REVIEW

What Do MicroRNAs Mean for Rheumatoid Arthritis?

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Introduction

MicroRNAs (miRNAs) are small noncoding RNA molecules that modulate the expression of multiple protein-encoding genes at the posttranscription level. They likely participate in nearly every developmental and physiologic process. Although the function of most mammalian miRNAs has yet to be determined, it appears that their aberrant expression may play a role in the pathogenesis of several pathologic conditions, including immune-mediated inflammatory disorders. Over the past 3 years, dysregulated expression of a dozen miRNAs has been reported in patients with rheumatoid arthritis (RA), in both the circulation and inflamed synovium; however, the pathogenic role of only a few of these has been investigated in experimental mouse models. Here, we provide an overview of the current understanding of miRNA biogenesis and of the activities identified for the miRNAs shown to be dysregulated in RA. We also discuss potential benefits to patients and clinicians, including identification of novel and much-required molecular biomarkers of RA, as well as the possible development of miRNA-based biologic agents.

Current understanding of miRNA biogenesis

MiRNAs are noncoding single-stranded RNAs of ~19–23 nucleotides (nt) in length that are conserved from worms to mammals and function as negative regulators of gene expression. In animals, miRNAs are involved in the regulation of multiple biologic pathways by posttranslationally repressing the expression of protein-encoding genes. The miRNAs achieve this by base-pairing to target messenger RNAs (mRNAs), thus influencing either their stability or rate of translation. Human miRNA genes are nonrandomly located throughout all chromosomes except the Y chromosome (1), are predominantly observed in introns (70%), and are frequently observed in cancer-associated genomic regions or fragile sites (52.5%) (2). Half of the known human miRNAs are found in clusters that may be functionally related by their targeting of the same gene or different genes that are involved in the same metabolic pathway. Interestingly, evolutionarily conserved miRNAs are thought to be conserved elements of a gene regulatory network involved in key biologic processes such as development and apoptosis. Thus, studying miRNAs in lower organisms should shed much light on evolutionary developmental biology (3) and human disorders.

In 2009, Hervé Seitz (Université de Toulouse, Laboratoire de Biologie Moléculaire Eucaryote, Toulouse, France) proposed a hypothesis that could reconcile several contradicting observations regarding the biologic role of miRNAs in animals (4). Recently, when Dr. Seitz was asked, “How can your work on model organisms help us to decide whether miRNAs are true mega regulators of cellular functions or just mini tuners with little impact on physiological conditions in humans,” he responded as follows:

“miRNA-mediated repression is usually modest (~2-fold), which is hard to reconcile with the robustness of biological pathways to fluctuations in gene expression (e.g., most genes in animals are haplo-sufficient: their physiological activity is not affected by a 2-fold repression in gene expression). Gene expression is also very variable between individuals in natural populations—it is hard to understand how miRNAs could do any-

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thing useful if they repress their targets less than these inter-individual variations. Yet, thousands of genes exhibit phylogenetically-conserved complementarity to miRNAs in many species, including humans: that number exceeds, by far, the estimations of the number of dose-sensitive genes. These observations can be reconciled if you consider that most of the predicted targets are actually not functionally targeted by miRNAs, but rather act as competitive inhibitors; they would be miRNA modulators, repression miRNAs by titrating them.

The only difference between "real targets" and such competitive inhibitors would be their sensitivity to small changes in gene expression, that depends on the architecture of their biological pathways (negative or positive feedback loops, stoichiometry of molecular complexes, etc.). Exploring these pathways with systems biology methodologies (assessing quantitatively the consequences of miRNA activity on integrated phenomena) requires genetic manipulation, which is only achievable in model organisms.”

