Review

Do microRNAs have a key epigenetic role in osteoarthritis and in mechanotransduction?

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ABSTRACT

Osteoarthritis (OA) is the most common degenerative disease affecting joint tissues. The pathogenesis of OA is complex and poorly understood, as well as the multiple factors contributing to its development and progression. Accumulating evidence has suggested that microRNAs (miRNAs) play an important role as regulators of cartilage biology and in the pathogenesis of OA. It has been demonstrated that mechanical loading, important for the regulation of cartilage metabolism, affects miRNAs expression. Furthermore, miRNAs present in human plasma and in synovial fluid could represent promising biological markers for OA.

Herein, we have reviewed the current state of research on miRNAs in cartilage homeostasis and OA pathogenesis and their potential clinical applications.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and a major contributor of functional impairment and disability in older people (1). The main feature of OA is destruction of articular cartilage leading to degeneration and apoptosis of chondrocytes. The structural breakdown of extracellular matrix components, such as proteoglycans (PG) and collagen, seems to be caused by the predominance of catabolic activities of cartilage-degrading enzymes, like matrix metalloproteinases (MMPs) and metalloproteinase with thrombospondin motif (ADAMTS), on anabolic activities typical of chondrocytes (2). The metabolic activity of these cells is regulated by several factors, such as cytokines, chemokines and growth factors, locally produced by the chondrocytes themselves and also by neighbouring tissues (3, 4). The pathogenesis of OA is complex and poorly understood, and multiple causes ranging from aging to biomechanical and biochemical stimuli contribute to the development and progression of the disease (5). Several studies have demonstrated a strong genetic component and heritability in primary OA, ranging from 40% to 65% depending on the joint site (6, 7).

In the last few years several studies have highlighted the role of microRNAs (miRNAs) as regulators of cartilage biology and OA (8-10).

MicroRNAs are an abundant class of evolutionarily conserved double-stranded RNA molecules of 22-25 nucleotides (nt), that have emerged as important post-transcriptional regulators of gene expression by binding specific sequences within target messenger RNAs (mRNA) (11).

The importance of miRNA epigenetic regulation in cellular function is becoming increasingly clear as new miRNAs and their targets are discovered. Nowadays 1881 precursors and 2588 mature miRNA are annotated in the database miRBase (12), that also defines their biological roles. MicroRNAs have been associated to control of important cellular processes such as lipid metabolism (13), apoptosis (14), differentiation (15) and organ development (16). Furthermore, alterations of miRNAs expression have been linked to clinicpathological features and several diseases outcome, particularly with cancer (17), but recently also with coronary syndromes (18) and neuropsychiatric disorders (19).

In this review article, we describe the current scientific evidence about the role of miRNAs in cartilage homeostasis and mechanotransduction in OA pathogenesis, as well as their potential clinical applications.

MiRNAs synthesis and function

The generation of miRNAs is a multi-step process that starts in the nucleus with the transcription of miRNAs genes by RNA polymerase II (pol II), producing primary (pri)-miRNAs, and finishes in the cytoplasm (Fig. 1). MiRNAs genes are located in the introns of protein-coding genes as well as of non-coding RNAs (ncRNA) (20), in intergenic region (21), or in exons of mRNA-like noncoding RNAs (mlncRNAs) (22).

A pri-miRNA is a long RNA with a single or several stem-loop structures, and harbours the future miRNA in the stem region (23, 24). In the canonical pathway the pri-miRNA is cleaved, in the nucleus, by a protein complex containing Drosha, a highly conserved Rnase-III-type enzyme, associated to its cofactor DiGeorge syndrome chromosomal region 8 (DGCR8). Cleavage reactions produce the pre-miRNA, a shorter stem loop miRNA precursor of 70-100 bp, with 2 nt overhanging at its 3' end (25, 26). Then the exportin-5 carries the pre-miRNA to the cytoplasm, where it is processed by an other Rnase III called Dicer, cooperating with its cofactor transactivation-response RNA-binding protein (TRBP) and the core component Argonaute-2 (Ago2). The result of this process is a miRNA duplex of 22 nucleotides, containing one strand that represents the mature miRNA, while the other one is the passenger strand, subsequently degraded. Then, the mature miRNA is incorporated into the effector RNA-induced silencing complex (RISC) (20). Strand selection seems to depend on the stability of the 5' end (27).

