Wnt3a knockdown promotes collagen type II expression in rat chondrocytes

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Abstract. Osteoarthritis (OA) is a chronic condition caused by cartilage degradation, and there are currently no effective methods for preventing the progression of this disease; gene therapy is a relatively novel method for treating arthritis. Decreased collagen type II (Col2) expression within the cartilage matrix is an important factor for the development of OA, and Wnt3a serves a significant role in cartilage homeostasis. The present study assessed whether Wnt3a knockdown promoted Col2 expression in chondrocytes. Lentivirus-introduced small interfering RNA was used to knock down the expression of Wnt3a in primary rat chondrocytes, and then IL-1β treatment was used to establish an OA chondrocyte model. The expression of target genes (Wnt3a, Col2, MMP-13 and β-catenin) was analyzed using reverse transcription-quantitative PCR, western blotting and immunocytochemistry. There was significantly less MMP-13 and β-catenin expression in the Wnt3a knockdown cells compared with the other controls. Col2 expression was significantly higher in the Wnt3a-knockdown cells compared with the control cells, indicating that knockdown of Wnt3a may promote Col2 expression. Consequently, Wnt3a was indicated to be an important factor in cartilage homeostasis, and Wnt3a knockdown may serve as a novel method for OA therapy.

Introduction

Osteoarthritis (OA) is a chronic refractory joint disease that places a heavy burden on health care systems, and there were $2046 in annual incremental expenditures for osteoarthritis in the USA. The annual incidence rate of OA was 0.18% worldwide in 2017 (1,2). Traditional treatments are primarily based on symptomatic management and include non-steroidal anti-inflammatory drugs and mind-body approaches (3,4). However, these therapies do not prevent OA deterioration.

Gene therapy has potential for treating OA. OA progression is closely associated with the TGF-β family (TGF-β1, TGF-β2 and TGF-β3), MAPK and Wnt signaling pathways (5-8). IL-1β is a major pro-inflammatory cytokine involved in the process of chondrocyte degradation that modulates the expression of Wnt-related proteins in chondrocytes (9,10). Wnt3a activates mesenchymal stem cell populations in autografts and modulates the articular chondrocyte phenotype (11,12). Wnt3a also promotes chondrocyte differentiation through $Ca2^{+}$/calmodulin-dependent protein kinase II and proliferation through β-catenin (13). Overexpression of Wnt3a can stimulate the expression of various matrix metalloproteinases (MMPs), including MMP-1, MMP-2, MMP-3 and MMP-13, and can significantly reduce the expression of cartilage matrix molecules, such as collagen type II (Col2) and aggrecan (14,15). However, to the best of our knowledge, the effect of Wnt3a knockdown on gene expression in chondrocytes has not yet been assessed.

OA arises from cartilage cataplasia (5). Col2 forms the backbone for the construction of cartilage, which has structural and biochemical properties (16,17). A decrease in Col2 abundance may initiate and promote chondrocyte hypertrophy and OA progression (18,19). IL-1β can upregulate MMPs in the chondrocyte matrix, and MMPs can cause Col2 degradation (20). In addition, Wnt3a expression is closely associated with the expression of MMPs in synovial cells *in vitro* (15). These findings suggest that Wnt3a may serve an important role in chondrocyte degradation. Therefore, it was hypothesized that knockdown of Wnt3a may be a potential therapeutic intervention for management of OA (Fig. 1).

The present study aimed to silence Wnt3a mRNA with lentiviral vector-mediated Wnt3a-specific siRNA (LV-Wnt3a-RNAi) to promote COL2 expression. First, a cell model of osteoarthritis was established using interleukin-mediated mechanisms *in vitro*. And then OA-like chondrocytes were transfected with LV-Wnt3a-RNAi. Finally, it was determined whether silencing Wnt3a mRNA could promote COL2 expression by determining the expression of COL2 proteins.

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Materials and methods

Animals. A total of five male Sprague-Dawley rats (age, 8 weeks; weight, 200±25 g) were obtained from the Animal Experiment Center of Shandong University (Jinan, China) and allowed to acclimate for 6 days before surgery. All operations were performed under anesthesia induced by 5% isoflurane in 1 l/min oxygen and maintained with 2.5% isoflurane in 0.5 l/min oxygen for the duration of endotracheal intubation and catheterization. Euthanasia was used in accordance with AVMA Guidelines for the Euthanasia of Animals (https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf). The best efforts were made to minimize suffering and distress. Animals were housed at home temperature, relative humidity ~40-70% and a 12-h light/dark cycle, and were provided fresh air, food and water *ad libitum*.

Volume-controlled ventilator (Anhui Zhenghua Biological Instrument Equipment Co., Ltd.) was used before endotracheal intubation and abdominal aorta catheterization via the femoral artery. Arterial blood pressure, respiration rate and heart rate were monitored continuously by telemetric devices (Anhui Zhenghua Biological Instrument Equipment Co., Ltd.). Euthanasia was performed using 100% CO_2 at a rate of 70% chamber volume displacement per minute. After heartbeat and respiration ceased, arterial blood pressure and heart rate were monitored for a further 2 min to confirm death. All the animal's vital signs were extinct after a minimum of 22 min and a maximum of 27 min of breathing carbon dioxide. The animal experiments were approved by the Ethics Committee of Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University (Shandong, China), and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (21).

