Original Paper

Long Noncoding RNA CIR Promotes Chondrocyte Extracellular Matrix Degradation in Osteoarthritis by Acting as a Sponge For Mir-27b

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Key Words

Osteoarthritis (OA) • LncRNA-CIR • MiR-27 • MMP13 • Chondrocytes

Abstract

Background/Aims: Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation. The degradation of the extracellular matrix (ECM) of chondrocyte is closely associated with the destruction of joints in OA patients. lncRNAs are non-coding segments of RNA that possess important regulatory functions at the cellular level and in a variety of pathophysiological processes. The present study was conducted to investigate whether lncRNA-CIR regulated the expression of MMP13 as a sponge of miR-27 in OA. **Methods:** Primary cultured chondrocytes were challenged by IL-1 β and TNF- α to simulate OA conditions. qRT-PCR was performed to detect the miR-27, lncRNA-CIR, MMP13 mRNA expression levels. Western blot was applied to detect MMP13 protein expression. Soluble sGAG secretion/formation was analysed by the dimethylmethylene blue (DMMB) assay. lncRNA-CIR overexpression or inhibition was performed using overexpression plasmid and small interfering RNAs (siRNAs), respectively. **Results:** lncRNA-CIR significantly up-regulated in OA patients, concomitantly down-regulated miR-27 and up-regulated MMP13. Bioinformatics analysis predicted miR-27 was the target of both lncRNA-CIR and MMP13. Overexpression of lncRNA-CIR significantly increased the expression of MMP13, while miR-27 remarkably suppressed the expression of MMP13, Accompanying with the increases of mRNA level, protein level and relative luciferase activity. **Conclusion:** The present findings indicated that lncRNA-CIR/miR-27/MMP13 axis involved in the degradation of the ECM of chondrocyte in OA.

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Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation and is the leading cause of pain, physical debilitation, and shortening of the adult working age throughout the world [1]. OA is associated with chronic joint injury, dysfunction of immune response, and inflammation, which is involved in the degradation

of the ECM of chondrocyte [2]. As the main ingredients of ECM in chondrocyte, collagen II and MMPs and a disintegrin and a metalloproteinase domain with thrombospondin motif family (ADAMTS) maintained homeostasis [3]. It has been reported that metalloproteinase expression is up-regulated in cartilage and challenged by inflammatory mediators such as IL-1 and TNF- α [2, 4]. However, the underlying mechanisms involved in articular cartilage degradation in OA have not been fully established. This partially explains why there is no approved treatment, which could ameliorate the disease progression, hampered therapeutic options for patients.

As we all know, Non-coding RNAs (ncRNAs), including tRNAs, snRNAs, snoRNAs, miRNAs and long ncRNAs, accounting for a large portion of human transcriptome compared to protein coding genes, are currently known to play crucial roles both in basic biology and in major pathologies such as cancer via regulating structural scaffolds, chromatin modification, and regulating gene expression at both the transcriptional and posttranscriptional levels [5]. Several studies have indicated that altered levels of miRNAs and lncRNAs could result in aberrant expressions of a gene that contributes to a variety of disease states and biological functions [6-8]. Literature has reported that alterations in non-coding genes can contribute to OA pathogenesis [9]. Synovial fluid microRNA signature is correlated with knee osteoarthritis stage, and microRNA-181a-5p and microRNA-4454 are known mediators of facet cartilage degeneration [10, 11]. In addition, recent evidence has shown that miR-27b and lncRNA-CIR contribute to ECM degradation and have key roles in the pathogenesis of OA [2, 6]. However, the role of lncRNAs in cartilage and their overall contribution to the degradation of ECM and the pathogenesis of OA has not been fully elucidated.

The present study was designed to investigate the interaction among miR-27b, lncRNA-CIR and MMP13 in the pathogenesis of OA and to determine their role in chondrocyte ECM degradation.