There is a class of miRNA, called 'mirtrons', that originate from short intronic hairpins through an alternative pathway; their synthesis, indeed, can bypass the cleavage by Drosha, but needs the action of the splicing machinery and lariat-debranching enzyme. The mirtrons rejoin the canonical miRNA biosynthesis pathway preceding cytoplasmatic export (28, 29).

In association with RISC, miRNAs bind the 3'-untraslated regions (UTRs) of the target mRNA to induce gene silencing: the target mRNA is cleaved or degraded

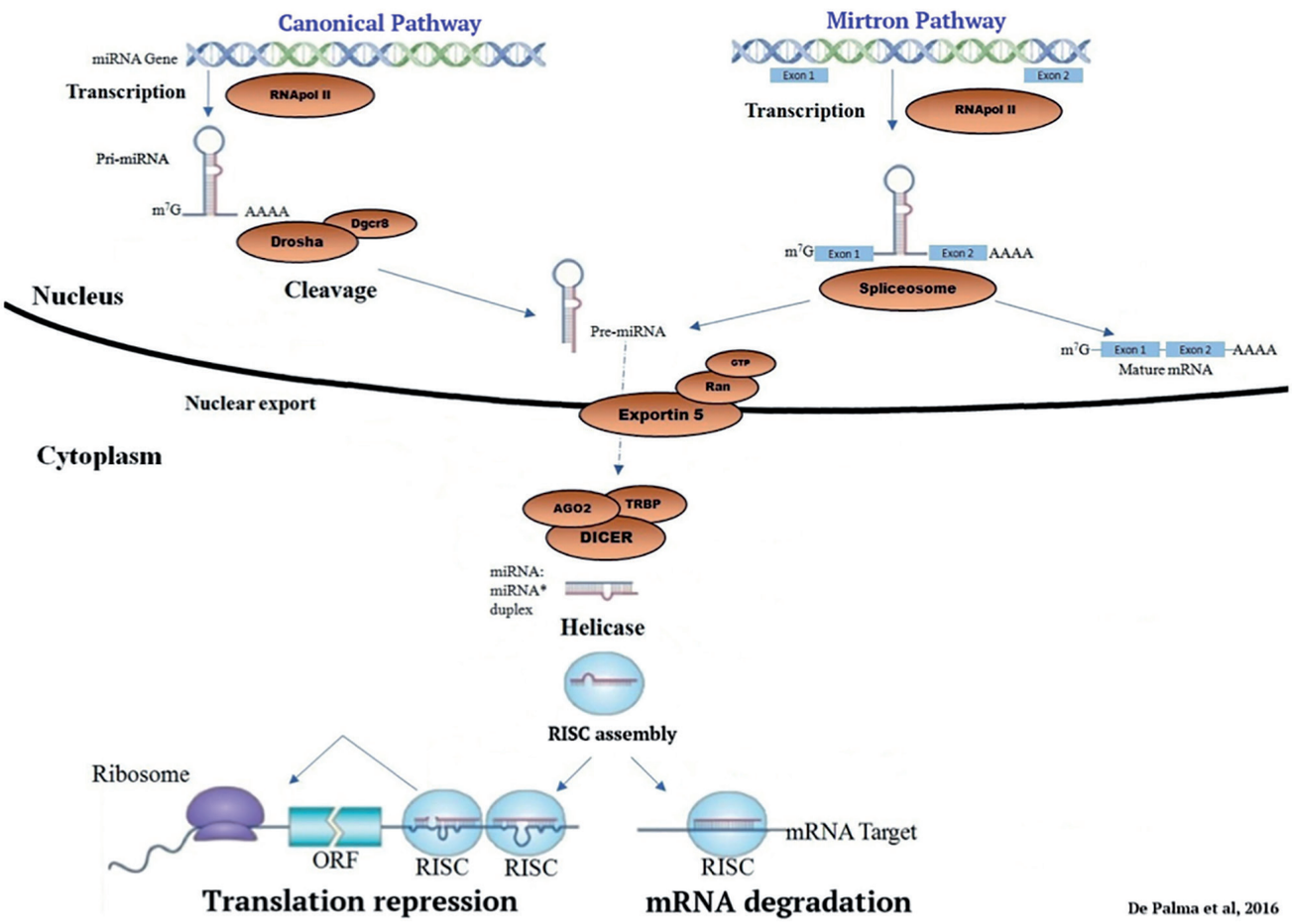

Fig. 1. MicroRNA biogenesis.

Canonical pathway: RNA polymerase II transcribes genomic sequences in stem-loop RNA molecules called pri-miRNA. Then, in the nucleus, the microprocessor complex composed of the Drosha RNase and the DiGeorge syndrome chromosomal region 8 (DGCR8) cofactor produces a pre-miRNA of 70-100 nucleotides. The pre-miRNA is exported by the Exportin-5 pathway into the cytoplasm, where it is further cleaved into a mature miRNA* duplex (~ 22 nt) by DICER, associated to its catalytic partner TAR-binding protein (TRBP) and to Argonaute (Ago2). One strand of miRNA* is assembled in the RNA-induced silencing complex (RISC) and binds a target mRNA: if there is a perfect match, the target miRNA is degraded, otherwise its translation is inhibited.

Alternative pathway: Mirtrons, the intron-derived miRNAs, are processed by spliceosome and debranched by lariat debranching enzyme (Ldbr) to fold into pre-miRNA, successively exported into the cytoplasm to be cleaved by DICER.

after deadenylation if the base-pairing is perfect, while translational repression occurs when their complementarity is only partial (11, 30).

MicroRNAs in osteoarthritis

MicroRNAs are necessary for normal skeletal development, playing an important role in regulating both prenatal and postnatal chondrogenesis and endochondral ossification (31, 32). The conditional knockout of Dicer in mouse cartilage, therefore an incomplete miRNAs biogenesis, leads to severe skeletal growth defects and premature death of mice. Furthermore, the deletion of Dicer gene in chondrocytes reduces their proliferation and increases the number of hypertrophic cells (33).

