Curr Osteoporos Rep (2014) 12:410–419
DOI 10.1007/s11914-014-0229-9

SKELETAL DEVELOPMENT (E SCHIPANI AND E ZELZER, SECTION EDITORS)

microRNAs in Cartilage Development, Homeostasis, and Disease

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Published online: 5 August 2014

Abstract microRNAs (miRNAs) regulate gene expression mainly at the posttranscriptional level. Many different miRNAs are expressed in chondrocytes, and each individual miRNA can regulate hundreds of target genes, creating a complex gene regulatory network. Experimental evidence suggests that miRNAs play significant roles in various aspects of cartilage development, homeostasis, and pathology. The possibility that miRNAs can be novel therapeutic targets for cartilage diseases led to vigorous investigations to understand the role of individual miRNAs in skeletal tissues. Here, we summarize our current understanding of miRNAs in chondrocytes and cartilage. In the first part, we discuss roles of miRNAs in growth plate development and chondrocyte differentiation. In the second part, we put a particular focus on articular cartilage and discuss the significance of variety of findings in the context of osteoarthritis, the most common degenerative joint disease.

Keywords Chondrocyte · microRNA · Growth plate · Articular cartilage · Osteoarthritis

Introduction

Cartilage-forming chondrocytes derive from mesenchymal progenitor cells. During embryonic development, mesenchymal progenitor cells proliferate, aggregate at mesenchymal condensations, and then start differentiating into chondrocytes to form cartilage templates for future bones and cartilage [1]. Chondrocytes secrete unique extracellular matrix (ECM) proteins, such as type II collagen and aggrecan, to generate cartilage matrix. The most common form of cartilage, hyaline cartilage, provides mechanical support. Hyaline cartilage is found in joints, ribs, and growth plates. Two types of hyaline cartilage play major roles in development and function of long bones. Growth plate cartilage drives long bone growth, and articular cartilage provides shock absorption and mobility in joints.

Distinct groups of chondrocytes exist in the growth plate. Resting chondrocytes, located at the most epiphyseal side of the bone, proliferate slowly and differentiate into proliferating chondrocytes. Proliferating chondrocytes, characterized by their vigorous proliferation, produce cartilage matrix proteins important for cartilage function, such as type II collagen and aggrecan, and then further differentiate into postmitotic hypertrophic chondrocytes that express type X collagen. Hypertrophic chondrocytes undergo mineralization and are eventually replaced by mineralized bone. This process leads to longitudinal bone growth. Tightly coordinated chondrocyte proliferation and differentiation, regulated by a variety of signaling molecules, such as Indian hedgehog (Ihh), parathyroid hormone-related peptide (PTHrP), bone morphogenetic proteins (BMP), and fibroblast growth factors (FGFs), is essential for normal bone growth. The transcription factor, sex determining region Y (SRY) box 9 (*Sox9*), plays an essential role for chondrocyte differentiation, function, and survival. *Sox9* directly regulates expression of Collagen II and aggrecan [2, 3]. The activity of *Sox9* is enhanced by the related molecules *Sox5* and *Sox6* [4]. Other transcription factors important for chondrocyte differentiation include myocyte enhancer factor 2 (MEF2) family transcription factors and Runx2. MEF2 transcription factors, negatively regulated by histone deacetylase 4 (HDAC4) and also possibly by other class IIa HDACs through direct binding, promote hypertrophic differentiation. Runx2, a transcription factor essential for osteoblast differentiation, is expressed in growth plate chondrocytes and stimulates chondrocyte hypertrophy [5, 6].

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These signaling molecules and transcription factors regulate chondrocyte-specific gene expression. In vivo and in vitro evidence has shown that miRNAs also play important roles in chondrocyte biology by suppressing undesired gene expression at the post-transcriptional level.

miRNAs are ~22 nucleotide-long, noncoding RNAs that directly bind to target RNAs in a sequence-complimentary manner to inhibit translation and facilitate degradation of their target transcripts. miRNAs are encoded in the genome, and are transcribed as long primary transcripts (pri-miRNAs). Pri-miRNAs are subsequently processed into small hairpin precursor miRNAs (pre-miRNAs), which are transported to the cytoplasm to be further cleaved by Dicer into mature and functional miRNAs [7].

A global reduction of miRNAs after *Dicer* deletion in multiple types of bone cells including chondrocytes causes significant defects in vivo, providing the basis for further investigation of individual miRNAs in bone and cartilage.

Role of miRNAs in Cartilage Development

In Vivo Studies

We previously showed that global reduction in miRNAs in chondrocytes by conditionally deleting the Dicer gene resulted in a significant growth defect and premature death [8]. Proliferation of growth plate chondrocytes was substantially reduced, while the number of hypertrophic chondrocytes was increased. These results suggest that miRNAs regulate proliferation and differentiation of growth plate chondrocytes. Expression of Hmga2, a well-characterized let-7 miRNA target, was upregulated in Dicer-deficient chondrocytes.

let-7 miRNAs are most abundant miRNAs in somatic tissues including chondrocytes. We have shown that let-7 family miRNAs are required for normal chondrocyte proliferation in the growth plate [9•]. Overexpression of the let-7 inhibitor Lin28a in chondrocytes suppressed let-7 miRNA expression. This reduced chondrocyte proliferation and led to mild growth impairment. Lin28a overexpression in chondrocytes upregulated predicted let-7 target genes, including cell division cycle 34 (*Cdc34*) and E2F transcription factor 5 (*E2f5*). The skeletal phenotype of Lin28a transgenic mice was mild, but compound miR140 deficient/Lin28a transgenic mice show a dramatic growth defect. As discussed below, miR-140 modulates mainly chondrocyte differentiation. Therefore, let-7 miRNAs and miR-140 coordinately regulate skeletal development by regulating chondrocyte proliferation and differentiation, respectively [9•].

miR-140 is expressed abundantly and relatively specifically in chondrocytes. miR-140 is encoded in an intronic sequence of the Wwp2 gene [10•]. Expression of miR-140 and *Wwp2* is regulated by *Sox9* [10•, 11•, 12]. An upstream region of the pri-miR-140 gene, present in an intron of the Wwp2 gene, possesses chondrocyte-specific promoter activity and is directly regulated by *Sox9* [4].

