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Role of MicroRNAs in Rheumatoid Arthritis

Merih Birlik, Ayşe Koçak and Duygu Harmancı

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Abstract

Rheumatoid arthritis (RA) is a common autoimmune disease. The hallmarks of RA are synovial inflammation and hyperplasia, autoantibody production, systemic features, and deformity. A lot of researchers have paid attention to the possibility that microRNAs (miRNAs) play a role in the pathogenesis of RA. miRNAs are a class of small noncoding RNAs, which have 18–25 nucleotides. These small RNAs modify gene expression by binding to target messenger RNA (mRNA), and they block the translation or induce the degradation of target mRNA. Biological relevance of miRNAs has been investigated in physiological and pathological conditions. A growing body of evidence suggests that miRNAs participate in the inflammatory disorders including RA. In this chapter, an overview of biogenesis and function of miRNAs has been presented to introduce researchers to the changes and functional regulation of the key miRNAs in RA and to provide current knowledge in miRNA and RA. It is important to understand the relationship between the key miRNAs and RA pathology as modulation of specific miRNA alterations could be of great pharmaceutical interest in the future.

Keywords: microRNA, miRNA, small noncoding RNA, rheumatoid arthritis, pathogenesis

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovial inflammation exemplified by cell hyperplasia, autoantibody production, systemic features, and deformity [1–3]. It affects approximately 0.36–2.0% of the human population worldwide and roughly three to four times more females than males [2, 4]. RA progression is a complex process and consists of many genes and environmental factors [3]. Accumulating clinical and

experimental evidence points out that the immune system plays a key role in disease pathogenesis. However, pathogenesis of RA is not entirely clear [1–3]. Generally, RA progression leads to joint destruction, functional disability, and cardiovascular comorbidities [5]. The immune system and different cell types such as macrophages, T and B cells, and synovial fibroblasts participate in the pathogenesis of RA. RA synovial fibroblasts and "fibroblast-like synoviocytes" (FLS) play an important role in RA [6, 7].

Genetic and environmental factors are associated with RA. These factors include infectious agents, exposure to cigarette smoke, air pollution, insecticides, and occupational exposures to mineral oil and silica. But so far, smoking is the only environmental risk factor that has been extensively studied and widely accepted [3]. When environmental factors are considered some of them can be controlled, but the control of genetic and epigenetic regulation is highly complex and difficult to predict. The contribution of epigenetic gene regulation is considerably less well established. Epigenetics is a mechanism that regulates gene expression independently of the underlying DNA sequence. In early 2000s, with the discovery of micro RNAs (miRNAs), researchers have started to discuss their roles in physiological and pathologic processes [8]. miRNAs are small noncoding RNAs 18–25 nucleotides in length that block the translation or induce the degradation of target mRNA [9]. The clinical application of epigenomic information has improved in recent years. A growing body of evidence suggests that miRNAs participate in the inflammatory disorders including RA [10–12].

In this chapter, we summarize the biogenesis and function of miRNA, with an emphasis of its role in the pathogenesis and the importance of miRNA in RA as a potential biomarker.

2. What are microRNAs?

The small RNAs are crucial for the cellular gene regulatory systems. Over the last decade, these small RNAs have become important regulators in eukaryotic genomes. These noncoding RNAs regulate genes and genomes. This regulation includes chromatin structure, transcription, RNA processing, RNA stability, and translation. Generally, the small RNAs show inhibitory activity on gene expression. One type of these small RNAs is microRNAs [13].

MicroRNAs (miRNAs) are small endogenous noncoding RNAs [13]. In 1993, Ambros and his collaborators discovered the first miRNA, lin-4 from *Caenorhabditis elegans*. Researchers studied the postembryonic development of *C.elegans* and found two noncoding RNAs which were produced by lin-4 with complementary sequences to the 3′UTR (un-translated region) of lin-14 mRNA [14]. These small transcripts could bind the 3′UTR of lin-14 mRNA and decrease protein levels without affecting mRNA stability. In early 2000s, Reinhart (and) et al discovered the second miRNA, let-7, and following this discovery, more than a dozen miRNAs were found in plant and animal species in 2000s [15–18]. The name "microRNA" was first used in 2001, and papers published in science reported that there were up to 50 miRNAs [19–22]. The brief history of miRNAs is reviewed in **Figure 1**.
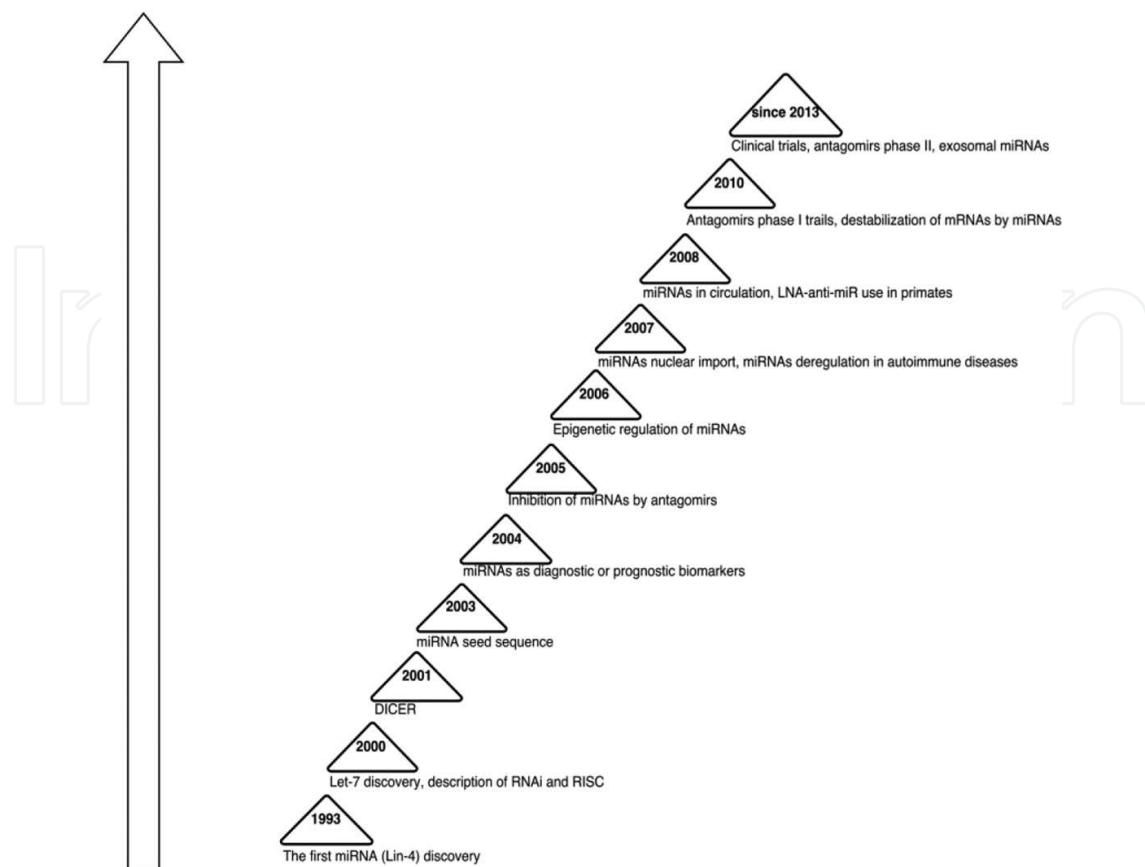
Figure 1. The brief history of miRNAs.