Accumulating evidence suggests that miRNAs are implicated in the pathogenesis of OA. Several studies of comparison between cartilage specimens isolated from patients with OA and those from healthy age matched control have pointed out miRNAs potentially involved in OA (Table I).

In 2008, Iliopoulos *et al*. integrated genetic, bioinformatic and proteomic approaches in order to identify new genes and their collaborative networks involved in OA pathogenesis. They tested the expression of 365 miRNAs in articular cartilage obtained from patients with OA undergoing knee replacement surgery and from normal individuals with no history of joint disease. 16 miRNAs showed a different expression in OA cartilage compared to normal controls: 9 miRNAs were significantly up-regulated (miR-16, miR-22, miR-23b, miR-30b, miR-103, miR-223, miR-377, miR-483 and miR-509) and 7 miRNAs were down-regulated (miR-25, miR-26a, miR-29a, miR-140, miR-210, miR-337 and miR-373). Five of these statistically correlated with body mass index (BMI), pointing out the potential role of miRNAs in obesity and inflammation; in particular miR-22 and miR-103 expression was positively correlated with BMI, while the correlation was negative for miR-25, miR-337 and miR-29a (34).

Another analysis of miRNAs expression profiling identified 17 miRNAs with a different expression between OA and normal cartilage, and 30 miRNAs that showed differential expression between OA and normal bone; in particular miR-9 and miR-98 were upregulated in both cartilage and bone tissue of OA, where they could be chondroprotective. In fact, their overexpression inhibited inflammatory signals in OA chondrocytes by reducing TNF-α production induced by IL-1β. Furthermore, miR-9 over-expression has been observed to reduce MMP-13 secretion (35).

