MicroRNAs as new player in rheumatoid arthritis

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microRNAs as new player in rheumatoid arthritis

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Abstract

MicroRNAs (miRNAs) are small non-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level. Currently, there are 939 mature human miRNA sequences listed in the Sanger updated miRNA registry. There are approximately 1500 predicted miRNAs in the human genome that may regulate the expression of one-third of our genes. By controlling the accumulation of the target protein(s) in cells, these regulatory RNA molecules participate in key functions in many physiological networks and their deregulation has been implicated in the pathogenesis of serious human disorders, such as cancer and infection. The implication of miRNAs in immune-mediated disorders such as rheumatoid arthritis (RA) has recently emerged suggesting that miRNA based therapeutic approaches may have a promising potential in these diseases. Here, we provide an overview of the state-of-the-art on miRNAs in RA, focusing on both systemic and local features of the pathology.

Key words: microRNA, rheumatoid arthritis, autoimmunity, inflammation, biomarkers
Introduction

Over the last decade, it has become clear that small ribonucleic acids (RNAs) are important components of the cellular gene regulatory networks. Among these, micro(mi)RNAs are a family of endogenous, small, noncoding single-stranded RNA of approximately 22 nucleotides in length that act as posttranscriptional gene regulatory elements. Currently, miRNAs have been found in virtually all species (animals, plants, and viruses) and there are over 900 mature human miRNA sequences listed in the Sanger updated miRNA registry (miRBase 15). There are approximately 1500 predicted miRNAs in the human genome that may regulate the expression of up to one-third of our genes. By controlling the accumulation of the target protein(s) in cells, these regulatory RNA molecules participate in key functions in many physiological networks and their deregulation has been implicated in the pathogenesis of serious human disorders, such as cancer and infection. The implication of miRNAs in immune-mediated disorders has emerged in 2007 with the identification of auto-antibodies directed against one component of the RNAi pathway, argonaute 2, in the serum of patients suffering from Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA). Since then, many more publications reinforced the promise that miRNAs possess great potential both at fundamental and clinical levels as a novel class of therapeutic targets, tool for molecular intervention, and diagnosis biomarkers in such diseases. Here we provide an overview of the current state-of-the-art on miRNAs in RA.
**MicroRNA biogenesis and function**

In human, the majority of mature miRNAs (70%) are transcribed from introns and/or exons, and approximately 30% are located in intergenic regions. Long primary transcripts, namely primary miRNA or pri-miRNA (Figure 1), are transcribed by RNA polymerase II from mono- or poly-cistronic miRNA genes. A single pri-miRNA may range from approximately 200 nucleotides (nt) to several kilobases (kb) in length and have both a 5’ 7-methylguanosine (m7) caps and a 3’ poly(A) tail. Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stems and the mature miRNA sequences are characteristically localized to regions of imperfect stem-loop sequences. In the nucleus, pri-miRNAs are processed by the RNase III DROSHA and its cofactor DGRC8 (Digeorge syndrome critical region gene 8), into 70-100 nt-long hairpin with a monophosphate at the 5’ terminus and a 2-nt overhang with a hydroxyl group at the 3’ terminus called pre-miRNA. Next, the pre-miRNAs are exported from the nucleus into the cytoplasm as complexes with Exportin-5 and RAN-GTP cofactor. Once in the cytoplasm, the pre-miRNA is further digested by the RNase III enzyme Dicer along with its partner protein TRBP (trans-activator RNA binding protein) to produce the mature miRNA duplex of 20-22 nt. The miRNA “guide” strand, currently identified as the 5’terminus that is energetically less stable, is then selected for incorporation into the RISC (RNA-induced silencing complex), while the “passenger” strand is released and degraded. Occasionally, both arms of the pre-miRNA hairpin give rise to mature
miRNAs as evidenced by recent data from the deep sequencing, suggesting a role played by both strands. The actions of the miRNA guide strand are mediated by the catalytic component of the ribonucleoprotein (miRNP) RISC complex formed by a member of the Argonaute family (Ago). The miRNA-RISC complex interacts with the complementary 3’- or 5’- untranslated region (UTR) of target messenger (m)RNAs through imperfect matching. Recent publications predict that miRNAs might also target the promoter and CDS region, suggesting that miRNAs might also play a transcriptional regulatory role. Any position on a target mRNA downstream of initiation is sufficient for a miRNA to exert repression of translation, the most stringent requirement being a contiguous and perfect base pairing of the miRNA nucleotides 2–8, representing the “seed” region, which nucleates the interaction. Another rule is the presence of mismatches (bulges) in the central region of the miRNA–mRNA duplex, precluding the Ago-mediated cleavage of mRNA. In the case of perfect complementary between a miRNA and its target, the mRNA is degraded through the RNA silencing mechanism in a process distinct from miRNA-mediated translation repression.

