Review Article

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MicroRNA – A New Dawn on Horizon for Osteoarthritis Assessment

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Abstract

The etiology of osteoarthritis (OA) is complex, with genetic, developmental, biochemical, and biomechanical factors contributing to the disease process. Chondrocytes in articular cartilage must express appropriate genes to achieve tissue homeostasis, and this is altered in OA given the role of microRNAs in mediating the translation of target mRNAs into proteins, the identification of differentially expressed microRNAs in OA tissue and the crucial contribution that microRNAs play in the progression of OA, microRNAs may have important diagnostic and therapeutic potential, and provide a novel means of treating OA.

Keywords: Osteoarthritis; Chondrocytes; Homeostas, microRNA.

Introduction

The etiology of osteoarthritis (OA) is complex, with genetic, developmental, biochemical, and biomechanical factors contributing to the disease process. Chondrocytes in articular cartilage must express appropriate genes to achieve tissue homeostasis, and this is altered in OA. One facet of the aberrant gene expression in OA is the replay of chondrocyte differentiation with the expression of genes associated with chondrocyte hypertrophy. The pattern of gene expression and the transcription factors that control chondrogenesis are known in some detail. Mechanisms that lead to altered gene expression in OA, however, are less well understood [1].

The importance of microRNAs in different biological processes has already been documented. Intensive research has established that are microRNAs powerful regulators of gene expression. These molecules, which are typically 22 nucleotide long, are produced from larger precursors that contain approximately 70 nucleotides, by enzymes belonging to the Argonaute family and the RNase III, Dicer. After incorporation of miR into the RNA-induced silencing complex (RISC), suppression of the translation or degradation of the target mRNA occurs, resulting in an inhibitory effect on the synthesis of protein product of the gene. The RISC complex is guided to its mRNA target by a single miR strand, which binds imperfectly to its complementary sequence in the 3'UTR of the target microRNA . Thus far, more than 2500 human mature miRs have been discovered [2].

Osteoarthritis

Osteoarthritis is a chronic degenerative joint disorder and a major cause of disability in the elderly. Approximately 10% of men and 18% of women over the age of 60 are affected with osteoarthritis. Approximately 80% of those affected with OA have significant movement limitations and 25% are unable to perform activities of daily living. OA is characterized by progressive structural changes in the articular cartilage, accompanied by new bone formation, changes in the subchondral bone and a low-grade synovitis [3]. The disease eventually leads to the loss of joint function, pain and immobility . Despite high frequency of the disease, its cause is still not completely elucidated [4]. Many factors may play a role in its onset and progression including: age, obesity, overuse or genetics. Articular cartilage undergoes several molecular changes during its lifespan, one of these being chondrocyte activity. Over time, chondrocytes synthesize less aggrecans

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and proteoglycans and become more susceptible to mechanical stress and joint loading [5].

Articular cartilage damage is characterized by degeneration of the extracellular matrix (ECM) [6]. Matrix degrading enzymes, such as the matrix metalloproteinases (MMP), and a disintegrin and a metalloproteinase with thrombospondin motifs (ADAMTS) play important roles in this process due to their ability to cleave type II collagen or aggrecans, which are two major components of the ECM [7].

MicroRNA synthesis and function

Microns are 20 – 22 nucleotides long, non-coding RNA molecules that were first discovered in 1993 [8]. Since then, numerous studies have discovered various microRNAs in almost all multicellular organisms. To date, the miRNA sequence database 'miRBase' includes over 8000 predicted miRNAs from many species of plants, animals and viruses [9]. For humans alone, miRBase lists over 800 predicted miRNAs and other bioinformatics predictions indicate that as many as one-third of all mRNAs might be regulated by miRNA [10]. In the past decade, the role of miRNA has received extensive interest. The importance of miRNA regulation in cellular function is becoming increasingly clear as new miRNA targets are discovered. Although the biosynthesis of microRNAs has now been well established, control of miRNA transcription is not fully understood [11,12] and regulatory mechanisms at the transcriptional level are beyond the scope of this review. Here, recent progress in elucidating the complexity of microRNA processing and posttranscriptional regulation is reviewed.