miRNAs are short (about 18–25 nucleotides long) noncoding RNAs that can affect messenger RNA (mRNA) processing at the post-transcriptional level [15]. They are endogenous and goal-oriented expressed products of an organism's own genome [13]. Chromosomal locations of miRNAs affect the expression and function themselves [23]. According to their genomic localizations, miRNAs can be classified into five groups [24]:

1. Intergenic miRNAs (miRNA genes are located between two consecutive protein-coding genes).

2. Intronic miRNAs in noncoding transcripts (miRNA genes are located in any intron of a noncoding RNA gene).

3. Intronic miRNAs in protein-coding transcripts (miRNA genes are located in any intron of a protein-coding RNA gene).

4. Exonic miRNAs in noncoding transcripts (miRNA genes are located in any exon of a noncoding RNA gene).

5. Exonic miRNAs in protein-coding transcripts (miRNA genes are located in any exon of a protein-coding RNA gene).

Some miRNAs are located in every second group because of alternative splicing [9]. Intergenic miRNAs are transcribed from their own promoters but intragenic miRNAs generally located in the same orientation as the host genes [24] (**Figure 2**).
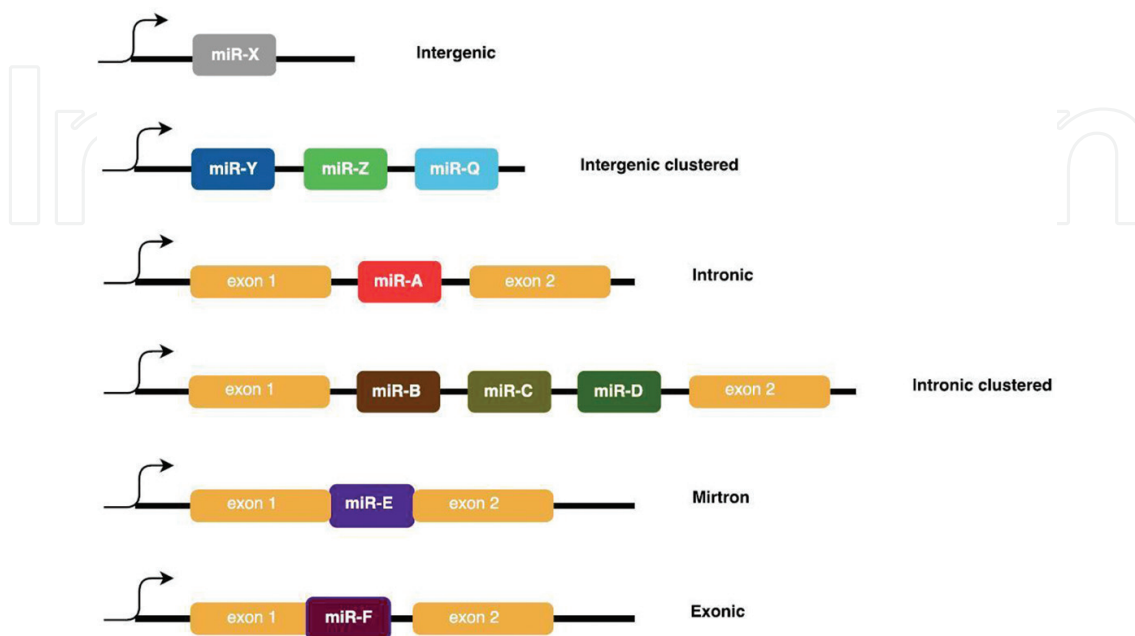

Figure 2. The genomic organization of miRNAs.

The scientific denomination rule is simple for the miRNAs. The mature miRNA is abbreviated with a capitalized "miR-" while the uncapitalized "mir-" indicates both the gene locus and precursor miRNA (pre-miRNA and pri-miRNA). Every miRNA is named according to discovery order, and "MIR" refers to the gene that encodes them. The numbering of miRNA genes is simply sequential. For example, miR-21 was discovered before the miR-155 [10]. In addition, three letters specific for each species precede each name. Thus "hsa-miR155" means that this miR origins from *Homo sapiens* while ggo-mir-155 is an example of gorilla's miRNA name. A letter after the number in the suffix is used to differentiate among multiple members of the same family (e.g., hsa-mir-155a and hsa-mir-155b). Today, miRNAs is still a popular subject for the researchers and have been studied in many organisms. Some miRNA databases (http://www.mirbase.org/, http://mirdb.org/miRDB/, http://www.microrna.org/microrna/home.do and so forth) are available and every moment the miRNA list is updated [25, 26].

3. miRNA biogenesis

In the early 2000s, the scientific world discovered small RNAs. They work as gene regulators, but their biogenesis is still unclear. Most mammalian miRNA genes are located in the intron region; some are located in the exon region and also approximately 30% are located in the intergenic regions. Since 2000, the miRNA biogenesis pathway has been studied and in 2001, Mello and his colleagues obtained the first data [17].

