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REVIEW ARTICLE

Is there a potential of circulating miRNAs as biomarkers in rheumatic diseases?

Klára Prajzlerová [a,*], Ladislav Šenolt [a,b], Mária Filková [a,b]

[a] *Institute of Rheumatology, Prague 12800, Czech Republic*
[b] *Department of Rheumatology, First Faculty of Medicine, Charles University, Prague 12800, Czech Republic*

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Abstract MicroRNAs (miRNAs) are small non-coding single-stranded RNAs of about 22 nucleotides in length that act as post-transcriptional regulators of gene expression. Depending on the complementarity between miRNA and target mRNA, cleavage, destabilization, or translational suppression of mRNA occurs within the RISC (RNA-induced silencing complex). As gene expression regulators, miRNAs are involved in a variety of biological functions. Dysregulation of miRNAs and their target genes contribute to the pathophysiology of many diseases, including autoimmune and inflammatory disorders. MiRNAs are also present extracellularly in their stable form in body fluids. Their incorporation into membrane vesicles or protein complexes with Ago2, HDL, or nucleophosmin 1 protects them against RNases. Cell-free miRNAs can be delivered to another cell *in vitro* and maintain their functional potential. Therefore, miRNAs can be considered mediators of intercellular communication. The remarkable stability of cell-free miRNAs and their accessibility in body fluid makes them potential diagnostic or prognostic biomarkers and potential therapeutic targets. Here we provide an overview of the potential role of circulating miRNAs as biomarkers of disease activity, therapeutic response, or diagnosis in rheumatic diseases. Many circulating miRNAs reflect their involvement in the pathogenesis, while for plenty, their pathogenetic mechanisms remain to be explored. Several miRNAs described as biomarkers were also shown to be of therapeutic potential, and some miRNAs are already tested in clinical trials.

* Corresponding author. Institute of Rheumatology, Na Slupi 4, Prague 12800, Czech Republic.
E-mail address: prajzlerova@revma.cz (K. Prajzlerová).
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Introduction

In 1993 Lee et al studied a gene *lin-4*, which regulates the postembryonic development of *Caenorhabditis elegans (C. elegans)*. They found that *lin-4* encodes small RNA with antisense complementarity to mRNA of gene *lin-14*, suggesting that *lin-4* regulates *lin-14* translation during the RNA—RNA interaction and thus prevents the larval development of *C. elegans*.[1] In the same model, another gene, *let-7*, was later described, which codes 21-nucleotide RNA regulating several genes involved in developmental timing in *C. elegans*.[2] These small RNAs were further named microRNAs (miRNAs).[3]

MiRNAs are small, non-coding RNAs about 22 nucleotides long that function as post-transcriptional regulators of gene expression. They belong to small non-coding RNAs involved in RNA interference.[4] Each miRNA can regulate several genes, and it is estimated that more than 50% of human genes are under selective pressure to maintain pairing to miRNAs.[5] Dysregulation of miRNAs and their target genes contribute to the physiological regulations and pathophysiology of many diseases.

MiRNAs are found not only intracellularly but also extracellularly in the body fluids. The remarkable stability of these extracellular (circulating) miRNAs and their easy accessibility from body fluids make them potential diagnostic or prognostic biomarkers of several diseases, including autoimmune disorders.[6] In recent years, miRNA-targeted therapeutics have been studied to manage cancer and other diseases.[7]

In this review, we provide an overview of the biogenesis of miRNAs, the secretory mechanisms of the circulating miRNAs, and the potential use of circulating miRNAs as biomarkers for rheumatic diseases.

Biogenesis of intracellular miRNAs

The biogenesis of miRNA is a complex process involving several steps (Fig. 1). Genes for miRNAs are primarily found in non-coding regions but may also be found in intron or exon regions of other protein-coding genes.[8,9] The intron gene for miRNA is often found in the same primary transcript as its target gene. The total amount of *miRNA* genes varies between chromosomes and does not correlate with their length.[10] In the nucleus, independent *miRNA* genes are transcribed by RNA polymerase II. The resulting primary hairpin-shaped transcript (pri-miRNA), up to 1000 nucleotides long, is capped with a specially modified nucleotide containing 7-methylguanosine triphosphate at the 5′ end as well as poly(A) tail at the 3′ end.[11] A minor group of miRNAs associated with Alu (*Arthrobacterus luteus*) repeats can be

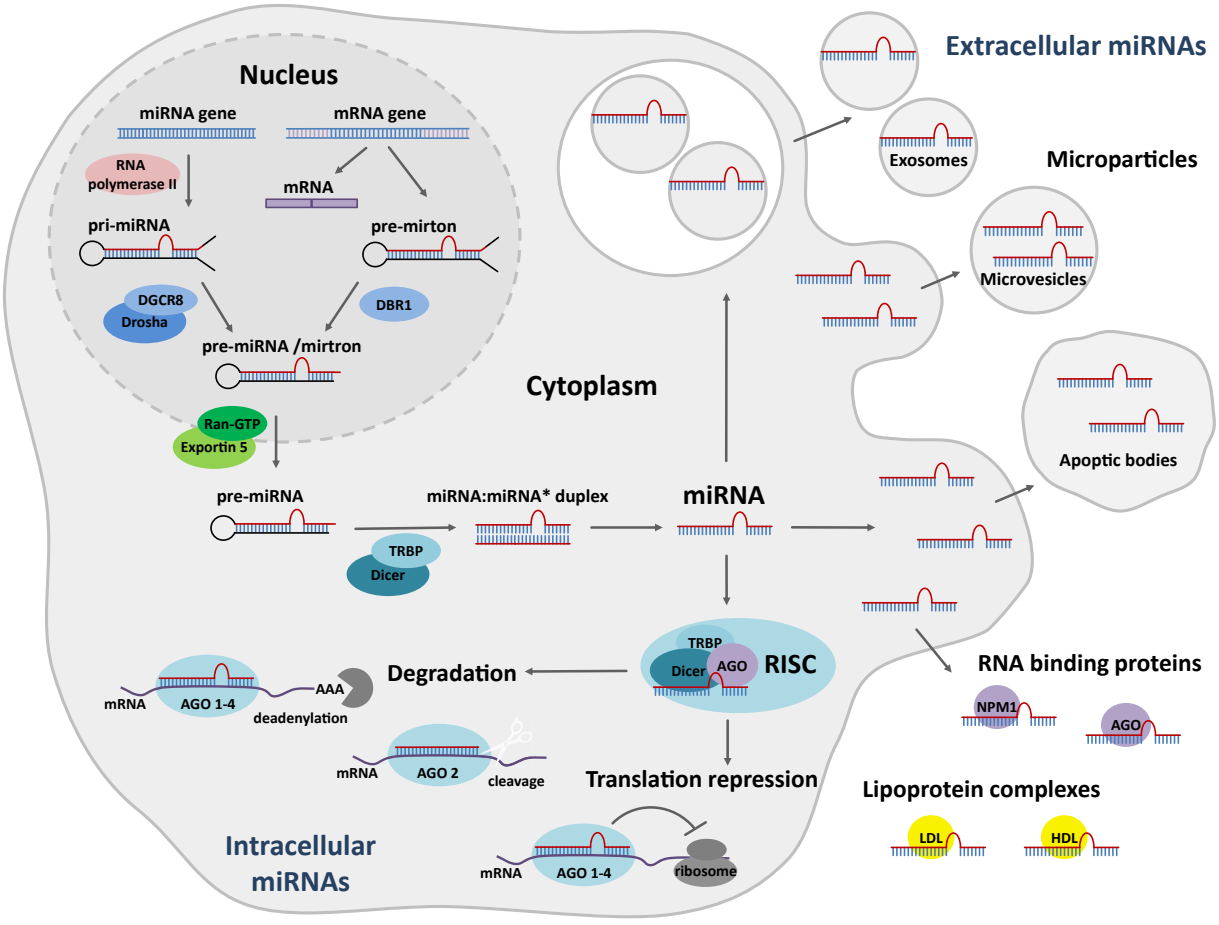

Figure 1 Biogenesis of intracellular miRNAs and secretory mechanisms of extracellular (circulating) miRNAs.

