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Original Article

MicroRNA-411 inhibited matrix metalloproteinase 13 expression in human chondrocytes

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Abstract: Osteoarthritis (OA) is the most common joint degenerative disease affecting the joint structure, leading to loss of joint function and tissue destruction. Recent studies have demonstrated that miRNAs are involved in many pathological conditions, including OA. The study was to investigate the role of miR-411 in the pathogenesis of OA. The expression of miR-411 was downregulated in OA cartilage compared with in normal cartilage. Conversely, the expression of MMP-13 was upregulated in OA cartilage compared with in normal cartilage. IL-1β treatment repressed miR-411 expression in chondrocytes. Moreover, we identified MMP-13 as a direct target gene of miR-411 in chondrocytes and overexpression of miR-411 inhibited the MMP-13 expression. Furthermore, overexpression of miR-411 increased the expression of type II collagen and type IV collagen expression in chondrocytes. MiR-411 is a crucial regulator of MMP-13 in chondrocytes and may response to the development of OA.

Keywords: Osteoarthritis, chondrocytes, miRNA, miR-411

Introduction

Osteoarthritis (OA) is a degenerative disease that causes physical disability among older persons worldwide [1, 2]. Available treatments are limited to pain care and joint replacement surgery in endstage OA patients [3, 4]. The etiology of osteoarthritis (OA) is complex, including genetic predisposition, failure of nutrient supply, abnormal mechanical loading and trauma [5, 6]. OA is characterized by imbalance between extracellular matrix (ECM) synthesis and degradation and resulted loss of movement, joint pain, and progressive dysfunction [1, 7]. Among these, MMP-13 has gained the most interest, due to its capacity to degrade collagens along with a wide range of matrix molecules [8-10]. Further investigation and understanding of OA pathology are needed and important to develop effective therapeutic targets to control OA.

MicroRNAs (miRNAs) are a class of 17-25 nucleotide small endogenous noncoding RNAs which regulate gene expression primarily through base paring to the 3'untranslated region (UTR) of target mRNAs (mRNAs) at posttranscriptional levels. MiRNAs leads to translation repression or mRNA cleavage [11-15]. Recent evidence has demonstrated that miRNAs play crucial role in cell development, proliferation, migration, invasion and differentiation [16-19]. The physiologic and pathogenic role of miRNAs in the maintenance of joint homeostasis and the development of arthritis is currently being elucidated [20-23]. Recent reports have also described a correlation of MMP-13 with specific miRNAs, such as miR-140, miR-126-5p and miR-27a [24-26].

In this study, miR-411 was significantly downregulated in OA cartilage compared with in normal cartilage. Conversely, MMP-13 was upregulated in OA cartilage compared with in normal cartilage. IL-1β treatment repressed miR-411 expression in chondrocytes. Moreover, we identified MMP-13 as a direct target gene of miR-411 in chondrocytes and overexpression of miR-411 inhibited the MMP-13 expression. Furthermore, overexpression of miR-411 increased the type II collagen and type IV collagen expression in chondrocytes.

Materials and methods

Specimen selection and cell culture

OA cartilage samples were isolated from 10 patients with OA (ages 56-64 years) who under-