Generally, mammalian miRNA biogenesis pathway can be divided into two main parts with regard to processing pri-miRNAs: canonical and noncanonical [27]. In the canonical miRNA biogenesis pathway, the pri-miRNA is processed to pre-miRNA in the nucleus. In the noncanonical miRNA biogenesis pathway, mirtrons participate in microprocessor processing as alternative miRNA precursors. There are some differences between pri-miRNAs and mirtrons. Mirtrons arise from the spliced-out introns and lack lower stem loop structure and the flanking single-stranded segments thus the mirtrons bypass Drosha processing [28, 29].

The canonical miRNA biogenesis pathway is Drosha and Dicer dependent. Drosha is a member of ribonuclease III enzyme family, and the core nuclease that initiates miRNA processing in the nucleus [16]. Dicer is also a member of RNAse III family. It cleaves double-stranded RNA (ds-RNA) and precursor miRNA (pre-miRNA) into short dsRNA fragments. Dicer forms the mature miRNA in the cytoplasm [16, 18]. The noncanonical miRNA biogenesis pathway is Drosha independent/Dicer dependent. Here, microprocessor is not required. Mirtrons are a type of miRNA that are direct Dicer substrates. A number of noncanonical pathways have been described, but most pathways remain unclear. Besides, mirtrons comprise small part of the miRNAs, so generally miRNAs are processed via canonical pathway [16, 18, 27].

Microprocessor components, Drosha, DiGeorge critical region 8 (DGCR8, also known as Pasha), and Dicer, are essential for the canonical miRNA biogenesis, and the biogenesis pathway is compartmentalized and stepwise [27]. The canonical mRNA biogenesis pathway starts with RNA Pol II-mediated transcription of genomic region containing miRNA genes. Transcripts (pri-miRNAs) are capped (at the 5′ end) and polyadenylated (at the 3′ end). The pri-miRNAs are cleaved to pre-miRNA hairpins by RNAse III enzyme Drosha in the nucleus. DGCR8 (Pasha) proteins accompany this processing. Then, pre-miRNAs are exported from the nucleus to cytoplasm by exportin-5 (XPO-5) via Ran-GTP–dependent mechanism. In the cytoplasm, pre-miRNA is processed into a mature miRNA. RNAse III Dicer cleaves the pre-miRNA together with trans-activation response RNA binding protein (TRBP)/protein activator of PKR (PACT) proteins to produce unstable, 18–22 nt asymmetric miRNA:miRNA* duplex.

miRNA represents guide RNA strand, and the other miRNA* means passenger RNA strand. The guide RNA strand incorporates into RNA-induced silencing complex (RISC), and passenger RNA strand is discarded. Argonaute (AGO) is one of the key components of RISC for this process [18, 27]. The canonical miRNA biogenesis pathway is summarized in **Figure 3**.

The alternative miRNA biogenesis pathways are named noncanonical pathways. These pathways do not require all enzymes and protein factors of the canonical pathway. Some miRNAs differ from the others in terms of structure and function and these miRNAs bypass one or more steps of miRNA canonical biogenesis [27, 29]. Dicer is required for both pathways—canonical and noncanonical, but Drosha and DGCR8 are only needed for the canonical pathway. If there is a mutation or deficiency of these enzymes, miRNA biogenesis goes on via the noncanonical pathway. The noncanonical miRNA biogenesis pathway can be divided mainly into two classes [27, 30].
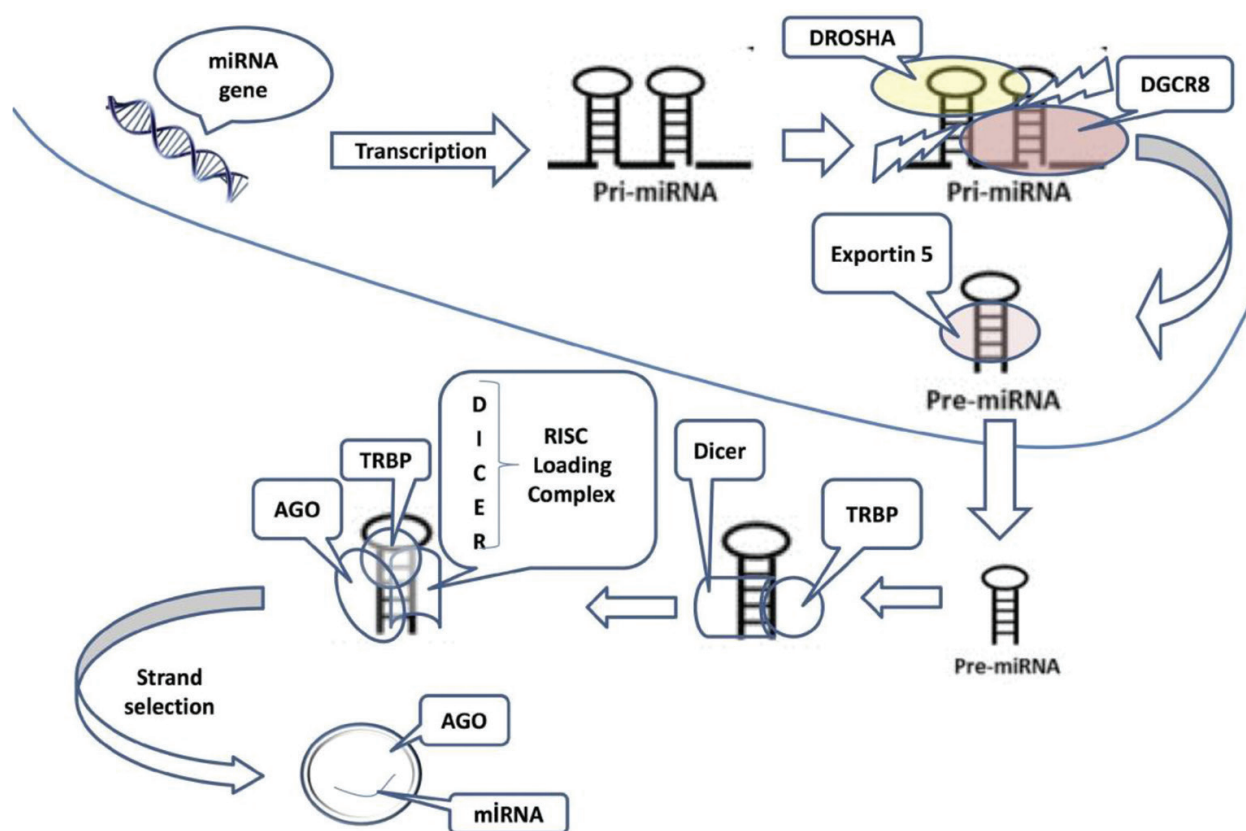
Figure 3. The canonical miRNA biogenesis pathway.

