Exploratory Research and Hypothesis in Medicine **2022** vol. 7(1) | 1–7
DOI: 10.14218/ERHM.2021.00029

Original Article

miR-34a/SIRT1 Axis Plays a Critical Role in Regulating Chondrocyte Senescence in Type 2 Diabetes Mellitus

De-Yan Li[1#], Feng-He Gao[2#], Chun-Fei Wu[2], Zu-Jian Liang[2*] and Wen-Hua Xiong[1*]

[1]Department of Orthopedics, Shi Yan People's Hospital, Shenzhen, Guangdong, China; [2]Department of Orthopedics, the Third Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China

Received: June 13, 2021 | **Revised:** September 08, 2021 | **Accepted:** September 14, 2021 | **Published:** October 11, 2021

Abstract

Background and objectives: Cartilage destruction is central in the pathogenesis of osteoarthritis (OA), an age-related chronic degenerative disease. Cartilage degeneration has been postulated to occur due to chondrocyte senescence. On the other hand, type 2 diabetes mellitus (T2DM) is an independent risk factor for OA initiation and progression, bringing about the concept of "diabetic OA (DM-OA)." Aberrant metabolic pathways in T2DM promote a chronic inflammatory environment that favors cell apoptosis and senescence. However, it is still unclear if the cartilage of diabetic OA patients contains a higher amount of senescent chondrocytes compared to nondiabetic patients.

Methods: We established mouse models of OA and DM-OA and determined the integrity of cartilage with and without OA induction with Safranin-O/Fast Green staining. MicroRNA-24a (miR-34a) and sirtuin 1 (SIRT1) cartilage expression was quantified using reverse transcription polymerase chain reaction. Chondrocytes were transfected with either miR-34a mimic or anti-miR-34a, and subsequent expression of SIRT1 and aging-related proteins was determined by western blotting and reverse transcription polymerase chain reaction and followed by quantification of senescent cells using a senescence-associated beta-galactosidase staining kit.

Results: This study showed that miR-34a expression was elevated in the cartilage of diabetic OA models. miR-34a may be critical for chondrocyte senescence, likely through its action on SIRT1 expression. This hypothesis was supported by reduced SIRT1 expression in the cartilage of diabetic OA models.

Conclusions: Our findings suggest that miR-34a/SIRT1 plays a critical role in the development and progression of diabetic OA. Targeting miR-34a/SIRT1 may function as a novel pathway for OA prevention through the elimination of senescent chondrocytes.

Keywords: Chondrocyte; miR-34a; Senescence; SIRT1; Type 2 diabetes mellitus.

Abbreviations: DM-OA, diabetic osteoarthritis; IL-1β, interleukin-1β; miRNA, microRNA; OA, osteoarthritis; PBS, phosphate-buffered saline; SIRT, sirtuin 1; STZ, streptozotocin; T2DM, type 2 diabetes mellitus.

[*]**Correspondence to:** Wen-Hua Xiong, Department of Orthopedics, Shi Yan People's Hospital, Shenzhen 518108, Guangdong, China. ORCID: https://orcid.org/0000-0003-3675-7534. Tel: +86-18948163968, Fax: +86-755-2760-1213, E-mail: xwhTGZY@163.com; Zu-Jian Liang, Department of Orthopedics, the Third Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China. ORCID: https://orcid.org/0000-0002-2833-573X. Tel: +86-137-5187-6166, Fax: +86-208-423-8775, E-mail: liangzujian@gzucm.edu.cn

[#]These authors contributed equally to this work.

How to cite this article: Li DY, Gao FH, Wu CF, Liang ZJ, Xiong WH. miR-34a/SIRT1 Axis Plays a Critical Role in Regulating Chondrocyte Senescence in Type 2 Diabetes Mellitus. *Explor Res Hypothesis Med* 2022;7(1):1–7. doi: 10.14218/ERHM.2021.00029.

Introduction

Osteoarthritis (OA) is an age-related chronic degenerative disease that features cartilage destruction. Chondrocytes are the only cells that secrete and convert extracellular matrix components. Chondrocyte apoptosis and senescence, therefore, confer significant effects on cartilage integrity.[1] Cellular senescence refers to the process of irreversible cell cycle arrest caused by various stresses, and is a major factor leading to age-related disease including OA.[2,3] A recent study demonstrated that injecting senescent chondrocytes into the knee joint triggers cartilage destruction, as seen in OA.[4] Other studies have shown that the elimination of senescent cells reduces

cartilage damage and pain in post-traumatic OA mouse models.[5] The implication of chondrocyte senescence in OA has been gaining significant attention in efforts to uncover its mechanism of action.

Type 2 diabetes mellitus (T2DM) and OA are the most common chronic diseases worldwide.[6,7] The concept of 'diabetic OA' has emerged over recent years and highlights T2DM as an independent risk factor for OA initiation and progression.[8] OA patients who are diabetic experience worse degrees of cartilage destruction.[9] Mechanistically, it has been demonstrated that T2DM accelerates OA development due to enhanced oxidative stress and inflammation.[10,11] Prolonged inflammation as a result of T2DM alters the chondrocyte homeostasis, resulting in increased favoring apoptosis and senescence.[3,12] However, whether there are higher numbers of senescent chondrocytes in the cartilage of diabetic OA patients has yet to be clarified.

It is well known that microRNAs (miRNAs) are crucial in OA initiation and progression. A previous study found that miR-140 is an important molecule that targets matrix metalloprotinase 13.[13] miR-34a was initially characterized as a tumor suppressor that can control malignant cell senescence, apoptosis, and proliferation. Specifically, miR-34a induces endothelial and endothelial progenitor cell senescence by acting on sirtuin 1 (SIRT1), a gene associated with longevity. Several types of aged organ tissues have increased miR-34a expression, and aged cardiac tissue with suppressed miR-34a expression results in ventricular remodeling and improved cardiac performance.[14] However, its role in regulating chondrocyte cellular senescence is largely unknown.

