European Review for Medical and Pharmacological Sciences 2021; 25: 1185-1197

MicroRNAs target on cartilage extracellular matrix degradation of knee osteoarthritis

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Abstract. – OBJECTIVE: Knee osteoarthritis (KOA) is currently indicated to be characterized by destruction of articular cartilage. The destruction can be described as an imbalance between synthesis and degradation of extracellular matrix (ECM) components. It is accompanied with changes of pro-inflammatory cytokines and degradation enzymes dominated by matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). Recent studies have revealed that microRNAs (miRNAs) are associated with synthesis and degradation of extracellular matrix (ECM). They play an important role in articular cartilage homeostasis of knee osteoarthritis (KOA). The related mechanisms include mediating the relevant enzymes and pro-inflammatory cytokines. The aim of this study is to reveal the potential microRNAs (miRNAs) and their corresponding upstream or downstream targeting on cartilage extracellular matrix (ECM) degradation in knee osteoarthritis (KOA).

MATERIALS AND METHODS: 7 databases were extensively searched with a theme of MicroRNAs (miRNAs) and knee osteoarthritis (KOA). The articles were searched regardless of publication status and language. The databases include PubMed, Cochrane Library, EMbase, China Biology Medicine (CBM), China National Knowledge Infrastructre (CNKI), WanFang Data and Chinese Scientific Journal Database (VIP).

RESULTS: This article reviews the microRNAs (miR-140, miR-146a, miR-25, miR-543, miR-19, miR-125b, miR-92a, miR-27b, miR-448, miR-558, miR-155) and their corresponding upstream or downstream in mediating cartilage extracellular matrix (ECM) degradation.

CONCLUSIONS: MicroRNAs (miRNAs) have been involved in the pathogenesis of KOA. They can directly regulate cartilage homeostasis by targeting on ECM degradation via corresponding upstream/downstream.

Key Words:
Knee osteoarthritis, MicroRNAs, Cartilage extracellular matrix, Matrix metalloproteinase 13.

Introduction

Knee osteoarthritis (KOA) is a chronic degenerative joint disease affecting millions of people worldwide[1]. It is indicated that approximately 6% adults aged above 30 will be suffered from symptomatic KOA[2]. While 80% people aged over 70 have been experiencing the harm brought by KOA. KOA is clinically characterized by joint pain, stiffness and varied degrees of functional disabilities. Factors as age, gender, obesity, trauma and genetics are all contributing to KOA development and progression[3,4]. However, the exact pathogenesis of KOA has not been fully illuminated. Current explored pathology of KOA is illustrated in Figure 1. Recent studies have found destruction of articular cartilage may be the key pathogenesis of KOA. It is caused by an imbalance between synthesis and degradation of extracellular matrix (ECM) components, such as type II collagen (Col2a1) and aggrecans (ACAN). At molecular level, ECM imbalance occurs accompanied with pro-inflammatory cytokines and degradation enzymes dominated by matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). ECM degradation can be divided into two phases in KOA. In the early on degradative phase, ADAMTS and MMP-13 digest the ECM in the presence of pro-inflammatory cytokines. Then in the compensative biosynthetic phase, the chondrocytes will proliferate to repair

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the damaged ECM. Once the compensation was disrupted by pro-inflammatory cytokines, the cartilage erosion will be accelerated subsequently.

Significant efforts have been made to understand the molecular mechanisms underlying KOA pathogenesis. Scholars[5,6] revealed that microRNAs (miRNAs) are involved in articular cartilage homeostasis of KOA. MiRNAs are a class of endogenous non-protein-coding small RNA identified as critical post-transcriptional regulators. MiRNAs exert their biological effects mainly through the regulation of post-transcriptional gene expression. Binding to 3'-untranslated region (3'UTR) of target gene, miRNAs can then play critical roles in cell proliferation, migration, invasion and differentiation. In regard to ECM degradation of KOA, dysregulation of several miRNAs is indicated to be involved in (miR-140, miR-146a, miR-25, miR-543, miR-19, miR-125b, miR-92a, miR-27b, miR-448, miR-558, miR-155) (Table I). Meanwhile, subsequent AMAMTS and MMP-13 changes are also one of the vital processes in ECM destruction. For example, ADAMTS-4 and ADAMTS-5 are downstream of miR-140 related to chondrocyte apoptosis. Up-regulated MMP-13 induced by down-regulated miR-27b is found to correlate with chondrocytes degradation. Although many studies have indicated the close relationship between miRNAs and ECM degradation in KOA, the corresponding upstream or downstream have not been illuminated clearly yet.

Thus, this review aimed at describing the miRNAs and associated upstream or downstream targeting on ECM degradation in KOA. The discussed pathology will involve chondrocyte, subsequent changes of MMP, ADAMTS and pro-inflammatory cytokines. It will also give new insight to therapeutic strategies in KOA clinically.

MicroRNA and Chondrocyte

Cartilage degradation is of great importance in KOA. Chondrocytes are responsible for synthetizing ECM components and maintain cartilage homeostasis. Iliopoulos et al[7] screened 365 miRNAs genes and found 16 ones varying in articular chondrocytes. Cong et al[8] identified 46 differentially expressed miRNAs involved in chondrocyte apoptosis, autophagy, differentiation and ECM degradation. In detail, miRNAs such as miR-140, miR-146a, miR-135b, miR-27b, miR-448, miR-558 are indicated to correlate with chondrocyte proliferation, differentiation and apoptosis. Above miRNAs are then subsequently involved in ECM degradation[9-13].

MiR-140

MiR-140 is one of research highlights among miRNA molecules in KOA. It is closely related to cartilage homeostasis including chondrocyte proliferation, differentiation, apoptosis, autophagy and inflammatory responses. MiR-140 is expressed only in cartilage during the development of embryonic cartilage[14-20]. The expression of miR-140 will increase in parallel with the differentiation and mature of chondrocytes[21,22]. MiR-140-deficient mice will obtain skeletal malformations and articular cartilage cells after birth. Moreover, miR-140$^{-/-}$ mice will obtain age-related KOA-like changes such as loss of specific proteoglycan and osteophyte in articular cartilage. Transgenic mice overexpressed with miR-140, however, will acquire resistance to pro-induced arthritis[23]. Thus, miR-140 is proved as an important factor in mediating chondrocyte homeostasis. In detail, miR-140 is indicated to influence chondrocyte degradation *via* chondrogenic factor transcription factor SRY-box-containing gene 9 (SOX9), ADAMTS, fucosyltransferase (FUT) and runt-related transcription factor (Runx2) regulators and pathways.

SOX9

SOX9 is the upstream of miR-140. The level of SOX9 is positively correlated with miR-140 as knocking down of SOX9 will significantly decrease miR-140. SOX9 is a key transcription factor in cartilage maturity[24]. Nakamura et al[18] found in zebrafish cartilage that SOX9 mutations will lead to absence of miRNA-140 transcription. In addition, Luciferase detection revealed that SOX9 could induce miR-140 expression binding to intron 10 of the WWP2 gene[25]. Yamashita et al[26] found that SOX5 and SOX6 can target on SOX9 and enhance the miR-140 expression in transgenic mice. Once when SOX9 increased, miR-140 would also be up-regulated. The expression of Col2a1 and ACAN will be promoted subsequently. Then, the equilibrium points of chondrocyte differentiation, Bap×1/ Nk×3.2, will be affected in order to influence chondrocyte differentiation[27]. Moreover, upregulated SOX9 can inhibit the expression of chondrocyte hypertrophy differentiation marker Col10a1 through miR-140[28]. MiR-140 can thus promote the expression of ACAN and Col2a1 and inhibit Col10a1 *via* SOX9. Then it is able to maintain the normal chondrocyte phenotype.