The function of most of the mammalian miRNAs has yet to be determined but it appears that deregulation of this genetic regulatory network can be important in the pathogenesis of serious human disorders, such as cancer and infection, and might therefore have a great therapeutic implication.

**miRNAs and the immune system**
The first evidences show that miRNAs represent an archaic immune system protecting plants, insects, nematodes and eukaryotes from viral infection; and growing literature identified several miRNAs as key regulators of the hematopoietic lineage differentiation and stability. Expression of miRNAs is initially controlled at the transcriptional level by transcription factors that regulate the production of miRNA-containing primary transcripts in specific cell types during development or in response to different environmental cues. There is evidence that some of these processes are influenced by immune challenges, inflammation or other forms of cellular stress. Examples of miRNA-mediated regulation of immune cells are reported in granulopoiesis, T and B cell development and maturation, in innate immunity, as well as in the adaptive immune response. Thanks to the identification of their target genes, miRNAs have been shown to regulate antigen-presentation, Toll-like receptor (TLR) signalling cascade and cytokine production, immunoglobulin class-switch recombination in B cells, and T cell receptor signaling, representing thus a negative feedback mechanism that attenuates the T-cell activation and innate immune responses, and limits excessive inflammatory response. Overall studies show that miR-181, miR-223 and miR-142s are preferentially expressed in hematopoietic tissues.

In vitro, miR-223 has been shown to regulate human granulopoiesis [1]. In human myeloid lineages, miR-146a, miR-155 and miR-132 are up-regulated in response to Toll-like receptor (TLR) signalling and pro-inflammatory cytokines, while miR-125b is down-regulated [2]. In response to LPS, miR-181c and let-7e are up-regulated through the
protein kinase Akt1, while miR-125b and miR-155 are down-regulated [3]. Recently, using microarray technologies, Luers and co-workers have identified a miRNA-based expression profile in human primary macrophages, and validated the expression levels for 8 out of 119 miRNAs [4].

During B cell differentiation, a distinct miRNA expression profile is displayed by each B-cell subpopulation, with miR-223 highly expressed in naïve and memory B cells and miR-125b up-regulated in GC lymphocytes [5]. However, miR-223 expression is mostly confined to bone marrow and myeloid (Gr-1+ and Mac-1+) lineages. miR-181a is strongly expressed in thymus, decreased during B cell development from the pro-B to the pre-B cell stage, and increased again from pre-B to mature B cell [6]. Ectopic expression of miR-181 or miR-223 in Lin- hematopoietic progenitors isolated from mouse bone marrow resulted in a doubling of cells in the B- and T-lymphoid lineage respectively [7]. miR-150 is expressed at low levels in B-cell progenitors and its up-regulation blocks B-cell differentiation. Human B-cell malignancies are frequently associated with high expression levels of miR-155 that impairs germinal center responses [8] and immunoglobulin class switch through deregulation of the AID (activation-induced cytidine deaminase) expression [9]. During B-cell development, miR-17-92 is highly expressed in progenitors, enhancing B-cell proliferation and survival through the silencing of the anti-apoptotic protein Bim, while the expression levels of the cluster decrease 2- to 3-fold upon maturation [10].
miR-181a has also been shown to modulate T cell receptor (TCR) signalling through multiple target genes, mainly encoding phosphatases such as SHP2, PTPN22, DUSP5, DUSP6, that act as negative regulators of T cell activation in response to antigens [11]. Ectopic expression of miR-17-92 cluster results in an expansion of both CD4 and CD8 T cells [10]. Although similarities exist between the miRNA profiles of natural T regulatory (Treg) cells and activated CD4 T cells, some miRNAs appear very specifically differentially expressed, such as miR-233 and miR-146 that are over-expressed in Treg but not in activated T cells, while miR-20b, -31, -99a, -100, -125b, -151, -335 and -365 have lower expression [12]. miR-155 is involved in the Treg homeostasis as its expression is directly regulated by Foxp3. Its up-regulation during thymic differentiation is important for increased Treg sensitivity to their principal growth factor, IL-2, and by targeting SOCS1, a negative regulator of IL-2R signalling, miR-155 regulates Treg survival [13]. Finally, miR-326 regulates Th-17 differentiation by targeting Ets-1 and its in vivo silencing results in fewer Th-17 cells and mild autoimmunity [14].

