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The suppression of miR-181 inhibits inflammatory responses of osteoarthritis through NF-κB signaling pathway

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Abstract. – OBJECTIVE: To investigate the role of micro ribonucleic acid (miR)-181 in the inflammatory responses of osteoarthritis (OA) and related mechanism.

MATERIALS AND METHODS: The rat model of OA was established by anterior cruciate ligament (ACL) transection, and an automatic immuno-analyzer was applied to detect the bone metabolism indexes in the serum. The levels of relevant inflammatory factors in the joint fluid and serum were measured using enzyme-linked immunosorbent assay (ELISA). The cartilage specimens were collected to determine the expression of miR-181 in OA and normal cartilage tissues. Meanwhile, isolated cartilage cells were cultured and transfected with miR-181 mimics and inhibitor separately, and a blank control group was also included. Quantitative Real-time polymerase chain reaction (qRT-PCR) was adopted to detect the messenger RNA (mRNA) expressions of inflammatory factors [tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)] in the cartilage cells. The expression levels of NF-κB and matrix metalloproteinase-13 (MMP-13) proteins related to the NF-κB signaling pathway were determined via Western blotting.

RESULTS: In OA model group, the content of serum osteocalcin (OSTEOC) and vitamin D (VD) declined markedly ($p<0.05$), the content of parathyroid hormone (PTH) increased notably ($p<0.05$), whereas the change of β-Cross Laps was not significant. ELISA results showed that the levels of TNF-α, IL-6 and MMP-9 were elevated remarkably in OA model group ($p<0.05$). Compared with that in normal cartilage tissues, miR-181 expression was increased evidently in OA cartilage tissues ($p<0.05$). Moreover, miR-181 expression was also significantly elevated in miR-181 mimics group after transfection ($p<0.05$). The expressions of inflammatory factors TNF-α and IL-6 in the cartilage cells were increased remarkably in miR-181 mimics group compared with those in control group ($p<0.05$). The miR-181 inhibitor could significantly lower the expressions of inflammatory factors TNF-α and IL-6 ($p<0.05$). According to the results of Western blotting, the protein expressions of MMP-13 and NF-κB were decreased notably in miR-181 inhibitor group ($p<0.05$), but were evidently up-regulated in miR-181 mimics group ($p<0.05$).

CONCLUSIONS: The decrease of miR-181 can reduce the expressions of inflammatory factors TNF-α and IL-6 through downregulating the NF-κB signaling pathway, thus repressing the occurrence of OA.

Key Words:
MiR-181, NF-ÐB signaling pathway, Osteoarthritis.

Introduction

Osteoarthritis (OA), a kind of joint disease affecting the articular cartilage and adjacent tissues[1], is caused by complex biochemical and biomechanical stimuli[2]. It has given rise to a worldwide health burden along with the rising obesity rate and population aging for the consequences of unbearable pain and even disability[3]. OA gradually leads to loss of functions in the patients, mainly manifesting as articular cartilage injury, narrowing space, synovitis and dyskinesia[4,5]. On the basis of its pathogenesis, currently, the studies on treatment of OA primarily involve endogenous hormones, oxygen free radicals, structure of calcified cartilage zone, autoimmunity and cytokines[6], but few of them can precisely define the molecular mechanism of OA progression. There is evidence that the etiology of OA is associated with heredity[7], and the gene expression in the cartilage cells is abnormally changed. For example,

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micro ribonucleic acids (miRNAs) participate in the synthesis and degradation of cartilage[8].