Primary chondrocytes were isolated from the articular cartilage of euthanized rats. A total of five rats were used. Briefly, cartilage tissue from femoral trochlea was cut into pieces and digested with 2.0 mg/ml collagenase type II (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C for 30 min with shaking, followed by incubation at 37˚C in a water bath for 4 h. Samples were then sifted three times through 200-mesh (SolelyBio-mall), and chondrocytes were collected, seeded in 60-mm culture dishes at a density of $8x10^5$ cells per dish and cultured in antibiotic-conditioned medium (penicillin: 100 U/ml; Streptomycin: 100 μg/ml; DMEM; Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.), and at 5% CO_2 and 37˚C. After the cells had grown to 90% confluence, the chondrocytes were subcultured; passage-2 cells were used for all subsequent experiments.

Chondrocyte isolation and identification. Chondrocyte identification was performed by detecting sulfated glycosaminoglycans using toluidine blue staining (MilliporeSigma). Briefly, cells were fixed in 4% paraformaldehyde at room temperature for 0.5 h. After three washes with PBS and blocking at room temperature for 5 min in 0.1% BSA (Beyotime Institute of Biotechnology), 0.2% Triton (MilliporeSigma; cat. no. T9284) in PBS was used to permeabilize the cells at room temperature for 20 min, after which toluidine blue staining was performed at room temperature for 30 min. To further confirm the chondrocyte properties, expression of Col2 protein in chondrocytes was determined using immunofluorescence. Briefly, samples were fixed in 4% paraformaldehyde at room temperature for 0.5 h. After three washes with PBS and blocking at room temperature for 5 min in 0.1% BSA (Beyotime Institute of Biotechnology), 0.2% Triton (cat. no. T9284; MilliporeSigma) in PBS was used to permeabilize the cells at room temperature for 20 min, after which the cells were washed with PBS and incubated overnight at 4˚C with an anti-Col2 antibody (1:500; cat. no. SAB4500366; Calbiochem; Merck KGaA) in PBS + 0.1% BSA. After three washes with PBS, the cells were incubated with allophycocyanin-conjugated goat anti-rabbit secondary antibody (1:500; cat. no. ab130805; Abcam) and DAPI (MilliporeSigma) for 1 h at room temperature. Cells were observed visually using a fluorescence microscope (magnification, x200).

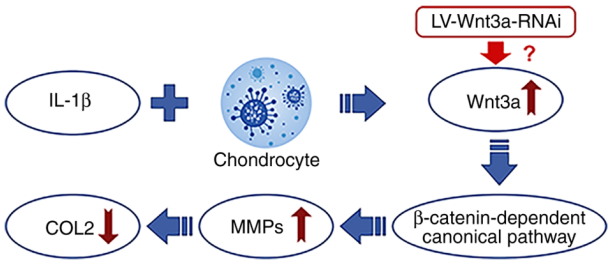

Figure 1. Putative effects of LV-Wnt3a-RNAi. IL-1β upregulates Wnt3a expression in chondrocytes, which activates the β-catenin-dependent canonical pathway to induce expression of MMPs and reduce Col2 expression. A LV-Wnt3a-RNAi was designed to knock down Wnt3a expression, which is hypothesized to result in an increase in Col2 expression. COL2, collagen type II; IL-1β, interleukin-1β; LV-Wnt3a-RNAi, lentivirus vector-mediated Wnt3a-specific RNA interference; MMPs, matrix metalloproteinases.

Establishment of an in vitro OA model. To construct an *in vitro* OA model, chondrocytes were cultured in DMEM without serum at 37˚C for 24 h and then with 10 ng/ml IL-1β (MilliporeSigma) at 37˚C for 1, 2 or 6 h. MMP-13 was revealed to be highly expressed in the chondrocytes stimulated with IL-1β for 6 h, which confirmed that the OA model was successfully established.

Lentivirus packaging and transfection. Lentivirus vector-mediated Wnt3a-specific RNA interference (LV-Wnt3a-RNAi; cat. no. GL27282; SS Sequence, GGAACUACGUGGAGA UCAUGC; AS Sequence, AUGAUCUCCACGUAGUUC CUG) was obtained from Shanghai GeneChem Co., Ltd., and used according to the manufacturer's recommendations. Lentiviral vector used was GFP-tagged and fluorescence staining was used to observe transfection. A preliminary experiment was first performed to determine the duration of transduction into OA-like chondrocytes, and MOI. OA-like chondrocytes were assigned to three groups: i) Cells incubated in complete media at 37˚C for 48 h (Control group); ii) cells incubated in complete media with the empty vector at 37˚C for 48 h (Empty vector group); and iii) cells incubated in complete media supplemented with LV-Wnt3a-RNAi at 37˚C for 48 h (LV-Wnt3a group). The amount of vector added to empty vector group was the same as that added to LV-Wnt3a group, several multiplicity of infection (MOI=20, 40, 60 and 100)

values was used. The optimal duration of transduction and MOI was 48 h and 60, respectively, which was consistent with manual recommendations Stable cell lines were not required for this experiment. Following 72 h of chondrocyte transfection, to determine the transfection efficiency, the expression of Wnt3a mRNA was detected using RT-qPCR.