Materials and Methods

Patients and specimens

Patients diagnosed with OA and health subjects (with normal joint) were included in this study. OA cartilage was isolated from the knee joints of 30 patients undergoing total knee arthroplasty (19 men and 11 women; age range 55-64 years; BMI range 20.3-23.2), and normal articular cartilage was isolated from the knee joints of 8 donors after death or trauma (4 men and 4 women; age range 32-38 years). All tissue was stained with Safranin O, and changes were graded according to a modified Mankin scale [12]. Informed consent was obtained from all tissue donors included in the current study. The study was approved by the Human Ethics Committee of Hunan Normal University (China).

Primary culture of chondrocytes and exposure to IL-1 β , TNF- α stress

The primary culture of the donor chondrocytes were conducted as following steps: digested with 0.25% trypsin for 30 min, incubated with 0.2% type II collagenase for 4 h at 37°C, maintained in Dulbecco's modified Eagle's medium (DMEM), which was supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 units/ml of streptomycin. Chondrocytes (5×10^5 /well) were grown on ProNectin F-coated Bioflex 6-well culture plates to 70% confluence. Then, cells were subjected to the stimulation of 10 ng/ml of IL-1 β or TNF- α for 12h.

Measurement of mRNA expression

Real-time PCR was used to analyze the miRNA, lncRNA and mRNA levels in the cartilage. Total RNA was extracted by using TRIzol reagent (TaKaRa, Dalian, China); the concentration and purity of RNA were determined spectrophotometrically. Two hundred ng of RNA from each sample was used for reverse transcription reaction conducted according to a transcription Kit (DRR037A; TaKaRa, Dalian, China). The real-time PCR was used for determining the miR-27, lncRNA-CIR, MMP13 mRNA expression levels by SYBR Premix Ex Taq (TaKaRa Dalian, China) at ABI 7300 quantitatively. The real-time PCR primers for MMP13, lncRNA-CIR and β -actin are displayed in Table 1. Data analysis was performed using the comparative Ct method, with ABI software. The result was adjusted by the ratio of MMP13mRNA to β -actin mRNA.

Table 1. Primers for real-time PCR

Gene	Forward primer	Reverse primer	Product size (bp)
MMP13	5'-AAGACTTCCCAGGAATTGGTGA-3'	5'-CAAGAAAGTTGGGTAACACCACA-3'	164
β -actin	5'-ACAGAGCCTCGCCTTTGC-3'	5'-CCACCATCACGCCCTGG-3'	150
lncRNA	5'-ACACTTGCAAGCCTGGGTAG-3'	5'-CCATTTCCTGTTGGTGCGG-3'	151

Determination of MMP13 protein expression

The total protein of each sample was extracted using a modified version of the method described by Zhang *et al.* 40 μ g protein was used for Western blot analysis according to the following steps: SDS-PAGE (10% gel), transferred to polyvinylidene fluoride (PVDF) membranes, incubated with rabbit anti-MMP13 (Santa Cruz, CA, USA) followed by horseradish peroxidase-conjugated secondary antibodies, determined the signals of bands by enhanced chemiluminescence (ECL kit, Amersham Biosciences, Piscataway, NJ, USA) through Molecular Imager ChemiDoc XRS System (Bio-Rad, Philadelphia, USA). The densitometric analysis was conducted with Image J 1.43 (National Institutes of Health). To ensure equal loading, blots were incubated with an antibody of mouse anti- β -actin (Millipore, Billerica, MA, USA).

Measurement of sulfated glycosaminoglycan (sGAG)

Dimethylmethylene blue (DMMB) assay was used for soluble sGAG secretion/formation of cells according to the method described by Farndale RW *et al.*, with a bit of modification [ENREF_1213]. Briefly, a mix was compared with 20 μ l of cell suspension and 200 μ l of DMMB reagent, and the absorbance was detected at a wavelength of 525nm. Total sGAGs were normalized to a standard curve, as well as the total protein content in cell lysates from each group, which was measured using a BCA protein assay kit.

