Chapter 1

MicroRNAs in Rheumatoid Arthritis: From Pathogenesis to Clinical Utility

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Additional information is available at the end of the chapter

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Abstract

Emerging evidence suggests that microRNAs (miRNAs) play a key role in the regulation of immunological functions including innate and adaptive immune responses, development and differentiation of immune cells, and the prevention of autoimmunity. The current state of our knowledge in this area is far from being complete, and continued investigations will be needed to reveal a better understanding of the miRNA network that is involved in the pathogenesis of rheumatoid arthritis (RA). In RA, miRNA plays an important role in many different cellular processes. It has been shown that they modulate inflammatory responses, proliferation of synoviocytes, and production of metalloproteinases in rheumatoid joins and affect the development, differentiation, effector, and regulatory functions of T and B cells and cytokine production. The specific circulating miRNA species may also be useful for the diagnosis, classification, and prognosis of diseases and prediction of the therapeutic response.

Keywords: pathogenesis, epigenetics, microRNA, function of miRNAs, polymorphisms

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune, polygenic disease, which affects millions of people worldwide [1]. Human genetic and epigenetic studies have shown that causal factors of disease include contributions from at least 40 different genes and the effect of environmental factors. Epigenetics represents a new aspect in autoimmunity, which regulates gene expression without alternating DNA sequence. The epigenetic mechanisms are implicated in the final interpretation of the encoded genetic information by regulating gene expression, and alterations in their profile influence the activity of the immune system. Although there

are controversies regarding the involvement of genetic and epigenetic factors in RA etiology, it is becoming obvious that the two systems (genetic and epigenetic) interact with each other and are ultimately responsible for RA development.

Recently, the list of molecules important for RA pathogenesis has been extended to microRNAs (miRNAs), which play a central role in regulating both adaptive and innate immune response, development and differentiation of immune cells, and the prevention of autoimmunity [2]. miRNAs regulate protein expression at the posttranscriptional level through reduction of mRNA stability and inhibition of translation [3]. Human miRNA genes are located on all chromosomes except the chromosome Y, are mainly observed in introns (70%), constitute only 3% of the human genome, and regulate about 90% of different genes [4, 5]. These molecules participate in the regulation of the expression of genes involved in the fundamental biological processes related to development, proliferation, and apoptosis, as well as pathological processes including autoimmunity [6, 7].

Although we are still at a very early stage in understanding their impact on immunity, miRNAs are changing the way we think about the development of the immune system and regulation of immune functions. While characterization of miRNA expression patterns in RA patients can have potential diagnostic use, new discoveries in cell type-specific miRNA expression profile, during the disease progression, may provide further understanding of RA pathogenesis. Because miRNAs are stable in plasma or serum, they may be use as biomarkers for the prediction of the disease activity, the differentiation of RA from other rheumatic diseases, or the monitoring of current therapy.

2. Biogenesis

Mature miRNAs are single-stranded, small, noncoding RNAs about 22 nt long. The biogenesis of miRNAs is under tight temporal and spatial control. It starts in nucleus where long primary miRNAs (pri-miRNAs) are transcribed by RNA polymerase II. miRNA sequences are located within various genomic contexts including both intronic and exonic regions. Pri-miRNA transcription is regulated by transcription factors like p53, MYC, ZEB1, ZEB2, and myoblast determination protein 1 (MYOD1). Additionally, epigenetic factors (DNA methylation and histone modifications) regulate this process. The resulting transcript is capped with a specially modified nucleotide at the 5' end (MGpppG) and polyadenylated with a poly(A) tail at 3' end. Pri-miRNA is subsequently cleaved by the so-called microprocessor complex and forms a precursor hairpin miRNA (pre-miRNA). Microprocessor complex is composed of RNase III enzyme—Drosha and of the RNA-binding protein DGCR8 (also known as Pasha). In the next step, pre-miRNA is exported from the nucleus to the cytoplasm in a process involving exportin-5 (Exp-5) and GTP-binding nuclear protein RAN•GTP. Following translocation to the cytoplasm, the pre-miRNA is cleaved, by the cytoplasmic RNase III enzyme Dicer, into 19- to 23-nucleotide mature miRNA duplexes. Although either strand of the duplex may potentially act as a functional miRNA, only one is usually loaded into the RNA-induced silencing complex (RISC) where the miRNA and its mRNA target interact (reviewed by Ref. [8]). miRNA

guides RISC complex to the 3'-untranslated region (3'-UTR) of target mRNA to prevent translation of the mRNA into protein. The other (termed the passenger) strand is degraded or released from the cell. There are few different mechanisms of miRNA export from the cell. miRNAs may be released from the cell by exosomes or microvesicles. Moreover, miRNAs can form complexes with high-density lipoproteins or RNA-binding proteins such as Ago2 and in that form circulate outside the cell. Exact mechanism of how these complexes are transported from the cell still remains unknown. They may be released passively, after cell death, or actively through specific membrane channels or proteins. Interestingly, other pathways for miRNA biogenesis are also emerging. Examples include those that are independent of Drosha or Dicer, "mirtrons"—some small nucleolar RNAs (snoRNAs) and endogenous short hairpin RNAs (shRNAs) [8–10].

3. Mechanism of action

Small noncoding miRNA has the potential to broadly influence various molecular pathways via suppression of unwanted mRNA transcripts. These highly conserved ~22-nucleotide long miRNAs recognize its mRNA target through "seed region" that is located at the 5' end of miRNA and span from nucleotide positions 2–7. It binds to mRNAs' complementary sequence usually located within 3'-untranslated region (3'-UTR). miRNAs silence its target expression via various mechanisms leading to mRNA degradation or preventing mRNA from being translated [11]. After specific mRNA sequence recognition, argonaute (AGO) protein recruits factors that induce translational repression or mRNA degradation. Target mRNA destination depends probably on the sequence complementarity level with miRNA—complete complementarity leads to mRNA degradation and partial complementarity to translational inhibition, although there are exceptions to this rule. Interestingly, some target mRNAs can be exclusively repressed by degradation or translational inhibition, but often it occurs by combination of these two processes (reviewed in Refs. [9, 12]).

3.1. Inhibition of translation

There are still many unknowns around mechanism of translational repression of target mRNA. It is not clear whether miRNA represses translation at the step of translational initiation or posttranslational level [13]. Significant number of studies support model of repression at posttranslational level (protein degradation) or at late stage of translation (premature termination or impaired elongation). These studies demonstrated that miRNA-repressed mRNAs maintained the same distribution pattern across polyribosomes compared with non-repressed mRNAs [14, 15]. However, other studies showed contradictory results, indicating that repression occurs at the translational initiation step. There are many models proposing mechanism of this process.