Cell viability detection. To demonstrate that the transfected chondrocytes were viable, Live-Dead staining was performed according to the manufacturer's instructions of LIVE/DEAD Viability/Cytotoxicity Assay kit (cat. no. L3224, Invitrogen; Thermo Fisher Scientific, Inc.) and as previously described (22). Briefly, calcein AM/ethidium homodimer-1 solution containing 2 mM calcein AM and 4 mM ethidium homodimer-1 were added to transfected chondrocytes for 30 min at 37˚C. After washing three times with PBS, the samples were observed using a fluorescence microscope with an excitation wavelength of 488 or 568 nm. Live and dead cells appeared green (calcein AM) or red (ethidium homodimer-1), respectively. Overall, ~200 cells were counted manually under a fluorescence microscope, with dead cells and living cells separately counted.

RT-qPCR. Cells were lysed using TRIzol® reagent (Thermo Fisher Scientific, Inc.). cDNA was then generated using a PrimeScript™ RT Reagent kit (Takara Bio, Inc.) according to the manufacturer's protocol. Table I presents the primer sequences for Wnt3a, MMP-13, Col2, β-catenin and β-actin. RT-qPCR was performed on a LightCycler 480 System (SYBR Green; Roche Diagnostics Ltd.). Amplification was performed with the following steps: Initial denaturation at 95˚C for 10 min; followed by denaturation at 95˚C for 5 sec, annealing at 55˚C for 30 sec and extension at 72˚C for 30 sec for a total of 40 cycles. Amplification of Wnt3a, MMP-13, Col2 and β-catenin was calculated using the $2^{-\Delta\Delta Cq}$ method (23), and normalized to β-actin. The procedure was repeated at least three times for each gene.

Western blotting. Following infection for 24 h, cells were lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentration was measured using a BCA assay (Thermo Fisher Scientific, Inc.). Subsequently, 10% SDS-PAGE was performed using an SDS-PAGE Gel Preparation Kit (Beyotime Institute of Biotechnology) to resolve the proteins (25 μg from each sample) by molecular weight, which were then transferred to PVDF membranes (MilliporeSigma). The membranes were blocked in 5% skimmed milk in TBS + 0.05% Tween (TBST) for 1 h at room temperature. After washing three times in TBST, membranes were incubated overnight at 4˚C with one of the following primary antibodies: Rabbit anti-Col2 (1:500; cat. no. SAB4500366; Calbiochem; Merck KGaA), rabbit anti-MMP-13 (1:500; cat. no. MA5-42462; Thermo Fisher Scientific, Inc.), rabbit anti-β-catenin (1:1,000; cat. no. MA5-34961; Thermo Fisher Scientific, Inc.), rabbit anti-Wnt3a (1:1,000; cat. no. 703666; Thermo Fisher Scientific, Inc.) or rabbit anti-β-actin antibody (1:1,000; cat. no. 20536-1-AP; ProteinTech Group, Inc.), followed by incubation for 1 h at room temperature with the goat anti-rabbit HRP secondary antibody (1:5,000; cat. no. sc-2054; Santa Cruz Biotechnology, Inc.). After washing with TBST, blots were visualized using Enhanced Chemiluminescence Reagent (MilliporeSigma), and ImageJ software version 1.8.0 (National Institutes of Health) was used for densitometric analysis.

Table I. Sequences of the primers used in reverse transcription-quantitative PCR.

Gene	Sequence (5'-3')
Wnt3a	F: GTCGGGTTCTTCTCTGGTCCT
	R: CTGGGCATGATCTCCACGTA
Col2	F: GGCCAGGATGCCCGAAAATTA
	R: ACCCCTCTCTCCCTTGTCAC
MMP-13	F: CAAGCAGCTCCAAAGGCTAC
	R: TGGCTTTTGCCAGTGTAGGT
β-catenin	F: GAAAATGCTTGGGTCGCCAG
	R: CATTTTCTGCAGCCCACCAG
β-actin	F: ACCCGCCACCAGTTCG
	R: GCTCGAAGTCTAGGGCAACA

Col2, collagen type II; F, forward; MMP, matrix metalloproteinase; R, reverse.

Immunocytochemistry. Following fixation with 4% paraformaldehyde at room temperature for 20 min, chondrocytes from the three groups were blocked with 5% BSA at 37˚C for 30 min in PBS and incubated overnight at 4˚C with a rabbit anti-Col2 antibody (1:500; cat. no. SAB4500366; Calbiochem; Merck KGaA). Samples were then washed three times with PBS, followed by incubation with a goat anti-rabbit HRP secondary antibody (1:5,000; cat. no. sc-2054; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h. After three washes in PBS, immunoreactivity was determined using 3,3'-diaminobenzidine (OriGene Technologies, Inc.). Counterstaining was performed with hematoxylin at room temperature for 15 sec. Stained cells were observed using light microscopy (magnification, x200), and Image-Pro Plus (version 7.0; Media Cybernetics, Inc.) was used to assess integrated optical density of the images and at least three fields were selected for each slide to evaluate the results.

