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**A mini-review: microRNA in arthritis**

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RECENTLY, microRNA (miRNA or miR) has attracted attention because of its role in human diseases. MiRNA has been shown to be one of the major regulators in a variety of biological processes such as cell cycle, immune function, and metabolism. Abnormal expression of miRNA has been implicated in the pathogenesis of human diseases including cancer, hematopoietic diseases, and viral infections (9, 10, 26, 40). MiRNA is a small, ~22-nucleotide class of noncoding RNAs that regulates gene expression posttranscriptionally (6, 7). Many miRNAs are evolutionarily conserved across phyla, identified from nematodes to humans (12). MiRNAs regulate gene expression by binding the 3′-untranslated region (UTR) of their target mRNAs leading to translational repression or mRNA degradation. Many investigators have attempted to elucidate the precise role of individual miRNAs in human disease to develop a new strategy to target miRNAs. The clarification that miRNAs participate in the pathogenesis of diseases, especially refractory diseases with unidentified mechanisms, might lead to a novel effective treatment.

Numerous patients in the world suffer from pain due to damaged joints. While there are various forms of arthritis, the most common form is osteoarthritis (OA). Other common arthritis forms are rheumatoid arthritis (RA), psoriatic arthritis, and other autoimmune diseases. Although these types of arthritis torment many patients, the pathogenesis of their arthritis has not been elucidated, and the most effective treatment has not been established yet.

While there have been many reports on the role of miRNA in human diseases, several reports specifically about miRNA-related arthritis have been published recently. This review article will focus on miRNA in arthritis, especially OA and RA. MiRNA is described as a novel therapeutic strategy for arthritis as a biomarker for diagnosis and prognosis and as a target for administration to regulate the endogenous miRNA.

**OA**

OA is the most widespread degenerative joint disease, characterized by progressive destruction of articular cartilage (2, 25, 37, 38). The common features of OA are joint pain, swelling, or synovial effusion, leading to the disability of mainly elderly people. Despite the high prevalence of OA, its etiology has not been found. One of the mechanisms of OA progression is the age-related loss of the homeostatic balance between anabolic factors, such as transforming growth factor-ß, and catabolic factors, such as matrix metalloproteinases (MMP) and aggrecanases in articular cartilage (3). Elucidation of the molecular mechanism in the homeostatic balance of articular cartilage has the potential to lead to a novel treatment for OA, and therefore several studies have been conducted to clarify miRNA as a new player participating in its mechanism.

Since many molecular events occur in OA chondrocytes during articular cartilage degeneration, several studies have been conducted to investigate the expression pattern of miRNAs. Iliopoulos et al. (19) investigated the miRNA profiling of patient-
derived OA cartilage compared with normal cartilage, and 16 miRNA with OA-specific signatures were identified. In these miRNAs, five miRNA (miR-22, miR-103, miR-25, miR-337, and miR-29a), statistically correlated with body mass index, suggesting the potential role of miRNA in lipid metabolism and the pathogenesis of OA. In particular, miR-22 regulated the expressions of PPARA and BMP7 as its direct target genes, subsequently inhibiting the inflammatory and catabolic changes in osteoarthritis chondrocytes.

Jones et al. (20) also demonstrated 17 miRNAs differentially expressed in late-stage human OA cartilage. They showed that miR-9, miR-25, and miR-98 were upregulated and that miR-146 was downregulated in OA cartilage. Functional pathway analysis of the predicted gene targets for these miRNAs was performed. These results suggested that miR-9, miR-98, and miR-146 might play an inflammatory role by regulation of IL-1β, TNF-α, and MMP-13.

Taganov et al. (44) reported that MiR-146a is induced in response to lipopolysaccharide (LPS) and proinflammatory mediators in THP-1 cells and that it is regulated by NF-κB. They proposed that miR-146 might regulate cytokine signaling by a negative feedback regulation loop involving downregulation of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6). Inflammatory cytokines play a crucial role as a catabolic factor in OA pathogenesis (15), and therefore miR-146 plays a role in the inflammatory process of OA progression. One report focused on the expression pattern of miR-146a in human OA cartilage (51). In that report, miR-146a is expressed intensely in cartilage with a low Mankin grade, and its expression decreased commensurately with the level of MMP-13 expression, declining as the Mankin grade became higher. MiR-146a was also expressed in all chondrocyte layers, especially in the superficial layers, while miR-146a-expressing cells were sparsely distributed in the deep zone, where the matrix appeared normal. The expression of miR-146a was remarkably increased by stimulation of IL-1β in normal chondrocytes. These results indicated that miR-146 expression might be induced by IL-1β stimulation when degenerative change begins and that it might play a role in the repression of catabolic factors through negative feedback in early-stage OA cartilage.