3.1. Drosha/DGCR8-independent/Dicer-dependent pathways

3.1.1. Mirtron pathway

Some introns form short RNA hairpins, and they are termed "mirtrons" that can be spliced resulting in a nonlinear intermediate. Investigations of the data of small RNAs from Drosophila melanogaster and Caenohabditis elegans led to the discovery of mirtrons [28]. They can be debranched into pre-miRNA hairpins and form the duplex. After this step, this product can join he (the) canonical pathway and is ready to be transported to the cytoplasm by exportin-5 (EXP-5) [29].

Depending on the splice donor and acceptor site, mirtrons can be categorized into three groups: 5'-tailed mirtron, regular mirtron, and 3'-tailed mirtron. Generally, 3'-tailed mirtrons have been found in Drosophila, and 5'-tailed mirtrons have only been described in vertebrates [28, 29]. Mirtrons being a new research area, there are many unanswered questions.

3.1.2. snoRNA-derived RNAs (sdRNAs)

Small-nucleolar RNAs are a class of noncoding RNAs. Generally, they show similar properties and structures to miRNAs. They are dependent on Dicer and can be bound with AGO acting as a miRNA [29, 31].

3.1.3. miRNAs from tdRNAs

The first (tdRNA) tRNA-derived RNA species to be discovered were isoleucine tRNA-derived miRNA [27]. Normally, they differ from regular miRNAs but they have RNA silencing function. tRNA-derived (tdRNA) precursors can be a potential substrate of Dicer because of their different folding form [29, 31]. In the light of this information, they have different properties from typical mammalian miRNAs, and their pattern of behavior remains a puzzle.

3.1.4. miRNAs from endogenous short hairpin RNAs (shRNAs)

Several studies have shown that some shRNAs could join the noncanonical miRNA-processing pathway. Normally, in the canonical miRNA pathway, pre-miRNAs hairpins are conserved and thus can be recognized by microprocessor complex. However, shRNA-derived miRNAs do not have the microprocessor sequence. For this reason, they are DiGeorge critical region-8 (DGCR-8) independent [27, 29]

3.2. Drosha/DGCR8-dependent/Dicer-independent pathways

Some miRNAs mature in a different way. One of them is miR-451. MiR-451 maturation occurs without Dicer. Initially, pri-miR-451 is cleaved by Drosha/DGCR8 to form pre-miRNA duplex. But it is too short to present to Dicer as a substrate, so directly it interacts with AGO proteins [29, 31]. The miRNA biogenesis pathways and their regulation have been studied extensively in last 10 years, but further information is required for miRNA processing.

4. miRNA function

The functions of miRNAs are post-transcriptional repression and degradation of mRNAs [13]. They have different expression patterns and regulate several biological and pathological processes [16, 32, 33]. The major function of miRNAs is protein synthesis inhibition. They can maintain this function either by inhibition of translation or by mRNA degradation [34, 35]. The miRNA-mRNA interaction is important for miRNA function, and each mature miRNA interacts with specific miRNA/miRNAs. Generally, the interaction takes place between the miRNA seed region and 3′ UTR of mRNA for the inhibition of target mRNA. There are a few exceptions about miRNA binding sites. In some cases, the miRNA-mRNA interaction occurs at the 5′-UTR. The second issue is miRNA's tissue specificity. MiRNAs are expressed in a tissue-specific fashion, and also, the action of miRNA is tissue specific [36–39].

Identification of miRNA target genes is crucial for the functional characterization of miRNAs. Recently, we can predict the target gene with the help of some bioinformatics algorithms and software. The most popular software is TargetScan [5, 10]. If in silico analysis is insufficient, further analysis can be needed. Some genetic or biochemical methods are preferable. The success of the prediction is important to understand miRNA function.

The miRNAs have a part as an adaptor for miRISC to recognize and regulate target mRNAs. MiRISC regulates the translation of AGO proteins by introducing silencing through mRNA deadenylation, degradation, or translation [13]. miRNAs which are integrated into the RISC complex control the gene expression the level of post-transcription by reducing miRNA stability or inhibiting translation of mRNA [9, 32, 40]. Besides, miRNAs can affect several epigenetic regulators (DNA methyltransferases and histone deacetylases) [16].

In addition to mRNAs repression, some miRNAs have positive effects on gene expression [9]. This can occur directly or indirectly. For instance, miR-373 binds to the promoter of E-cadherin directly and increases the function of RNA polymerase. If its target mRNA does not consist of poly (A)-tail, AGO2 activates the gene expression [8, 9].

The selectivity in gene regulation mechanism depends on the degree of the match occurred between target mRNA and miRNA. Exact match is usually found in plants. miRISC complex which make partial matches is required to make an exact match with the seed region in the related to miRNA to inhibit the translation [41, 42]. Although many miRNAs cause of gene silencing by reducing the target mRNA stability, some miRNAs lead to gene silencing by inhibiting target mRNA translation, but there is not enough evidence about what stage they repress. In general, studies suggest that miRNAs repress the translation with four different mechanisms: (1) suppression of protein synthesis, (2) a drop of premature mRNAs in ribosomes, (3) slowing down during the alongation step of protein synthesis, (4) in parallel with protein synthesis the fragmentation of protein [40, 43, 44].