Generally, the generation of microRNA is a multistep process that starts in the nucleus and finishes in the cytoplasm. First, microRNA genes are transcribed by RNA polymerase II or RNA polymerase III to form long RNA precursors, which contain a single or several stem loops [13, 14]. This structure is called primary (pri)-miRNA; [13, 14] it has a hairpin appearance, with partially complementary sequences in the stem region, which harbours the future miRNA. The primiRNA is subjected to cleavage by an microRNA processor – a protein complex composed of Drosha (a highly conserved RNase-III-type enzyme) associated with DGCR8 (DiGeorge syndrome critical region gene 8) – to form a shorter precursor microRNA called pre-miRNA, characterized by a stem loop or hairpin structure of 70 – 100 nucleotides in the nucleus [14, 15]. Alternatively, many miRNAs are found in polycistronic units that encode more than one miRNA and these miRNAs are also formed in the same way. Additionally, some miRNAs are generated from introns of mRNA, such as *miR-140*, though a not-fully understood mechanism that involves a spliceosome.A smallnumber of pre-miRNAs, named mirtrons – which are directly formed from pri-miRNA processing by a spliceosome instead of Drosha have also been reported [14].

In the processing of these mirtrons, the microRNA processor activity is not required. Pre-microRNAs are exported to the cytoplasm through the exportin-5 pathway and are sliced by another RNase III, Dicer, and its cofactor transactivation-response RNA-binding protein. This results in a double-stranded microRNA duplex that is approximately 22 nucleotides in length, which contains the mature microRNA and the passenger microRNA strand [14]. The passenger microRNA strand is degraded, while the mature microRNA enters the RNA-induced silencing complex (RISC), of which the main components are Argonaute proteins (Agos). Although both strands can generate two mature microRNAs, it is usually only the one with the thermodynamically less stable 52 -end that is incorporated into RISC, while the other strand is degraded. microRNA induces gene silencing through translation repression or targeted mRNA cleavage, depending on the degree of base-pairing complementarity between the microRNA and the 32 -untranslated regions (32 -UTRs) of the target mRNA. microRNA causes cleavage or degradation of target mRNA when perfect base-pairing between microRNAs and their targets occurs [7, 14, 15].

Role of microRNA in cartilage function and its involvement in OA

Although the precise role of microRNA is unclear, its importance in cartilage and chondrocytes has been established. Dicer, an essential component for microRNA biogenesis, is essential for normal skeletal development [32]. Dicer deficiency in chondrocytes results in a reduction in the number of proliferating chondrocytes by two distinct mechanisms: decreased proliferation and accelerated differentiation into postmitotic hypertrophic chondrocytes. Recently, Kobayashi *et al* [32] demonstrated that microRNAs are important for cartilage function. In that study, Dicer-deficient chondrocytes in Dicer-null mice resulted in skeletal growth defects and premature death. Because Dicer is a crucial component in microRNA synthesis, these findings indicated the

indirect involvement of microRNA in the biological roles of chondrocytes [20,21].

At the same time, Iliopoulos *et al*. [23] tested the expression of 365 miRNAs in articular cartilage obtained from patients with OA and total knee arthroplasty, and from normal individuals with no history of joint disease. They identified 16 miRNAs that were differentially expressed in osteoarthritic cartilage versus normal cartilage, which can be used to distinguish osteoarthritic from normal chondrocytes. Thus, accumulating evidence suggests that miRNA deregulation can have effects in OA, and may also be involved in obesity and inflammation.43 Additionally, Jones *et al*. [24] investigated the expression of 157 human miRNAs and identified several that were differentially expressed in human OA cartilage and bone, compared with normal tissue. Here, some typical miRNAs in determining the complex gene expression patterns of OA chondrocytes are introduced, and their roles in transcription regulation and possible demethylation mechanisms that might be applicable to OA are discussed.