transcribed by RNA polymerase III.[12] A single pri-miRNA often contains sequences for several different miRNAs, so-called clusters. About 25% of the miRNA genes are stored in these clusters.[8] In the next step of miRNA maturation, the pri-miRNA is cleaved by RNase III-type nuclease Drosha and cofactor DGCR8 (the DiGeorge syndrome critical region gene 8) protein,[13] forming the microprocessor complex.[14] DGCR8 binds directly to a double-stranded region of pri-miRNA. It determines where the nuclease Drosha cleavage occurs and processes pri-miRNAs to 70 nucleotide hairpins known as pre-miRNAs with a 2–3 nucleotide overlap on its 3′ end.[13] Pre-miRNA hairpins are exported to the cytoplasm by the Exportin-5, which recognizes and binds a two-nucleotide overhang at the 3′ end of the pre-miRNA hairpin. Binding occurs only in the presence of the Ran-GTP cofactor. In the cytoplasm, pre-miRNAs are cleaved near the terminal loop by enzyme Dicer cooperating with protein TRBP (the human immunodeficiency virus transactivating response RNA-binding protein), releasing 20 nucleotide miRNA:miRNA* duplex.[15,16] It seems that TRBP also contributes to the formation of the RNA-induced silencing complex (RISC).[17] Argonaute 2 (AGO2) with RNase H-like endonuclease activity (endonuclease enzyme with a hydrolytic mechanism of function) can support Dicer processing by cleaving the 3′ arm of some pre-miRNAs.[18] Next, one strand of miRNA:miRNA∗ duplex, acts as a mature miRNA and is incorporated into a miRNA-induced silencing complex (miRISC), whereas the other strand (passenger strand, labeled as ∗ throughout the text) is usually degraded. The retained guide strand is the one from the miRNA:miRNA∗ duplex with the less thermodynamically stable base-paired 5′ end.[19,20] However, sometimes both arms of the pre-miRNA mature to the miRNAs.[4] The new nomenclature has introduced the terms miR-3p/miR-5p. Although produced from the exact transcript, the -3p and -5p miRNAs present only a partial complementary overlap with different spectrums of targets.[21] Interestingly, several studies have also demonstrated the functional passenger strand. For example, the role of miR-34a∗ in the resistance of rheumatoid arthritis (RA) synovial fibroblasts (RASF) to apoptosis was described.[22] In another study, miR-155 and miR-155∗ had the opposite effects on the regulation of type I interferon production by plasmacytoid dendritic cells.[23]

In addition to the classical biogenesis described above, in the alternative mirtron pathway, hairpin-shaped miRNAs precursors originate from the intronic regions of protein-coding genes. Spliced pre-mirtron is subsequently cleaved by lariat debranching enzyme (DBR1) into the mature mirtron, is exported out of the nucleus by Exportin-5, and later processed the same as pre-miRNA.[24]

Mechanisms of gene expression regulation by miRNAs

The RISC has an essential role in gene silencing. The most important components of the RISC are argonaute proteins (AGO1–AGO4). AGO protein contains 2 conserved RNA binding domains: a PAZ (Piwi Argonaut and Zwille protein) domain binding the 3′ end of mature miRNA and a PIWI (p-element induced wimpy) domain interacting with the 5′ end of the mature miRNA. The seed region of miRNA (base 2–8) is exposed outwards and binds to the 3′ untranslated region (3′UTR) of target messenger RNA (mRNA).[25] MiRNAs usually pair imperfectly with target mRNA; only bases 2–7 of the miRNA are required to match a target and initiate translational repression.[26] There are 2 mechanisms of gene silencing caused by miRNA: 1) degradation of mRNA in case of perfect complementarity between the miRNA and target mRNA, or 2) repression of mRNA translation in case of partial complementarity. Only AGO2 can cleave and degrade mRNA,[4] AGO1, AGO3, and AGO4 lack slicer activity.[27] Cleavage always occurs between the target nucleotides that pair with bases 10 and 11 of miRNA.[25] More common than direct cleavage is the destabilization of mRNA. This is caused by deadenylation of the poly(A) tail followed by hydrolysis of 5′ cap due to interaction between AGO and P-body component GW182, followed by exonucleolytic digestion of mRNA.[28] P-bodies are granules that consist of many enzymes and proteins involved in the post-transcriptional regulation of gene expression.[29] In addition to their function in regulating mRNA by interacting with miRNA or siRNA (small interfering RNA), AGO proteins also ensure the stability of miRNAs and protect them from degradation by exonucleases.[30] The siRNAs are double-stranded RNAs that, like miRNAs, after incorporated into RISC, prevent mRNA translation.[31] The main difference from miRNAs is that each siRNA has only one target mRNA that is always entirely complementary.[32]

Through the regulation of gene expression, miRNAs are involved in many cellular processes, including proliferation, differentiation, apoptosis, and development.[33]

Several mechanisms can affect miRNA expression like chromosomal abnormalities, mutations in the primary transcripts or polymorphisms (SNPs), and defects in the miRNA biogenesis machinery (e.g., modification of Drosha and DBCR8 activity). In general, most miRNAs serve as tumor suppressors, and their down-regulation often results in cancer development.[5] For example, genes for *miR-15a* and *miR-16* are located at chromosome 13q14, in the region frequently deleted in B cell chronic lymphocytic leukemia.[34] Both miRNAs negatively regulate the expression of the anti-apoptotic protein Bcl2 (B cell lymphoma 2) and therefore induce apoptosis.[35]

The aberrant expression of miRNA can also be affected by epigenetic modifications (changes in gene expression not caused by changes in the nucleotide sequence of DNA), such as DNA methylation that inhibits transcription or histone acetylation that promotes transcription.[36] About half of the miRNA genes are associated with CpG islands, regions with a high density of CpG dinucleotides that can be easily methylated.[37] Higher expression of miR-203 in RASF is associated with hypomethylation of the *miR-203* gene promoter.[38] Increased histone deacetylase 3 (HDAC3) observed in peripheral blood mononuclear cells (PBMC) from patients with ankylosing spondylitis (AS) resulted in a decrease of miR-130 by downregulation of the histone acetylation of its promoter.[39] Some miRNAs may affect the biogenesis of miRNAs. For example, miRNA let-7 can negatively affect the formation of new miRNAs by direct targeting the enzyme Dicer.[40] One miRNA can also affect the expression of another miRNA. For example, mouse miR-709 can suppress the maturation of miR-15a/16-1 by binding to their pri-miR in the nucleus.[41]