In mammals, miRNA genes are transcribed by RNA polymerase II as monocistronic or polycistronic, long, primary transcripts named primary miRNA or pri-miRNA (5,6) that range from ~200 nt to several kilobases in length and are folded into hairpin structures containing imperfectly base-paired stems. In the nucleus, these pri-miRNAs are processed by the RNase III type endonuclease Drosha, along with its partner protein DGCR8 (DiGeorge syndrome critical region gene 8), into 70–100-nt–long pre-miRNA (7). The pre-miRNAs are then transported to the cytoplasm by exportin 5 (8), where they are cleaved by the RNase III endonuclease Dicer, along with its partner protein TRBP (human immunodeficiency virus transactivating response RNA-binding protein), to produce a miRNA/miRNA+ duplex of 20–22 nt (9). This duplex is unwound by an helicase, and the miRNA “guide” strand is then selected for incorporation into the multiproteic RNA-induced silencing complex (RISC) to function as a so-called mature miRNA, while the “passenger” strand (miRNA+) is released and degraded (10,11).

High-throughput sequencing studies have provided evidence for both arms of some pre-miRNAs generating 2 different regulatory small RNAs, suggesting that miRNA+ strands may also sometimes regulate gene expression (12,13). Currently, much debate still surrounds how the guide strand is selected. The silencing action of the mature miRNA is mediated by the catalytic component of the miRISC (RISC with incorporated miRNA) complex and a member of the Argonaute family Ago, together with a number of accessory factors. It is believed that most miRISC complexes silence gene expression by mechanisms of either mRNA cleavage or translational repression, depending on the perfect or incomplete complementarity between the miRNA guide strand and its mRNA target. Only Ago2 has been shown to cleave the targeted mRNA by endonuclease activity, while other Ago proteins are thought to mediate translational repression (12). In that case, changes in the expression of targeted genes are evidenced only at the protein level, without any obvious decrease in mRNA levels. The miRNA binding sites were originally thought to be principally located in the 3′-untranslated regions (3′-UTRs) of target mRNAs; however, recent studies have suggested that miRNAs might also interact with complementary sequences residing in the 5′-UTR or the promoter and even the coding sequence (CDS) regions of target genes (14–16).

To date, efforts have mainly been focused on the regulatory function of miRNAs, with little knowledge being sought on the regulation of expression of the miRNA genes themselves. Moreover, our current understanding of the precise processing of miRNAs and how they act to silence target mRNAs remains incomplete (17), and many exceptions to the rules likely exist.

Our current knowledge on miRNAs and RA

Rheumatoid arthritis is a chronic inflammatory autoimmune disorder affecting millions of people worldwide. It commonly affects the joints, with progressive systemic inflammatory manifestations that can then affect many organs throughout the body and lead to substantial morbidity. Although a better understanding of the pathophysiology of RA has enabled the development of numerous efficient targeted therapies, RA pathogenesis remains largely unsolved. The discovery of a potential link between miRNAs and the pathophysiology of RA has stimulated research toward obtaining a more integrated view of the networks that are dysregulated in RA and perhaps accessing a key pivotal mediator of pathogenesis.

The first evidence for miRNAs playing a role in RA emerged in 2007 with the identification, in the serum of patients with RA, of autoantibodies directed against GW bodies (18), which are cytoplasmic structures for mRNA storage and/or degradation, including the RNA interference machinery (19). Three studies over the following year demonstrated the up-regulation of specific miRNAs either in the circulation (20) or
within the inflamed joints of patients with RA (21,22); these specific miRNAs are miR-16, miR-132, miR-146a, and miR-155. Since then, the abnormal expression of a dozen miRNAs has been documented in patients with RA (23); most of these miRNAs are up-regulated (Figure 1).

Not surprisingly, among the first miRNAs investigated in RA samples were miR-146a and miR-155, both of which are involved in the development of innate and adaptive immune cells, are up-regulated under inflammatory conditions and in response to a variety of microbial components, and are overexpressed in several immune-mediated inflammatory disorders (24–26). Both miRNAs are thought to finely tune the immune response and the inflammatory response through negative feedback loops on molecules downstream of Toll/interleukin-1 (IL-1) receptor (TIR) signaling (27).