This study evaluated miR-34a expression in the cartilage of diabetic OA, and investigated the regulatory role of miR-34a/SIRT1 in mediating chondrocyte senescence and OA development. The results provide evidence that targeting miR-34a/SIRT1 signaling effectively attenuates OA progression by suppressing cellular senescence.

Methods

Establishment of OA and DM-OA models

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. This study was conducted after approval by the Animal Research Ethics Committee of Jinan University (Guangzhou, China). Twelve-week-old mice were reared in specific pathogen-free conditions under suitable temperatures with 70% humidity. In establishing mouse models of OA, the animals were subjected to anterior cruciate ligament and medial collateral ligament transection surgery over their right knee joints as previously described.[15] Control animals (sham group) were only subjected to resection of the skin over their right knee joints. DM was induced by feeding mice a combination of streptozotocin (STZ), high-sugar, and high-fat diets over a short period of time.[15] Consequently, 8-week feeding of a high-fat and high-sugar diet plus a single intraperitoneal injection of STZ at a dose of 40 mg/kg was used to mimic T2DM. The control group was injected with an equal dose of citrate buffer vehicle. Six weeks after the procedure, samples from the right knee joint were harvested.

Assessment of blood glucose level

After 1 week of acclimatization, the DM-OA group was induced with DM via a single intraperitoneal injection of 40 mg/kg STZ (S0130; Sigma, St. Louis, MO, USA), while the OA and sham groups were given phosphate-buffered saline (PBS) vehicle at the same time. On day 3 after STZ injection, to confirm the induction of DM, mice were fasted for 6 h and tail vein blood was sampled using Optium Xceed (Abbott Diabetes Care Ltd., Witney, UK). Following administration of a glucose bolus (500 mg/kg intraperitoneal), glucose levels were measured immediately postinjection and at 15, 30, 60, 90, and 120 min. On day 7 after STZ injection, mice were fasted for 6 h and tail vein blood was sampled. Following administration of insulin (1 IU/kg IP, S6955; Selleck Chemicals, Houston, TX, USA), glucose levels were measured immediately postinjection and at 15, 30, 60, 90, and 120 min. To quantify metabolic status, the net area under the curve was calculated from the glucose tolerance test and insulin tolerance test curve of each mouse (GraphPad Prism 7; GraphPad Software, San Diego, CA, USA). Hyperglycemic induction was confirmed when blood glucose levels were ≥16.7 mmol/L with abnormal levels in the glucose tolerance test and insulin tolerance test.[16,17]

Human cartilage specimens

Human cartilage specimens from OA patients were included in this study. Knee OA was diagnosed based on clinical and radiological evaluation, and its severity was classified according to the Kellgren and Lawrence x-ray criteria. Diabetic patients were enrolled according to the diagnostic criteria of guideline. All donors provided written informed consent, with study approval granted by Jinan University.[18]

Histological and immunochemical examination

Mice knee joint samples were fixed in 10% formalin and decalcified for 14 days with 10% EDTA (G1105; Wuhan Servicebio Technology, Wuhan, China) prior to embedding samples in paraffin. 5 μm sections were treated with a combination of hematoxylin and eosin (H&E, Heagene, China) as well as Safranin-O/Fast Green dyes. The Osteoarthritis Research Society International scoring system was used to evaluate cartilage integrity.[19] Immunohistochemical staining was performed based on established guidelines.[20] A Senescence β-Galactosidase Staining Kit (Beyotime, Shanghai, China) was used to determine the number of senescent cartilage cells in compliance with the manufacturer's protocols. Anti-SIRT1 primary antibody was used (1:50, D1D7; Cell Signaling Technology, Danvers, MA, USA). A light microscope (390335; Leica, Wetzlar, Germany) was used to image all samples in order to calculate the percentage of stained cells.

Cell culture

Primary chondrocytes were isolated from human cartilage tissues harvested from nondiabetic OA patients who underwent a total knee replacement. Briefly, the articular cartilage tissue was finely minced, rinsed with PBS (HyClone, Logan, UT, USA), and digested with 2 mg/mL collagenase II (Sigma) for 8–10 h after treatment with 0.25% trypsin-EDTA (Gibco, Gaithersburg, MD, USA) for 30 min at 37°C. The isolated cells were cultured until cells reached 80–90% confluence. All cells were maintained in a humidified incubator containing 5% CO_2 at 37 °C. Cells between the third and seventh passages were used for subsequent experiments. Human interleukin-1β (IL-1β, (Cell Signaling Technology, 8900SC, Beverly, MA, USA) was used at a working concentration of 20 ng/mL.

Western blot analysis

Western and immunoprecipitation buffer (P0013; Beyotime) containing protease inhibitor cocktail (Roche, Penzberg, Germany) was used to lyse cells. Between 20 and 50 μg of protein was subjected to 8–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by analyses of immunoreactive bands using enhanced chemiluminescence reagents with the Tanon 5200 Luminescent Imaging Workstation (Tanon Science & Technology, Shanghai, China). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to analyze all images.[21] SIRT1 antibodies at a concentration of 1:1,000 were obtained from Cell Signaling Technology.

Senescence β-galactosidase staining

A Senescence β-Galactosidase Staining Kit (Beyotime) was used to detect chondrocyte senescence in compliance with the manufacturer's protocols. In brief, 6-well plates were used to culture primary chondrocytes for 72 h. Cells were then fixed at room temperature for 20 min with 1 mL fixative solution. Cells were rinsed twice with PBS, and then incubated overnight at 37 °C with a staining solution mixture containing X-gal. Ten random fields that had at least more than 500 cells were selected to determine the proportion of senescent cells.

