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Experimental Study on the Improvement of Osteoarthritis Cartilage Degeneration by miR129-3p through Regulating Autophagy

Wei Huacheng[1,a], Li Xinwu[2,b,*]

[1]*Department of Orthopedics and Joint Surgery, Nanning First People's Hospital, Nanning, Guangxi, China*
[2]*Surgery Department of Spinal Diseases, Baise People's Hospital, Baise City, Guangxi, China*
[a]*4040830311@qq.com*, [b]*lixinwu1983@163.com*
[*]*Corresponding author*

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Abstract: Osteoarthritis (OA) is a chronic degenerative joint disease characterized by cartilage degeneration and persistent inflammatory responses. This study aimed to investigate the expression of miR129-3p in OA and explore its regulatory role in autophagy and inflammation, thereby clarifying the molecular mechanism by which miR129-3p improves cartilage degeneration through autophagy regulation. Articular cartilage tissues from patients with OA and normal control cartilage tissues were collected, and the expression levels of miR129-3p, inflammatory factors (IL-6 and MMP-13), and autophagy-related proteins (LC3B and Beclin-1) were detected using molecular biology techniques. An in vitro OA chondrocyte model was established by IL-1β stimulation, followed by transfection with mimic-miR129-3p and inhibitor-miR129-3p to evaluate the effects of miR129-3p on autophagy and inflammatory responses. The results showed that miR129-3p expression was significantly decreased in OA cartilage tissues compared with normal tissues, while IL-6 and MMP-13 levels were significantly increased, and the expression of LC3B and Beclin-1 was significantly reduced ($P < 0.05$). In the cell experiments, upregulation of miR129-3p significantly increased the expression of LC3B and Beclin-1 and reduced the levels of IL-6 and MMP-13, whereas inhibition of miR129-3p produced the opposite effects. These findings indicate that miR129-3p is downregulated in OA and that its decreased expression is associated with reduced autophagy and enhanced inflammatory responses. Upregulation of miR129-3p can activate autophagy and suppress inflammation, thereby alleviating cartilage degeneration. miR129-3p may therefore serve as a potential molecular target for the treatment of osteoarthritis.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by articular cartilage degeneration, subchondral bone remodeling, and synovial inflammation, and is one of the leading

causes of disability in the elderly [1]. With the intensifying aging of the population, the incidence of OA is rising annually, severely affecting patients' quality of life and imposing a substantial socioeconomic burden. Currently, it is believed that abnormal mechanical loading, inflammatory responses, cellular senescence, and metabolic disorders collectively contribute to the pathogenesis and progression of OA, with chondrocyte functional imbalance being a key factor driving cartilage degeneration [2]. As a crucial intracellular mechanism for material turnover, autophagy plays a significant role in maintaining chondrocyte homeostasis and clearing damaged organelles and protein aggregates [3]. However, numerous studies indicate that autophagic activity is diminished in OA chondrocytes, and impaired autophagy accelerates chondrocyte apoptosis and extracellular matrix degradation, thereby promoting disease progression [4,5]. Therefore, identifying key molecules capable of regulating chondrocyte autophagic activity is of great value for elucidating the pathogenic mechanisms of OA and discovering novel therapeutic targets. MicroRNAs (miRNAs) are a class of non-coding small RNAs widely present in cells; through post-transcriptional regulation, they participate in the modulation of various signaling pathways and are closely associated with processes such as inflammation, apoptosis, and metabolic abnormalities [6]. Emerging research suggests that certain miRNAs are involved in the development of OA and influence the biological behavior of chondrocytes [7]. However, the role of miR129-3p in OA remains unclear, particularly whether it affects articular cartilage degeneration by modulating autophagy, which lacks experimental evidence. Through detecting the expression changes of miR129-3p, autophagy-related proteins, and inflammatory cytokines in cartilage tissues of OA patients and an in vitro cell model, and further observing its biological effects by upregulating or inhibiting miR129-3p expression, this study aims to elucidate whether miR129-3p improves cartilage degeneration by regulating autophagy, thereby providing experimental evidence for OA molecular mechanism research and targeted therapeutic strategies.