Since 2008, the majority of studies investigating the expression and role of miRNAs in RA have focused on miR-146a (20–22,28–30). These studies showed that miR-146a is strongly up-regulated in synovial tissue, fibroblast-like synoviocytes (FLS), macrophages, B cells, and CD3+ T cells along the superficial and sublining layers, as well as in CD4+ T cells from the synovial fluid of patients with RA compared with healthy donors and patients with osteoarthritis (OA). The expression of miR-146a is also increased in plasma, peripheral blood mononuclear cells (PBMCs), and CD4+ T cells isolated from patients with RA compared with healthy donors, but not compared with patients with OA (20,30). Both locally and systemically, monocyte/macrophages more than lymphocytes seem to represent the major cellular source contributing to the increased miR-146a expression levels in patients with RA. Although Niimoto and colleagues (30) did demonstrate that miR-146a expression colocalizes with IL-17 staining in RA synovium, this demonstration does not formally prove that miR-146a is expressed by IL-17–producing T cells (30). Other cell types, including macrophages, also express IL-17.

Interestingly, using comparative analysis of miRNA concentrations in various fluids and cell types from patients with RA and control subjects, Murata et al showed that miR-146a expression levels in synovial fluid mimic those in synovial tissue/fibroblasts, and that high plasma concentrations are inversely correlated with tender joint counts (29). Similarly, Pauley et al observed a correlation between high miR-146a expression levels and disease activity based on C-reactive protein and erythrocyte sedimentation rate values, providing further support for the potential usefulness of miR-146a as a biomarker of RA disease activity (20).

Other studies have shown increased expression of miR-155 in RA synovium compared with samples of both noninflamed synovial fluid (29) and noninflamed tissue (22) from patients with OA, which is mainly attributable to the constitutive expression of miR-155 in RA FLS and in CD14+ cells from synovial fluid. Obtaining a clear picture from studies assessing miR-155

Figure 1. Tissue distribution of microRNAs that are dysregulated in rheumatoid arthritis. CD4+ = T cell lymphocytes positive for the CD4 phenotypic marker; RA-FLS = rheumatoid arthritis fibroblast-like synoviocytes; PBMC = peripheral blood mononuclear cell; DC = dendritic cell; NK = natural killer cell. Figure 1 was produced using Servier Medical Art.
expression in the circulation is, however, more cumbersome. Indeed, although miR-155 is detectable in plasma, the concentrations are comparable between patients with RA, patients with OA, and healthy donors (29). However, miR-155 expression levels in PBMCs isolated from patients with RA are higher than those in patients with OA and healthy control subjects (20,30). As observed with miR-146a, Pauley et al also showed that the adherent cells in blood from patients with RA (monocyte/macrophages) expressed higher levels of miR-155 than did the nonadherent fraction (lymphocytes) (20).

The abnormal abundance of miR-16 in blood and RA joints has also been reported. Similar to the expression of miR-155, the expression levels of miR-16 in plasma are comparable among patients with RA, patients with OA, and healthy individuals (29), but miR-16 expression levels are higher in PBMCs isolated from patients with RA versus healthy control subjects (20). The expression levels of miR-16 in synovial fluid are higher in patients with RA than in patients with OA. Because miR-16 is ubiquitously expressed, this observation might reflect higher cellularity within inflamed tissues and the circulation of patients with RA compared with control subjects. Pauley et al observed a correlation between miR-16 expression levels in PBMCs and RA disease activity (based on the C-reactive protein level and the erythrocyte sedimentation rate), while Murata et al showed that plasma miR-16 concentrations were inversely correlated with the Disease Activity Score in 28 joints (DAS28) (31).

Marked overexpression of another miRNA, miR-223, in patients with RA, both systemically and within inflamed joints, has been shown by 3 different groups of investigators (29,32,33). Hematopoietically specific, this miRNA is abundantly expressed in blood and serum. Although no detectable variations of plasma concentrations were measured in RA samples compared with OA and control samples (29), Fulci and coworkers showed that miR-223 is strikingly up-regulated in naive CD4+ T lymphocytes from the blood of patients with RA compared with that from healthy donors (32); however, no correlation between miR-223 levels in CD4+ T cells and clinical parameters was observed. Nakamachi et al observed stronger expression of miR-223 in RA FLS than in OA FLS (33), which was then confirmed at the synovial fluid level by Murata et al (29). Interestingly, this comparative study by Murata et al on secreted miRNAs by different cell types also showed that, in addition to synovial tissue that appears to be the main source of the miRNAs detected in synovial fluid, mononuclear cells infiltrating the synovial fluid also strongly contribute to the levels of miR-223 in synovial fluid. Finally, Murata et al observed an inverse correlation between the plasma miR-223 level and the tender joint count (29).