Almost all proposed models consider GW182 interaction with one of the Ago proteins as a first step of translational repression. In first model, GW182 disrupts the association between

eIF4G and poly-A-binding protein (PABP) and prevents the circularization required for efficient translation. In the second model, formation of the mRNA 40S preinitiation complex occurs, but miRISC complex prevents 60S ribosome from joining it, resulting in translation repression. The third scenario assumes that ribosome is detached earlier leading to premature translation termination. Another possible proposed mechanism assumes that target mRNA is accumulated in so-called processing bodies (P-bodies) that prevents from interaction with translational machinery [12, 16].

3.2. Destabilization of target mRNA

There are two mechanisms of miRNA-mediated mRNA degradation-specific cleavage and non-cleavage mRNA degradation. mRNA degradation by miRNA requires Ago, GW182, and the cellular decapping and deadenylation machinery. miRNA sufficient complementarity to target mRNA leads to its specific cleavage. The process is facilitated through Ago protein slicer activity. Ago cleavage site is determined by miRNA sequence, not mRNA paring residues, and occurs within mRNA pairing site to the 10th and 11th residues of miRNA. Therefore, the degree of complementarity at the 3' end of miRNA is more important than at the 5' end. After cleavage miRNA remains intact and is able to mediate another mRNA silencing [17, 18]. Non-cleavage degradation of target mRNA involves GW182. GW182 forms a bridge between RISC and CCR4-NOT deadenylase complex leading to mRNA deadenylation and decapping [19, 20].

4. Biological function of microRNAs

MicroRNAs as endogenous regulatory molecules are present in plant, animal, and human cells, and they are essential for proper development and functioning of the organism. Their main role is associated with posttranscriptional regulation of the expression of numerous genes in both pathogenic and pathological aspects of diseases (**Figure 1**) [21, 22]. miRNAs together with Argonaute (AGO) proteins, forming a core of the miRNA-induced silencing complex (miRISC) which mediates gene silencing [23]. Each miRNA has several different targets, and many of mRNAs are subject to regulation by more than one miRNA [24]. Over 60% of human genes are controlled by miRNAs, which indicates the great importance of these molecules [23].

The continuous miRNA-mRNA interaction is essential for miRNA function. miRNA genes are present either as independent transcription units or located in introns and exons of other genes. Gene silencing can be done either through degradation of a specific mRNA or as a result of the inhibiting of the transcript translation. Furthermore, the mechanisms by which miRNA complementarity to its target mediates repression are varied [25, 26]. In some cases the miRNA binds with complementarity in the seed region (nucleotides 2–8 of the miRNA) within the 3'-untranslated region (UTR) of mRNA and leads to inhibit target gene translation or less commonly within the 5'-UTR region that enhancement of translation [25, 27]. But on the other hand, the miRNA-mRNA interaction occurs in the central region (bases ~9 to 12)

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leading to the mRNA cleavage and subsequent degradation [26]. Generally, miRNAs do not act to completely silence their targets, but rather decrease expression. However, the identification of the type of interaction between miRNA and mRNA remains a very important focus to understand control in gene expression [26, 27].

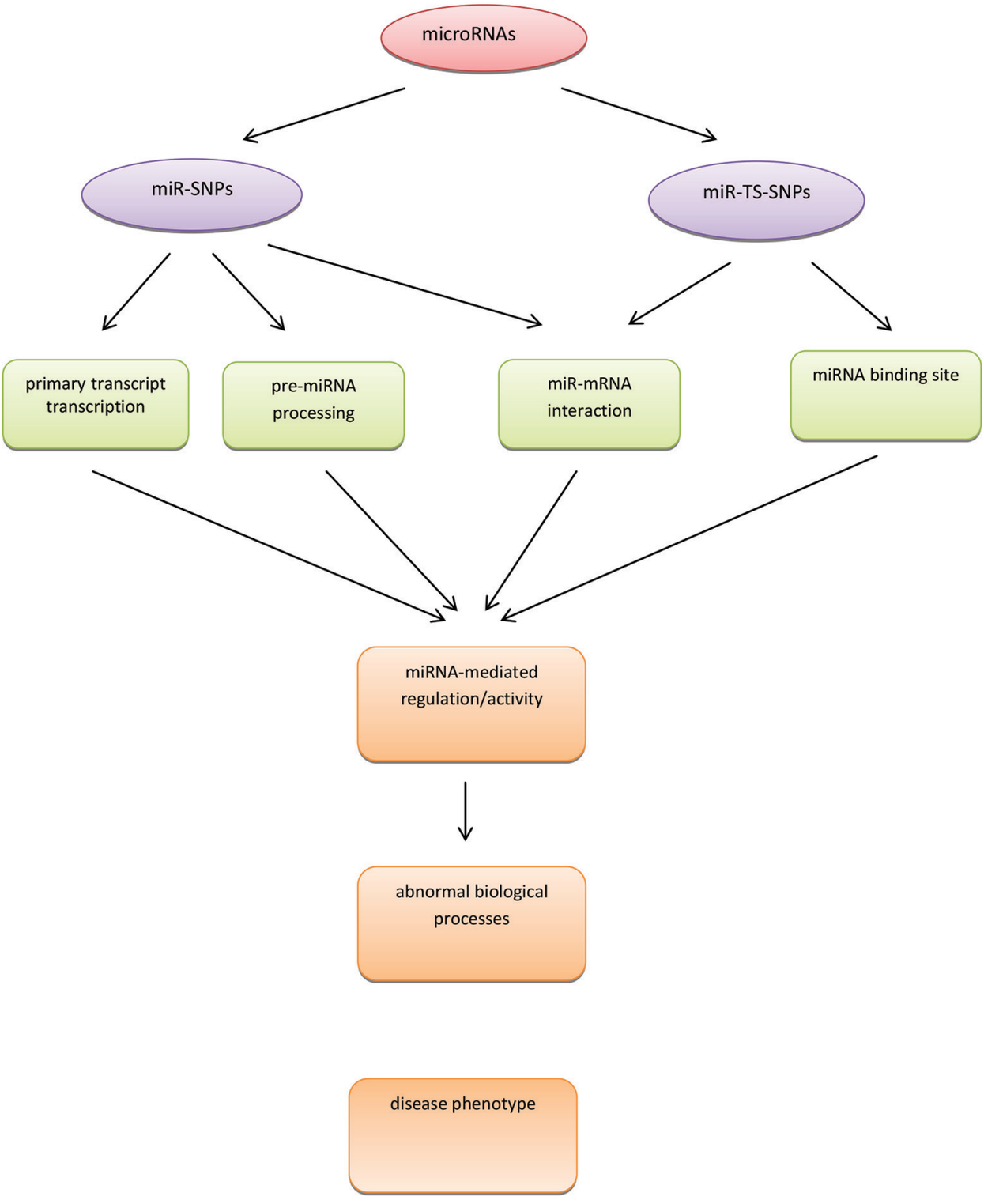

Figure 1. Biological functions of microRNAs.