2. Materials and Methods

2.1 Study Subjects and Sample Sources

This study collected articular cartilage tissues from 5 patients with osteoarthritis (OA) who underwent knee replacement surgery to serve as the OA group. Additionally, normal articular cartilage tissues from 5 patients who underwent amputation due to trauma or other non-joint diseases were selected as the control group. All samples were obtained under aseptic conditions during surgery and were immediately stored at low temperatures for subsequent molecular biological testing. This study strictly adhered to the Helsinki Declaration. All participants signed informed consent forms, and the relevant research protocol was reviewed and approved by the Hospital Ethics Committee.

2.2 Chondrocyte Culture and Model Establishment

For the in vitro experiments, cultured human chondrocytes were used as the research subjects. Cell growth was maintained under conventional culture conditions, and an osteoarthritis (OA) chondrocyte model was established via stimulation with IL-1β. Following successful model construction, OA chondrocytes were transduced with mimic-miR129-3p and inhibitor-miR129-3p, respectively. Corresponding negative control groups were established simultaneously. After transduction, the cells were cultured further and subsequently harvested for downstream assays to evaluate the impact of miR129-3p expression changes on the biological behavior of chondrocytes.

2.3 Detection of miR129-3p Expression

Real-time quantitative PCR (qRT-PCR) was employed to detect the expression levels of miR129-3p in OA cartilage tissues and normal cartilage tissues. Additionally, the expression changes of miR129-3p in OA chondrocytes following different treatments were examined to elucidate its expression characteristics in osteoarthritis and the transfection efficiency.

2.4 Detection of Autophagy- and Inflammation-Related Proteins

Western blot analysis was utilized to measure the expression levels of the inflammatory cytokines IL-6 and MMP-13, as well as the autophagy-related proteins LC3B and Beclin-1. Differences in the expression of these markers between OA tissues and normal cartilage tissues were compared. Furthermore, changes in these indicators following the upregulation or downregulation of miR129-3p expression were observed to evaluate the role of miR129-3p in the regulation of the inflammatory response and autophagy.

2.5 Observation Indicators and Analytical Strategy

This study conducted analyses at both the tissue and cellular levels. First, we compared the expression differences of miR129-3p, the autophagy-related proteins LC3B and Beclin-1, and the inflammatory factors IL-6 and MMP-13 between OA cartilage tissues and normal cartilage tissues. Subsequently, through in vitro cell transfection experiments, we observed the dynamic changes in autophagy and inflammation indicators following the upregulation or inhibition of miR129-3p, thereby inferring the association between miR129-3p and the regulation of autophagy and inflammatory responses.

2.6 Statistical Analysis

All experimental data are expressed as bar{x} pm s. Statistical analysis was performed using SPSS 26.0 software. Intergroup comparisons were conducted using the t-test, and a P-value < 0.05 was considered statistically significant. All experiments were repeated at least three times to ensure the stability and reliability of the results.

3. Results

3.1 Expression of miR129-3p is downregulated in osteoarthritic cartilage tissue and accompanied by elevated inflammatory cytokines