Diaz-Prado *et al*. more recently analysed the expression profile of 723 miRNAs in normal and OA chondrocytes. 7 miRNAs showed a statistically significant differential expression, among these 1 (miR-483-5p) was upregulated, and 6 (miR-149, miR-582-3p, miR-1227, miR-634, miR-576-5p and miR-641) were downregulated in OA chondrocytes (36).

In 2009 Miyaki *et al*., through microarray analysis, compared miRNA expression between human normal and OA chondrocytes, and bone marrow-derived mesenchymal stem cells (MSCs). MiR-140 was found expressed in a different manner between the analysed cellular types and specifically, it was downregulated in OA chondrocytes. IL-1β, the principal cytokine involved in the pathogenesis of OA, reduced miR-140 expression in OA chondrocytes cultures (37). Deletion of miR-140 gene predisposed mice to the development of age-related OA-like changes, probably due to the increased levels of ADAMTS-5, directly regulated by miR-140 (38). Additionally, it was demonstrated that transfection of chondrocytes with miR-140 mimic down-regulated IL-1β -induced ADAMTS-5 expression (39). Other target genes of this miRNA are MMP-13 and insulin-like growth factor binding protein 5 (IGFBP-5), that are involved in OA (40,41). In consequence of these observations miR-140 could be considered a regulator of chondrocyte differentiation and of cartilage homeostasis, with an important role in the pathogenesis of OA; this issue is underlined by its cartilage-specific expression in mouse embryos and zebrafish (42, 43).

Another microRNA involved in the regulation of chondrocyte functions is miR-27. The human genome contains 2 miR-27 genes, miR-27a and miR-27b, localised in chromosome 19 and 9 respectively, and their major products differ by only 1 nucleotide in the 3' region. A study has demonstrated that miR-27a was downregulated in OA compared to normal chondrocytes and indirectly it downregulated both MMP-13 and IGFBP-5 expression levels (41). An *in vitro* experiment investigated the role of miR-27b in normal and OA chondrocytes stimulated with IL-1β. The results showed that at basal conditions the expression of miR-27b was lower in OA cartilage in comparison to non-diseased tissue; when the cells were stimulated with IL-1β, the expression of miR-27b was significantly downregulated, and the production of MMP-13 protein was enhanced. These results indicate that miR-27b is a post-transcriptional regulator of MMP-13 expression in OA chondrocytes (44).

MiR-146a might also participate in the chondrocytes anabolic/catabolic balance and its expression is dysregulated in OA cartilage. Some authors showed that miR-146a was downregulated in OA cartilage, and that its overexpression in human chondrocytes reduced IL-1β induced TNF-α production, suggesting that this miRNA is a negative regulator of inflammation in OA (35, 45). On the contrary Yamasaki *et al*., by observing that miR-146 was expressed at very low levels in normal cartilage, intensely expressed in low grade OA cartilage and reduced in late OA stages, hypothesised that miR-146 may be involved or in degenerative processes of early OA (46). The upregulation of miR-146a in human OA cartilage and in experimental OA animal models has been detected also by other studies (47, 48). The direct target of this miRNA is Smad4, a mediator of the anabolic TGF-β pathway; Smad4 inhibition resulted in upregulation of vascular endothelial growth factor (VEGF), activation of cellular matrix degradation pathway and apoptosis of chondrocytes (48). Data about pattern of miR-146 expression in OA are thus opposing, so further experiments are needed to understand how it is involved in the pathogenesis of the disease.

Chondrocyte death by apoptosis, necrosis, chondroptosis, or by a combination of these processes has been implicated in the pathogenesis of OA (49, 50). Several studies reported a possible link between some microRNAs (miR-370, miR-373, miR-195, miR-210) and apoptotic process (51, 52).

The last microarray screening of human OA subchondral bone has discovered four novel microRNA involved in bone remodelling and OA development: miR-211-5p, miR-199a-5p, miR-199a-3p and miR-590-5p. They were downregulated in OA animal models, in a time-dependent manner, while their overexpression restored normal bone mineralisation (53).

