Published in final edited form as:

Nat Rev Rheumatol. 2010 July ; 6(7): 391–398. doi:10.1038/nrrheum.2010.81.

MicroRNAs as biomarkers in rheumatic diseases

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Summary

MicroRNAs (miRNAs) are endogenous, noncoding, single-stranded RNAs of 19–25 nucleotides in length. They regulate gene expression and are important in a wide range of physiological and pathological processes. MiRNAs are attractive as potential biomarkers because their expression pattern is reflective of the underlying pathophysiologic processes and they are specific to various disease states. Moreover, miRNAs can be detected in a variety of sources, including tissue, blood and body fluids; they are reasonably stable and appear to be resistant to differences in sample handling, which increases their appeal as practical biomarkers. The clinical utility of miRNAs as diagnostic or prognostic biomarkers has been demonstrated in various malignancies and a few nonmalignant diseases. There is accumulating evidence that miRNAs have an important role in systemic rheumatic diseases and that various diseases or different stages of the same disease are associated with distinct miRNA expression profiles. Preliminary data suggest that miRNAs are promising as candidate biomarkers of diagnosis, prognosis, disease activity and severity in autoimmune diseases. MiRNAs identified as potential biomarkers in pilot studies should be validated in larger studies specifically designed for biomarker validation.

Introduction

MicroRNAs (miRNAs), which are endogenous noncoding RNAs of 19–25 nucleotides in length, are involved in the regulation of gene expression and are important in a wide range of physiological and pathological processes¹. The first gene encoding an miRNA (*lin-4*) was discovered in 1993 in *Caenorhabditis elegans*² and was shown to negatively regulate LIN-14 protein expression at a specific time during development. Cloning of *lin-4* revealed the presence of a 22 nucleotide sequence, complementary to a 3'-untranslated region (UTR) repeated sequence element of the *lin-4* mRNA transcript, and led to the hypothesis that short nucleic acid sequences control protein expression by an antisense RNA–RNA interaction². The term miRNA was not introduced until 2001 with the simultaneous publication of three related articles^{3–5}. Since then, an exponentially increasing number of publications have shed light on various aspects of miRNAs, ranging from their biogenesis to their functional roles in physiological and disease processes. In this article, we will briefly describe the biogenesis and functions of miRNAs and review recent advances in our understanding of their role in immunity. We will summarize the accumulated clinical evidence of their potential use as biomarkers in rheumatic diseases and discuss their future potential, based on the available data for miRNA biomarkers in nonrheumatic conditions.

Biogenesis of microRNAs

The first step in the biogenesis of mammalian miRNAs is the generation of primary miRNA transcripts (pri-miRNAs) usually transcribed by RNA polymerase II⁶ (Figure 1). Pri-miRNAs are of variable sizes and can be tens of thousands of nucleotides long⁷. Pri-miRNAs are cleaved in the nucleus to hairpin pre-miRNAs (typically 70–100 nucleotides in length)⁸ and transported to the cytoplasm⁹ for further processing to become double stranded RNAs of 19–25 nucleotides long. One strand is then loaded into a protein complex called RISC (RNA-induced silencing complex)^{1,10} which facilitate the binding of miRNAs to their mRNA targets. MiRNA binding can lead either to mRNA degradation or to repression of translation; degradation occurs due to mRNA deadenylation and destabilization, and repression of translation occurs during protein synthesis either at the initiation or post-initiation phase¹¹. It should be noted that one miRNA can control the translation of hundreds of genes simultaneously having an important role in fine tuning protein expression^{12, 13}

Mechanisms of action of microRNAs

As the first miRNAs to be discovered (*lin-4* and *let-7*) were shown to exert their effect on the 3'UTR of target mRNAs, it was widely believed that miRNAs exerted their effects through a perfect or imperfect complementarity with sequences in the 3'UTR only. The imperfect complementarity required perfect target matching of the 2nd through the 7th nucleotides (seed sequence) starting from the 5' end of the miRNA. Primarily through the seminal work of the Rigoutsos group¹⁴, however, it was realized that miRNAs can also target sequences within the 5'UTR and the coding sequences of mRNAs. This has been confirmed by research showing that genes involved in embryonic stem cell differentiation in mice (*Nanog*, *Oct4*, and *Sox2*) are downregulated by miRNAs targeting their coding sequence¹⁵. Similarly, miRNA-148 targets the coding sequence of DNA methyltransferase-3b, an enzyme important for the *de novo* methylation of DNA¹⁶. There is also evidence that miRNAs might target splice variants of specific genes, as exon–exon junctions have been shown to be specific miRNA targets¹⁵. The implications of such a possibility are stunning and will add another layer of complexity to the regulation of expression of specific isoforms within a system.