Emerging roles for miRNAs in autoimmunity.

Hematopoietic-specific miRNA expression patterns have been identified in physiological and pathological conditions and first evidences of an implication of miRNAs in immune-mediated disorders has emerged a decade ago. Thus, the exploration of the roles of miRNAs in the context of autoimmunity is only being started and it is not surprising that recent studies reveal links between miRNA functions and
autoimmunity. The first miRNA-deficient animals generated were the miR-155 KO mice [8]. They displayed severe immunodeficiencies, particularly impaired B-cell responses and skewed Th2 cell responses. Down-regulation of miR-155 does not significantly compromise Treg function in vitro or in vivo, although miR-155-deficient mice have impaired development of Treg lineage. Mice with increased expression of miR-17-92 in lymphocytes developed autoimmune disease resembling to SLE [10]. Recently, Du and co-workers identified a Th17 cell-associated miRNA, miR-326, whose expression is highly correlated with disease severity in patients with MS and the corresponding mouse EAE model. Finally, crossing the FoxP3-GFP-hCre transgenic (Tg) mice with the conditional Dicer knockout mice (Dicer lox/lox) to selectively inactivate miRNAs generation in Treg cells, Zhou and collaborators showed that the Treg cells developed normally in the thymus, but changed in their differentiation and functional capacities in the periphery. The Dicer-deficient Treg cells failed to remain stable and altered expression of multiple genes and proteins associated with the Treg cell fingerprint, including FoxP3, was observed. In addition to their instability, mice rapidly develop spontaneous systemic autoimmune disease similar to the disease observed in Treg cell-deficient FoxP3 KO mice [15]. Liston et al. also found that Treg cell-mediated tolerance is critically dependent on miRNA pathway as miRNA depletion within the Treg cell lineage resulted in fatal autoimmunity indistinguishable from Treg cell-deficient mice [16]. Overall, the ability of miRNAs to modulate both innate and adaptive immunity, and both T and B cell biology, strongly suggested that miRNAs will represent an
additional class of genes involved in the development of autoimmune disorders, that might represent novel innovative targets for molecular intervention.

The first clinical data linking RNAi pathways to autoimmunity showed that specific anti-SU auto-antibodies detected in the serum of patients suffering from Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA), are directed towards Argonaute 2 and other components of the GW/P bodies, a unique cytoplasmic structure involved in mRNA processing and RNAi [17]. In 2008, using a genome-wide disease phenocode analysis, Glinsky determined relationships between disease-linked SNPs, miRNAs and mRNAs expression patterns in PBMCs of 16 common human disorders, and identified components of the nuclear import and inflammasome pathways to be associated with human genotypes of RA and Crohn’s disease populations [18]. Since then, the list of miRNAs deregulated in autoimmune disorders and shown to be involved in disease pathogenesis is constantly implemented (Table 1).

miRNAs in rheumatoid arthritis.

Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune disorder, primarily affecting the articular structures and synovial membranes of the multiple joints. Recent studies have uncovered dysregulated miRNA expression in RA patients, suggesting that abnormalities in miRNA expression may contribute to the molecular mechanisms of the disease. In the following sections, we will overview the current data on differential
miRNAs expression in RA, both in the afflicted joints and the circulation (Table 2).