Statistical analysis. An analysis of variance was conducted with one-way analysis of variance, followed by Tukey's test for all experiments performed in triplicate. The data is presented as the mean ± standard deviation. The analysis was conducted using SPSS software version 23 (IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of chondrocytes and construction of OA-like cells. Green fluorescence specific to Col2 and toluidine blue-stained cytoplasm as well as Col2-specific green fluorescence expression indicated that the obtained cells were chondrocytes (Fig. 2A); thus, these cells were used in subsequent experiments. qPCR and western blotting were used to

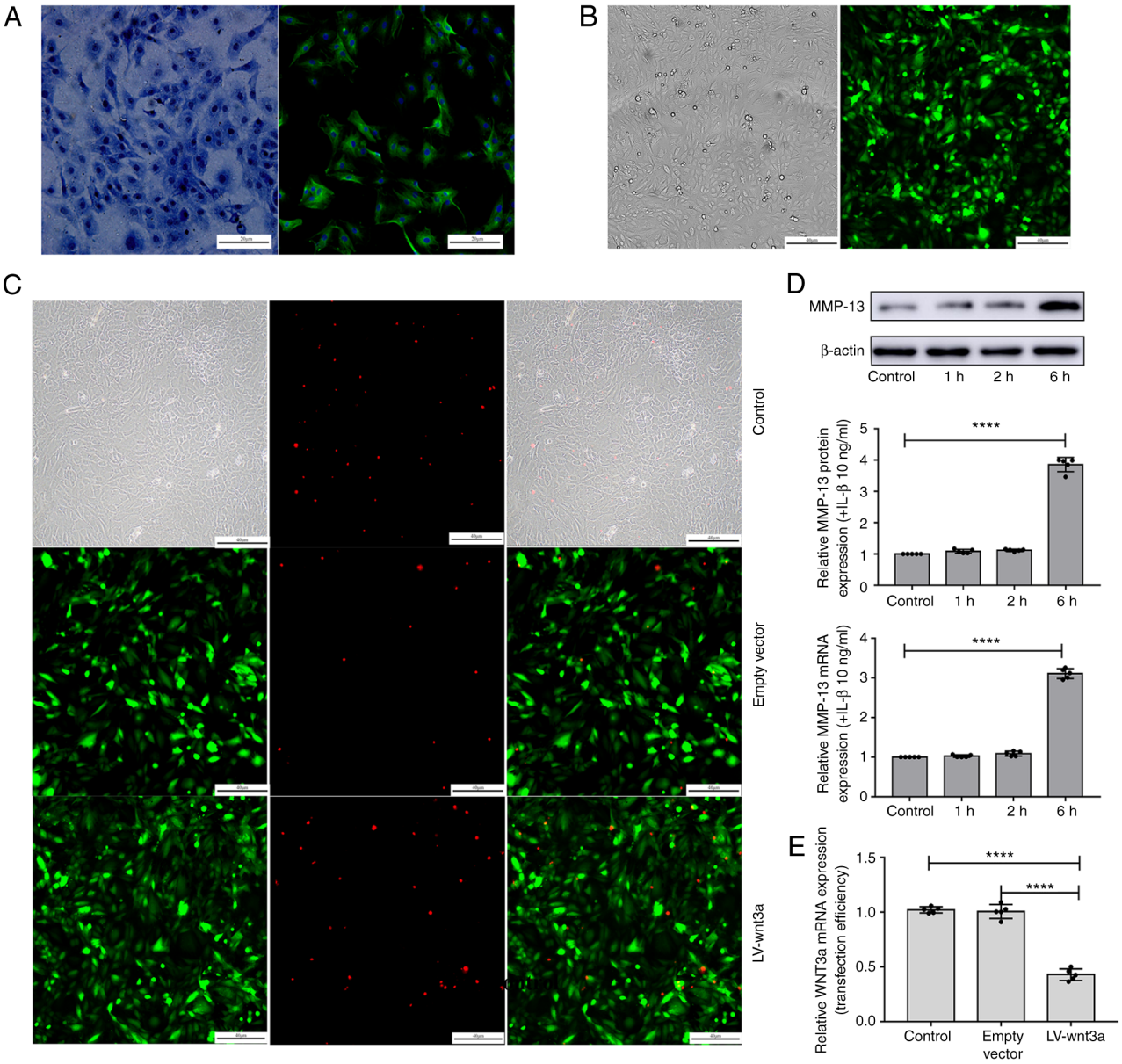

Figure 2. Characteristics of the cultured chondrocytes. (A) Cultured chondrocyte cells were stained with toluidine blue. Cultured cells were also incubated with anti-collagen II antibody, and signal was detected using green fluorescence (magnification, x200). (B) Determination of transfection. OA-like chondrocytes were observed by optical microscopy after LV-Wnt3a-RNAi transfection. Transfected cells were confirmed using fluorescence microscopy, which indicated the optimum multiplicity of infection to be 60 (magnification, x100). (C) Live-Dead staining was used to assess the viability of transfected chondrocytes. Transfected chondrocytes treated with calcein AM (green) and ethidium homodimer-1 (red) demonstrated viable chondrocytes with only a minority of dead cells. The merged image indicates that viable chondrocytes accounted for 96% of the total cells (magnification, x100). (D) Chondrocytes were cultured in DMEM with 10 ng/ml IL-1β for 1, 2 or 6 h. MMP-13 gene expression and protein abundance at 6 h were significantly higher compared with that in other groups. (E) Quantitative PCR was used to assess transfection efficiency. The experiment was divided into three groups: control group, empty vector group and LV-wnt3a group. Relative Wnt3a mRNA expression was detected. The experiments were performed on five samples per group. ****P<0.0001. IL-1β, interleukin-1β; LV-Wnt3a-RNAi, lentivirus vector-mediated Wnt3a-specific RNA interference; MMP, matrix metalloproteinase; OA, osteoarthritis.

investigate whether the cell model was successfully established after IL-1β stimulation. As shown in Fig. 2D, expression of MMP-13 was significantly higher in the 6 h-group compared with other groups, which indicated that osteoarthritis model was successfully established.

