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REVIEW

microRNA-mediated regulation of innate immune response in rheumatic diseases

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Abstract

miRNAs have been shown to play essential regulatory roles in the innate immune system. They function at multiple levels to shape the innate immune response and maintain homeostasis by direct suppression of the expression of their target proteins, preferentially crucial signaling components and transcription factors. Studies in humans and in disease models have revealed that dysregulation of several miRNAs such as miR-146a and miR-155 in rheumatic diseases leads to aberrant production of and/or signaling by inflammatory cytokines and, thus, critically contributes to disease pathogenesis. In addition, the recent description of the role of certain extracellular miRNAs as innate immune agonist to induce inflammatory response would have direct relevance to rheumatic diseases.

Introduction

miRNAs are small endogenous noncoding RNAs, discovered nearly two decades ago. Our understanding of the biological importance of miRNAs has grown exponentially recently owing to the tremendous breakthrough in research in the last several years. Mature miRNAs exhibit robust regulatory roles in almost all biological processes by modulating the expression of their target genes. Not surprisingly, emerging studies have demonstrated the active role of miRNAs in regulating the development and function of immune cells and the association of aberrant expression of miRNA with disorders of the immune system. In this review, we will first discuss several noteworthy features and new findings in biology of miRNA, then focus on the function of miRNA in regulating innate immune response, and, finally, touch the evidence of dysregulation of this process in connection with rheumatic diseases.

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New findings in miRNA biology

miRNA biogenesis and action processes are subject to dynamic regulation

miRNA genes are prevalent in multicellular organisms. These genes often form clusters encoding multiple mature miRNAs that cooperatively regulate the same mRNA target or functionally related targets [1]. Most miRNAs are transcribed by RNA polymerase II. The cell type-specific or spatiotemporal expression patterns of miRNAs are primarily determined at the transcriptional level [2]. The primary transcripts of miRNA genes are sequentially processed by two nucleases, Drosha and Dicer, whose activities are assisted by a number of other protein cofactors, to generate the ~22 nucleotide-long miRNA duplexes [1]. Regulation of the expression and activity of these miRNA processors during different developmental stages or in response to environmental stimuli thus represents an intriguing post-transcriptional control of the miRNA expression profile that accommodates the needs of shaping protein expression in a given cell [2]. For instance, activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway mediates phosphorylation of a critical partner of Dicer, TRBP, which leads to increased stability of the processing complex and enhanced miRNA production [3]: a component of the processing complexes that regulates the biogenesis of a subset of miRNAs is the KH-type splicing regulatory protein (KSRP) [4]; the activity of KSRP can be induced by signaling in an innate immune system [5,6]. Once cleaved, the guide strand of the miRNA duplex gives rise to mature miRNA, which is preferentially incorporated into the RNA-induced silencing complex (RISC) and guides the complex to its target mRNAs. The passenger strand of the miRNA duplex gives rise to the rarely expressed star-form miRNA (miRNA*); however, the ratio of miRNA to miRNA* can be dynamically controlled in response to stimuli. miRNA* species also exhibit important regulatory function [6,7]. In most cases, the miRNA:target interaction is primarily

mediated by base-pairing of the miRNA seed region (nucleotides 2 to 7) to the 3' UTR of the mRNA, resulting in target degradation and/or translational repression [1]. Such a short sequence signature of individual miRNAs is readily found in the genomic transcripts, endowing them with the potential to target dozens or even hundreds of different mRNAs. Moreover, multiple miRNAs can bind to the same mRNA and coordinate its expression [2]. In addition, several other features affect miRNA:target interaction, including local AU content, the position of the binding site in the 3' UTR, and pairing at the 3' end of miRNA [8].

Similar to manipulation of miRNA biogenesis by regulating the components of the processing complex, miRNA effects can be enhanced or attenuated by positively or negatively regulating the levels and activity of RISC components [2]. For instance, in response to stress, the mitogen-activated protein kinase/p38 kinase signaling pathway mediates phosphorylation of serine-387 in AGO2, the core component of RISC, increasing its recruitment to processing bodies [9]. Another intriguing regulation of miRNA function on specific mRNAs depends on the interplay between RISC and other RNA binding proteins. The AU-rich element binding protein HuR is required by let-7/RISC for efficient inhibition of c-Myc expression [10], whereas in other stress conditions HuR relieves miR-122-mediated repression of *CAT-1* mRNA by promoting dissociation of RISC from the target RNA [2,11]. The released mRNA is recruited to polysomes for active translation, suggesting miRNA-mediated repression is reversible [11].

Mature miRNA polymorphisms

A great number of polymorphisms other than those transcribed from genetic variants have been identified in mature miRNA species [12]. First, Drosha and Dicer cleavage of some precursor molecules is not uniform and yields miRNA isoforms with shifting termini [2]. Second, the double-stranded segments in many miRNA precursor molecules are subject to RNA editing (adenosine to inosine) by adenosine deaminases that act on RNA. Those that occur in the mature miRNA corresponding region alter the sequence of the final miRNA product [12,13]. RNA editing may also affect protein binding and, thus, alter the processing efficiency or nuclear export of miRNA precursors [13]. Third, deep sequencing has revealed an abundance of untemplated additions of adenosine or uracil residues to the 3' end of miRNAs [12]. Such polymorphisms can greatly affect the stability of mature miRNAs, and may direct the miRNA to different target mRNAs if located in the seed region [2]. New technologies, such as deep sequencing, will promote the identification of functional isoforms originating from a given miRNA gene and that precise quantification of their expression levels in both physiological and disease settings.