ADAMTS

ADAMTS-4 (Aggrecanase-1) and ADAMTS-5 (Aggrecanase-2), mainly responsible for ACAN

Table I. MicroRNAs and associated molecular regulators.

Key MicroRNA in KOA	Tissue	Changes in KOA	Biological Targets Involved	Upstream of the MicroRNA	Downstream of the MicroRNA
Chondrocyte					
miR-140[21,22]	Chondrocyte	↓	Chondrocyte proliferation/differentiation/ apoptosis/autophagy ECM homeostasis	SOX9[24] (SOX5/SOX6) WWP2 gene[25]	Bap × 1 / Nk × 3.2 [27] Col10a1[28] Col2a1 and ACAN
				IL-1	TIMP-ADAMTS4/5 balance[30] Col2a1 and ACAN
				/	FUT1 [33] (LC-3II/Atg5/ Beclin-1 protein) Col2a1 and ACAN
				/	Runx2 [42-44] (Smad1/Smad3) (ADAMTS/ Col10a1)
miR-146a[45]	Chondrocyte Synoviocytes	↓	Chondrocyte proliferation/apoptosis ECM homeostasis	/	Smad4→VEGF
miR-25[48]	Chondrocyte	↓	Chondrocyte proliferation/apoptosis	IL-1β[49] MAPK 4 (p38)[54]	ATF-2/ MEF-2C [55] Col2a1 and ACAN
miR-543[56]	Chondrocyte	↓	ECM homeostasis	IL-1β	MDM4 (TP53/Cytochrome C) [57] Akt1 (mTOR) [58] Bcl-2 [58]
miR-19b[59]	Chondrocyte	↓	Chondrocyte proliferation/apoptosis	IL-1β	GRK6 p65 (NF-κB) Col2a1 and ACAN
MMP					
miR-27b[66]	Chondrocyte	↓	ECM homeostasis	NF-κB	MMP-13 [70]
miR-146a[71]	Chondrocyte/ Synoviocytes	↓	ECM homeostasis	NF-κB	IRAK1/TRAF6 MMP-13
miR-140[99]	Chondrocyte	↓	ECM homeostasis	Estrogen/E2	MMP-13
miR-448[82]	Chondrocyte	↑	Chondrocyte apoptosis ECM homeostasis	/	Matrilin-3 MMP-13 Col2a1 and ACAN
miR-558[85]	Colorectal cells fibroblasts	↓	Chondrocyte apoptosis ECM homeostasis	NF-κB (TNF-α/IL-1/ IL-6)	COX2/PGE2 MMP-13
miR-155[88]	Chondrocyte	↑	Chondrocyte apoptosis ECM homeostasis	NF-κB/JNK	AP-1 MMP-13
Pro-inflammatory Cytokines					
miR-146a[93]	Chondrocyte/ Synoviocytes	↓	Chondrocyte inflammation	/	TLR4/TRAF6/IRAK1

degradation, are the downstream of miRNAs. Expressions of ADAMTS are negatively correlated with the level of miR-140[29]. Compared with normal chondrocytes, in response to IL-1 stimulation, miR-140 was markedly decreased while ADAMTS4/5 overexpressed. Overexpressed ADAMTS-4 and ADAMTS-5 will significantly decrease ACAN release/catabolism *via* affecting the protease/tissue inhibitor of metalloproteinases (TIMP)[30]. That is to say, the balance between TIMP-3 and ADAMTS-4/5 will be disturbed. The delicate ECM balance will then be destroyed and result in chondrocyte degradation. As listed above, miR-140 can indeed mediate chondrocyte degradation *via* regulating downstream ADAMTS-4/5. However, the mechanism how ADAMTS-4 and ADAMTS-5 are regulated by miR-140 is still unexplored. MiR-140 is indicated to regulate ADAMTS-4/5 *via* interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) or transforming growth factor β (TGF-β). Song et al[31] found that ADAMTS-4 could be regulated by the growth arrest-specific 5 gene (GAS5) involved in stimulating chondrocyte apoptosis. Moreover, the fibroblast growth factor-2 (FGF2), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinases (MAPK) activation are also associated with ADAMTS-4/5 regulation at molecular levels.

FUT1

FUT1 is the direct downstream of miR-140 in chondrocyte regulation. It is negatively correlated with miR-140. FUT1, a fucosylated synthase, plays an important role in mediating inflammatory cell adhesion and chondrocyte proliferation[32]. Wang et al[33] identified that FUT1 was aberrant upregulated in KOA cartilage tissues. Meanwhile, miR-140 was significantly down-regulated in KOA patients compared with the healthy group. Study showed that overexpressed miR-140 inhibited apoptosis and promoted proliferation of primary chondrocytes *via* down-regulating FUT1. On the contrary, the downregulation of miR-140 would inhibit chondrocyte proliferation and contribute to ECM degradation *via* upregulating FUT1[33]. The luciferase assay further verified miR-140 could directly mediate FUT1 by bounding to its 3'-UTR in chondrocytes. Besides, in terms of corresponding protein levels, the Western blot showed decreased miR-140 could significantly reduce the expressions of LC-3II, Atg5, and Beclin-1 protein levels. Thus, miR-140, FUT1 and associated proteins might serve as prospective

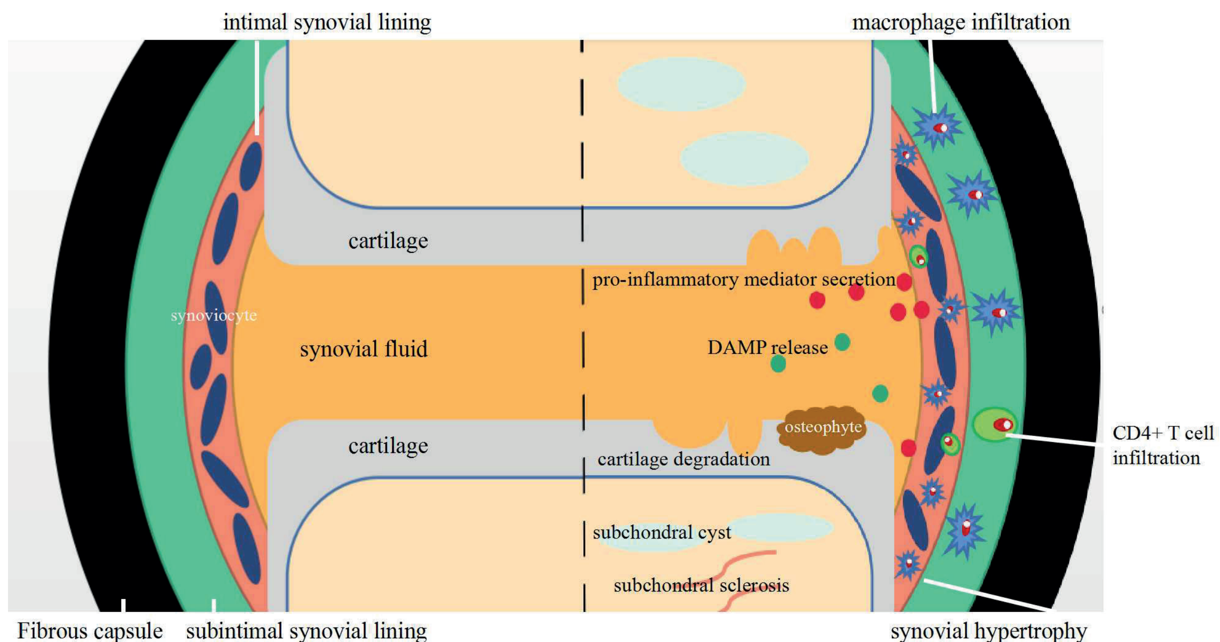

Figure 1. Current explored pathology of knee osteoarthritis. The left one stands for healthy knee joint. The right one stands for the joint of knee osteoarthritis. Inflammatory cells infiltrate the synovium resulting in the release of pro-inflammatory mediators and chronic inflammation. The cartilage and bone are degraded and remodeled with subchondral sclerosis, osteophyte and cyst formation.

biomarkers and potential therapeutic targets in future KOA treatment.