In the last years, a large number of studies examined cellular miRNA profiles, where expression changes under stress conditions, in cancer, and in other diseases. Distortion of miRNA expression may result in abnormalities in the course of numerous cellular processes, such as development, differentiation, proliferation, inflammation, cell fate determination, apoptosis, signal transduction, organ development, hematopoietic lineage differentiation, host-viral interactions, osteogenesis, and stem cell and germ line proliferation [22, 24, 25, 27–30]. These discoveries may help to clarify molecular mechanisms of diseases and based on this knowledge and may help to develop new drugs. However, since the amount of miRNA may be differ, its total quantity in the different type of cells and gene silencing efficiency depends on the concentration of mRNA targets interpretation of miRNA expression, as well as profiles, including clinical studies, should be performed with attention.

While the majority of miRNAs are found intracellularly, miRNAs may also function beyond RISC, as an extracellular, in virtually all known body fluids [31, 32]. The extracellular miRNAs are secreted into exosomes or microvesicles [26]. However, little is known about the origin of body fluid miRNAs in both healthy and disease conditions or what factors affect their levels in serum/plasma [31]. One hypothesis suggests that blood cells are the major contributors to extracellular miRNAs in the serum or plasma, but another hypothesis speculates that organs may also contribute to extracellular miRNAs because tissue-enriched miRNAs (e.g., miR-122 in the liver, miR-133 in the muscle, miR-208 in the heart, and miR-124 in the brain) are also detected in serum or plasma [31]. Independently of existing hypotheses, explanation of the origin of the extracellular miRNA would increase our understanding of their potential pathophysiological significance. In addition, regardless of their origin, extracellular miRNAs are extremely stable, and their concentration did not significantly change with prolonged incubation of blood plasma at room temperature [23]. The level and composition of extracellular miRNAs in body fluids, such as serum or plasma, are correlated with several pathological conditions, including cancer, diabetes, inflammatory diseases, as well as tissue injury [31]. Moreover, expression patterns of miRNAs are also different between disease state and normal tissue [26] and appear to be tissue specific [21]. However, current knowledge about the biological function of extracellular miRNAs is in its infancy stage. Nevertheless, it seems that extracellular miRNAs may act as signaling molecules and mediate distant cell-cell communication. Cross talk mediated by these miRNAs may provide new insights into the understanding of the mechanisms underlying various dysfunctional conditions. Cross talk through these miRNAs may provide new look at the understanding of the mechanisms underlying the various dysfunctional states [31].

5. Rheumatoid arthritis as a chronic civilization disease

Rheumatoid arthritis is one of the most prevalent chronic inflammatory diseases and is characterized by progressive joint inflammation, by destruction of articular cartilage and underlying bone, and by synovial hyperplasia. RA can affect any joint, with a joint cavity lined by synovial membrane (SM) [33, 34]. The disease process is highly variable, with periods of exacerbation and remission, and uncontrolled inflammation leads to progressive joint damage, pain, long-term disability, and reduced quality of life in many patients [34, 35].

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Although RA affects people all over the world, women are affected three times more than men with the peak of incidence between 35 and 55 years of age [33, 36–38]. The incidence of rheumatoid arthritis is relatively constant in most European and North-American populations and is estimate between 0.5% and 1%. RA incidence decreases from north to south and from urban to rural areas. A positive family history increased the risk of RA, and we can see that the familial RA occurrences are about 10–30% of patients, whereas the concordance rate is 12–15% in monozygotic twins and 3–4% in dizygotic twins [39].

RA is pathologically heterogeneous and it is classified among systemic autoimmune disorders because of the presence of rheumatoid factor (RF) and other autoantibodies such as anticitrullinated protein autoantibodies (ACPAs) [35, 40, 41]. RF is present in about 60–85% patients with RA; ACPAs appear to be equally sensitive (75%) and highly specific: 94% for the established disease and 61% for early RA, which may be able to serve as an early diagnostic marker and prognostic factor of joint destruction [42].

Although the exact etiology remains an active area of worldwide research, it is generally accepted that the RA is a multifactorial disease of which pathogenesis is defined by genetic and environmental factors [21]. Genetic predisposition is responsible for 50–60% of the risk of developing RA [43, 44]. A genetic link to RA was established through the observation of familial clustering of RA and large-scale genome-wide association studies (GWAS) [45]. Moreover, it is suggested that only the specific combination of genetic factors in an individual might determine the outcome of the disease [7]. HLA-DRB1 alleles, which share a common sequence, known as the "shared epitope" account for approximately 30% to 50% of overall genetic susceptibility to RA. Probably some other genes associated with components of the innate and adaptive immune system and other mechanisms such as epigenetics may also play a role in the pathogenesis of RA. Epigenetic mechanisms are engaged in the final interpretation of the genetic information by regulation of the gene expression, and changes in their profile influence the activity of the immune system [46]. The change in epigenetic mechanisms is implicated in the final interpretation of the encoded genetic information by regulating gene expression; on the other hand, genetic mutations in epigenetic regulators may alter the epigenetic profile. Consequently, epigenetics and genetics can be two sides of the same coin, as has been determined in the field of rheumatoid arthritis research [46]. Future challenges are understanding and leveraging of the role of genetics and epigenetic modifications, as well as their functional characteristics in RA pathogenesis and translation of fundamental discoveries into clinical practice [47].

