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miRNA-335-5p relieves chondrocyte inflammation by activating autophagy in osteoarthritis

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ABSTRACT

Aims: Osteoarthritis (OA) is a chronic and degenerative joint disease prevalent in the elderly, which is characterized by hypertrophy and reactive hyperplasia of articular cartilage. Autophagy has been reported to inhibit inflammation and reduce chondrocyte apoptosis in OA. As the microRNA (miRNA)-335-5p has been linked to both inflammation and autophagy, this study aimed to investigate its potential role in regulating autophagy during the pathogenesis of OA.

Main methods: Quantitative real-time PCR (qRT-PCR) was used to detect miRNA-335-5p expression in normal and OA human chondrocytes. Following transfection of human OA chondrocytes with double-stranded miRNA-335-5p mimic/inhibitor, qRT-PCR, western blotting, and immunofluorescence were used to determine expression levels of the inflammatory mediators IL-1β, IL-6, and TNF-α, and the autophagic markers Beclin-1, autophagy-related protein 5 (ATG5), and ATG7. The autophagy inhibitor 3-methyladenine (3-MA) was used to link the anti-inflammatory effects of miRNA-335-5p to autophagy.

Key findings: The expression of miRNA-335-5p was significantly lower in OA chondrocytes than in normal chondrocytes. Transfection of human OA chondrocytes with the miRNA-335-5p mimic led to a remarkable increase in viability, a significant increase in autophagy-related factors, and a reduction in inflammatory mediators. Importantly, treatment of miRNA-335-5p-overexpressing OA chondrocytes with the autophagy inhibitor 3-MA restored the expression of inflammatory mediators.

Significance: We conclude that miRNA-335-5p can significantly alleviate inflammation in human OA chondrocytes by activating autophagy. Therefore, miRNA-335-5p has potential for future use in the clinical diagnosis and treatment of OA.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative articular cartilage disease that affects humans worldwide. Initially manifesting as joint pain and stiffness, OA can progress to joint deformity at the more advanced stages. The disease is most common in the middle-aged and elderly, with a reported incidence of over 65% in people aged over 65 years. OA seriously affects the quality of life of patients [1], and its high incidence places a significant economic burden on society [2]. It has been reported that immune responses and the secretion of inflammatory factors both play important roles in the pathological process of OA [3]. However, the specific mechanisms underlying the complex pathogenesis of OA are still unclear, meaning that current therapeutic options are limited mainly to short-term symptomatic relief. This highlights the need for a deeper understanding of the mechanisms underlying OA pathogenesis, which will allow the development of more targeted approaches.

MicroRNAs (miRNAs) are a class of multifunctional non-coding RNA molecules of 22–25 nucleotides in length which are encoded by endogenous genes. By binding to non-coding genetic regions, miRNAs inhibit splicing and translation, thereby regulating target gene expression and downstream pathways. In this way, miRNAs have been shown to regulate various biological processes, such as proliferation, differentiation, inflammation, and apoptosis [4–6]. Importantly, recent

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studies have described anti-inflammatory and anti-apoptotic effects of several miRNAs (miR-200a, miR-155, miR-146a, miR-144, and miR-217) [7,15–18].

miRNA-335-5p is another miRNA that has been shown to have a significant anti-inflammatory effect. Moreover, it has been shown to activate AMP-activated protein kinase (AMPK) [8], an important factor in the mechanistic target of rapamycin (mTOR) pathway that plays a crucial role in autophagy activation [9]. Autophagy, a critical method of self-repair via the orderly degradation and recycling of cellular components [10], has gained much interest in OA treatment research. We hypothesized that miRNA-335-5p reduces the inflammatory response of OA chondrocytes through activation of autophagy, thus preventing the pathological process of OA. As the role of miRNA-335-5p in OA has not previously been studied, we investigated the potential protective effect of miRNA-335-5p on human OA chondrocytes with the aim of providing potential targets for the clinical diagnosis and treatment of OA.

2. Materials and methods

2.1. Materials and reagents

Type II collagenase and high glucose Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (USA); penicillin-streptomycin, fetal bovine serum, and trypsin were purchased from Zhejiang Tianhang Biotechnology Co., Ltd. (China); miRNA-335-5p, the corresponding blank control, and a transfection reagent were purchased from Shanghai GenePharma Co., Ltd. (China); 3-methyladenine (3-MA), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma (USA); TRIzol reagent and a real-time quantitative polymerase chain reaction (qPCR) kit were purchased from Magen (China); an immunohistochemistry kit (ZSGb Bio, China) was purchased from ZSGB-BIO (China).

3. Methods

3.1. Cartilage tissue collection and chondrocyte isolation

OA Patients involved in this study were diagnosed according to the OA diagnostic criteria of the Chinese Orthopedic Association of the Chinese Medical Association and received a total knee replacement. OA articular cartilage specimens were obtained under aseptic conditions in the operating room of the Department of Orthopedics at the First Affiliated Hospital of Guangxi Medical University. Normal articular cartilage specimens were obtained from patients who underwent phalangectomy at the First Affiliated Hospital of Guangxi Medical University, China. All operating procedures were performed in compliance with the requirements of the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, with written informed consent obtained from the patients or their family members. Chondrocytes were obtained as previously described [15]. Briefly, fresh cartilage specimens were digested with 0.25% trypsin for 20 min and then cut into pieces of approximately 0.5 cm^2 using ophthalmic scissors, followed by the addition of 0.2% type 2 collagenase and incubation for 4 h. The resulting suspended chondrocytes were then cultured in high-glucose DMEM containing 15% fetal bovine serum and 2% penicillin-streptomycin at 37 °C with 5% CO_2. The medium was changed every two days, and cells were passaged every four days. Second-generation chondrocytes were used for experiments.