Circulating miRNA in the body fluids

The presence of the circulating miRNAs in the body fluids

The discovery of miRNAs in body fluids in 2008 became a significant research challenge.[42] Circulating miRNAs are very stable and easily accessible in body fluids, which makes them potential biomarkers and leads to the hypothesis about their function in intercellular communication. The presence of miRNAs was described in serum, plasma, bronchial lavage, cerebrospinal and peritoneal fluid, pleural fluid, tears, urine, and other body fluids.[42,43] MiRNAs in exosomes isolated from whole human saliva serve as an ideal non-invasive biomarker of various salivary gland pathologies, for example, Sjögren's syndrome.[44] High levels of immune-related miRNAs were found during the first 6 months of lactation in breast milk.[45] A high concentration of miRNAs in tears may help offer an early diagnosis for glaucoma, macular degeneration, and malignancies of the eye.[46,47]

Circulating miRNAs are incorporated into microparticles (depending on their size, known as exosomes of diameter 30—150 nm, microvesicles of diameter 100—1000 nm, or the biggest apoptotic bodies of diameter 50—5000 nm) or associated with RNA-binding proteins (AGO2, NPM1-nucleophosmin) or lipoprotein complexes. Such incorporation protects them against endogenous RNase activity and makes them very stable. The stability of circulating miRNAs allows for regulating gene expression of distant cells and functioning in cell-to-cell communication.[48—50]

The presence of the miRNAs was described in the microparticles from several human cell lines. Secreted exosomes were isolated for the first time in 1983 from sheep reticulocytes.[51] However, the following analysis of the supernatants revealed that most miRNAs are exported from the cell in exosome-independent forms.[52] Most (potentially 90%) of circulating miRNAs were co-fractioned with protein complexes, mostly AGO2 proteins.[53] In addition, AGO1, AGO3, and AGO4 might also be associated with circulating miRNAs.[54] Circulating miRNAs can be found in the complex with high-density lipoproteins (HDL). HDL remove fat molecules from cells and are therefore essential in protection against atherosclerosis.[55] Patients with familial hypercholesterolemia exhibit a significantly different HDL-miRNA profile than normal subjects, including differences in content and its abundances.[56] The presence of miRNAs was also described in low-density lipoproteins (LDL) but in a lower concentration than in HDL. However, the proinflammatory miR-155 was in 4 times higher quantities in LDL than HDL.[57] Another protein able to bind miRNA is NPM1,[52] a phosphoprotein highly expressed in proliferating cells serving as a regulator of ribosome export.[58]

Secretory mechanisms of circulating miRNA

Ceramide-secretory mechanisms and neutral sphingomyelinase (nSMase) are involved in the extracellular secretion of miRNAs. Ceramide is a second messenger in the sphingomyelin pathway, a signaling system linking a specific set of cell surface receptors to the nucleus.[59] While the inhibition of nSMase did not alter levels of intracellular miRNAs, it reduced their expression in exosomes.[60] On the other hand, overexpression of nSMase caused an increase in extracellular miRNAs.[61] The principle of selection in which miRNA gets into the circulation remains unknown. Post-transcriptional modifications may affect miRNA excretion. While miRNAs with adenylated 3′ end are relatively enriched in cells, miRNAs with uridylated 3′ end are more abundant in exosomes.[62] Specific miRNA sequences (EXO-motif) can be recognized by RNA binding proteins that control their localization into exosomes. For example, sumoylated heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) binds to the GGAG region of miRNAs and directs their transport into exosomes and then out of the cell.[63] Some miRNAs are released to the circulation non-specifically, for example, after cell death, and are incorporated into the apoptotic bodies.[64]

There are significant differences in the spectrum of miRNAs in microparticles or miRNAs associated with proteins. Some miRNAs (e.g., let-7a) are associated predominantly with microvesicles, and some miRNAs (e.g., miR-122) are detected only in protein-associated fractions.[53] *In vitro* experiments showed that some miRNAs are expressed at higher levels in cells, while others are released from the cells to supernatants.[60,65] Moreover, some miRNAs are detected exclusively in exosomes.[60] MiRNAs originate from different cell types and could reflect cell type-specific miRNA expression and release mechanisms. For example, liver-specific miR-122 may be released by hepatocytes through the protein carrier pathway, whereas let-7a might originate from cell types known to generate vesicles such as reticulocytes or platelets.[53]

Circulating microRNAs as biomarkers in rheumatic diseases

The function of the intracellular miRNA and their potential as biomarkers in rheumatic diseases were thoroughly described in many publications, but there is significantly less data on extracellular miRNAs. Here, we provide an overview of circulating miRNAs described as diagnostic markers and markers reflecting disease activity or treatment response (Table 1).

Rheumatoid arthritis

RA is a chronic inflammatory autoimmune disease typically affecting the small joints of the hands. As a result of progressive joint destruction, non-treated RA leads to a loss of joint function and reduces the quality of life.

A comparison of plasma and synovial fluid miRNAs revealed higher levels of miR-16, miR-132, miR-146a, and miR-223 in plasma than in synovial fluid of patients with established RA and osteoarthritis (OA). There was no correlation between plasma and synovial fluid levels of miRNAs. Synovial fluid levels of miR-16, miR-146a, miR-155, and miR-223 were significantly higher in RA than in OA. Plasma levels of miR-132 were significantly higher in healthy controls (HC) than the RA and OA. Based on ROC (receiver operating characteristics) analysis, miR-132 can help distinguish patients with RA and OA from HC with high sensitivity and specificity.[66] The miR-155 plays an essential

Table 1 Summary of circulating miRNAs as biomarkers in rheumatic diseases.