The function of miRNAs has been studied since the first two microRNAs discovered. miRNAs are considered key regulators to physiological processes, such as cellular and tissue differentiation, apoptosis, innate, and adaptive immune responses and development [13, 17, 35, 45, 46]. Thus, miRNAs are crucial for the regulation of biological processes [17]. Deregulation of this regulatory network can lead to the initiation of undesired pathological processes, such as cancer, fibrotic diseases, and autoimmune diseases [17, 35, 45, 46].

miRNAs have essential roles in immune system as they arrange innate and adaptive immune systems and have pivotal roles in the regulation of inflammation, by controlling the differentiation of B cells, conventional T cells, and regulatory T cells [11, 39].

4.1. Innate immunity and miRNAs

The innate immune system fights microorganisms. It has many cell types, which are physical epithelial barriers, phagocytic leukocytes, dendritic cells, natural killer (NK) cells, and circulating plasma proteins. Pattern recognition receptors (PRRs) recognize microorganisms and include toll-like receptors (TLRs), C-type lectin-like receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like-receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). This process starts to intracellular signaling pathways. TLR has 10 subtypes such as TLR1, TLR2, TRL3, TLR4, TLR5, TLR6, TRL7, TRL8, TRL9, and TLR10 [47–50]. TLR4 identify lipopolysaccharides (LPSs). miR-146a/b, miR-132, and miR-155 are overexpressed for the LPS-mediated inflammatory responses. miR-146 upregulation causes interleukin-1 receptor-associated kinase (IRAK) 1 and genes expression and tumor necrosis factors

receptor–associated factor (TRAF) 6 [51]. miR-146 was investigated in *in vitro* model of mouse macrophages, and it is a negative regulator of IRAK1, IRAK2 and TRAF6 [52]. On the other hand, miR-155 and miR-125b levels are related to TNF-α secretion in mouse macrophages. Regulation of miR-155 and miR-125b is LPS/TNF-α dependent; therefore, it is suggested that they are new targets of drug design. miR-155 increases TNF-α translation, Fas-associated death domain protein (FADD), IkappaB kinase epsilon (IKKepsilon), and TNFR superfamily-interacting serine–threonine kinase 1 (Ripk1) [53]. One study showed that miR-147 increased in murine macrophages after stimulation with TLR2, TLR3, and TLR4 ligands and in the lungs of mice after LPS exposure [54]. Also, NF-κB is regulated by miR-1303. Mir-1303 regulates the process of autophagy related to mycobacteria by targeting Atg2B [55]. Besides, miR-146a and miR-155 affect IFN-type I synthesis by TLR-7 and TLR-9, some miRNAs such as miR-126, miR-21, miR-146a, miR-155, and miR-1246 can mediate regulation with epigenetic modification [56].

4.2. Adaptive immunity and miRNAs

T and B-lymphocytes are major cellular components of the adaptive immunity. As previously described, Dicer enzymes are important in the biogenesis of miRNA. In B cells, miR-181b and miR-148a play role as miR-148a inhibits the expression of the autoimmune suppressor Gadd45α [57, 58]. In T cell early development, decrease of Dicer leads to reduction of T cell numbers both in the thymus and peripheral lymphoid organs [59]. In early B cell development, miR-17 deficiency induced by ablation of Dicer in pro-B cells affected B cell antibody diversity and B cell survival [60]. miR-326 and miR-155 are related to IL-17 [61]. Moreover, miR-155 expression related to Th17 cell production and miR-155 downregulated the LPS-induced inflammatory process [62, 63]. miR-146 has an essential role in the adaptive immune system. In the Treg cells miR-146 is an activator of STAT1 transcription that is associated with Th1-mediated autoimmunity [64].

Many studies in animal (many researches on animal olabilir) models and clinical studies showed that miRNAs participate in the inflammation and autoimmune diseases mechanisms. In humans, loss of miRNA expression related of the many autoimmune conditions. Especially, multiple sclerosis (MS), osteoarthritis, and rheumatoid arthritis (RA) are most studied (are the most).

5. Expression profiles of miRNAs in rheumatoid arthritis patients

RA, characterized by chronic joint inflammation, is an autoimmune disease. Small joints of the hands and feet are affected most (more or the most) often; however, the disease is known to vary among individuals. In the general population, RA affects roughly three times more women (females) than men (males) and is more common between 35 and 50 years and in older individuals [65–68]. Persistent inflammation over time leads to joint damage and disability [69]. This disease is characterized by autoimmunity with multiple joint lesions and systemic inflammation. Also, patients can develop complications, which result in lifelong disability and increased mortality. Accordingly, RA is a growing public health concern as it is associated with significant social and economic burden [70].

In genetically susceptible people, various environmental factors can act as a trigger for inducing autoimmunity [71]. Recent experimental data showed that the pathogenesis of RA is associated with epigenetic regulation. Over the past few years, it has become clear that different microRNAs (miRNAs) expression levels, particularly in RA, contribute to the development of autoimmunity. miRNAs plays (play) multiple roles in regulation of pro-inflammatory cytokine secretion and development of immune cells, and they work in the induction or suppression of autoimmunity and ECM remodeling. Thus, dysregulated miRNA expression seems to promote the molecular mechanisms of the RA process [72–77]. Stanczyk et al. described for the first time the dysregulation of miRNAs in RA in 2008 [78]. They reported increased miR-155 and miR-146a expression in RA synovial fibroblasts compared to those in osteoarthritis (OA) patients. miRNAs expression is detected in several cell types in RA, and miRNAs expression profiles can regulate many physiological actions in RA (**Table 1**) [79, 80].

Cell type	miRNA		Refs.
	Downregulated	**Upregulated**	
Synovial tissue	miR-22	miR-146a	[80–86]
	miR-23b	miR-146b	
	miR-30a	miR-150	
		miR-155	
		miR-223	
Synovial fibroblasts	miR-124a	miR-133a	[85–91]
	miR-34a	miR-142-3p	
	miR-22	miR-142-5p	
	miR-152	miR-146a	
	miR-375	miR-155	
		miR-203	
		miR-221	
		miR-222	
		miR-323-3p	
Synovial fluid	miR-152	miR-16	[86, 92, 93]
	miR-375	miR-146a	
		miR-155	
		miR-223	
Synovium macrophages		miR-146a miR-155	[81, 94]
Whole blood		miR-99	[95, 96]
		miR-100	
		miR-125b	
		miR-146a	

Cell type	miRNA		Refs.
	Downregulated	Upregulated	
		miR-155	
Peripheral blood mononuclear cells (PBMCs)	miR-21	miR-26a	[82, 96–98]
		miR-132	
		miR-146a	
		miR-146b	
		miR-150	
		miR-155	
		miR-16	
Plasma and serum	miR-16	miR-16	[91, 95, 98–100]
	miR-125a-3p	miR-21	
	miR-126-3p	miR-24	
	miR-132	miR-26a	
	miR-146a	miR-125a-5p	
	miR-155	miR-125b	
		miR-126-3p	
		miR-223	
		miR-451	

Table 1. Expression of miRNAs in RA.