Functions of microRNAs

MiRNAs have been implicated in development, physiological functions and disease processes. Embryonic stem cell fate has been shown to be tightly regulated by the temporal expression of specific miRNAs^{15, 17–19}. Skin morphogenesis²⁰, pancreas development²¹, muscle differentiation²², cardiac growth^{23, 24} and neural development²⁵ are all processes in which miRNAs have been implicated. A large number of malignancies have been associated with altered miRNA expressions^{26, 27}. Other nonmalignant diseases where miRNAs have been implicated include Alzheimer's disease²⁸, neuropsychiatric disorders²⁹, viral infections such as hepatitis³⁰ and AIDS³¹, primary biliary cirrhosis³² and rheumatic diseases including systemic lupus erythematosus (SLE)^{33–35} and rheumatoid arthritis (RA)^{36–39}.

Roles in the immune system

As master regulators of gene expression, miRNAs are instrumental in regulating immune system development, normal immune function and autoimmunity. Expression profiles of hematopoietic cells have identified a number of miRNAs that are differentially expressed in subsets of hematopoietic cells, the expression of which is tightly regulated during hematopoiesis and lineage differentiation⁴⁰. There are several reviews on the role of miRNAs in immunity, and in autoimmune and rheumatic diseases,^{41–44} therefore in this

article we will focus on summarizing some new developments relating to the potential use of miRNAs as biomarkers.

The immune response in autoimmunity is characterized by increased immune cell activation and failed or inefficient immune regulation, with type 17 T helper (T_H17) cells promoting autoimmunity and regulatory T (T_{REG}) cells exerting a protective effect. Most likely it is a functional imbalance of these subtypes that leads to pathologic responses in autoimmune diseases⁴⁵. The fundamental role of miRNAs in regulating these cells has been shown in several studies. MiR-326 was identified in lymphocytes as a miRNA associated with polarization towards the T_H17 cell lineage; the expression of this miRNA correlated with disease activity and severity in multiple sclerosis and experimental autoimmune encephalomyelitis⁴⁶. In contrast, mice unable to generate miRNAs in their T_{REG} cells develop severe autoimmune disease similar to mice deficient for T_{REG} cells, due to their inability to suppress activated T cells under inflammatory conditions⁴⁷. MiR-155 has been shown to be important for maintenance of suppressor cell activity⁴⁸. Interestingly miR-155 also downregulates lipopolysaccharide-induced inflammatory pathways in human monocyte-derived dendritic cells⁴⁹ and together with miR-181b, suppresses activation-induced cytidine deaminase expression, which is required for immunoglobulin gene diversification by B cells^{50, 51}. Adaptive B cell immunity is required for naïve B cells to differentiate into effector B cells, such as memory and plasma cells. Two papers, using slightly different approaches, described the role of known and hitherto unknown miRNAs in B cell differentiation^{52, 53}. In one of these studies miRNA signatures of various lymphomas were analyzed and were shown to be predictive of lineage origin of lymphomas in over 95% of cases⁵³.

Regulation of microRNAs

Aberrant expression of miRNAs can be caused by chromosomal abnormalities, insertion of foreign genetic material such as viral genomes, mutations or single nucleotide polymorphisms (SNPs), as well as epigenetic changes, and defects in miRNA biogenesis pathways²⁶. Sequence homology profiling of approximately 250 SNPs, which were unequivocally associated with 15 common human disorders, identified 72 SNPs and 18 miRNAs with an apparent propensity to target mRNA sequences derived from a single protein-coding gene, *KPNA1* (karyopherin alpha 1, also known as importin alpha 5), a component of the nuclear import pathway. Each miRNA in this elite set appeared linked to at least three common human diseases and had potential protein-coding mRNA targets among the principal components of the nuclear import pathway⁵⁴.