Transfection of chondrocytes with LV-Wnt3a-RNAi and viability assays. A total of 48 h after transfection with the lentiviral vector, LV-Wnt3a-RNAi (at MOI=60), transfection was confirmed by fluorescence staining (Fig. 2B). Live-Dead staining was used to assess the viability of transfected chondrocytes, and the results demonstrated that Wnt3a-knockdown chondrocytes remained viable, ~200 cells were counted manually under a fluorescence microscope, with dead cells and living cells separately counted. and the cell survival rate of all the groups was ≥96% (Fig. 2C and Table II). As shown in Fig. 2E, RT-qPCR analysis demonstrated that expression of Wnt3a was significantly lower in the LV-Wnt3a group compared with the control group (non-transfected cells) and empty vector-transfected group. Therefore, the 72 h Wnt3a-knockdown chondrocytes were used in the subsequent experiments.

Table II. Cell counting of Live-Dead staining.

Group name	First counting	Second counting	Third counting	Dead cell ratio (mean)	Live cell ratio (mean)
Control	7/200	8/200	7/200	3.67%	96.33%
Empty vector	6/200	8/200	9/200	3.83%	96.17%
LV-wnt3a	7/200	9/200	8/200	4.0%	96.0%

LV-wnt3a, lentivirus vector-mediated Wnt3a-specific RNA interference; N/200, number of dead cells/200.

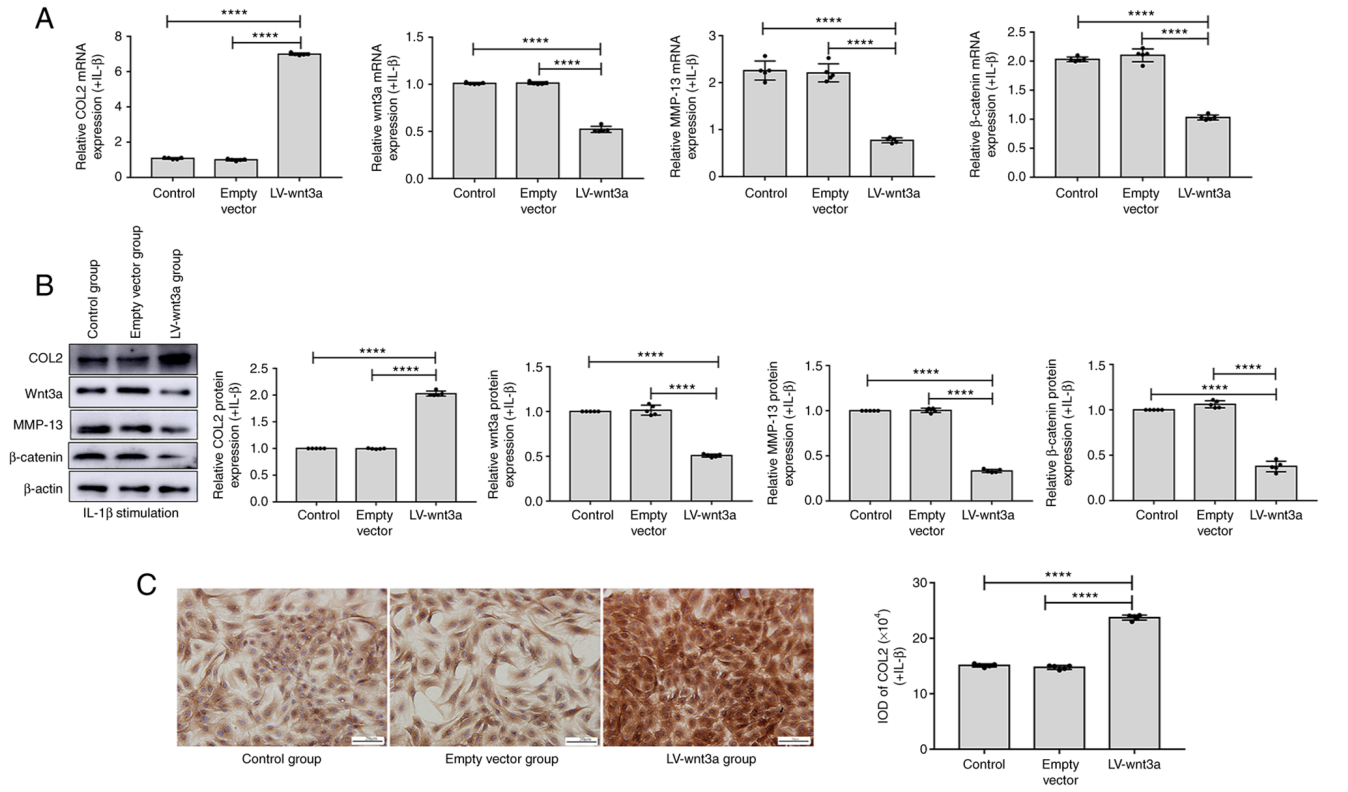

Figure 3. Wnt3a knockdown increases Col2 expression and decreases MMP-13 and β-catenin expression. (A) Quantitative PCR analysis: Quantitative PCR was performed to detect expression of Wnt3a, Col2, MMP-13 and β-catenin following Wnt3a knockdown. Wnt3a, MMP-13 and β-catenin expression in LV-wnt3a group was significantly lower than that in control group and empty vector group, while expression of Col2 in LV-wnt3a group was significantly higher than that in other groups. (B) Western blotting analysis. Western blot was performed to detect expression of Wnt3a, Col2, MMP-13 and β-catenin following Wnt3a knockdown. Protein expression data were normalized to the β-actin loading control. Wnt3a, MMP-13 and β-catenin expression in LV-wnt3a group was significantly lower than that in control group and empty vector group, while expression of Col2 in LV-wnt3a group was significantly higher than that in other groups. (C) Immunocytochemistry analysis: Representative images of immunocytochemical staining in all groups(magnification, x200). IOD analysis of the immunocytochemical images indicated that Col2 expression was significantly higher in LV-wnt3a group compared with control group and empty vector group. The experiments were performed on five samples per group. ****P<0.0001. COL2, collagen type II; IL-1β, interleukin-1β; IOD, integrated optical density; MMP, matrix metalloproteinase.