6. The roles of miRNAs in RA

Several miRNAs are found to be upregulated or downregulated in synovial tissue and cells of joint or blood compartment of patients with RA. The bone and joint destruction occurring in RA is accompanied by specific changes in cellular miRNAs that can affect epigenetic regulation. The current literature demonstrates that a variety of miRNAs is frequently dysregulated in RA patients.

miR-15/miR-16: miR-15a/16-1 and miR-15b/16-2 are highly protected in mammalian species. miR-15a is downregulated in arthritic synovial tissue, which has a potential role in apoptosis [95]. Nagata et al showed that in synovium miR15a, expression levels are lower in diseased mice than in healthy controls [101]. miR-15 plays a role in apoptosis; it has a negative regulatory effect on Bcl-2. Sera of patients with early RA show differential levels of miR-16-1 and miR-16-2, which are increased in peripheral blood mononuclear cells (PBMC) of RA patients [86, 95, 98]. Besides, miR-16 is a good candidate as a marker of disease activity. It is one of the key pro-inflammatory (proinflammatory) mediators in RA: mir-16 being able to target "tumor necrosis factor-α" (TNF-α) may be the biomarker of the RA.

miR-17-92 cluster: It is a controller of apoptosis, lots of research groups found de-regulated expression of miRNAs within this cluster in the context of RA [102].

miR-18: miR18a is upregulated in fibroblast-like synoviocytes (FLS) of RA patients. It is important in inflammation, because miR-18 contributes to NFκBmediated cartilage destruction and chronic inflammation in the joint through a positive feedback loop involving silencing of the "nuclear factor kappa-light-chain-enhancer of activated B cells" (NFκB) inhibitor TNFinduced protein-3 [103].

miR-21: In RA patients, levels of miR-21 are raised in plasma [99]; on the other hand, one study showed that miR-21 level is decreased in peripheral blood mononuclear cells (PBMCs). Downregulation of miR-21 is related to upregulation of "signal transducer and activator of transcription 3" (STAT-3) expression and decreased "forkhead box P3" (FOXP 3) mRNA levels in RA patients [104].

miR-22: Lin and his colleagues showed that expression of miR-22 is down regulated and negatively correlated with Cyr61 expression in synovial tissue of RA patients [85]. CYR61 expression is high in RA FLS and stimulates RA synovial fibroblast proliferation and interleukin (IL)-6 secretion in an autocrine-paracrine manner [85]. CYR61 may encourage synovial tissue hyperplasia alongside IL-6-dependent Th17 differentiation, which is critical for joint lesions in RA.

miR-23b: miR-23b is reported to be decreased in RA. miR-23b expression level is reduced and correlated with the high concentration of IL-17 in tissue samples from RA patients [83]. IL-17 suppresses the expression of miR-23b in human primary FLS. miR-23b regulates IL-1β, TNF-α, and IL-17 signaling and decreased levels of miR-23b in RA may be responsible for the induction of autoimmune inflammation.

miR-24: In RA, increased levels of plasma miR-24 have been reported, also its level significantly increased compared to OA [100]. Furin enzyme is important for the processing of latent TGF-β1, which may increase the inflammation by miR-24. Furin is a protein that is encoded by the FUR gene in humans. It was named FUR because it is in the upstream region of FES oncogene. Furin is also known as paired basic amino acid cleaving enzyme (PACE).

miR-26a: Murata and Nimoto worked with this molecule, and they showed that miR-26a is overexpressed in PBMCs and plasma of RA patients. Thus, it may be biomarker for the diagnosis of RA [82, 100]. miR-26a is upregulated during differentiation of IL-17 and generation of CD4+ cells. CD4+ cells are very important in the pathology of RA [82].

miR27a and miR27b: These miRNAs' expression is decreased in OA chondrocytes and coincides with increased expression of "Insulin Like Growth Factor Binding Protein 5" (IGFBP5) and Matrix Metallopeptidase-13 (MMP13) [105, 106].

miR-30a: miR-30a has been shown to be downregulated in RA synovial tissue and miR-30a-3p has a critical role in the regulation of "B lymphocyte activating factor of the tumor necrosis factor family" (BAFF) expression, which has a major impact in the regulation of the autoimmune responses occurring in RA [107].

miR-34a/miR-34b: miR-34a and miR-34b family are investigated in RA. miR34a is downregulated in RA synovial fibroblasts. The "X-linked inhibitor of apoptosis protein" (XIAP) is identified as a direct target of miR-34a [89]. So, miR-24 may contribute to the impaired apoptosis of activated RA synovial fibroblasts. On the other hand, miR-34b is overexpressed in RA T cells.

miR-124a: In 2009, Nakamachi et al. identified decreased expression levels of miR-124 in RA FLS. They showed that miR-24a targets the "monocyte chemoattractant protein-1" (MCP-1) mRNA expression and "cyclin-dependent kinase-2" (CDK-2) and decreases their protein levels [88]. miR-124 controls inflammatory process in RA, indirectly. The curtailed expression of miR-124a can contribute to RA pathogenesis via increased RA synovial fibroblast proliferation, angiogenesis, and leukocyte chemotaxis [89].

miR-125a-3p/miR-125a-5p/miR-125b: The family of miR-125 is dysregulated in the peripheral blood of RA patients. Kim et al. found that miR-125a-5p and miR-125b activate the pro-inflammatory NF-κB signaling pathway [108]. Thus, they may have roles in the induction of excessive inflammation in RA. Because of that, miRNA levels are monitored in serum from RA patients [95]. MiR-125b has a specific role in the regulation of normal and abnormal immune functions. Hruskova et al. suggested that the expression of miR-125b in those patients may present a novel biomarker for monitoring the treatment outcome during the early phase of RA [109].