3.2. Transfection of OA chondrocytes with double-stranded (ds) miRNA-335-5p mimic/inhibitor

Second-generation OA chondrocytes were seeded in 12-well plates (1×10^5 cells/well) and incubated for 24 h. Cells were then transfected with the miRNA-335-5p mimic (100 nM) or the corresponding miRNA-335-5p inhibitor (100 nM) according to the protocol provided with the transfection reagent (Shanghai GenePharma). The transfection efficiency was verified 48 h later, before performing further experiments.

3.3. Viability of OA chondrocytes

Primary normal or OA chondrocytes (untransfected or transfected with ds-miRNA-335-5p mimic/inhibitor) were seeded in 96-well plates (3×10^3 cells/well, 10 replicates per group). After incubation for 24 h, 20 μL of MTT reagent (5 mg/mL) was added, followed by incubation at 37 °C for 4 h. The supernatant was aspirated, and 200 μL of DMSO was added followed by shaking for 15 min. The absorbance was measured with a spectrophotometer at a wavelength of 490 nm.

3.4. Cytotoxicity assay

Primary normal or OA chondrocytes (untransfected or transfected with ds-miRNA-335-5p mimic/inhibitor) were cultured in 96-well plates at the concentration of 3×10^3 cells/well. CCK-8 reagent (Gibco, USA) was added after incubation for 24 h in the incubator. Cells were then incubated with CCK-8 reagent at 37 °C for 4 h. Absorbance of culture medium was measured using a spectrophotometer (Thermo, USA) at a wavelength of 490 nm. The test is repeated three times.

3.5. Glycosaminoglycan measurement

The DNA content of chondrocytes was detected using Hoechst 33258 (Sigma, USA) as previously described [16]. Briefly, primary normal or OA chondrocytes (untransfected or transfected with ds-miRNA-335-5p mimic/inhibitor) were seeded in 6-well plates (5×10^5 cells/well) and cultured at 37 °C with 5% CO_2 for 24 h. A 1,9-dimethylmethylene blue assay (Sigma, USA) was used to determine the GAG content according to the manufacturer's instructions. Absorbance was measured at a wavelength of 525 nm using a fluorescence microplate reader.

3.6. Safranin-O staining

Primary normal or OA chondrocytes (untransfected or transfected with ds-miRNA-335-5p mimic/inhibitor) were seeded in 24-well plates (5×10^4 cells/well) and incubated for 24 h The medium was then aspirated, and the cells were washed three times with PBS, followed by incubation with a 0.2 mg/mL safranin-O staining solution (Solarbio, China) for 5 min in the dark. The cells were washed three times with PBS and photographed under a fluorescence microscope (Olympus BX53, Japan).

3.7. Immunohistochemistry

Immunohistochemistry staining was applied by SP9000 (ZSGb Bio, China). Briefly, chondrocytes were quenched with 3% hydrogen peroxide for 15 min followed by tryptic antigen for 15 min. The chondrocytes were then overlaid with 10% normal goat blocking serum for 15 min at room temperature and incubated antibody for TNF-α (Abcam, USA) overnight for 4 °C. The cells were then incubated with goat anti-rabbit IgG antibody (Abcam, USA) for 1 h at room temperature. Finally, images were captured by fluorescence imaging microscope (Olympus BX53, Japan).

3.8. Transmission electron microscopy (TEM)

Chondrocytes transfected with miRNA-335-5p mimic were collected and centrifuged at 1000 rpm for 5 min. The pelleted chondrocytes were fixed using 2.5% (v/v) glutaraldehyde for 24 h and then 1% (v/v) osmium tetroxide for 3 h. The chondrocytes were then dehydrated by

incubation for 10 min in each of the following concentrations of alcohol (30%, 50%, 75%, 90%, 99%, 100%) and embedded in resin after thoroughly washing with PBS. The samples were sectioned and stained with 2% aqueous uranyl acetate for 1 h in the dark. Images were taken using a TEM (Hitachi H-7650, Japan).

3.9. Autophagy flux detection

Autophagy flux in chondrocytes was assessed using the Cyto-ID Autophagy Detection Kit (Enzo Life Sciences, USA) [17]. Briefly, cells on coverslips were washed three times with PBS and then incubated with Cyto-ID (Enzo Life Sciences, USA) and Hoechst 33258 in the dark for 15 min. Excessive dye was washed off with PBS, and images were taken using a fluorescence microscope (Olympus BX53, Japan).

3.10. Treatment of OA chondrocytes with 3-MA

OA chondrocytes transfected with the ds-miRNA-335-5p mimic were seeded in 6-well plates (5×10^6 cells/well). After attachment to the wells, the transfected chondrocytes were cultured in complete medium containing 5 mM 3-MA. After 24 h, the cells were collected and used for subsequent experiments.