Expanding the rules of miRNA behavior

Deep sequencing also revealed numerous miRNA binding sites that reside in coding sequences [14], in line with previous sporadic reports of functional miRNA target sites within the protein-coding region [15,16]. miRNA targeting can also be mediated by 11 to 12 contiguous perfect match to the center of the miRNA [17]. There are also reports on miRNAs functioning as activators of translation [18,19]. In a recent study, a role for miR-328 as an RNA decoy to directly bind hnRNP E2 was ascribed, thus interrupting the protein's binding capacity and regulatory function towards select mRNAs [20]. Interaction between miRNAs and long noncoding RNAs or transcribed pseudogenes has also been reported [21,22]; such transcripts, along with mRNAs [23,24], can reciprocally control the level and function of miRNAs by dynamic binding to the same miRNA [25]. A growing body of evidence also shows the existence of miRNAs in body fluids, which may be transferable and functional in recipient cells [26,27]. However, more studies are warranted to fully understand the miRNA regulatory network in maintaining homeostasis and the implication in human diseases.

Regulation of innate immune response by miRNAs

Since the initial observation of distinct miRNA expression patterns across the hematopoietic lineage [28], extensive studies have established critical roles for specific miRNAs in immune cell development and, equally importantly, in regulating their function during both innate and adaptive immune response [29-31]. Particularly, cells of the innate immune branch, such as monocyte/macrophages, dendritic cells (DCs), and granulocytes, constitute the first line of defense against invading pathogens. Toll-like receptors (TLRs), which constitute the major pathogen detection system, initiate rapid signaling upon engagement in innate immune cells to induce the transcription of a set of inflammatory cytokines, such as TNFα and type I interferons, and to subsequently prime specific adaptive immune responses. Engagement of other pattern-recognition receptors such as nucleotide-binding oligomerization domain-like receptors (NLRs) and subsequent inflammasome assembly leads to caspase-1 activation and, hence, the production of IL-1β and IL-18 to mediate inflammatory response. Discoveries of novel miRNA players in regulation of innate immunity continue to emerge (Table 1).

miRNA regulates innate immune cell development

A circuitry involving mutual repression between three miRNAs and a key protein regulator has been described

Table 1. miRNA players in regulation of innate immunity

	Target						
miRNA	**Ligand/ cytokine**	**Receptor**	**Signaling component**	**Transcription factor**	**Others**	**Regulated process**	**References**
let-7e		TLR4				Endotoxin sensitivity and tolerance	[53]
miR-106a	IL-10			AML1		Monocyte differentiation, IL-10 production	[32,77]
miR-125b	TNFα					TNFα production by macrophages	[55]
miR-142-3p	IL-6					IL-6 production by DCs	[76]
miR-145			MAL			TLR2-mediated and TLR4-mediated signaling	[63]
miR-146a			IRAK1, IRAK2, TRAF6	IRF5, STAT1		NF-κB-mediated inflammatory response, type I interferon induction and signaling	[39-41,43, 44,47]
miR-148a/b			CaMKIIα			DC activation/antigen presentation	[66]
miR-15			IKKα			Monocyte differentiation	[34]
miR-152			CaMKIIα			DC activation/antigen presentation	[66]
miR-155			IKKε MyD88 TAB2	PU.1, C/EBPβ, c-Fos	KPC1 SOCS1 SHIP1	DC development/maturation granulocyte/ monocyte expansion endotoxin sensitivity and tolerance	[6,38,45, 50-60,92]
				BCL6		NF-κB-mediated inflammatory response, AKT signaling and inflammation, type I interferon induction and signaling	
miR-155*					IRAKM	Type I interferon induction	[6]
miR-16			IKKα			Monocyte differentiation	[34]
miR-17-5p				AML1		Monocyte differentiation	[32]
miR-187				IκBζ		IL-10-driven anti-inflammatory response	[71]
miR-19a/b		TLR2				TLR2-triggered inflammatory response	[73]
miR-200b/c			MyD88			NF-κB-mediated inflammatory response	[65]
miR-20a				AML1		Monocyte differentiation	[32]
miR-21	JAG1				PDCD4	DC development/maturation, NF-κB-mediated inflammatory response	[35,64]
miR-221					p27	DC development/maturation	[36,59]
miR-223			IKKα, NLRP3	MEF2C STAT3		Monocyte differentiation, granulocyte proliferation/activation, TLR-induced IL-6/IL-1β production	[34,37,69, 74,75]
						NLRP3 inflammasome response	
miR-23b			TAB2, TAB3, IKKα			IL-17-triggered, TNFα-triggered, and IL-1β-triggered inflammatory response	[67]
miR-29	IFNγ					IFNγ production and NK cell activation	[78]
miR-34a	JAG1 WNT1					DC development/maturation	[35]
miR-424				NFI-A		Monocyte differentiation	[33]
miR-9				NFKB1		NF-κB-mediated inflammatory response	[70]