**miR-15/16**

Using the autoantibody-mediated arthritis model, Nagata and colleagues showed that the expression levels of miR-15a are lower in the synovium of diseased mice than in healthy controls [19]. Knowing that miR-15a induces cell apoptosis by negatively regulating the expression of Bcl-2 [20], they transiently over-expressed miR-15a into the synovium of arthritic mice thanks to the intra-articular electrotransfer of miR-15a-atelocollagen complexes. They showed a decreased Bcl-2 protein expression and increased expression of caspase 3, as compared with that the control group. Unfortunately, no data on the clinical effect was reported in this study. MiR-15a/16-1 and miR-15b/16-2 clusters are highly conserved among mammalian species [21] and have been shown to play very important roles in regulating cell proliferation and apoptosis by targeting cell cycle proteins and the anti-apoptotic genes (Table 2). Several groups evidenced dysregulated expression of miR-15/16 in various lymphoid and myeloid malignancies, further emphasizing their important roles in growth signaling networks. Interestingly, Pauley and colleagues showed that expression levels of miR-16 are increased in PBMC isolated from RA patients, compared with healthy donors, and are correlated with disease activity, suggesting that miR-16 might be used as biomarker of disease activity [22]. This observation was confirmed by the recent study from Murata et al [23] showing a significant correlation of plasma miR-16 with DAS28. Moreover,
synovial fluid concentrations of miR-16 are significantly higher than those of OA and of RA plasma, and mainly reflect secretions by synovial tissues. Interestingly, miR-16 levels are lower in OA than in healthy donors. From other work, miR-16 is known to be expressed by human primary macrophages [4]. Finally, mice transgenic for the hTNF gene but lacking the 3’UTR that contains sequences for the ARE binding protein TTP and miR-16 overexpress TNF-α protein and develop spontaneous severe arthritis syndrome because of impaired regulation of TNF biosynthesis [24].

**miR-124a**

Recently published profiling data from miRNA expression in RASF versus OASF revealed several novel differentially expressed miRNAs. Particularly, miR-124a is down-regulated in RASF as compared with OASF, and its enforced expression in RASF suppresses their proliferation [25]. The authors showed that miR-124a targets the 3’UTR of CDK-2 and MCP-1 mRNAs and decreases their protein levels. Using reporter gene analyses, IκBζ appeared to be another target gene for miR-124a, suggesting its involvement in the fine-tuning of NFκB-mediated gene expression [26]. miR-124a has been identified as specifically expressed in the pancreas and brain, participating to metabolic regulation [27, 28]. Depending on the malignancy studied, miR-124a is either a tumor suppressive miRNA or a metastasis-associated miRNA.

**miR-132**
In RA and OA, synovial fluid concentrations of miR-132 were significantly lower than their plasma concentrations, and plasma miR-132 were significantly lower than that of healthy donors [23]. These authors also showed that plasma miR-132 well differentiated healthy from patients with RA or OA, while synovial fluid miR-132 differentiated RA from OA patients, suggesting that detection of miR-132 expression levels might be used as diagnosis marker. These data are in contradiction with the report from Pauley showing that PBMC isolated from RA blood display increased expression of miR-132 compared with healthy controls [22]. miR-132 has been mainly studied in brain and neurotransmission, but it also plays anti-inflammatory functions [29]. Its expression in monocytes and role in the regulation of antiviral immunity has been recently reported [30].

miR-146a

In five studies, miR-146 is shown up-regulated in all RA samples investigated. In four of them, an increased basal expression of miR-146a was reported in RASF compared with OASF [31, 32] and RASF are shown to be more sensitive than OASF to LPS- or IL-1β-induced miR-146a expression. In two out of these 5 studies, 2 groups also quantified miR-146a locally, either directly within the synovial fluid [23] or in CD4+ T cells isolated from synovial fluid [33], and found higher concentrations in RA than in OA, correlated with disease activity. Finally, in three of these studies, miR-146 is up-regulated in the circulation of RA patients compared with healthy donors; data were generated either
from the plasma [23], or from PBMC [22] and CD4+ T cells isolated from the blood of RA patients. As mentioned by Murata et al., the plasma levels of miR-146a were comparable between RA and OA, and Pauley found that increased miR-146a expression levels correlated with active disease in RA patients.