6. miRNAs in the pathogenesis of rheumatoid arthritis

Up to date about 2,500 different miRNAs were discovered in humans. From all of these miRNAs, some may play an important role in the regulation of the innate and adaptive immune responses in patients with RA. Increasing number of studies has shown that abnormal miRNA expression in different fluids or cells from RA patients may lead to inflammation, cytokine signaling, bone degradation, and invasive behavior of resident cells [6]. In addition, there is growing evidence that miRNAs as crucial mediators of inflammation may also regulate the

plasticity and the effector functions of differentiated Th-cell subsets [24, 48]. miRNAs on the one hand have an important role in defining and maintaining the gene expression programmer of differentiated Th17 cells, but on the other hand, the miRNA expression in regulatory T cells (Treg cells) is important for the maintenance of self-tolerance. And as we know, abnormally activated inflammatory Th cells and defective Treg cells play primary role in the pathogenesis of RA. Moreover, miRNA expression may be also diverse in the different stages of RA progression, allowing miRNAs to help monitor disease activity and help to understand its pathogenesis [6]. Understanding the potential link between miRNAs and the pathogenesis of RA may lead to future insights into disease diagnosis and treatment [4, 22].

The first evidence that miRNAs may be involved in the molecular mechanisms of RA arose in 2007 with the recognition, in the serum of RA patients, of autoantibodies directed against GW bodies that are cytoplasmic structures for storage and/or degradation of mRNA [4]. The first miRNAs that shown abnormal expression within the inflamed joints of RA patients includes miR-16, miR-146a, and miR-155 [4, 49]. These multifunctional miRNAs play a central role in the inflammatory response and immune dysfunction, in response to microbial components, and are upregulated after immune cell maturation [4]. miR-16 and miR-146a play a role in the regulation of the tumor necrosis factor alpha (TNF-α) signaling, which is one of the key cytokines involved on the pathogenesis of RA [2, 50]. Furthermore, miR-155 could suppress matrix metalloproteinases (MMPs) through the interaction with proinflammatory cytokines and Toll-like receptor (TLR) ligands and lead to modulation of joint inflammation [2, 51]. miR-146a and miR-155 are responsible for the maintenance of immune homeostasis, and their expression changes in RA may alert cytokine secretion from different immune cells [30]. Moreover, "miR-146a and miR-155 are considered the 'yin and yang' of inflammation as these miRNAs are negative and positive regulators of inflammatory responses, respectively" [30]. miR-155 promotes T-cell–mediated tissue inflammation by the regulation of the Th1- and Th17-cell response, and it also through the targeting of SOCS1, a negative regulator of IL-2 signaling, controls Treg cell homeostasis [24]. Like miR-155, miR-146a is highly expressed in Treg cells. Furthermore, in contrast to miR-155, miR-146a required for the suppressive activity of Treg cells is essential for the ability of Treg cells to restrain IFN-γ-mediated pathogenic Th1 responses and associated inflammation [24, 50]. Whereas miR-146a rather reduces the proinflammatory immune responses, miR-155 enhances inflammatory processes [48]. The main advantage of the detection of miR-16 and miR-146a in serum/plasma is that this opens the possibility to test as a biomarker to monitoring disease activity/course, because both have shown positive correlation with C-reactive protein (CRP) and disease activity score in 28 joints (DAS28) [4, 48].

Other tissue and/or extracellular miRNAs have been also shown to be associated with RA (**Table 1**), and those miRNAs can regulate specific cytokine pathways, thus leading to inflammation [27]. Characteristics of miRNA profile in patients with RA suggest putative functions of these factors in cytokine secretion and signaling during chronic inflammatory processes in the joints. The miR-10a, miR-16, miR-23b, and miR-203 may serve to fine-tune cytokine signaling, and they may be responsible for the induction of autoimmune inflammation [24, 50]. Upregulation of miR-16 and miR-203 in synovium of RA patients is responsible for the regulation of the TNF-α signaling and for increased matrix metalloproteinases (MMPs), as well as

IL-6 production, respectively, thus indicating that these miRNAs may plays a role as proinflammatory and joint destructive factors [27, 50]. In contrast, miR-10a and miR-23b, which are downregulated in RA synovial tissue, regulate signaling pathway of proinflammatory cytokines such as IL-1β, TNF-α and IL-6, IL-8, and IL-17, as well as MCP-1 and MMPs, and they may be a strong pathological factors that play a critical role in the onset and progression of RA [50, 52]. The cytokines are also a key regulator of Th17/Treg imbalance, which participate in the immune dysfunction. The immune dysfunction in RA patients is found to be induced by decreased expression of miR-21, which may suppress Treg development while promoting Th17 differentiation. Moreover, this miRNA may serve as a novel regulator of Th17/Treg balance in autoimmune diseases including RA [53]. Another miRNA, which participate in the balance between Th17 and Treg cells, is miR-10a, which exhibits high expression in natural Treg cells, limiting the conversion of inducible Treg cells into follicular helper T cells (Tfh cells) and inhibiting Th17 differentiation [54].

miRNAs	Localization	Target gene	Changes	Function
miR-10a	PBMCs, DCs, nTreg cells	STAT - Signal transducer and activator of transcription Foxp3, Bcl-6, Ncor2	Downregulation	- Control IL-6 and IL-21 signaling - Regulate the stability of Treg cells - Limit the conversion of iTreg cells into Tfh cells - Inhibit Th17 differentiation
miR-16	PBMCs, plasma, synovial fluid	TNF-α	Upregulation	- Marker of disease activity
miR-17-92	RASFs, FLS, B cells	MMP-1, IL-6, IL-8, MCP-1, RANTES	Downregulation	- Modulate apoptosis, proliferation - Joint inflammation and destruction - Regulated proinflammatory cytokine production
miR-21	plasma, PBMCs, $CD4^+T$	Foxp3 STAT3	Upregulation	- Regulate th17/Treg balance
miR-22	RASFs	CYR61	Downregulation	- Synovial tissue hyperplasia - Th17 differentiation
miR-23b	ST	IL-1β TNF-α IL-17	Downregulation	- Induction of autoimmune inflammation
miR-24	plasma	TGF - transforming growth factor-β1	Upregulation	- Marker of disease activity - Enhance the inflammation
miR-26a	PBMCs, plasma		Upregulation	- Diagnostic biomarker
miR-30a	ST	BECN1 - beclin 1	Downregulation	- Reduce apoptosis

miRNAs	Localization	Target gene	Changes	Function
miR-34a	RASFs	XIAP	Downregulation	- Contribute to apoptosis
miR-124a	RASFs, synoviocytes	CDK-2 MCP-1 VEGF	Downregulation	- Regulator of RASFs and autoimmune inflammation - Contribute to angiogenesis
miR-125b	serum, blood	NF-κB	Downregulation	- Induce of excessive inflammation
miR-146	SF, ST, RASFs, PBMCs, blood, serum, T cells, B cells, monocytes, macrophages	TLR4, NOX4 - NADPH oxidase 4,	Upregulation	- Marker of disease activity - Inhibit Th1-mediated responses - Suppress activity of Treg cells - Modulated apoptosis
miR-155	ST, SF, RASFs, PBMCs, blood, serum, macrophages	SHIP-1, SOCS-1MyD88, MAP3K10 - Mitogen-Activated Protein Kinase Kinase Kinase 10	Upregulation	- Regulate cytokine expression - Require for homeostasis and function of Treg cells
miR-203	RASFs	MMP-1, NF-κB	Upregulation	- Participate in joint inflammatory state
miR-223	PBMCs, RASFs, ST, plasma, macrophages, monocytes, T cells		Upregulation	- Regulate osteoclastogenesis
miR-451	T cells, neutrophils	p38 MAPK - mitogen-activated protein kinases, CPNE3 - copine 3	Downregulation	- Role in inflammation