miR-140 deletion causes a mild skeletal developmental defect. miR-140-null mice show short endochondral bones and reduced longitudinal growth of the skull [13••, 14••]. We have previously shown that miR-140-deficiency causes premature hypertrophic chondrocyte differentiation and delayed differentiation of resting chondrocytes to proliferating chondrocytes [14••]. In chondrocytes, *Dnpep*, a gene encoding for an aspartyl aminopeptidase, was identified as a direct miR-140 target. Other miR-140 target genes, such as *Pdgfra*, *HDAC4*, *Smad3*, and *Rala*, have been identified in other studies [15–18]. The in vivo significance of the regulatory role miR-140 for each individual target gene remains to be determined.

miR-199a, miR-199a* and miR-214 are expressed in mesenchymal cells, perichondrial cells and periarticular chondrocytes. These miRNAs are generated from the long RNA transcript, *Dnm3os*, transcribed from the Dynamin 3 gene (*Dnm3*) locus. Mutation of the *Dnm3os* locus by LacZ insertion reduced expression of all three miRNAs [19]. This resulted in growth retardation, craniofacial hypoplasia, dorsal vertebral hypoplasia, and osteopenia, suggesting that these miRNAs play physiologically significant roles in skeletal development. In vitro studies have shown that miR-199 is upregulated upon chondrocyte differentiation in mesenchymal stem cells [20–22].

In Vitro Studies

In addition to the relatively limited in vivo studies discussed above, a large number of studies have investigated roles of specific microRNAs in vitro.

In vitro assays have identified miRNAs that regulate chondrocyte function and differentiation. Several miRNAs are downregulated upon chondrocyte differentiation and negatively regulate the process. miR-145 was found to be downregulated during chondrocyte differentiation of mouse C3H10T1/2 cells [23]. miR-145 inhibited chondrocyte differentiation; miR-145 directly targets and suppresses the expression of *Sox9*, which leads to reduced mRNA levels of chondrocyte markers such as *Col2a1*, *Col9a1*, *Col11a1*, and *Acan* (aggrecan).

Ohgawara et al showed that miR-18a targets and suppresses connective tissue growth factor (Ccn2/Ctgf), a molecule important for endochondral bone formation, in human chondrocytic HCS-2/8 cells [24]. miR-18a overexpression also suppressed aggrecan expression in this study.

miR-1 was found to be downregulated upon hypertrophic differentiation of chondrocytes [25]. miR-1 overexpression in HCS-2/8 cells and chicken primary chondrocytes reduced

aggrecan expression. However, the mechanism by which miR-1 decreased aggrecan levels was unclear.

In chicken limb mesenchymal cells, miR-375 was also downregulated upon chondrocyte differentiation [26]. miR-375 inhibition increased chondrogenic differentiation in these cells. Additionally, miR-375 inhibition increased migration of chondrocyte progenitor cells and stimulated chondrocyte differentiation in a wound healing assay, which mimics the process of mesenchymal condensation. Cadherin-7 was proposed as the miR-375 target responsible for these effects.

miR-1247 was reported to be expressed abundantly in mouse chondrocytes [27]. *Sox9* negatively regulates miR-1247 expression, while miR-1247 itself targets *Sox9*. This creates a negative feedback loop; therefore, miR-1247 may function as part of the autoregulatory system of *Sox9* function.

Sumiyoshi et al found that miR-181a, a miRNA highly expressed in chicken chondrocytes, directly targets and suppresses the pro-chondrogenic gene, CCN family member 1(*Ccn1*) and aggrecan [28].

In chicken cells, miR-221 expression increases upon inhibition of chondrocyte differentiation [29]. Inhibition of miR-221 increases proliferation of limb mesenchymal cells. miR-221 suppresses chondrocyte proliferation by targeting and suppressing Mdm2, a ubiquitin ligase that facilitates degradation of p53 as well as the transcription factor, Slug, a negative regulator of chondrocyte progenitor proliferation.

In MSCs, miR-449 negatively regulates chondrocyte differentiation [30]. In human bone marrow MSCs and also in human chondrosarcoma cells, miR-449 suppresses expression of Lef1, a critical component of the canonical Wnt signaling pathway. This suppression downregulates *Col2a1* and *Sox9* expression, and reduces proteoglycan production.

While many miRNAs negatively regulate chondrogenesis as discussed above, there are also miRNAs that promote chondrogenesis in vitro. During chondrogenic differentiation of mesenchymal stem cells (MSCs), miR-574-3p is highly upregulated [31]. miR-574-3p shows an expression pattern similar to that of miR-140. miR-574-3p targets retinoid X receptor alpha (*Rxra*) whose expression is progressively reduced during chondrocyte differentiation. *Sox9* binds to the promoter of the miR-574 gene and increases its transcription in chondrocytes. Since *Rxra* was previously shown to inhibit *Sox9* activity, miR-574-3p may be part of a positive feedback loop that promotes chondrogenesis.

Expression of miR-335, co-expressed with its host gene *Mest*, increases upon chondrocyte differentiation of mouse MSCs [32]. miR-335 overexpression in MSCs promotes chondrocyte differentiation. miR-335-5p targets *Daam1* and *Rock1*, negative regulators of *Sox9*. *Sox9* downregulates expression of miR-29a and miR-29b, which, in turn, suppress *Mest* expression. Therefore, this regulatory relay may form a positive feedback loop to enhance chondrogenesis. In another study using human MSCs, miR-29a was identified as one of the most downregulated miRNAs during chondrogenesis [33]. Consistent with the aforementioned study, *Sox9* was again found to downregulate miR-29a. Overexpression of miR-29a inhibited chondrogenesis. In this study, *Foxo3a* was identified as a direct target of miR-29a.