It has been previously reported that miR-146a/b is induced in response to a variety of microbial components and pro-inflammatory cytokines, such as LPS, CpG, IFN-α and TNF-α [2]. Conversely, ectopic expression of miR-146a in PBMCs enhances the function of Th1 cells and induces the expression of TNF-α, MCP-1, NF-κB p65 [34]. In RA, Li et al. showed that the level of miR-146a expression is positively correlated with levels of TNF-α. Induction of miR-146a expression is regulated by NF-κB and miR-146a targets TNF receptor–associated factor 6 (TRAF6), IL-1 receptor–associated kinase 1 (IRAK1) and IRAK2, FADD, IRF-5, Stat-1, and PTC1 genes [35-38]. Thus, miR-146 is among the genes playing a role in the fine-tuning of innate immune responses through negative feedback loops on genes induced by inflammatory cascades. Despite the increased expression of miR-146a in RA PBMC, TRAF6 and IRAK-1 are equally expressed in PBMC isolated from both RA patients and healthy controls [22]. Furthermore, enforced expression of miR-146a impairs both activator protein 1 (AP-1) activity and interleukin-2 (IL-2) production [35], and Fas associated factor 1 (FAF1) [33], thus suggesting a role in the modulation of TCR engagement and T cell apoptosis. While miR-146a levels are low in monocytes and non detectable in neutrophiles, they are constitutive high in langerhans cells, in which it regulates cell sensitivity to inappropriate TLR2 activation by
commensal bacteria [39]. Finally, it has been shown that ectopic expression of miR-146a in primary human fibroblasts suppresses IL-6 and IL-8 secretion through decreased IRAK1, suggesting that miR-146a might be part of a compensatory network aiming at restraining inflammation [40].

miR-155
The first miRNA profiling experiment performed in TNF-stimulated RA synovial fibroblasts (RASF) with the intention to mimic inflammatory conditions in the RA joint, revealed significant up-regulation of miR-155 [32]. Stanczyk and colleagues showed a marked up-regulation (8-fold) of miR-155 in RASF compared to synovial fibroblasts isolated from OA patients, as well as in the whole synovial tissue of RA patients. Interestingly, Murata and coworkers showed that miR-155 is secreted by synovial tissues in the synovial fluid, and confirmed that synovial fluid concentrations of miR-155 are higher in RA than those in OA [23]. In the periphery, miR-155 is up-regulated in both plasma and PBMC from RA patients compared to healthy donors (1.8 to 2.6 fold) [22, 23]. Moreover, compared with monocytes from RA PBMC, RA synovial fluid monocytes displayed higher levels of miR-155 [32]. Finally, the functional analysis indicated the involvement of miR-155 in the control of expression of MMP-3 and MMP-1 under basal as well as inflammatory conditions. Numerous target genes have been identified for miR-155, such as c-Maf, Bach1, PU.1, c/EBP and SHIP1. Recently, McCoy C et al. [41] showed that, in bone marrow-derived macrophages, the anti-inflammatory
cytokine IL-10 inhibits the LPS-induced transcription of miR-155 from the BIC gene, in a STAT3 dependent manner, leading to an increased expression of SHIP1, a miR-155 target gene.

**miR-223**

As for other miRNAs investigated by Murata and colleagues, concentrations of miR-223 in the synovial fluid are lower than those in plasma, for both RA and OA patients, and are mainly due to miR-223 secretion by MNC infiltrating inflamed joints [42]. Indeed, miR-223 is rarely secreted by RA-FLS and synovial tissues. Interestingly, miR-223 levels were higher in RA samples than in OA, and inversely correlated with tender joint count. Fulci and coworkers showed that miR-223 is the only miRNA that is markedly up-regulated in peripheral T-lymphocytes from RA patients compared with healthy donors, mostly in naive CD4+ T lymphocytes [42]. An up-regulation of miR-223 in leukemia is widely reported in the literature. Serum miR-223 has been also proposed as a potential biomarker for sepsis [43]. miR-223 regulates glucose metabolism [44], and granulopoiesis by targeting E2F1 and CEBPα [1].