PBMCs, peripheral blood mononuclear cells; RASFs, RA synovial fibroblasts; FLS, fibroblast-like synoviocytes; MMP-1, matrix metalloproteinase-1; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; ST, synovial tissue; XIAP, X-linked inhibitor of apoptosis protein; CDK-2, cyclin-dependent kinase 2; VEGF, vascular endothelial growth factor; SF, synovial fluid; SHIP-1, Src homology 2-containing inositol phosphatase-1

Table 1. Candidate miRNAs for the RA pathogenesis.

Several of different cells, such as T cells, B cells, or macrophages, have been identified as the sources leading to local joint deformation [55]. These cells are the sources of some miRNAs that, through the regulation of the osteoclastogenesis and differentiation of articular chondrocytes, are implicated in the renewal of cartilage and bone degradation [50]. The best known miRNAs involved in joint destruction are miR-146a and miR-155. However, recently marked expression of other miRNAs, miR-223 and miR-19a/b, have been shown within inflamed joint in patients with RA [3, 4, 51, 56]. miR-223 overexpression suppressed the production of osteoclasts and expression of osteoclast-marked genes [50]. Moreover, an inverse correlation between the

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plasma miR-223 levels and the tender join count was observed [4]. The expression of the miR-19 was downregulated in RA fibroblast-like synoviocytes (FLS), and this plays a role in joint destruction, in response to TLR stimulation [57]. miR-19a and miR-19b negatively regulate the synthesis of IL-6 and regulate the expression of MMP3, suggesting a role of both these miRNAs in protecting patients with RA from joint inflammation and consequently destruction [57].

The above-addressed miRNAs are not only important positive/negative regulators of inflammatory response and immune dysfunction, but they also may be useful for the prediction of the disease course and identification of early pathological events, as well as beneficial for the development of new therapeutic strategies [50].

7. Therapeutic potential of miRNA in treatment of rheumatoid arthritis

The discovery of the miRNA as regulators of gene expression was followed by a wave of interest and intensive studies for elucidating their role both in physiological and pathological conditions. The obtained results raised hopes for the possibility of developing a number of therapeutic uses of these molecules. miRNAs are characterized by many features that make them excellent candidates for biomarker drug entity. These molecules are small with sequence well-known and often conserved among species, which are very attractive features from a drug development standpoint.

Some alterations of miRNA expression have been associated with many human disorders including autoimmune diseases, metabolic disorders, and genetic diseases. By targeting cells affected with improper miRNA expression, the normal balance of the expression can be restored. Novel systems to achieve targeted modulation of specific miRNA in vivo were developed. By controlling the miRNA that regulate mRNAs in cells, they can be used for therapeutic treatment for certain cellular disorders. Individual miRNAs can be targeted using specifically modified antisense oligonucleotides—anti-miRNAs. By virtue of specific modifications, anti-miRNAs gain improved binding affinity, nuclease resistance, and in vivo delivery. For example, 2′-O-methoxyethyl phosphorothioate (MOE) or N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine (ZEN) oligonucleotide modifications were generated to improve binding affinity and block exonuclease degradation. Other examples are anti-miRNAs conjugated to cholesterol to improve its uptake into the cell through the membrane. Furthermore, the great potential seems to lie in the ability of individual miRNAs to regulate the expression of many related genes, which could lead to the regulation of whole signaling pathways or metabolic modulation using one or more miRNA molecules. On the other hand, effect on multiple targets also involves high risk due to the unexpected side effects. Nevertheless, important role of microRNA in regulation of all cellular processes and constant tissue-specific expression indicates its high potential for diagnostic and therapeutic usage [9]. Moreover, miRNA properties and characteristics indicate their excellent potential to be clinical biomarkers. It is well-known that perfect biomarker should be stable in a sample, easy to assess, specific, and sensitive to relevant changes, and miRNA fulfil these criteria. Although cell endogenous miRNA is not very stable and easily degraded, it was discovered that miRNA detected in the blood [21] (plasma, platelets, nucleated blood cells) and synovial fluid [32] is stable and

in the case of miRNA in the blood even resistant to harsh condition like high temperature, high and low pH, or multiple freeze-thaw cycles. miRNAs require this surprising stability due to microparticles (exosomes, microvesicles, and apoptotic bodies), RNA-binding proteins (like Ago2), or lipoprotein complexes [high-density lipoprotein (HDL)] that protect them from degradation. The value of miRNA as molecular biomarkers for diagnosis, prognosis, and prediction of therapeutic response is widely documented in cancer. Another advantage that raises interest in the use of circulating miRNAs as clinical biomarkers is fact that there are highly sensitive and specific miRNA detection methods in a quantitative manner, such as real-time PCR, sequencing, and microarrays. In that respect, circulating miRNAs offer many features to make them an attractive class of biomarkers [10].

7.1. miRNA as potential therapeutic agent for RA treatment

The involvement in the pathophysiology of RA coupled with specific characteristics of miRNAs has triggered the scientific community to start exploring the possibilities of viewing miRNAs as therapeutic entities. Especially the potential of a single miRNA to modulate several genes and affect multiple distinct disease-regulatory pathways simultaneously makes miRNAs particularly attractive candidate targets for chronic inflammatory disease as RA. However, only a limited number of groups have explored the therapeutic potential of miRNAs in RA.

Among many approaches considering belief that one miRNA is able to control several genes and pathways, targeting miR-155 represents the most encouraging miRNA-based therapeutic strategy today.