Reverse transcription and quantitative polymerase chain reaction

Total chondrocyte RNA was extracted using the TRIzol Plus RNA Purification Kit (Life Technologies, Carlsbad, CA, USA) before it was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Primers used for *SIRT1* amplification were as follows: 5′-CAACGGTTTC-CATTCGTGTG-3′ (sense) and 5′-GTTCGAGGATCTGTGC-CAAT-3′ (antisense). *GAPDH* was used as an endogenous control. miRNA isolation was performed using the miRcute miRNA Isolation Kit (DP501, Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Then 100 μg miRNA was reverse transcribed using the miRcute Plus miRNA First-Strand cDNA Kit (KR211; Tiangen Biotech). The miRcute Plus miRNA qPCR Kit (FP411; Tiangen Biotech) was used to quantify miRNA expression level. The relative fold change ($2^{-\Delta\Delta CT}$) was used to calculate relative gene expression. The DNA engine CFX96 Real-Time PCR Amplification System (CFX96 Touch; Bio-Rad Laboratories, Feldkirchen, Germany) was used to perform all experiments in triplicate.

miR-34a and anti-miR-34a transfection

miRNAs were purchased from RiboBio (Guangzhou, China). miR-34a, mimic negative control (micrON™ Mimic Negative Control #22, Intergrated Biotech Solutions, miR01101, Shanghai, China), miR-34a mimic (micrON™ hsa-miR-34a-5p mimic, Intergrated Biotech Solutions, miR10000255, Shanghai, China), inhibitor negative control (micrON™ Inhibitor Negative Control #22, miR02101), and anti-miR-34a (micrOFF™ hsa-miR-34a-5p Inhibitor, miR20000815) were used to increase and suppress miR34a expression. Briefly, a 6-well plate (Costar3516; Corning, Corning, NY, USA) was used to culture primary chondrocytes for 24 h at a density of 2×10^5 cells/per well. The aforementioned miRNAs were transfected using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 72 h. Then cells were rinsed and lysed for protein analyses.

Statistical analyses

All data are expressed as the mean ± standard deviation. GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA) was used for all statistical analyses. Intergroup variances were calculated with the Student's *t*-test. Comparisons among three groups were evaluated using one-way analysis of variance with the Dunnett's test. $P < 0.05$ was considered statistically significant.

Results

Diabetic OA cartilage exhibits raised miR-34a expression

It is increasingly recognized that miR-34a exerts significant functions in OA development and progression by regulating chondrocyte apoptosis.[22,23] Yet its role in diabetic OA, a condition associated with more severe cartilage destruction, is largely unknown. Therefore, we evaluated miR-34a expression in diabetic OA after establishing an OA model using the classical surgical destabilization of the medial meniscus (DMM) method. DM-OA mice were generated by feeding OA mice with diets rich in sugar and fat in addition to a single intraperitoneal injection of STZ.[24] As shown in Figure 1a and 1b, more severe cartilage destruction was observed in the DM-OA group compared to the sham and OA group, as assessed by Safranin O staining. Interestingly, miR-34a expression increased with increasing OA severity (Fig. 1c). Next, we assessed the expression of miR-34a in human cartilage specimens, and our results also demonstrated elevated miR-34a in DM-OA in contrast to OA (Fig. 1d, e). We conclude that OA development appears to be mediated by chondrocyte expression of miR-34a.

miR-34a regulates the senescence of chondrocytes

The cartilage of OA patients undergoing joint replacement has a high quantity of senescent cells. Therefore, researchers have proposed targeting senescent cells as a promising treatment for OA.[1,2,12,25] Our subsequent experiments determined how miR-34a is involved in regulating chondrocyte senescence. As shown in Figure 2a, the lateral and medial cartilages of DM-OA patients exhibited a higher percentage of senescent cells. Primary chondrocytes underwent higher rates of cellular senescence in the *in vitro* culture. We demonstrated that a miR-34a mimic enhanced the expression of senescent markers, including p16 and p21, on both days 3 and 7 (Fig. 2b), while anti-miR-34a markedly inhibited their expressions (Fig. 2c). Consistently, anti-miR-34a significantly suppressed the proportion of senescence-associated β-galactosidase-stained senescent cells (Fig. 2d, e). Taken together, these findings support the critical role of miR-34a in modulating chondrocyte senescence.