There are several other miRNAs whose expression is regulated upon chondrocyte differentiation of mouse MSCs. miR-24 and miR-199b were upregulated by more than five-fold upon differentiation into chondrocytes and osteoblasts [20]. miR-101, miR-124a and miR-199a were also upregulated, while miR-18 and miR-96 were downregulated. In human MSCs, miR-199a was upregulated by four-fold upon chondrocyte differentiation [21]. These miRNAs may regulate lineage determination during MSC differentiation.

Role of microRNA in Articular Cartilage Homeostasis and Osteoarthritis

In addition to growth plate cartilage, chondrocytes form permanent cartilage in ribs and joints. Permanent cartilage has distinct and unique properties compared with growth plate cartilage. Impairment of function of permanent cartilage, particularly synovial joint cartilage, limits skeletal mobility and poses significant health risks especially to the elderly. In this section, we discuss roles of miRNAs in synovial joint cartilage in relation to osteoarthritis (OA), the most common cartilage disease.

OA is characterized by destruction of the articular cartilage caused by an imbalance between synthesis and degradation of extra-cellular matrix (ECM) components, such as type II collagen and proteoglycans. Age-related chondrocyte dysfunction likely reduces ECM synthesis, while various stresses induce aberrant expression of matrix-degrading enzymes, including matrix metalloproteinases (Mmps) and a disintegrin and a metalloproteinase domain with thrombospondin motif (Adamts) family enzymes [34]. It is also recognized that inflammation plays an important role in OA progression. Significant efforts have been made to understand the molecular mechanisms underlying OA pathogenesis. In recent years, studies have revealed that microRNAs are involved in articular cartilage homeostasis and OA. Because there have been published a relatively large number of studies on this subject, we concisely summarize the relevant literature in Table 1. We also individually discuss several miRNAs of particular interest.

Maintenance of Articular Chondrocytes and Cartilage

As in the growth plate chondrocytes, miR-140 is highly expressed in human articular chondrocytes. miRNA profiling of patient-derived osteoarthritic chondrocytes has demonstrated

Table 1 Summary of microRNAs in OA

microRNAs	Expression change in OA	Target gene(s)	Function	Study models
miR140	Decreased	*Adamts5*	ECM homeostasis	Human articular chondrocytes and human bone marrow-derived mesenchymal stem cells (MSCs) [43].
	Decreased	*Adamts5*	ECM homeostasis	Cartilage-specific miR-140 overexpression and miR-140 deletion in mice. Mouse models with surgically induced OA and antigen-induced arthritis (AIA) [13].
	Decreased	*Igfbp5*	ECM homeostasis	Human OA cartilage from patients undergoing total knee arthroplasty (TKA) [37].
	Decreased	*Mmp13*	ECM homeostasis	The human cartilage cell line C28/I2 treated with IL-1β to mimic an osteoarthritic environment [42].
	Increased	*Adamts5*, *Dnpep*	Maintenance of chondrocytes and ECM homeostasis	Human articular cartilage of femoral heads from patients undergone total hip replacement surgery [39].
	Increased	ND	Maintenance of chondrocytes and ECM homeostasis	Human articular chondrocytes from OA patients with knee replacement and biopsy samples of normal cartilage [38].
	Increased	*Mmp13*	ECM homeostasis	Human articular chondrocyte C28/I2 cells [42].
miR-145	Increased	*Sox9*	Maintenance of chondrocytes and ECM homeostasis	OA articular cartilage from the knee [44].
	Increased	*Smad3*	Maintenance of chondrocytes and ECM homeostasis	Human articular cartilage [45].
miR-27b	Decreased	*Mmp13*	ECM homeostasis	Full-thickness cartilage and subchondral bone from OA patients [41].
miR-27a	Decreased	*Igfbp5*	ECM homeostasis	Human OA cartilage of the knee [36].
miR-21	Increased	ND	aging	Normal cartilage of the metacarpophalangeal joints of eight horses; four young donors (4 yr old) and four old donors (>15 yr old) [48].
	Increased	*Gdf5*	ECM homeostasis	Human articular chondrocytes (CH8 cell lines). Ten paired osteoarthritic tissues and matched normal tissues from traumatic amputees [64].
miR-23b	Increased	ND	Chondrocyte differentiation	Synovial fluid samples obtained from 10 osteoarthritis patients by joint puncture. SFMSCs (synovial fluid-derived mesenchymal stem cells) were isolated from synovial fluid [51].
miR-9	Decreased (61), Increased (54)	*Protogenin* (*61*), *Mmp13* (54)	Chondrocyte survival	Human articular cartilage. Overexpression of miR-9 in cartilage tissue by lentivirus injection into mouse knee joints [61].
miR-34a	Increased with IL-1β stimulation	*Col2a1* and *iNOS* (indirect effect)	Chondrocyte apoptosis	Articular chondrocytes isolated from rat knee joints [62].
miR-101	Decreased	*Dnmt3b*	Chondrocyte differentiation	Wing mesenchymal cells. Overexpression of miR-101 in cartilage tissue by injecting miR-101-expressing lentiviruses into mouse knee joints [47].
	Increased with IL-1β stimulation	*Sox9*	Maintenance of chondrocytes and ECM homeostasis	IL-1β-stimulated rat primary chondrocytes [46].
miR-199a	Decreased	*Cox-2*	Inflammatory response	Cartilage samples from patients with OA [63].
miR-199a-3p	Increased with age	ND (Not detected)	Maintenance of chondrocytes and ECM homeostasis	Human articular cartilage [50].
miR-146	Decreased	*Traf6*, *Irak1*	Inflammatory response	Human OA cartilage [53, 54].