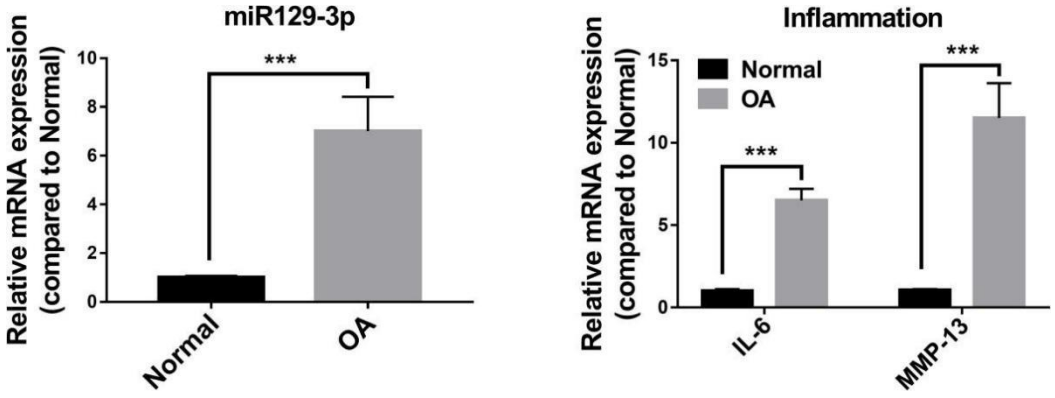

Figure 1: Expression Analysis of miR129-3p, IL-6, and MMP-13

Compared with normal cartilage tissue, the expression level of miR129-3p was significantly decreased in osteoarthritis (OA) cartilage tissue, whereas the expression of inflammatory cytokines IL-6 and MMP-13 was markedly increased. The differences between groups were statistically significant ($P<0.05$). These results suggest that low expression of miR129-3p may be associated with enhanced inflammatory responses. See Figure 1 for results.

3.2 Decreased Expression of Autophagy-Related Proteins in Osteoarthritic Cartilage Tissue

Further detection revealed that the expression levels of the autophagy-related proteins LC3B and Beclin-1 in the cartilage tissue of OA patients were significantly lower than those in the normal control group, and the differences were statistically significant ($P < 0.05$). See Figure 2 for results.

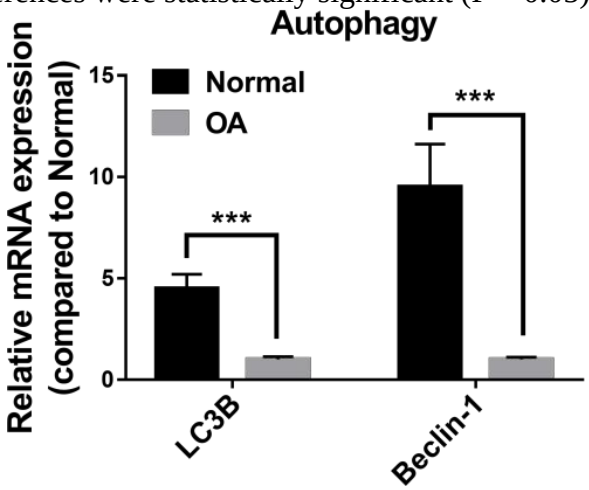

Figure 2: Expression Analysis of Autophagy-Related Proteins LC3B and Beclin-1

3.3 miR129-3p Regulates Autophagy and Inflammatory Factor Expression in Chondrocytes