Another characteristic of miRNAs is the presence of mutations and polymorphisms not only in the sequences of the mature miRNAs⁵⁵ but also in the sequences of the pri-miRNAs and the pre-miRNAs⁵⁶. Those sequence changes can affect the biogenesis of the miRNAs or the action of mature miRNAs on target sequences. Variations in both the 5' and 3' end of the mature miRNAs, which lead to variations in the length of miRNAs, have also been identified by sequencing⁵⁷. This has an important implication for the use of miRNAs as biomarkers since the assays used should clearly discriminate between those miRNAs with polymorphisms and those without to allow the precise measurements of the levels of the mature miRNAs. A SNP of the pre-miR-146a has been shown to modify the proapoptotic function of miR-146a, by decreasing its expression levels, thus decreasing the well established inhibition of miR-146a target genes such as *IRAK1* (encodes interleukin-1 receptor-associated kinase 1) and *TRAF6* (encodes TNF-receptor-associated factor 6)⁵⁸. This polymorphism possibly contributes to the genetic predisposition to papillary thyroid carcinoma⁵⁹ and is associated with the risk for hepatocellular carcinoma⁶⁰. The study of

associations between polymorphic or mutated miRNAs and disease is at an early stage, but it seems promising.

Biomarkers in rheumatic diseases

Rheumatology encompasses a wide variety of diseases ranging from those affecting primarily the joints, such as osteoarthritis, gout and the inflammatory arthritides to systemic diseases, such as SLE, vasculitis or systemic sclerosis, which typically affect multiple organs. Many of these diseases have a heterogeneous and often unpredictable clinical course. Despite this heterogeneity some features, such as systemic inflammation, are common to many diseases, whereas others, such as the formation of autoantibodies, are restricted to specific conditions or a subset of patients with a certain disease.

A biomarker is as a physical sign or cellular, biochemical, molecular or genetic alteration by which a normal or abnormal biologic process can be recognized or monitored, or both, and that might have diagnostic or prognostic utility⁶¹. Biomarkers have several potential applications in rheumatic diseases (Table 1). Genetic markers can predict or quantify the risk or the severity of diseases in populations or individuals. Classical examples include the increased risk of ankylosing spondylitis in carriers of the *HLA-B27* allele⁶² and the association between the ‘shared epitope’ and RA⁶³. An increasing number of genetic polymorphisms have been identified as risk factors for autoimmune diseases in general^{64, 65}, as well as for specific diseases⁶⁶. Autoantibodies are frequently used to establish or confirm a diagnosis. Some, such as anti-cyclic citrullinated peptide (CCP) antibodies in RA, are fairly specific for a particular disease⁶⁷ whereas others, such as antinuclear antibodies and anti-Ro (SSA) antibodies are present, albeit at different frequencies and levels, in a number of conditions⁶⁸. Once a diagnosis is established, some biomarkers provide prognostic information regarding disease progression and severity. For example, the combination of anti-CCP positivity and the presence of the shared epitope defines a subset of patients with a severe form of RA^{69, 70}, whereas the presence of specific autoantibodies define distinct subsets of inflammatory myopathies⁷¹. Other biomarkers are used to monitor the degree of immunologic activity or inflammation. Measures of levels of complement activation and of anti-double stranded DNA autoantibodies are commonly used to monitor disease activity in lupus nephritis⁷², and nonspecific markers of inflammation, such as the erythrocyte sedimentation rate or C-reactive protein levels, are measured in many diseases. Some of the biggest challenges, especially in the late stages of most chronic rheumatic diseases, include distinguishing between ongoing inflammation and irreversible organ damage, and assessing the impact of comorbidities or the side effects of treatments. Probably most needed are biomarkers that predict response to a particular therapy. Such markers could be used to optimize the risk to benefit ratio of a treatment in individual patients. If a strong correlation between a biomarker and a change in clinical activity can be established, a biomarker could act as a surrogate marker of a clinically important endpoint. Monitoring that surrogate endpoint could permit the use of targeted preemptive therapy, if the measurement predicts relapse, or could be used as a guide to discontinue therapy, if it denotes remission⁶¹.