As endogenous non-coding RNAs, miRNAs target the messenger RNA (mRNA) transcripts and silence their gene expression through complementary sequences, so they can serve as key regulatory factors for gene expression[9]. In view of such characteristics as evolutionary conservation and stability, miRNAs can be regarded as novel diagnostic markers for cartilage diseases[10]. It has already been proven that miRNAs play vital roles in many human diseases, including OA[11]. Song et al discovered[12] that the expression of miR-9 in OA cartilage cells was significantly lower than that in normal control group. Later, research testified that miR-221 and miR-483-5p responded to the loss of interaction between cartilage cells and matrix by modulating proliferation and matrix synthesis[13]. Previous studies have manifested that miR-146a had a close correlation with the pathophysiology of OA, and it was lowly expressed in OA cartilage, which is induced by the stimulation of interleukin-1 beta (IL-1β). MiR-181 originates from the gene family in Urochordata. It was previously demonstrated that the up-regulation of miR-181 in the liver accelerated the growth, migration and invasion of hepatocellular carcinoma cells[14,15]. In addition, some studies[16,17] on the expression and function of miR-181 in OA cartilage cells have been conducted. Moreover, it has been revealed in research that miR-9 can bind to corresponding genes such as nuclear factor-kappa B1 (NF-κB1), because the activation of NF-κB1 signaling pathway will trigger the release of pro-inflammatory cytokines that exert crucial effects on cartilage injury. Among the aforementioned genes, emphasis should be placed on NF-κB for the activated NF-κB signaling pathway that will induce the release of pro-inflammatory cytokines such as IL-1β and tumor necrosis factor-alpha (TNF-α), which perform essential functions in altering the degree of cartilage injury and balance of bone metabolism[18]. Therefore, the present study aims to investigate the influences of miR-181 on the inflammatory responses of OA, which is conducive to developing new diagnosis and treatment strategies for the disease.

Materials and Methods

Establishment of OA Model

Sprague-Dawley (SD) rats were utilized to establish the OA model by intraperitoneal injection of chloral hydrate (3.5 mL/kg). A 1.5 cm-long longitudinal incision was separately made on the medial collateral ligament and anterior cruciate ligament of knee joint, and then the medial meniscus was resected. It is worth noting that the operation should be performed under sterile conditions as much as possible, so as to establish the OA model. Traction test was adopted to confirm that the ligament was completely fractured. Subsequently, the rats were fed separately and injected with penicillin every day to diminish inflammation. Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Jinan Central Hospital.

Measurement of Bone Metabolism Indexes

The whole blood was collected to isolate the serum, which was then stored at 4°C. An automatic biochemistry analyzer and detection (Kinghawk, Jinan, Shandong, China) were applied to determine the content of osteocalcin (OSTEOC), vitamin D (VD), β-CrossLaps and parathyroid hormone (PTH).

Measurement of Inflammatory Factors Via Enzyme-Linked Immunosorbent Assay (ELISA)

The rats were fixed after anesthesia, whose joint fluid was drawn using a syringe after local disinfection and placed into a sterile centrifuge tube. Next, the venous blood was drawn to separate the serum by means of centrifugation, and the levels of relevant inflammatory factors in the joint fluid and serum were measured using the ELISA kit (R&D Systems, Minneapolis, MN, USA), and a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to measure the absorbance in each group.

Culture and Transfection of Cartilage Cells

The articular cartilage samples were taken out under sterile conditions, some were used to extract the total RNA in cartilage cells for detection of miR-181 expression, and others were utilized for extraction of cartilage cells. Next, the cells were put in a medium containing fetal bovine serum and dual antibodies, and cultured in an incubator. The medium was changed every 48 hours, and the cartilage cells in the logarithmic growth phase were harvested for experiments. After that, the cells were transfected using the transfection kit, and the transfection efficiency was verified.

Table I. PCR primers.

MRNA	Sequence
MiR-181	F: 5'-TGCGGGTGCTCCGCTTCGGCAGC-3'
	R: 5'-CAGTGCAGGGTCCGAGGT-3'
NF-κB	F: 5'-CTGAACCAGGGCATACCTGT-3'
	R: 5'-GAGAAGTCCATGTCCGCAAT-3'
MMP-13	F: 5'-CCCCAACCCTAAACATCCAA-3'
	R: 5'-AACAGCTCCGCATCAACCT-3'
IL-6	F: 5'-CAATGAGGAGACTTGCCTGG-3'
	R: 5'-GCACAGCTCTGGCTTGTTCC-3'
TNF-α	F: 5'-CGCTACGACCGCCAGATTG-3'
	R: 5'-ACACCGTTCACCAGCAAGTC-3'
GAPDH	F: 5'-TGACTTCAACAGCGACACCCA-3'
	R: 5'-CACCCTGTTGCTGTAGCCAAA-3'