Currently available data clearly show that miR-155 is crucial for the development of arthritis by increasing production of proinflammatory cytokines and promoting differentiation of antigen-specific T cells and antigen-specific antibodies. Interestingly Kurowska-Stolarska et al. observed that miR-155 deficiency is protective against development of collagen-induced arthritis (CIA) in mice. Inhibition of miR155 was associated with impaired production of proinflammatory cytokines, development of the autoreactive Th17 cells, production of anti-collagen II antibodies, and lack of articular inflammation. MiR-155-deficient mice display reductions in both joint inflammation and bone erosion [58]. Beside miR-155, several miRNAs have been implicated in diseases that affect cartilage or have been identified as essential in cartilage homeostasis or repair. For example, the expression of miR-26a was shown to be reduced in splenic cells isolated from rats with induced arthritis. Treatment with methotrexate restored the expression miR-26a to levels comparable to those of non-arthritic controls, and repeated miR-26a injections stabilized disease severity and reduced synovitis, but had no effect on joint destruction [59]. miR-26a can affect bone formation, through angiogenesis and osteogenesis-positive regulation. Overexpression of miR-26a resulted in enhanced bone regeneration coordinated with improved vascularization in mouse, leading to complete repair of the defect [60]. Nakasa et al. demonstrated in vivo and in vitro that miR146a is also able to prevent bone/cartilage destruction. They observed that in $CD14^+$ human cells transfected by dsmiR-146a and treated with M-CSF and TNFα or Receptor Activator for Nuclear Factor κ B Ligand (RANKL) for osteoclastogenesis induction—tumor necrosis factor receptor-associ-

ated factor 6 (TRAF6) protein levels—and the number of Tartrate-resistant acid phosphatase (TRAP)-positive cells were significantly reduced compared to cells transfected with nonspecific dsRNA. Also osteoclast function was significantly inhibited in those cells. Furthermore they tested the efficacy of ds miR-146a inhibition of bone destruction in vivo and observed that the extent of bone and cartilage destruction was reduced in arthritic mice injected with ds miR-146a compared to mice treated with nonspecific dsRNA. However, despite the inhibition of bone/cartilage destruction by ds miR-146a injection, there was no significant difference in the arthritis score between the ds miR-146a-treated group and the nonspecific dsRNA-treated group suggesting that administration of ds miR-146a alone may not be a sufficient treatment for RA [61]. Another group that observed underexpression of miR-451 in neutrophils isolated from patients with RA used a systemic administration to restore normal expression level of miR-451 in mice with monogenic model of autoimmune arthritis (SKG mice). This approach led to reduction in the number of neutrophils in synovium, by suppressing their migration. This "miR-451" treatment resulted in reduced severity of arthritis in SKG mice suggesting its potential as therapeutic agent in RA [62]. miR-124 was also found to negatively regulate osteoclastogenesis of mouse bone marrow macrophages. In rats with adjuvant-induced arthritis (AIA), miR-124 expression level was significantly decreased. Nakamachi et al. observed that injection of miR-124 into rat AIA joints reduces disease severity. It is probably due to miR-124-mediated targeting of NFATC1, a master transcription factor of osteoclast differentiation. This suggests a potential of miR-124 as future therapeutic entity used for arthritis amelioration [63, 64].

Studies on miRNA-based treatment strategies are rare, but we can expect that these therapies will be developed in the near future. Although mentioned above preclinical studies demonstrate the potential of delivering therapeutic miRNAs, numerous challenges still remain in designing effective and safe delivery of these agents in a cell- or tissue-specific manner. We are still lacking evidence of similar miRNA deregulation in human RA and of the ex vivo correction of disease phenotypes in patient-derived cell or tissue samples. Furthermore, the cellular mechanisms that could explain observed clinical benefits of miRNA therapies still need to be characterized in depth.

7.2. miRNA as RA diagnostic biomarkers

The knowledge about potential value of miRNA as molecular biomarkers for diagnosis, prognosis, and prediction of therapeutic response in RA has been expanded. Several abnormally expressed miRNAs in circulation or inflamed joints in RA have been identified. That suggests theirs potential to be used as biomarkers that help to establish or confirm a diagnosis, monitor the degree of immunologic activity or inflammation, or provide prognostic information regarding disease progression and severity. There are few studies that referred the possibility to use plasma and synovial miRNAs as diagnostic biomarkers that distinguish healthy people from patients with RA or another disease with similar symptoms. For example, upregulation of miR-146a, miR-155, and miR-223 has been shown in various compartments such as serum, blood, synovial fluid, and tissues in patients with RA. Many studies observed elevated levels of expression of miR-146a in different tissues from RA patients; some of them confirm that miR146a level is correlated with disease activity that highlights its significance in RA patho-

genesis and diagnostic biomarker potential [51, 65–67]. Elsayed et al. confirmed significant upregulation of miR-146a expression in the whole blood of patients with RA and that miR-146a in diagnostic performance was better than anti-CCP and RF. miR-146a expression level allowed for distinguishing RA patients from healthy controls (HCs) with high sensitivity and specificity (96 and 100%, respectively). Moreover, its expression level was positively correlated with disease activity [68]. It is important to note that these studies are limited by a relatively small number of patients and were conducted in patients from different races and ethnicities. Similarly Kriegsmann et al. observed that miR-146a, miR-155, and miR-223 were significantly elevated in RA compared to osteoarthritis (OA) synovial tissues. The sensitivity and specificity for the detection of RA were 0.76/0.80 for miR-146a, 0.80/0.95 for miR-155, and 0.86/0.81 for miR-223. In combination these miRNAs gave even better results as RA diagnostic biomarker with a sensitivity and specificity of 0.84/0.91, respectively [69]. Murata et al. performed comprehensive array study to find out which miRNAs in plasma samples significantly differentiate patients with RA (patients with high disease activity, who had never received any biological therapy such as the anti-TNF agents) from HCs. They tested eleven preselected candidate miRNAs in 102 patients with RA and 104 HCs and found out that miR-125a-5p, miR-26a, and miR-24 expression levels gave the best results. For miR-24, miR-26a, and miR-125a-5p, the values of sensitivity and the specificity were 63.7 and 89.5%, 53.9 and 94.3%, 64.7 and 89.5%, respectively. These results suggest that plasma miR-24, miR-26a, and miR-125a-5p can be diagnostic biomarkers with high specificity. The combinations of miR-24, miR-30a-5p, and miR-125a-5p (termed ePRAM for "estimated probability of RA by plasma miRNAs") with sensitivity 78.4% and specificity 92.3% are more efficient for RA differentiation and have higher diagnostic significance as biomarkers in RA. These selected miRNAs correlate with disease activity (besides miR-125a-5p) erythrocyte sedymentiation rate (ESR), CRP, RF, ACPA, or DAS28 and not only can be markers for diagnosis of RA but also for disease activity of RA [70]. In a small study, Murata et al. assessed the diagnostic usefulness of plasma miR-132 for patients with RA or OA. They showed that plasma miR-132 differentiate RA and OA patients from healthy controls (83.8% of sensitivity and 80.7% of specificity for RA and 84.0% of sensitivity and 81.2% of specificity for OA) but failed to distinguish them from each other. Interestingly they observed that miRNAs in synovial fluid (miR-16, miR-146a, miR-155, and miR-223) from RA patients were significantly higher than those of patients with OA, suggesting that synovial fluid miRNAs could be a useful tool for diagnosis of RA from OA; however, they did not correlate with clinical variables of RA including DAS28 [32].