RUNX2

Runx2 is the downstream protein which can be directly affected by miR-140. Runx2 is required for the differentiation of prehypertrophic chondrocytes to hypertrophic chondrocytes[34-36]. Runx2$^{-/-}$ mice lack hypertrophic chondrocytes in most of the skeleton[37,38]. Runx2 usually works as Runx2-Smads complex in chondrocyte regulation. Among the Smads family, Smad1 and Smad3 can bind with Runx2 competitively. The former activates Runx2 while the latter inhibits Runx2[39,40]. Komori[41] has showed that level of Runx2 is negatively correlated with miR-140. Down-regulated miR-140 will promote Smad1 expression in KOA. Runx2 will be activated and overexpressed due to up-regulation of Smad1 subsequently. Then the chondrocyte maturation in whole cartilage will be accelerated. Impaired joint and chondrocyte will be formed as a result. Moreover, Runx2 can induce the expression of ADAMTS5 which will further disrupt the ECM synthesis[42-44]. Col10a1, the chondrocyte hypertrophy differentiation marker, will also be activated when Runx2 overexpressed. The chondrocyte degradation will be quickened subsequently.

MiR-146a

MiR-146a is markedly expressed in KOA cartilage. Actually, miR-146a is involved in human chondrocyte apoptosis in response to mechanical injury. It may contribute to chondrocytes degradation by increasing vascular endothelial growth factor (VEGF) levels *via* Smad4 in cartilage. Saito et al[45] found that VEGF, one of the major regulators in KOA pathology, was downstream of miR-146a. VEGF and its receptors can be significantly up-regulated and involved in chondrocytes vascularization and inflammatory response[46]. Once the balance between anti-angiogenic factors and pro-angiogenic factors mediated by VEGF was disrupted, blood vessels would then grow from subchondral bone into articular chondrocyte. Hypertrophy, degradation and apoptosis of chondrocytes will then be led to consequently[47]. Moreover, Smad4 was identified as a direct target of miR-146a by harboring its binding sequence in the 3'-untranslated region (3'-UTR). In KOA, decreased miR-146a could up-regulate VEGF *via* Smad4 in KOA development and contribute to chondrocyte degradation[47].

MiR-25

Recent studies have showed that transfected miR-25 will significantly decrease the apoptotic rate and improve proliferation rate of chondrocytes. Meanwhile, the transfected miR-25 inhibitors will reverse above condition. This indicates that miR-25 can protect chondrocytes from degradation[48]. It decreased significantly in KOA chondrocyte.

IL-1Đ

IL-1β is a major inflammatory cytokine playing a vital role in pathogenesis and development of KOA [49]. It is the downstream of miR-25. Meanwhile, IL-1β is negatively correlated with miR-25. This cytokine can not only inhibit the chondrocyte proliferation and differentiation, but also induce chondrocytes degradation and apoptosis[49-53]. Increased IL-1β means down-regulated production of ACAN and Col2a1 in ECM. As an anti-IL-1β factor, miR-25 can effectively slow down chondrocyte degradation *via* down-regulating IL-1β.

MAPK

MAPK plays an important role in KOA development. MAPK4 acts as the activator of intracellular phosphorylation cascade signaling pathway. Proved by targeting binding gene sequence, MAPK 4 gene is indicated to be miR-25 upstream[54]. Activated MAPK4 will significantly decrease miR-25 expression. Meanwhile, MAPK4 is also a significant activator of 38 kDa protein kinase (p38) [55]. P38 can further activate intracellular activated transcription factor-2 (ATF-2) and the transcription factor myocyte enhancement factor (MEF-2C) in chondrocyte apoptosis. Moreover, activated ATF-2 and MEF-2C can increase related inflammatory genes expressions in KOA and promote ECM degradation[55]. Therefore, activated upstream MAPK4 gene and p38 can decrease miR-25 and contribute to ECM degradation.

MiR-543

MiR-543 may also be involved in chondrocyte homeostasis[56]. MiR-543 can promote chondrocytes proliferation. The expression of miR-543 in KOA chondrocyte decreased significantly. This suggests that normal expression of miR-543 may be of great significance in maintaining normal physiological function of chondrocytes. Research has indicated that proteins such as MDM4, Bcl-2

and Akt1 may be downstream of miR-543. MiR-543 can reduce the release of pro-apoptotic protein MDM4 and increase the levels of apoptosis resistant proteins like Bcl-2 and Akt1[57]. MDM4 downstream TP53 mitochondrial and cytochrome C apoptosis are thus declined in order to protect chondrocytes from degradation. Additionally, Akt1 can reduce chondrocyte apoptosis by regulating the mTOR signaling pathway, thereby maintaining the balance of ECM[58].

MiR-19b

MiR-19b is indicated to play a protective role in chondrocyte as its mimic can dramatically increase the viability of chondrocyte and suppress cell apoptosis. Meanwhile, miR-19b up-regulation can substantially restore the ECM homeostasis. Luciferase reporter assay showed that G protein-couple receptor kinase 6 (GRK6) gene was a direct downstream of miR-19b. MiR-19b exerts its protective role *via* NF-κB/GRK6 signaling pathway. GRK6 is a member of the GRK family related to various inflammatory processes in chondrocyte degradation[59]. MiR-19b was significantly decreased in KOA chondrocytes. The level of miR-19b was in paralleled with decreased ACAN, Col2a1 expressions and elevated GRK6 levels[60]. Decreased miR-19b in paralleled with increased GRK6 significantly suppressed cell viability and promoted chondrocyte apoptosis[61]. Moreover, NF-κB pathway, which plays a significant role in chondrocyte differentiation and apoptosis, was also correlated with miR-19b. Decreased miR-19b could no longer suppress the expression of p65 and resulted in increase of pro-inflammatory cytokines such as IL-6 and IL-8. Thus, local inflammatory responses were aggravated and chondrocyte degradation accelerated. Taken together, these results indicated that miR-19b could affect chondrocyte homeostasis *via* GRK6 accompanied with NF-κB pathway.

MicroRNA and MMP

Except for directly affecting chondrocytes, miRNAs can also mediate MMP transcription in inducing ECM degradation. MMPs are Zn2+ dependent endopeptidase, which have high specificity to collagen. MMP-1, MMP-3, MMP-8, MMP-9, MMP-13 and MMP-14 are all found associated with chondrocyte degradation[62]. However, among above MMPs, MMP-13 is considered to be the most important collagenase. Conditional expression of MMP-13 in murine cartilage induces spontaneous cartilage degradation[63]. Whilst MMP $13^{-/-}$ mice are protected from cartilage erosion in a surgical KOA model[64]. As a marker of chondrocyte hypertrophy and differentiation, it has the strongest ability to break down Col2a1[65]. Recent studies have reported miRNAs can mediate MMP-13 transcription in regard to pro-inflammatory cytokines as TNF-α/IL-1β/NF-κB (miR-27b, miR-146a, miR-155, miR-124), hormones changes (miR-140) and proteins or enzymes (miR-448, miR-558).