Table 1 (continued)

microRNAs	Expression change in OA	Target gene(s)	Function	Study models
	Decreased (late stage OA), Increased with IL-1β stimulation	*Mmp13*	ECM homeostasis	Human OA cartilage [55].
miR-146a	Increased	*Smad4*	Inflammatory response and mechanical stress	Rat OA cartilage, surgically induced OA rats [56], human chondrocytes with pressure-mediated mechanical stress [57].
	Increased	*IL-1β*, *Col13a1*, *Smad4*	Inflammatory response	miRNA expression profiles in the plasma of patients with OA of the knee in comparison with those of healthy subjects [59].
miR-149	Decreased	ND	Inflammatory response	Human OA cartilage [60].
miR-22	Increased	*PPARα*, *Bmp7*	ECM homeostasis and BMI	OA knee articular cartilage obtained from patients [43].
miR-455	Increased	*Smad2*, *Acvr2b*, *Chrdl1*	Maintenance of chondrocytes and ECM homeostasis	SW-1353, C3H10T1/2, and 3 T3 cells, human articular cartilage obtained from femoral head [39].
miR-885-5p	Increased	ND	ND	miRNA expression profiles in the plasma of patients with OA [59]. Primary human articular chondrocytes [65].
miR-24	Decreased	*p16INK4a* (*Cdkn2a*)	aging	IL-1beta-treated primary OA chondrocytes, mesenchymal stem cells differentiated into chondrocytes [66].
miR-148a	Decreased	*Col10a1*, *Mmp13*, *Adamts5*	ECM homeostasis	Chondrocytes isolated from OA articular cartilage of the knee [67].
miR-127-5p	Decreased	*Mmp13*	ECM homeostasis	Normal and osteoarthritic (OA) human cartilage [68].
miR-29c	Increased	*Col3a1*	ECM homeostasis	miRNA expression profiles in the plasma of patients with OA of the knee in comparison with those of healthy subjects [59].
miR-16	Increased	*Vegfa*, *Fgfr1*	Inflammatory response	miRNA expression profiles in the plasma of patients with OA of the knee in comparison with those of healthy subjects [59].
miR-93	Increased	*Vegfa*	Inflammatory response	miRNA expression profiles in the plasma of patients with OA of the knee in comparison with those of healthy subjects [59].
miR-483	Increased	*Mmp13*, *Sox5 and Bmp1*	Maintenance of chondrocytes and ECM homeostasis	The surgical OA mouse models by resection of the medial collateral ligament and medial meniscus of the knee joint [69].
miR-483*	Increased	*Igf1*, *Igf2 and Il1r1*	Inflammatory response	The surgical OA mouse models [69].
miR-558	Decreased	*Cox2*, *NF-κB*,	Inflammatory response	OA cartilage obtained from knee joints from patients, human chondrogenic SW1353 cells [70].
miR-125b	Decreased	*Adamts4*	ECM homeostasis	Chondrocytes isolated from knee cartilage and treated with interleukin-1 beta [71].
miR-181b	Increased	*Mmp13* (indirect effect)	Cartilage development	Human chondrocytes prepared from macroscopically severely damaged zones of osteoarthritic knee joints or biopsy specimens of normal cartilages, surgically induced mouse OA [72].
MiR-675	Increased	*Col2a1* (indirect effect)	Chondrocyte differentiation and maintenance	OA cartilage isolated from knee joints of patients with primary or secondary osteoarthritis [73].
miR-194	Increased	*Sox5*	Chondrocyte differentiation and maintenance	Articular chondrocytes isolated from the knee joint of juvenile rats [74].

Table 1 (continued)

microRNAs	Expression change in OA	Target gene(s)	Function	Study models
miR-488	Decreased	*ZIP8*/*Slc39a*, *Mmp13* (indirect)	ECM homeostasis	Human articular chondrocytes isolated from biopsy samples of normal cartilages [38].
miR-455	Increased	*Smad2*, *Acvr2b*, *Chrdl1*	Chondrocyte differentiation and maintenance	ATDC5 cells [39].
miR-203	ND	*Trpv4*	Inflammatory response	Mandibular condylar cartilage [75].
miR-320c	Decreased	*Adamts5*	Aging	Cartilaginous tissue obtained from 17 OA patients [50].
miR-193b	Increased	*Col2a1*, *aggrecan*, *Sox9*	Maintenance of chondrocytes and ECM homeostasis	Human articular cartilage [50].
miR-222	Increased	ND	Articular cartilage mechanotransduction	Bovine articular cartilage [76].
miR-221	Increased	ND	Articular cartilage mechanotransduction	Bovine articular cartilage [76].
miR-365	ND	Hdac4	Mechanical stimulation of chondrocyte differentiation	Primary chicken chondrocytes cultured in three-dimensional collagen scaffoldings under cyclic loading (1 Hz, 5 % elongation) [77].

downregulation of miR-140 in chondrocytes of osteoarthritic cartilage compared with normal human cartilage [35–37], although some studies also show increased miR-140 expression in OA cartilage [38, 39]. A recent study also reported that the expression level of miR-140 in the knee joint synovial fluid was inversely correlated with the severity of OA in patients [40].

miR-140 is expressed in the surface and middle zones of articular cartilage of the mouse knee joint, exhibiting an expression pattern similar to that of *Col2a1* [11•]. Miyaki et al showed that miR-140-deficiency caused early onset of OA in aging mice and accelerated development of OA in OA-induced mice [13••]. Conversely, transgenic mice overexpressing miR-140 in articular cartilage under a *Col2a1* promoter were resistant to proteoglycan and type II collagen loss after antigen-induced joint inflammation. In this study, *Adamts5*, an aggrecan-degrading enzyme, was identified as a direct target of miR-140 in articular cartilage.

Tardif et al reported significantly decreased miR-140 expression in chondrocytes isolated from patients with OA [36]. Insulin-like growth factor binding protein-5 (Igfbp5) appears to be a direct target of miR-140. In this study, miR-27a was also modestly decreased in OA chondrocytes. Inhibition of miR-27a also significantly increased *Igfbp5* as well as *Mmp13* expression. A separate study showed that treatment of chondrocytes with Interleukin-1 beta (IL-1β), a major mediator of inflammation in OA, significantly downregulated miR-27b expression. These authors found that miR-27b directly targeted *Mmp13* to regulate its expression [41]. miR-140 was also shown to suppress IL-1β-induced *Mmp13* expression in the human chondrocytic C28/I2 cells by directly targeting *Mmp13* mRNA [42]. Mechanisms controlling miR-140 expression were studied in OA chondrocytes [37]. *Smad3*, a downstream mediator of the transforming growth factor beta (TGF-β) pathway, reduces expression of miR-140, but not its host gene, *Wwp2*. This regulation is mediated by direct binding of *Smad3* to a regulatory sequence unique to the miR-140 gene. This study also showed that nuclear factor of activated T-cells (NFAT) transcription factors, NFAT5 and 3, regulated miR-140 expression likely through the same regulatory element.