Wnt3a knockdown increases Col2 expression and decreases MMP-13 and β-catenin expression. RT-qPCR, immunocytochemistry and western blotting were used to investigate whether knockdown of Wnt3a by LV-Wnt3a-RNAi could promote Col2 expression. Results from RT-qPCR analysis demonstrated that expression of Wnt3a was significantly lower in LV-wnt3a group (LV-Wnt3a-RNAi-trasfected cells) compared with control group (non-transfected cells) and empty vector group (empty vector-transfected cells) (both P<0.0001; Fig. 3A). In addition, Col2 expression was significantly higher in LV-wnt3a group compared with control group and empty vector group (both P<0.0001), and MMP-13 and β-catenin expression levels were significantly lower in LV-wnt3a group compared with control group and empty vector group (all P<0.0001) (Fig. 3A). Western blotting demonstrated that Col2 protein expression was significantly higher in LV-wnt3a group compared with control group and empty vector group (both P<0.0001), and that MMP-13 and β-catenin expression levels were significantly lower in LV-wnt3a group compared with control group and empty vector group (all P<0.0001) (Fig. 3B). Immunocytochemical analysis demonstrated that Col2 expression was higher in LV-wnt3a group than in control group and empty vector group (Fig. 3C). By contrast, there was no significant difference in

Col2 expression between groups A and B (Fig. 3B). These results of IOD of immunocytochemical images were consistent with the western blotting analysis. Thus, these results indicated that knockdown of Wnt3a promoted Col2 expression in chondrocytes.

Discussion

OA is a common degenerative disease amongst older individuals (>60 years old) and is a significant burden on healthcare resources. Therapeutic regimens have primarily focused on symptomatic relief and typically involve non-steroidal anti-inflammatory drugs and rehabilitation exercises (2,24). There are few treatments available to delay the degeneration of articular cartilage; gene therapy may be an effective treatment method.

Chondrogenic dedifferentiation is closely associated with various signaling pathways, including TGF-β, MAPK and Wnt (7,8,22). Cartilage matrix and chondrocyte degeneration are aggravated by increased levels of IL-1β. For example, in osteoarthritic chondrocytes, IL-1β upregulates MMP-3 and tumor necrosis factor-stimulated gene 6, and downregulates aggrecan (ACAN) (25,26). Similar results were observed for chondrocytes from patients with rheumatoid arthritis. Continuous IL-1β exposure has been indicated to efficiently suppress synthesis of Col2 and Col4, but increase the mRNA levels of Col1 and Col3. The differential expression of collagen proteins in cartilage may potentially promote the breakdown of the cartilage matrix (26,27). IL-1β-related pathways are complex; IL-1β can modulate Wnt3a expression, and may therefore induce β-catenin nuclear accumulation to increase expression of MMPs (28,29). Differential expression of MMPs results in the decrease of Col2 and Col4, which contributes to OA (30). Overexpression of Wnt3a can induce differential expression of MMPs and can significantly reduce the expression of the cartilage matrix molecules, Col2 and ACAN (14,15). Therefore, it was hypothesized that Wnt3a knockdown may promote Col2 expression in chondrocytes and the appropriate repair of cartilage matrix.

A Wnt3a-targeting small interfering (si)RNA packaged in a lentiviral vector was used in the present study to knock down Wnt3a. This method was supported by a previous transgenic mouse study that demonstrates the effectiveness of lentiviral vectors to deliver siRNA (31). To explore whether Wnt3a knockdown could increase Col2 expression, the levels of Col2 in chondrocytes were determined. Col2 mRNA and protein expression levels were significantly higher in the Wnt3a-knockdown group compared with the control groups. These results were consistent with the immunocytochemistry results, where the levels of Col2 were markedly higher in the Wnt3a-knockdown group compared with the control groups. Wnt3a-knockout also significantly increased the protein expression of Col2 in OA-like chondrocytes.

The specific molecular mechanisms for this effect can be associated with the β-catenin-dependent canonical Wnt pathway (32). A decrease in Wnt3a levels, combined with a decrease in the expression levels of Frizzled receptors and low-density lipoprotein receptor-related receptor (LRP)5 and LRP6, disrupts the accumulation of β-catenin, which further increases Col2 gene transcription (32,33). Similarly, Held *et al* (34) reported that canonical Wnt inhibitors can regulate the expression of characteristic chondrocyte genes (Sox9, Col2 and ACAN) by blocking Wnt3a receptors. In *in vivo* experiments, Bertrand *et al* (35) came to the same conclusion that Wnt3a can modulate cartilage matrix maker genes (Sox9, Col2 and ACAN) through LRP6 phosphorylation and stabilization of β-catenin. In addition, Thomas *et al* (36) reported that Wnt3a-loaded exosomes can promote cartilage repair in the knees of mice. These exosomes may regulate circumarticular synovial tissues in addition to cartilage. The precise mechanisms of cross-talk and cross-regulation between different tissues requires further study. In the present study, knockdown of Wnt3a can promote protein expression of Col2 *in vitro*, which may be an effective therapy for management of OA. One concern about the findings was that transfected viral vector (GFP-tagged) and calcein-AM staining have the same green fluorescence properties, which may affect the intensity of the fluorescence. But it had very little impact on the count of living or dead cells. Furthermore, the present study chose naked eye observation to determine the optimal MOI value. Although visual observation is not very accurate, subsequent transfection efficiency and cell viability confirmed that MOI can be applied. Further animal experiments should be performed to verify the effectiveness of Wnt3a knockdown.