3.11. Detection of OA-related gene expression by qRT-PCR

Total RNA was extracted from chondrocytes using TRIzol and the concentration was determined. The RNA was reverse transcribed into complementary DNA, and then qPCR was performed according to the FastStart Universal SYBR Green master mix protocol. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal standard to normalize gene expression levels. Target gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [18]. The primer sequences are shown in Table 1.

3.12. Detection of OA-related protein expression by western blotting (WB)

Total protein was extracted from chondrocytes using a protein extraction kit (BOSTER, China). Protein (60 μg per sample) was separated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) on a 10% gel and transferred to a polyvinylidene difluoride membrane (PVDF, Millipore, USA). After blocking in 5% non-fat milk in TBS containing 0.1% Tween-20, the membrane was incubated with a primary antibody for IL-1β, IL-6, TNF-α, Beclin-1, ATG5, or ATG7 (BOSTER, China) for 24 h, followed by incubation in HRP-conjugated secondary antibody for 2 h. GAPDH primary antibody (BOSTER, China) was used as a standard control for protein expression levels. Protein signal was detected using an enhanced chemiluminescence (ECL) kit.

3.13. Statistical analysis

Statistical analysis was performed using SPSS V64.0 software (IBM, USA). All data are expressed as the mean ± standard deviation (SD). Data comparison was carried out using Student's *t*-test or analysis of variance, and differences with $P < 0.05$ were considered statistically significant.

4. Results

4.1. Expression of miRNA-335-5p in human OA and normal cartilage tissues

To investigate the potential role of miRNA-335-5p in OA, we first examined the expression of miRNA-335-5p in OA chondrocytes and normal chondrocytes using qPCR. As shown in Fig. 1a, the expression of miRNA-335-5p in OA chondrocytes was significantly lower, at only 11.3% of its expression level in normal chondrocytes ($P < 0.05$). These results indicate that miRNA-335-5p expression is suppressed in an OA microenvironment.

4.2. Assessment of miRNA-335-5p transfection efficiency

In order to allow us to manipulate miRNA-335-5p levels in chondrocytes, we next tested the transfection efficiency of ds-miRNA-335-5p mimic and inhibitor in human OA chondrocytes using qPCR at 24 h post-transfection. As can be seen in Fig. 1b, miRNA-335-5p expression was significantly elevated by transfection with the ds-miRNA-335-5p mimic compared to untransfected or ds-miRNA-335-5p inhibitor-transfected OA chondrocytes ($P < 0.05$). The miRNA-335-5p expression level in the mimic-transfected cells was approximately 14.45-fold higher than that in untransfected OA chondrocytes. These results confirm that miRNA-335-5p transfection into human OA chondrocytes was successful.

4.3. Effect of miRNA-335-5p on the viability of human OA chondrocytes

The effect of miRNA-335-5p mimic transfection on the viability of human OA chondrocytes was evaluated using the MTT assay, the results of which are shown in Fig. 1c. The absorbance values of human OA chondrocytes were significantly lower than those of normal chondrocytes ($P < 0.05$), indicating reduced viability of OA chondrocytes. However, following transfection of the miRNA-335-5p mimic, the absorbance values of OA chondrocytes significantly increased compared with those of untransfected human OA chondrocytes (2.32-fold, $P < 0.05$), indicating that miRNA-335-5p enhances the viability of human OA chondrocytes. Similar results also appeared in the CCK-8 test for cytotoxicity (Fig. 1d).

4.4. Effect of miRNA-335-5p on the glycosaminoglycan content of human OA chondrocytes

The effect of miRNA-335-5p mimic transfection on the glycosaminoglycan (GAG) content of human OA chondrocytes was examined using a glycosaminoglycan (GAG) kit. As can be seen in Fig. 1e, the GAG content in human OA chondrocytes was significantly lower, at only 22.24% of that in normal chondrocytes ($P < 0.05$). However, transfection with the miRNA-335-5p mimic significantly increased the

Table 1
Primer sequences used in qRT-PCR experiments (human).

Gene name	Forward primer	Reverse primer
miR-335-5p	5′-GCGGCGGTCAAGAGCAATAACG-3′	5′-ATCCAGTGCAGGGTCCGAGG-3′
GAPDH	5′-AGGGCCCTGACAACTCTTTT-3′	5′-AGGGGTCTACATGGCAACTG-3′
IL-1β	5′-TGAGCTCGCCAGTGAAATGA-3′	5′-CATGGCCACAACAACTGACG-3′
TNF-a	5′-TCAGAGGGCCTGTACCTCAT-3′	5′-GGAGGTTGACCTTGGTCTGG-3′
IL-6	5′-TCTGCGCAGCTTTAAGGAGT-3′	5′-CCCAGTGGACAGGTTTCTGA-3′
ATG5	5′-AGAAGCTGTTTCGTCCTGTG-3′	5′-AGGTGTTTCCAACATTGGCT-3
Beclin-1	5′-TCCGGGCTCCCGAGG-3′	5′-GGGGGATGAATCTGCGAGAG-3′
ATG7	5′-TGGTTACAAGCTTGGCTGCT-3′	5′-TCAAGAACCTGGTGAGGCAC-3′