Diagnosis	Sample	MiRNAs	Biomarker type			Reference
			Diagnosis	Disease activity	Prediction of treatment response	
RA	Plasma	↓miR-132	yes	No	no	66
	Serum	↑miR-223	yes	No	no	68
	serum	↓miR-10a	yes	No	yes	70
	serum exosomes	↓miR-548a-3p	yes	yes	no	72
	Serum	↑miR-448, ↑miR-551b, ↓miR-124	yes	no	no	73
	Plasma	↑miR-16, ↑miR-21, ↑miR-223, ↑miR-451	yes	no	no	79
	Plasma	↑miR26a, ↑miR-125a-5p	yes	no	no	80
	Plasma	↑miR-24	yes	yes	no	80
	Plasma	↑miR-23b	yes	yes	no	83
	Serum	miR-125b	no	no	yes	92
	Serum	miR-16-5p, miR-125b, miR-126-3p, miR-146a-5p	no	yes	no	95
	Serum	miR-23-3p, miR-223-3p	no	yes	yes	95
	Serum	↑miR-5196	yes	yes	yes	143
ERA	serum	miR-16, miR-223	no	no	yes	84
	plasma	miR-99b-5p, miR-143-3p, miR-145-5p	no	yes	no	85
	plasma	miR-27a-3p	no	no	yes	88
	plasma	↓miR-125b	yes	no	no	91
OA	serum	let-7e, miR-454, miR-885-5p	no	yes	no	97
	serum	↑miR-146a-5p	yes	no	no	99
	plasma	↑miR-92a, ↑miR-320b,	yes	no	no	100
	plasma	miR-19b-3p, miR-122-5p, miR-486-5p	yes	yes	no	100
	plasma	↑miR-93, ↑miR-126, ↑miR-146a, ↑miR-184, ↑miR-186, ↑miR-195, ↑miR-345, ↑miR-885-5p	yes	no	no	103
	plasma	miR-126-5p, miR-320a, miR-146a-5p	no	yes	no	105
	synovial fluid	↑miR-210	yes	no	no	107
SLE	plasma	↑miR-16, ↑miR-21, ↑miR-126, ↑miR-223, ↑miR-451	yes	no	no	79
	serum	↑miR-448, ↑miR-551b, ↓miR-124	yes	no	no	73
	serum	↓miR-146a, ↓miR-155	yes	yes	no	111
	urine	↑miR-146a	yes	no	no	111
	serum	↓miR-192, ↓miR-200a, ↓miR-200b, ↓miR-200c, ↓miR-205, ↓miR-429	yes	yes	no	112
	urine	↓miR-141, ↓miR-192, ↓miR-200a, ↓miR-200c, ↓miR-429,	yes	no	no	112
	serum exosomes	↓miR-146a	yes	no	no	113
	plasma	↓miR-16, ↓miR-20a, ↓miR-22, ↓miR-23a, ↓miR-27a, ↓miR-92a, ↓miR-103, ↓miR-150, ↓miR-223, ↓miR-181a	yes	no	no	116
	plasma	↓miR-15b, ↓miR-19b, ↓miR-25, ↓miR-93	yes	yes	no	116
	plasma	↑miR-142-3p, ↑miR-181a, ↓miR-17, ↓miR-20a, ↓miR-92a, ↓miR-106a, ↓miR-203	yes	no	no	117
	plasma	↑miR-107-3p, ↑miR-125b-5p,↑miR-145-5p, ↑miR-153-3p, ↑miR-199a-5p, ↑miR-323b-3p,↑miR-369-5p, ↑miR-410-3p,↑miR-485-5p, ↑miR-543, ↑miR-584-5p, ↑miR-589-3p, ↑miR-1260b, ↑miR-3942-5p, ↑miR-6087, ↑miR-7977,↓miR-106a-5p, ↓miR-183-5p, ↓miR-375-3p, ↓miR-550a-5p, ↓miR-550b-2-5p, ↓miR-4511, ↓miR-4732-3p, ↓miR-6741-3p	yes	no	no	118
	plasma	↑miR-21, ↑miR-423, ↓miR-150	yes	no	no	119

(*continued on next page*)

Table 1 (*continued*)

Diagnosis	Sample	MiRNAs	Biomarker type			Reference
			Diagnosis	Disease activity	Prediction of treatment response	
	plasma	↓miR-141-5p, ↓miR-200b-5p, ↓miR-200c-5p	yes	no	no	[120]
	plasma	↑miR-21	yes	no	no	[123]
SSc	serum	↑miR-448, ↑miR-551b, ↓miR-124	yes	no	no	[73]
	serum	↑miR-92a	yes	no	no	[124]
	serum	↑miR-142-3p	yes	no	no	[125]
	serum	↓miR-30b	yes	yes	no	[126]
	serum	↑miR-4484	yes	no	no	[127]
	serum	↑miR-5196	yes	yes	no	[128]
	serum	↑miR-483-5p	yes	yes	no	[131]
SpA	serum	↑miR-146a	yes	no	no	[132]
	serum	↑miR-155	yes	yes	no	[132]
	serum	↓miR-214	yes	yes	no	[133]
	plasma	↑miR-151-3p, ↓miR-150-5p, ↓miR-451a	yes	no	no	[135]
	plasma	↑miR-22-3p, ↑miR-125a-5p, ↑miR-146a-5p	yes	yes	no	[135]
	plasma	↑miR-32, ↑miR-34a, ↓miR-10b, ↓miR-16, ↓miR-30a, ↓miR-150, ↓miR-154	yes	no	no	[136]
	plasma	↓miR-29a-3p, ↓miR-146a-5p, ↓miR-222-3p, ↓miR-625-3p,	yes	yes	no	[139]
	serum	↑miR-5196	yes	yes	no	[143]
	plasma	miR-145	no	no	yes	[144]
IIM	serum	↑miR-7b*, ↑miR-498, ↑miR-4310, ↓miR-1234, ↓miR-3679-5p, ↓miR-4299	yes	no	no	[147]
	serum	miR-877*, miR-3676, miR-3907	no	yes	no	[147]
	serum	↓miR-223	yes	yes	no	[148]
	plasma	↑miR-4442	yes	yes	no	[149]
	serum	↑miR-21	yes	no	no	[150]
	plasma	↑miR-21, ↓miR-7	yes	no	no	[151]

ERA, early RA; IIM, idiopathic inflammatory myopathy; OA, osteoarthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; SSc, systemic sclerosis.

role in the pathogenesis of RA as miR-155-deficient mice did not develop CIA and showed significantly reduced local bone destruction.[67] In a different study, serum levels of miR-223 were increased while serum levels of miR-155 were unchanged in RA patients compared to HC.[68] MiR-223 is overexpressed in synovial tissue of patients with RA, and its up-regulation is implicated in stimulating osteoclast differentiation through repressing the expression of its target, nuclear factor 1A (NF-1A). Moreover, lentivirus-mediated silencing of miR-223 reduced the disease severity of experimental arthritis.[69] Therefore, high levels of both miR-155 and miR-223 may reflect an inflammatory and erosive nature of RA. Serum levels of miR-10a were lower in RA patients than HC and were increased in RA patients treated with MTX compared to untreated RA. ROC analysis proves that miR-10a expression may predict therapeutic response in RA patients treated with MTX.[70] It was previously described that miR-10a inhibits the production of proinflammatory cytokines via inhibition of NF-κB, and the downregulation of miR-10a observed in RA promotes the proliferation and migration of RASF involved in the pathogenesis of RA.[71] Similarly, serum exosome levels of miR-548a-3p were lower in RA patients than in HC, and their levels were negatively correlated with CRP (C-reactive protein), ESR (erythrocyte sedimentation rate), and RF (rheumatoid factor). MiR-548a-3p in macrophage-like cells downregulates TLR4-mediated inflammatory response and inhibits the generation of proinflammatory cytokines IL-6 and TNF-α *in vitro*.[72] Thus, lower levels of miR-10 and miR-548a-3p in the serum of RA patients may reflect their contribution to the proinflammatory state in RA. In a different study, serum levels of miR-551b and miR-448 were increased, and levels of miR-124 decreased in RA compared to HC.[73] *In vitro* overexpression of miR-488 significantly decreased cell apoptosis and caspase-3 activity in RASF.[74] As mentioned above, miR-124 reduced osteoclast differentiation and seemed a promising therapeutic target for RA.[75–78] Dysregulated circulating levels of miR-488 and miR-124 may therefore reflect their local pathogenetic role in RA.