**miR-346**

Alsaleh and coworkers described another miRNA to be induced by LPS and in turn to counteract the stimulatory effect of LPS on the IL-18 production [45]. This study demonstrated that miR-346 indirectly impacts on IL-18 release by inhibiting LPS-
induced Bruton’s tyrosine kinase expression in RASF. A target sequence for miR-346 was found in the 5′UTR of RIP140 mRNA (receptor-interacting protein 140), suggesting a regulatory role in hormonal and metabolic processes [46].

Finally, **miR-363** and **miR-498** are down-regulated in CD4+ T cells isolated from RA synovial fluid, as compared to normal peripheral blood CD4+ T cells, respectively [33].

**Conclusion**

Identification of biological markers of disease is a major area of research, especially in RA. Due to the simplicity of getting a blood sample, easily testable, biomarkers found in blood or serum would have great potential for clinical application. Mitchell and co-workers showed that microRNAs are present in the blood and serum of healthy donors in remarkably stable forms [47]. They are well protected from RNases and remain stable after being subjected to harsh conditions. The progression of pregnancy has also been correlated with placental-associated circulating miRNAs. Such peripheral blood-based miRNA signatures are due to the presence of circulating microparticules and exosomes, as it was demonstrated in the plasma and sera of normal subjects and ovarian cancer-bearing patients. The majority of these microvesicles were shown to derive from peripheral blood platelets and mononuclear phagocytes [48] in healthy donors, and from tumor cells shedding into the blood stream of patients with ovarian cancer [47]. From the above cited works, three studies confirmed that, miR-16, miR-132, miR-146a, miR-155,
and miR-223 are up-regulated in RA circulation compared to controls. Although most of these miRNAs are also deregulated in other immune-mediated disorders and cancers, the definition of a blood-based miRNA signature containing these miRNAs, and others to be yet identified, might serve as biomarker for RA diagnosis in a close future.

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No conflict of interest to be declared.
Bibliographic references


Table 1: miRNAs involved in autoimmune diseases.

<table>
<thead>
<tr>
<th>Up-regulated miRNA</th>
<th>Down-regulated miRNA</th>
<th>Associated Diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21, miR-61, miR-78, miR-142-3p, miR-189, miR-198, miR-298, miR-299-3p, miR-342, miR-371-5p, miR-423-5p, miR-638, miR-663</td>
<td>miR-146, miR-17-5p, miR-112, miR-141, miR-184, miR-196a, miR-383, miR-409-3p, miR-146a</td>
<td>SLE</td>
<td>[38, 49, 50]</td>
</tr>
<tr>
<td>miR-493, miR-599, miR-326, miR-34a, miR-155, miR-326</td>
<td>miR-18b</td>
<td>MS</td>
<td>[14, 51, 52]</td>
</tr>
<tr>
<td>miR-146a, miR-203, miR-221, miR-205, miR-21</td>
<td></td>
<td>Psoriasis</td>
<td>[53, 54]</td>
</tr>
<tr>
<td>miR-16, miR-132, miR-146a, miR-155, miR-203, miR-223, miR-346</td>
<td>miR-124a, miR-15a, miR-498, miR-363</td>
<td>RA</td>
<td>[19, 22, 23, 25, 32, 33, 42, 45]</td>
</tr>
</tbody>
</table>