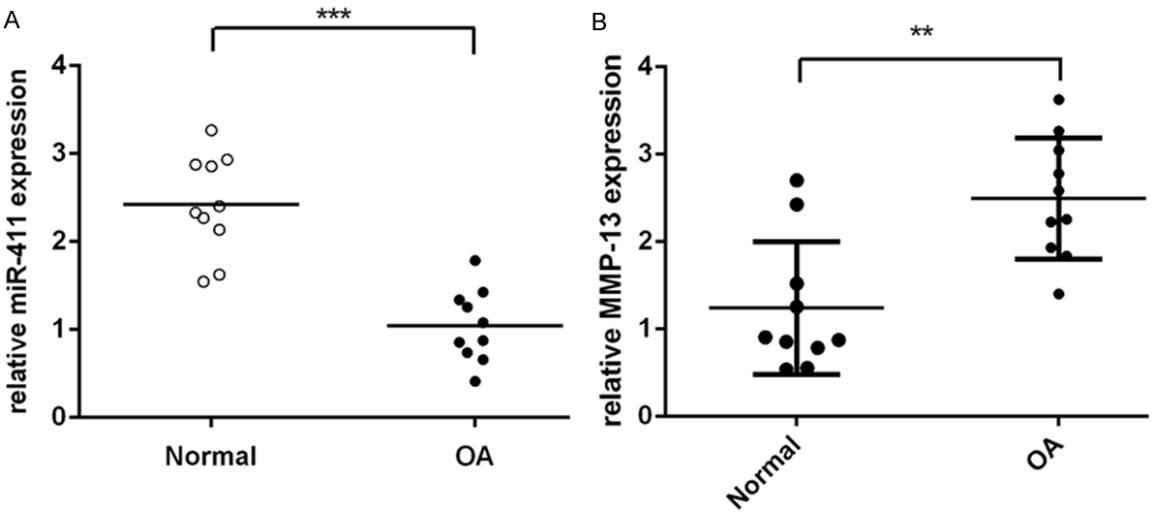

Figure 1. The expression of miR-411 was downregulated in OA cartilage and the expression of MMP-13 was upregulated in OA cartilage. A. The expression of miR-411 was measured in OA cartilage and normal cartilage using qRT-PCR. B. The expression of MMP-13 was measured in OA cartilage and normal cartilage used qRT-PCR.

went total knee replacement surgery. The diagnosed of patients were made according to the American College of Rheumatology criteria. Normal knee cartilage was obtained from 10 patients who underwent amputation due to trauma with no history of rheumatoid arthritis or OA. All patients have given written informed consent and agreed to involve in this study. The consent and study was approved by the ethical board of the institute of Affiliated Hospital of Jining Medical College and complied with Declaration of Helsinki.

Oligonucleotides, cell culture and transfections and cell proliferation

The human immortalized juvenile costal chondrocytes cell line C28/I2 was cultured in DMEM/F12 according previously protocol [27, 28]. miR-411 mimics and scramble were synthesized by GenePharma (Shanghai, China) and were transfected into the cells with DharmaFECT1 reagent (Dharmacon, TX, USA) with a final oligonucleotide concentration of the 20 nmol/L. Cell proliferation was performed using the CCK-8 (Dojin Laboratories, Japan) at different time after transfection. Absorbance was measured at 450 nm.

RNA extraction and quantitative RT-PCR

Total RNA was extracted using the mirVana miRNA isolation kit (Ambion). miR-411 expression was detected by TaqMan qRT-PCR with TaqMan microRNA assay kits (Ambion) according to manufacturer's instruction. The expression of miR-411 was normalized to U6 expression. The expression levels of MMP13, Ki-67, type II collagen and type IV collagen were measured by SYBR Green qRT-PCR. Primer sequences are shown in Table S1.

Dual luciferase assays

Cells were transfected with reporter construct, pGL-3 control vector, and either miR-411 or a scramble. The luciferase values were measured by the Dual Luciferase Assay following to manufacturer's suggestion. Firefly luciferase value was normalized to the Renilla signal and the ratio of the Firefly/Renilla values was detected.

Western blot

Western blot was done according to standard methods. Proteins were resolved by 12% SDS-PAGE and transferred to PVDF membrane (Millipore). Membranes were blocked with 5% milk and probed with primary antibody (MMP-1, type II collagen and type IV collagen, Abcam) at a 1:2000 dilution. After washing, the membranes were measured using HRP-conjugated secondary antibodies and visualized by an enhanced chemiluminescence kit.

Statistical analysis

Statistical analyses were performed using SPSS 18.0 software (IBM). Data are expressed