Detection of Expressions of Inflammation-Related Genes in Cartilage Cells Via Quantitative Real-Time Polymerase Chain Reaction (Qrt-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was applied to extract the total RNA in the cartilage tissues of knee joint in normal rats and OA model group as well as that in each group of transfected cells. After the purity and concentration of RNA were determined, the total RNA was reversely transcribed into cDNA. The primer sequences of target genes and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an internal reference, were designed according to those on GenBank and synthesized by Sangon (Shanghai, China). The expression levels of target genes were determined via qRT-PCR (TaKaRa, Otsu, Shiga, Japan). The sequences are shown in Table I.

Detection of Expressions of The NF-Kb Signaling Pathway-Related Proteins in Each Group of Cartilage Cells Via Western Blotting

After the medium was discarded, the cartilage cells were washed with phosphate-buffered solution (PBS) for 3 times. Next, the lysis buffer prepared at an appropriate proportion was added to sufficiently lyse the cells and release the proteins, followed by collection of supernatant via centrifugation. The protein concentration was detected in accordance with the instructions of bicinchoninic acid (BCA) kit. After that, samples and gel were prepared and loaded for electrophoresis, followed by membrane transfer and sealing. Next, primary antibody (Abcam, Cambridge, MA, USA) was added for incubation overnight, and secondary antibody (Abcam, Cambridge, MA, USA) for incubation for 1 hour. Next, the protein bands were scanned and quantified using an Odyssey membrane scanner (LI-COR Biosciences, Lincoln, NE, USA), and the level of proteins to be detected was corrected via Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Image Lab software (Bio-Rad, Hercules, CA, USA) was employed to quantify the bands of Western blotting. The expression levels of corresponding proteins in each group were calculated.

Statistical Analysis

All the raw data were processed using SPSS 22.0 software (IBM, Armonk, NY, USA) and presented as mean ± standard deviation. *t*-test was performed for comparison of data between groups, and $p<0.05$ suggested that the difference was statistically significant.

Results

Detection Results of Bone Metabolism Indexes in the Serum

The bone metabolism indexes including OSTEOC, VD, β-CrossLaps and PTH in the serum were detected, in order to provide an important reference for the early diagnosis of OA. As shown in Table II, the content of serum OSTEOC and VD was significantly reduced ($p<0.05$), with statistical rise of PTH level ($p<0.05$), while level of β-CrossLaps was not significantly changed between two groups, suggesting that the decrease of VD level has feedback regulation on PTH and stimulates osteogenesis in OA patients.

Table II. Detection results of bone metabolism indexes in the serum (ng/mL).

Group	VD	OSTEOC	β-CrossLaps	PTH
Normal group	24.3±1.1	26.5±0.8	0.57±0.7	24.8±0.6
Model group	15.9±0.8[a]	12.9±0.5[a]	0.68±0.9[a]	67.9±0.5[a]

Note: The content of serum OSTEOC and VD decline remarkably ($p<0.05$), while PTH content rises evidently in model group ($p<0.05$). a: $p<0.05$ vs. normal group.

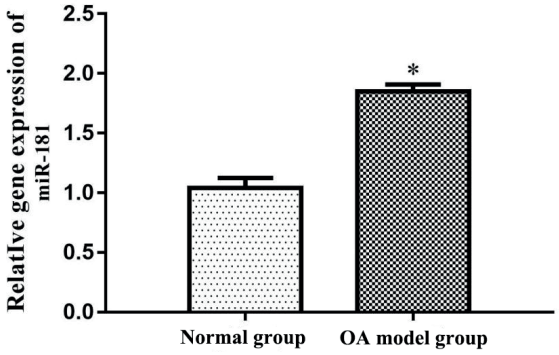

Figure 1. Expression of miR-181 in OA model group and normal cartilage tissues. The expression of miR-181 is increased obviously in the cartilage cells in OA model group ($p<0.05$). *$p<0.05$ *vs.* normal group.