MiR-140 has been reported as cartilage-specific miRNA, which is expressed in cartilage during limb development, inhibiting HDAC4 expression as a direct target gene of miR-140 (49). Using microarray analysis, Miyaki et al. (28) showed that miR-140 is expressed in normal human articular cartilage and that its expression is significantly reduced with the progression of OA stages. Furthermore, IL-1β treatment for normal human chondrocytes suppresses miR-140 expression, and overexpression of miR-140 can downregulate IL-1β-induced ADAMTS5 expression and rescued IL-1β-dependent repression of aggrecan gene expression. Furthermore, using mice genetics in vivo, this group showed that miR-140 plays a critical role in the pathogenesis of OA. MiR-140−/− mice showed age-related OA-like changes, characterized by proteoglycan loss and fibrillation of articular cartilage (29). Conversely, overexpression of miR-140 in vivo was shown to inhibit degeneration from OA. Finally, they revealed that miR-140 regulates ADAMTS5 expression as the direct target of miR-140 through OA pathogenesis. This study directly revealed that miRNA plays a crucial role in OA pathogenesis.

Another group, Tardif et al. (47), also showed that miR-140 expression decreases during OA. This group focused on MMP-13 and IGFBP-5 expression in OA cartilage and predicted a functional binding site within the 3′-UTR sequences of the MMP-13 and IGFBP-5 via several bioinformatic approaches. MiR-140 and miR-27a have the potential to bind the 3′-UTRs of the MMP-13 and IGFBP-5, and Tardif et al. (47) showed that both miRNAs are expressed in normal chondrocytes, decreasing their expression in OA chondrocytes.

MiR-27b has been reported as regulating the expression of MMP-13 in human OA chondrocytes. MiRNA expression profiles using the RNA from chondrocytes stimulated with or without IL-1β were evaluated, and miR-27b has been shown to be downregulated in IL-1β-stimulated chondrocytes. In silico analyses revealed that 3′-UTR sequences of MMP-13 had a beneficial effect on the seed sequence of miR-27b (4). The study demonstrated that increased expression of MMP-13 correlated with downregulation of miR-27b and MMP-13 is the direct target of miR-27b.

In these miRNA reports about OA cartilage (including miRNA profiling analyses), there are a few overlapping results and discrepancies. The expression of miR-25 was downregulated in one study (19) but upregulated in the other (20). One of the reasons for this discrepancy is the stage of the OA cartilage. Most cartilage was obtained at the time of joint replacement surgery. However, in histological analysis, the degree of degeneration of cartilage was partially different. The histological evaluation of cartilage degeneration from which RNA was extracted, is also important.

Recent studies have revealed that miRNAs might play a role in regulating the gene expression in OA pathogenesis, especially in their effect on catabolic factors such as MMPs and ADAMTS. There is increasing evidence that miRNA in articular cartilage plays an important role, and this evidence promises to provide a breakthrough in OA treatment.

RA

RA is characterized as being an autoimmune disease with chronic inflammation of synovial tissue, causing subsequent irreversible joint destruction (14). In RA synovial tissue, infiltration of macrophages, T cells, and B cells plays a crucial role in RA pathogenesis, including proliferation of the lining cells and production of inflammatory cytokines such as TNF-α and IL-1β (8, 22). However, the pathogenesis of RA has not been fully elucidated. Recently, it has become clear that miRNAs play a role in the pathogenesis of RA. Since abnormal expression of miRNAs may contribute to the molecular mechanisms of RA just as it does with other human diseases, synovial tissue (the important tissue in RA), especially synovial fibroblasts, and peripheral blood mononuclear cells (PBMC) were analyzed.