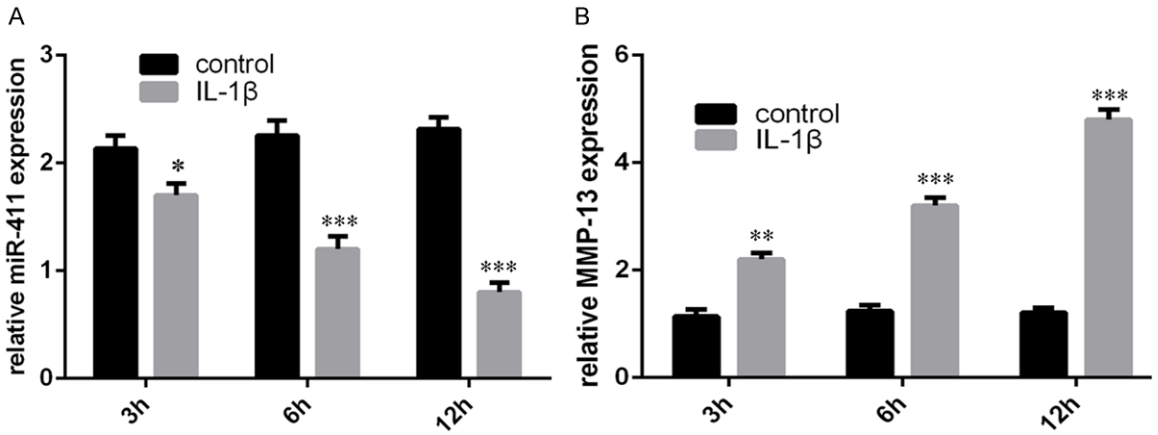

Figure 2. IL-1β treatment decreased expression of miR-411 and increases MMP-13 expression in chondrocytes. A. The expression of miR-411 was measured after treated by IL-1β (10 ng/ml) using qRT-PCR. B. The expression of MMP-13 was measured after treated by IL-1β (10 ng/ml) using qRT-PCR.

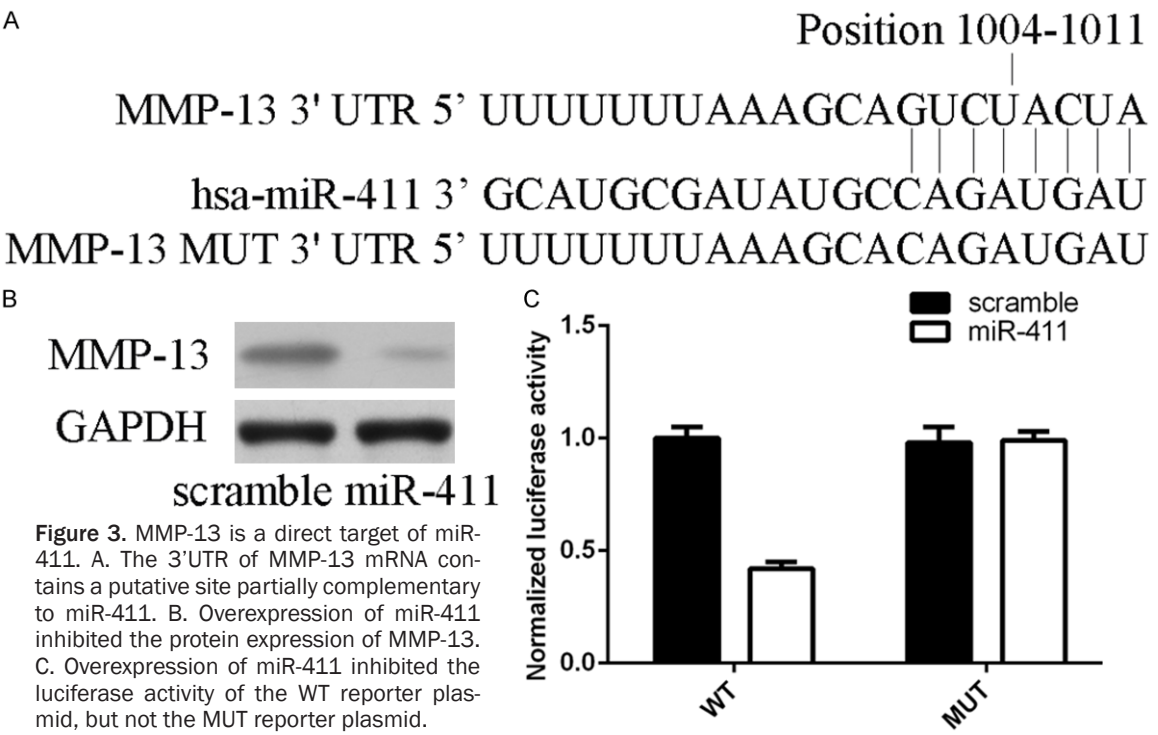

Figure 3. MMP-13 is a direct target of miR-411. A. The 3'UTR of MMP-13 mRNA contains a putative site partially complementary to miR-411. B. Overexpression of miR-411 inhibited the protein expression of MMP-13. C. Overexpression of miR-411 inhibited the luciferase activity of the WT reporter plasmid, but not the MUT reporter plasmid.

as the mean±SD and were analyzed using one-way ANOVA or Student's t test. $P<0.05$ was considered statistically significant.

Results

miR-411 was downregulated in OA cartilage and upregulated in OA cartilage

As shown in **Figure 1A**, the expression of miR-411 was downregulated in OA cartilage compared with in normal cartilage. Conversely, the expression of MMP-13 was upregulated in OA cartilage compared with in normal cartilage (**Figure 1B**).