AML1, acute myeloid leukemia-1; C/EBPβ, CCAAT/enhancer binding protein beta; DC, dendritic cell; IFN, interferon; IRAK, IL-1 receptor-associated kinase; IRF, IFN regulatory factor; IKK, I-kappa-B kinase; MAL, MyD88 adapter-like; NK, natural killer; NLRP3, NLR family PYD-containing protein 3; SHIP1, SH2 domain-containing inositol phosphatase-1; STAT, signal transducer and activator of transcription; TAB, TAK1-binding protein; TLR, toll-like receptor; TRAF, TNF receptor-associated factor.

to control monocyte differentiation. AML1, the key transcription factor for the process, is directly targeted by miR-17-5p, miR-20a and miR-106a. Expression levels of the three miRNAs are thus downregulated during monocytic differentiation, allowing for the accumulation of AML1, which, in turn, can transcriptionally repress the expression of these miRNAs and promote cell differentiation [32]. By contrast, miR-424 is upregulated by PU.1, another crucial transcription factor for monocyte/macrophage differentiation, and facilitates the expression of differentiation-specific genes by suppressing the protein level of the inhibitory transcription factor NFI-A [33]. Another study reported an increase in IKKα protein level during monocyte/macrophages differentiation owing to

substantial decrease in the expression of miR-15a, miR-16, and miR-223, which contributes to p52 production and prevention of the new macrophage from becoming overactivated [34].

Monocytes can also differentiate into DCs (monocyte-derived DCs), and miR-21 and miR-34a were shown to be important for this process by cooperatively targeting the mRNAs encoding JAG1 and WNT1 [35]. In addition, a handful of miRNAs are differentially expressed across DC subsets and regulate their fate decision, as miR-221 and miR-222 expression favors conventional DC development, whereas inhibition of the miRNAs skewed precursor cells toward plasmacytoid dendritic cell (pDC) commitment [36]. miRNAs also play important roles in granulocytes and natural killer (NK) cell development [30,37]. miR-155 is enriched in hematopoietic stem cells compared with more mature hematopoietic cells; enforced expression of the miRNA in mouse bone marrow cells caused granulocyte/monocyte expansion [38]. However, when miR-146a is depleted, proliferation myeloid cell lineage is observed [39,40].

miR-146a as a crucial negative regulator of innate immune response

miR-146a and miR-155 are the first miRNAs induced during immune activation and profoundly regulate the innate immune response. In human and murine innate immune cells, transcription of both miRNAs is induced by engagement of several TLRs and inflammatory cytokines or upon viral infection, although the extent and peak induction time may vary [6,41-45]. miR-146a effectively suppresses NF-κB activation and downstream cytokine production (for example, IL-8 induction by IL-1) by various stimuli via a negative feedback loop [41,46]. The signaling adaptor proteins TNF receptor-associated family (TRAF)-6 and IL-1 receptor-associated kinase (IRAK)-1 were identified as direct targets of miR-146a [41]. Because these molecules are also critical signaling components for type I interferon production, miR-146a has also been shown to be able to dampen type I interferon induction by TLR7 and the intracellular sensor retinoic acid-inducible gene-I pathway [43,47]. In this context, the transcription factor interferon regulatory factor-5 and another adaptor IRAK2 were also proved to be miR-146a targets, respectively [43,47]. miR-146a thus inhibits the type I interferon production by simultaneously targeting multiple key components of the induction pathway. In addition, miR-146a suppresses the expression of type I interferon-inducible genes in Akata cell line [44] and in peripheral blood mononuclear cells (PBMCs) via targeting signal transducer and activator of transcription (STAT)-1 [43]. Since these classes of molecules play essential roles in receiving and interpretation of the activation signals, relatively small reductions in their levels could greatly affect the functional response [48]. The overall regulation of the type I interferon induction and action by miR-146a via multiple targets can thus produce great impact, although the inhibitory effect towards each individual target may be modest. Consistent with the *in vitro* findings, miR-146a-deficient mice display chronic NF-κB activation and develop autoimmune-like disease in aging animals [39,40].

Dual role for miR-155 and opposing action of *miR-155/miR-155** in different settings

There is extensive evidence supporting miR-155 as a negative regulator of innate immune or inflammatory response. The adaptor protein MyD88 and the kinase IKKε were identified as potential targets of miR-155 [49-51]. In monocyte-derived DCs, miR-155 attenuates TLR/IL-1R inflammatory pathway activation by directly targeting the signaling molecule TAB2 [52]. On the contrary, miR-155 promotes inflammatory response of macrophages and also type I interferon signaling via direct inhibition of the canonical negative regulator suppressor of cytokine signaling-1 [45,53]. Another study confirmed SH2 domain-containing inositol phosphatase-1 (SHIP1) as a direct target of miR-155; repression of endogenous SHIP1 by miR-155 resulted in increased activation of the kinase AKT during macrophage response to lipopolysaccharide (LPS) [54]. Concordantly, miR-155 transgenic mice produced higher levels of TNFα when exposed to LPS [55]. During acute inflammatory response, induction of miR-155 leads to the suppression of CCAAT/enhancer binding protein beta, which may be responsible for the upregulation of granulocyte colony-stimulating factor [56]. The proinflammatory role of miR-155 was also evident in atherosclerotic plaques, where specific expression of miR-155 in macrophages directly inhibits the transcriptional repressor BCL6, leading to the expression of the chemokine CCL2, and thus recruitment of monocytes to the inflammatory site [57]. In addition, several other proteins have been identified as miR-155 targets in DCs, such as PU.1 [58], KPC1 [59], and c-Fos [60], indicating that miR-155 regulates many aspects of DC biology (reviewed in [61]). Indeed, miR-155-deficient DCs fail to effectively activate T cells [61,62], exemplifying a role for miRNA in regulating the priming of adaptive immune response.

