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 pairing 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 expression of 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-
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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.

Figure 1.

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
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-
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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-
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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 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. These results suggest that miR-411 plays 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.

![Figure 5](image)

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.
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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.

Acknowledgements

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References

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### Table S1. Primer sequence

<table>
<thead>
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<th>Name</th>
<th>Sequence (5’-3’)</th>
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<td>MMP-13</td>
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<td>Type II collagen</td>
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