Plasma levels of miR-16, miR-21, miR-223, miR-451 and miR-126 were higher in systemic lupus patinets than in HC. The first 4 miRNAs were higher also in RA patients compared to HC.[79] Another study showed that upregulated plasma levels of miR-24, miR-26a, and miR-125a-5p distinguished patients with RA from HC with high specificity. The combination of miR-24 and miR-125a-5p was described as a potential diagnostic marker of RA. Moreover, miR-24 positively correlated with CRP, VAS (visual analog scale of general health), and DAS28 (disease activity score), suggesting its use as a marker of disease activity.[80] In the synovium of CIA rats, the expression of miR-24 positively correlated with anti-apoptotic protein Bcl2.[81] MiR-125a activates the NF-κB signaling by targeting the TNFAIP3 (a negative regulator of NF-κB).[82] Therefore, increased circulating levels of miR-24 and miR-125a may reflect their abovementioned pathogenetic role in RA. Recently, the correlation of miR-23b with ESR, CRP, and DAS28 was described in RA plasma samples. Levels of miR-23b were higher in RA patients than in HC and decreased significantly after therapy with disease-modifying anti-rheumatic drugs (DMARDs) and/or non-steroidal anti-inflammatory drugs (NSAIDs) in patients who achieved at least ACR20 (Americal College of Rheumatology criteria) response while did not change in non-responders.[83]

In patients with early rheumatoid arthritis (ERA), serum levels of miR-16, miR-146a, and miR-155 were significantly lower than in patients with established RA. In ERA patients treated with DMARDs, the increase of miR-16 from baseline to month 3 correlated with decreasing DAS28 from month 3 to month 12. Moreover, higher levels of miR-223 before treatment correlated with CRP and DAS28 and disease activity reduction in the following 9 months.[84] Plasma levels of miR-99b-5p, miR-143-3p, and miR-145-5p were significantly higher in ERA patients with bone erosions than in non-erosive ERA patients.[85] Previously *in vitro* experiments on the mouse and human monocytes showed that miR-99b-5p stimulates osteoclastogenesis.[86,87] Moreover, increased baseline expression of miR-99b-5p predicted progression of erosions at month 12 [85]. Therefore, high levels of miR-99b-5b in plasma may reflect the erosive course of the disease. High plasma levels of miR-27a-3p served as a potential predictive biomarker of remission in patients with ERA treated with anti-TNF therapy (adalimumab) combined with conventional synthetic DMARDs.[88] MiR-27a inhibits migration and invasion of RASF and differentiation of Th1 and Th17 cells, which are all involved in the pathogenesis in RA.[89,90] We hypothesize that higher levels of miR-27a could reflect its protective potential and be a marker for less destructive ERA. The expression of miR-125b in plasma of treatment-naïve ERA patients was lower than HC and increased after 3 months of anti-TNF therapy.[91] Higher serum levels of miR-125b in RA patients were associated with good clinical response to treatment with rituximab. In contrast, patients with low miR-125b had less chance of clinical improvement after 3 months of therapy.[92] MiR-125b downregulates T cell differentiation[93] and TNF-α production.[94] So, higher levels of miR-125b after treatment in ERA patients may explain more effective suppression of inflammation and improvement of the therapeutic response. In another study, serum levels of miR-125b along with miR-16-5p, miR-23-3p, miR-126-3p, miR-146a-5p, and miR-223-3p increased after 6 months of anti-TNF/DMARDs combination therapy in RA patients with a good response to treatment. ROC analysis showed a combination of miR-23-3p and miR-223-3p as an ideal predictor of treatment response.[95] As all these miRNAs have been previously described to act as regulators of immune cell development and key players in inflammatory response,[96] their aberrant expression before and after anti-TNF/DMARDs therapy has the potential to serve as a novel biomarker for predicting treatment response.

Osteoarthritis

OA is the most common long-term chronic joint disease that progresses slowly over the years. Damage to hyaline cartilage and underlying bone remodelation cause pain and loss of joint function resulting in impaired quality of life.

Circulating let-7e was described as a negative predictor for total joint arthroplasty in patients with OA. While miR-885-5p in serum was positively associated with OA severity, miR-454 was inversely correlated with severe knee and hip OA requiring joint replacement.[97] MiR-454 is a negative regulator of RUNX2, a key transcription factor associated with osteoblast differentiation.[98] We, therefore, hypothesize that low levels of miR-454 in patients with severe OA may reflect higher osteoblastogenesis leading to osteophyte formation. Expression of miR-146a-5p in serum OA samples was increased compared to controls and correlated with increased expression of miR146a-5p in cartilage.[99] Expressions of miR-19b-3p, miR-92a, miR-122-5p, miR-320b, and miR-486-5p were higher in plasma samples of patients with knee OA than HC. Further ROC analysis showed upregulated plasma levels of miR-19b-3p, miR-122-5p, and miR-486-5p as a predictor of knee OA development. Their combination was described as a potential biomarker for diagnosis and disease severity assessment.[100] It was previously described that by the positive regulation of NF-κB signaling, miR-19b increases the production of proinflammatory cytokines[101] that contribute to OA pathogenesis.[102] Higher levels of miR-19b may reflect inflammatory exacerbation of the disease. A different study revealed 8 miRNAs (miR-93, miR-126, miR-146a, miR-184, miR-186, miR-195, miR-345, and miR-885-5p) overexpressed in plasma of patients with OA compared to HC. *In silico* analysis showed that at least 6 target genes of these miRNAs play a role in the pathogenesis of OA.[103] For example, miR-146a inhibition of Smad4 resulted in upregulation of vascular endothelial growth factor (VEGF) and apoptosis of chondrocytes, contributing to the OA development.[104] Plasma levels of miR-126-5p and miR-320a increased, while levels of miR-155-5p and miR-146a-5p decreased in OA patients after 6 weeks of treatment by selective COX-2 inhibitor celecoxib. Moreover, changes in levels of miR-126-5p, miR-320a, and miR-146a-5p correlate with treatment response to celecoxib.[105] *In vitro* experiments on macrophages showed that miR-155 increases the production of proinflammatory cytokines involved in RA as well as OA pathogenesis (including IL-6 and TNF-α) by

downregulation of SHIP-1 [106]. Therefore, increased expression of miR-155 after treatment may correspond with the reduction of the proinflammatory state in OA patients. In synovial fluid samples, levels of miR-210 were higher in both early and late-stage OA patients than in HC.[107]

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease. The basis of pathogenesis is the production of autoantibodies against self-proteins by polyclonal activation of B-lymphocytes. Autoantibodies subsequently contribute to the development of inflammation and tissue damage.