In conclusion, Wnt3a siRNA was successfully packaged into a lentiviral vector and used to effectively knock down Wnt3a expression. Wnt3a-knockdown may delay the degeneration of articular cartilage; thus, LV-Wnt3a-RNAi may be a novel tool for the management of OA.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SSu conceived and designed the study, and revised the manuscript. SSh drafted the manuscript and performed the experiments. ZM collected and analyzed data. SSu and ZM confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments were approved by the Ethics Committee of Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University (approval number 2019-08, Shandong, China) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Menon J and Mishra P: Health care resource use, health care expenditures and absenteeism costs associated with osteoarthritis in US healthcare system. Osteoarthritis Cartilage 26: 480-484, 2018.
2. Safiri S, Kolahi AA, Smith E, Hill C, Bettampadi D, Mansournia MA, Hoy D, Ashrafi-Asgarabad A, Sepidarkish M, Almasi-Hashiani A, *et al*: Global, regional and national burden of osteoarthritis 1990-2017: A systematic analysis of the global burden of disease study 2017. Ann Rheum Dis 79: 819-828, 2020.
3. Brosseau L, Taki J, Desjardins B, Thevenot O, Fransen M, Wells GA, Imoto AM, Toupin-April K, Westby M, Gallardo ICÁ, *et al*: The Ottawa panel clinical practice guidelines for the management of knee osteoarthritis. Part one: Introduction, and mind-body exercise programs. Clin Rehabil 31: 582-595, 2017.
4. Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J, Callahan L, Copenhaver C, Dodge C, Felson D, *et al*: 2019 American college of rheumatology/arthritis foundation guideline for the management of osteoarthritis of the hand, hip, and knee. Arthritis Rheumatol 72: 220-233, 2020.
5. Jenei-Lanzl Z, Meurer A and Zaucke F: Interleukin-1β signaling in osteoarthritis-chondrocytes in focus. Cell Signal 53: 212-223, 2019.
6. Finnson KW, Chi Y, Bou-Gharios G, Leask A and Philip A: TGF-b signaling in cartilage homeostasis and osteoarthritis. Front Biosci (Schol Ed) 4: 251-268, 2012.
7. Abusarah J, Benabdoune H, Shi Q, Lussier B, Martel-Pelletier J, Malo M, Fernandes JC, de Souza FP, Fahmi H and Benderdour M: Elucidating the role of protandim and 6-gingerol in protection against osteoarthritis. J Cell Biochem 118: 1003-1013, 2017.
8. Kovács B, Vajda E and Nagy EE: Regulatory effects and interactions of the Wnt and OPG-RANKL-RANK signaling at the bone-cartilage interface in osteoarthritis. Int J Mol Sci 20: 4653, 2019.
9. Bai M, Ge L, Chen H and Jin Q: Calcitonin protects rat chondrocytes from IL-1β injury via the Wnt/β-catenin pathway. Exp Ther Med 18: 2079-2085, 2019.
10. Miyatake K, Kumagai K, Imai S, Yamaguchi Y and Inaba Y: Sclerostin inhibits interleukin-1β-induced late stage chondrogenic differentiation through downregulation of Wnt/β-catenin signaling pathway. PLoS One 15: e0239651, 2020.
11. McBride JD, Rodriguez-Menocal L, Guzman W, Candanedo A, Garcia-Contreras M and Badiavas EV: Bone marrow mesenchymal stem cell-derived CD63^{+} exosomes transport Wnt3a exteriorly and enhance dermal fibroblast proliferation, migration, and angiogenesis in vitro. Stem Cells Dev 26: 1384-1398, 2017.
12. Yasuhara R, Ohta Y, Yuasa T, Kondo N, Hoang T, Addya S, Fortina P, Pacifici M, Iwamoto M and Enomoto-Iwamoto M: Roles of β-catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells. Lab Invest 91: 1739-1752, 2011.
13. Nalesso G, Sherwood J, Bertrand J, Pap T, Ramachandran M, De Bari C, Pitzalis C and Dell'accio F: WNT-3A modulates articular chondrocyte phenotype by activating both canonical and noncanonical pathways. J Cell Biol 193: 551-564, 2011.
14. Hou L, Shi H, Wang M, Liu J and Liu G: MicroRNA-497-5p attenuates IL-1β-induced cartilage matrix degradation in chondrocytes via Wnt/β-catenin signal pathway. Int J Clin Exp Pathol 12: 3108-3118, 2019.
15. van den Bosch MH, Blom AB, van de Loo FA, Koenders MI, Lafeber FP, van den Berg WB, van der Kraan PM and van Lent PL: Brief report: Induction of matrix metalloproteinase expression by synovial Wnt signaling and association with disease progression in early symptomatic osteoarthritis. Arthritis Rheumatol 69: 1978-1983, 2017.