IL-1β treatment decreased expression of miR-411 and increases MMP-13 expression in chondrocytes

IL-1β (10 ng/ml) treatment repressed miR-411 expression in chondrocytes (**Figure 2A**). In par-

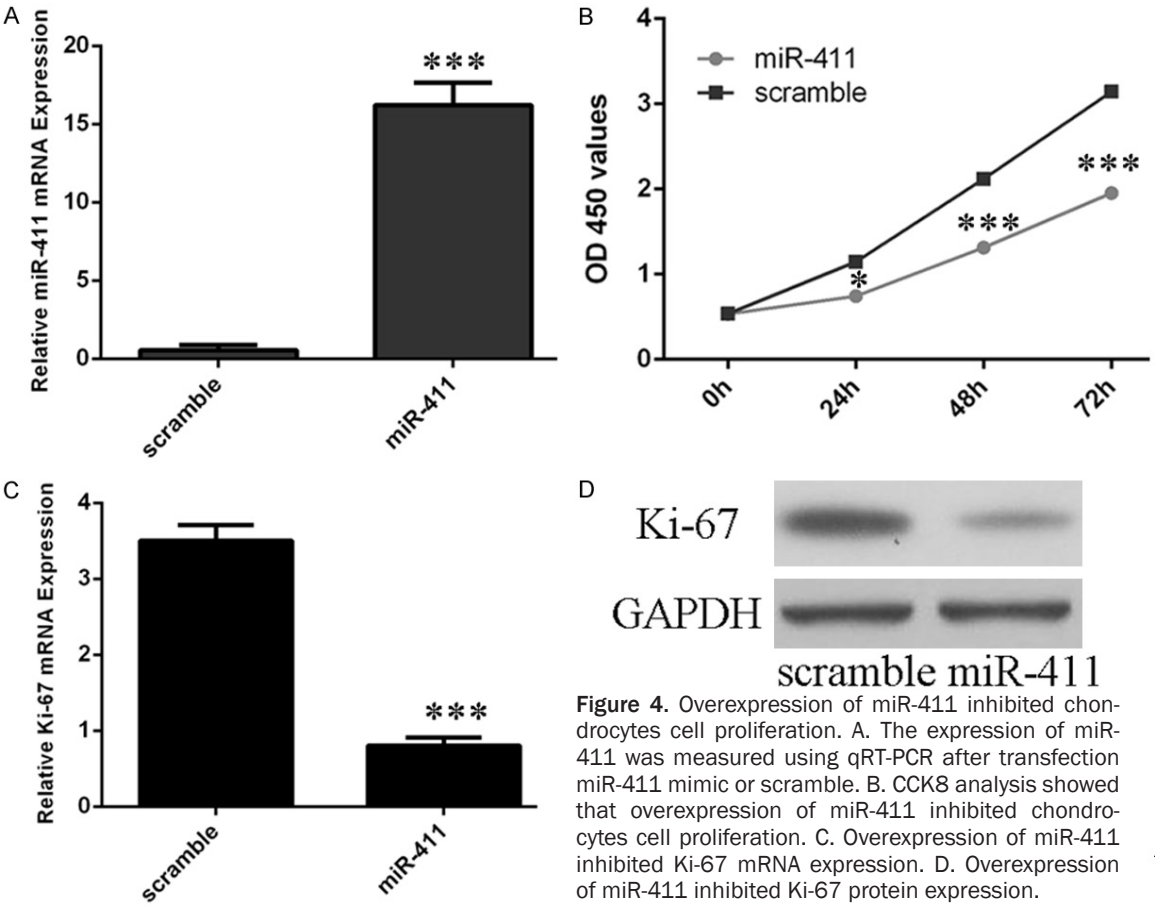

Figure 4. Overexpression of miR-411 inhibited chondrocytes cell proliferation. A. The expression of miR-411 was measured using qRT-PCR after transfection miR-411 mimic or scramble. B. CCK8 analysis showed that overexpression of miR-411 inhibited chondrocytes cell proliferation. C. Overexpression of miR-411 inhibited Ki-67 mRNA expression. D. Overexpression of miR-411 inhibited Ki-67 protein expression.

allel with the decrease of miR-411 expression, treatment of IL-1β stimulated MMP-13 expression (**Figure 2B**).

MMP-13 is a direct target of miR-411

We found a potential miR-411 binding sequence in the 3'UTR of MMP-13 by using miRNA target prediction software (**Figure 3A**). Overexpression of miR-411 inhibited the protein expression of MMP-13. Luciferase reporter assay showed that treatment with miR-411 mimic repressed reporter activity (**Figure 3C**).