SIRT1 expression is reduced in diabetic OA

Given the critical role of miR-34a/SIRT1 signaling in regulating

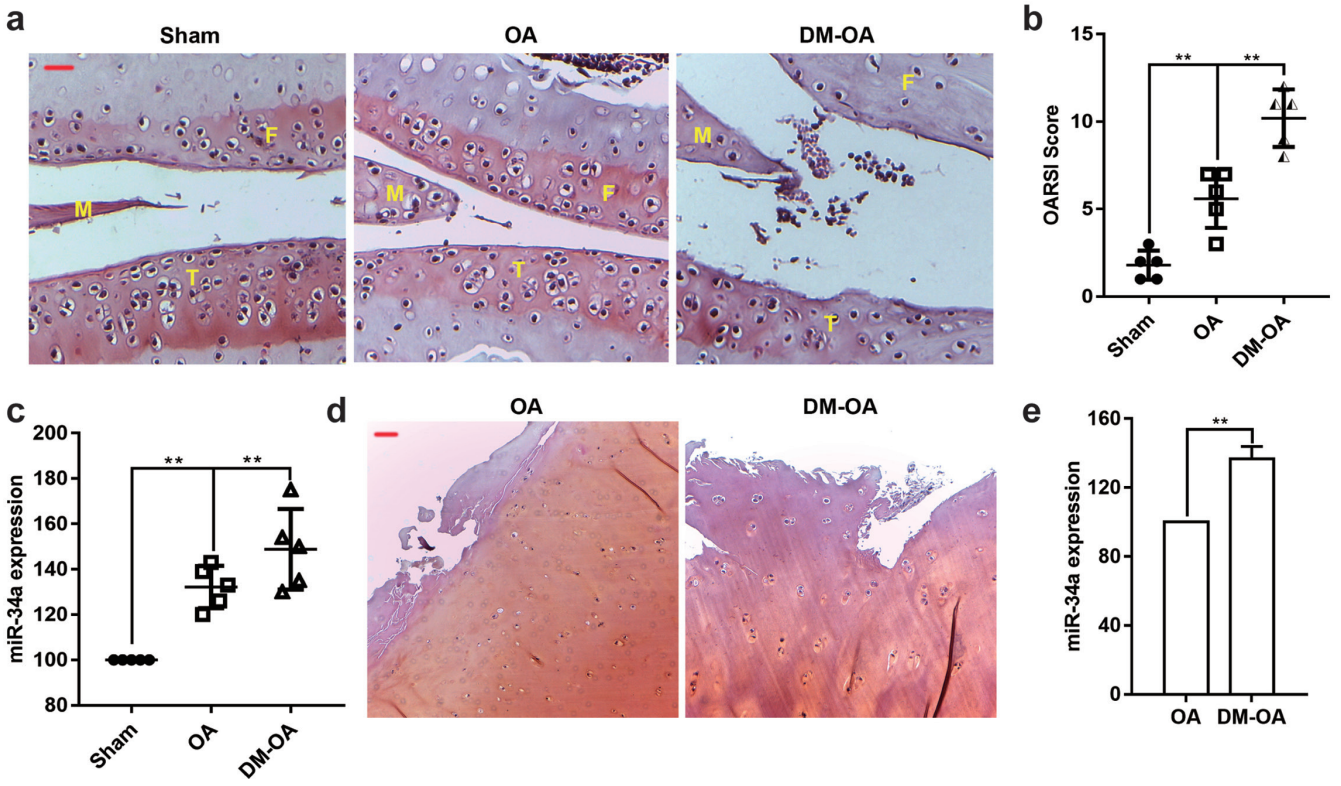

Fig. 1. MicroRNA-34a expression is elevated in diabetic osteoarthritis cartilage. (a) Integrity of the cartilage before and after osteoarthritis (OA) and diabetic OA (DM-OA) induction in mice was evaluated by Safranin-O/Fast Green staining. F: Femur, M: meniscus, T: tibia. Scale bar = 50 μM. (b) Osteoarthritis Research Society International score in A was determined. **, $P < 0.01$. (c) MicroRNA-34a (miR-34a) expression in the cartilage of the indicated group were quantified by quantitative polymerase chain reaction (qPCR) (n = 5) and normalized against U6 levels. Data are depicted expressed as the mean ± standard deviation (**, $P < 0.01$). (d) The integrity of cartilage in OA and DM-OA patients was evaluated by Safranin-O/Fast Green staining. Scale bar = 50 μM. (e) miR-34a expression in D was quantified by qPCR (n = 6). **, $P < 0.01$.

chondrocyte senescence, as well as the fact that diabetic OA patients exhibit enhanced numbers of senescent cells, we hypothesized that SIRT1 is involved in the development of diabetic OA. By assessing the expression of SIRT1 in a series of OA model using quantitative polymerase chain reaction, we found that the DM-OA group had lower SIRT1 expression than the Sham and OA groups (Fig. 3a). Immunohistochemistry also demonstrated similar decreases of SIRT1 expression in cartilage specimens of DM-OA mice (Fig. 3b). We conclude that miR-34a/SIRT1 signaling plays a critical role in the development and progression of diabetic OA.

SIRT1 is involved in miR-34a-triggered chondrocyte senescence

SIRT1 plays a large role in enhancing the longevity and cellular senescence of several organisms;[3,11,26] however, little is known regarding its role in DM-OA. We demonstrated that transfection of miR-34a mimics into primary chondrocytes significantly reduced SIRT1 mRNA and protein expression, while anti-miR-34a restored SIRT1 expression (Fig. 4a, b). This was followed by an investigation regarding the function of SIRT1 in miR-34a induced cellular senescence. As shown in Figure 4c, anti-miR-34a markedly inhibited IL-1β-induced chondrocyte senescence. However, knockdown of SIRT1 by its corresponding siRNA restored the percentage of senescent cells induced by IL-1β (Fig. 4d). Collectively, these results highlight the essential role of SIRT1 in miR-34a-triggered chondrocyte senescence.

Discussions

Our studies showed that miR-34a was highly expressed in diabetic OA cartilage, while SIRT1 expression was decreased in diabetic OA model cartilage. miR-34a may be a key molecule in adjusting and controling chondrocyte senescence by regulating the expression of SIRT1. Meanwhile, further studies have shown that miR-34a /SIRT1 plays a regulatory role in mediating chondrocyte senescence and OA development.

Senescent chondrocytes can participate in OA occurrence and development by inducing oxidative stress and the senescence-related secretion phenotype.[25,27] These changes alter the chondrocyte microenvironment, resulting in altered synthesis and degradation of cartilage matrix components. Senescent cells also lose their ability to trigger repair signals, leading to aggravated articular cartilage degeneration and increased OA incidence.[3,28,29] In recent years, T2DM was found to accelerate OA development due to enhanced oxidative stress and inflammation.[30] However, whether increased numbers of senescent cells are present in the cartilage of diabetic OA patients and models has remained unknown. We sought to bridge this knowledge gap and found that the degree of cartilage destruction observed in the DM-OA group was more severe and the numbers of senescent cells were higher in contrast to the sham and OA groups. Our study is the first to show higher numbers of senescent cells in the cartilage of DM-OA patients and models.