The *miR-140* gene is located between exons 16 and 17 of the E3 ubiquitin protein ligase gene *Wwp2* on murine chromosome 8 and the small arm of chromosome 16 in humans [23]. Tuddenham *et al*. [26] reported that *miR-140* was specifically expressed in cartilage tissues of mouse embryos during long and flat bone development, and they detected that histone deacetylase 4 was down-regulated by this miRNA. Miyaki *et al* [25, 27]. compared gene-expression profiling using miRNA microarrays and quantitative polymerase chain reaction in human articular chondrocytes and human mesenchymal stem cells (MSCs). They demonstrated that miR-140 had the largest difference in expression between chondrocytes and MSC [25,27]. An *in vitro* study showed that interleukin (IL)-1β can suppress miR-140 expression in chondrocyte. Transfection of chondrocytes with ds-miR- 140 also inhibits IL-1âβ - induced *ADAMTS5* expression and rescues IL-1β-dependent repression of *aggrecan* gene expression. ADAMTS5 plays an important role in the process of OA; this evidence indicates that miR-140 regulates cartilage development and homeostasis, and its loss could contribute to the development of age-related OA-like changes [27]. Tardif *et al*. [28] used miR-140 and miR-27a to manipulate two significant factors – insulin-like growth factor- binding protein 5 (IGFBP-5) and MMP-13 – in human OA chondrocytes. They found that IGFBP-5 was present in human chondrocytes at a significantly lower level than in OA. These data suggest that IGFBP-5 is a direct target of miR-140; nevertheless, miR-27a indirectly downregulates MMP-13 and IGFBP-5 [28]. Additionally, Kim *et al [29]*. found that miR-27 a suppressed adipocyte differentiation through targeting peroxisome proliferator activated receptor-ã and, therefore, downregulation of miR-27a might be connected with adipose tissue dysregulation in obesity. Obesity is a strong risk factor for OA [29]. Some weight-bearing joints, particularly the knee and hip, are readily affected by OA as a result of increased joint loading [30, 31]. Adipose tissue is a true endocrine organ that can release cytokines: for example, IL-1 and tumour necrosis factor (TNF)-α [33]. Recently, an *in vitro* study of IL-1β stimulation of chondrocytes demonstrated that a sequence in the 32 -UTR of MMP-13 mRNA is complementary to the seed sequence of miR- 27b [34]. Increased expression of *MMP-13* correlates with down-regulation of miR-27b. This illustrates that miR-27b plays a role in regulating the expression of *MMP-13* in human chondrocytes, which could open up novel avenues for OA therapeutic strategies [34]. Another study, by Ohgawara *et al*. [35], demonstrated that miRNA-18a is connected to chondrocyte differentiation and confirmed the functionality of an miRNA-18a target in the 32 -UTR of connective tissue growth factor (CCN2) mRNA, which had been predicted in computer models. Studies have revealed a regulatory role for miR-18a in chondrocyte through CCN2, which is a central conductor of endochondral ossification [36-40]. Increasing evidence has suggested that *miR-146* is a novel gene that has an independent effect on immunemediated diseases. For example, Taganov *et al*. [41] found that *miR-146a/b* is a nuclear factor (NF)-*k*B-dependent gene, which can inhibit expression of the *IRAK1* gene (encoding IL-1-receptor-associated kinase 1) and the *TRAF6* gene (encoding TNF-receptorassociated factor 6) by binding to the 32 -UTR of their mRNA; *miR-146a/b* expression is mediated by inflammatory cytokines.

Additionally, when Yamasaki *et al*. [42] investigated the expression pattern of *miR- 146a* in cartilage from patients with OA, they showed that *miR-146a* was intensely expressed in low-grade OA cartilage, and that its expression was induced by stimulation with IL-1. The results suggest that *miR-146a* has target genes that play a role in OA cartilage pathogenesis. In the early stages of OA, according to Jones *et al*. [24] *miR-146* is associated with a substantial number of genes within the NF-*k*B pathway.

These authors suggested that *miR-146* is down-regulated in late-stage OA cartilage and that reduced

miR-146 expression could be a factor in the promotion of inflammatory OA.Investigations of the role of *miR-34a* in chondrocytes have indicated that its expression is significantly up-regulated by IL-1β. That study revealed that silencing of *miR-34a* might be a novel intervention for OA treatment, through prevention of cartilage degradation [43].

An increasing number of studies in macrophages, monocytes and whole animals have shown that activation through the Toll/IL-1 and TNF-α receptors leads to rapid up-regulation of many miRNAs, including miR-9. In addition, one of the targets of miR-9 is to downregulate proteins that are involved in the TIR signalling pathway. miR-9 can also be induced by Toll-like receptor (TLR)2 and TLR7/8 agonists, and by the pro-inflammatory cytokines TNF-α and IL-1β [44-46]. Each zone has a different pattern of gene expression that has a particular role in articular cartilage development and maintenance. Dunn *et al*. showed that miR-222 expression in articular cartilage is greater in the weight bearing anterior medial condyle than in the posterior non-weight bearing medial condyle. These data indicate that miR-222 is a potential regulator of an articular cartilage mechanotransduction pathway, which may lead to novel ways to treat OA [46].