Interestingly, although miR-155 and miR-155* originate from the same precursor, they display opposite effects on the regulation of type I interferon production by pDCs [6]. In the initial stage of pDC stimulation by TLR7 agonist, the transcriptional activation of the *miR-155/miR-155** gene leads to rapid production of mature miR-155* versus miR-155. This results in the degradation of its target IRAKM, a negative regulator that blocks TLR7 pathway activation in resting pDCs, thereby

facilitating type I interferon production. Simultaneously, both TLR7 stimulation and the autocrine/paracrine signaling of IFNα/β lead to gradual accumulation and activation of KSRP, which promote miR-155 maturation at the post-transcriptional level. In the later stage of activation, therefore, expression of miR-155 dominates whereas miR-155* levels decrease sharply. Targeting TAB2 by miR-155 in pDCs confers negative regulation of the activation signaling, thus maintaining type I interferon production and pDC activation at a proper level [6].

Other miRNAs targeting innate immune signaling molecules

miRNA-mediated regulation of other molecules in the TLR signaling cascades can also effectively control or fine-tune the innate immune response. miR-145 was shown to target the bridging adaptor MAL [63]. miR-21 inhibits the expression of proinflammatory regulator PDCD4 after TLR4 engagement [64]. A couple of signaling proteins in the TLR4 pathway were predicted as potential targets for miR-200 family members (miR-200a/b/c); however, a reporter gene screening showed that only the MyD88 3' UTR was targeted by miR-200b/c, which was confirmed by mutational analysis [65]. Consequently, overexpression of miR-200b/c inhibited NF-κB reporter activity and TLR4-induced inflammatory cytokine expression [65]. CaMKIIα is a major downstream effector of calcium and plays an important role in promoting TLR signaling-induced DC maturation and function. Upregulation of members of the miR-148 family (miR-148a/b and miR-152) in DCs by several TLR agonists leads to targeted inhibition of CaMKIIα, which results in suppression of cytokine production, reduced MHC II surface expression and DC-initiated antigen-specific T-cell proliferation [66], demonstrating a role for miRNAs other than miR-155 in regulating antigen-presenting capacity of DCs.

In a recent study, the concomitant regulation of TAB2, TAB3, and IKKα by miR-23b was reported, which is responsible for the critical suppression of NF-κB activation and inflammatory cytokine production induced by IL-17, TNFα, or IL-1β [67]. Importantly, miR-23b is downregulated by IL-17 stimulation [67]. Despite the recent research focus on IL-17 as a T-cell-secreted cytokine, many innate immune cell populations release a high amount of IL-17 at the early stage of an immune response, which is central to the initiation of IL-17-dependent immune responses [68]. Downregulation of miR-23b by IL-17 would therefore play a profound role in regulating the innate immune activation.

miRNA-mediated regulation is also dictated to targeting transcription factors that are instrumental in generating an innate immune response, as exemplified by miR-146a (targeting interferon regulatory factor-5 and STAT1) discussed above. In macrophages, downregulation of miR-223 by TLR3 and TLR4 agonists results in derepression of its target STAT3 and, thus, in enhanced production of IL-6 and IL-1β but not TNFα [69]. miR-9 is induced by LPS in both monocytes and neutrophils and directly targets *NFKB1* mRNA, representing another important feedback control of NF-κB-dependent responses [70]. In the IL-10-driven anti-inflammatory response, miR-187 was shown to be induced to downregulate the production of several inflammatory cytokines by activated monocytes. One relevant target identified for miR-187 is IκBζ, which is a key transcriptional regulator of IL-6 and IL-12p40 [71].

miRNAs have also been shown to directly target mRNAs encoding individual TLRs [72]. TLR4 expression is thus inhibited by let-7e in macrophages [53], whereas TLR2 is targeted by miR-19a/b [73]. The miRNA-mediated regulation of TLR signaling pathways is summarized in Figure 1.

In the context of NLR-mediated inflammatory response, two very recent studies independently reported the direct regulation of NLR family PYD-containing protein 3 (NLRP3) and, consequently, inhibition of IL-1β production from the NLRP3 inflammasome by miR-223 [74,75].

Direct targeting of cytokine mRNAs

In addition to the preferential regulation via signaling molecules, several cytokine mRNAs also fall into direct control by miRNA [72]. In addition to indirect suppression of IL-6 and IL-12p40 by miR-187 discussed above, this miRNA also directly inhibits TNFα mRNA expression and translation in monocytes [71]. The 3' UTR of TNFα mRNA also harbors a binding site for miR-125b, and downregulation of the miRNA by LPS stimulation may help stabilize TNFα expression [55]. IL-6 mRNA is directly targeted by miR-142-3p; silencing of miR-142-3p leads to enhanced IL-6 production both in immature DCs and following LPS activation [76].