IL-1β treatment suppresses miR-140 expression and increases *Adamts5* expression in OA chondrocytes [43]. miR-140 overexpression downregulated IL-1β-induced *Adamts5* expression.

A recent study has shown that miR-145 is increased in OA chondrocytes and in response to IL-1β stimulation [44]. miR-145 inhibition was able to reverse the downregulation of type II collagen and aggrecan expression caused by IL-1β treatment. This study identified *Smad3* as a direct miR-145 target. An independent study using human articular chondrocytes has shown that miR-145 targets *Sox9* [45]. miR-145 overexpression decreased *Sox9* expression and subsequently reduced the cellular phenotype of differentiated chondrocytes and decreased miR-140 expression.

Sox9 is also targeted by miR-101 [46]. Silencing of miR-101 in rat articular chondrocytes reversed the IL-1β-induced downregulation of collagen type II and aggrecan. Kim, et al. reported that miR-101 was expressed at a lower level in severely damaged OA regions than in non-OA regions of human articular cartilage [47]. Lentivirus-mediated miR-101 overexpression significantly reduced cartilage destruction in OA-induced mouse knee joints. miR-101 targeted DNA methyltransferase 3 beta (*Dnmt3b*); this appeared to suppress integrin-α1 expression. Using a surgically-induced mouse OA model, this group also demonstrated that overexpression of miR-101 or integrin-α1 prevented OA progression.

In a study to identify genes associated with aging, miR-21 was found increased in aged horse articular cartilage [48]. Since miR-21 promotes proliferation and matrix synthesis in rat chondrocytes embedded in atelocollagen gels [49], miR-21 upregulation upon aging could be a counter-regulatory mechanism protecting against aging-associated impairment of chondrocyte function. By microarray analysis on human articular chondrocytes, miR-199a-3p and miR-193b were found to be upregulated with age, and miR-320c was found to be downregulated with age [50]. Overexpression of miR-199a-3p or miR-193b in chondrocytes downregulated type II collagen, aggrecan, and *Sox9* expression, and decreased proliferation. Therefore, miR-199a-3p and miR-193b may accelerate the aging process of chondrocytes. However, the precise mechanisms by which these miRNAs regulate chondrocyte genes are unclear.

Song et al found that miR-23b was upregulated in human OA chondrocytes [38]. miRNA-23b overexpression induced chondrogenic differentiation by downregulating protein kinase A (PKA) signaling by targeting *Prkacb* that encodes a catalytic subunit of PKA, in synovial fluid-derived mesenchymal stem cells [51]; miR-23b may contribute to OA progression by inducing chondrocyte differentiation.

Inflammatory Response

miR-146a and miR-146b appear to be key microRNAs in the inflammatory response [52]. miR-146a and miR-146b were regulated by the transcription factor NF-κB, a critical mediator of inflammatory cytokines. These miRNAs were shown to directly target IL-1 receptor-associated kinase 1 (*Irak1*) and TNF receptor-associated factor 6 (*Traf6*), upstream regulators of NF-kB [53]. Therefore, miR-146a and miR-146b may be components of a negative feedback loop that regulates cytokine signaling. Jones et al determined the expression profile of 157 miRNAs in late-stage human OA cartilage [54]. Among 17 miRNAs that showed more than 4-fold differential expression between OA and normal cartilage, miR-146a and miR-149 were the top two most downregulated miRNAs in late-stage OA cartilage. Overexpression of miR-146a in isolated human chondrocytes reduced IL-1β-induced TNF-α production, suggesting that this miRNA is a negative regulator of inflammation in OA.

Yamasaki et al found that miR-146a was expressed abundantly in early-stage human OA cartilage and that its expression was reduced as OA advances [55]. In situ hybridization analysis revealed that miR-146a was expressed in the superficial layer with degenerative changes in early stage OA. Consistent with previous reports that miR-146a expression was regulated by cytokine signaling, in vitro stimulation of human normal chondrocytes by IL-1β markedly increased miR-146a expression [55]. Another study also shows upregulation of mIR-146a by IL-1β treatment in primary rat chondrocytes [56]. Surgically-induced instability of rat knee joints also increased miR-146a expression. miR-146a was found to target and downregulate *Smad4*, which subsequently induced VEGF expression and increased articular chondrocytes apoptosis. Another in vitro study using human articular chondrocytes has also shown that miR-146a is induced in chondrocytes by pressure-mediated mechanical stress [57]. This was accompanied by *Smad4* downregulation, VEGF upregulation, and increased chondrocyte apoptosis. miR-146a expression is also induced by HDAC inhibitor treatment in synovial fibroblasts isolated from OA patients [58]. This study showed that HDAC inhibitor treatment increased NF-κB binding to the promoter region of the miR-146a gene. It is therefore possible that miR-146a expression is also epigenetically regulated. Interestingly, miRNA expression profiling showed increased levels of miR-146 in OA patients [59]. This finding suggests that plasma miR-146 can be used as a marker for assessing OA severity.

miR-149 is downregulated in OA chondrocytes [60]. miR-149 targets TNFα, IL1β, and IL6; miR-149 downregulation might contribute to OA progression. Iliopoulos et al analyzed miRNA and protein expression profiles in OA cartilage [35]. They found that 16 miRNAs and 76 proteins were differentially expressed between OA and control cartilage. Many upregulated proteins were involved in inflammatory pathways, such as IL-1β and IL-6, while downregulated proteins were involved in lipid metabolism, such as peroxisome proliferator-activated receptor α (Pparα). This study also found that two upregulated miRNAs (miR-22 and miR-103) were highly correlated with body mass index (BMI) in OA patients. They showed that miR-22 directly targeted Pparα and Bmp7. miR-22 overexpression, or suppression of either Pparα or Bmp7 increased IL-1β and Mmp13 levels in chondrocytes, suggesting that miR-22 may promote OA-related inflammation by modulating lipid metabolism.