Abbreviations: SLE, Systemic lupus erythematosus; MS, Multiple sclerosis; RA, Rheumatoid arthritis.
**Table 2:** Validated targets and functions of microRNAs dysregulated in rheumatoid arthritis.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Targets</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mir-15/miR-16</td>
<td>BCL2, DLK1, PDCD4, TIA1, RASSF5, Bim-1, Cyclin D1, cyclin E1, MYB</td>
<td>Apoptosis, Proliferation</td>
<td>[20, 55-60]</td>
</tr>
<tr>
<td>miR-124a</td>
<td>CDK-2, MCP-1, IκBζ</td>
<td>Apoptosis, Inflammation</td>
<td>[25, 26]</td>
</tr>
<tr>
<td>miR-146a</td>
<td>IRAK1, IRAK2, TRAF6, FADD, IRF-5, STAT1, PTC1, FAF-1</td>
<td>Inflammation, Apoptosis</td>
<td>[2, 33, 35-38]</td>
</tr>
<tr>
<td>miR-155</td>
<td>MMP-1, MMP-3, c-Maf, Bach-1, PU.1, CEBP, SHIP-1, ZIC3, HIVEP2, ZNF652, ARID2, SMAD5</td>
<td>Remodeling, inflammation, Antiviral immunity</td>
<td>[32, 41]</td>
</tr>
<tr>
<td>miR-223</td>
<td>E2F1, CEBP</td>
<td>Granulopoiesis, Glucose metabolism</td>
<td>[1, 44]</td>
</tr>
<tr>
<td>MiR-346</td>
<td>Btk, RIP140</td>
<td>Inflammation, Hormonal, metabolism processes</td>
<td>[45, 46]</td>
</tr>
</tbody>
</table>
This table lists all the miRNAs that have been deregulated in rheumatoid arthritis, as well as their validated targets and functions.

<table>
<thead>
<tr>
<th>miR-132</th>
<th>ND</th>
<th>Neurotransmission</th>
<th>[29, 30, 33]</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-363</td>
<td></td>
<td>Inflammation</td>
<td></td>
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<tr>
<td>mir-498</td>
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</table>

Abbreviations: BCL2, B-cell CLL/lymphoma 2; DLK1, delta-like 1 homolog; PDCD4, programmed cell death 4; TIA1, T-cell-restricted intracellular antigen-1; RASSF5, Ras association domain family member 5; Bim-1, BCL2-like 11; MYB, v-myb myeloblastosis viral oncogene homolog; CDK2, cyclin-dependent kinase 2; MCP-1, monocyte chemoattractant protein; IkBζ, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta; IRAK, IL-1 receptor activated kinase; TRAF, TNF receptor-associated factor; STAT, signal transducer and activator of transcription; IRF, Interferon regulatory factor; IKK, IkappaB kinase; FADD, Fas-associated via death domain; PTC1, patched homolog 1; FAF1, Fas (TNFRSF6) associated factor 1; MMP, matrix metallopeptidase; C-Maf, v-maf musculoaponeurotic fibrosarcoma oncogene homolog; Bach-1, BTB and CNC homology 1, basic leucine zipper transcription factor 1; PU.1, spleen focus forming virus (SFFV) proviral integration oncogene spi1; CEBP, CCAAT/enhancer binding protein; SHIP-1, inositol polyphosphate-5-phosphatase; ZIC3, Zic family member 3; HIVEP2, human immunodeficiency virus type I enhancer binding protein 2; ZNF652, zinc finger protein 652; ARID2, AT rich interactive domain 2; SMAD5, E2F1, E2F transcription factor 1; Btk, Bruton agammaglobulinemia tyrosine kinase; RIP140, nuclear receptor interacting protein 1.
**Table 3:** Tissue distribution of miRNAs dysregulated in RA.

<table>
<thead>
<tr>
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<th>BLOOD</th>
<th>JOINT</th>
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<tbody>
<tr>
<td></td>
<td>PBMC</td>
<td>Serum/plasma</td>
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<tr>
<td>miR-15a</td>
<td></td>
<td></td>
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<tr>
<td>miR-16</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>miR-124a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mir-132</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Mir-146a</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Mir-155</td>
<td>X</td>
<td>X</td>
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<td>Mir223</td>
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<td>X</td>
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<td>Mir346</td>
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<td>Mir-363</td>
<td>X</td>
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<td>Mir-498</td>
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</table>

Abbreviations: PBMC, Peripheral Blood Mononuclear Cell; ST, synovial tissue; RA-FLS, fibroblast-like synoviocytes from RA patients; SF, synovial fluid.