The results of another study revealed direct inhibition of IL-10 expression by miR-106a [77]. Ma and colleagues found that NK cells activated *in vivo* (that is, from mice infected with an intracellular pathogen) or *in vitro* by the innate immune ligand poly(I:C) downregulated their expression of miR-29 while producing a large amount of IFNγ [78]. They further showed a direct interaction between miR-29 and IFNγ mRNA: in addition to evidence that mutation of the predicted miR-29 binding sites abolished its inhibitory effect on IFNγ 3'-UTR reporter gene activity, the authors detected elevated association of IFNγ mRNA with the Ago2-containing complex in cells transfected with synthetic miR-29a mimic using an immunoprecipitation approach with an

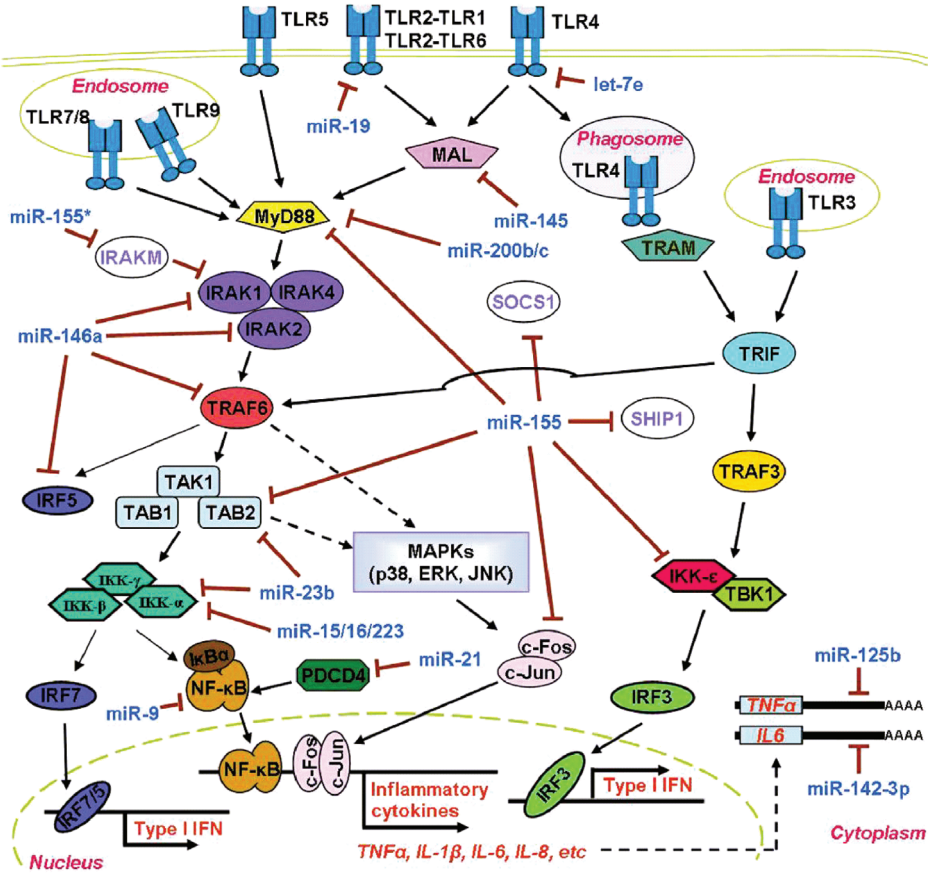

Figure 1. Schematic presentation of select miRNAs that regulate toll-like receptor signaling pathways. During innate immune response, toll-like receptors (TLRs) are activated by various pathogens and initiate signaling transduction to induce the production of type I interferons (IFNs) and other inflammatory cytokines. miRNAs exert pronounced control of the pathway activation at multiple levels to ensure the generation of proper immune response. The miRNAs preferentially target the common signaling components and transcription factors, but also directly act on receptors and cytokine mRNAs. In most cases, decreases in the concentrations of miRNA target proteins achieve effective negative regulation and therefore avoid detrimental immune activation. However, if the target protein itself is a negative regulator (IL-1 receptor-associated kinase (IRAK)-M, suppressor of cytokine signaling-1 (SOCS1), SH2 domain-containing inositol phosphatase-1 (SHIP1)), miRNA-mediated regulation will facilitate TLR signaling and the production of inflammatory cytokines. ERK, extracellular signal-regulated kinase; IFR, interferon regulatory factor; IKK, I-kappa-B kinase; MAL, MyD88 adapter-like; MAPK, mitogen-activated protein kinase; TAB, TAK1-binding protein; TAK, transforming growth factor-beta activated kinase; TBK, TANK-binding kinase; TRAM, TRIF-related adapter molecule; TRAF, TNF receptor-associated factor; TRIF, TIR domain containing adaptor inducing IFNβ.

antibody against Ago2 [78]. The importance of miR-29 in regulating the immune response to intracellular bacterial infection (via targeting IFNγ) was further demonstrated *in vivo* by competitive inhibition of miR-29 by transgenic expression of a sponge target [78].

miRNAs take action in host–virus interaction

Compelling evidence demonstrates that miRNAs are directly incorporated into host–virus interactions, providing another layer to the innate immune response [31,79]. For instance, host cell miR-32 can recognize and bind to five viral mRNAs, contributing to the repression of the replication of the retrovirus primate foamy virus type 1 [80]. In addition to the induction of a plethora of well-known antiviral proteins, IFNβ is also found to stimulate the expression of several miRNAs that target the genome of hepatitis C virus [81]. Simultaneously, IFNβ suppresses the expression of miR-122, a host miRNA that is utilized by hepatitis C virus to promote its replication.