MicroRNAs as biomarkers

An ideal biomarker should be measurable in a reproducible way, have high sensitivity and specificity for the clinical outcome of interest, and should reflect an important pathogenetic process⁶¹. MiRNAs are exciting as potential biomarkers because they fulfill many of these criteria (Figure 2 and Table 2). There are established technologies for measuring miRNA levels reliably: global miRNA expression profiles can be determined by miRNA microarray analysis using arrays containing all known human and many viral miRNA sequences,

whereas selected miRNAs are best measured by quantitative PCR⁷³. MiRNAs have been shown to have the specificity and sensitivity required for clinical applications in some diseases⁷⁴

Similar to mRNA or protein expression profiles, miRNAs levels reflect the underlying physiological state of cells and tissues, but have several advantages over mRNAs or proteins as biomarkers. A single mRNA is usually translated into a single protein; however, a single miRNA is capable of regulating the translation of a multitude of genes; therefore, it is expected that compared with mRNA expression analysis a limited number of miRNAs can be used as biomarkers so reducing the complexity of global gene expression analysis. Importantly, miRNAs appear to be long lived *in vivo*,⁷⁵ and, in comparison with mRNAs, are stable⁷⁶ and relatively resistant to degradation by nucleases⁷⁷ *in vitro*. This stability, which is probably due to their small size and protection in miRNA–protein complexes either intracellularly or in microvesicles such as exosomes in blood and other biologic fluids⁷⁸, allows them to be detected in clinical specimens from which mRNA isolation is hampered because of the sensitivity of mRNA to degradation. In fact, miRNAs can be measured reproducibly from various sources, such as blood components, biologic fluids (urine⁷⁹, saliva⁸⁰) and tissue samples⁸¹. The presence of miRNAs in biological fluids dramatically increases their potential biomarker applications, and means that invasive procedures might eventually be unnecessary. Good correlations between serum and tissue miRNA profiles have already been shown for several cancers^[Chen, 2008 #205, 82, 83]. Furthermore, in contrast to mRNAs, miRNAs can be readily detected in formalin fixed paraffin embedded (FFPE) samples by microarray profiling or *in situ* hybridization. Formalin has been shown to crosslink nucleic acids that are larger than the mature miRNAs and causes chemical modification to the nucleic acids making their detection difficult. In contrast, due to their small size miRNAs, are modified to a lesser extent than mRNAs and can readily be amplified from formalin fixed tissues⁸⁴. Importantly, the expression levels of miRNAs isolated from frozen and from FFPE samples correlate closely with each other⁸⁵. Arguably, using existing paraffin samples, which are a source of well preserved miRNAs, with their associated clinical information, such as disease outcome might lead to expedited biomarker discovery.

MicroRNAs in rheumatic diseases

Rapidly accumulating evidence supports a central role for miRNAs in normal and abnormal immune processes, but the exploration of their role in rheumatic diseases is in its infancy. A few studies have been published about the differential expression of miRNAs and the role they might have in the etiology of rheumatic diseases, which we will discuss here; the potential of miRNAs as biomarkers in rheumatic diseases is a new area of research for which currently available data are limited (Table 1).

Rheumatoid arthritis

Several reports have described altered miRNA expression in the synovium of patients with RA. Lipopolysaccharide-activated RA fibroblast-like synoviocytes (FLS) overexpressed miR-346, which has been shown to negatively regulate the interleukin (IL)-18 response of these cells³⁷. In comparison with FLS from patients with osteoarthritis (OA), RA FLS expressed a considerably lower level of miR-124a, which was identified as a key player in the regulation of proliferation and chemokine production by RA FLS³⁶. MiR-146a has been shown to be highly expressed in human RA synovial tissue and its expression is induced by the proinflammatory cytokines tumor necrosis factor and IL-1 β ³⁹. Interestingly, the cells with the highest levels of miR-146a were CD68⁺ macrophages, CD3⁺ T cells and CD79a⁺ B cells in the superficial and sublining layers of the synovium³⁹. Levels of miR-146, miR-155 and miR-16 were found to be upregulated in the peripheral blood of patients with RA, and

miR-146 and miR-16 levels were higher in patients with active ($n=8$) rather than inactive ($n=3$) disease,³⁸ suggesting that these miRNAs could be potential markers of disease activity if these results are confirmed in a larger number of patients.