Jones et al found that several miRNAs, including miR-9, miR-98, and miR-34, were upregulated in OA cartilage [54]. Overexpression of miR-9 and miR-98 in isolated human chondrocytes reduced IL-1β-induced TNF-α production, and inhibited secretion of Mmp13. Interestingly, another study reported a significant decrease of miR-9 expression in OA chondrocytes [61]. The reason for this contradiction is unclear; possible sample heterogeneity may be to blame because both studies used human OA samples. In the latter study, Protogenin (*Prtg*), that activates caspase-3 signaling and promotes chondrocyte apoptosis, was found as a direct target of miR-9.

Abouheif et al reported that expression of the p53-regulated miRNA, miR-34a, was upregulated in rat primary chondrocytes upon IL-1β stimulation [62]. Silencing of miR-34a significantly prevented the IL-1β-induced *Col2a1* downregulation, iNOS (inducible nitric oxide synthase) upregulation, and apoptosis in rat chondrocytes.

IL-1β increases cyclooxygenase-2 (*Cox2*) expression in OA, and IL-1β signaling is mediated also by mitogen activated

protein kinases (MAPKs). Akhtar et al showed that IL-1β-induced activation of p38-MAPK signaling reduced miR-199a expression in OA chondrocytes [63]. They showed that miR-199a directly targeted Cox2. Treatment of OA chondrocytes with the p38-MAPK inhibitor, SB202190, increased miR-199a levels and significantly decreased Cox2 expression.

In addition to the miRNAs that we have discussed, many more miRNAs appear to play important roles in various aspects of cartilage homeostasis and OA pathogenesis (Table 1). Understanding the complex miRNA regulatory networks is expected to provide scientific bases for developing novel diagnostic methods and therapeutic strategies for OA.

Conclusions

These studies have demonstrated that various miRNAs regulate many aspects of cartilage development, homeostasis and pathology through diverse mechanisms. Our understanding about roles of miRNAs in chondrocytes and cartilage has significantly increased in the past decade. However, arriving at a comprehensive view on the miRNA-mediated regulatory network in cartilage from the plethora of data obtained from highly heterogeneous systems still requires further coordinated efforts. Testing these findings in more uniform and physiologic settings will clarify the roles of individual miRNAs and their targets, and serve as a strong foundation for translating these findings to help patients with osteoarthritis.

Acknowledgments We thank Dr. Marc Wein for helpful discussion. This work was supported by the National Institutes of Health [AR054500 and AR056645 to T.K.]

Compliance with Ethics Guidelines

Conflict of Interest F. Mirzamohammadi, G. Papaioannou, and T. Kobayashi all declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent All studies by the authors involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Kronenberg HM. Developmental regulation of the growth plate. Nature. 2003;423:332–6.
2. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. Nat Genet. 1999;22:85–9.
3. Lefebvre V, Huang W, Harley VR, Goodfellow PN, de Crombrugghe B. SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. Mol Cell Biol. 1997;17:2336–46.
4. Yamashita S, Miyaki S, Kato Y, et al. L-Sox5 and Sox6 proteins enhance chondrogenic miR-140 microRNA expression by strengthening dimeric Sox9 activity. J Biol Chem. 2012;287:22206–15.
5. Yoshida CA, Yamamoto H, Fujita T, et al. Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes Dev. 2004;18:952–63.
6. 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;15:467–81.
7. Tetreault N, De Guire V. miRNAs: their discovery, biogenesis and mechanism of action. Clin Biochem. 2013;46:842–5.
8. Kobayashi T, Lu J, Cobb BS, et al. Dicer-dependent pathways regulate chondrocyte proliferation and differentiation. Proc Natl Acad Sci U S A. 2008;105:1949–54.
9.• Papaioannou G, Inloes JB, Nakamura Y, Paltrinieri E, Kobayashi T. let-7 and miR-140 microRNAs coordinately regulate skeletal development. Proc Natl Acad Sci U S A. 2013;110:E3291–300. *This in vivo study shows the importance of let-7 miRNAs in growth plate chondrocyte proliferation. It also demonstrates that simultaneous reductions in let-7 miRNAs and miR-140 in chondrocytes cause a substantial growth defect due to reduced proliferation and altered differentiation in growth plate chondrocytes*.
10.• Yang J, Qin S, Yi C, et al. MiR-140 is co-expressed with Wwp2-C transcript and activated by Sox9 to target Sp1 in maintaining the chondrocyte proliferation. FEBS Lett. 2011;585:2992–7. *These studies show the chondrocyte-specific expression of miR-140 and its regulation by Sox9*.
11.• Nakamura Y, He X, Kato H, et al. Sox9 is upstream of microRNA-140 in cartilage. Appl Biochem Biotechnol. 2012;166:64–71. *These studies show the chondrocyte-specific expression of miR-140 and its regulation by Sox9*.
12. Nakamura Y, Yamamoto K, He X, et al. Wwp2 is essential for palatogenesis mediated by the interaction between Sox9 and mediator subunit 25. Nat Commun. 2011;2:251.
13.•• Miyaki S, Sato T, Inoue A, et al. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev. 2010;24:1173–85. *This in vivo study describes mouse phenotypes caused by miR-140-loss in mice. miR-140-deficiency causes a mild growth defect, craniofacial abnormalities, and accelerated development of osteoarthritis in aged or surgically manipulated mice*.
14.•• Nakamura Y, Inloes JB, Katagiri T, Kobayashi T. Chondrocyte-specific microRNA-140 regulates endochondral bone development and targets Dnpep to modulate bone morphogenetic protein signaling. Mol Cell Biol. 2011;31:3019–28. *This in vivo study independently shows that miR-140-loss causes a mild growth defect due to altered chondrocyte differentiation in mice. This study experimentally identified Dnpep as a miR-140 target*.
15. Eberhart JK, He X, Swartz ME, et al. MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis. Nat Genet. 2008;40:290–8.
16. Tuddenham L, Wheeler G, Ntounia-Fousara S, et al. The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. FEBS Lett. 2006;580:4214–7.
17. Pais H, Nicolas FE, Soond SM, et al. Analyzing mRNA expression identifies *Smad3* as a microRNA-140 target regulated only at protein level. RNA. 2010;16:489–94.
18. 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 SOX9 and ACAN. Stem Cells Dev. 2014;23:290–304.