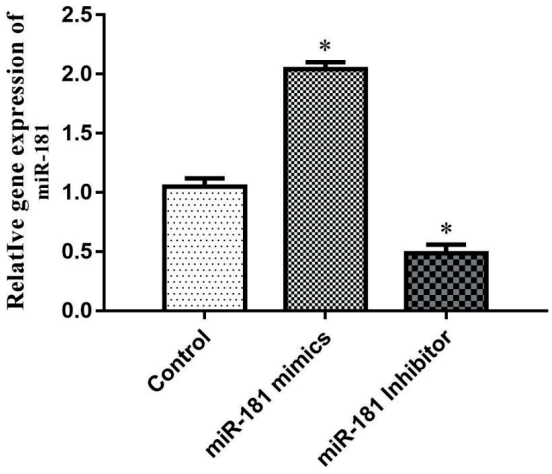

Figure 2. Expression of miR-181 in each group of cartilage cells after transfection. The expression of miR-181 is increased prominently in miR-181 mimics group and decreased notably in miR-181 Inhibitor group. *$p<0.05$ *vs.* control group.

Detection Results of Inflammatory Factors in the Joint Fluid and Serum

The content of inflammatory factors in the joint fluid and serum of rats in normal group and OA model group was measured using ELISA. It was indicated that the levels of TNF-α, IL-6 and MMP-9 in the joint fluid and serum were elevated markedly in OA model group ($p<0.05$) (Table III), implying that massive inflammatory factors are produced in OA patients.

Expression of MiR-181 in OA Model Group and Normal Cartilage Tissues

RT-qPCR was performed to determine the expression of miR-181 in the cartilage tissues of OA rats. The results manifested that in comparison with that in normal cartilage tissues, miR-181 expression was significantly increased in the cartilage cells in OA model group ($p<0.05$) (Figure 1).

Verification of Transfection Efficiency in Each Group of Cartilage Cells

The relative expression of miR-181 in the cartilage cells transfected with miR-181 mimics and miR-181 Inhibitor is shown in Figure 2. The

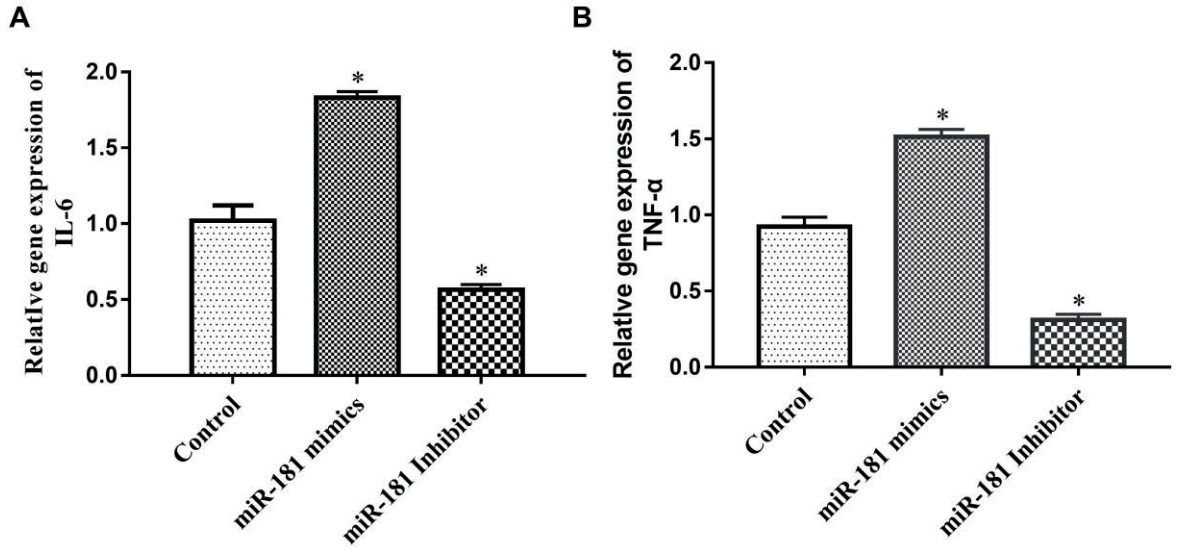

Figure 3. Expressions of inflammation-related genes in cartilage cells. The expressions of inflammatory factors in the cartilage cells are increased obviously in miR-181 mimics group. *$p<0.05$ *vs.* control group.