MiR-146 is recognized as a negative regulator of inflammatory response. MiR-146 is induced by NF-κB and plays a role in fine-tuning innate immune responses through negative feedback, including downregulation of TRAF6 and IRAK1 genes (44). In human lung alveolar epithelial cells, miR-146a/b can negatively regulate acute inflammation through inhibition of the release of proinflammatory cytokines IL-8 and RANTES (39). MiR-155 also plays an important role in the innate immune response in macrophages and lymphocytes. MiR-155...
expression is induced by proinflammatory cytokines in dendritic cells and directly controls the level of TAB2, which is an important signal transduction molecule in the TLR/IL-1 signal cascade (11, 44). Moreover, miR-155 participates in the maturation of human dendritic cells and modulates pathogen binding by directly targeting the transcription factor PU.1 (24). As for RA, miR-146 and miR-155 are more intensely expressed in the RA synovial fibroblasts than in the OA synovial fibroblasts, and miR-155 expression is higher in synovial tissue and synovial fluid monocytes in RA than in OA (42). Overexpression of miR-155 in RA synovial fibroblasts can downregulate the expression of MMP-3. MiR-146 is more highly expressed in RA synovial tissue than in OA, and an in situ hybridization study revealed that miR-146-expressing cells in synovial tissue were dominantly CD68+ macrophages, but also CD3+ T cells and CD79+ B cells (33).

Proliferation of RA synovial fibroblasts plays a crucial role in the RA disease process, because RA synovial fibroblasts produce inflammatory cytokines, chemokines, and angiogenic factors. In these functions of RA synovial fibroblasts, it is clear that miRNAs assume an important role. There was a significantly greater decrease in the MiR-124a expression level of RA synovial fibroblasts than of OA synovial fibroblasts (32). MiR-124a regulates the proliferation of RA synovial fibroblasts by suppression of the production of the CDK-2 and MCP-1 proteins. In a study using microarray analysis, MiR-346 was found to be upregulated in LPS-activated RA synovial fibroblasts, and it was also determined that the IL-18-releasing mechanism in LPS-activated RA synovial fibroblasts through inhibition of Bruton’s tyrosine kinase by miR-346 (5). In RA synovial tissue, miR-146, miR-155, and miR-346 are upregulated and miR-124a is downregulated by inflammatory cytokines, and these altered expressions of miRNAs contribute to the proliferation of RA synovial fibroblasts, inflammatory response, and production of MMPs, and subsequently to joint destruction.

Peripheral blood is easily obtained and is usually analyzed to diagnose RA and evaluate the activity of RA. PBMC from RA patients exhibit higher expression levels of miR-146a, miR-155, miR-132, and miR-16 than healthy and disease control individuals (35). Also, the expression level of miR-16 and miR-146 correlated with RA disease activity. Higher levels of MiR-223 were expressed in T-lymphocytes from RA patients, especially naïve CD4+ lymphocytes (13). Synovial tissue in RA patients is infiltrated with macrophages and naïve CD4+ lymphocytes; therefore, miR-223 might also participate in RA pathogenesis. It has been reported that miR-223 is also the critical regulator of osteoclastogenesis, suggesting that miR-223 might play a role in bone destruction in RA (42). Recently, the importance of IL-17 in RA pathogenesis has been well discussed. IL-17 is a proinflammatory cytokine that induces other cytokines, such as TNF-α, IL-1β, IL-6, IL-23, and G-CSF (1, 50). In addition, IL-17 plays a role in osteoclastogenesis via activation of RANKL. IL-17 is recognized as being a key factor in inflammation and bone destruction in RA (40, 45). Microarray analysis by Niimoto et al. (35) demonstrated that six miRNAs, let-7a, miR-26, miR-146a/b, miR-150, and miR-155 are significantly upregulated in IL-17-producing T cells. Moreover, the study showed that miR-146a and IL-17 are intensely expressed in PBMC in patients with a low score on the Larsen scale and high disease activity. In RA synovium, miR-146a is expressed intensely in the synovium with hyperplasia and high expression of IL-17 from patients with high disease activity, and IL-17-producing cells expressed miR-146a.