Changes in DNA methylation are likely to be important in determining the complex gene expression patterns in OA chondrocytes, their role in transcriptional regulation and possible demethylation mechanisms that might be applicable to OA. Preliminary evidence suggests that changes in DNA methylation, together with cytokines, growth factors and matrix composition, are likely to be important in determining the complex gene expression patterns that are observed in OA chondrocytes [47]. This is because primary and secondary OA are characterized by the abnormal expression of cartilage-degrading proteases that correlate with epigenetic DNA demethylation of CpG sites in the promoter regions of these enzymes [47,48]. Wu *et al*. [49] believe that DNA methylation may be mediated by an miRNA. In their study, miRNAs directed DNA methylation at the same loci from which they were produced, as well as in the *trans* regions of their target genes, and were able to affect gene regulation. 68 Considered together, their findings define an miRNA pathway that mediates DNA methylation [48,49]. As more studies are performed on different miRNAs, a better understanding will be gained of their pro-inflammatory and catabolic/anabolic roles in the pathophysiology of OA.

Conclusion

In summary, given the role of microRNAs in mediating the translation of target mRNAs into proteins, the identification of differentially expressed microRNAs in OA tissue and the crucial contribution that microRNAs play in the progression of OA, microRNAs may have important diagnostic and therapeutic potential, and provide a novel means of treating OA.

References

1. Trzeciak T , Czarny-Ratajczak M. MicroRNAs: Important Epigenetic Regulators in Osteoarthritis. *Curr Genomics,* 2014*; 15:* 481-484.
2. Lee, Y.; Jeon, K.; Lee, J.T.; Kim, S.; Kim, V. N. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J.,* 2002; *21*: 4663-4670.
3. Diaz-Prado S.; Cicione C.; Muinos-Lopez E.; Hermida-Gomez T.; Oreiro N.; Fernandez-Lopez, C.; Blanco, F.J. Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes. *BMC Musculosceletal Disor.*, 2012; *13*: 144.
4. Cheung, K.S.; Hashimoto, K.; Yamada, N.; Roach, H.I. Expression of ADAMTS-4 by chondrocytes in the surface zone of human osteoarthritic cartilage is regulated by epigenetic DNA demethylation. *Rheumatol. Int.*, 2009, *29*(5), 525-534.
5. Buckwalter, J.A.; Martin, J.A. Osteoarthritis. *Adv. Drug. Deliev. Rev*., 2006; *58*(2): 150-167.
6. Goldring MB, Goldring SR: Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann NY Acad Sci* 2010; 1192: 230–237.
7. Yu C, Chen WP, Wang XH. MicroRNA in Osteoarthritis J of Int Med Res 2011; 39: 1–9.
8. Lee RC, Feinbaum RL, Ambros V: The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993; 75: 843 – 854.

9. Griffiths-Jones S: miRBase: the microRNA sequence database. *Methods Mol Biol* 2006; 342: 129 – 138.

10. Lewis BP, Burge CB, Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; 120: 15 – 20.

11. Diederichs S, Haber DA: Sequence variations of microRNAs in human cancer: alterations in predicted secondary structure do not affect processing. *Cancer Res* 2006; 66: 6097 – 6104.

12. Yanaihara N, Caplen N, Bowman E, *et al*: Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; 9: 189 – 198.

13. Lee Y, Ahn C, Han J, *et al*: The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003; 425: 415 – 419.

14. Winter J, Jung S, Keller S, *et al*: Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009; 11: 228 –234.

15. Lagos-Quintana M, Rauhut R, Lendeckel W, *et al*: Identification of novel genes coding for small expressed RNAs. *Science* 2001; 294: 853 – 858.

16. Lau NC, Lim LP, Weinstein EG, *et al*: An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 2001; 294: 858 – 862.

17. Lee RC, Ambros V: An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* 2001; 294: 862 – 864.

18. Zeng Y, Cullen BR: Sequence requirements for micro RNA processing and function in human cells. *RNA* 2003; 9: 112 – 123.

19. Kobayashi T, Lu J, Cobb BS, *et al*: Dicerdependent pathways regulate chondrocyte proliferation and differentiation. *Proc Natl Acad Sci USA* 2008; 105: 1949 – 1954.

20. Thai TH, Calado DP, Casola S, *et al*: Regulation of the germinal center response by microRNA-155. *Science* 2007; 316: 604 – 608.

21. Cobb BS, Nesterova TB, Thompson E, *et al*: T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. *J Exp Med* 2005; 201: 1367 – 1373.

22. Kanellopoulou C, Muljo SA, Kung AL, *et al*: Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev* 2005; 19: 489 – 501.

23. Iliopoulos D, Malizos KN, Oikonomou P, *et al*: Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One* 2008; 3: e3740.

24. Jones SW, Watkins G, Le Good N, *et al*: The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-á and MMP13. *Osteoarthritis Cartilage* 2009; 17: 464 – 472.