Results from studies on miR-132 expression levels appear to be conflicting, with levels being increased in PBMCs isolated from the blood of patients with RA (20) while being significantly decreased in plasma samples obtained from patients with RA (29) compared with healthy controls. This apparent discrepancy between expression levels in plasma and PBMCs isolated from blood, also found with miR-155 (see above), might be explained by the fact that not all the miRNAs expressed by blood cells are necessarily retrieved in plasma.

The dysregulation of other miRNAs has been shown in RA synovium: miR-124a expression is decreased, while miR-203, miR-133a, miR-142-3p, and miR-142-5p are up-regulated in RA FLS compared with OA samples (33,34). The expression of miR-363 and miR-498a is decreased in CD4+ T cells isolated from the blood and synovial fluid of patients with RA (28). Importantly, none of the miRNAs identified as abnormally expressed in RA tissues are specific for RA.

According to an abundance of reports in the literature, the expression of some miRNAs is altered in human pathologic conditions including immune-mediated inflammatory disorders other than RA, but the main questions remaining are as follows: what does the altered expression of these miRNAs in RA mean in terms of the impact on patients, and what is its significance to clinicians and researchers? Based on our current knowledge, we have attempted in the following sections to provide readers with initial answers to these fundamental questions.

What hopes do miRNAs carry for RA?

A novel class of biomarkers fulfilling unmet medical needs in RA? RA is a heterogeneous disorder with a fluctuating clinical course and an unpredictable prognosis. Although a large panel of very effective biotherapies is now available to clinicians, the most challenging issue remains: the identification of biomarkers for early disease diagnosis and to predict therapeutic outcome that would enable clinicians to treat RA patients as early as possible with the most optimal biologic therapy. Among molecules that are able to fulfill this requirement, miRNAs certainly represent a strong candidate. Numerous studies in cancer demonstrate that miRNAs represent highly specific and sensitive noninvasive biomarkers that are able to provide miRNA-
MicroRNAs IN RHEUMATOID ARTHRITIS

15

based fingerprints for disease diagnosis or to predict drug response. Correlations between miRNA expression levels and the development of malignancies, disease severity and aggressiveness, metastatic potential, therapeutic response, and survival rate have all been reported in various cancer types (35). In 2008, concomitant to the first evidence revealing the abnormal expression of specific miRNAs in RA tissue, the first reports demonstrating the presence and good stability of miRNAs in body fluids were published (36,37). Thereafter, the search for unique expression patterns of circulating miRNAs in RA patients for diagnostic/prognostic purposes became clinically realistic. However, very few groups of investigators are addressing this issue.

In only one study was investigation of the concentrations of miRNAs in plasma and synovial fluid as diagnostic markers for RA reported (29). Murata and colleagues showed that, among the 5 miRNAs investigated (miR-16, miR-132, miR-146a, miR-155, and miR-223), only low plasma levels of miR-132 can differentiate patients with RA from healthy control subjects. However, plasma miR-132 levels cannot discriminate between RA and OA, which means that none of the 5 miRNAs quantified is able to differentiate between RA and OA.

So far, no plasma miRNA has been formally identified as being specific for RA and thus useful for diagnosis. Murata et al did, however, reveal that plasma levels of miR-16, miR-146a, miR-155, and miR-223 are inversely correlated with disease activity (tender joint count and/or DAS28) and thus represent biomarkers for disease activity that might be useful for treatment follow-up. Those investigators showed that synovial fluid levels of miR-16, miR-146a, miR-155, and miR-223 can discriminate between patients with RA and those with OA. Although they suggested that the detection of these 4 miRNAs might be useful for RA diagnosis, further studies comparing these results with those for patients with other rheumatic diseases and healthy donors should first be conducted. Pauley et al suggested that analyzing miRNA expression in purified PBMCs may also be useful as a biomarker of disease activity, because low expression levels of miR-16 and miR-146a in PBMCs correlate with disease inactivity (DAS28 and tender joint counts) and vice versa, independently of age, ethnicity, or treatment (20).

