelling, improved cartilage histological structure, suppressed the expression of the inflammation-related protein MMP-13, and upregulated the autophagy-related genes LC3B and Beclin-1. These findings

indicate that miR129-3p exerts chondroprotective effects in vivo, likely through enhancing autophagy and inhibiting inflammation-mediated extracellular matrix degradation. Furthermore, the PAA-mPEG-MSNs nanocarrier provides a promising and efficient delivery strategy for miRNA-based therapies. Overall, this study provides experimental evidence supporting miR129-3p as a potential therapeutic target for osteoarthritis and highlights the translational potential of nanodelivery systems in nucleic acid-based treatment strategies.

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