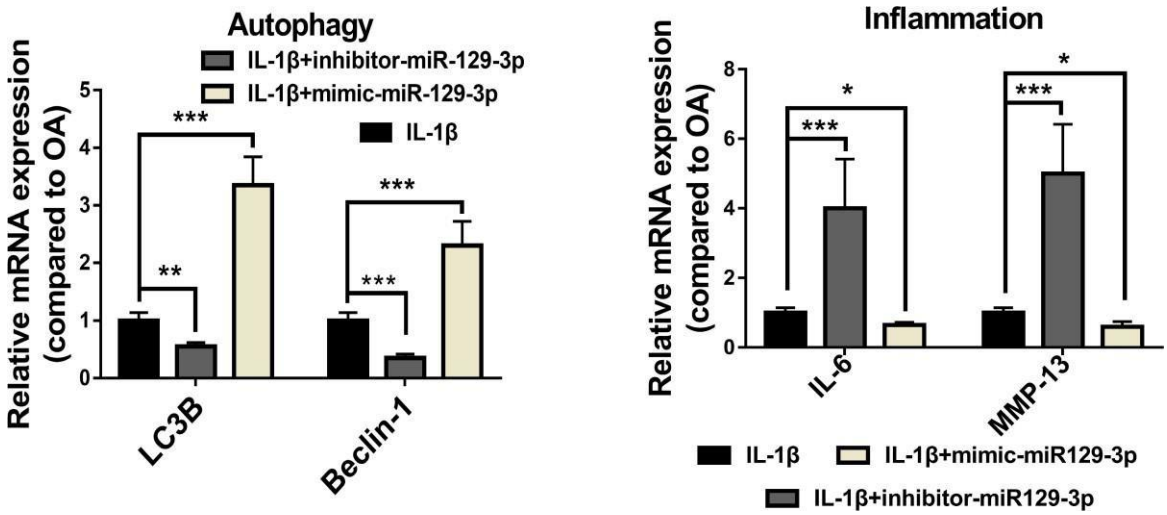

Figure 3: Cell Infection Assay

In vitro cell experiments demonstrated that following transfection with mimic-miR129-3p, the expression levels of LC3B and Beclin-1 in OA chondrocytes were significantly elevated. Conversely, transfection with inhibitor-miR129-3p led to a marked decrease in the expression of these proteins. Furthermore, upregulation of miR129-3p significantly reduced the expression of IL-6 and MMP-13, whereas inhibition of miR129-3p resulted in a significant increase in their

expression. All aforementioned differences were statistically significant ($P < 0.05$), suggesting that miR129-3p participates in the regulation of the inflammatory response by modulating autophagy activity. The results are shown in Figure 3.

4. Discussion

This study first confirmed that miR129-3p expression is significantly reduced in osteoarthritic cartilage tissue, accompanied by elevated levels of the inflammatory cytokines IL-6 and MMP-13. This suggests that miR129-3p may be involved in the pathogenesis and progression of osteoarthritis. Existing research posits that various microRNAs play crucial regulatory roles in the OA process, with functions spanning inflammation regulation, cellular senescence, and extracellular matrix metabolism [8-10]. The findings of this study further suggest that miR129-3p may belong to a class of protective microRNAs. Its downregulation may impair the homeostatic regulatory capacity of chondrocytes, thereby promoting degenerative changes in cartilage and the persistence of inflammatory responses.

In recent years, autophagy has been confirmed as one of the key mechanisms for maintaining chondrocyte homeostasis, and the decline in autophagic function is considered a significant contributing factor to the progression of OA [11,12]. This study found that the expression levels of LC3B and Beclin-1 were decreased in OA cartilage tissue, indicating suppressed autophagic activity, which aligns with the trend of miR129-3p downregulation. Further cellular experimental results demonstrated that upregulating miR129-3p expression significantly increased the expression of LC3B and Beclin-1, whereas inhibiting miR129-3p produced the opposite effect. This suggests that miR129-3p participates in the regulation of the autophagy pathway to a certain extent, thereby influencing chondrocyte homeostasis. Based on the findings of this study, it is speculated that the low expression of miR129-3p may be one of the important molecular causes of the decline in autophagy levels in OA.

Inflammation is a key pathological process that exists from the early stages of osteoarthritis (OA) and persists throughout the disease course. This study demonstrates that upregulation of miR129-3p significantly reduces the expression of IL-6 and MMP-13, whereas its inhibition leads to a further elevation of inflammatory cytokines. This suggests that miR129-3p plays a negative regulatory role in the OA inflammatory response. Previous studies have confirmed that the inflammatory microenvironment promotes chondrocyte apoptosis and extracellular matrix degradation, while autophagy possesses the ability to suppress inflammatory responses [13-15]. Therefore, it can be inferred that miR129-3p may promote the restoration of autophagy, thereby indirectly lowering the level of inflammatory response and forming an "autophagy-inflammation mutual balance" mechanism. This finding is highly consistent with the results of the present study.

This study systematically evaluated the expression characteristics of miR129-3p in osteoarthritis (OA) and its regulatory effects on autophagy and inflammatory responses at both the tissue and cellular levels. The results indicate that miR129-3p possesses potential protective effects during the pathogenesis of OA, providing significant experimental evidence for elucidating the molecular mechanisms of OA and identifying novel therapeutic targets. However, this research is primarily based on in vitro experiments and tissue sample analysis, and thus lacks further in vivo validation. Additionally, the specific target genes and signaling pathways of miR129-3p remain to be fully elucidated. Future studies could combine animal models with clinical samples to expand the sample size and further clarify the regulatory network at the molecular level, thereby providing a more robust theoretical foundation for the precision treatment of OA.