Overexpression of miR-411 inhibited chondrocytes cell proliferation

qRT-PCR analysis demonstrated that miR-411 mimic can enhance the miR-411 expression in chondrocytes (**Figure 4A**). CCK8 analysis showed that overexpression of miR-411 inhibited chondrocytes cell proliferation (**Figure 4B**). qRT-PCR and western blot analysis demonstrated overexpression of miR-411 inhibited mRNA and protein expression of Ki-67 in chondrocytes (**Figure 4C** and **4D**).

Overexpression of miR-411 increased the expression of the type II collagen and type IV collagen

RT-PCR and western blot analysis demonstrated overexpression of miR-411 increased mRNA and protein expression of type II collagen in chondrocytes (**Figure 5A** and **5B**). Overexpression of miR-411 also enhanced mRNA and protein expression of type IV collagen in chondrocytes (**Figure 5C** and **5D**).

Discussion

In this study, miR-411 was downregulated in OA cartilage compared with in normal cartilage. Conversely, MMP-13 was upregulated in OA cartilage compared with in normal cartilage. IL-1β treatment repressed miR-411 expression in chondrocytes. Moreover, we identified MMP-13 as a direct target gene of miR-411 in chondrocytes and overexpression of miR-411 inhibited the MMP-13 expression. Furthermore, overexpression of miR-411 increased the expression of the type II collagen and type IV collagen in chondrocytes. Taken together, miR-