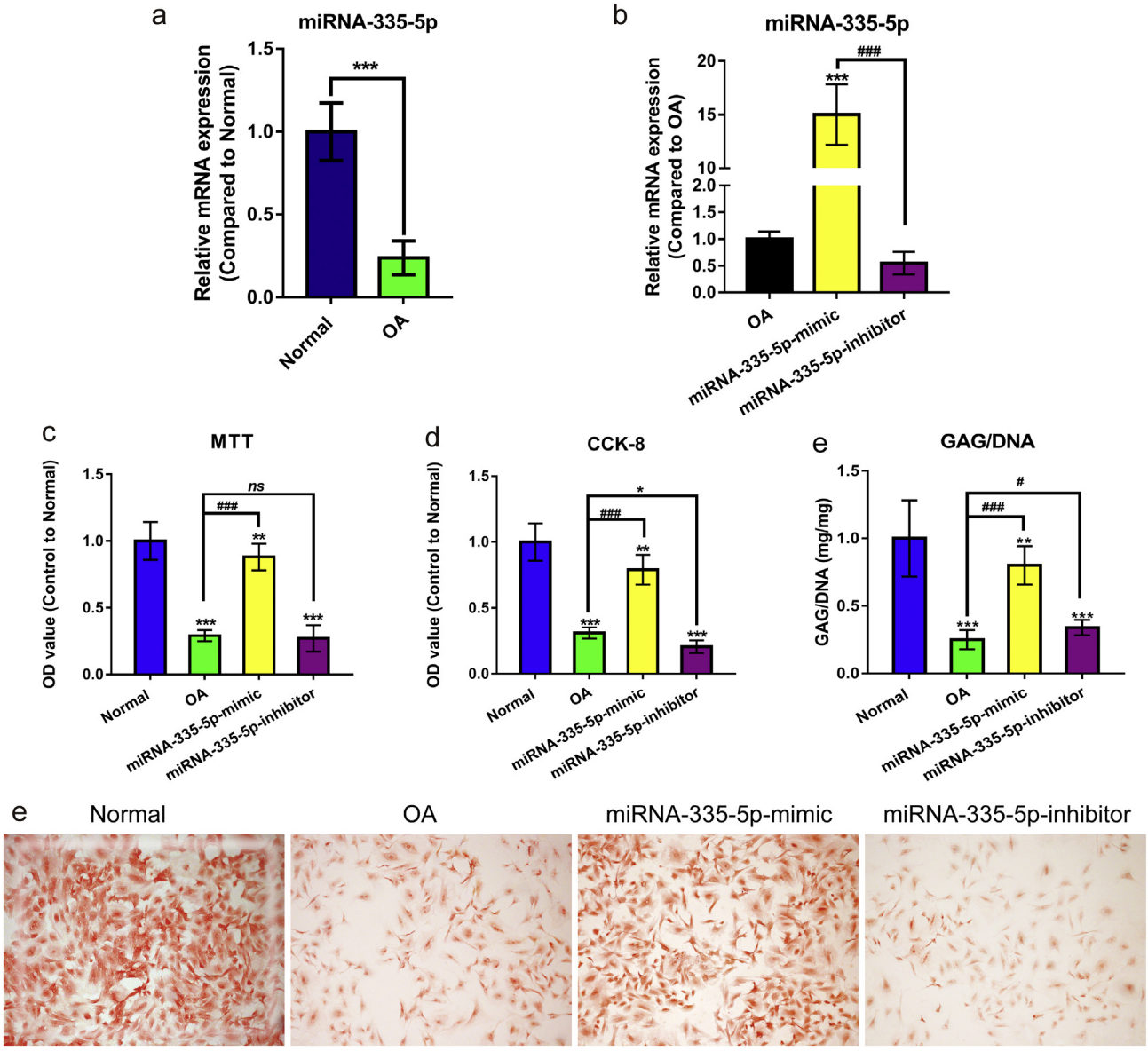

Fig. 1. miRNA-335-5p expression is decreased in OA, and its re-expression increases OA chondrocyte viability. (a) miRNA-335-5p expression in normal and OA chondrocytes was detected by qRT-PCR. (b) Second-generation OA chondrocytes were transfected with 100 nM miRNA-335-5p mimic or inhibitor, and miRNA-335-5p expression was detected by qRT-PCR. (c) The effect of miRNA-335-5p on the viability of human chondrocytes was measured using an MTT assay (data are expressed as the mean ± SD, $n = 6$, $^{***}P < 0.001$). (d) The effect of miRNA-335-5p on the cytotoxicity of human chondrocytes was measured using an CCK-8 assay (data are expressed as the mean ± SD, $n = 6$, $^{***}P < 0.001$). (e) The effect of miRNA-335-5p on intracellular GAG production in human chondrocytes was measured using a GAG assay. (e) Safranin-O staining was used to assess GAG production. Data are expressed as the mean ± SD, n = 6, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ relative to respective control groups; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

GAG content in the OA chondrocytes compared to the untransfected control ($P < 0.05$).