Plasmid construction, RNA interference, and transfection

The full-length lncRNA-CIR was introduced into the pEGFP-C1 vector, the correct constructs were verified using DNA sequencing, and then designated as plncRNA-CIR. Small interfering RNAs (siRNAs) against lncRNA-CIR (named si-CIR) were designed and synthesized by RiboBio. For miRNA transfection, miR-27 mimics, miR-27 inhibitors and negative control (RiboBio) were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Luciferase assay

lncRNA-MSR that contains the putative binding site for miR-27 and its identical sequence with a mutation of the miR-27 seed sequence, were inserted between the restrictive sites Xho I and Not I of the pmiR-RB-ReportTM and validated using sequencing. The pmiR-RB-ReportTM luciferase reporter vectors of the lncRNA-CIR and MMP13 3'-UTR were constructed as above. Chondrocyte cells were transfected with wild type or mutated reporter vectors, miRNA mimics or negative control. Lysates were harvested 24 h after transfection. Renilla luciferase activities were consecutively measured according to the dual-luciferase assay manual (Promega).

Statistical analysis

SPSS software (Version 11, SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Data were expressed as mean $\pm$ SEM. Differences in measured values among the multiple groups were analyzed by the analysis of variance with Bonferroni's multiple comparison tests. The χ^2 test was used to compare the data of frequency distributions between the groups. Differences were considered significant when $P < 0.05$.

Results

Differences in expression of genes in cartilage in OA patients

The expression of significantly upregulated lncRNA-CIR in chondrocytes in OA patients compared to health controls is shown in Fig. 1A. To explore the function of lncRNA-CIR in OA, we conducted a bioinformatics analysis to predict its potential target miRNA. We found miR-27 can bind to lncRNA-CIR or MMP13 in a complementary manner. Thus we measured

Fig. 1. Some differently expressed genes in cartilage in OA patients. A. lncRNA-CIR expression level; B. miR-27 expression level; C. MMP13 mRNA expression level; D. MMP13 protein expression level. OA: osteoarthritis. All values were expressed as means $\pm$ SEM. * $P < 0.005$ vs control.

Fig. 2. Effect of IL-1, TNF- α on the expression of gene in cartilage. A. lncRNA-CIR expression level; B. miR-27 expression level; C. MMP13 mRNA expression level; D. MMP13 protein expression level. OA: osteoarthritis; +IL-1: Interleukin (10ng/ml); +TNF- α : tumor necrosis factor α (10ng/ml). All values were expressed as means $\pm$ SEM. * $P < 0.05$ vs control.

the expression of miR-27 and MMP13 as well. As shown in Fig. 1, miR-27 remarkably downregulated, and MMP13 obviously upregulated at both mRNA and proteins in OA, when compared to controls (Fig. 1 B, C and D).

Effect of IL-1, TNF- α on the expression of gene in cartilage

To confirm the speculation that inflammation could induce chondrocyte injury by degradation of extracellular matrix of cartilage, we added 10 ng/ml of IL-1 or TNF- α into the culture to simulate the environment of chondrocyte in OA patients. Then we measured the gene expressions. In line with the results of Fig. 1, cells in conditions of inflammation cause an upregulation lncRNA-CIR and MMP13, and downregulated miR-27 compared to cells without cytokines challenge, as shown in Fig. 2. This suggests that inflammation may

Fig. 3. The effects of lncRNA-CIR on the expression of gene and the degradation of the ECM in chondrocytes. lncRNA-CIR expression level; B miR-27 expression level; C. MMP13 mRNA expression level; D. lncRNA-CIR expression level; E. miR-27 expression level; F. MMP13 mRNA expression level; G and H. sGAG content in culture. OA: osteoarthritis; +NC siRNA: negative control siRNA; +CIR siRNA: lncRNA-CIR siRNA; +NC vector: negative control vector; +lncRNA-CIR: lncRNA-CIR overexpression plasmid. All values were expressed as means $\pm$ SEM. *P < 0.05 vs control. **P < 0.01 vs control.

be an important cause for osteoarthritis. Additionally, the lncRNA-CIR/ miR-27/MMP13 axis may be involved in chondrocyte survival.