Osteoarthritis

As mentioned, MiR-146a has been detected in the synovium and FLS of patients with OA, albeit at a lower level than seen in patients with RA⁸⁶. Levels of this miRNA were considerably higher in OA cartilage in comparison with normal cartilage, although the levels decreased with the severity of cartilage degeneration⁸⁷. The levels of miR146-a correlated inversely with the levels of matrix metalloproteinase-13, which is known to have a pathogenic role in the cartilage destruction in OA, but a direct causal relationship between the two molecules was not demonstrated. Therefore, the function of miR-146 in the pathogenesis of OA remains unclear.

Systemic lupus erythematosus

There have been three main studies of miRNA expression profiles in patients with SLE. In the first study, peripheral blood mononuclear cell (PBMC) miRNA profiles in healthy controls ($n=10$) and patients with SLE ($n=23$) or idiopathic thrombocytopenic purpura (ITP, $n=10$) were compared. In comparison with the controls, 16 of the 331 human miRNAs tested were differentially expressed (7 were downregulated and 9 upregulated) in SLE³⁵, whereas 19 miRNAs were differentially expressed in ITP (14 downregulated and 5 upregulated). From the differentially expressed miRNAs, 13 had the same expression pattern in SLE and ITP, 6 were downregulated in ITP only, and 3 (miR-184, miR-198 and miR-21) were up or downregulated in SLE with no change in ITP. When comparing SLE patients with active (SLEDAI ≤ 12) and inactive (SLEDAI ≥ 15) disease, eight miRNAs were downregulated in the more active group; interestingly, no miRNA was expressed at higher levels in the active disease group. Unfortunately, there are no longitudinal data to assess how well these miRNAs correlate with disease activity in individuals over time. Using a cutoff of two-fold change in a second study, the same group identified 36 miRNAs which were upregulated and 30 which were downregulated in the kidneys of patients with WHO Class II lupus nephritis compared with normal controls³⁴.

The third study found that, of 156 miRNAs analyzed, 42 were differentially expressed between controls and SLE patients, with 7 being more than 6-fold lower in SLE. Since type I Interferon (IFN) is known to have a role in the etiology of SLE, the authors explored the association between the activation of the type I IFN pathway and the altered expression of miR-146a, which has been reported to negatively regulate the innate immune system. Overexpression of miR-146a in primary PBMCs resulted in inhibition of Toll-like receptor-7 mediated IFN α and IFN β production. In addition, transfection with synthetic miRNA-146a hairpin inhibitor to decrease endogenous miRNA expression led to increased type I IFN production. The study also suggested that miR-146a expression negatively correlated with SLEDAI and renal SLEDAI scores³³; however, the correlation was rather weak (r values -0.28 and -0.38 , respectively).

Sjögren's syndrome

We have generated miRNA signatures from the minor salivary glands of patients with Sjögren's syndrome and normal controls; analysis of these expression profiles makes it possible to distinguish between these two populations as well as between subsets of patients with Sjögren's syndrome with low or high grade inflammation⁸⁸. We have also explored the presence of miRNAs in exosomes isolated from parotid and submandibular saliva of Sjögren's syndrome patients. Preliminary results have been published and showed that miRNAs can be identified in saliva, which is of interest as this means it could be possible to

obtain information from this target organ without the need for invasive methods such as biopsies⁷⁸.

MicroRNA biomarkers in other diseases

Malignancies

The study of miRNA biomarkers is most advanced in oncology. The data accumulated to date demonstrate the enormous potential of this field. Initial reports showed that cancer cells and tissues have different miRNA profiles than normal cells and tissues, suggesting that miRNA profiles could be used for the diagnosis of cancer²⁷. These were followed by larger studies demonstrating that miRNA profiles can distinguish between normal and malignant tissues, including breast^{89, 90}, lung^{91, 92}, liver^{60, 93, 94}, pancreas^{95, 96} and colon⁹⁷ cancers and lymphomas^{98–101}. In addition to diagnosis, miRNA analysis has also been tested as a tool for the early detection of cancer or cancer recurrence, cancer classification and also as a marker of prognosis or response to therapy. Expression profiling of 217 miRNAs successfully identified the origin of 12/17 poorly differentiated tumors of unknown origin, whereas simultaneous profiling of 16,000 mRNAs did not accurately classify the tumors¹⁰². In another study, hierarchical clustering of miRNA expression data from 540 samples from the 6 most frequent human cancers, miRNA successfully identified their tissue origin, thus demonstrating the specificity of miRNA profiling⁹⁰.