In the context of viral exploitation of miRNAs for their own advantage, some virus-encoded miRNAs target host mRNAs to evade immune surveillance or dampen the immune response. For instance, hcmv-miR-UL112 represses the expression of histocompatibility complex class I-related chain B and consequently impairs NK cell activation and killing infected cells [82]. The Epstein–Barr virus-encoded miRNA, miR-BART15, represses the

expression of NLRP3, and thus IL-1β production [74]. Furthermore, this viral miRNA can be secreted and transferred via exosomes to inhibit the NLRP3 inflammasome capacity in noninfected cells [74]. Such direct interactions between virus-encoded and host-encoded nucleic acids provide another dimension to innate immunity [79]. Since viral infection has also been implicated in rheumatic disease onset or flare and Epstein–Barr virus is considered a major environmental risk factor for systemic lupus erythematosus (SLE) [83], the involvement of miRNA in host–virus interaction may also have some relevance to rheumatic disease pathogenesis.

Dysregulation of miRNA and innate immune response in rheumatic diseases

It becomes evident now that miRNAs mediate dynamic regulation at multiple levels that essentially controls innate immune cell development and activation, inflammatory cytokine production and signaling, and antigen presentation. Dysregulated miRNA expression or function could severely affect the duration and extent of innate immune response and be detrimental. Indeed, emerging data underscore the role of excessive or protracted innate immune signaling in the pathogeneses of autoimmune and autoinflammatory rheumatic diseases [83-85], which has been linked to dysregulation of critical miRNAs.

Systemic lupus erythematosus

SLE is a prototypical autoimmune disease with a hallmark of chronic immune activation and multiple immunologic alterations. To identify dysregulated miRNAs in SLE, a profiling analysis of 156 miRNAs was undertaken to compare their expression levels in the peripheral blood leukocytes from patients with SLE and healthy subjects. This led to the identification of underexpression of miR-146a in SLE [43], which appeared to be a primary defect caused by lupus-associated germline variant in miR-146a promoter [86], rather than a consequence of disease onset or medication [43]. In the same study, a reverse correlation of miR-146a levels with disease activity and with interferon score was identified, which reflects the magnitude of type I interferon pathway activation in patients with SLE. This indicates that decreased expression of miR-146a would result in inadequate regulation of the multiple target proteins and consequently overproduction of type I interferons and unabated downstream activation [43]. Importantly, enforced expression of miR-146a in PBMCs from patients with active SLE attenuated the mRNA levels of several interferon-inducible genes [43], strongly supporting the contribution of miR-146a dysregulation to such molecular phenotype of SLE.

Other miRNAs with a known role in innate immune response and dysregulated in SLE include miR-21, miR-142-3p, miR-148a/b, and miR-155, all of which are upregulated in PBMCs in patients with SLE [87,88]. However, the contribution of these miRNAs in SLE pathogenesis via dysregulated innate immune response still needs to be determined. Stagakis and colleagues reported that the expression of the miR-21 target gene PDCD4 [64] is correspondingly decreased in active SLE, but suggested that their interaction affects aberrant T-cell responses in SLE in humans [88].

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes irreversible joint damage. Investigation of the expression and contribution of miRNAs in RA is very active and has revealed the dysregulation of several miRNAs in various cells/tissues, including PBMCs, the synovial tissue, isolated fibroblast-like synoviocytes (FLS), and cell-free synovial fluid (reviewed in [89]). The joint resident cells, FLS are unique for RA in that they, like innate immune cells, express several TLRs, are implicated in inflammatory response, and play critical roles in osteoarticular destruction [73]. Stimulation of RA FLS with LPS or bacterial lipoprotein strongly induced TLR2 expression while suppressing the levels of miR-19a/b, which directly targets TLR2 mRNA [73]. Supporting a role for miR-19a/b in regulating RA inflammation, transfection of the miRNA mimic significantly downregulated the release of IL-6 and matrix metalloproteinase-3 by TLR2-activated RA FLS [73]. Secretion of IL-6 and matrix metalloproteinase-1 also appears to be indirectly regulated by miR-203, which is highly expressed in RA FLS [90].

Compared with osteoarthritis, miR-155 is significantly upregulated in RA FLS, whose expression can be further induced by TNFα, IL-1β, and by ligands of TLR2 through TLR4. This indicates that the inflamed milieu may be responsible for the altered expression of miR-155 in these cells [91]. Moreover, miR-155 is also highly expressed in synovial fluid-derived monocytes/macrophages compared with the peripheral blood counterparts from patients with RA [91,92], whereas both mRNA and protein levels of the miR-155 target SHIP1 are decreased [92]. Incubation of peripheral blood $CD14^+$ cells with RA synovial fluid stimulated the expression of miR-155 and release of TNFα; the cytokine production was abrogated by transfection of miR-155 inhibitor [92]. Direct evaluation of the regulation in RA synovial $CD14^+$ cells revealed inhibition of miR-155 augmented SHIP1 expression and downregulated TNFα production when these cells were reactivated by LPS [92]. Moreover, the authors of this study and another group independently showed that miR-155 knockout mice did not develop collagen-induced arthritis (CIA) where significantly lower production of many proinflammatory cytokines was observed [92,93]. One should note here that, in addition to the

essential regulation of monocyte/macrophage activation and of DCs in priming the adaptive immune response, miR-155 is also directly required for proper function of T cells and B cells [30,62]. Clearly, the protective role of miR-155 deficiency in the CIA model resulted from the combinatory effect on both innate and adaptive immune responses [92,93].