High concentrations of cell-free miRNAs originating from the primary tumor have been observed in the plasma of cancer patients, and tumor-associated miRNomes appear highly tissue-specific. In RA, one could thus speculate that abnormal miRNA expression in plasma might reflect rheumatoid synovium, and that miRNAs specific for pathologic synovial tissue might be retrievable in the circulation. However, according to the in-depth study performed by Murata et al, there is no correlation between plasma miRNA concentrations and synovial fluid miRNA levels. Those investigators showed that miRNAs detected in synovial fluid and plasma have different origins, with the expression pattern of synovial fluid miRNAs, but not that of plasma, being similar to that of the miRNAs secreted by the synovial tissue. Further investigations are required to identify the joint-associated miRNome in the circulation of patients with RA. Yet, for prognostic purposes, a biomarker does not necessarily need to be disease specific. In addition, because RA is a systemic chronic inflammatory disorder in which organs other than joints may be affected, a blood-based miRNA signature is of great interest.

The circulation of miRNAs within microparticles confers high stability due to their resistance to drastic conditions and protection from endogenous RNase activity and allows their detection thanks to the expression of tissue-specific markers. This means that one can track the cellular origin of a miRNA-containing vesicle found in body fluids (38). Because blood is easily accessible, plasma or serum miRNA–based diagnostics is of great interest (39). Despite this, regardless of the disease studied, very few reports describe the use of miRNAs in serum or plasma as biomarkers. This is certainly attributable to technical hurdles relating to the extraction, detection, and quantification of the miRNAs in these fluids. Indeed, few miRNAs are detectable in serum or plasma, and they are often recovered at low concentrations, thus limiting their use for the establishment of solid profiling. Mainly for these technical reasons, whole-blood profiling should be preferred for future biomarker discovery programs, as opposed to plasma- or serum-based signatures alone.

Another way to unravel RA pathophysiology?
The pathogenesis of RA has not yet been fully elucidated. Because the abnormal expression of a dozen miRNAs has been reported in patients with RA, and because miRNAs are known to modulate several processes, their dysregulation would be expected to reflect their pathologic role, as for instance has been shown in cancer. As such, increasing interest surrounds investigating miRNAs as a novel pathway to shedding light on the molecular mechanisms involved in RA pathophysiologic processes. Investigating the role of miRNA dysregulation in RA is, however, not so simple, as each miRNA is thought to regulate multiple genes, and multiple
miRNAs may regulate a single mRNA through multiple target sites.

Following their discovery in *Caenorhabditis elegans*, miRNAs were thought to form part of a primitive immune system resulting from the race between host and pathogens (40). In accordance with this, plant and animal cells direct RNA interference against invading viruses to prevent or slow down viral replication. Conversely, some viruses have evolved to produce viral proteins or miRNAs that interfere with the host gene silencing machinery to attenuate antiviral defenses (41,42). More studies followed showing the contribution of miRNAs in development (embryology, tissue and cell differentiation), and diseases such as cancer, with most of the miRNAs studied being involved in the control of proliferation, apoptosis, or cell fate. To date, it is clear that miRNAs are involved in a broad range of biologic functions, and it is believed that those that are phlogenetically conserved are likely to be of regulatory importance in key cellular functions.

Some miRNAs are widely expressed, but others can exhibit tissue-, lineage-, or developmental stage–specific expression patterns. Because of the impact of miRNAs on the expression of protein-encoding genes, their abnormal expression in specific cell types during disease development should indicate certain gene pathways, or even cells, playing a detrimental role in specific stages of the disease of interest.