There is emerging evidence that miRNAs can be used as prognostic markers. In chronic lymphocytic leukemia, a unique signature of 13 miRNAs distinguished between patients with good or poor prognoses,¹⁰³ and in breast cancer differentially expressed miRNAs were associated with invasive pathology⁸⁹ and were identified as independent predictors of survival⁹². MiRNA expression pattern analysis was validated in a large study specifically designed to test miRNAs as prognostic markers in colon adenocarcinomas⁹⁷: high levels of miR-21 were associated with a poor therapeutic outcome and with poor survival in both the training (hazard ratio, 2.5; 95% confidence interval, 1.2–5.2) and validation cohorts (hazard ratio, 2.4; 95% confidence interval, 1.4–3.9), independent of clinical covariates, including tumor staging⁹⁷. Using a similar approach, hsa-miR-205 was identified as a marker for squamous cell lung carcinoma, with a sensitivity of 96% and specificity of 90%⁸¹.

The first commercial miRNA-based diagnostic assays in oncology have recently become available in the US. Based on the high discriminating power of miRNAs, these assays can be used to differentiate between malignant pleural mesotheliomas, peripheral adenocarcinoma of the lung, and metastatic carcinomas involving the lung and pleura, or to subclassify nonsmall cell lung carcinomas into squamous and nonsquamous histology subtypes¹⁰⁴. In addition, by simultaneously measuring the levels of 48 miRNAs, these assays allow for the identification of the tissue of origin of metastatic cancers of unknown origin. In this context, elevated levels of various serum miRNAs have been detected in prostate⁸³, ovarian¹⁰⁵ and lung^{77, 91, 106–108} cancers in comparison with normal serum.

Several papers have described the use of circulating exosomal miRNAs as biomarkers in cancer. Exosomes are small membrane-bound vesicles of endocytic origin released by cells, which are an important source of cell-free miRNA in serum and other bodily fluids such as urine⁷⁹ and saliva⁷⁸. Comparisons between miRNAs derived from circulating exosomes and miRNA derived from tumors indicated that the miRNA signatures from these two sources were not considerably different¹⁰⁷. If this is confirmed, it is possible that exosomal miRNAs from body fluids could be analyzed instead of miRNAs from tissue or tumor samples, so again meaning that invasive procedures could be avoided.

Transplantation

Rejection of organ transplants is an immune-mediated process and in many respects at the cellular and molecular level resembles inflammatory rheumatic diseases. A study in patients with acute kidney rejection, showed that renal allograft function could be predicted with a high level of precision using intragraft levels of miRNAs as an indicator¹⁰⁹.

Multiple sclerosis

In patients with multiple sclerosis (MS), peripheral blood levels of miRNA-326 have been shown to be considerably higher in patients with the relapsing remitting form of the disease than in healthy controls⁴⁶ or patients with neuromyelitis optica, another demyelinating disease with overlapping clinical features but a different etiology to MS⁴⁶. The levels of miRNA-326 were high in patients in the relapsing but not in the remitting phase of MS, and was due to an increased expression of this miRNA-326 in T_H17 cells⁴⁶.

Conclusions

MiRNAs possess many features of an ideal biomarker and, therefore, they represent a novel group of biomarker candidates for rheumatic diseases (Figure 2). Some MiRNAs have already been validated as diagnostic or prognostic biomarkers in various malignant diseases demonstrating their clinical utility. Given the central role of miRNAs in the regulation of physiologic and pathologic processes, we can expect an exponential increase in publications about miRNAs in various rheumatic diseases. Some of these miRNAs will undoubtedly be associated with distinct clinical characteristics, such as diagnosis or disease activity, making them interesting candidates for further evaluation and testing in larger studies specifically designed to validate them as biomarkers.