4.5. Effect of miRNA-335-5p on the expression of inflammatory factors in human OA chondrocytes

We next assessed the effect of miRNA-335-5p on the expression of inflammatory factors in human OA chondrocytes by qRT-PCR and WB. OA chondrocytes transfected with miRNA-335-5p showed a marked reduction in the expression of genes encoding the inflammatory factors interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α), compared with the untransfected control (Fig. 2a, $P < 0.05$). A similar effect was also observed at the protein level, as assessed by WB and immunohistochemistry (Fig. 2b, c). These results indicate that miRNA-335-5p inhibits inflammation in human OA chondrocytes.

4.6. Effect of miRNA-335-5p on the expression of autophagy-related factors in human OA chondrocytes

Next, we investigated the effect of miRNA-335-5p on autophagy-related gene and protein expression in human chondrocytes using qRT-PCR, WB, and Cyto-ID Green dye analysis. The expression of genes encoding the autophagy-related proteins Beclin-1, ATG5, and ATG7 was significantly lower in human OA chondrocytes than in normal chondrocytes ($P < 0.05$). However, miRNA-335-5p-transfected OA chondrocytes exhibited significantly enhanced expression levels of the autophagy-related genes compared with the untransfected control ($P < 0.05$) (Fig. 3a). The effect of miRNA on autophagy related protein expression in human OA chondrocytes obtained by WB is the same as that obtained by qRT-PCR (Fig. 3b). In line with the results of transmission electron microscopy, a large number of autophages were