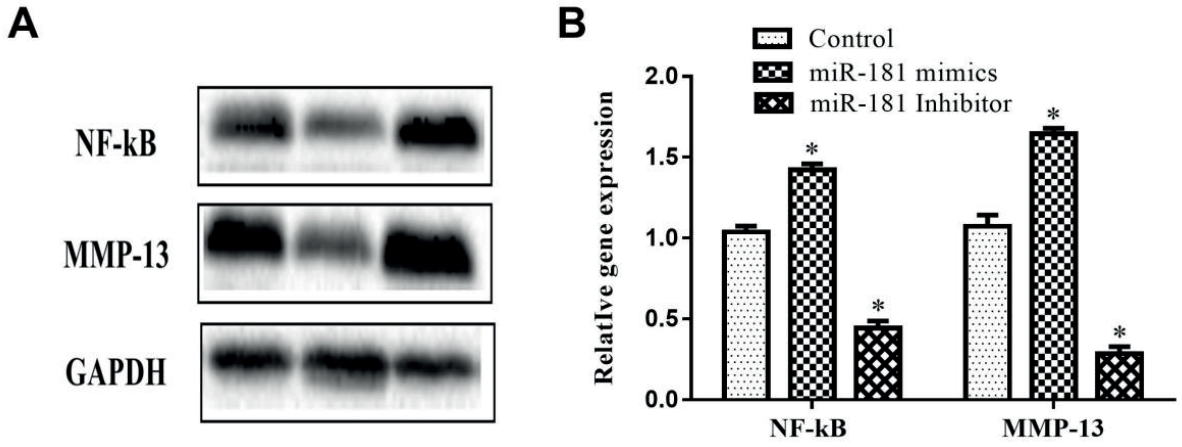

Figure 4. Expressions of the NF-κB signaling pathway-related proteins in cartilage cells. The protein expressions of MMP-13 and NF-κB are decreased notably in miR-181 Inhibitor group. *p<0.05 *vs.* control group.

expression of miR-181 in the cartilage cells was increased prominently in miR-181 mimics group (p<0.05) and decreased notably after the treatment of miR-181 inhibitor (p<0.05).

Expressions of Inflammation-Related Genes in Cartilage Cells

According to the results of RT-qPCR (Figure 3), miR-181 mimics induced significantly higher expressions of inflammatory factors (TNF-α and IL-6) in the cartilage cells than control group (p<0.05), and the miR-181 inhibitor could significantly reduce the expressions of those inflammatory factors (p<0.05), indicating that the results are consistent with those of ELISA.

Expressions of the NF-κb Signaling Pathway-Related Proteins Targeted by Mir-181

The results of Western blotting analysis displayed that the protein expressions of MMP-13 and NF-κB were decreased notably due to the suppression of miR-181 level (p<0.05), while they were increased evidently in miR-181mimics group (p<0.05) (Figure 4).

Discussion

OA is a complicated inflammatory disease affecting the whole joint, whose further progression will lead to cartilage degeneration and injury of knee joint, ultimately resulting in disability[19]. However, few researchers are capable of thoroughly explaining its underlying mechanism. There is evidence that genetic constitution is associated with the etiology of osteoarthrosis, and some gene expressions in the cartilage cells are involved in the synthesis and degradation of cartilage[2]. MiRNAs, recognized as potential biomarkers, participate in the pathogenesis of OA by controlling the target genes and fine-tuning the gene expressions. Several studies have demonstrated that miRNAs (miR-9, miR-27, miR-140, etc.) and genes (NF-κB, IL-6, MMAP-13, etc.) are abnormally expressed in OA patients. Wu et al[20] emphasized the functions and roles of miRNAs in controlling cartilage cell differentiation and OA progression. Hence, the present study aims to explore the influences of miR-181 on the inflammatory responses of OA by targeting NF-κB, so

Table III. Detection results of inflammatory factors in the joint fluid and serum in each group.