MiR-27b

MiR-27b is negatively correlated with the expression of MMP-13. MMP-13 is the downstream of miR-27b. KOA chondrocytes with overexpressed miR-27b produced 38% less MMP-13 protein while inhibited miR-27b resulted in an increase of 13% in MMP-13 protein expression[66]. Besides, compared with non-transfected controls, KOA chondrocytes transfected with miR-27b inhibitor alone produced 51% more MMP-13 protein[66]. MiR-27b significantly decreased in KOA patients induced by activated upstream NF-κB signaling pathway directly[67,68]. NF-κB is a protein complex which plays a crucial role in inflammatory responses and chondrocyte apoptosis. The activation of NF-κB signaling pathways will trigger release of pro-inflammatory cytokines. The degree of cartilage damage and the balance of ECM metabolism will then be altered[69,70]. Previous studies have shown that NF-κB can up-regulate the levels of p38 MAPK[70]. Apart from the effect derived from NF-κB, activated p38 MAPK can also decrease miR-27b and increase MMP-13 expression. Meanwhile, down-regulated miR-27b could directly increase MMP-13. That is to say, the activated NF-κB could up-regulate the expression of MMP-13 but act as a negative regulator of miR-27b in promoting chondrocytes degradation.

MiR-146a

MiR-146a is a negative upstream of MMP-13 in KOA cartilage. It can regulate MMP-13 *via* a feedback loop. This loop involves miR-146a, MMP-13, IL-1 receptor–associated kinase 1 (IRAK1) and TNF receptor associated factor 6 (TRAF6). First, Taganov et al[71] reported miR-146a could directly inhibit the expression of IRAK1 and TRAF6 binding to the 3'-UTR. Second, miR-146a was markedly decreased in human chondrocytes in parallel with significantly increased MMP-13. MiR-146a was inferred to regulate MMP-13 through IRAK1 and TRAF6. In primary KOA compensatory stage, miR-146a

could still maintain the delicate balance of ECM homeostasis through the feedback loop. However, during decompensated period, the negative feedback loop was destroyed. Significantly decreased miR-146a could not inhibit IRAK1 and TRAF6 and thus resulted in MMP-13 upregulation. Once MMP-13 was up-regulated, ECM homeostasis would be destroyed.

MiR-140

MiR-140 is also a negative regulator of MMP-13 in chondrocyte degradation[72]. However, the mechanism underlies miR-140-mediated regulation on MMP-13 is currently unclear. Recent studies have showed the possible correlations among miR-140, estrogen and MMP-13. Sex hormone is considered as an important etiology of KOA. The increased prevalence in women with menopausal transition is considered as a result of estrogen levels reduction[73,74]. Both estrogen receptor (ER) and MMP-13 are expressed in joint tissue and chondrocytes, indicating that chondrocytes can respond to estrogen levels[75,76]. MMP-13 levels were significantly suppressed by 17-β-estradiol (E2) in chondrocytes in female patients[66]. Moreover, E2 is indicated to be the upstream of miR-140. E2 can directly up-regulate the expression of miR-140 in chondrocyte. Meanwhile, E2 has the capacity to down-regulate MMP-13 transcription and protein levels in KOA-diseased chondrocytes. MiR-140 can also down-regulate MMP-13 as already known. Thus, miR-140 is a crucial regulator in E2-mediated cartilage homeostasis as miR-140 can enable E2 to suppress MMP-13 production. The chondrocytes are thus protected from degradation. Taken together, these findings provide new insight into the mechanism of menopausal caused KOA. The E2/miR-140/MMP-13 axis is indicated to be a potential target for therapeutic interventions for KOA.

MiR-448

It has been reported that miR-448 is broadly involved in chondrocyte apoptosis[77,78]. In chondrocyte regulation, miR-448 is positively correlated with MMP-13 levels. Recent studies have indicat-

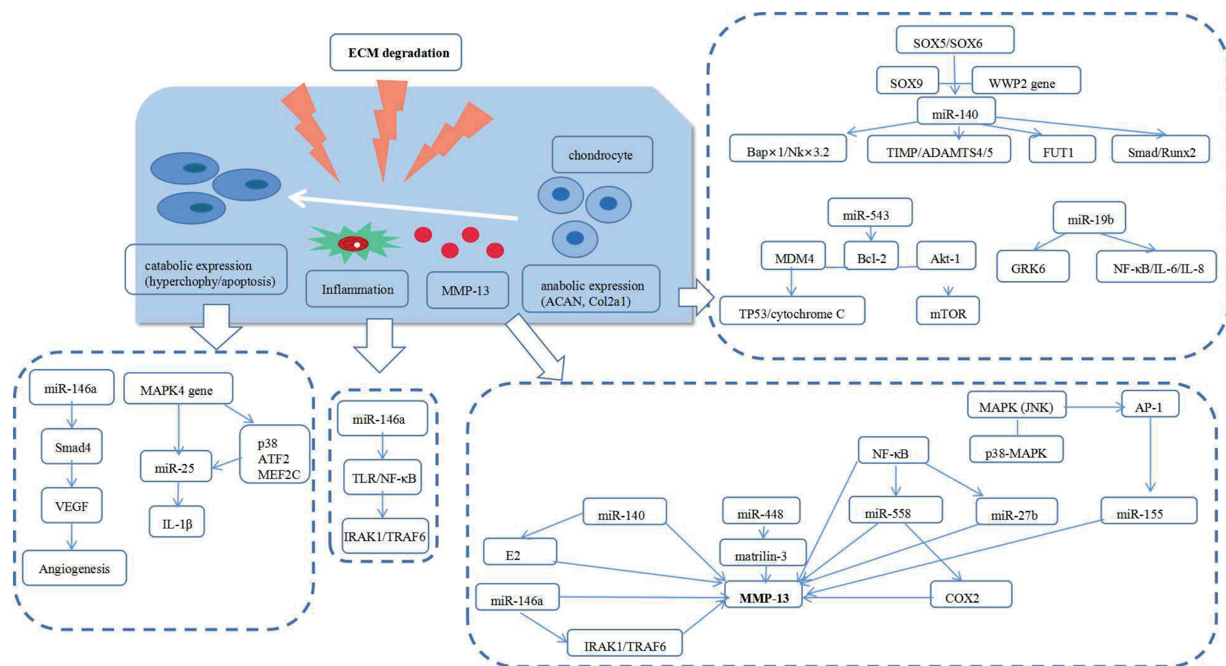

Figure 2. MicroRNAs and associated regulators in ECM degradation. Cartilage degradation is considered to be the central pathogenesis of KOA. The imbalance between catabolic and anabolic expression accompanied with MMP-13 and inflammation contributes to the ECM degradation. MicroRNAs such as miR-140, miR-146a, miR-25, miR-543, miR-19b can directly regulate chondrocyte and destroy the balance established in ECM by affecting their upstream/downstream regulators or pathways including ADAMTS, SOX9, p38 MAPK, VEGF, FUT1 and RUNX2. Besides, miRNAs can regulate the activation of key enzymes such as MMP-13 in order to degrade chondrocytes. For example, miRNAs such as miR-27b, miR-146a, miR-558, miR-155, miR-140 and miR-448 can up-regulate the expression of MMP-13 via NF-κB, estrogen and matrilin-3 pathways. Meanwhile, miRNAs play a significant role in regulating inflammation. MiRNAs such as miR-146a can increase inflammatory responses via various inflammatory cytokines especially through TLR4/NF-κB pathways.