19. Watanabe T, Sato T, Amano T, et al. Dnm3os, a non-coding RNA, is required for normal growth and skeletal development in mice. Dev Dyn. 2008;237:3738–48.
20. Suomi S, Taipaleenmaki H, Seppanen A, et al. MicroRNAs regulate osteogenesis and chondrogenesis of mouse bone marrow stromal cells. Gene Reg Syst Biol. 2008;2:177–91.
21. Laine SK, Alm JJ, Virtanen SP, Aro HT, Laitala-Leinonen TK. MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. J Cell Biol. 2012;113:2687–95.
22. Lin EA, Kong L, Bai XH, Luan Y, Liu CJ. miR-199a, a bone morphogenic protein 2-responsive MicroRNA, regulates chondrogenesis via direct targeting to Smad1. J Biol Chem. 2009;284:11326–35.
23. Yang B, Guo H, Zhang Y, Chen L, Ying D, Dong S. MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. PLoS One. 2011;6:e21679.
24. Ohgawara T, Kubota S, Kawaki H, et al. Regulation of chondrocytic phenotype by micro RNA 18a: involvement of Ccn2/Ctgf as a major target gene. FEBS Lett. 2009;583:1006–10.
25. Sumiyoshi K, Kubota S, Ohgawara T, et al. Identification of miR-1 as a micro RNA that supports late-stage differentiation of growth cartilage cells. Biochem Biophys Res Commun. 2010;402:286–90.
26. Song J, Kim D, Chun CH, Jin EJ. MicroRNA-375, a new regulator of cadherin-7, suppresses the migration of chondrogenic progenitors. Cell Signal. 2013;25:698–706.
27. Martinez-Sanchez A, Murphy CL. miR-1247 functions by targeting cartilage transcription factor SOX9. J Biol Chem. 2013;288: 30802–14.
28. Sumiyoshi K, Kubota S, Ohgawara T, et al. Novel role of miR-181a in cartilage metabolism. J Cell Biol. 2013;114:2094–100.
29. Kim D, Song J, Jin EJ. MicroRNA-221 regulates chondrogenic differentiation through promoting proteosomal degradation of slug by targeting Mdm2. J Biol Chem. 2010;285:26900–7.
30. Paik S, Jung HS, Lee S, Yoon DS, Park MS, Lee JW. miR-449a regulates the chondrogenesis of human mesenchymal stem cells through direct targeting of lymphoid enhancer-binding factor-1. Stem Cells Dev. 2012;21:3298–308.
31. Guerit D, Philipot D, Chuchana P, et al. Sox9-regulated miRNA-574-3p inhibits chondrogenic differentiation of mesenchymal stem cells. PLoS One. 2013;8:e62582.
32. Lin X, Wu L, Zhang Z, et al. MiR-335-5p promotes chondrogenesis in mouse mesenchymal stem cells and is regulated through two positive feedback loops. J Bone Min Res. 2013;29:1575–85.
33. Guerit D, Brondello JM, Chuchana P, et al. FOXO3A regulation by miRNA-29a Controls chondrogenic differentiation of mesenchymal stem cells and cartilage formation. Stem Cells Dev. 2014;23: 1195–205.
34. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. Arthritis Res Ther. 2009;11:224.
35. 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.
36. 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.
37. Tardif G, Pelletier JP, Fahmi H, et al. NFAT3 and TGF-beta/SMAD3 regulate the expression of miR-140 in osteoarthritis. Arthritis Res Ther. 2013;15:R197.
38. Song J, Kim D, Lee CH, Lee MS, Chun CH, Jin EJ. MicroRNA-488 regulates zinc transporter SLC39A8/ZIP8 during pathogenesis of osteoarthritis. J Biomed Sci. 2013;20:31.
39. Swingler TE, Wheeler G, Carmont V, et al. The expression and function of microRNAs in chondrogenesis and osteoarthritis. Arthritis Rheum. 2012;64:1909–19.
40. Zhang M, Liu L, Xiao T. Guo W [Detection of the expression level of miR-140 using real time fluorescent quantitative PCR in knee synovial fluid of osteoarthritis patients]. Zhong nan da xue xue bao Yi xue ban. 2012;37:1210–4.
41. 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;62:1361–71.
42. Liang ZJ, Zhuang H, Wang GX, et al. MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1beta-stimulated human articular chondrocyte C28/I2 cells. Inflamm Res. 2012;61:503–9.
43. Miyaki S, Nakasa T, Otsuki S, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum. 2009;60:2723–30.
44. Yang B, Kang X, Xing Y, et al. Effect of microRNA-145 on IL-1beta-induced cartilage degradation in human chondrocytes. FEBS Lett. 2014;588:2344–52.
45. Martinez-Sanchez A, Dudek KA, Murphy CL. Regulation of human chondrocyte function through direct inhibition of cartilage master regulator SOX9 by microRNA-145 (miRNA-145). J Biol Chem. 2012;287:916–24.
46. Dai L, Zhang X, Hu X, Zhou C, Ao Y. Silencing of microRNA-101 prevents IL-1beta-induced extracellular matrix degradation in chondrocytes. Arthritis Res Ther. 2012;14:R268.
47. Kim D, Song J, Han J, Kim Y, Chun CH, Jin EJ. Two non-coding RNAs, MicroRNA-101 and HOTTIP contribute cartilage integrity by epigenetic and homeotic regulation of integrin-alpha1. Cell Signal. 2013;25:2878–87.
48. Peffers MJ, Liu X, Clegg PD. Transcriptomic signatures in cartilage ageing. Arthritis Res Ther. 2013;15:R98.
49. Kongcharoensombat W, Nakasa T, Ishikawa M, et al. The effect of microRNA-21 on proliferation and matrix synthesis of chondrocytes embedded in atelocollagen gel. Knee Surg Sports Traumatol Arthro. 2010;18:1679–84.
50. Ukai T, Sato M, Akutsu H, Umezawa A, Mochida J. MicroRNA-199a-3p, microRNA-193b, and microRNA-320c are correlated to aging and regulate human cartilage metabolism. J Orthop Res. 2012;30:1915–22.
51. Ham O, Lee CY, Song BW, et al. Upregulation of miR-23b enhances the autologous therapeutic potential for degenerative arthritis by targeting PRKACB in synovial fluid-derived mesenchymal stem cells from patients. Mol Cells. 2014;37:449–56.
52. Sonkoly E, Stahle M, Pivarcsi A. MicroRNAs and immunity: novel players in the regulation of normal immune function and inflammation. Semin Cancer Biol. 2008;18:131–40.
53. 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;103:12481–6.
54. Jones SW, Watkins G, Le Good N, et al. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. Osteoarthr Cartil. 2009;17:464–72.
55. Yamasaki K, Nakasa T, Miyaki S, et al. Expression of MicroRNA-146a in osteoarthritis cartilage. Arthritis Rheum. 2009;60:1035–41.
56. Li J, Huang J, Dai L, et al. miR-146a, an IL-1beta responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. Arthritis Res Ther. 2012;14:R75.
57. Jin L, Zhao J, Jing W, et al. Role of miR-146a in human chondrocyte apoptosis in response to mechanical pressure injury in vitro. Int J Mol Med. 2014;34:451–63.
58. Wang JH, Shih KS, Wu YW, Wang AW, Yang CR. Histone deacetylase inhibitors increase microRNA-146a expression and enhance negative regulation of interleukin-1beta signaling in osteoarthritis fibroblast-like synoviocytes. Osteoarthr Cartil. 2013;21: 1987–96.