Previous studies have identified a considerable number of dif-

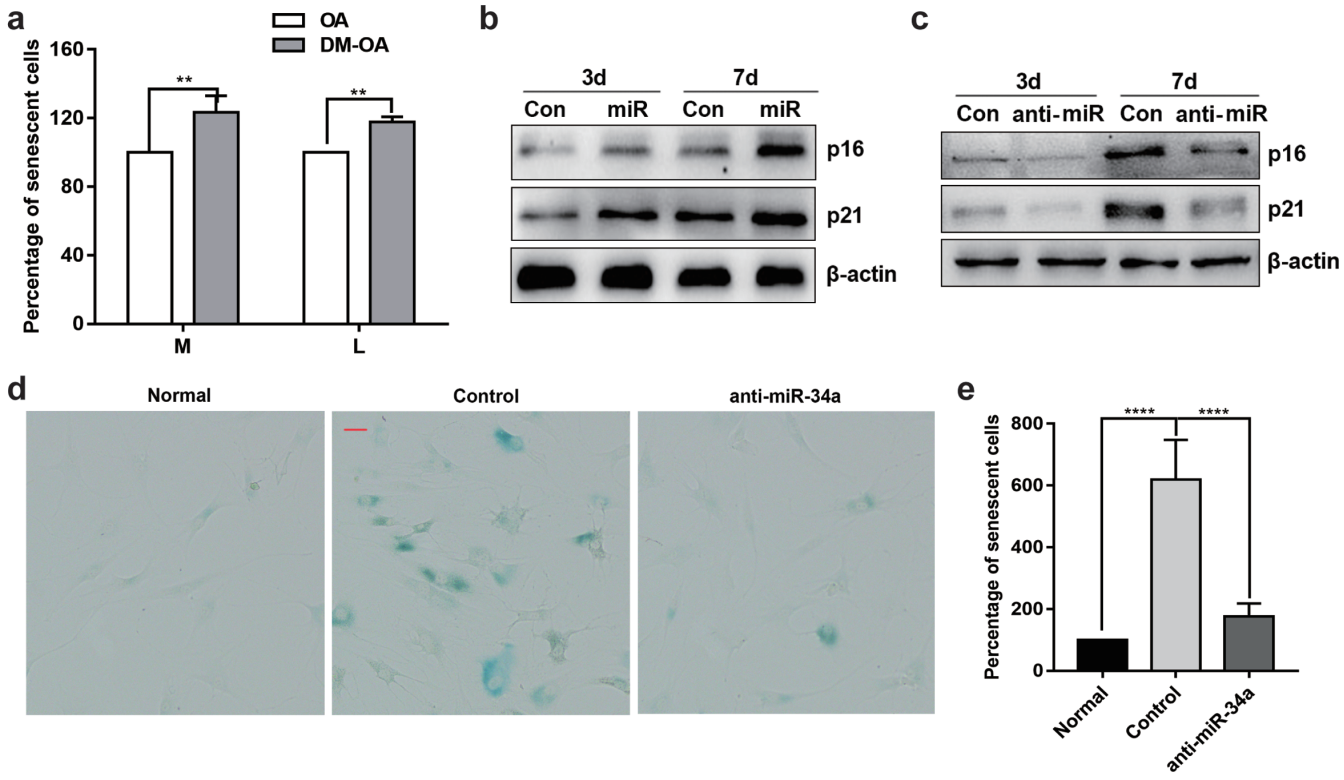

Fig. 2. MicroRNA-34a is a critical regulator of chondrocyte senescence. (a) The percentage of senescent cells in the medial (M) and lateral (L) sides of cartilage in osteoarthritis (OA) and diabetic OA (DM-OA) was analyzed. **, $P < 0.01$. (b and c) Chondrocytes were transfected with either microRNA-34a (miR-34a) mimic (miR) or anti-miR-34a (anti-miR) for 3 or 7 days prior to western blot analysis of the expression of p16, p21, and β-actin. (d) Chondrocyte senescence was induced by interleukin 1 beta, followed by a 3-day transfection period with either control or anti-miR-34a. The proportion of senescent cells were assessed with a senescence-associated beta-galactosidase staining kit. Scale bar = 20 μm. (e) Senescent cell proportions in D were analyzed. ****, $P < 0.0001$.

ferentially expressed miRNAs involved in the development and progression of OA. miR-140 was previously found to negatively regulate matrix metalloproteinase 13 expression, thus controlling chondrocyte phenotype. Here, we found that miR-34a plays a role in OA progression by regulating chondrocyte senescence (Figs. 1 and 2). This is in line with previous reports showing that miR-34a is required for regulating chondrocyte apoptosis. Moreover, we identified SIRT1 as a likely downstream target of miR-34a in chondrocyte senescence induction. Several studies have found that SIRT1 exerts its effects on chondrocyte-regulated extracellular matrix protein expression. For instance, SIRT1 silencing in chondrocytes results in the enhanced expression of OA genes,[31] and the chondrocyte SIRT1