16. Cinque L, Forrester A, Bartolomeo R, Svelto M, Venditti R, Montefusco S, Polishchuk E, Nusco E, Rossi A, Medina DL, *et al*: FGF signalling regulates bone growth through autophagy. Nature 528: 272-275, 2015.
17. Onuora S: Osteoarthritis: UCMA links cartilage and bone in OA. Nat Rev Rheumatol 13: 130, 2017.
18. van der Kraan PM and van den Berg WB: Chondrocyte hypertrophy and osteoarthritis: Role in initiation and progression of cartilage degeneration? Osteoarthritis Cartilage 20: 223-232, 2012.
19. Du G, Xiang C, Sang X, Wang X, Shi Y, Wang N, Wang S, Li P, Wei X, Zhang M, *et al*: Histone deacetylase 4 deletion results in abnormal chondrocyte hypertrophy and premature ossification from collagen type 2α1-expressing cells. Mol Med Rep 22: 4031-4040, 2020.
20. Park KW, Lee KM, Yoon DS, Park KH, Choi WJ, Lee JW and Kim SH: Inhibition of microRNA-449a prevents IL-1β-induced cartilage destruction via SIRT1. Osteoarthritis Cartilage 24: 2153-2161, 2016.
21. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals: Guide for the Care and Use of Laboratory Animals. 8th edition. National Academy of Sciences, Washington, DC, 2011.
22. Praxenthaler H, Krämer E, Weisser M, Hecht N, Fischer J, Grossner T and Richter W: Extracellular matrix content and WNT/β-catenin levels of cartilage determine the chondrocyte response to compressive load. Biochim Biophys Acta Mol Basis Dis 1864: 851-859, 2018.
23. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
24. Sun X, Zhen X, Hu X, Li Y, Gu S, Gu Y and Dong H: Osteoarthritis in the middle-aged and elderly in China: Prevalence and influencing factors. Int J Environ Res Public Health 16: 4701, 2019.
25. Stöve J, Huch K, Günther KP and Scharf HP: Interleukin-1beta induces different gene expression of stromelysin, Aggrecan and tumor-necrosis-factor-stimulated gene 6 in human osteoarthritic chondrocytes in vitro. Pathobiology 68: 144-149, 2000.
26. Attur M, Zhou H, Samuels J, Krasnokutsky S, Yau M, Scher JU, Doherty M, Wilson AG, Bencardino J, Hochberg M, *et al*: Interleukin 1 receptor antagonist (IL1RN) gene variants predict radiographic severity of knee osteoarthritis and risk of incident disease. Ann Rheum Dis 79: 400-407, 2020.
27. Li GS, Cui L and Wang GD: miR-155-5p regulates macrophage M1 polarization and apoptosis in the synovial fluid of patients with knee osteoarthritis. Exp Ther Med 21: 68, 2021.
28. Zhou ZB, Huang GX, Fu Q, Han B, Lu JJ, Chen AM and Zhu L: circRNA.33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p. Mol Ther 27: 531-541, 2019.
29. Haltmayer E, Ribitsch I, Gabner S, Rosser J, Gueltekin S, Peham J, Giese U, Dolezal M, Egerbacher M and Jenner F: Co-culture of osteochondral explants and synovial membrane as in vitro model for osteoarthritis. PLoS One 14: e0214709, 2019.
30. Xia C, Wang P, Fang L, Ge Q, Zou Z, Dong R, Zhang P, Shi Z, Xu R, Zhang L, *et al*: Activation of β-catenin in Col2-expressing chondrocytes leads to osteoarthritis-like defects in hip joint. J Cell Physiol 234: 18535-18543, 2019.
31. Merentie M, Rissanen R, Lottonen-Raikaslehto L, Huusko J, Gurzeler E, Turunen MP, Holappa L, Mäkinen P and Ylä-Herttuala S: Doxycycline modulates VEGF-A expression: Failure of doxycycline-inducible lentivirus shRNA vector to knockdown VEGF-A expression in transgenic mice. PLoS One 13: e0190981, 2018.
32. Hernández AR, Klein AM and Kirschner MW: Kinetic responses of β-catenin specify the sites of Wnt control. Science 338: 1337-1340, 2012.
33. Estarás C, Hsu HT, Huang L and Jones KA: YAP repression of the WNT3 gene controls hESC differentiation along the cardiac mesoderm lineage. Genes Dev 31: 2250-2263, 2017.
34. Held A, Glas A, Dietrich L, Bollmann M, Brandstädter K, Grossmann TN, Lohmann CH, Pap T and Bertrand J: Targeting β-catenin dependent Wnt signaling via peptidomimetic inhibitors in murine chondrocytes and OA cartilage. Osteoarthritis Cartilage 26: 818-823, 2018.
35. Bertrand J, Kräft T, Gronau T, Sherwood J, Rutsch F, Lioté F, Dell'Accio F, Lohmann CH, Bollmann M, Held A and Pap T: BCP crystals promote chondrocyte hypertrophic differentiation in OA cartilage by sequestering Wnt3a. Ann Rheum Dis 79: 975-984, 2020.
36. Thomas BL, Eldridge SE, Nosrati B, Alvarez M, Thorup AS, Nalesso G, Caxaria S, Barawi A, Nicholson JG, Perretti M, *et al*: WNT3A-loaded exosomes enable cartilage repair. J Extracell Vesicles 10: e12088, 2021.