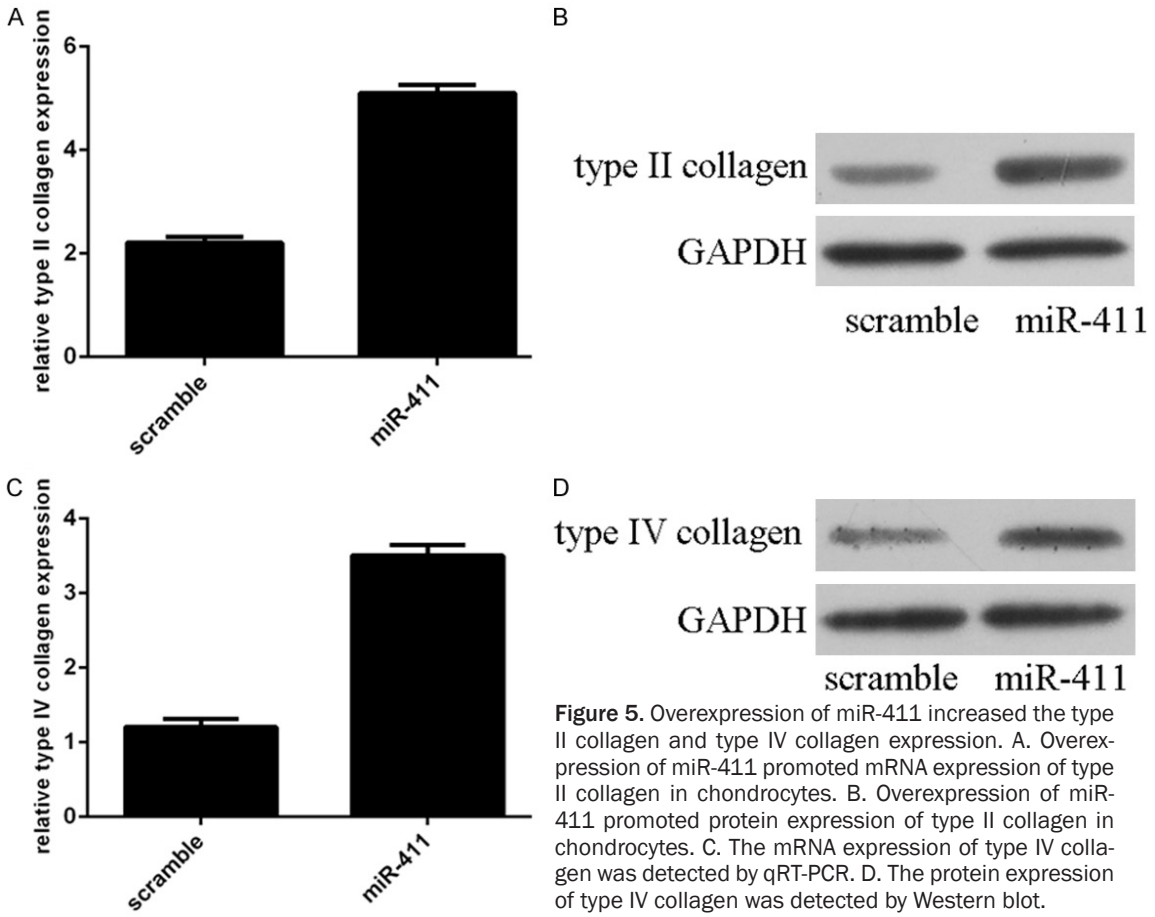

Figure 5. Overexpression of miR-411 increased the type II collagen and type IV collagen expression. A. Overexpression of miR-411 promoted mRNA expression of type II collagen in chondrocytes. B. Overexpression of miR-411 promoted protein expression of type II collagen in chondrocytes. C. The mRNA expression of type IV collagen was detected by qRT-PCR. D. The protein expression of type IV collagen was detected by Western blot.

411 acts as a crucial regulator of the MMP-13 and ECM synthesis and degradation in human chondrocytes.

Harafuji et al [29] reported that the expression of miR-411 was increased in primary immortalized and facioscapulohumeral muscular dystrophy (FSHD) myoblasts in comparison with in control myoblasts. They also identified YAF2 as a direct target of miR-411 and overexpression of miR-411 inhibited Myod, Myh1, and myogenin in C2C12 cells. Xia et al [30] demonstrated that miR-411 was downregulated in hepatocellular carcinoma cells and tissues. Overexpression of miR-411 increased hepatocellular carcinoma cells anchorage-independent growth and proliferation by regulating ITCH expression. However, the role of miR-411 in OA remains unknown. In our study, miR-411 was downregulated in OA cartilage compared with normal cartilage. Moreover, IL-1β treatment repressed miR-411 expression in chondrocytes. There results suggest that miR-411 play important roles in pathogenesis and development of OA.

Proteolytic degradation of cartilage by matrix-degrading enzymes is a hallmark of OA [2, 31]. MMp-13 is a critical MMP collagenase and belongs to a family of extracellular matrix-degrading endopeptidases [8, 32, 33]. Previous studies demonstrated that MMP-13 was at low levels in articular cartilage during physiologic ECM turnover and is overexpression in human OA [34-36]. MMP-13 could degrade type 2 collagen and aggrecan [37]. Several cytokines are capable to stimulate MMP-13 expression in human OA [38, 39]. In line with previous data, we demonstrated that MMP-13 was upregulated in human OA compared with normal cartilage. Moreover, treatment of IL-1β stimulated MMP-13 expression and repressed miR-411 expression in chondrocytes. Furthermore, we identified MMP-13 as a direct target gene of miR-411 and overexpression of miR-411 inhibited the MMP-13 expression. We also showed that ectopic expression of miR-411 inhibited chondrocytes cell proliferation and increased the type II collagen and type IV collagen expression.

In conclusion, miR-411 was downregulated in human OA, acting as a crucial regulator of the MMP-13 and catabolic signaling pathways in chondrocytes. Our data provide an insight into the roles of miRNA in OA pathogenesis and provide the possibility of miR-411 as a therapeutic target for the treatment of OA.

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References

[1] Huang K and Wu LD. Aggrecanase and aggrecan degradation in osteoarthritis: a review. J Int Med Res 2008; 36: 1149-1160.

[2] Li W, Wu M, Jiang S, Ding W, Luo Q and Shi J. Expression of ADAMTs-5 and TIMP-3 in the condylar cartilage of rats induced by experimentally created osteoarthritis. Arch Oral Biol 2014; 59: 524-529.

[3] Cutolo M, Berenbaum F, Hochberg M, Punzi L and Reginster JY. Commentary on recent therapeutic guidelines for osteoarthritis. Semin Arthritis Rheum 2015; 44: 611-617.

[4] Campos GC, Rezende MU, Pasqualin T, Frucchi R and Bolliger Neto R. Lateral wedge insole for knee osteoarthritis: randomized clinical trial. Sao Paulo Med J 2015; 133: 13-19.

[5] Brody LT. Knee osteoarthritis: Clinical connections to articular cartilage structure and function. Phys Ther Sport 2015; 16: 301-16.

[6] Pathak NN, Balaganur V, Lingaraju MC, Kant V, Kumar D, Sharma AK and Tandan SK. Effect of atorvastatin, a HMG-CoA reductase inhibitor in monosodium iodoacetate-induced osteoarthritic pain: Implication for osteoarthritis therapy. Pharmacol Rep 2015; 67: 513-519.