	Joint fluid			Serum		
Group	TNF-α (pg/mL)	IL-6 (pg/mL)	MMP-9 (ng/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)	MMP-9 (ng/mL)
Normal group	22.3±1.1	42.3±0.9	32.1±0.7	4.5±0.3	8.7±0.5	6.3±0.2
Model group	67.8±0.8[a]	119.6±0.7[a]	78.6±0.6[a]	17.3±0.2[a]	31.6±0.1[a]	15.4±0.3[a]

Note: The content of serum OSTEOC and VD decline remarkably (p<0.05), while PTH content rises evidently in model group (p<0.05). [a]: p<0.05 *vs.* normal group.

as to reveal the etiology of OA. According to the results, miR-181 can repress the inflammatory responses of OA through regulating the expressions of inflammatory factors and NF-κB signaling pathway-related proteins.

In the present study, it was revealed that miR-181 expression in OA cartilage tissues was increased prominently. To our knowledge, the expression of miRNAs, including miR-23b, miR-140, miR-181 and miR-210, is closely correlated with the formation of cartilage cells[17]. It has been reported that miR-181 is involved in such biological processes as cell proliferation, apoptosis and metabolism[21]. The members of miR-181 family can regulate the persistent differentiation of mature cartilage cells[22]. Special studies have been conducted for miR-181 in the context of immunocyte differentiation. In the present work, the cartilage cells were transfected with miR-181 mimics and miR-181 inhibitor, and it was shown that miR-181 mimics could significantly elevate the expressions of inflammatory factors TNF-α and IL-6 in the cartilage cells. A previous report on miR-9 indicated that both IL-6 and MMP-13 are overexpressed in OA cartilage tissues. Besides, the expressions of IL-6 and MMP-13 decline markedly after the transfection of cartilage cells with NF-κB1 siRNA, elaborating that the decreased expressions of IL-6 and MMP-13 are triggered by NF-κB expression regulated by miR-9[23]. MMPs can stimulate the pro-inflammatory cytokines, and the fully activated MMPs may be conducive to cartilage destruction in OA. In addition, some components of inflammatory factors are able to specifically stimulate the cartilage cells and enhance the expression of epithelium-specific transcription factors by modifying NF-κB[24].

To elaborate the impacts of miR-181 and NF-κB on downstream molecules, the protein expressions of MMP-13 and NF-κB were compared between miR-181 mimics group and miR-181 inhibitor group. It was concluded that those expressions were elevated remarkably in miR-181 mimics group. Jones et al[25] have revealed that miR-9 regulates the secretion of MMP-13 and inhibits tumorigenesis by repressing the activity of IL-6[26]. Moreover, NF-κB1 and IL-6, acting as matrix-degrading enzymes, as well as MMP-13, serving as a catabolic marker, are overexpressed in patients with knee OA[27,28]. The NF-κB signaling pathway can participate in the occurrence and progression of inflammations and tumors in the body through the transcription factor NF-κB. Multiple investigations have testified that NF-κB can activate the genes maintaining cell proliferation. For instance, the inhibition of NF-κB impairs HeLa cell proliferation, while the up-regulated NF-κB may indirectly cause the proliferation of H-ras oncogene-induced cells[29,30]. NF-κB is indeed capable of controlling various genes with different functions (anti-apoptotic and anti-proliferative effects) at the same time[31]. Compared with anti-proliferative genes, NF-κB may be more prone to promote the expression of anti-apoptotic genes in the cartilage cells but exert the opposite effect on other cells. Researchers have discovered that miR-181 can repress the proliferation and promote the apoptosis of OA cartilage cells, and the expressions of Caspase-3 and poly ADP-ribose polymerase (PARP), apoptosis-related proteins, are increased after miR-181 is up-regulated, illustrating that miR-181 is likely to act on OA cartilage cells by regulating Caspase-3 and PARP. In the present study, the in-depth exploration of the NF-κB signaling pathway combined with ingenuity pathway analysis makes the roles of miR-181 and NF-κB in OA progression more credible, which may serve as the potential indicators, along with the CTX-III and microRNA-98, for the diagnosis of osteoarthritis[32].