5. Conclusions

In conclusion, this study demonstrates that miR129-3p is significantly downregulated in osteoarthritic cartilage and is closely associated with decreased autophagy activity and enhanced inflammatory responses. Upregulation of miR129-3p promotes the expression of autophagy-related proteins LC3B and Beclin-1 while suppressing inflammatory factors such as IL-6 and MMP-13, thereby alleviating cartilage degeneration. These findings suggest that miR129-3p exerts a protective role in osteoarthritis through the regulation of the autophagy–inflammation axis. Therefore, miR129-3p may serve as a promising molecular target for the development of novel therapeutic strategies for osteoarthritis. However, further in vivo studies and mechanistic investigations are required to validate its clinical application potential.

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References

[1] Tang S, Zhang C, Oo WM, et al. Osteoarthritis. Nat Rev Dis Primers. 2025,11(1):10.
[2] Zou Z, Hu W, Kang F, et al. Interplay between lipid dysregulation and ferroptosis in chondrocytes and the targeted therapy effect of metformin on osteoarthritis. J Adv Res. 2025,69:515-529.
[3] Andrei C, Mihai DP, Nitulescu GM, Nitulescu G, Zanfirescu A. Modulating Autophagy in Osteoarthritis: Exploring Emerging Therapeutic Drug Targets. Int J Mol Sci. 2024,25(24):13695.
[4] Liao J, Yu X, Chen J, et al. Knowledge mapping of autophagy in osteoarthritis from 2004 to 2022: A bibliometric analysis. Front Immunol. 2023,14:1063018.
[5] Liu N, Ma Y, Gong W, et al. Osteocyte-derived extracellular vesicles mediate the bone-to-cartilage crosstalk and promote osteoarthritis progression. Nat Commun. 2025,16(1):4746.
[6] Zang K, Brossard M, Wilson T, Ali SA, Espin-Garcia O. A scoping review of statistical methods to investigate colocalization between genetic associations and microRNA expression in osteoarthritis. Osteoarthr Cartil Open. 2024,6(4):100540.
[7] Wilson TG, Baghel M, Kaur N, et al. Circulating miR-126-3p is a mechanistic biomarker for knee osteoarthritis. Nat Commun. 2025,16(1):2021.
[8] Xie W, Jiang L, Huang X, You W, Sun W. Hsa_circ_0004662 Accelerates the Progression of Osteoarthritis via the microRNA-424-5p/VEGFA Axis. Curr Mol Med. 2024,24(2):217-225.
[9] Paisan M, Papadopoulos KI, Papadopoulou A, Prasongchean W, Wattanaarsakit P. Intertwined roles of microRNA-155 and metformin in osteoarthritis: Novel potential diagnostic, prognostic, and therapeutic modulators. World J Orthop. 2025,16(12):110992.
[10] Wang J, Li X, Guo X, et al. MicroRNA-34a-5p promotes the progression of osteoarthritis secondary to developmental dysplasia of the hip by restraining SESN2-induced autophagy. J Orthop Res. 2024,42(1):66-77.
[11] Chen X, Gong W, Shao X, et al. METTL3-mediated m6A modification of ATG7 regulates autophagy-GATA4 axis to promote cellular senescence and osteoarthritis progression. Ann Rheum Dis. 2022,81(1):87-99.
[12] Yu Y, Zhao J. Modulated Autophagy by MicroRNAs in Osteoarthritis Chondrocytes. Biomed Res Int. 2019,2019:1484152.
[13] Li YS, Zhang FJ, Zeng C, et al. Autophagy in osteoarthritis. Joint Bone Spine. 2016,83(2):143-148.
[14] Vinatier C, Dom ńguez E, Guicheux J, Caram és B. Role of the Inflammation-Autophagy-Senescence Integrative Network in Osteoarthritis. Front Physiol. 2018,9:706.
[15] Wang Y, Eapen VV, Liang Y, et al. WSTF nuclear autophagy regulates chronic but not acute inflammation. Nature. 2025,644(8077):780-789.