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MicroRNA in autoimmunity and autoimmune diseases

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ABSTRACT

MicroRNAs (miRNAs) are small conserved non-coding RNA molecules that post-transcriptionally regulate gene expression by targeting the 3′ untranslated region (UTR) of specific messenger RNAs (mRNAs) for degradation or translational repression. miRNA-mediated gene regulation is critical for normal cellular functions such as the cell cycle, differentiation, and apoptosis, and as much as one-third of human mRNAs may be miRNA targets. Emerging evidence has demonstrated that miRNAs play a vital role in the regulation of immunological functions and the prevention of autoimmunity. Here we review the many newly discovered roles of miRNA regulation in immune functions and in the development of autoimmunity and autoimmune disease. Specifically, we discuss the involvement of miRNA regulation in innate and adaptive immune responses, immune cell development, T regulatory cell stability and function, and differential miRNA expression in rheumatoid arthritis and systemic lupus erythematosus.
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1. Introduction

MicroRNAs (miRNAs) are 20–22 nucleotide long non-coding RNA molecules that were first discovered in 1993 [1,2]. Currently, the known function of miRNAs is the post-transcriptional regulation of certain subsets of messenger RNAs (mRNAs) by binding to their 3′ untranslated region (UTR) thus targeting them for degradation or translational repression [3].

The importance of miRNA regulation to cellular functions is becoming increasingly clear as new miRNA targets are revealed. Currently, miRNA is known to regulate cellular processes such as apoptosis, differentiation, cell cycle, and immune functions. To date, the miRNA sequence database, miRBase, includes over 8000 predicted miRNAs in numerous species of plants, animals, and viruses [4,5]. For humans alone, miRBase lists over 800 predicted miRNAs, and other bioinformatics predictions indicate that as much as one-third of all mRNAs may be regulated by miRNA [6].

2. Biogenesis and maturation of miRNA

miRNAs are first transcribed from the genome by RNA polymerase II as primary miRNA (pri-miRNA) transcripts [7,8]. In animals, miRNA maturation is then achieved by two main processing steps involving two ribonuclease III (RNase III) enzymes, Drosha and Dicer. First, Drosha and its partner protein DGCR8 (DiGeorge syndrome critical region 8) process the nuclear pri-miRNA into ∼70 nucleotide precursor miRNA (pre-miRNA) molecule [9–13]. The pre-miRNA is then exported from the nucleus to the cytoplasm by Exportin 5/RanGTP which specifically recognizes the structure of pre-miRNA molecules [14–16]. Once in the cytoplasm, pre-miRNA is cleaved by Dicer along with its partner protein TRBP (trans-activator RNA binding protein) into a ∼21 nucleotide miRNA duplex and one strand is selected to be loaded into the RNA-induced silencing complex (RISC) in a dynamic process that is not yet fully understood [3,17]. Once loaded into RISC, the miRNA will bind to its target mRNA in the 3′UTR resulting in degradation or translational repression via several proposed mechanisms including cotranslational protein degradation, inhibition of translational elongation, premature termination of translation, and inhibition of translation initiation (reviewed in Ref. [18]).

Recently, an alternative Drosha-independent pathway for miRNA maturation has been reported in *Caenorhabditis elegans*, *Drosophila melanogaster*, and mammals [19–21]. In this pathway, short hairpin introns are spliced into pre-miRNA mimics referred to as "mirtrons" (pre-miRNAs/introns) that can enter the miRNA-biogenesis pathway without Drosha-mediated cleavage. However, mirtrons represent a relatively small number of miRNAs to date and the majority of miRNAs are processed by the Drosha dependent pathway.