The downregulation of several microRNA observed in OA cartilage causes the transcriptional activation of their direct or indirect target genes, generally encoding for extracellular matrix degrading enzymes. In fact, bioinformatics analysis identified many candidate genes, besides miRNAs, involved in OA, that in turn could be regulated by some miRNAs (54). MiR-148a, downregulated in OA cartilage compared to normal, could be a potential disease-modifying compound in OA. Its overexpression in OA chondrocytes decreased COL10A1, MMP-13 and ADAMTS-5 gene expression, stimulated the production of collagens, specifically type II collagen, and enhanced the retention and deposition of collagen and proteoglycans (55).

During articular cartilage degradation the reduction of expression levels of miR-105 has been observed; this microRNA targets Runx2, essential for maintaining cartilage homeostasis by regulation of MMP-13 and ADAMTS-5 synthesis (56). It has been demonstrated that MMP-13 gene expression is directly regulated by miR-411 and miR-320, both of them being downregulated in human OA chondrocytes compared to normal cells and repressed by IL-1β stimulation (57,58). Pro-inflammatory cytokines, such as IL-1β and TNF-α, have an important role in the activation of signal pathway for OA progression. Santini *et al*. demonstrated the reduction of miR-149 levels in OA chondrocytes stimulated by pro-inflammatory cytokines, and observed that this miRNA targeted TNF-α 3'UTR. Thus they hypothesised a negative regulation circuitry involved in OA pathogenesis, in which pro-inflammatory cytokines could trigger miRNA downregulation and enhance their own expression through the inhibition of post-transcriptional control (59). In support of this hypothesis, a recent report observed a pivotal role of miR-130a, expressed at low levels in human OA chondrocytes, in regulating the expression of TNF-α (60). Two microRNAs, miR-199a and miR-558, by regulating cyclooxygenase-2 (COX-2) expression, thus by enhancing the inflammation, strongly contribute to cartilage degeneration in OA (61, 62).

The list of miRNA that are upregulated in OA is very wide; among the most recently discovered ones, there is miR-33a, a regulator of cholesterol and fatty acid metabolism that works by targeting Smad7. Transfection of normal chondrocytes with this miRNA increased MMP-13 expression levels, promoting OA pathogenesis (63). Also overexpression of miR-138 could have a role in OA; in fact it induced a decrease of cartilage extracellular matrix components, such as COL2A1, and an increase of the catabolic MMP-13 (64).

Obesity constitutes one of the major risk factors for OA, presumably due to excessive joint loading; however, several studies demonstrated that obesity is also a risk factor for non-weight-bearing joints (65). Obesity is nowadays considered as a chronic low-grade inflammatory status which is closely related to the release, by white adipose tissue, of many factors, most of them of pro-inflammatory nature (66). Recent studies have identified the expression profiles of miRNAs related to both cartilage and adipose tissue (34). In obesity, high levels of non-esterified fatty acid (NEFA) in circulation might induce NF-κB activity which suppresses miR-26a expression in chondrocytes; as result there is an increased secretion of pro-inflammatory and chondrodestructive cytokines normally downregulated by miR-26a (67). The regulative functions of miR-26a and the control of the cholesterol efflux genes by the above mentioned miR-33a in OA chondrocytes, highlight the relationship between metabolic syndrome and OA.

In addition to the miRNAs here discussed, many more miRNAs appear to play important roles in various aspects of cartilage homeostasis and OA pathogenesis (Table I). Understanding these complex miRNA regulatory networks can provide scientific bases for developing novel biomarkers and therapeutic strategies for OA.

MicroRNAs and mechanotransduction

The appropriate mechanical stress has been demonstrated to be required for cartilage homeostasis, but an excessive mechanical load represents one of the most important risk factors for OA. Various *in vitro* studies showed the importance of mechanical compression or hydrostatic pressure (HP) as a modulator of cartilage metabolism and morphology (68–71). Several studies demonstrated that microRNAs may be implicated in mechanotransduction pathway. MiR-221 and miR-222 might be potential regulators of this pathway, since their expression patterns in bovine articular cartilage are higher in the weight-bearing area as compared to the non-weight-bearing area (72). MiR-146a seems to be upregulated, in a time-dependent manner, in human chondrocytes subject to mechanical injury, besides in OA chondrocytes, contributing to cell death and cartilage degradation (73). MiR-365 was expressed at high levels in OA cartilage and more recently has been identified as a mechanically induced mediator of osteoarthritic cartilage destruction. In fact it is overexpressed following cyclic mechanical overloading (1Hz) in growth plate chondrocytes through NF-κB signal pathway, causing the increase of MMP-13 and Col X, by targeting histone deacetylase (HDAC)-4 (74).