Previous reports have suggested that inflammatory cytokines and the miR-146a pathway play a role in RA and OA, although the etiology of RA and OA are different. Most papers have shown that the expression of miR-146 is higher in RA than in OA. MiR-146 is expressed in several cells as well as in the tissue constituents of joints. The expression of miR-146 is induced by inflammatory cytokines in RA synovial fibroblasts and chondrocytes (33, 51). Inflammatory cytokines play a greater role in the pathogenesis of RA than of OA. On the other hand, mechanical factors play a crucial role in the pathogenesis of OA, although inflammatory cytokines such as IL-1β contribute to cartilage degeneration. The degree of dependence of inflammatory cytokines in pathogenesis might determine the difference in the expression levels of miR-146 in RA versus in OA. The function of miR-146 in arthritis has not been explained by previous reports. In the past several years, while the expression analyses of miRNA in arthritis have increased, functional analysis has been barely reported. The functional role of miRNA in arthritis should be clarified to enable development of a novel treatment.

**AS A NOVEL BIOMARKER**

MiRNA existing in human body fluid such as plasma, urine, and saliva in a stable form has the potential to be a novel diagnostic and prognostic biomarker of various diseases, especially cancer (16–18, 27, 53). RA can be difficult to diagnose, but it is important to diagnose RA earlier and to start treatment, to prevent joint destruction. Therefore, more useful tools to diagnose RA are needed.

MiRNA expression was detected in plasma and synovial fluid from RA and OA patients (30). MiR-132 expression in RA and OA patients was significantly lower than in healthy individuals, and expression of miR-16, miR-146a, miR-155, and miR-223 in synovial fluid of RA was significantly greater than in OA. Interestingly, miR-16 and miR-146a in plasma significantly correlated with tender joint counts and the 28-joint Disease Activity Score. Exosomes including miRNA are secreted from cells and contribute to the cell-to-cell interaction (27). The expression of exosomal miRNAs has a pattern distinct from those of intracellular miRNAs, and therefore these results are inconsistent with previous reports that have analyzed the expression pattern of miRNAs in PBMC in RA patients (36). The function and the origin of miRNAs in plasma and synovial fluid should be determined, to enable interpretation of the relationship between the expression pattern of miRNAs in plasma and synovial fluid and to facilitate analysis of miRNA’s clinical status as a biomarker. MiRNAs in plasma and synovial fluid will become a useful biomarker in clinical use, because they are reported to be remarkably stable and are not degraded from the endogenous RNase.

**NOVEL TARGET FOR TREATMENT**

The evidence that miRNAs play a role in human diseases will potentially open the door to develop a novel therapeutic strategy. Recently, therapeutic trials aimed at targeting miRNA in vivo have been conducted (21, 23, 34, 45, 48, 52). As for...
arthritis, Nagata et al. (31) demonstrated the possibility of miRNA therapy for arthritic joints. They showed that an intra-articular injection of double-stranded miR-15a successfully induces cell apoptosis by inhibiting the translation of BCL2 protein in the synovium in arthritic mice. This suggests that an intra-articular injection of synthetic miRNAs can regulate the endogenous miRNAs in arthritic synovia. This evidence suggests that arthritis may be treated by regulating miRNAs that play a significant role in the pathogenesis of RA. However, there are many problems to be overcome in the clinical setting. For example, an effective delivery method for the administration of synthetic miRNA is needed. In particular, it is supposed that uptake of synthetic miRNA into chondrocytes surrounding the abundant matrix would be difficult in the treatment of damaged cartilage. Development of a more effective drug delivery system is expected.

In the treatment of RA, biological reagents such as TNF-α, IL-1, and IL-6 blockers have been shown to successfully cure RA patients. However, these reagents have limitations such as side-effects, nonresponders, and cost. On the other hand, by targeting miRNA, miRNAs do not secrete protein such as inflammatory cytokines. The combination of a biological reagent and disease-modified antirheumatic drugs together with miRNA treatment might be more effective and conducive to a cure.

Another concern in clinical settings is side-effects. The regulation of certain miRNA might have a carcinogenic effect because several miRNAs play a role as oncogenes. Sufficient examination of miRNA therapy should be conducted before clinical use, to prevent unexpected side-effects.

In future, more evidence about the role of miRNA in arthritis and more knowledge about RNA interference technology in vivo will enable a new advanced strategy toward arthritis treatment.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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miRNA IN ARTHRITIS