[7] Yan D, Chen D, Hawse JR, van Wijnen AJ and Im HJ. Bovine lactoferricin induces TIMP-3 via the ERK1/2-Sp1 axis in human articular chondrocytes. Gene 2013; 517: 12-18.

[8] Piecha D, Weik J, Kheil H, Becher G, Timmermann A, Jaworski A, Burger M and Hofmann MW. Novel selective MMP-13 inhibitors reduce collagen degradation in bovine articular and human osteoarthritis cartilage explants. Inflamm Res 2010; 59: 379-389.

[9] Lee YJ, Lee EB, Kwon YE, Lee JJ, Cho WS, Kim HA and Song YW. Effect of estrogen on the expression of matrix metalloproteinase (MMP)-1, MMP-3, and MMP-13 and tissue inhibitor of metalloproternase-1 in osteoarthritis chondrocytes. Rheumatol Int 2003; 23: 282-288.

[10] Kalva S, Saranyah K, Suganya PR, Nisha M and Saleena LM. Potent inhibitors precise to S1' loop of MMP-13, a crucial target for osteoarthritis. J Mol Graph Model 2013; 44: 297-310.

[11] Li M, Yu M, Liu C, Zhu H, He X, Peng S and Hua J. miR-34c works downstream of p53 leading to dairy goat male germline stem-cell (mGSCs) apoptosis. Cell Prolif 2013; 46: 223-231.

[12] Li Z, Yu X, Shen J, Wu WK and Chan MT. MicroRNA expression and its clinical implications in Ewing's sarcoma. Cell Prolif 2015; 48: 1-6.

[13] Luo X, Dong Z, Chen Y, Yang L and Lai D. Enrichment of ovarian cancer stem-like cells is associated with epithelial to mesenchymal transition through an miRNA-activated AKT pathway. Cell Prolif 2013; 46: 436-446.

[14] Ohdaira H, Sekiguchi M, Miyata K and Yoshida K. MicroRNA-494 suppresses cell proliferation and induces senescence in A549 lung cancer cells. Cell Prolif 2012; 45: 32-38.

[15] Fu LL, Zhao X, Xu HL, Wen X, Wang SY, Liu B, Bao JK and Wei YQ. Identification of microRNA-regulated autophagic pathways in plant lectin-induced cancer cell death. Cell Prolif 2012; 45: 477-485.

[16] Yu X, Li Z and Liu J. MiRNAs in primary cutaneous lymphomas. Cell Prolif 2015; 48: 271-277.

[17] Li Z, Lei H, Luo M, Wang Y, Dong L, Ma Y, Liu C, Song W, Wang F, Zhang J, Shen J and Yu J. DNA methylation downregulated mir-10b acts as a tumor suppressor in gastric cancer. Gastric Cancer 2015; 18: 43-54.

[18] Li Z, Yu X, Wang Y, Shen J, Wu WK, Liang J and Feng F. By downregulating TIAM1 expression, microRNA-329 suppresses gastric cancer invasion and growth. Oncotarget 2015; 6: 17559-17569.

[19] Yu X, Li Z, Shen J, Wu WK, Liang J, Weng X and Qiu G. MicroRNA-10b Promotes Nucleus Pulposus Cell Proliferation through RhoC-Akt Pathway by Targeting HOXD10 in Intervetebral Disc Degeneration. PLoS One 2013; 8: e83080.

[20] Yu C, Chen WP and Wang XH. MicroRNA in osteoarthritis. J Int Med Res 2011; 39: 1-9.

[21] Steck E, Boeuf S, Gabler J, Werth N, Schnatzer P, Diederichs S and Richter W. Regulation of H19 and its encoded microRNA-675 in osteoarthritis and under anabolic and catabolic in vitro conditions. J Mol Med (Berl) 2012; 90: 1185-1195.

[22] Swingler TE, Wheeler G, Carmont V, Elliott HR, Barter MJ, Abu-Elmagd M, Donell ST, Boot-Handford RP, Hajihosseini MK, Munsterberg A,

Dalmay T, Young DA and Clark IM. The expression and function of microRNAs in chondrogenesis and osteoarthritis. Arthritis Rheum 2012; 64: 1909-1919.

[23] Li X, Gibson G, Kim JS, Kroin J, Xu S, van Wijnen AJ and Im HJ. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. Gene 2011; 480: 34-41.

[24] Wu Z, Yin H, Liu T, Yan W, Li Z, Chen J, Chen H, Wang T, Jiang Z, Zhou W and Xiao J. MiR-126-5p regulates osteoclast differentiation and bone resorption in giant cell tumor through inhibition of MMP-13. Biochem Biophys Res Commun 2014; 443: 944-949.