Conclusions

We demonstrated that the expression of miR-181 in OA tissues was increased prominently compared with that in normal tissues, and the expressions of TNF-α, IL-6, NF-κB and MMP-13 in miR-181 mimics group were relatively higher. The decrease of miR-181 can reduce the expressions of inflammatory factors TNF-α and IL-6 by targeting NF-κB and thus repress the occurrence of OA. Therefore, miR-181 and NF-κB may become diagnostic biomarkers and therapeutic targets for OA patients.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Bennell KL, Wrigley TV, Hunt MA, Lim BW, Hinman RS. Update on the role of muscle in the genesis and management of knee osteoarthritis. Rheum Dis Clin North Am 2013; 39: 145-176.

2) Detert J, Klaus P, Listing J, Hohne-Zimmer V, Braun T, Wassenberg S, Rau R, Buttgereit F, Burmester GR.

Hydroxychloroquine in patients with inflammatory and erosive osteoarthritis of the hands (OA TREAT): study protocol for a randomized controlled trial. Trials 2014; 15: 412.

3) Helmick CG, Felson DT, Lawrence RC, Gabriel S, Hirsch R, Kwoh CK, Liang MH, Kremers HM, Mayes MD, Merkel PA, Pillemer SR, Reveille JD, Stone JH, National Arthritis Data Workgroup. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum 2008; 58: 15-25.
4) Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. Osteoarthritis Cartilage 2015; 23: 1966-1971.
5) Yu SP, Hunter DJ. Managing osteoarthritis. Aust Prescr 2015; 38: 115-119.
6) Roos EM, Arden NK. Strategies for the prevention of knee osteoarthritis. Nat Rev Rheumatol 2016; 12: 92-101.
7) Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. Nat Rev Cancer 2011; 11: 289-301.
8) Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007; 213: 626-634.
9) Zhang D, Wang J, Wang Z, Zhang T, Shi P, Wang X, Zhao F, Liu X, Lin X, Pang X. miR-136 modulates TGF-beta1-induced proliferation arrest by targeting PPP2R2A in keratinocytes. Biomed Res Int 2015; 2015: 453518.
10) van der Kolk JH, Pacholewska A, Gerber V. The role of microRNAs in equine medicine: a review. Vet Q 2015; 35: 88-96.
11) Le LT, Swingler TE, Clark IM. Review: the role of microRNAs in osteoarthritis and chondrogenesis. Arthritis Rheum 2013; 65: 1963-1974.
12) Song J, Kim D, Chun CH, Jin EJ. MicroRNA-9 regulates survival of chondroblasts and cartilage integrity by targeting protogenin. Cell Commun Signal 2013; 11: 66.
13) Yang M, Zhang L, Gibson GJ. Chondrocyte miRNAs 221 and 483-5p respond to loss of matrix interaction by modulating proliferation and matrix synthesis. Connect Tissue Res 2015; 56: 236-243.
14) Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, Li C, Deng C, Wauthier E, Reid LM, Ye QH, Qin LX, Yang W, Wang HY, Tang ZY, Croce CM, Wang XW. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. Hepatology 2009; 50: 472-480.
15) Wang B, Hsu SH, Majumder S, Kutay H, Huang W, Jacob ST, Ghoshal K. TGFbeta-mediated upregulation of hepatic miR-181b promotes hepatocarcinogenesis by targeting TIMP3. Oncogene 2010; 29: 1787-1797.
16) Song J, Lee M, Kim D, Han J, Chun CH, Jin EJ. MicroRNA-181b regulates articular chondrocytes differentiation and cartilage integrity. Biochem Biophys Res Commun 2013; 431: 210-214.