Plasma levels of miR-16, miR-21, miR-126, miR-223 and miR-451 were higher in SLE patients than in HC, as mentioned above. Next, bioinformatics analysis confirmed that these miRNAs regulate transduction pathways and cellular interaction in SLE.[79] A previous study on $CD4^+$ T cells from patients with SLE showed that miR-126 downregulated DNA methylation and contributed to higher T cell autoreactivity by targeting DNA methyltransferase 1 (Dnmt1), which causes DNA methylation.[108] MiR-21 as a direct target of inhibitor PDCD4 (programmed cell death protein 4) contributes to aberrant T cell phenotype in human SLE patients.[109] As T cells play an important role in the pathogenesis of SLE,[110] higher levels of miR-126 and miR-21 may reflect their contribution to the SLE pathogenesis mentioned above. Also, serum levels of miR-448 and miR-551b were increased, and levels of miR-124 decreased in SLE compared to HC.[73] In different studies, both serum and urinary levels of miRNAs were compared. Serum levels of several miRNAs (miR-146a, miR-155, miR-192, miR-200a, miR-200b, miR-200c, miR-205, and miR-429) were lower in SLE patients compared to HC. Urinary miR-141, miR-192, miR-200a, miR-200c, and miR-429 were lower, while urinary levels of miR-146a were higher in SLE patients than in HC. Serum levels of miR-146a, miR-155, miR-192, miR-200b, miR-200c, miR-205, and miR-429 correlated with renal function (eGFR), serum levels of miR-146a and miR-200a negatively correlated with SLE disease activity index (SLEDAI) and with proteinuria.[111,112] Moreover, the expression of miR-146a was also decreased in serum exosomes of SLE patients compared to HC.[113] MiR-146a is a negative regulator of the IFN pathway, and its deficiency contributes to the pathogenesis of SLE.[114] Low levels of miR-146a could, therefore, correspond with the high activity of the IFN pathway in SLE.[115] Several miRNAs in plasma were decreased in SLE patients compared to HC. The expression of 6 of these miRNAs (miR-16, miR-19b, miR-23a, miR-27a, miR-92a, and miR-223) was significantly lower than RA and could distinguish SLE patients from RA patients.[116] Among other differently expressed miRNAs in SLE patients, several miRNAs were significantly decreased (e.g., miR-20a, miR-141-5p, miR-150, miR-200b-5p, miR-200c-5p, miR-223, miR-342-3p and 758-3p) or increased (e.g., miR-21, miR-375-3p, and miR-423) in plasma of SLE patients with active nephritis.[117–120] MiR-150 inhibits B cell development by targeting c-Myb,[121] transcription factor important during B-lymphopoiesis.[122] Thus low expression of miR-150 implicates B cell activation in SLE patients. A recently performed meta-analysis confirmed that circulating miRNA, especially miR-21, might serve as a diagnostic biomarker in SLE.[123]

Systemic sclerosis

Systemic sclerosis (SSc) is a chronic connective tissue characterized by fibrosis of the skin and internal organs. The main pathological processes include inflammation, vasculopathy, and fibrosis.

Serum levels of several miRNAs (miR-92a, miR-142-3p, miR-448, miR-551b, miR-4484, and miR-5196) were higher in SSc patients compared to HC, while levels of miR-30b and miR-124 were lower.[73,124–128] The overexpression of miR-92a in dermal fibroblasts resulted in the down-regulation of MMP-1,[129] the enzyme that breaks down the collagens.[124] It is hypothesized that high levels of miR-92a in serum reflect its local expression that plays a role in excessive collagen accumulation in SSc via the down-regulation of MMP-1. In addition to low miR-30b levels mentioned above, levels of miR-30b correlated negatively with the severity of the disease activity assessed by the modified Rodnan Skin Score, and levels of miR-5196 correlated positively with CRP but not with Rodnan skin score.[126,128] The direct target of miR-30b is platelet-derived growth factor (PDGF),[126] playing an essential role in the pathogenesis of fibrosis by stimulating the replication, survival, and migration of myofibroblasts.[130] Downregulation of circulating miR-30b observed in SSc patients may therefore mirror local profibrotic potential. Recently, higher expression of miR-483-5p was described in SSc patients, even in the patients with early SSc compared to HC and its correlation with the modified Rodnan Skin Score. Its overexpression in fibroblasts and endothelial cells modulated the expression of fibrosis-related genes. It indicates that miR-483-5p plays a profibritic role in SSc and may serve as a biomarker of the early stages of the disease.[131]

Spondyloarthritis

Spondyloarthritis (SpA) is a group of chronic immune-mediated rheumatic diseases affecting the axial skeleton (spine and sacroiliac joints), peripheral joints, and entheses and having several extra-articular manifestations (psoriasis, uveitis, inflammatory bowel disease). It causes mainly low back pain and reduced spinal mobility.

Levels of miR-146a and miR-155 were significantly elevated in patients with ankylosing spondylitis (AS) compared to HC. Based on ROC analysis, these miRNAs may serve as diagnostic biomarkers to distinguish patients with AS from HC. Moreover, miR-155 correlated with disease activity assessed by BASDAI (Bath Ankylosing Spondylitis Disease Activity Index).[132] As mentioned above, miR-155 increases the production of proinflammatory cytokines also involved in AS pathogenesis, so higher expression of miR-155 may reflect the inflammatory milieu of the disease.[106] Recently, lower expression of miR-214 in serum of AS patients compared to HC was described.[133] MiR-214 promotes osteoclast differentiation, so its decreased expression in serum may reflect its local participation in excessive new bone formation in AS patients by inhibiting osteoclast formation.[134] The expression of several miRNAs in plasma samples of AS patients was increased (miR-22-3p, miR-32, miR-34a, miR-125a-5p, miR-146a-5p and miR-151-3p) or decreased (miR-10b, miR-16, miR-30a, miR-150-5p, miR-154 and miR-451a) in comparison to HC. Moreover, expression of miR-22-3p, miR-125a-5p, and miR-146a-5p were upregulated in active AS patients compared to non-

active AS. Comprehensive analysis of potential gene targets suggested that some of these miRNAs could be involved in the signaling pathways related to inflammation and bone remodeling.[135,136] MiR-22 contributes to the activation of antigen-presenting cells and Th17 responses through the activation of AP-1 transcription factor and the histone deacetylase HDAC4.[137] Higher levels of circulating miR-22 in AS could therefore correspond with the inflammatory nature of active AS as Th17 cells produce IL-17, the key cytokine playing a role in the pathogenesis of AS.[138] Comprehensive analysis of plasma miRNAs in patients with SpA revealed several differentially expressed miRNAs according to progressing spinal involvement. Levels of miR-625-3p were lower in individuals with non-radiographic SpA compared to HC. MiR-29a-3p, miR-146a-5p, or miR-222-3p were associated with spinal changes and/or disease activity assessed by BASDAI in AS patients, including miR-625-3p reflecting disease activity in AS with spinal involvement. Expression of miR-29a-3p was lower in patients with SpA compared to HC and in patients with radiographic impairment compared to non-radiographic SpA.[139] TGF-β, an important stimulator of bone formation,[140] inhibits miR-29a expression.[141] We hypothesize that advanced-stage AS patients with extensive bone formation have higher levels of TGF-β, which ultimately results in miR-29a suppression and increased bone formation.