ed that miR-448 can regulate MMP-13 by directly targeting on matrilin-3[79]. Matrilin-3 is a non-collagenous oligomeric ECM protein, belonging to one of the four members of the Matrilin family[80]. As an ECM protein, matrilin-3 can interact with collagen fibrils, multiple proteoglycans and other glycoproteins. It significantly contributes to the formation of filamentous matrix network[81]. In KOA, it was discovered that miR-448 was significantly increased while matrilin-3 decreased[82]. Overexpression of miR-448 would up-regulate MMP-13 level and down-regulate ACAN and Col2a1 expression. Reversely, knockdown of miR-448 significantly increased the expressions of matrilin-3, ACAN and Col2a1. The expression of MMP-13 was thus decreased subsequently compared with osteoarthritis chondrocytes. These data indicated that overexpression of miR-448 could up-regulate MMP-13 *via* matrili-3 in ECM degradation. This also indicates that matrilin-3 may be a potential highlight for future research.

MiR-558

MiR-558 was first identified in human colorectal cells and then found to be up-regulated in irradiated fibroblasts. It is now noted for targeting genes involved in chondrocyte apoptosis[83,84]. MMP-13 and COX2 are downstream of miR-558[85]. COX-2 is a major prostaglandin E2 (PGE2) synthetic enzyme involved in the pathogenesis of chronic inflammation and pain in KOA. COX-2 can be directly down-regulated by miR-558 overexpression by binding to COX-2 3'UTR in human chondrocytes[85]. NF-κB acts as the upstream of miR-558, the activation of which can significantly inhibit miR-558 expression. In human KOA chondrocyte, miR-558 was reported to be significantly down-regulated by upstream NF-κB activation[86]. Decreased miR-558 up-regulated COX-2 mRNA levels and promoted the expression of COX2. Meanwhile, MMP-13 expression was significantly increased due to miR-558 decrease. Then, Col2a1 synthesis decreased and ECM degraded as a result of above condition. These findings demonstrated that chondrocyte homeostasis could be influenced by miR-558 *via* upstream NF-κB and downstream COX/MMP-13.

MiR-155

It has been shown that miR-155 plays an important role in chondrocyte degradation *via* upstream MAPK pathway[87,88]. MAPKs are central regulators in chondrocyte proliferation, survival control and matrix synthesis. MAPKs have three signal groups which are extracellular signal regulated kinase (ERK), Jun-N terminal kinase (JNK) and 38 kDa protein kinase (p38). Impairment of JNK signal transmission, directly targeting on miR-155, contributes to the chondrocyte development, growth and apoptosis[89]. Moreover, JNK can regulate MMP production[89]. In KOA chondrocyte, activated JNK level will increase Activator protein-1 (AP-1) and then up-regulate miR-155 in parallel with MMP-13. Chondrocyte degradation was thus accelerated due to MMP-13 upregulation. Therefore, miR-155 was indicated to regulate chondrocyte *via* JNK, miR-155, MMP-13 paticipation[90]. MAPK and AP-1 can be seen as therapeutic target in alleviating KOA.

MicroRNA and Pro-inflammatory Cytokines

Recent evidence suggests that pro-inflammatory cytokines/pathways contribute to the abnormal inflammatory network within KOA[91]. Toll-like receptors (TLRs) are indicated to be controlled post-transcriptionally by specific miRNAs[92]. Previous studies have demonstrated that overexpression of miR-146a could decrease TLR4-mediated inflammation through inhibition of TLR4/NF-κB signaling[93]. However, the detailed mechanism has not been illuminated. Current research showed that abnormal inflammation is associated with danger-associated molecular patterns (DAMPs). Innate immune responses can be activated not only against pathogens but also by DAMPs released upon pathological conditions in KOA[94]. DAMPs are recognized by pattern recognition receptors such as TLRs. They will lead to activation of signaling adaptor proteins (IRAK1 and TRAF-6). Subsequently, they will result in nuclear translocation of the central pro-inflammatory transcriptional factor NF-κB and mediate the cytokine responses[94].

The increased production of pro-inflammatory cytokines observed in KOA is thought to be mediated by the activation of TLR4[95]. Upon DAMPs stimulation, overexpressed TLR4 in KOA chondrocytes initiates the downstream signaling pathway and leads to inflammatory responses *via* NF-κB activation[95].

MiR-146a

MiR-146a has been described as one of the key molecules in inflammatory responses. It will lead to pain and abnormal immune responses in KOA. Taganov et al[71] reported that miR-146a could be induced by inflammatory cytokines. Moreover, it

was a NF-κB dependent gene which could inhibit IRAK1 and TRAF6 expressions.

TLR4/IRAK1/TRAF6 pathway is downstream of miR-146a. MiR-146a can regulate inflammatory responses *via* above signaling pathway. TLR4 is indicated to be the only common KOA-related target gene of miR-146a involved in KOA inflammatory response[96]. Overexpression of miR-146a can result in a strong negative effect on TLR4 expression in chondrocyte. Meanwhile, overexpression of miR-146a can result in significant reduction of NF-κB phosphorylation levels and related gene expressions, as well as IL-1β, IL-6 and TNF-α[97]. Moreover, IRAK1 and TRAF6 expressions can be regulated by TLR4 and negatively correlated with miR-146a as mentioned. Compared with normal chondrocytes, the expression of miR-146a decreased in KOA. Decreased miR-146a would make TLR4 dependent signaling over-activated. Meanwhile, pro-inflammatory cytokines levels such as IL-6 and TNF-α induced by NF-κB were also up-regulated. The directly targeted IRAK1 and TRAF6 would also be up-regulated subsequently[97]. Thus, decreased chondrocyte proliferation and increased chondrocyte apoptosis were led in KOA progression. That is to say, decreased miR-146a could lead to chondrocyte apoptosis and inhibit proliferation by promoting TLR4 activation and expression of IRAK1 and TRAF6. Moreover, recent studies showed that miR-146a has suppressed the expressions of some proteins related to ECM in chondrocytes[98-101]. However, the detailed mechanisms at the protein molecular levels are remained unclear. It has then pointed out a future potential research field for KOA.

Conclusions

There are increasing studies indicating miRNA has been involved in the pathogenesis of KOA. MiRNAs can directly regulate chondrocyte homeostasis targeting on ECM degradation by affecting their upstream/downstream or pathways (Figure 2). Moreover, as KOA is an inflammation-related disease, miRNAs also play a significant role in regulating inflammation. To date, the interactions among above factors in the pathogenesis and development of KOA have been less understood. Further studies should be focused on exploring the following aspects. First, the complex network involving miRNAs, proteins and corresponding pathways should be analyzed in order to make clear the specific mechanism of KOA. Second, the value of miRNAs in clinics serving as potential new drugs should be paid attention to. For example, the development of nano carriers based on miRNAs and the preparation of corresponding drugs have broad application prospects. In fact, miRNAs, especially their application in exosomes, are likely to make a breakthrough. In a recent study, a platelet rich plasma derived exosome was used to interfere with the inflammatory model of chondrocytes. It was showed that exosomes could alleviate the inflammatory response and promote cell proliferation. Moreover, intra-articular administration of the exosomes and miRNA has already been used in the treatment of KOA. It is of great significance for the treatment of osteoarthritis.