17) Gabler J, Ruetze M, Kynast KL, Grossner T, Diederichs S, Richter W. Stage-specific miRs in chondrocyte maturation: differentiation-dependent and hypertrophy-related miR clusters and the miR-181 family. Tissue Eng Part A 2015; 21: 2840-2851.
18) Toegel S, Wu SQ, Otero M, Goldring MB, Leelapornpisid P, Chiari C, Kolb A, Unger FM, Windhager R, Viernstein H. Caesalpinia sappan extract inhibits IL1beta-mediated overexpression of matrix metalloproteinases in human chondrocytes. Genes Nutr 2012; 7: 307-318.
19) Rainbow R, Ren W, Zeng L. Inflammation and joint tissue interactions in OA: implications for potential therapeutic approaches. Arthritis 2012; 2012: 741582.
20) Wu C, Tian B, Qu X, Liu F, Tang T, Qin A, Zhu Z, Dai K. MicroRNAs play a role in chondrogenesis and osteoarthritis (review). Int J Mol Med 2014; 34: 13-23.
21) Su R, Lin HS, Zhang XH, Yin XL, Ning HM, Liu B, Zhai PF, Gong JN, Shen C, Song L, Chen J, Wang F, Zhao HL, Ma YN, Yu J, Zhang JW. MiR-181 family: regulators of myeloid differentiation and acute myeloid leukemia as well as potential therapeutic targets. Oncogene 2015; 34: 3226-3239.
22) Ohgawara T, Kubota S, Kawaki H, Kondo S, Eguchi T, Kurio N, Aoyama E, Sasaki A, Takigawa M. Regulation of chondrocytic phenotype by micro RNA 18a: involvement of Ccn2/Ctgf as a major target gene. FEBS Lett 2009; 583: 1006-1010.
23) Gu R, Liu N, Luo S, Huang W, Zha Z, Yang J. MicroRNA-9 regulates the development of knee osteoarthritis through the NF-kappaB1 pathway in chondrocytes. Medicine (Baltimore) 2016; 95: e4315.
24) Vincenti MP, Brinckerhoff CE. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1beta. Arthritis Res 2001; 3: 381-388.
25) Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL, Brockbank SM, Needham MR, Read SJ, Newham P. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. Osteoarthritis Cartilage 2009; 17: 464-472.
26) Zhang J, Jia J, Zhao L, Li X, Xie Q, Chen X, Wang J, Lu F. Down-regulation of microRNA-9 leads to activation of IL-6/Jak/STAT3 pathway through directly targeting IL-6 in HeLa cell. Mol Carcinog 2016; 55: 732-742.
27) Levinger I, Levinger P, Trenerry MK, Feller JA, Bartlett JR, Bergman N, McKenna MJ, Cameron-Smith D. Increased inflammatory cytokine expression in the vastus lateralis of patients with knee osteoarthritis. Arthritis Rheum 2011; 63: 1343-1348.
28) Tao R, Xu X, Sun C, Wang Y, Wang S, Liu Z, Zhai L, Cheng H, Xiao M, Zhang D. KPNA2 interacts with P65 to modulate catabolic events in osteoarthritis. Exp Mol Pathol 2015; 99: 245-252.
29) Kaltschmidt B, Kaltschmidt C, Hehner SP, Droge W, Schmitz ML. Repression of NF-kappaB impairs

HeLa cell proliferation by functional interference with cell cycle checkpoint regulators. Oncogene 1999; 18: 3213-3225.

30) Jo H, Zhang R, Zhang H, McKinsey TA, Shao J, Beauchamp RD, Ballard DW, Liang P. NF-kappa B is required for H-ras oncogene induced abnormal cell proliferation and tumorigenesis. Oncogene 2000; 19: 841-849.

31) Gu R, Liu N, Luo S, Huang W, Zha Z, Yang J. MicroRNA-9 regulates the development of knee osteoarthritis through the NF-kappaB1 pathway in chondrocytes. Medicine (Baltimore) 2016; 95: e4315.

32) Zheng WD, Zhou FL, Lin N, Liu J. Investigation for the role of CTX-III and microRNA-98 in diagnosis and treatment of osteoarthritis. Eur Rev Med Pharmacol Sci 2018; 22: 5424-5428..