25. Miyaki S, Sato T, Inoue A, *et al*: MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev* 2010; 24: 1173 – 1185.

26. 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 – 4217.

27. 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 – 2730.

28. Tardif G, Hum D, Pelletier JP, *et al*: 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.

29. Kim SY, Kim AY, Lee HW, *et al*: miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARã expression. *Biochem Biophys Res Commun* 2010; 392: 323 – 328.

30. Felson DT, Anderson JJ, Naimark A, *et al*: Obesity and knee osteoarthritis. The Framingham Study. *Ann Intern Med* 1988; 109: 18 – 24.

31. Oliveria SA, Felson DT, Cirillo PA, *et al*: Body weight, body mass index, and incident symptomatic osteoarthritis of the hand, hip,and knee. *Epidemiology* 1999; 10: 161 – 166.

32. Kobayashi T, Lu J, Cobb BS, *et al*: Dicer dependent pathways regulate chondrocyte proliferation and differentiation. *Proc Natl Acad Sci USA* 2008; 105: 1949 – 1954.

33. Kershaw EE, Flier JS: Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; 89: 2548 – 2556.

34. Akhtar N, Rasheed Z, Ramamurthy S, *et al*: MicroRNA-27b regulates the expression of matrix metalloproteinase 13 in human

osteoarthritis chondrocytes. *Arthritis Rheum* 2010; 62: 1361 – 1371.

35. 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 – 1010.
36. Perbal B: The CCN family of cell growth regulators: a new family of normal and pathologic cell growth and differentiation regulators: lessons from the first international workshop on CCN gene family. *Bull Cancer* 2001; 88: 645 – 649 [in French].
37. Takigawa M, Nakanishi T, Kubota S, *et al*: Role of CTGF / HCS24 / ecogenin in skeletal growth control. *J Cell Physiol* 2003; 194: 256 – 266.
38. Ivkovic S, Yoon BS, Popoff SN, *et al*: Connective tissue growth factor coordinates chondrogenesis and angiogenesis during skeletal development. *Development* 2003; 130: 2779 – 2791.
39. Nakanishi T, Nishida T, Shimo T, *et al*: Effects of CTGF / Hcs24, a product of a hypertrophic chondrocyte-specific gene, on the proliferation and differentiation of chondrocytes in culture. *Endocrinology* 2000; 141: 264 – 273.
40. Nishida T, Kubota S, Nakanishi T, *et al*: CTGF / Hcs24, a hypertrophic chondrocytespecific gene product, stimulates proliferation and differentiation, but not hypertrophy of cultured articular chondrocytes. *J Cell Physiol* 2002; 192: 55 – 63.
41. Taganov KD, Boldin MP, Chang KJ, *et al*: NF-êBdependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* 2006; 103: 12481 – 12486.
42. Yamasaki K, Nakasa T, Miyaki S, *et al*: Expression of MicroRNA-146a in osteoarthritis cartilage. *Arthritis Rheum* 2009; 60: 1035 – 1041.
43. Abouheif MM, Nakasa T, Shibuya H, *et al*: Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model *in vitro*. *Rheumatology (Oxford)* 2010; 49: 2054 – 2060.
44. Bazzoni F, Rossato M, Fabbri M, *et al*: Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. *Proc Natl Acad Sci USA* 2009; 106: 5282 – 5287.
45. Dudek KA, Lafont JE, Martinez-Sanchez A, *et al*: Type II collagen expression is regulated by tissue-specific miR-675 in human articular chondrocytes. *J Biol Chem* 2010; 285: 24381 – 24387.
46. Dunn W, DuRaine G, Reddi AH: Profiling microRNA expression in bovine articular cartilage and implications mechanotrans-duction. *Arthritis Rheum* 2009; 60: 2333 – 2339.
47. Roach HI, Aigner T: DNA methylation in osteoarthritic chondrocytes: a new molecular target. *Osteoarthritis Cartilage* 2007; 15: 128 – 137.
48. da Silva MA, Yamada N, Clarke NM, *et al*: Cellular and epigenetic features of a young healthy and a young osteoarthritic cartilage compared with aged control and OA cartilage *J Orthop Res* 2009; 27: 593 – 601.
49. Wu L, Zhou H, Zhang Q, *et al*: DNA methylation mediated by a microRNA pathway. *Mol Cell* 2010; 38: 465 – 475.
50. Bayne EH, Allshire RC: RNA-directed transcriptional gene silencing in mammals. *Trends Genet* 2005; 21: 370 – 373.