Our previous studies demonstrated that a cyclical HP (1-5 MPa, frequency of 0.25 Hz) within the physiological range of the human joint could limit the articular damage of OA (69, 75, 76). We recently used this HP to evaluate possible changes of expression levels of miR-27a, miR-27b, miR-140, miR-146 and some of their target genes, in particular

Table I. Summary of microRNAs (miRNAs) implicated in osteoarthritis (OA).

miRNA	Target gene(s)	Function (s)/pathways involved
	UP-REGULATED IN OA	
miR-9	MMP-13	Inflammatory response
miR-16	SMAD3	Cartilage homeostasis
miR-21	GDF-5	Inhibition of chondrocytes proliferation
miR-22	PPARα, BMP7	Inflammatory response, aging
miR-23	ADAMTS-5, BMPR2, CHUK	Cartilage homeostasis and inflammatory response
miR-29c	Col1a1	Extracellular matrix production
miR-30b	ERG	Chondrocyte differentiation
miR-33a	Smad7	Cholesterol and fatty acids metabolism
miR-93	Oncostatin M	Inflammatory response
miR-98	TNF-α	
miR-103	ACOX1	Lipid metabolism pathways
miR-126	LRP6, PI3KCD	Inflammatory response
miR-138	Sp-1, HIF-2α	Chondrocyte phenotype
miR-145	SOX-9, SMAD3	Cartilage homeostasis
miR-146 (opposing data)	VEGF, SMAD4	Chondrocyte apoptosis
miR-148	COL2A1, COL10A1, MMP13, ADAMTS-5	Cartilage homeostasis
miR-181b	MMP-13 (indirect effect)	Cartilage development
miR-184		
miR-186		
miR-193b	COL2a1, Aggrecan, SOX-9	Cartilage homeostasis
miR-195	HIF-1α	Chondrocyte apoptosis
miR-223	CHUK, PIK3C2A	Peroxisomal dysfunction in OA and chondrocyte apoptosis
miR-345		
miR-365	HDAC-4	Cartilage homeostasis and mechanotransduction
miR-377	CART1	Cartilage homeostasis and structure
miR-455	SMAD2, ACVR2B, CHRDL1	Chondrocyte differentiation and maintenance
miR-483	ACAN	Cartilage homeostasis and structure
miR-483-5p	IGF2	TGF-β, Wnt, Erb and mTOR signalling
miR-675	COL2A1 (indirect effect)	Chondrocyte differentiation and maintenance
miR-509	SOX-9,	Cartilage homeostasis and structure
miR-885-5p		
	DOWN-REGULATED IN OA	
miR-24	CDKN2A	Aging
miR-25	ITAGA5	Biomechanic pathways
miR-26a	ASPN	Cartilage homeostasis and structure
miR-27a/b	MMP-13, IGFBP-5	Cartilage homeostasis
miR-29a	LEP	Lipid metabolism pathways
miR-105	Runx2	Cartilage homeostasis
miR-125b	ADAMTS-4	
miR-127-5p	MMP-13	
miR-130a	TNF-α	TNF-α expression
miR-140	ADAMTS-5, IGFBP-5, MMP-13	Cartilage homeostasis
miR-146 (opposing data)		
miR-149	TNF-α	TGF-β, Wnt, Erb and mTOR signalling
miR-195	HIF-1α	Apoptotic mechanisms
miR-199a	COX-2	Inflammatory response
miR-199a-3p		
miR-199a-5p	SOX9, aggrecan, type 2 collagen	Chondrocytes senescence
miR-210	HIF-3α	Chondrocyte proliferation and cartilage homeostasis
miR-211-5p	BMPR2, IL6R, PTEN, TGFbeta3	Subchondral bone mineralisation
miR-320	ADAMTS-5, MMP-13	Aging
miR-337	RETN	Lipid metabolism pathways
miR-370 miR-373	SHMT-2, MECP-2	Apoptotic mechanisms
miR-411	MMP-13	Cartilage homeostasis
miR-558	COX-2	Inflammatory response
miR-576-5p		TGF-beta, Wnt, Erb and mTOR signalling
miR-582-5p		
miR-590-3p		TGF-beta, Wnt, Erb and mTOR signalling
miR-590-5p	MMP-3, MMP-9	Cartilage homeostasis and osteogenic differentiation
miR-634	PIK3R1	Chondrocyte survival and homeostasis
miR-641		TGF-beta, Wnt, Erb and mTOR signalling
miR-1227		