miR-126-3p: miR-126-3p is significantly irregular in the plasma of RA patients. There is conflicting evidence regarding miR-126-3p. Wang et al. showed that miR-126-3p is lower in the plasma of RA patients [99], while another research group showed that the expression level of circulated miR-126-3p is markedly elevated in RA [100]. miR-126 can inhibit PI3K/AKT signaling pathway. It may clinically be helpful for RA patients [110].

miR-132: The expression of miR-132 of PBMCs from RA patients was twice higher as compared to controls [45]. However, miR-132 concentrations were found to be lower in RA plasma than in the plasma of healthy individuals [86]. Plasma concentrations of miR-132 differentiated patients with RA or OA from healthy controls, though plasma and synovial fluid miR-132 failed to differentiate RA from OA. Besides, plasma miR-132 or its SF/PB ratio (ratio of concentration of synovial fluid miRNA to plasma miRNA) correlated with tender joint count. These results indicate that miR-132 may have a role in systemic conditions with joint inflammation, such as RA [86]. miR-132 studies have mainly focused on central nervous system and neurotransmission; however, further analyses are required to determine the importance of miR-132 in inflammatory diseases [111].

miR-133a: MiR-133a is upregulated in RA synovial fibroblasts. Also, miR-133a is known as a negative regulator of the "Runt-related transcription factor 2" (Runx2), which is essential for the osteoblast differentiation [112].

miR146a/miR-146b: Studies showed that miR-146 is upregulated in all RA samples. One study found an increased basal expression of miR-146a in RA synovial fibroblasts compared with OA synovial fibroblasts [81, 87, 113]. In addition, increased expression levels of miR-146a have been observed in synovial fluid [85, 86, 97], synovial tissue [82, 83, 99], PBMCs [96, 97, 99, 114, 115],

and whole blood [96] of RA patients. Expression of miR-146a is detected in many cell types, T cells, B cells, monocytes, macrophages, and IL-17 producing CD4+ cells [81, 82, 97, 114, 115]. However, plasma or serum miR-146a levels in patients with RA are comparable to that seen in healthy controls [86, 98], and two studies demonstrated that miR-146a was markedly decreased in plasma from patients with established RA [99]. miR-146a can inhibit T-helper 1-mediated responses and is required for the suppressive activity of Treg cells, which are both important in RA pathogenesis [64, 115]. Nevertheless, the increasing expression of miR-146a is not specific to RA, as it is also reported in OA cartilage after stimulation by IL-1b [116].

Pauley and Abou-Zeid found that peripheral blood mononuclear cell expression of miR-146a was positively correlated with CRP, ESR, DAS28 index, and TNF-α concentration [97, 115]. There are two known gene targets of miR-146a, that is, TNF-α/TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1). Also, miRNA-146a can regulate genes such as FAF1, IRAK2, FADD, IRF-5, STAT-1, and PTC-1. However, miR-146a has also been reported to correlate inversely with the tender joint count [97, 115]. In addition, miRNA-146a expression levels in the peripheral blood of RA patients were comparable to the levels seen in synovial tissue and fibroblastic cells of those patients [97]. miR-146a/b is induced in response to a variety of microbial components and pro-inflammatory cytokines, such as LPS, CpG, IFN-β, and TNF-α [51]. miR-146a in PBMCs enhances the function of Th1 cells and induces the expression of TNF-α, MCP-1, and NFκB p65 [117]. miR-146b was reported to increase in RA synovial tissue and in PBMCs of RA patients [82]. Upregulated miR-146b is related with infiltration of IL-17-producing T cells in RA synovium and higher expression of IL-17 within T cells expanded from RA PBMCs. So, it suggested that miR-146a and miR-146b are strongly implicated in RA development.

miR-152: Decreased expression of miR152 is reported in FLS of arthritic rats. It leads to activation of the Wnt-signaling pathway through direct targeting of "DNA (cytosine-5)-methyltransferase-1" DNMT1 and "encoding Frizzled8" (FZD8) by turn [92, 93].

miR-155: This is the most widely studied miRNA in RA. miR-155 expression profiles in RA synovial fibroblasts can also be induced by TNF-α [118]. The first miRNAs found to be abnormally expressed in FLS with RA were miR146a and miR155, both previously known as important regulators of inflammation. Also, miR146a and miR155 are two miRNAs, which are negative and positive regulators of inflammatory responses, respectively. miR-155 has been shown to express strongly in RA synovial tissue, synovial fluid and synovial fluid CD14+ cells [118]. Nonetheless, miR-155 concentrations are significantly lower as compared to controls in RA serum [98, 99]. The increased level of miR-155 in synovial fluid CD14+ cells and in synovial tissue macrophages is associated with decreased expression of the miR-155 target, a potent inhibitor of inflammation, called Src homology 2-containing inositol phosphatase-1 (SHIP-1), in patients with RA [94]. In addition, targets of miR-155 are the genes ZNF652, matrix metalloproteinase (MMP)-1 MMP-3, SHIP-1, c-Maf, PU.1, CEBP, ZIC3, HIVEP2, ARID2, SMAD5, and Bach-1. These pathways are all play a role in RA.

miR-203: One study showed that miR-203 is higher in RA synovial fluid in comparison with OA synovial fluid. Expression of miR-203 in the RA and OA synovium may be explained

by differences in cellular composition of synovial tissue among RA patients [67, 78, 91]. Overexpression of miR-203 in RA synovial fluid resulted in higher release of MMP-1 and secretion of IL-6 via the NF-κB pathway and NF-κB-dependent production of IL-6, thus contributing to the RA synovial fluid activated phenotype and joint inflammatory state. Also, miR-203 plays a role in oncogenic transformation, proliferation, migration, and invasiveness of tumor cells. This mechanism is unknown in RA.

miR-221/miR-222: miR-221 and miR-222 are expressed together. miR-221/miR-222 are overexpressed in synovial fluids, blood serum, and synovial tissues of RA patients. MiR-221 plays a role in increasing production of pro-inflammatory cytokines, RA synovial fibroblast activation and migration. It also has a role in increasing resistance to apoptosis [119].