[25] Liang ZJ, Zhuang H, Wang GX, Li Z, Zhang HT, Yu TQ and Zhang BD. MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1beta-stimulated human articular chondrocyte C28/I2 cells. Inflamm Res 2012; 61: 503-509.

[26] Tardif G, Hum D, Pelletier JP, Duval N and Martel-Pelletier J. Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes. BMC Musculoskelet Disord 2009; 10: 148.

[27] Goldring MB. Immortalization of human articular chondrocytes for generation of stable, differentiated cell lines. Methods Mol Med 2004; 100: 23-36.

[28] Lago R, Gomez R, Otero M, Lago F, Gallego R, Dieguez C, Gomez-Reino JJ and Gualillo O. A new player in cartilage homeostasis: adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes. Osteoarthritis Cartilage 2008; 16: 1101-1109.

[29] Harafuji N, Schneiderat P, Walter MC and Chen YW. miR-411 is up-regulated in FSHD myoblasts and suppresses myogenic factors. Orphanet J Rare Dis 2013; 8: 55.

[30] Xia K, Zhang Y, Cao S, Wu Y, Guo W, Yuan W and Zhang S. miR-411 regulated ITCH expression and promoted cell proliferation in human hepatocellular carcinoma cells. Biomed Pharmacother 2015; 70: 158-163.

[31] Morris KJ, Cs-Szabo G and Cole AA. Characterization of TIMP-3 in human articular talar cartilage. Connect Tissue Res 2010; 51: 478-490.

[32] Neuhold LA, Killar L, Zhao W, Sung ML, Warner L, Kulik J, Turner J, Wu W, Billinghurst C, Meijers T, Poole AR, Babij P and DeGennaro LJ. Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. J Clin Invest 2001; 107: 35-44.

[33] Higashiyama R, Miyaki S, Yamashita S, Yoshitaka T, Lindman G, Ito Y, Sasho T, Takahashi K, Lotz M and Asahara H. Correlation between MMP-13 and HDAC7 expression in human knee osteoarthritis. Mod Rheumatol 2010; 20: 11-17.

[34] Niebler S, Schubert T, Hunziker EB and Bosserhoff AK. Activating enhancer binding protein 2 epsilon (AP-2epsilon)-deficient mice exhibit increased matrix metalloproteinase 13 expression and progressive osteoarthritis development. Arthritis Res Ther 2015; 17: 119.

[35] Lim H, Min DS, Kang Y, Kim HW, Son KH and Kim HP. Inhibition of matrix metalloproteinase-13 expression in IL-1beta-treated articular chondrocytes by a steroidal saponin, spicatoside A, and its cellular mechanisms of action. Arch Pharm Res 2015; 38: 1108-1116.

[36] Yammani RR and Loeser RF. Brief report: stress-inducible nuclear protein 1 regulates matrix metalloproteinase 13 expression in human articular chondrocytes. Arthritis Rheumatol 2014; 66: 1266-1271.

[37] Nummenmaa E, Hamalainen M, Moilanen T, Vuolteenaho K and Moilanen E. Effects of FGF-2 and FGF receptor antagonists on MMP enzymes, aggrecan, and type II collagen in primary human OA chondrocytes. Scand J Rheumatol 2015; 44: 321-30.

[38] Long DL, Ulici V, Chubinskaya S and Loeser RF. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is increased in osteoarthritis and regulates chondrocyte catabolic and anabolic activities. Osteoarthritis Cartilage 2015; 23: 1523-1531.

[39] Zhong Y, Huang Y, Santoso MB and Wu LD. Sclareol exerts anti-osteoarthritic activities in interleukin-1beta-induced rabbit chondrocytes and a rabbit osteoarthritis model. Int J Clin Exp Pathol 2015; 8: 2365-2374.

Table S1. Primer sequence

Name	Sequence (5'-3')
Ki-67	TCCTTTGGTGGGCACCTAAGACCTG
	TGATGGTTGAGGTCGTTCCTTGATG
MMP-13	TGCTTCCTGATGACGATGTAC
	TCCTCGGAGACTGGTAATGG
GAPDH	GACTCATGACCACAGTCCATGC
	AGAGGCAGGGATGATGTTCTG
Type II collagen	AAGGTGCTTCTGGTCCTGCTG
	GGGATTCCATTAGCACCATCTTTG
Type IV collagen	ACTCATTCCAACCGTCTGTCAGC
	GCAAATCATTGACAGTGGCGTCTA