3. Cell biology and autoimmune targeting of the miRNA pathway

The key components of RISC are the argonaute (Ago) family of proteins. In mammals, there are four Ago proteins (Ago1–4), but

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only Ago2 is known to function in the miRNA and siRNA pathways. Ago2 has been shown to cleave mRNA targeted by miRNA or small interfering RNA (siRNA) and is known as the catalytic enzyme of RNA interference (RNAi) [22,23]. In addition to Ago proteins, many other proteins are required for miRNA functioning including GW182 and Rck/p54, and these proteins all localize in discrete cytoplasmic foci known as GW bodies (GWB). In mammals, these foci were discovered in 2002 using an autoimmune serum from a patient with motor and sensory neuropathy [24], and most subsequent GWB reactive sera have been identified from patients with neurological symptoms (33%), Sjögren's syndrome (31%), and various other autoimmune disorders including systemic lupus erythematosus (SLE, 12%), rheumatoid arthritis (7%), and primary biliary cirrhosis (10%) [25]. About the same time period, similar foci were discovered in yeast and referred to as processing bodies (P bodies) or Dcp-containing foci [26–28].

In 1994, Satoh et al. characterized autoantigens of 100/102 and 200 kDa recognized by anti-Su autoantibodies [29]. Autoantibodies that immunoprecipitated the 100/102 and 200 kDa proteins were detected in sera of up to 20% of patients with SLE, scleroderma, and overlap syndromes [29]. In 2006, Jakymiw et al. reported that anti-Su autoantibodies from human patients with rheumatic diseases and a mouse model of autoimmunity recognize the catalytic enzyme in the RNAi/miRNA pathways, Ago2, as well as Ago1, 3, 4 and Dicer [30]. Additionally, by immunofluorescence, the anti-Su autoantibodies were shown to recognize GWB [30]. Recently, an investigation into the clinical and serological features of patients with autoantibodies to GWB revealed that the most common clinical presentations of these patients were neurological symptoms, Sjögren's syndrome, SLE, rheumatoid arthritis and primary biliary cirrhosis [25]. The most common autoantigens targeted by these patients were Ge-1/Hedls (58%), GW182 (40%), and Ago2 (16%) and 18% of GWB reactive sera did not react to any of the antigens analyzed indicating that there are other target autoantigens yet to be discovered [25]. These data demonstrate an autoimmune response to key components of the RNAi/miRNA pathways which could indicate the involvement of the miRNA pathway in the induction and production of autoantibodies.

4. Role of miRNA in normal immune functions

Regulation of the immune system is vital to prevent many pathogenic disorders including autoimmune disease and cancers, and mammals have developed a complex system of checks and balances for immune regulation in order to maintain self tolerance while allowing immune responses to foreign pathogens, most of which are not fully understood. Recently, it has become evident that miRNAs play an important role in regulating immune response, as well as immune cell development. To date, a relatively small number of specific miRNAs have been revealed as important regulators of the immune system. Their functions are discussed below and summarized in Table 1.

4.1. miRNA response to toll like receptor stimulus

In 2006, three miRNAs, miR-146a, miR-155, and miR-132, were found to be upregulated in LPS-stimulated human monocytic THP-1 cells [31]. Expression of miR-146a was also found to be inducible by TNF-α and IL-1β, and further analysis revealed that this induction was NFκB-dependent [31,32]. Two targets of miR-146a were confirmed, TNF receptor-associated factor-6 (TRAF6) and IL-1 receptor-associated kinase-1 (IRAK-1), which are key components in the TLR4 signaling pathway [31]. Interestingly, miR-146a expression was only inducible by cell-surface TLR (TLR2, TLR4, TLR5) signaling and not intracellular TLR (TLR3, TLR7, TLR9) signaling, indicating that miR-146a plays a role in regulating the innate immune response to bacterial pathogens, but not viral pathogens [31]. In human lung alveolar epithelial cells increased miR-146a expression was found to negatively regulate the release of the proinflammatory chemokines IL-8 and RANTES [32]. Overall, these data suggest that miR-146a functions to downregulate the inflammatory response to bacterial pathogens.