The effects of lncRNA-CIR on the expression of gene and the degradation of the ECM in chondrocytes

To investigate the role of lncRNA-CIR in osteoarthritis, we constructed lncRNA-CIR over-expression plasmid to increase the expression of lncRNA-CIR. We also designed a small interfering RNA to specifically suppress the expression of lncRNA-CIR. Then, we observed the effect of overexpression lncRNA-CIR and siRNA-CIR on gene expression and the degradation of the extracellular matrix in chondrocytes. As shown in Fig. 3 A, B and C, siRNA of lncRNA-CIR significantly suppressed the expression of lncRNA-CIR and obviously increased the expression of miR-27, concomitantly with downregulation MMP13 expression. However, overexpression of lncRNA-CIR by transfection of lncRNA-CIR plasmid significantly increased the expression of lncRNA-CIR, remarkably downregulated the expression of miR-27, and accompanied overt upregulation of MMP13 (Fig. 3 D, E, F). Meanwhile, we also measured the content of sulfated glycosaminoglycan (sGAG) secreted by chondrocytes in culture, as shown in Fig. 3 G and H, cells challenged by siRNA of lncRNA-CIR with higher sGAG content

Fig. 4. The effects of lncRNA-CIR on MMP13 expression as a ceRNA in chondrocytes. A and B. relative luciferase activity; C. MMP13 mRNA expression level; D. lncRNA-CIR expression level; E, H and I. MMP13 protein expression level; F and G. MMP13 mRNA expression level. +NC vector: negative control vector; +CIR-WT: report gene containing wild type lncRNA-CIR 3'UTP; +CIR-MU: report gene containing mutated lncRNA-CIR 3'UTP; +miR-27: miR-27 mimics; +MMP13-WT: report gene containing wild type MMP13 3'UTP; +MMP13-MU: report gene containing mutated MMP13 3'UTP; +NC miRNA: negative control miRNA; +CIR-siRNA: specific siRNA of lncRNA-CIR; +lncRNA-CIR: lncRNA-CIR overexpression plasmid. All values were expressed as means $\pm$ S.E.M. * $P < 0.05$ vs control. ** $P < 0.01$ vs control.

compared to controls, and cells transfected with overexpression plasmid of lncRNA-CIR with lower (compared to controls) content of sGAG. The aforementioned results suggest that lncRNA-CIR is involved in the degradation of the ECM in chondrocytes.

The effects of lncRNA-CIR on MMP13 expression as a ceRNA in chondrocytes

To confirm the interaction of miR-27 with lncRNA-CIR or MMP13, luciferase report gene were constructed. Chondrocytes were cotransfected with both luciferase report gene of lncRNA-CIR or MMP13 and miR-27 mimics. Relative luciferase activity was measured, and we found that miR-27 significantly suppressed the luciferase activity in cells challenged by lncRNA-CIR or MMP13 report gene compared to negative vector or mutated lncRNA-CIR or MMP13 report gene (Fig. 4A and B). We then measured the expression of lncRNA-CIR and MMP13, consistently with the results of relative luciferase activity: miR-27 significantly suppressed the expression of lncRNA-CIR and MMP13, in contrast to the miR-27 mimics intervening experiment, in which the miR-27 inhibitor slightly increased the expression of lncRNA-CIR and MMP13 (Fig. 4 C, D and E), but without statistical significance. Then we

conducted a cotransfection experiment, in which cells were transfected with both lncRNA-CIR and miR-27 mimics, or both lncRNA-CIR siRNA and miR-27 inhibitor. As shown in Fig. 4, sole transfection of lncRNA-CIR remarkably upregulated the expression of MMP13, and cotransfection of lncRNA-CIR and miR-27 mimics significantly downregulated the expression of MMP13 (Fig. 4F, H). In contrast, sole transfection of lncRNA-CIR siRNA obviously downregulated the expression of MMP13. miR-27 inhibitor overtly reversed the expression of MMP13, as shown in Fig. 4 G and I.