Contributors

Sihui Li and Qiaofeng Wu designed the manuscript together. Sihui Li drafted while Qiaofeng Wu revised the manuscript. All authors have read, revised and approved this version of the manuscript.

Acknowledgements

We would like to thank Tong Feng and Weishang Hu Wu for the discussion during the manuscript drafted.

Funding

This work was supported by the National Key R&D Program of China (No.2019YFC1709001); National Natural Science Foundation of China (No.81873383,81873386); Fund of Science and Technology Department of Sichuan Province, China (No. 2018JY0095); Fund of Sichuan Provincial Department of Education, China (No. 18TD0018).

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Wieland HA, Michaelis M, Kirschbaum BJ, Rudolphi KA. Osteoarthritis-an untreatable disease? Nat Rev Drug Discov 2005; 4: 331-344.
2) Cao PK, Wang XM, Bai WX, Niu YZ, Wang F. Interpretation of 2018 edition of osteoarthritis diagnosis and treatment guide. J Hebei Med Univ 2018; 39: 1241-1243
3) Felson DT. Developments in the clinical understanding of osteoarthritis. Arthritis Res Ther 2009; 11: 203.

4) Heinegard D, Saxne T. The role of the cartilage matrix in osteoarthritis. Nat Rev Rheumatol 2011; 7: 50-56.
5) Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004; 303: 83-86.
6) Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 2005; 33: 1290-1297.
7) Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. PLoS One 2008; 3: e3740.
8) Cong L, Zhu Y, Tu G. A bioinformatic analysis of microRNAs role in osteoarthritis. Osteoarthritis Cartilage 2017; 25: 1362-1371.
9) Zhou MW, Guo TF, Li SH, Sun FQ, Mu HX. Research progress of the effect of microRNA on the metabolism of bone tissue. Chin J Osteoporosis 2014; 20: 84-90.
10) Wang JC, Yi Z. Correlation between miRNA and pathological development of osteoarthritis. Chin J Tissue Eng Res 2019; 23: 3875-3881.
11) Ji QB, Xu YM, Wang Y. Research progress of microRNA in osteoarthritis cartilage matrix degradation. Chin J Repar Reconstr Surg 2016; 30: 1431-1436.
12) Yu Q, Li HP, Guo X. The mechanism advance of microRNA in cartilage injury and degradation. Chin J Orthop Traumatol 2012; 25: 530-534.
13) Malemud CJ. MicroRNAs and osteoarthritis. Cells (Basel) 2018; 7: 1-10.
14) Araldi E, Schipani E. MicroRNA-140 and the silencing of osteoarthritis. Genes Dev 2010; 24: 1075-1080.
15) Li X, Gibson G, Kim JS, Kroin J, Xu S, van Wijnen AJ, Im HJ. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. Gene 2011; 480: 34-41.
16) Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno H, Asahara H. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev 2010; 24: 1173-1185.
17) Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, Kato Y, Sato T, Lotz MK, Asahara H. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum 2009; 60: 2723-2730.
18) Nakamura Y, He X, Kobayashi T, Yan YL, Postlethwait JH, Warman ML. Unique roles of microRNA140 and its host gene WWP2 in cartilage biology. J Musculoskelet Neuronal Interact 2008; 8: 321-322.
19) Nicolas FE, Pais H, Schwach F, Lindow M, Kauppinen S, Moulton V, Dalmay T. mRNA expression profiling reveals conserved and non-conserved miR-140 targets. RNA Biol 2011; 8: 607-615.
20) Tardif G, Hum D, Pelletier JP, Duval N, 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: 148-158.
21) Buechli ME, Lamarre J, Koch TG. MicroRNA-140 expression during chondrogenic differentiation of equine cord blood-derived mesenchymal stromal cells. Stem Cells Dev 2013; 22: 1288-1296.
22) Karlsen TA, Jakobsen RB, Mikkelsen TS, Brinchmann JE. microRNA-140 targets RALA and regulates chondrogenic differentiation of human mesenchymal stem cells by translational enhancement of SOX9and ACAN. Stem Cells Dev 2014; 23: 290-304.
23) Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno H, Asahara H. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev 2010; 24: 1173-1185.
24) Sive JI, Baird P, Jeziorsk M, Watkins A, Hoyland JA, Freemont AJ. Expression of chondrocyte markers by cells of normal and degenerate intervertebral discs. Mol Pathol 2002; 55: 91-97.
25) Yang B, Kang X, Xing Y, Dou C, Kang F, Li J, Quan Y, Dong SW. Effect of microRNA-145 on IL-1beta-induced cartilage degradation in human chondrocytes. FEBS Lett 2014; 588: 2344-2352.
26) Yamashita S, Miyaki S, Kato Y, Yokoyama S, Sato T, Barrionuevo F, Akiyama H, Scherer G, Takada S, Asahara H. L-Sox5 and Sox6 proteins enhance chondrogenic miR-140 microRNA expression by strengthening dimeric Sox9 activity. J Biol Chem 2012; 287: 22206-22215.
27) Li CF, Chen Z, Yang J, Zhou ZK, Kang PD, Pei FX, Shen B. Expression and function of miR-140 in different stage chondrocytes of patients with osteoarthritis. J Pract Orthopaedics 2017; 23: 995-999.
28) Zhou XH, Wang M, Ji YH, Jiang Y, Liu Q. Expression of miRNA-140 in chondrocytes of patients with early osteoarthritis and its function. Acad J Second Mil Med Univ 2014; 35: 610-615.
29) Bursavich MG, Gilbert AM, Lombardi S, Georgiadis KE, Reifenberg E, Flannery CR, Morris EA. Synthesis and evaluation of aryl thioxothiazolidinone inhibitors of ADAMTS-5 (Aggrecanase-2). Bioorg Med Chem Lett 2007; 17: 1185-1188.
30) Huang K, Wu LD. Aggrecanase and aggrecan degradation in osteoarthritis: a review. J Int Med Res 2008; 36: 1149-1160.
31) Song RH, Tortorella MD, Malfait AM, Alston JT, Yang Z, Arner EC, Griggs DW. Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. Arthritis Rheum 2007; 56: 575-585.
32) Ma B, Simala-Grant JL, Taylor DE. Fucosylation in prokaryotes and eukaryotes. Glycobiology 2006; 16: 158-184.
33) Wang Z, Hu J, Pan Y, Shan Y, Jiang L, Qi X, Jia L. miR-140-5p/miR-149 affects chondrocyte proliferation, apoptosis, and autophagy by targeting FUT1 in osteoarthritis. Inflammation 2018; 41: 959-971.
34) Enomoto H, Enomoto-Iwamoto M, Iwamoto M, Nomura S, Himeno M, Kitamura Y, Kishmoto T, Komori T. Cbfa1 is a positive regulatory factor in