miR-223: miR223 is increased in T cells from patients with RA compared to levels in T cells from healthy donors [120]. miR-223 overexpressed in peripheral blood CD3+ and CD4+ naive T-lymphocytes of RA patients contributes to pathogenesis of the disease. CD14+ monocytes from RA synovia also overexpress miR223 and modulate differentiation of myeloid precursors into osteoclasts [121].

miR-323-3p: miR-323-3p expression is higher in RA synovial fibroblasts than OA synovial fibroblasts [90]. This miRNA may increase the Wnt-cadherin pathway and decrease the levels of target protein "β-transducin repeat containing E3 ubiquitin protein ligase" (BTRC) that contains an inhibitor of β-catenin [90]. It has been shown that miR-323-3p may have pro-inflammatory role in RA.

miR-375: miR-375 regulates the pathogenesis of adjuvant-induced arthritis rat model through the canonical Wnt signaling pathway [93].

miR-451: miR451, most preserved in vertebrates, regulates cell proliferation, invasion, and apoptosis. Neutrophils are important players in RA pathogenesis. miR451, significantly overexpressed in RA serum, peripheral blood T cells from patients with active RA and downregulated in neutrophils from patients with RA. Besides, it negatively regulates the migration of neutrophils by silencing CPNE3 (encoding copine3) and RAB5A (encoding Rasrelated protein Rab5A) [122]. In RA, miR-451 may reduce neutrophil chemotaxis through p38 MAPK (p38 mitogen-activated protein kinases) [122].

miR-455-3p: miR-455-3p can drive cartilage degradation by suppressing the expression of key molecules involved in cartilage formation. Increased expression of miR4553p in OA cartilage contributes to its destruction by targeting ACVR2B (encoding activin receptor type2B), SMAD2 (encoding MAD homology 2), CHRDL1 (encoding chordinlike protein 1), and by suppressing TGF-β signaling [118].

miR-498: miR-498 is associated with neurotransmission and inflammation. miR-498 is downregulated in CD4+ T cells from synovial fluid and peripheral blood [85].

miR-886-3p: A recent study reported that the combination of low expression of miR-22 and high expression of miR-886-3p were predictive of a good clinical response to adalimumab treatment in patients with early RA [123].

7. The importance of miRNAs in RA

MicroRNAs (miRNAs) are involved in the pathophysiological mechanisms underlying human diseases states. During the last decade, researchers have obtained a growing body of evidence regarding miRNAs in many human autoimmune diseases, such as RA, multiple sclerosis, psoriasis and systemic lupus erythematosus. miRNAs-mediated regulation is very clear from all of the present data and can play an important role in the development of various inflammatory conditions. So, dysregulation of miRNA expression (loss or downregulation of miRNA because of mutation, miRNA promoter region mutation, or overexpression of miRNA, epigenetic activation etc.) can lead to a variety of diseases.

For example, upregulation of miR-146 and miR-203 were observed in psoriasis [11]. Also, serum miR-1266 levels were highly significant in patients with psoriasis [124, 125]. Aberrations of miR-223, miR-143, miR-142, miR-378, miR-100, miR-21, and miR-31 levels were also found in these patients [126–128]. In systemic lupus erythematosus, some miRNAs, miR-146, miR-17-5p, miR-112, miR-141, miR-184, miR-196a, miR-383, and miR-409-3p, are downregulated, but miR-21, miR-61, miR-78, miR-142-3, miR-189, miR-198, miR-298, miR-299-3p, and miR-342 are upregulated in PBMCS from patients as compared to healthy controls [11, 127–131]. In multiple sclerosis, miR-18b, miR-599, and miR-96 are dysregulated in PBMCs in those patients [11, 132].

RA is a polygenic disease with multiple effects and miRNAs are post-transcriptional regulators of gene expression. In RA, high miRNA expressions (high mirna expressions mı olacak?) have been identified in different sites and cells, such as synovial tissue, synovial fibroblasts, PBMCs, plasma, synovial fluid, and activated immune cells within injured joints. The identified miRNA candidates for RA play a substantial role for key molecular mechanisms. These mechanisms are especially cytokine signaling pathways and inflammation. So, miRNAs are upregulated or downregulated in RA patients, relative to other diseases and healthy controls.

miRNAs, as diagnostic tools for RA, have many advantages. As such, miRNAs are stable and can be isolated from different parts of bodies (synovial fibroblasts, blood, plasma). miRNAs can be detected in circulating blood without the need for biopsies. Finally, miRNAs expression levels can be estimated by PCR.

8. Conclusion and perspectives

Since the discovery that microRNAs (miRNAs) take part in biological and pathological processes, it has become important to determine the roles.

In this chapter, we have summarized the role of miRNAs in RA. RA is a systemic autoimmune disorder characterized by chronic inflammation of the synovial tissue, and many studies suggest that miRNAs play role on autoimmunity in RA, as miR-16, miR-146b, miR-132, and miR-155 are upregulated in RA circulation. This suggests that miRNAs contribute to

RA pathogenesis. miR-124a is downregulated in RA synovium tissue [88] while miR-146a, miR-155 and miR-203 are upregulated in RA synovium tissue [51, 91, 133]. As we mentioned above, miR-146 and miR-203 are also upregulated in psoriasis; for this reason, they are questionable as possible biomarkers. miR-16, miR-132, miR-146, and miR-155 are upregulated in RA PBMCs, and miR-223 is upregulated in CD4+ naïve T-lymphocytes of RA patients [121].

Recently, the understanding of the molecular mechanism, prediction, or identification of miRNA targets is the main subjects of research in this area. This contribution may be lead to consider miRNAs as therapeutic targets. However, some miRNAs mentioned above are not specific for RA, as they are also dysregulated in the other immune disorders or cancers.

In the past few years, some experimental models have been used to study the therapeutic potential of miRNAs in RA, but still they are insufficient. Further studies are necessary.

In summary, each miRNA that is identified for RA opens the door slightly in terms of diagnostic and/or prognostic marker. Today, the available evidence shows that miRNAs are not diagnostic markers yet, but they can be used for therapy in the future.

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