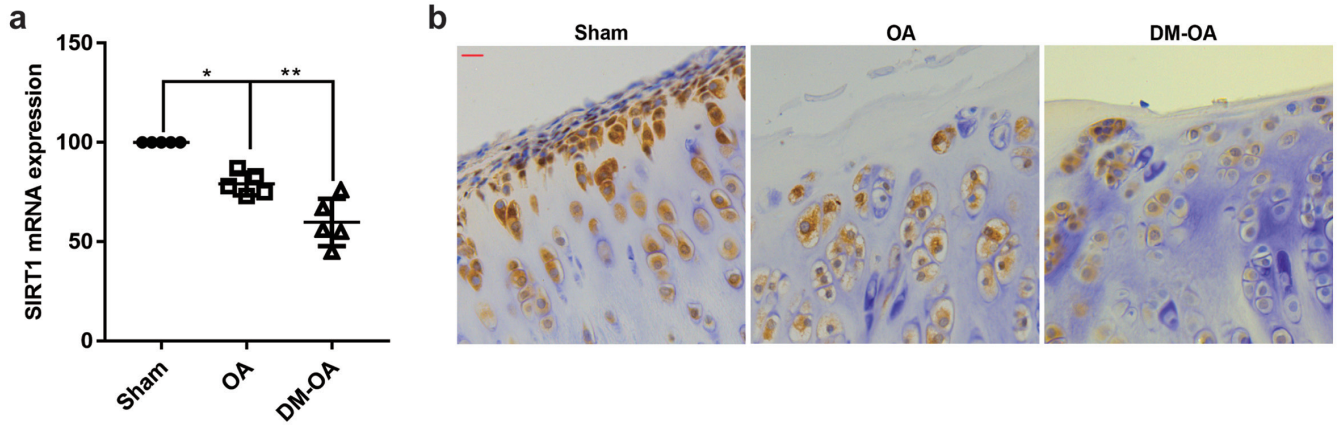

Fig. 3. Sirtuin 1 expression is reduced diabetic osteoarthritis cartilage. (a) The mRNA expression of sirtuin 1 (SIRT1) in osteoarthritis (OA) and diabetic OA (DM-OA) was determined by quantitative polymerase chain reaction. *, $P < 0.05$; **, $P < 0.01$. (b) The expression of SIRT1 in cartilage before and after OA and DM-OA induction in mice was examined by immunohistochemistry. The relevant images depicting cartilage SIRT1 expressio are shown. Scale bar = 50 μm.

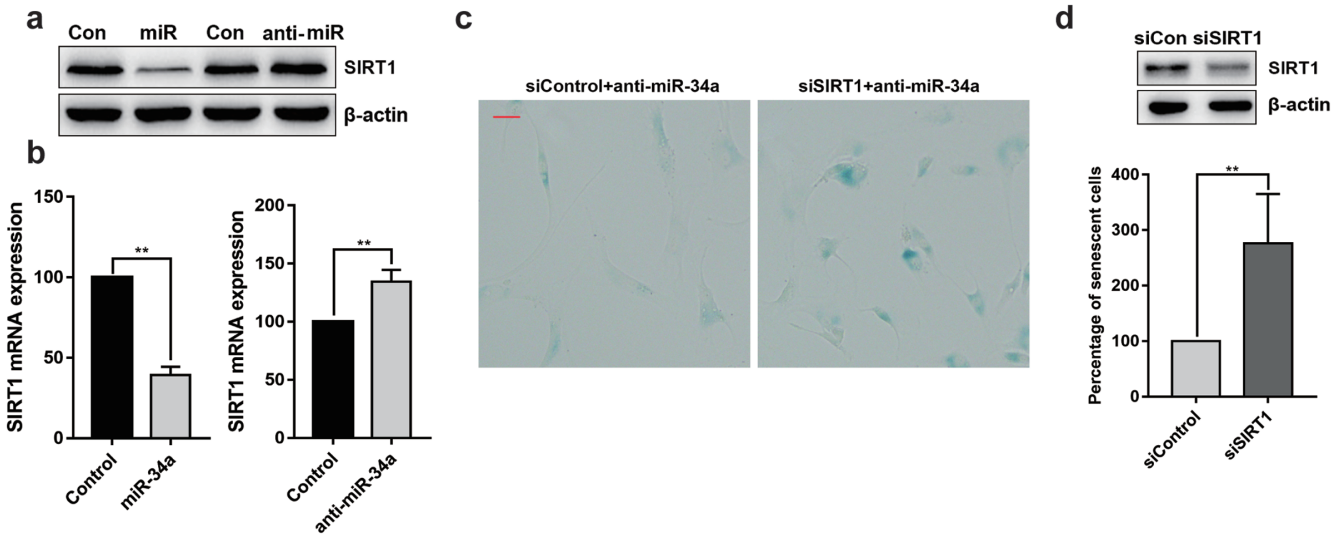

Fig. 4. MicroRNA-34a targets sirtuin 1 expression and is required for chondrocyte senescence. (a) Chondrocytes were transfected with either microRNA-34a (miR-34a) mimic (miR) or anti-miR-34a (anti-miR) for 3 days, followed by western blot analysis of SIRT1 and β-actin expression. (b) Chondrocytes were transfected with miR-34a mimic (miR) or anti-miR-34a (anti-miR) for 3 days. Then, mRNA expression of SIRT1 was determined by quantitative polymerase chain reaction. **, $P < 0.01$. (c) Chondrocytes were transfected with either siControl or siSIRT1 along with the anti-miR-34a for 3 days. The proportion of senescent cells were determined with a senescence-associated beta-galactosidase staining kit. Scale bar = 20 μm. (d) Senescent cell proportions in C were analyzed. **, $P < 0.01$.

knockout mouse model exhibits enhanced development of OA.[32,33]

Cellular senescence is marked by the formation of a senescence-related secretion phenotype and accumulation of senescence-associated β-galactosidase. Cellular senescence occurs primarily through activation of the retinoblastoma 1/p16 and p53/p21 pathways, although this phenomenon may also take place via p53-independent pathways.[34] This series of investigations demonstrate that miR-34a/SIRT1 signaling coordinates the p16 and p21 pathways in inducing chondrocyte senescence.

In conclusion, we found that miR-34a expression is elevated in the cartilage of diabetic OA, and that miR-34a is essential in modulating chondrocyte senescence. Mechanistically, we show that SIRT1 acts as a miR-34a target and is essential for miR-34a-mediated chondrocyte senescence and likely OA progression. Our findings raise the possibility of targeting the miR-34a/SIRT1 signaling pathway for OA prevention by reducing the numbers of senescent chondrocytes.