As mentioned above, miR-146a inhibits osteoclastogenesis, and *in vivo* intravenous administration of miR-146a prevents joint destruction in arthritic mice.[142] Although in a different disease, we hypothesize that low levels of this miRNA may be associated with the proinflammatory condition in AS. Levels of miR-5196 in serum were higher in AS patients as well as RA than in HC and significantly decreased after anti-TNF therapy. Moreover, changes in its expression positively correlated with disease activity improvement.[143] Similarly, expression of miR-145 in plasma samples decreased after 3 months of anti-TNF therapy and predicted future disease activity improvement in AS patients.[144] MiR-145 inhibits osteoblast differentiation[145] and promotes bone erosion by increasing osteoclastogenesis.[146] Therefore decline of miR-145 after TNF treatment may indicate a shift from the active erosive phase to the later repair process associated with bone formation.

Idiopathic inflammatory myopathies

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of disorders characterized by inflammation of skeletal muscles associated with characteristic skin manifestation and multiple organ involvement. The main characteristic is proximal muscle weakness and elevated levels of muscle enzymes.

The expression of miR-7b*, miR-498, and miR-4310 was increased in serum of patients with IIM, while the expression of miR-1234, miR-3679-5p, and miR-4299 was decreased compared to HC. Moreover, 3 miRNAs (miR-877*, miR-3676, and miR-3907) had higher expression in patients with highly active disease compared to patients with low disease activity assessed using VAS.[147] Expression of serum miR-223 was decreased in patients with IMM, particularly in patients with clinically amyopathic dermatomyositis compared to HC. Moreover, the time between symptom onset and the first hospital visit was significantly shorter in patients with decreased miR-223 levels, so patients with decreased miR-223 could have more severe symptoms. *In vitro* inhibition of miR-223 in human epidermal keratinocytes increased its direct target PKCϵ (effective promoter of keratinocyte growth) and induced keratinocyte proliferation. Therefore, low expression of miR-223 in amyopathic dermatomyositis may be associated with hyperkeratosis observed in Gottron's papules typical in dermatomyositis.[148] Plasma levels of miR-4442 were significantly higher in IIM patients compared to RA or SLE patients and HC, correlated with disease activity, and decreased after prednisone treatment.[149] The expression of miR-21 was increased in patients with IIM compared to HC.[150] Recently, the down-regulated expression of miR-7 and upregulated expression of miR-21 in plasma samples of patients with IIM compared to HC was described. Moreover, miR-7 may distinguish IIM patients with intestinal lung disease (ILD) from IIM patients without ILD.[151]

Analysis of circulating miRNAs

Despite the advance in the knowledge of circulating miRNAs, their clinical use as biomarkers is affected by many factors, including specimen collection and processing, RNA isolation method, quality control and normalization, methods of detection, and analysis of the data. The exact recommendations are still lacking and need future agreement.

Preanalytical phase

Serum and plasma both include circulating miRNAs, their levels strongly correlate with each other, and both are suitable for investigating miRNAs as blood-based biomarkers.[43] It is recommended to process blood samples within 4 h of collection. When using plasma, it is necessary to avoid using a heparin tube because it potentially inhibits polymerase chain reaction (PCR).[152]

Circulating miRNAs are very stable. Their stability was confirmed in plasma samples after repetitive freeze—thaw cycles and samples left at room temperature for 24 h.[153] In plasma samples stored for 2 weeks at 4 °C or 5 years at −20 °C, miRNAs were degraded significantly compared to fresh samples. However, concentrations of exosome miRNAs did not differ during 5 years of storage. Similarly, repeated freeze/thaw cycles affected exosome miRNAs less than all plasma miRNAs.[154] Serum miRNAs remained stable after 10 freeze—thaw cycles, boiling, and low/high pH exposure with no significant differences compared to non-treated samples.[42] In different experiments, the stability of miRNAs in serum was confirmed after 4 h at room temperature; however, it was affected by 2 freeze—thaw cycles.[155] The stability of miRNAs in synovial fluid samples was confirmed after 7 days stored at −20 °C; however, repeated freeze—thaw cycles slightly decreased the concentration of miRNAs.[66]

Cellular contamination of the serum and plasma samples can be a source of variation in miRNA levels. In collecting

plasma, it is therefore recommended to spin the samples twice. This step is not necessary for serum because of the coagulation of specimen.[156] Several miRNAs (miR-15b, miR-16, and miR-24) are highly expressed in plasma compared to serum samples. Since the concentration of miR-15b and miR-24 decreased after the second spin, their higher plasma concentrations might be caused by cellular contaminants.[153]

Hemolysis increased several circulating miRNAs, for example, miR-15b, miR-16, and miR-451.[153,157] The expression of miR-16 and miR-451 is up to 8-fold higher in hemolysed than non-hemolysed samples.[157] Potential hemolysis of serum and plasma samples should be monitored, e.g., using a spectrophotometer measuring oxyhemoglobin absorbance at $\lambda = 414$ nm.[156] Another method is evaluating levels of miRNAs that regulate erythroid development, such as miR-144 and miR-451.[158] For the correct assessment of hemolysis, Blondal et al advised relating levels of miR-451 to miR-23a, which is not affected by hemolysis. Significantly higher levels of miR-451 than miR-23a (delta Ct miR-23a-miR-451 higher than 8) indicated an increased risk of hemolysis.[156] Another miRNA not affected by blood cell count is liver-specific miR-122.[159]

Sample to sample normalization

Even using the same amount of input material, the amount of isolated RNA may vary. Therefore, an appropriate control for normalization of their levels should be used. While the use of non-coding small nuclear RNAs MammU6, RNU24, RNU43, RNU44, RNU48, and RNU6B is possible for the normalization of cellular miRNAs, their concentration is not consistently detected in serum and plasma samples, and they might not be suitable for normalizing circulating miRNAs.[54,160] Instead, adding synthetic miRNAs corresponding to *C. elegans* during the RNA isolation process could be more appropriate. The sequences of these miRNA do not coincide with any other miRNA of human origin.[43] Another possibility is to use a miRNA commonly present in the samples with constant concentration. For example, miR-16 and miR-223 are both expressed at high levels in plasma and serum and are relatively unvarying across large numbers of samples[152]; however, miR-16 was shown to be affected by hemolysis.[153,157] Normalization to the average of several detected miRNA is another option. In serum samples of RA patients, normalization to the average of Ct of 6 miRNAs (miR-142-3p, miR-142-5p, miR-24, miR-181d, miR-15b, and miR-125b) has been described.[92] Plasma miRNAs from microparticles or associated with (lipo)protein complexes may require a different normalization procedure.[49] Each normalization method used so far has its drawback, and the ideal way to normalize circulating miRNAs is still lacking.

Methods of detection

MiRNAs can be isolated together with total RNA by phenol-chloroform extraction[84] or commercially available kits for circulating miRNA extraction. Three primary methods are used to study miRNAs' expression: PCR, microarray, and sequencing. One of the most commonly used methods for the quantification of miRNAs is PCR.[161] The PCR reaction is preceded by reverse transcription, the method in which target RNA is transcribed into cDNA (complementary DNA) amplified exponentially by DNA polymerase. The synthesized product is quantified using fluorescent-labeled probes. The increase in fluorescence signal can be compared to the miRNA selected for normalization. Microarray methods allow the wide-range analysis of defined miRNAs simultaneously, but the disadvantage is the high cost and the greater susceptibility to errors. Next-generation sequencing is a modern high-throughput method enabling identify up to millions of sequences. This method has discovered several miRNAs, but it is very time-consuming and expensive for routine use.[162]

Functional consequences of circulating miRNAs - is there a future for their therapeutic use?