59. Borgonio Cuadra VM, Gonzalez-Huerta NC, Romero-Cordoba S, Hidalgo-Miranda A, Miranda-Duarte A. Altered expression of circulating microRNA in plasma of patients with primary osteoarthritis and in silico analysis of their pathways. PLoS One. 2014;9: e97690.
60. Santini P, Politi L, Vedova PD, Scandurra R, Scotto d' Abusco A. The inflammatory circuitry of miR-149 as a pathological mechanism in osteoarthritis. Rheumatol Int. 2014;34:711–6.
61. 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.
62. Abouheif MM, Nakasa T, Shibuya H, Niimoto T, Kongcharoensombat W, Ochi M. Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro. Rheumatology. 2010;49:2054–60.
63. Akhtar N, Haqqi TM. MicroRNA-199a* regulates the expression of cyclooxygenase-2 in human chondrocytes. Ann Rheumatol Dis. 2012;71:1073–80.
64. Zhang Y, Jia J, Yang S, Liu X, Ye S, Tian H. MicroRNA-21 controls the development of osteoarthritis by targeting GDF-5 in chondrocytes. Exp Mol Med. 2014;46:e79.
65. Beyer C, Zampetaki A, Lin NY, et al. Signature of circulating microRNAs in osteoarthritis. Ann Rheumat Dis. 2014;0:1–7.
66. Philipot D, Guerit D, Platano D, et al. p16INK4a and its regulator miR-24 link senescence and chondrocyte terminal differentiation-associated matrix remodelling in osteoarthritis. Arthritis Res Ther. 2014;16:R58.
67. Vonk LA, Kragten AH, Dhert WJ, Saris DB, Creemers LB. Overexpression of hsa-miR-148a promotes cartilage production and inhibits cartilage degradation by osteoarthritic chondrocytes. Osteoarthr Cartil. 2014;22:145–53.
68. Park SJ, Cheon EJ, Lee MH, Kim HA. MicroRNA-127-5p regulates matrix metalloproteinase 13 expression and interleukin-1beta-induced catabolic effects in human chondrocytes. Arthritis Rheum. 2013;65:3141–52.
69. Qi Y, Ma N, Yan F, et al. The expression of intronic miRNAs, miR-483 and miR-483*, and their host gene, Igf2, in murine osteoarthritis cartilage. Int J Biol Macromol. 2013;61:43–9.
70. Park SJ, Cheon EJ, Kim HA. MicroRNA-558 regulates the expression of cyclooxygenase-2 and IL-1beta-induced catabolic effects in human articular chondrocytes. Osteoarthr Cartil. 2013;21:981–9.
71. Matsukawa T, Sakai T, Yonezawa T, et al. MicroRNA-125b regulates the expression of aggrecanase-1 (ADAMTS-4) in human osteoarthritic chondrocytes. Arthritis Res Ther. 2013;15:R28.
72. 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;431:210–4.
73. Steck E, Boeuf S, Gabler J, et al. Regulation of H19 and its encoded microRNA-675 in osteoarthritis and under anabolic and catabolic in vitro conditions. J Mol Med. 2012;90:1185–95.
74. Xu J, Kang Y, Liao WM, Yu L. MiR-194 regulates chondrogenic differentiation of human adipose-derived stem cells by targeting Sox5. PLoS One. 2012;7:e31861.
75. Hu F, Zhu W, Wang L. MicroRNA-203 up-regulates nitric oxide expression in temporomandibular joint chondrocytes via targeting TRPV4. Arch Oral Biol. 2012;58:192–9.
76. Dunn W, DuRaine G, Reddi AH. Profiling microRNA expression in bovine articular cartilage and implications for mechanotransduction. Arthritis Rheum. 2009;60:2333–9.
77. Guan YJ, Yang X, Wei L, Chen Q. MiR-365: a mechanosensitive microRNA stimulates chondrocyte differentiation through targeting histone deacetylase 4. FASEB J. 2011;25:4457–66.