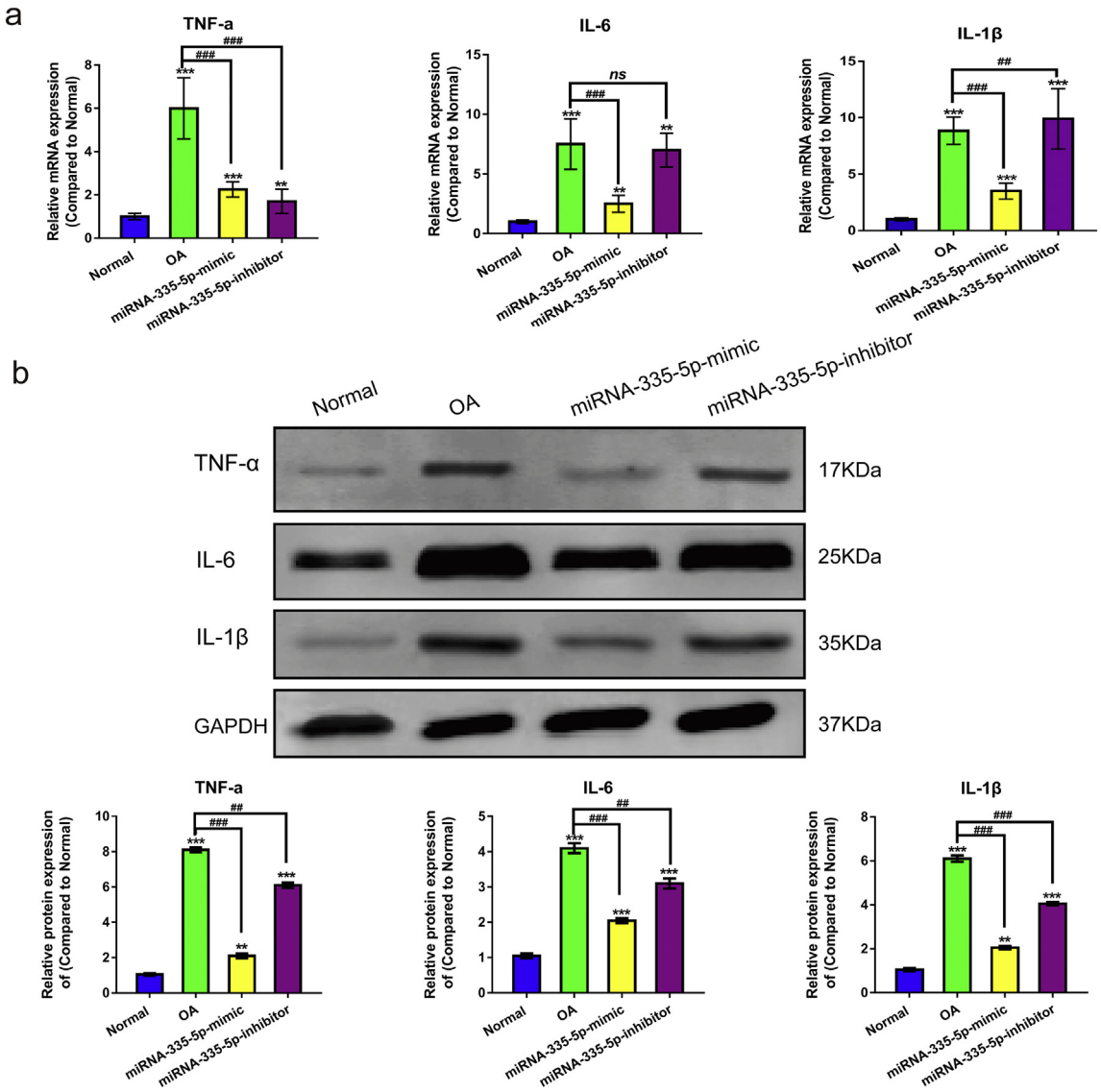

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Fig. 2. miRNA-335-5p reduces the expression of inflammatory factors in human OA chondrocytes.
Human OA chondrocytes were transfected with 100 nM miRNA-335-5p mimic or inhibitor for 24 h. (a) The mRNA expression levels of TNF-α, IL-6, and IL-1β were assessed by qRT-PCR. (b) The protein expression levels of TNF-α, IL-6, and IL-1β were determined by WB and subsequently quantified by densiometry analysis. (c) Immunofluorescence staining revealed the presence of TNF-α. Representative images are shown. Data are expressed as the mean ± SD of three independent experiments. n = 6, *P < 0.05, **P < 0.01, ***P < 0.001 relative to respective control groups; #P < 0.05, ##P < 0.01, ###P < 0.001.

observed in chondrocytes transfected with miRNA 335-5p (Fig. 3c). In addition, analysis of autophagic flow revealed an increase in autofluorescence intensity (shown in green) following transfection with miRNA-335-5p relative to the untransfected OA chondrocytes (Fig. 3d). These results demonstrate that miRNA-335-5p activates autophagy in human OA chondrocytes.

4.7. The autophagy inhibitor 3-MA restores expression of inflammatory factors in miRNA-335-5p-transfected human OA chondrocytes

To further investigate the mechanism of the effect of miRNA-335-5p on inflammation in OA chondrocytes, we made use of the autophagy inhibitor 3-MA. Human OA chondrocytes transfected with miRNA-335-5p were treated with 3-MA, followed by assessment of the expression of inflammatory factors by qRT-PCR and WB. Similar to the result shown in Fig. 2, the mRNA expression of IL-1β, IL-6, and TNF-α was significantly lower in miRNA-335-5p-transfected human OA chondrocytes than in untransfected OA chondrocytes ($P < 0.05$). However, 3-MA treatment reversed this change, causing a dramatic increase in the expression of the inflammatory factors IL-1β, IL-6, and TNF-α in miRNA-335-5p-transfected human OA chondrocytes (P < 0.05) (Fig. 4a). WB confirmed that 3-MA treatment also greatly increased the expression of IL-1β, IL-6, and TNF-α at the protein level in miRNA-335-5p-transfected human OA chondrocytes (Fig. 4b). The finding that 3-MA abolishes the protective effect of miRNA-335-5p on OA chondrocytes strongly suggests that miRNA-335-5p protects human OA chondrocytes through activation of autophagy.

5. Discussion

OA is a debilitating disease that results in high morbidity in the middle-aged and elderly population. Its pathophysiology mainly includes massive cartilage matrix loss, chondrocyte apoptosis, and chronic inflammation in parts of a joint, such as the synovial membrane [19]. Progression of OA to advanced stages can cause severe joint pain, deformity, and dysfunction, significantly reducing patients' quality of life [20]. The current clinical treatment for OA is mainly based on symptomatic therapy, with nonsteroidal anti-inflammatory drugs commonly prescribed to temporarily relieve joint pain [21]. However, this type of treatment does not treat the root cause of OA, and can cause serious side effects in the gastrointestinal tract, blood, and immune system [22,23]. With the current and ongoing increase in the aging population, the morbidity rate associated with OA continues to rise. Therefore, there is an increasing need to find more effective and longer-lasting treatments for OA.

MiRNAs are a class of multifunctional non-coding microRNA molecules that regulate the expression of many important genes and have been shown to participate in the genetic regulation of many pathophysiological processes [24]. In recent years, there have been multiple reports of the involvement of miRNAs in the pathological process of OA [25]. For example, miR-34a was shown to target delta-like protein 1 (DLL1), leading to apoptosis and senescence of chondrocytes and promoting the progression of OA [26]. Conversely, miRNA-92a-3p was shown to promote the expression of a proteoglycan in articular cartilage and relieve OA progression [27]. Furthermore, miR-146a was reported to target C–X–C chemokine receptor type 4 (CXCR4) to suppress the secretion of inflammatory factors in OA [28]. The present study revealed that miRNA-335-5p expression is significantly downregulated in human OA (Fig. 1), and that its re-expression remarkably enhances viability (Fig. 3) and significantly reduces the expression of inflammatory factors (IL-1β, IL-6, and TNF-α) (Fig. 4) in OA chondrocytes. In summary, we conclude that miRNA-335-5p may exert protective effects on human OA chondrocytes.

Autophagy is a process by which damaged proteins and organelles are phagocytosed and degraded to meet the body's metabolic needs and support organelle renewal [29]. When cells are under stress, such as hypoxia, starvation, and accumulation of reactive oxygen species, autophagy is activated to prevent apoptosis and maintain cellular homeostasis [30–32]. Multiple studies have demonstrated that autophagy is significantly suppressed in the microenvironment of OA [33]. Here, we confirmed that expression of the autophagy-related genes encoding Beclin-1, ATG5, and ATG7 was significantly downregulated in human OA chondrocytes (Fig. 1a), and demonstrated that this was reversed by miRNA-335-5p overexpression. Consistent with this, miRNA-335-5p overexpression also significantly reduced the expression of inflammatory factors in OA chondrocytes. Significantly, we showed that the expression of inflammatory factors (IL-1β, IL-6, and TNF-α) in miRNA-335-5p-overexpressing cells was increased by treatment with 3-MA, a selective phosphoinositide 3-kinase (PI3K) inhibitor [34] that has been widely used to inhibit autophagy. Although further studies will be required to confirm our findings in a more physiological setting, our study provides strong evidence that miRNA-335-5p alleviates inflammation in OA chondrocytes through activation of autophagy.