Monsef Benkirane (Institut de Génétique Humaine, Laboratoire de Virologie Moléculaire, Montpellier, France), an expert in immunology and virology, heads a laboratory aiming at understanding cellular factors involved in the regulation of human immunodeficiency virus 1 (HIV-1) gene expression (43). Recently, when Dr. Benkirane was asked, “Knowing that pathogen-associated molecular patterns are activated in inflamed RA tissues and that target prediction software predicts components of the Toll-like receptor pathways for miRNAs abnormally expressed in RA tissues, how could the intertwined race existing between host and pathogens that you showed for HIV-1 replication and the RNA interference [RNAi] pathway help us to understand RA etiology?” he responded as follows:

“Recent advances have revealed an implication of innate antiviral immunity in many autoimmune disorders. Antiviral immunity is triggered by innate sensors that induce inflammatory cytokine production required for the activation of protective innate and adaptive immunity. This response must be tightly regulated to avoid excessive inflammation. Many susceptibility genes are regulators of pathogen-sensing pathways and sensor activation is observed in many autoimmune disorders including RA, suggesting a role of antiviral innate responses in autoimmunity. However the implication of viral infection in the etiology of these disorders is unclear. While viral infection in a susceptible genetic background has been [shown] to induce inflammatory disease, aberrant activation of this innate sensor and inflammatory cytokine production in the absence of viral infection is also observed and results in autoimmune disorders that resemble viral infection.

Future work will have to define the role of the RNAi pathway or RNAi effectors in the regulation of endogenous retroelements and the metabolism of endogenous RNA that can trigger innate sensor activation and inflammation. Recent work has revealed an important role of the microRNAs in inflammation by targeting pathogens sensing signaling. Could viral infection-mediated deregulation of the RNAi pathway result in excessive innate immunity activation that could result in autoimmune pathology?”

Considering the dozen miRNAs dysregulated in RA tissues and the few mRNAs validated as their direct targets, hematopoietic activities clearly predominate, and most of the miRNAs concerned are involved in hematopoietic-related cancers (Figure 2). At least half of them have either high basal expression levels in tissues (such as miR-16 or miR-223) or play important roles in the inflammatory response (miR-132, miR-142-3p, miR-146a, miR-155, and miR-223), or both. Not surprisingly, most are also dysregulated in other types of immune-mediated inflammatory disorders (25,40), thus appearing to reflect a loss of homeostatic regulation of inflammatory events rather than an RA-specific pathogenic process. Indeed, several of the miRNAs found to be dysregulated in RA are common players in the homeostatic regulation of immune function and inflammation. They are induced by TIR activation in innate immune cells and target mRNAs encoding components of the TIR signaling system; thus, they represent components of a negative feedback loop (27).

Typically, miR-146a and miR-155 have been a particular focus for investigators, because both are induced by proinflammatory stimuli (IL-1β, tumor necrosis factor α, and Toll-like receptors); both are detected in synovial fibroblasts, T cells and B cells, as well as cells of myeloid lineage; and both have multiple targets and represent a link between innate and adaptive immunity. Numerous publications have demonstrated their critical
role in hematopoietic differentiation and function (44). As an example, miR-155 is the prototype of a multi-functional miRNA that controls the development of inflammatory Th1 and Th17 cells as well as FoxP3+/H11001 regulatory T cells, the IgG class switch in B cells, the maturation of dendritic cells, and the susceptibility of CD4+/H11001 T cells to natural Treg cell–mediated suppression (45). The second critical cellular function that is revealed when searching for the validated target genes of the miRNAs that are abnormally expressed in RA is apoptosis/proliferation. The miRNAs concerned (miR-16, miR-124a, miR-133, miR-155, miR-203, and miR-223) were observed to be dysregulated in synovial fibroblasts and in both circulating and infiltrating PBMCs known to play a role in the pathogenesis of RA due to an imbalance between proapoptotic and antiapoptotic factors (46). Other studies have implicated miRNAs in cancer; for example, miR-155 has been referred to as an oncomiR, and miR-223 (the prototype of myeloid-specific miRNA) is well documented in myeloid leukemia (47).