miR-223 is also significantly overexpressed in RA FLS and synovial fluid [89,94,95]. Intriguingly, when a lentiviral vector expressing the miR-223 target sequence was intraperitoneally administrated to mice with CIA to abrogate miR-223 function, the severity of experimental arthritis was markedly reduced. This suggested a potential therapeutic strategy [95], although the extent and contribution of miR-223 silencing in distinct cell types needs detailed examination.

To identify novel miRNAs associated with RA pathology, Pandis and colleagues started with a different approach. They first applied deep sequencing to examine the miRNA expression profile of FLS isolated from the human TNF transgenic mouse model (TghuTNF) [96]. A number of dysregulated miRNAs were identified, including miR-155 and miR-223 that are known to be upregulated in FLS of RA patients. The expression levels of select miRNAs were further quantified in patient biopsies, and the upregulation of miR-221, miR-222 and miR-323-3p was also consistently found to be associated in human RA [96].

Interestingly, miR-23b was found to be underexpressed in RA synovial tissue and in the joints of mice with CIA, in the kidneys of patients with SLE and the MRL/lpr mouse model, and in experimental autoimmune encephalomyelitis mice, which may be a result of IL-17-mediated transcriptional inhibition [67]. The results of this study provide *in vivo* evidence that miR-23b could suppress autoimmune disease pathogenesis, although the expression of this miRNA in resident cells in inflammatory lesions appears to be vital in this regard [67].

Upregulated expression of miR-146a in rheumatoid arthritis, Sjögren's syndrome, and myositis

In contrast to the decreased expression of miR-146a in SLE, patients with RA display higher expression of miR-146a in both FLS [91,97] and PBMCs [98], with a hint that the alteration primarily occurs in monocytes/macrophages [98]. Although miR-146a does target IRAK1 and TRAF6 mRNA for degradation [99], their expression in PBMCs in patients with RA is similar to that in healthy subjects [98]. The results of a recent study revealed overexpression of miR-146a in PBMCs of patients with Sjögren's syndrome, which is also observed in PBMCs and the salivary glands in an animal model of the disease [99]. In another study, increased expression of both miR-146a and miR-146b in patients with Sjögren's syndrome was observed [100]. In PBMCs from patients with Sjögren's syndrome, the mRNA level of IRAK1 is decreased while that of TRAF6 is increased when examined in a small number of subjects (n = 9 for patients and n = 10 for healthy subjects, respectively [100]). Altered miRNA levels were also identified in patients with myositis; the expression of miR-146a was found to be elevated, probably due to leukocyte infiltration [101]. Although the 31 patients with myositis examined displayed a general signature of type I interferon pathway activation, six out of eight patients with dermatomyositis examined had reverse correlation between miR-146a levels and type I interferon gene signature [101]. Further studies are warranted to explore the reason for increased expression of miR-146a and its contribution to such rheumatic diseases.

Scleroderma

Plenty of studies have also been performed by Ihn's group to identify scleroderma-associated miRNAs – several miRNAs including miR-29a and miR-196a were found to be dysregulated, either in skin biopsy or fibroblast samples, or in the serum from scleroderma patients [102,103]. Given that recent studies have provided new insights into the role of the innate immune system in scleroderma [104], the potential contribution of miRNA-mediated dysregulation of innate immune response to scleroderma pathology is yet to be explored.

Extracellular miRNAs: a missing link between innate immune response and rheumatic disease?

The presence of miRNAs in body fluids attracts a lot of attention. There are examples of the extracellular miRNAs entering into and maintaining their regulatory function in recipient cells [26,27], but further evidence is awaited. Another major focus of current studies is the identification of certain circulating miRNAs as disease biomarkers.

Nevertheless, the results of two recent studies suggested an unconventional role for miRNAs and an intriguing link between miRNAs in body fluids and innate immune signaling in disease settings. Lehmann and colleagues identified an increase in let-7b levels in the cerebrospinal fluid from individuals with Alzheimer's disease and provided *in vivo* evidence that extracellular let-7 acts as an RNA ligand to activate neuronally expressed TLR7 and induce neurodegeneration [105]. The results of another study showed that miR-21 and miR-29a in the cancer cell-derived exosomes are able to bind murine TLR7 and human TLR8 and to induce a prometastatic inflammatory response [106].

Although the exact structural features in the sequence of such miRNAs that confer their capacity to activate TLR7/8 require more studies, they all appear to harbor a GU-rich motif, which is known to be present in

TLR7/8-stimulating virus-derived RNAs [105,106]. Supporting this idea, miR-599, miR-147, and miR-574-5p, which also contain GU-rich motifs, similarly induce TLR7/8-dependent cytokine production [105,106]. Given the more direct relevance of such an innate immune pathway to rheumatic diseases, and given the dysregulated miRNA levels in the body fluids of patients with such disorders systemically (in serum) and/or locally (such as in RA synovial fluid) [89], it would be interesting to examine such a link in a specific rheumatic disease.