MMP-13, ADAMTS-4, ADAMTS-5 and IGFBP-5, in normal and OA chondrocytes. We observed a significant increase of the miR-27a, miR-27b, and miR-140 expression, that are downregulated in OA cartilage (37, 41, 44), and a significant reduction of MMP-13, ADAMTS-4 and ADAMTS-5 expression in OA chondrocytes, but not in normal cells. Furthermore, our data indicated a lower expression of miR-146 in OA chondrocytes compared to normal cells, and that its expression was significantly increased following HP (data not published).

A better understanding of the different roles of miRNAs in mechanotransduction pathways, activated by both a physiological and pathological loading, might be one way of approaching cartilage homeostasis and OA pathogenesis.

Clinical application

MicroRNAs as biomarkers of OA

The search for biochemical markers capable of diagnosing early stages of OA, predicting and assessing disease progression, and monitoring treatments effects has been intensified over recent years. A significant number of biochemical markers has been studied, but few of them have shown substantial ability in predicting OA, and none has yet entered into clinical routine (77).

Circulating miRNAs are easily accessible and stable, since they resist harsh conditions that would normally degrade most RNAs, including boiling, extreme pH, long-time storage and repeated freeze-thaw cycles; furthermore they are present in human plasma in a remarkably stable form resistant to endogenous RNase activity (78, 79). MiRNAs have been detected in also dried biological fluids such as semen, saliva, vaginal secretions, menstrual blood and synovial fluid (80).

Recent studies suggest that miRNAs in plasma can be biomarkers for the diagnosis of lung, colorectal and prostate cancer (81, 82). Plasma miRNAs are also considered to be potential biomarkers for drug induced liver injury, and myocardial injury (83, 84).

Murata *et al*. measured concentrations of miR-16, miR-132, miR-146a, miR-155 and miR-223 in plasma and in synovial fluid from patients with RA and OA and in plasma from healthy controls. In RA and OA, synovial fluid concentrations of miR-16, miR-132, miR-146a, and miR-223 were significantly lower than their plasma concentrations, and there was no correlation between plasma and synovial fluid miRNAs, implying their distinct origin. Synovial fluid concentrations of miR-16, miR-146a, miR-155 and miR-223 of RA were significantly higher than those of OA and they could be used for differential diagnosis of RA and OA (85).

A set of 380 circulating miRNA in plasma from patients with primary knee OA was measured by Borgonio Cuadra *et al*. revealing that 12 miRNA, eight of them (miR-29c, -93, -126, -184, -186, -195, -345, and -885-5p) not previously known to be associated with OA, were overexpressed compared to healthy controls (47).

In 2015 Beyer *et al*. published a prospective population-based survey exploring, for the first time, the relationship between serum miRNAs levels and the development of severe OA of the knee and hip joint in 816 Caucasian individuals. After a microarray screen, the Authors validated 12 miRNAs by real-time PCR in the entire cohort; three of these were associated with severe knee and hip OA. MiRNA-454 and miRNA-885-5p respectively showed a trend towards a negative and positive correlation with arthroplasty, though this was not significant after appropriate adjustments. let-7e, indeed, was inversely correlated in a dose-dependent manner, with the risk of joint replacement, and this association was independent of age, sex and BMI. For this reason miRNA let-7e may represent a predictive biomarker for severe OA (86).