chondrocyte maturation. J Biol Chem 2000; 275: 8695-8702.
35) Takeda S, Bonnamy JP, Owen MJ, Ducy P, Karsenty G. Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice. Genes Dev 2001; 15: 467-481.
36) Ueta C, Iwamoto M, Kanatani N, Yoshida C, Liu Y, Enomoto-Iwamoto M, Ohmori T, Enomoto H, Nakata K, Takata K, Kurisu K, Komori T. Skeletal malformations caused by overexpression of Cbfa1 or its dominant negative form in chondrocytes. J Cell Biol 2001; 153: 87-100.
37) Kim IS, Otto F, Zabel B, Mundlos S. Regulation of chondrocyte differentiation by Cbfa1. Mech Dev 1999; 80: 159-170.
38) Inada M, Yasui T, Nomura S, Miyake S, Deguchi K, Himeno M, Sato M, Yamagiwa H, Kimura T, Yasui N, Ochi T, Endo N, Kitamura Y, Kishimoto T, Komori T. Maturational disturbance of chondrocytes in Cbfa1-deficient mice. Dev Dyn 1999; 214: 279-290.
39) Chen CG, Thuillier D, Chin EN, Alliston T. Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis. Arthritis Rheum 2012; 64: 3278-3289.
40) Javed A, Afzal F, Bae JS, Gutierrez S, Zaidi K, Pratap J, Wijnen AJ, Stein JL, Stein GS, Lian JB. Specific residues of RUNX2 are obligatory for formation of BMP2-induced RUNX2-SMAD complex to promote osteoblast differentiation. Cells Tissues Organs 2009; 189: 133-137.
41) Komori T. Molecular mechanism of Runx2-dependent bone development. Mol Cells 2020; 43: 168-175.
42) Hess J, Porte D, Munz C, Angel P. AP-1 and Cbfa/runt physically interact and regulate parathyroid hormone-dependent MMP-13 expression in osteoblasts through a new osteoblast-specific element 2/AP-1 composite element. J Biol Chem 2001; 276: 20029-20038.
43) Hirata M, Kugimiya F, Fukai A, Saito T, Yano F, Ikeda T, Mabuchi A, Sapkota BR, Akune T, Nishida N, Yoshimura N, Nakagawa T, Tokunaga K, Nakamura K, Chung U, Kawaguchi H. C/EBPbeta and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2alpha as the inducer in chondrocytes. Hum Mol Genet 2012; 21: 1111-1123.
44) Takahashi A, de Andres MC, Hashimoto K, Itoi E, Otero M, Goldring MB, Oreffo RO. DNA methylation of the RUNX2 P1 promoter mediates MMP-13 transcription in chondrocytes. Sci Rep 2017; 7: 7771-7780.
45) Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Ohba S, Nishida N, Akune T, Yoshimura N, Nakagawa T, Nakamura K, Tokunaga K, Chung U, Kawaguchi H. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. Nat Med 2010; 16: 678-686.
46) Franses RE, McWilliams DF, Mapp PI, Walsh DA. Osteochondral angiogenesis and increased protease inhibitor expression in OA. Osteoarthritis Cartilage 2010; 18: 563-571.
47) Zhang Q, Yang J, Chen JY, Li Z, Zhang P, Bi MN, Zhang SS, Zhu JW, Lu MS. The mechanism of miRNA 146a regulating VEGF in the degenerative process of OA chondrocyte. J Pract Orthopaedics 2016; 22: 237-241.
48) Wu HS, Huang SF, Sun K, Cai F, Liao Q. MicroRNA-25 induced the synthesis of ACAN and Col2a1 of OA chondrocyte. Sci Technol Eng 2016; 16: 143-148.
49) Feldmann M, Maini SR. Role of cytokines in rheumatoid arthritis: an education in pathophysiology and therapeutics. Immunol Rev 2008; 223: 7-19.
50) Hashimoto M, Nakasa T, Hikata T, Asahara H. Molecular network of cartilage homeostasis and osteoarthritis. Med Res Rev 2008; 28: 464-481.
51) Maier R, Bilbe G, Rediske J, Lotz M. Inducible nitric oxide synthase from human articular chondrocytes: cDNA cloning and analysis of mRNA expression. Biochim Biophys Acta 1994; 1208: 145-150.
52) Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. Osteoarthritis Cartilage 2006; 14: 403-412.
53) Wojdasiewicz P, Poniatowski LA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014; 66: 127-132.
54) Rasheed Z, Akhtar N, Haqqi TM. Advanced glycation end products induce the expression of interleukin-6 and interleukin-8 by receptor for advanced glycation end product-mediated activation of mitogen-activated protein kinases and nuclear factor-kappaB in human osteoarthritis chondrocytes. Rheumatology (Oxford) 2011; 50: 838-851.
55) Rasheed Z, Akhtar N, Haqqi TM. Pomegranate extract inhibits the interleukin-1beta-induced activation of MKK-3, p38alpha-MAPK and transcription factor RUNX-2 in human osteoarthritis chondrocytes. Arthritis Res Ther 2010; 12: R195-197.
56) Song N, Liu H, Ma X, Zhang S. Placental growth factor promotes metastases of ovarian cancer through MiR-543-regulated MMP7. Cell Physiol Biochem 2015; 37: 1104-1112.
57) Mancini F, Pieroni L, Monteleone V, Luca R, Fici L, Luca E, Urbani A, Xiong S, Soddu S, Masetti R, Lozano G, Pontecorvi A, Moretti F. MDM4/HIPK2/p53 cytoplasmic assembly uncovers coordinated repression of molecules with anti-apoptotic activity during early DNA damage response. Oncogene 2016; 35: 228-240.
58) Luo FQ, Lin Y, Xu J. Study on the mechanism of effect of microRNA-543 on osteoarthritis chondrocytes in rats. Chin Foreign Med Res 2019; 17: 1-3.
59) Zhou Y, Li RJ, Li M, Liu X, Zhu HY, Ju Z, Miao XH, Xu GY. Overexpression of GRK6 attenuates neuropathic pain via suppression of CXCR2 in rat dorsal root ganglion. Mol Pain 2016; 12: 1744806916646381.
60) Duan L, Duan D, Wei W, Sun Z, Xu H, Guo L, Wu XH. MiR-19b-3p attenuates IL-1beta induced extracellular matrix degradation and inflammato-