Discussion

Our present results demonstrated that endogenous lncRNA-CIR serves as sponge of miR-27b and is involved in the expression regulation of MMP13. Overexpression of lncRNA-CIR and MMP13 and low expression of miR-27b were found in OA patients, which suggests that there is a close relationship among them. Through gaining of function and loss of function approaches *in vitro*, we demonstrated that miR-27b directly binds with the 3'UTR of both lncRNA-CIR and MMP13. This study found that lncRNA-CIR/miR-27/MMP13 axis plays an important role in the degradation of chondrocyte ECM.

Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation and is the leading cause of physical disability [14]. Despite its high prevalence and substantial public health impact, OA etiology is not fully understood. Previous studies have reported that degradation of extracellular matrix of chondrocytes is the common cause for OA, and MMP overexpression is involved in such degradation [2]. But the detailed mechanism of ECM degradation is still not well understood. Previous studies have shown that aberrant expressions of cytokines and growth factors were closely related to OA and lead to degradation of the ECM [15-19], which is consistent with our present study. As we know, OA is a disease associated with age-related loss of homeostatic balance. At the same time, ECM degradation induced chondrocyte death is the main pathophysiological mechanism for the destruction of cartilage cellularity. However, the process of degradation of the ECM is complicated, for it involves genetic, developmental, biochemical, and biomechanical factors [20]. MMP-13 is an ECM-degrading enzyme produced in osteoarthritic joints. Under physiological situations, MMP-13 acts as an important regulator of tissue modelling and repair. Upon pathophysiological situations, such as inflammation, overexpression of MMP-13 causes pathological changes through excessive ECM degradation [7, 21]. In present study, we found that high expression of MMP13 is closely associated with OA. Therefore, clarifying the exact mechanism responsible for the expression regulation of MMP13 will lead to better understanding of the degradation of ECM of chondrocyte.

Several lines of evidence have reported that miR-27b is a potential therapeutic target in tumors for its involvement in tumor angiogenesis, proliferation, metastasis, and drug resistance [22, 23]. However, it has been well established that miRNAs are involved in OA. Several miRNAs have been identified as having aberrant expression levels in osteoarthritis, such as miR-9, miR-27, miR-34a, miR-140, miR-146a, miR-558 and miR-602 [7, 19, 24]. Increasing amounts of evidence have indicated that there is an existing link between miRNAs and osteoarthritis in the pathogenesis of OA. Further study has confirmed that miR-9 and miR-98 were upregulated in both cartilage and bone from OA patients when compared to non-OA specimens. Subsequent functional studies have verified involvement of both miR-9 and miR-98 in the inflammatory pathway via inhibition of IL-1 β induced MMP-13 protein release [7]. In present study, we found that miR-27b significantly downregulated in OA patients, and that its expression was inversely related to the expression of MMP13. Thus we speculated that there exists a close relationship between miR-27b and MMP13. Subsequent data found that miR-27b suppresses MMP13 by directly targeting its 3'UTR, which is consistent with previous reports [6]. This suggests that microRNAs including miR-27b, which act as an important therapy target for OA, are involved in the pathogenesis of OA. In view of this, we speculate that targeting miR-27b as a treatment for OA prevention and treatment will potentially provide a new therapeutic strategy.

It has been widely accepted that endogenous lncRNAs play important roles in the expression regulation of miRNAs and are involved in a variety of biological processes, such as cell cycle control, immune response regulation, cell differentiation and maintenance of embryonic stem cell pluripotency [25]. lncRNAs are also involved in the pathogenesis of OA. Several lines of evidence have demonstrated that lncRNAs, such as H19[26], MEG3, PCGEM1[27], GAS5[28], HOTAIR, CIR, UFC1, GM4419 ameliorate cartilage injury and degradation. Emerging evidence has supported the theory that lncRNAs might act as promising therapeutic targets of OA. Literatures has reported that TMSB4 pseudogene lncRNA functions as a competing endogenous RNA to promote cartilage degradation in human osteoarthritis [2]. Meanwhile, other studies have revealed that long noncoding RNA UFC1 promotes proliferation of chondrocyte in osteoarthritis by acting as a sponge for miR-34a [29]. In the present study, we found that lncRNA-CIR upregulates in OA patients, which is consistent with previous studies [1]. Further functional study demonstrates that miR-27b inhibits lncRNA-CIR by directly targeting its 3'UTR. Based on the aforementioned data, we speculate that lncRNA-CIR plays pivotal role in OA. As a matter of fact, many reports have found that lncRNA-CIR is involved in ECM degradation [1, 30]. Thus, it is essential to fully understand the detailed mechanism of lncRNA-CIR in OA.