Moreover, the measurement of miRNAs in the peripheral blood mononuclear cells (PBMCs) could be a useful method in developing new biomarkers for OA. The expression patterns of miR-146a, 155, 181a, and 223 in PBMCs of OA patients were significantly higher than those of cells from healthy controls, especially in the early stages of OA (87).

Thus, miRNAs in body fluid are promising biomarkers in OA, although none are yet proposed for daily clinical use.

Therapeutical potential of microRNAs in OA

Pharmacologic non-surgical treatment of OA has been largely confined to analgesic or nonsteroidal anti-inflammatory drugs (NSAIDs) which are only symptom-modifying agents. These drugs control pain but do not have known disease-modifying effects. Studies have been performed to identify agents that can prevent or delay/stabilise the pathological changes that occur in OA joints, thereby limiting disease progression (88). Currently, there are no disease modifying therapies available for OA (89, 90). Given that miRNAs play pivotal roles in maintaining the homeostasis of articular cartilage and contribute to the pathogenesis and progression of OA, the development of therapeutic strategies either restoring or repressing miRNAs expression and activity of miRNAs has attracted much attention. Depending on the expression status of the target miRNA, miRNA therapeutic approaches can be classified into two categories: 1) miRNA inhibition therapy when the miRNA is over expressed and 2) miRNA replacement therapy when the miRNA is repressed (89). Although miR-based strategies for OA are emerging as a highly promising therapeutic approach, their systemic delivery still remains a great challenge. For this purpose both viral vectors and non viral delivery systems can be developed (91–94).The ideal delivery system is expected to transport miRNAs to the right place without being degraded by endogenous RNases. Viral vectors are effective delivery agents, but their clinical use is limited by toxicity and immunogenicity. In this regard, many others non viral delivery strategies, such as nanoparticles, have been developed for biological and medical application (95, 96).

Several clinical trials are already ongoing for miRNA-based treatment of hepatitis C, liver cancer, and heart failure (97–99). MiRNA-based treatment for joint diseases can be difficult due to the relative inaccessibility of cartilage, and unfortunately at the moment there is no

trial for OA. Few evidences are available in animal models of OA (100, 101). In particular, Dai *et al*. demonstrated the therapeutical effect of miR-101 silencing *in vivo*: adenovirus (Ad)-miR-101 inhibitor injection into knee joints of mono-iodoacetate-induced arthritis (MIA) rats model of OA reduced cartilage degradation, while Ad-miR-101 mimic increased articular damage (101). Manipulation of miRNA expression could have important applications in OA therapy with tissue engineering-based regenerative strategies; for this purpose Lolli *et al*. have recently discovered that, through miR-221 silencing, human mesenchymal stromal cells (hMSCs) differentiated in chondrocytes and promoted cartilage repair when implanted in nude mice with cartilage defects (102).

Conclusion

MicroRNAs play an important role in the pathogenesis of OA. The number of reports about specific miRNAs and their contribution to cartilage metabolism is constantly increasing. Microarray miRNA analysis in human OA and normal cartilage specimens has revealed different miRNAs expression profiles, and interestingly, some dysregulated miRNAs were functionally associated with cartilage homeostasis or inflammatory pathways. MiRNA expression is regulated by mechanical stimuli, demonstrating their involvement in mechanotransduction pathways that participate to control chondrocytes functions. Recently, the role of miRNAs as diagnostic biomarkers in body fluids (plasma and in synovial fluid) has gained enormous research interest. Furthermore, the development of therapeutic strategies either restoring or repressing miRNAs expression and activity has attracted much attention. Significant progress has been made in the delivery systems of miRNAs, but substantial improvements will still be necessary in this area.

Further studies are needed to search for additional miRNAs that contribute to the pathophysiology of OA and for their clinical application as new possible biomarkers and novel therapeutic agents for OA.

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