Although, evidence that supports the therapeutic potential of miRNA-based strategies is growing, further investigations are required to find suitable miRNAs for utilizing as biomarkers for diagnosis, predicting drug efficacy in order to plan optimal management of RA patients.

7.3. miRNA as response to treatment biomarkers

Almost all newly diagnosed RA patients enter their treatment with methotrexate monotherapy. If it fails (and it is ineffective in 66% of patients), other disease-modifying antirheumatic drugs (DMARD) and then one of biologic agents are prescribed. Considering that each type of biology treatment targets different inflammatory mechanisms, sometimes it may take some time to find the best therapy for a patient. Although biological medicines significantly

improved RA treatment, there are still some stumbling blocks to be overcome. The major problems of the treatment with biologics are serious potential side effects and high cost of biologics. Currently clinical and serological markers that sufficient predict disease outcome are unavailable. Therefore one of the most challenging issues is the identification of biomarkers that will predict therapeutic outcome and thus would improve patient care and medical cost-effectiveness. Potential value of miRNAs as molecular biomarker for prognosis treatment response is still unexplored in RA. However, there are several reports demonstrating possibility to use them as therapy outcome predictors [6, 71]. Duroux-Richard and Jorgensen proposed hsa-miR-23a-3p and hsa-miR-223-3p as predictors of therapy response and biomarkers of response to anti-TNFα/DMARDs combination therapy. Strong increase in hsa-miR-23a-3p and hsa-miR-223-3p expressions indicated good response to treatment. Moreover those changes significantly correlated with clinical and inflammatory parameters (such as DAS28, CRP, or ESR). Most importantly expression level of these miRNAs showed its potential to predict therapy outcome. hsa-miR-23-3p and hsa-miR-223-3p levels were predictors of nonresponse to anti–TNF-α/DMARD combination treatment with a sensitivity of 62.5 and 57.1% and a specificity of 86.4 and 90.2%, respectively. The combination of these two miRNAs demonstrated an increase in both the sensitivity (62.5%) and specificity (91.5%) in relation to those given by each miRNA alone [6].

Another circulating miRNAs suggested to be a potential predictive biomarker of response to RA treatment was miR-125b. Duroux-Richard et al. observed that miR-125b expression significantly distinguish RA patients from healthy donors and OA patients but not from patients with other rheumatic disorders like tumor necrosis factor receptor-associated periodic syndrome (TRAPS) or spondyloarthritis (SpA). Results obtained in this study also showed that high expression of miR-125b was associated with good response to rituximab therapy. Serum expression levels of tested miRNA before treatment were significantly higher in good responders than in nonresponders. However, it was not correlated with disease activity parameters. These data suggest miR-125b might be considered as a biomarker that predicts therapy response but is not useful as disease activity marker [72].

The realization of personalized medicine requires comprehensive understanding of genetic and nongenetic factors that may be responsible for drug response. Although several miRNAs appear to be attractive molecular biomarkers, discovery of perfect biomarkers for RA diagnosis and prediction of treatment outcome is still in the future. Better understanding of the role of miRNAs in RA pathogenesis and identification of specific miRNA expression patterns in RA may provide novel molecular prognostic and diagnostic biomarker markers and new gene therapy strategies for treating RA.

8. miRNA polymorphisms

As mentioned, RA is a common, but heterogeneous, disease with the heritability about 60 % [73]. As genetic variants are present at birth, genetic studies can help us identify the key genes and pathways that contribute to both occurrence and severity of disease [74]. The last 60 years provided evidence for the genetic basis of rheumatoid arthritis by identifying genetic

susceptibility variants. Three basic approaches were applied to recognize these susceptibility loci: candidate single nucleotide polymorphisms (SNPs), linkage, and genome-wide association studies (GWAS), which genotype thousands to millions of SNPs in large human samples. However, more than 10 years after the completion of the human genome sequencing project and numerous GWA studies, we still do not fully understand the genetic basis of RA [74, 75].

Since miRNAs are an important class of regulators of gene expression, genetic polymorphisms located in miRNA genes (miR-SNPs) and/or in miRNA binding sites of target genes (miR-TS-SNPs) can act as regulatory SNPs through modifying the activity of miRNAs to affect the susceptibility to and/or severity of disease (**Figure 2**) [76, 77]. The miR-SNPs are thought to affect function in one of three ways. First, genetic alternation in miRNA genes affects its expression through the transcription of the primary transcript; second, via altering pri-miRNA and pre-miRNA processing; and third, by affecting the miRNA-mRNA interactions [77–79]. In contrast to SNPs in miRNA genes, the miR-TS-SNPs, which are present at or near a miRNA binding site in 3'-untranslated region (UTR) of the target gene, are abundant in the human genome [80]. These SNPs can potentially alter miRNA function by creating as well as destroying a miRNA binding site [78]. Moreover, the functional impact of miR-TS-SNPs on disease phenotype largely depends on whether the corresponding miRNA is expressed in a particular tissue and the expression of other possibly compensatory miRNAs [77]. Prediction of SNPs that could alter the expression of a miRNA or its complementarily with the target genes, and as a result the normal function of the involved molecular pathway, seems to be of great importance [79]. Moreover, the polymorphisms associated with miRNAs may have a more important role in RA disease onset and progression than originally suspected. However, to date only a limited number of studies have identified miR-SNPs and/or miR-TS-SNPs relevant to RA (**Table 1**) [81, 82]. In a genome-wide interaction analysis, miR-146a and miR-499 have received much attention in this field. Up today, miR-146a, the most studied miRNAs in patients with RA, is encoded by chromosome 5q33 and can bind to the 3'-UTR of many target mRNAs, including interleukin-1 receptor-associated kinase 1 (IRAK-1), tumor necrosis factor receptor-associated factor 6 (TRAF-6), and other transcripts associated with inflammatory signaling [83, 84]. The common miR-146a polymorphism [85, 85], which may affect the stability of the miRNA, thereby influencing its expression level [82, 86], is not associated with susceptibility to RA [81, 85, 87], but the miRNA-146a rs2910164 variant might increase the risk of RA in the female population and may influence disease activity [84]. Moreover, it remains unclear which allele is associated with a significantly higher level of mature miR-146a. On the other hand, miR-499, the gene of which is located on 20q11.22 chromosome, targets IL-17 receptor B (IL-17RB), IL-23a, IL-2 receptor B (IL-2Rβ), IL-6, IL-2, B- and T-lymphocyte attenuator, IL-18 receptor (IL-18R), IL-21, peptidyl arginine deiminase type 4 (PADI4), and regulatory factor X 4 (influences human leukocyte antigen class II expression) [83, 84]. The miR-499 polymorphism at position rs3746444T > C, located within the stem region of the miR-499 gene is closely associated with the inflammation of RA and can serve a potential diagnostic biomarker for RA [81, 84]. To date, very little is known about the role of miRNA SNPs in the pathogenesis of RA, and even less is known about whether it could affect miRNA-mRNA interactions. That way, the mechanism by which sequence variations—due to genetic or posttranscriptional changes—in miRNAs and/or in their target genes that modulate miRNA binding characteristics or miRNA