Future directions

Considering the irreversible nature of osteoarthritis, it is important to explore the underlying mechanism of its occurrence and development. In this article, a series of studies evaluated the expression of miR-34a in diabetic OA cartilage and subsequently investigated the regulatory role of miR-34a /SIRT1 in mediating chondrocyte senescence and OA development. The results indicate that targeting miR-34a /SIRT1 signaling can effectively reduce OA progression by inhibiting cell senescence. The present study demonstrates a critical role of miR-34a/SIRT1 in DM-OA, providing miR-34a inhibition and SIRT1 activation as novel strategies in clinical management of DM-OA. In the following studies, we will focus on the influence on other markers of senescence in DM-OA. As well as sequencing of clinical samples, to obtain the differentially expression of microRNA profiles in DM-OA.

Acknowledgments

The authors would like to acknowledge the technical support and critical comments of all members of the Institute of Orthopedic Diseases laboratory (Department of Bone and Joint Surgery, The First Affiliated Hospital, Jinan University).

Funding

The authors appreciate the funding support from the Science and Technology Plan of Baoan District, Shenzhen (20180404115502902); and the Natural Science Foundation of Guangdong (2021A1515011469).

Conflict of interest

The authors have no potential conflicts of interest to declare.

Author contributions

Concept and design (DTL, FHG), performed the experiments (DYL, FHG), data analyses and interpretation (FHG, CFW), writing and review of the manuscript (DYL, ZJL, WHX). All authors approved the final version to be published.

Ethical statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. This study was conducted af-

ter approval by the Animal Research Ethics Committee of Jinan University. Human cartilage specimens from OA patients were included in this study. All donors provided written informed consent, with study approval granted by Jinan University.

Data sharing statement

No additional data are available.

References

[1] Martin JA, Brown TD, Heiner AD, Buckwalter JA. Chondrocyte senescence, joint loading and osteoarthritis. Clin Orthop Relat Res 2004;427(Suppl):S96–103. doi:10.1097/01.blo.0000143818.74887.b1.

[2] McCulloch K, Litherland GJ, Rai TS. Cellular senescence in osteoarthritis pathology. Aging Cell 2017;16(2):210–218. doi:10.1111/acel.12562.

[3] Hou A, Chen P, Tang H, Meng H, Cheng X, Wang Y, *et al*. Cellular senescence in osteoarthritis and anti-aging strategies. Mech Ageing Dev 2018;175:83–87. doi:10.1016/j.mad.2018.08.002.

[4] Xu M, Bradley EW, Weivoda MM, Hwang SM, Pirtskhalava T, Decklever T, *et al*. Transplanted Senescent Cells Induce an Osteoarthritis-Like Condition in Mice. J Gerontol A Biol Sci Med Sci 2017;72(6):780–785. doi:10.1093/gerona/glw154.

[5] Jeon OH, Kim C, Laberge RM, Demaria M, Rathod S, Vasserot AP, *et al*. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med 2017;23(6):775–781. doi:10.1038/nm.4324.

[6] Handelsman Y, Bloomgarden ZT, Grunberger G, Umpierrez G, Zimmerman RS, Bailey TS, *et al*. American association of clinical endocrinologists and american college of endocrinology - clinical practice guidelines for developing a diabetes mellitus comprehensive care plan - 2015 - executive summary. Endocr Pract 2015;21(4):413–437. doi:10.4158/EP15672.GL.

[7] Park J, Hwang I, Kim SJ, Youn SW, Hur J, Kim HS. Atorvastatin prevents endothelial dysfunction in high glucose condition through Skp2-mediated degradation of FOXO1 and ICAM-1. Biochem Biophys Res Commun 2018;495(2):2050–2057. doi:10.1016/j.bbrc.2017.08.023.

[8] Schett G, Kleyer A, Perricone C, Sahinbegovic E, Iagnocco A, Zwerina J, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. Diabetes care 2013;36(2):403–409. doi:10.2337/dc12-0924.

[9] Onur T, Wu R, Metz L, Dang A. Characterisation of osteoarthritis in a small animal model of type 2 diabetes mellitus. Bone Joint Res 2014;3(6):203–211. doi:10.1302/2046-3758.36.2000244.

[10] Laiguillon MC, Courties A, Houard X, Auclair M, Sautet A, Capeau J, *et al*. Characterization of diabetic osteoarthritic cartilage and role of high glucose environment on chondrocyte activation: toward pathophysiological delineation of diabetes mellitus-related osteoarthritis. Osteoarthritis Cartilage 2015;23(9):1513–1522. doi:10.1016/j.joca.2015.04.026.

[11] Qu ZA, Ma XJ, Huang SB, Hao XR, Li DM, Feng KY, *et al*. SIRT2 inhibits oxidative stress and inflammatory response in diabetic osteoarthritis. Eur Rev Med Pharmacol Sci 2020;24(6):2855–2864. doi:10.26355/eurrev_202003_20649.

[12] Toh WS, Brittberg M, Farr J, Foldager CB, Gomoll AH, Hui JH, *et al*. Cellular senescence in aging and osteoarthritis. Acta Orthop 2016;87(sup363):6–14. doi:10.1080/17453674.2016.1235087.

[13] Liang ZJ, Zhuang H, Wang GX, Li Z, Zhang HT, Yu TQ, *et al*. MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1beta-stimulated human articular chondrocyte C28/I2 cells. Inflamm Res 2012;61(5):503–509. doi:10.1007/s00011-012-0438-6.

[14] Piegari E, Cozzolino A, Ciuffreda LP, Cappetta D, De Angelis A, Urbanek K, *et al*. Cardioprotective effects of miR-34a silencing in a rat model of doxorubicin toxicity. Sci Rep 2020;10(1):12250. doi:10.1038/s41598-020-69038-3.