lncRNA-CIR plays an important role in gene expression regulation in OA. Deciphering the precise molecular mechanisms of lncRNA-CIR function in OA will be critical to understanding the pathogenesis of OA and exploring new potential types of therapy. In conclusion, to our best knowledge, this study was the first to find that lncRNA-CIR acts as a sponge of miR-27b, and that the lncRNA-CIR/miR-27b/MMP13 pathway is involved in the degradation of ECM in OA.

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Disclosure Statement

The authors state no conflict of interest.

References

- Pearson MJ, Philp AM, Heward JA, Roux BT, Walsh DA, Davis ET, Lindsay MA, Jones SW: Long intergenic noncoding rnas mediate the human chondrocyte inflammatory response and are differentially expressed in osteoarthritis cartilage. *Arthritis Rheumatol* 2016;68:845-856.
- Liu Q, Zhang X, Dai L, Hu X, Zhu J, Li L, Zhou C, Ao Y: Long noncoding rna related to cartilage injury promotes chondrocyte extracellular matrix degradation in osteoarthritis. *Arthritis Rheumatol* 2014;66:969-978.
- Diaz-Prado S, Cicione C, Muinos-Lopez E, Hermida-Gomez T, Oreiro N, Fernandez-Lopez C, Blanco FJ: Characterization of microrna expression profiles in normal and osteoarthritic human chondrocytes. *BMC Musculoskelet Disord* 2012;13:144.
- Blain EJ, Mason DJ, Duance VC: The effect of thymosin beta4 on articular cartilage chondrocyte matrix metalloproteinase expression. *Biochem Soc Trans* 2002;30:879-882.
- Cui Z, Ren S, Lu J, Wang F, Xu W, Sun Y, Wei M, Chen J, Gao X, Xu C, Mao JH: The prostate cancer-up-regulated long noncoding rna plncrna-1 modulates apoptosis and proliferation through reciprocal regulation of androgen receptor. *Urol Oncol* 2013;31:1117-1123.
- Akhtar N, Rasheed Z, Ramamurthy S, Anbazhagan AN, Voss FR, Haqqi TM: Microrna-27b regulates the expression of matrix metalloproteinase 13 in human osteoarthritis chondrocytes. *Arthritis Rheum* 2010;62:1361-1371.
- Nugent M: Micrornas: Exploring new horizons in osteoarthritis. *Osteoarthritis Cartilage* 2016;24:573-580.
- Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y,

- Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY: Long non-coding rna hotair reprograms chromatin state to promote cancer metastasis. *Nature* 2010;464:1071-1076.
- 9 Kim D, Song J, Han J, Kim Y, Chun CH, Jin EJ: Two non-coding rnas, microRNA-101 and hottip contribute cartilage integrity by epigenetic and homeotic regulation of integrin-alpha1. *Cell Signal* 2013;25:2878-2887.
- 10 Nakamura A, Rampersaud YR, Sharma A, Lewis SJ, Wu B, Datta P, Sundararajan K, Endisha H, Rossomacha E, Rockel JS, Jurisica I, Kapoor M: Identification of microRNA-181a-5p and microRNA-4454 as mediators of facet cartilage degeneration. *JCI Insight* 2016;1:e86820.
- 11 Li YH, Tavallae G, Tokar T, Nakamura A, Sundararajan K, Weston A, Sharma A, Mahomed NN, Gandhi R, Jurisica I, Kapoor M: Identification of synovial fluid microRNA signature in knee osteoarthritis: Differentiating early- and late-stage knee osteoarthritis. *Osteoarthritis Cartilage* 2016;24:1577-1586.
- 12 Mankin HJ, Dorfman H, Lippiello L, Zarins A: Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg Am* 1971;53:523-537.
- 13 Farndale RW, Buttle DJ, Barrett AJ: Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* 1986;883:173-177.
- 14 Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, Crish JF, Bebek G, Ritter SY, Lindstrom TM, Hwang I, Wong HH, Punzi L, Encarnacion A, Shamloo M, Goodman SB, Wyss-Coray T, Goldring SR, Banda NK, Thurman JM, Gobeze R, Crow MK, Holers VM, Lee DM, Robinson WH: Identification of a central role for complement in osteoarthritis. *Nat Med* 2011;17:1674-1679.
- 15 Goldring MB: Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. *Best Pract Res Clin Rheumatol* 2006;20:1003-1025.
- 16 Kuhn K, D'Lima DD, Hashimoto S, Lotz M: Cell death in cartilage. *Osteoarthritis Cartilage* 2004;12:1-16.
- 17 Goldring MB: The role of the chondrocyte in osteoarthritis. *Arthritis Rheum* 2000;43:1916-1926.
- 18 Lotz M: Cytokines in cartilage injury and repair. *Clin Orthop Relat Res* 2001:S108-115.
- 19 Chen Z, Jin T, Lu Y: Antimir-30b inhibits tnfr-alpha mediated apoptosis and attenuated cartilage degradation through enhancing autophagy. *Cell Physiol Biochem* 2016;40:883-894.
- 20 Zhang F, Wang J, Chu J, Yang C, Xiao H, Zhao C, Sun Z, Gao X, Chen G, Han Z, Zou W, Liu T: MicroRNA-146a induced by hypoxia promotes chondrocyte autophagy through bcl-2. *Cell Physiol Biochem* 2015;37:1442-1453.
- 21 Cong L, Zhu Y, Tu G: A bioinformatic analysis of microRNAs role in osteoarthritis. *Osteoarthritis Cartilage* 2017;25:1362-1371.
- 22 Ding L, Ni J, Yang F, Huang L, Deng H, Wu Y, Ding X, Tang J: Promising therapeutic role of mir-27b in tumor. *Tumour Biol* 2017;39:1010428317691657.
- 23 Ye J, Wu X, Wu D, Wu P, Ni C, Zhang Z, Chen Z, Qiu F, Xu J, Huang J: Mirna-27b targets vascular endothelial growth factor c to inhibit tumor progression and angiogenesis in colorectal cancer. *PLoS One* 2013;8:e60687.
- 24 Chen WP, Xiong Y, Hu PF, Bao JP, Wu LD: Baicalein inhibits mmps expression via a mapk-dependent mechanism in chondrocytes. *Cell Physiol Biochem* 2015;36:325-333.
- 25 Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL: Many human large intergenic noncoding rnas associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A* 2009;106:11667-11672.
- 26 Steck E, Boeuf S, Gabler J, Werth N, Schnatzer P, Diederichs S, 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.
- 27 Kang Y, Song J, Kim D, Ahn C, Park S, Chun CH, Jin EJ: Pcgem1 stimulates proliferation of osteoarthritic synoviocytes by acting as a sponge for mir-770. *J Orthop Res* 2016;34:412-418.
- 28 Song J, Ahn C, Chun CH, Jin EJ: A long non-coding rna, gas5, plays a critical role in the regulation of mir-21 during osteoarthritis. *J Orthop Res* 2014;32:1628-1635.
- 29 Zhang G, Wu Y, Xu D, Yan X: Long noncoding rna ufc1 promotes proliferation of chondrocyte in osteoarthritis by acting as a sponge for mir-34a. *DNA Cell Biol* 2016;35:691-695.
- 30 Jiang SD, Lu J, Deng ZH, Li YS, Lei GH: Long noncoding rnas in osteoarthritis. *Joint Bone Spine* 2017;84:553-556.