Various human cells actively export miRNAs in microparticles or protein complexes, and these miRNAs can be delivered to another cell and remain functional in that new location.[52,56,65] B-lymphocytes infected by Epstein—Barr virus (EBV) secrete exosomes containing EBV miRNAs. These EBV-miRNAs are transferred to noninfected dendritic cells and regulate their target genes.[163]

Since the exosomes transport miRNAs to specific target cells, circulating miRNAs could serve as a promising tool in treating several diseases such as malignancies, autoimmune rheumatic disorders, diabetes mellitus, infection diseases, etc. However, the functional potential of circulating miRNAs in target cells has only been demonstrated so far for miRNAs contained in microparticles.

The immune-modulating potential of exosomes was confirmed by several *in vivo* experiments. Exosome-derived miRNAs from T cells transported into the antigen-presenting cells modulate gene expression in recipient cells.[164] MiRNAs secreted from tumor cells in exosomes bind to Toll-like receptors in HEK-293 (human embryonic kidney) cells, induce pro-metastatic inflammatory cytokines, and in *in vivo* experiments, cause tumor growth and metastasis.[165] Another experiment confirmed miRNA's function in intracellular communication: exosomal tumor suppressor miR-16 delivered its inhibitory ability to the recipient cells in the *in vivo* model.[166]

Normal liver cells produce miR-26a, which is a suppressor of the cell cycle in the tumors, and its decreased expression was observed in carcinomas. Administration of this miRNA in a mouse model of hepatocellular carcinoma resulted in the induction of apoptosis and inhibition of proliferation in cancer cells.[167]

Similarly, miRNAs can also be used to treat rheumatic diseases. Synovial fibroblasts and other cells involved in the pathogenesis of RA are resistant to apoptosis.[168] MiR-15a induces cell apoptosis by targeting the anti-apoptotic protein Bcl-2 in the MEG-01 leukemia-derived cell line.[35] The expression of miR-15a is significantly decreased in synovial fluid in a mouse model of collagen-induced arthritis (CIA). *In vivo* application of this miRNA into the knee joint of the CIA mice resulted in down-regulation of Bcl-2 protein and consequent apoptosis in synovial tissue determined using immunohistochemical analysis.[169] MiR-146a is a negative

regulator of immune processes and is highly expressed in patients with RA both locally in synovial tissue and systemically in PBMC.[170,171] In *in vitro* experiments, miR-146a inhibits osteoclastogenesis, and *in vivo* intravenous administration of miR-146a prevents joint destruction in arthritic mice.[142] MiR-223 stimulates osteoclastogenesis and is upregulated in the synovial tissue of RA patients. Inhibition of miR-223 in mice with CIA reduced bone erosion and improved local inflammation.[69] Expression of miR-140-3p and miR-140-5p is lower in synovial tissue and synovial fibroblasts of RA patients compared to osteoarthritis (OA) patients and in the CIA mice after the induction of arthritis. Intraarticular administration of these miRNAs in the CIA mouse model reduces the symptoms of arthritis and decreases the count of synovial fibroblasts. Moreover, these miRNAs induce apoptosis a reduce migration and proliferation of synovial fibroblasts.[172] MiR-21 regulating apoptosis and cell proliferation is upregulated in B and T cells in a mouse model of systemic lupus erythematosus (SLE) with splenomegaly. *In vivo* inhibition of this miRNA resulted in reducing the spleen and suppressing autoimmune manifestations of the disease.[173]

MiR-124 inhibits the expression of transcription factor NFATc1, which is involved in osteoclast differentiation. Injection of pre-miR-124 into the ankle joints of adjuvant-induced arthritis (AIA) rats reduced NFARc1 levels in synovial tissue, reduced bone destruction, and ameliorated disease symptoms.[75] Combination of miR-124 with methotrexate (MTX) in hybrid micelles (M-PHMs/miR-124) injected into the AIA rats specifically targeted activated macrophages in the joints, decreased serum levels of proinflammatory cytokines (e.g., TNF-α, IL-1β, and IL-17) and induced remission.[76] In a recent placebo-control phase II clinical trial (NCT03093259 and NCT03368118), oral administration of ABX464, which upregulates the production of miR-124, increased the chance of achieving clinical remission in patients with moderate-to-severe ulcerative colitis.[77] An ongoing phase IIa clinical trial (NCT03813199) aims to explore the safety and tolerability of ABX464 administered in combination with MTX in RA patients with inadequate response to MTX and/or to anti-TNF (tumor necrosis factor) therapy; however, the final data are not available yet.[78]

The therapeutic application of miRNA was also described in diabetes mellitus. MiR-103 and miR-107 are negative regulators of insulin sensitivity by reducing insulin sensitivity and increasing glucose production in the liver. *In vivo* administration of these miRNAs deteriorated glucose tolerance and insulin sensitivity, while their inhibition improved glucose homeostasis and insulin sensitivity in obese mice.[174]

Phase II clinical trial (NCT01200420) explored the safety, tolerability, pharmacokinetics, and antiviral activity of the miR-122 antagonist in treating hepatitis C.[175,176] This clinical trial was based on the fact that liver-specific miR-122 plays an essential role in the hepatitis C virus (HCV) replication. In 2005, the increased expression of miR-122 in human liver cells was found. The inactivation of this miRNA in Huh7 cells derived from human hepatocytes by transfection with 2′-O-methylated RNA oligonucleotide with complementarity to miR-122 reduced the amount of HCV RNA by approximately 80%.[177] Moreover, *in vivo* therapeutic silencing of miR-122 in primates with chronic HCV infection inhibited viral replication and improved HCV-induced liver pathology.[178] An *in vitro* study in human cell cultures using the miR-122 blocker (Miravirsen) confirmed its antiviral effect on HCV used alone or in combination with other available antiviral drugs.[176] Clinical trials in patients with chronic HCV infection have previously confirmed that Miravirsen is safe, and its administration reduces the load of HCV RNA.[175]

Conclusion

MiRNAs contribute to the pathophysiology of several diseases. The high stability of circulating miRNAs and easy accessibility from body fluids makes them ideal diagnostic or prognostic biomarkers and potential therapeutic targets. In this systematic review, we summarized the current knowledge about the use of circulating miRNA as biomarkers of disease activity, therapeutic response, or diagnosis of several rheumatic inflammatory or non-inflammatory diseases. Moreover, we tried to put them into context with their potential relationship to the pathogenesis of these diseases, even though there are still plenty of miRNAs with unknown pathogenetic mechanisms that need further research. Many of the miRNAs described as biomarkers were also shown to be of therapeutic potential, and some miRNAs are already tested in clinical trials. Verification in larger studies and establishment of the standardized analytical approach, including systematic reviews and meta-analyses, are essential before the use of circulating miRNAs is a part of routine clinical practice.

Author contributions

Klára Prajzlerová: literature search, writing — original draft preparation. Ladislav Šenolt: writing - review & editing. Mária Filková: conceptualization, writing - review & editing. All authors contributed to this manuscript. All authors read and approved the final manuscript.

Conflict of interests

All authors declare no conflict of interests.

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