[15] Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. J Diabetes Investig 2014;5(4):349–358. doi:10.1111/jdi.12235.

[16] Obrosov A, Shevalye H, Coppey LJ, Yorek MA. Effect of tempol on peripheral neuropathy in diet-induced obese and high-fat fed/low-dose streptozotocin-treated C57Bl6/J mice. Free Radic Res 2017;51(4):360–367. doi:10.1080/10715762.2017.1315767.

[17] Han HS, Choi BH, Kim JS, Kang G, Koo SH. Hepatic Crtc2 controls whole body energy metabolism via a miR-34a-Fgf21 axis. Nat Commun 2017;8(1):1878. doi:10.1038/s41467-017-01878-6.

[18] Doyle-Delgado K, Chamberlain JJ, Shubrook JH, Skolnik N, Trujillo J. Pharmacologic Approaches to Glycemic Treatment of Type 2 Diabetes: Synopsis of the 2020 American Diabetes Association's Standards of Medical Care in Diabetes Clinical Guideline. Ann Intern Med 2020;173(10):813–821. doi:10.7326/M20-2470.

[19] Pritzker K, Gay S, Jimenez S, Ostergaard K, Pelletier JP, Revell PA, *et al*. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14(1):13–29. doi:10.1016/j.joca.2005.07.014.

[20] Chen Y, Lin S, Sun Y, Guo J, Lu Y, Suen CW, *et al*. Attenuation of subchondral bone abnormal changes in osteoarthritis by inhibition of SDF-1 signaling. Osteoarthritis Cartilage 2017;25(6):986–994. doi:10.1016/j.joca.2017.01.008.

[21] Zhang HT, Gui T, Sang Y, Yang J, Li YH, Liang GH, *et al*. The BET Bromodomain Inhibitor JQ1 Suppresses Chondrosarcoma Cell Growth via Regulation of YAP/p21/c-Myc Signaling. J Cell Biochem 2017;118(8):2182–2192. doi:10.1002/jcb.25863.

[22] Chen HJ, Wang JX, Hu B, Wu XD, Chen Y, Li RH, *et al*. MiR-34a promotes Fas-mediated cartilage endplate chondrocyte apoptosis by targeting Bcl-2. Mol Cell Biochem 2015;406(1-2):21–30. doi:10.1007/s11010-015-2420-4.

[23] Zhang W, Hsu P, Zhong B, Guo S, Zhang C, Wang Y, *et al*. MiR-34a Enhances Chondrocyte Apoptosis, Senescence and Facilitates Development of Osteoarthritis by Targeting DLL1 and Regulating PI3K/AKT Pathway. Cell Physiol Biochem 2018;48(3):1304–1316. doi:10.1159/000492090.

[24] Gui T, Lin YK, Huan SW, Li YH, Wang BH, Yang J, *et al*. Elevated expression of ICAM-1 in synovium is associated with early inflammatory response for cartilage degeneration in type 2 diabetes mellitus. J Cell Biochem 2019;120(8):13177–13186. doi:10.1002/jcb.28592.

[25] Benderdour M, Martel-Pelletier J, Pelletier JP, Kapoor M, Zunzunegui MV, Fahmi H. Cellular Aging, Senescence and Autophagy Processes in Osteoarthritis. Curr Aging Sci 2015;8(2):147–57. doi:10.2174/1874609808666150727111530.

[26] Ni S, Xu C, Zhuang C, Zhao GY, Li CK, Wang YJ, *et al*. LncRNA LUADT1 regulates miR-34a/SIRT1 to participate in chondrocyte apoptosis. J Cell Biochem 2021;122(9):1003–1008. doi:10.1002/jcb.29637.

[27] Park J, Kim J, Chen YQ, Song HC, Chen YB, Zheng M, *et al*. CO ameliorates cellular senescence and aging by modulating the miR-34a/Sirt1 pathway. Free Radic Res 2020;54(11-12):848–858. doi:10.1080/10715762.2019.1710142.

[28] Kelly PN. Targeting senescence to combat osteoarthritis. Science 2017;356(6338):595–596. doi:10.1126/science.356.6338.595-b.

[29] Jeon OH, Wilson DR, Clement CC, Rathod S, Cherry C, Powell B, *et al*. Senescence cell-associated extracellular vesicles serve as osteoarthritis disease and therapeutic markers. JCI Insight 2019;4(7):e125019. doi:10.1172/jci.insight.125019.

[30] Brunner AM, Henn CM, Drewniak EI, Lesieur-Brooks A, Machan J, Crisco JJ, *et al*. High dietary fat and the development of osteoarthritis in a rabbit model. Osteoarthritis Cartilage 2012;20(6):584–592. doi:10.1016/j.joca.2012.02.007.

[31] Gabay O, Zaal KJ, Sanchez C, Dvir-Ginzberg M, Gagarina V, Song YJ, *et al*. Sirt1-deficient mice exhibit an altered cartilage phenotype. Joint Bone Spine 2013;80(6):613–620. doi:10.1016/j.jbspin.2013.01.001.

[32] Cheng HL, Mostoslavsky R, Saito S, Manis JP, Gu YS, Patel P, *et al*. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. Proc Natl Acad Sci USA 2003;100(19):10794–10799. doi:10.1073/pnas.1934713100.

[33] Chai B, Zheng ZH, Liao X, Li KY, Liang JS, Huang YX, *et al*. The protective role of omentin-1 in IL-1β-induced chondrocyte senescence. Artif Cells Nanomed Biotechnol 2020;48(1):8–14. doi:10.1080/21691401.2019.1699803.

[34] Wu D, Prives C. Relevance of the p53-MDM2 axis to aging. Cell Death Differ 2018;25(1):169–179. doi:10.1038/cdd.2017.187.