ry injury in chondrocytes by targeting GRK6. Mol Cell Biochem 2019; 459: 205-214.
61) Pan L, Liu D, Zhao L, Wang L, Xin M, Li X. Long noncoding RNA MALAT1 alleviates lipopolysaccharide-induced inflammatory injury by upregulating microRNA-19b in murine chondrogenic ATDC5 cells. J Cell Biochem 2018; 119: 10165-10175.
62) Shi J, Li T, Zuo CH, Zhi L, Wei R, Wang WM, Liu YJ. Influence of interleukin-1beita on microRNA-27b and matrix metalloproteinase-13 expression in rat chondrocytes. Chin J Tissue Eng Res 2016; 20: 4277-4283.
63) Neuhold LA, Killar L, Zhao W, Sung ML, Warner L, Kulik J, Turner J, Wu W, Billinghurst C, Meijers T, Poole AR, 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.
64) Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, Shah M, Thompson EW. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum 2009; 60: 3723-3733.
65) Knauper V, Lopez-Otin C, Smith B, Knight G, Murphy G. Biochemical characterization of human collagenase-3. J Biol Chem 1996; 271: 1544-1550.
66) 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.
67) Lotz M. Cytokines in cartilage injury and repair. Clin Orthop Relat Res 2001; 391: S108-S115.
68) 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.
69) Fu YC, Zhao SR, Zhu BH, Guo SS, Wang XX. MiRNA-27a-3p promotes osteogenic differentiation of human mesenchymal stem cells through targeting ATF3. Eur Rev Med Pharmacol Sci 2019; 23: 73-80.
70) Chen J, Crawford R, Xiao Y. Vertical inhibition of the PI3K/Akt/mTOR pathway for the treatment of osteoarthritis. J Cell Biochem 2013; 114: 245-249.
71) Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 2006; 103: 12481-12486.
72) Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. Physiol Rev 2001; 81: 1535-1565.
73) Liu YP, Li J, Xin SB. Study the relevance between inflammatory factors and estradiol and their association with knee osteoarthritis in postmenopausal women. Eur Rev Med Pharmacol Sci 2018; 22: 472-478.
74) Ushiyama T, Ueyama H, Inoue K, Ohkubo I, Hukuda S. Expression of genes for estrogen receptors alpha and beta in human articular chondrocytes. Osteoarthritis Cartilage 1999; 7: 560-566.
75) Braidman IP, Hainey L, Batra G, Selby PL, Saunders PT, Hoyland JA. Localization of estrogen receptor beta protein expression in adult human bone. J Bone One Miner Res 2001; 16: 214-220.
76) Lee YJ, Lee EB, Kwon YE, Lee JJ, Cho WS, Kim HA, Song WY. 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.
77) Lv Y, Lei Y, Hu Y, Ding W, Zhang C, Fang C. MiR-448 negatively regulates ovarian cancer cell growth and metastasis by targeting CXCL12. Clin Transl Oncol 2015; 17: 903-909.
78) Shen L, Liu L, Ge L, Xie L, Liu S, Sang L, Zhan TT, Li WH. miR-448 downregulates MPPED2 to promote cancer proliferation and inhibit apoptosis in oral squamous cell carcinoma. Exp Ther Med 2016; 12: 2747-2752.
79) Feldmann M, Maini SR. Role of cytokines in rheumatoid arthritis: an education in pathophysiology and therapeutics. Immunol Rev 2008; 223: 7-19.
80) Klatt AR, Nitsche DP, Kobbe B, Morgelin M, Paulsson M, Wagener R. Molecular structure and tissue distribution of matrilin-3, a filament-forming extracellular matrix protein expressed during skeletal development. J Biol Chem 2000; 275: 3999-4006.
81) Jayasuriya CT, Goldring MB, Terek R, Chen Q. Matrilin-3 induction of IL-1 receptor antagonist is required for up-regulating collagen II and aggrecan and down-regulating ADAMTS-5 gene expression. Arthritis Res Ther 2012; 14: R197-205.
82) Yang H, Wu D, Li H, Chen N, Shang Y. Downregulation of microRNA-448 inhibits IL-1β-induced cartilage degradation in human chondrocytes via upregulation of matrilin-3. Cell Mol Biol Lett 2018; 23: 7-18.
83) Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LJ, Sjoblom T, Barad O, Bentwish Z, Szafranska AE, Labourier E, Raymond CK, Roberts BS, Juhl H, Kinzler KW, Vogelstein B, Velculescu VE. The colorectal microRNAome. Proc Natl Acad Sci U S A 2006; 103: 3687-3692.
84) Maes OC, An J, Sarojini H, Wu H, Wang E. Changes in MicroRNA expression patterns in human fibroblasts after low-LET radiation. J Cell Biochem 2008; 105: 824-834.
85) Park SJ, Cheon EJ, Kim HA. MicroRNA-558 regulates the expression of cyclooxygenase-2 and IL-1beta-induced catabolic effects in human articular chondrocytes. Osteoarthritis Cartilage 2013; 21: 981-989.
86) Attur M, Al-Mussawir HE, Patel J, Kitay A, Dave M, Palmer G, Pillinger MH, Abramson SB. Prostaglandin E2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the EP4 receptor. J Immunol 2008; 181: 5082-5088.
87) Sheedy FJ, O'Neill LA. Adding fuel to fire: microRNAs as a new class of mediators of inflammation. Ann Rheum Dis 2008; 67: i50-i55.
88) Ceppi M, Pereira PM, Dunand-Sauthier I, Barras E, Reith W, Santos MA, Pierre P. MicroRNA-155

modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. Proc Natl Acad Sci U S A 2009; 106: 2735-2740.
89) Bogoyevitch MA, Ngoei KR, Zhao TT, Yeap YY, Ng DC. c-Jun N-terminal kinase (JNK) signaling: recent advances and challenges. Biochim Biophys Acta 2010; 1804: 463-475.
90) Sowers MR, McConnell D, Jannausch M, Buyuktur AG, Hochberg M, Jamadar DA. Estradiol and its metabolites and their association with knee osteoarthritis. Arthritis Rheum 2006; 54: 2481-2487.
91) Kalaitzoglou E, Griffin TM, Humphrey MB. Innate immune responses and osteoarthritis. Curr Rheumatol Rep 2017; 19: 45-50.
92) Bayraktar R, Bertilaccio M, Calin GA. The interaction between two worlds: MicroRNAs and toll-like receptors. Front Immunol 2019; 10: 1053-1069.
93) Liu W, Wu YH, Zhang L, Xue B, Wang Y, Liu B, Liu XY, Zuo F, Yang XY, Chen FY, Duan R, Cai Y, Zhang B, Ji Y. MicroRNA-146a suppresses rheumatoid arthritis fibroblast-like synoviocytes proliferation and inflammatory responses by inhibiting the TLR4/NF-kB signaling. Oncotarget 2018; 9: 23944-23959.
94) Scanzello CR, Plaas A, Crow MK. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? Curr Opin Rheumatol 2008; 20: 565-572.
95) Herrero-Beaumont G, Perez-Baos S, Sanchez-Pernaute O, Roman-Blas JA, Lamuedra A, Largo R. Targeting chronic innate inflammatory pathways, the main road to prevention of osteoarthritis progression. Biochem Pharmacol 2019; 165: 24-32.
96) Chen Y, Zeng Z, Shen X, Wu Z, Dong Y, Cheng JC. MicroRNA-146a-5p negatively regulates pro-inflammatory cytokine secretion and cell activation in lipopolysaccharide stimulated human hepatic stellate cells through inhibition of toll-like receptor 4 signaling pathways. Int J Mol Sci 2016; 17: 1076-1083.
97) Papathanasiou I, Balis C, Trachana V, Mourmoura E, Tsezou A. The synergistic function of miR-140-5p and miR-146a on TLR4-mediated cytokine secretion in osteoarthritic chondrocytes. Biochem Biophys Res Commun 2020; 522: 783-791.
98) Al-Modawi RN, Brinchmann JE, Karlsen TA. Multi-pathway protective effects of MicroRNAs on human chondrocytes in an in vitro model of osteoarthritis. Mol Ther Nucleic Acids 2019; 17: 776-790.
99) Liang Y, Duan L, Xiong J, Zhu W, Liu Q, Wang D, Liu W, Li ZG, Wang DP. E2 regulates MMP-13 via targeting miR-140 in IL-1β-induced extracellular matrix degradation in human chondrocytes. Arthritis Res Ther 2016; 18: 105-115.
100) Ding JN, Zhang YF, Ding YL. MiRNA-146b-5p inhibits the malignant progression of gastric cancer by targeting TRAF6. Eur Rev Med Pharmacol Sci 2020; 24: 8837-8844.
101) Zhang M, Meng QC, Yang XF, Wu MD. TGF-β1/WISP1/Integrin-α interaction mediates human chondrocytes dedifferentiation. Eur Rev Med Pharmacol Sci 2020; 24: 8675-8684.