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expression can affects the inflammatory processes requires further functional studies. With the development of large-scale RNA-sequencing methods, a new light could be shed on these mechanisms [80].

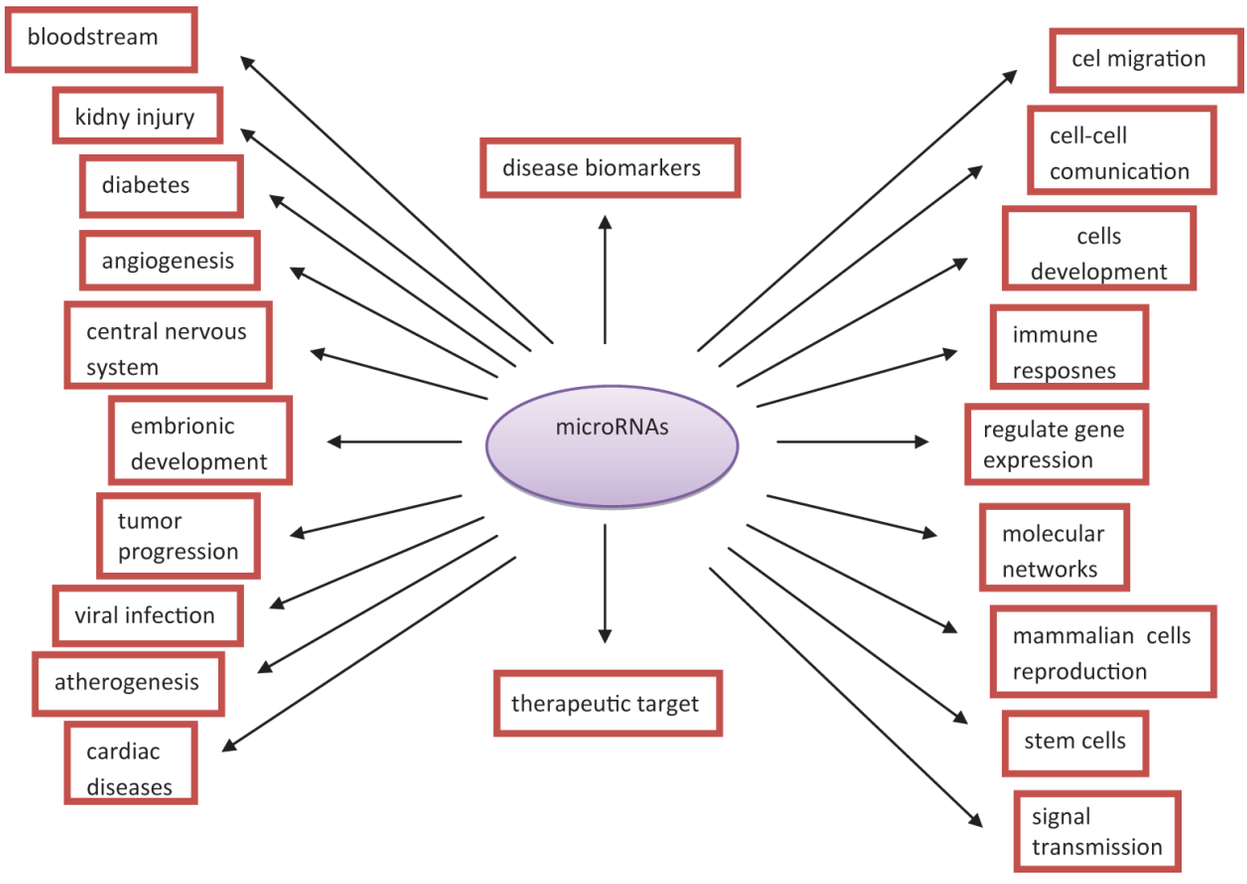

Figure 2. Single nucleotide polymorphisms located in miRNAs.

9. Conclusion

RA is a syndrome in which many different elements of the immune system become activated. A growing number of GWAS demonstrated that genetic polymorphisms, the standard genetic marker to identify associated alleles, make a substantial but incomplete contribution to the risk of RA developing. Now it is widely accepted that epigenetic mechanisms such as miRNAs, a class of powerful and major gene regulators, are also involved in the coordination of the immune processes in the rheumatoid arthritis. Both tissue and extracellular miRNAs are not only key molecules in the molecular mechanisms of the disease but also are very important biomarkers for several pathological conditions and represent a promising therapeutic approach in future. Circulating miRNAs may be suitable for clinical use as they are stable present in body fluids such as plasma or serum. Recent evidence has suggested that miRNA levels in serum or plasma are often higher than in the synovial fluid. In addition, miRNA expression profiles in synovial fluids were similar to those in synovial tissues. These findings

demonstrated that synovial tissues and infiltrating cells are the primary source of synovial fluid miRNAs. In contrast, miRNAs that are presented in the serum or plasma are released by several tissues and cells different from those in synovium [32]. Because rheumatoid arthritis is a systemic disease which can have not only a joint involvement but also other organs may be affected, cell-free circulating miRNA signature in serum or plasma is of great interest.

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