Conclusion

miRNA appears to preferentially target signaling proteins and transcription factors (Figure 1), molecules that are instrumental for dictating the extracellular stimuli and driving the development and activation of innate immune cells. Some miRNAs simultaneously regulate the expression of multiple proteins (for example, targeting of IRAK1, TRAF6, interferon regulatory factor-5, and STAT1 by miR-146a), thus effectively controlling the activation of innate immune signaling cascade. Some other miRNAs bind to the same site (for example, targeting of CaMKIIα by miR-148 family members) or separate ones (for example, targeting of IKKα by miR-15a, miR-16, and miR-223) within a single mRNA and coordinately control the expression of a common target. In addition, miRNAs also directly target mRNAs encoding innate immune receptors, such as TLR4 and TLR2, or inflammatory cytokines, such as TNFα, IL-6 and IFNγ. miRNAs may also exert their regulation through inhibition of some relevant targets that are previously not linked to the innate immune response or that display an important function in other cellular pathways, and thus one might expect a careful analysis of putative targets to lead to the identification of novel genes involved in some aspects of innate immunity or to provide a missing link between innate immune and other cellular pathways [107].

In many cases, the expression of specific miRNAs is upregulated via transcriptional activation (for instance, NF-κB-dependent induction) to decrease the concentration of their target proteins. In other scenarios, the miRNA expression is downregulated to allow for the accumulation of its target (for example, decreased miR-29 expression with increased IFNγ production during NK cell activation). The post-transcriptional regulation adds another layer of control of miRNA expression during an innate immune response, enabling selective modulation of levels of certain mature miRNAs, and ensuring miRNA-mediated regulation to be exerted more precisely, as in the case of KSRP-promoted maturation of miR-155 during pDC activation. Molecules mediating signaling activation are not only targeted by miRNAs, but several negative regulators are also under miRNA-mediated control (for example, targeting of suppressor of cytokine signaling-1 and SHIP1 by miR-155). miRNAs thus regulate innate immune response at multiple levels. Depending on the nature of the target proteins, miRNAs can either suppress or facilitate distinct aspects of immune activation, and ultimately maintain the balance of innate immune response. Altered expression of critical miRNAs, such as miR-146a and miR-155, thus profoundly contributes to the pathogeneses of rheumatic diseases, where dysregulation of their target proteins leads to unabated inflammatory cytokine production and signaling, and aberrant priming of adaptive immune response. With the application of new technologies, such as deep sequencing, one would expect that more miRNAs or functional isoforms will be identified to have a role in regulating innate immune response and dysregulation in rheumatic diseases. This would particularly provide insight into autoinflammatory disorders, where activation of the innate immune system alone is sufficient to induce the disease [83].

On the contrary, although the importance of miRNA-mediated regulation of innate immune response should be highly appreciated, one should notice that a considerable fraction of miRNAs discussed here are also critical regulators of adaptive immune response (for example, miR-155, which also regulates T-cell and B-cell function, as evidenced by knockout mice). This is particularly important in disease settings, because dysregulation of adaptive immune response is considered indispensable in the pathogenesis of autoimmune rheumatic diseases [83]; in many studies, altered expression of miRNAs in PBMCs or diseased tissue with leukocyte infiltration, instead of purified innate immune cells, was observed. Moreover, some miRNAs may even simultaneously regulate processes beyond immune system but essentially related to disease pathogenesis. For instance, miR-155, miR-223 and miR-21 promote osteoclastogenesis [93, 108-110] while miR-146a inhibits it [111]. The effect of these miRNAs on local bone destruction in RA has been demonstrated in animal models [93,111]. miRNA knockout mice would thus provide unambiguous evidence for the physiological and pathological roles of specific miRNAs in the innate immune system and in other processes.

One should still pay attention to the strategy applied, however, as both the target miRNA and its star form partner will be depleted. For example, it would be interesting to hypothesize whether there is any phenotype reported for miR-155 deficiency actually attributable to loss of miR-155*. This query would require a thorough investigation of the distinct contribution of critical targets of each miRNA, as occurred for demonstration of targeting activation-induced cytidine deaminase by miR-155 *in vivo* [112,113]. With a better understanding of the contribution of dysregulation of miRNAs to the

aberrant immune activation and, consequently, pathogeneses of rheumatic diseases, we would further explore the promise that miRNAs hold for developing new therapeutic targets.

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Abbreviations
CIA, collagen-induced arthritis; DC, dendritic cell; FLS, fibroblast-like synoviocytes; IFN, interferon; IL, interleukin; IRAK, IL-1 receptor-associated kinase; KSRP, KH-type splicing regulatory protein; LPS, lipopolysaccharide; miRNA, microRNA; miRNA*, star-form miRNA; NF, nuclear factor; NK, natural killer; NLR, nucleotide-binding oligomerization domain-like receptor; NLRP3, NLR family PYD-containing protein 3; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; RA, rheumatoid arthritis; RISC, RNA-induced silencing complex; SHIP1, SH2 domain-containing inositol phosphatase-1; SLE, systemic lupus erythematosus; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TNF, tumor necrosis factor; TRAF, TNF receptor-associated factor; UTR, untranslated region.

Competing interests
The authors declare that they have no competing interests.

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