6. Conclusion

In summary, this study demonstrated that miRNA-335-5p normally displays reduced expression in human OA chondrocytes. However, by re-expressing miRNA-335-5p, we demonstrated its ability to promote proliferation, inhibit apoptosis, and reduce the expression of inflammatory factors through activation of autophagy in human OA chondrocytes. Thus, for the first time we have revealed the important role of miRNA-335-5p in the pathological process of OA, the clinical impact of which will be revealed by further studies. Our results provide the basis for investigation into the potential of miRNA-335-5p as a new molecular marker for OA diagnosis and a new target for gene therapy.

Acknowledgements

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.03.071.

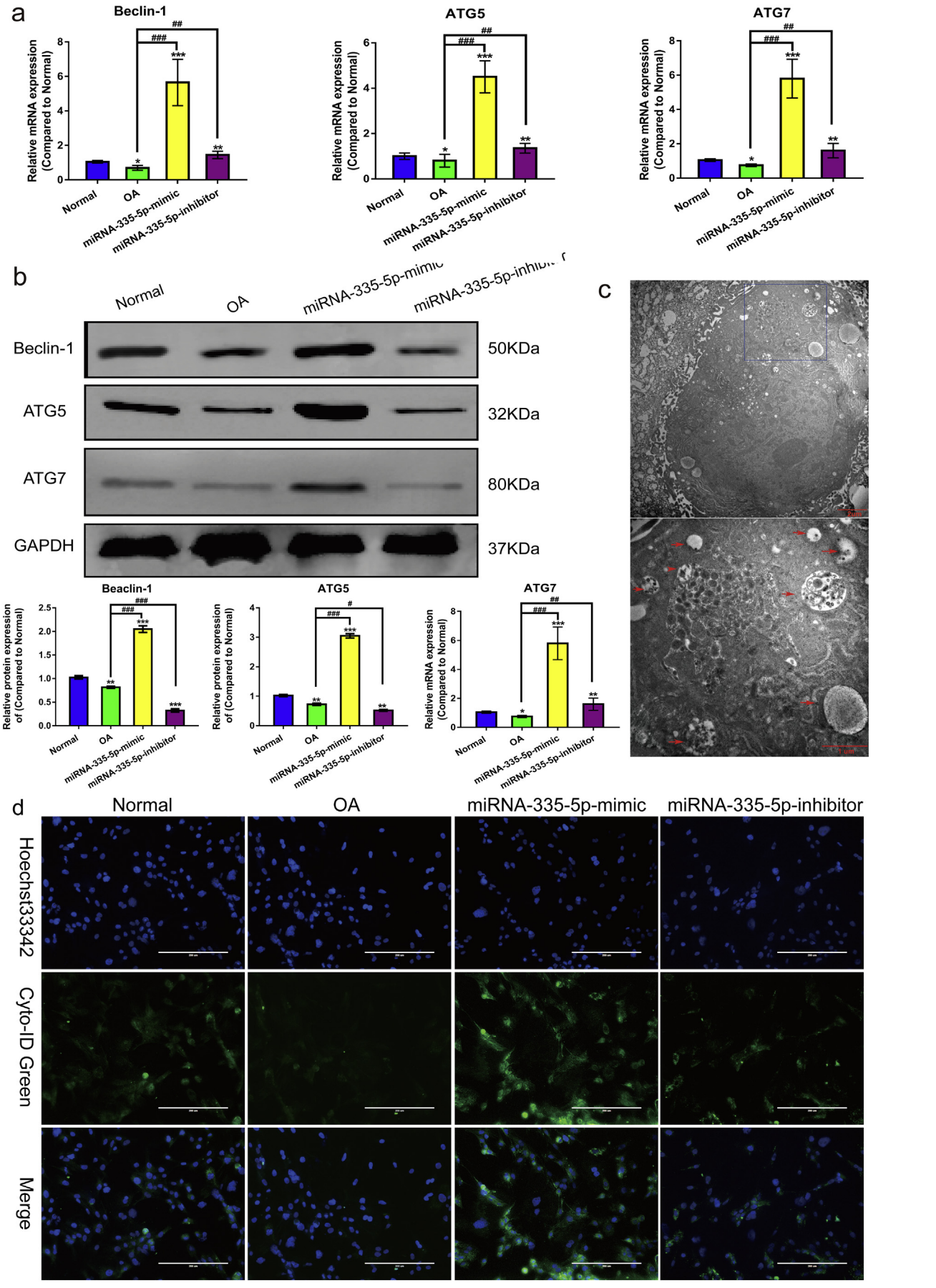

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Fig. 3. miRNA-335-5p activates autophagy in human OA chondrocytes.
Human OA chondrocytes were transfected with 100 nM miRNA-335-5p mimic or inhibitor for 24 h. (a) The mRNA expression levels of ATG7, ATG5, and Beclin-1 were assessed by qRT-PCR. (b) The protein expression levels of ATG7, ATG5, and Beclin-1 were determined by WB and subsequently quantified by densiometry analysis. (c) TEM was used to analyze the uptake of DM nanoparticles and intracellular autophagy. Representative images are shown. (d) Immunofluorescence staining was used to reveal the presence of TNF-α. Representative images are shown. Data are expressed as the mean ± SD, n = 6, *P < 0.05, **P < 0.01, ***P < 0.001 relative to respective control groups; #P < 0.05, ##P < 0.01, ###P < 0.001.