The identification of miRNAs playing a key causal role in RA pathogenesis would be expected to lead to the development of a novel efficient therapeutic strategy. Many more functional analyses would be initially required before planning to develop miRNA-based treatments.

**Novel therapeutic strategies?** Since miRNAs are a novel class of highly effective, highly specific, and sometimes even tissue- and developmental stage–specific regulators of gene expression, they represent very attractive candidates for the design of innovative therapeutic strategies in several important human disorders. Considering the dogma that miRNAs are important regulators not only for single genes but also for whole gene networks, they theoretically present enormous advantages over current drug design strategies. For clinical development, however, the use of miRNA-based therapeutics would first need to overcome several major hurdles, mainly those concerning targeted delivery and safety issues (48). In addition, several requirements should be met, as follows: the protection of pre/antago-miRNAs from serum degradation during transport; high delivery and uptake by target tissues and/or cells; and internalization of the vehicle with release of antago-miRNAs into the cytosol for direct access to the RNA interference pathway or with introduction of miRNA-expressing vectors into the nucleus for long-term expression.

Thus, although the in vivo targeting of miRNAs

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**Figure 2.** Comprehensive overview of the functional activities targeted by microRNAs that are dysregulated in rheumatoid arthritis. AC9 = adenylate cyclase 9; LMO2 = T cell translocation gene 2; E2F1 = E2F transcription factor 1; CEBP = cytoplasmic polyadenylation element binding protein 1; P300 = E1A binding protein p300; IL13Ra1 = interleukin-13 receptor α1; SOCS1 = suppressor of cytokine signaling 1; SHIP-1 = SH2 domain–containing inositol-5′-phosphatase; RIP1 = receptor-interacting protein 1; ZIC3 = zinc finger protein 203; HIVEP2 = human immunodeficiency virus type 1 enhancer binding protein 2; ZNF652 = zinc finger protein 652; ARID2 = AT-rich interactive domain 2; MCP1 = chemokine (C-C motif) ligand; CDK-2 = cyclin-dependent kinase 2; IRAK-1 = interleukin-1 receptor–associated kinase 1; TRAF6 = tumor necrosis factor receptor–associated factor 6; IRF-5 = interferon regulatory factor 5; FAF-1 = Fas-associated protein factor 1; FADD = Fas-associated death domain; PTC1 = patched homolog 1; Bmi1 = Bmi1 polycomb ring finger oncogene; CCND1 = cyclin D1; MYB = v-myb myeloblastosis viral oncogene homolog; PDCD4 = programmed death 4 protein; RASSF5 = Ras association domain–containing family member 5; DLK1 = delta-like 1 homolog; TIA1 = TIA1 cytotoxic granule-associated RNA binding protein; GSTP1 = glutathione S-transferase P1; TAGLN2 = transgelin 2; CAV1 = caveolin 1; FSCN1 = fascin homolog, actin-bundling protein 1; AKT-2 = v-akt murine thymoma viral oncogene homolog.
has been validated in many experimental models, mostly cancer, the greatest challenge now is to achieve effective inhibition or overexpression of specific miRNAs in targeted cell types and compartments without inducing side effects, using clinically applicable technologies. To overcome these hurdles, different vehicles have been, and are still being, developed by groups focusing on gene therapy delivery systems. The growing interest in miRNAs among rheumatology researchers, especially concerning their potential as novel therapeutic candidates, should energize the field of gene therapy in RA.

In 2008, Santaris Pharma initiated the first clinical trial aimed at targeting miRNA in vivo. In a double-blind, randomized, dose-escalation, phase I study, 48 healthy male volunteers were injected with miravirsen (SPC3649; Santaris Pharma), a locked nucleic acid (LNA)–based antisense molecule against miR-122 that was developed for the treatment of hepatitis C virus (HCV). This liver-specific miR-122 is essential for HCV replication and, as expected, antagonizing it by injecting LNA-modified oligonucleotides in chimpanzees with chronic infection down-regulated IFN-regulated genes and improved HCV-induced liver pathology (49), thus providing proof-of-concept for the development of a new type of antiviral intervention. In 2010, a phase IIa clinical trial with miravirsen was launched to assess its safety and tolerability in treatment-naive patients with chronic HCV infection.