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Biographies

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Figure 1. Biogenesis of mammalian microRNAs

The first step entails the generation of pri-miRNAs, usually transcribed by RNA polymerase II. Pri-miRNAs are cleaved in the nucleus to hairpin pre-miRNAs (typically 70–100 nucleotides) by Drosha, which is part of two multiprotein complexes including RNA helicases, heterogeneous nuclear ribonucleoproteins and a double-stranded RNA-binding protein⁸. Pre-miRNAs are then actively transported to the cytoplasm by the nuclear pore exportin-5⁹ for further processing by the cytoplasmic endonuclease Dicer, which, in collaboration with RNA binding proteins, cleaves both strands of the pre-miRNA hairpin duplex generating a double stranded RNA, 19–25 nucleotides long. One strand is then loaded into a protein complex called RISC¹, which is a ribonucleoprotein complex consisting of an RNA helicase A and proteins including Argonaute-2 and TRBP,¹⁰ which facilitate the binding of miRNAs to their mRNA targets. MiRNA binding can lead either to mRNA degradation or to repression of translation.

Abbreviations: pri-miRNAs, primary miRNA transcripts; RISC, RNA-induced silencing complex; TRBP, trans-activation-response RNA-binding protein.

Figure 2. MicroRNA biomarkers in rheumatic diseases

Genetic alterations in the genes encoding miRNAs, their processing machinery or their binding sites could predispose to an autoimmune response or end-stage organ damage, or both. Similarly, various environmental exposures are associated with specific miRNA expression patterns that might contribute to the pathogenesis of autoimmune diseases. MiRNAs can be detected in a wide range of readily accessible biologic sources. Different microarray platforms or sequencing can be used to analyze global miRNA expression patterns, whereas real-time PCR and *in situ* hybridization allows for the targeted detection of selected miRNAs. The central role miRNAs have in the regulation of physiologic and pathologic processes, combined with their relative stability and the availability of technology for their detection, makes them promising biomarker candidates for diagnosis, prognosis and disease activity in autoimmune diseases. In addition, studying the biologic impact of differences in miRNA expression between controls and various disease states will improve our insight of the pathogenesis of these conditions.

Abbreviation: FFPE, formalin fixed paraffin embedded

Table 1

Potential application of microRNA biomarkers in rheumatic diseases

Application	Potential source of miRNA	Studied/validated as biomarker	
		Rheumatic disease	Nonrheumatic disease
Susceptibility or prognosis	DNA	Yes/No	Yes/No
Diagnosis	Tissue [*] , blood, urine, synovial fluid, CSF, saliva, exosomes	Yes/No	Yes/Yes
Severity or prognosis		No/No	Yes/Yes
Inflammatory activity		Yes/No	Yes/No
End-stage organ damage		No/No	No/No

* Fresh, frozen or formalin-fixed.

Abbreviation: CSF, cerebrospinal fluid.

Table 2

Characteristics of microRNAs as biomarkers

Characteristic	Features	Potential advantage
Stability	MiRNAs are relatively resistant to nucleases and have been isolated from various sources including some, such as saliva ⁸⁰ , where mRNA stability is low ⁴ Can be isolated and evaluated from FFPE samples ¹¹⁰	Withstands differences in sample processing – increased clinical utility Potential for retrospective studies on large number of samples
Tissue specificity	MiRNA expression profiles are specific to tissue origin ¹¹¹ Exosomal miRNA might reflect tissue of origin	Distinguish between inflammation and organ damage “Remote diagnosis” of tissue pathology Replacement of biopsy
Representative of physiology	MiRNAs have been associated with specific physiological and pathological states Many physiological states are under the control of few miRNAs	Decreased complexity compared with mRNA and/or protein expression
Discriminatory power	In cancer studies miRNA-based classifiers performed better than mRNA-based classifiers in identifying the tissue of origin of poorly differentiated tumors with nondiagnostic histologic appearance ¹⁰²	Increased diagnostic accuracy Distinguish between active disease and organ damage

Abbreviations: FFPE, formalin fixed paraffin embedded; miRNA, microRNA