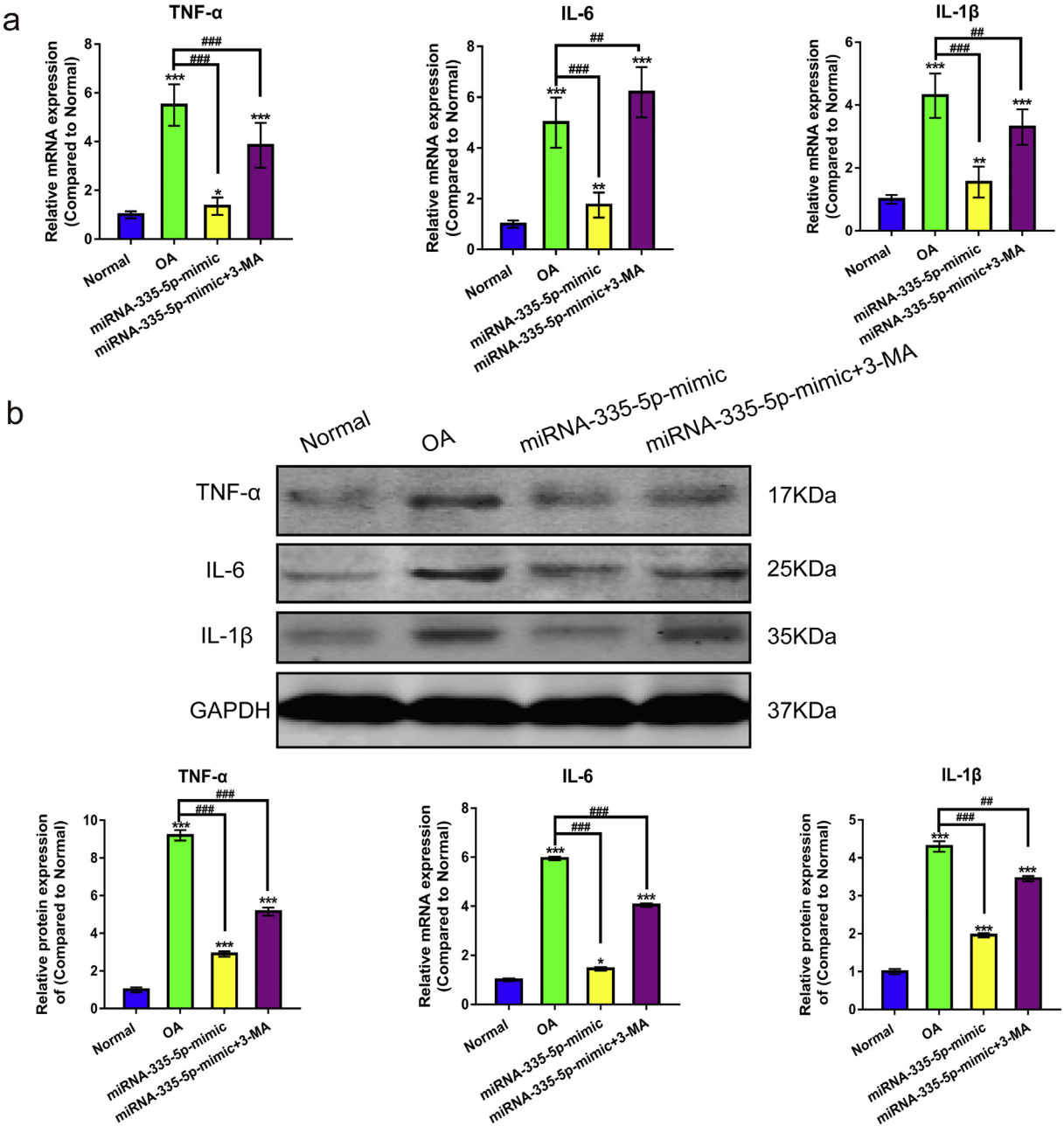

Fig. 4. MiRNA-335-5p alleviates inflammation by activating autophagy in human OA chondrocytes.
Human OA chondrocytes were transfected with 100 nM miRNA-335-5p mimic with or without 3-MA treatment for 24 h. (a) The mRNA expression levels of TNF-α, IL-6, and IL-1β were assessed by qRT-PCR. (b) The protein expression levels of TNF-α, IL-6, and IL-1β were determined by WB and subsequently quantified by densiometry analysis. Data are expressed as the mean ± SD, n = 6, *P < 0.05, **P < 0.01, ***P < 0.001 relative to respective control groups; #P < 0.05, ##P < 0.01, ###P < 0.001.

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Further reading

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