Surprisingly, despite the fact that both preclinical and clinical studies based on the modulation of miRNA activity hold great promise for future drug development, only few publications report data supporting the therapeutic potential of miRNA-based treatment in RA. The first data concerning the role of miR-15a in a preclinical model of RA were published in 2009 (50). Using repetitive intraarticular injections of double-stranded miR-15a atelocollagen complex into the knee joints of mice with autoantibody-mediated arthritis, Nagata and colleagues showed that enforced local expression of miR-15a was able to induce synovial membrane cell apoptosis by negatively regulating the local expression of Bcl-2. However, because the investigators presented no data on the clinical effect of local miR-15a overexpression in experimental arthritis, it remains unclear as to whether this approach represents a valuable design strategy for treating RA.

In contrast, 2 recently published reports (51,52) do provide evidence for a miRNA-based therapeutic strategy in RA. Again, although they are not specific for RA, both miR-146a and miR-155 have been a particular focus for investigators, because they are induced by inflammatory conditions, are overexpressed in RA, and play a broad regulatory role in the immune response and inflammation. As was previously shown in the mouse model of experimental allergic encephalitis (51), mice deficient for miR-155 are protected from collagen-induced arthritis (CIA) and consequently show an impaired generation of pathogenic autoreactive T cells and B cells, as assessed by a marked reduction of an anticol-lagen antibody response and an impaired antigen-specific T cell response (52). However, using the K/BxN mouse model of arthritis that depends primarily on innate immune system cells and molecules, the lack of miR-155 expression only reduced bone erosion without impacting inflammation, thus suggesting a role of miR-155 in osteoclast differentiation.

The same observation (i.e., prevention of joint destruction but no effect on inflammation) was made when miR-146a was systemically overexpressed in mice with CIA (53). No information about the cell type(s) mediating the effect of the injected miR-146a atelocollagen was provided in that report. Importantly, considering that miR-146a is overexpressed in RA tissues and plays a key role in inflammatory responses, it would have been more logical to investigate the clinical effect of inhibiting miR-146a in mice with CIA rather than enforcing its expression. Overall, in both studies, modulation of the expression levels of miR-146a or miR-155 in experimental arthritis models proved that both miRNAs play important roles in disease pathogenesis; however, neither study convincingly proved that targeting these specific miRNAs would fulfill expectations for RA treatment.

More knowledge about the functional roles of other miRNAs is needed before miRNA-based therapeutics for the treatment of RA can undergo further development. One route that remains insufficiently studied and could provide an alternative strategy for modulating miRNA expression is the regulation of their transcription. Given that RA is a disease that affects predominantly female individuals, and that estrogens are shown to regulate miRNAs in the immune system, we should also consider sex hormone regulation of miRNAs in inflammatory responses.

**Conclusion**

Over the past 3 years, although a dozen miRNAs have been reported to be dysregulated in RA, most studies are descriptive, and in-depth functional analyses are still lacking. Only the very recent studies performed in animal models of preclinical arthritis have (or have
not) provided evidence that some miRNAs actively contribute to the molecular mechanisms underlying RA. Thus, although the identification, characterization, and modulation of miRNA expression in RA is a matter of great interest, little evidence exists supporting miRNAs as novel candidates for the development of immunomodulatory drugs. The potential of miRNAs is, however, promising, and rapid progress toward providing strong proof of concept in preclinical models for the use of miRNA-based therapeutic strategies in RA should now be expected. Nevertheless, as with all gene therapy strategies developed over the past few decades, the principal hurdles are likely to be more related to vector limitations than to the identification of a key miRNA in a specific cellular subset.

Overall, considering specificity and safety issues, the main challenge now remains the development of optimized vehicles for in vivo miRNA-based therapies that avoid off-target effects while providing efficient cell targeting, and increasing our understanding of the cellular functions of dysregulated miRNAs.

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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