In mouse macrophages, miR-155 expression was upregulated by IFN-β and polyriboinosinic:polyribocytidylic acid [poly(I:C)] and as well as a variety of TLR ligands [33,34]. These data indicate the involvement of miR-155 in the regulation of both bacterial and viral innate immune responses. Additionally, miR-125b expression was decreased in response to LPS in mouse macrophages [34]. Since miR-125b was shown to be capable of targeting the 3′UTR of TNF-α mRNA, this miRNA may be downregulated to allow for LPS-induced production of TNF-α [34].

4.2. miR-155 required for normal immune function, germinal center response, and generation of Ig class-switched plasma cells

Given the abundance of miRNAs expressed in humans, it is surprising that a single miRNA, miR-155, plays a role in regulating several vital immune functions. In addition to its regulatory role in innate immunity, miR-155 has been shown to be a key factor for normal adaptive immune responses. miR-155 is processed from the non-coding RNA known as bic, which is now known to be pri-miR-155 [35,36]. Increased expression of bic/miR-155 can be detected in activated B and T cells [37,38] as well as in activated macrophages [31,33] and is associated with B cell malignancies [39–41].

In 2007, Rodriguez et al. found that mice deficient in bic/miR-155 exhibited diminished adaptive immune responses and were unable to develop immunity to *Salmonella typhimurium* after intravenous immunization [42]. This diminished adaptive immune response was found to be due to impaired B and T cell functioning as well as defective antigen presentation by dendritic cells [42]. These data suggest that miR-155 is essential for normal functioning of B and T lymphocytes and dendritic cells.

Concurrently, Thai et al. reported that miR-155 regulates the germinal center response [43]. Initially, they demonstrated that germinal center B cells upregulate miR-155 expression during the course of normal germinal center response. Using bic/miR-155-deficient mice, they determined that miR-155 regulates the germinal center response at least in part at the level of cytokine production [43].

Later in 2007, it was reported that miR-155 is also responsible for regulating the generation of immunoglobulin (Ig) class-switched plasma cells [44]. In this study, miR-155-deficient B cells failed to generate high-affinity IgG1 antibodies [44]. Over expression of Pu.1, a transcription factor targeted by miR-155, leads to the production of fewer IgG1 cells, indicating that miR-155 regulation of Pu.1 may be responsible for the normal generation of Ig class-switched plasma cells [44].

4.3. Role of miRNAs in immune cell development

Several studies have reported the involvement of miRNAs in immune cell development [45–47]. One of the first miRNAs described to have a role in immune cell development was miR-181a which is highly expressed in thymus cells and expressed at a lower level in the heart, lymph nodes, and bone marrow [48,49]. In bone marrow-derived B cells, miR-181a expression was shown to decrease during B cell development from the pro-B to the pre-B cell stage [48]. Also, miR-181a may have a role in regulating lymphocyte development based on evidence that expression of miR-181a in hematopoietic stem and progenitor cells resulted in an increase in

Table 1
MicroRNAs in normal immune functions.[a]

miRNA	Known target(s)	Pathway	References
miR-146a	TRAF6/IRAK-1	Innate immune response, TLR signaling	[31,32]
miR-125b	TNF-α	Innate immune response, TLR signaling	[34]
miR-155	Pu.1	Innate/adaptive immune responses, germinal center response, IgG class-switch	[33,34,37,38,42–44]
miR-181a	Not determined	B cell development, T cell receptor signaling	[48,49]
miR-181b	AID	Class switch recombination in activated B cells	[50]
miR-223	Not determined	Granulopoiesis	[51,52]
miR-150	Not determined	B cell differentiation	[47,53]

[a] Data obtained from cell culture and animal model studies.

CD19+ B cells and a decrease in CD8+ T cells [48]. miR-181a has also been shown to modulate T cell receptor (TCR) signaling, thus affecting the sensitivity of T cells to antigens [49].

Recently, miR-181b was reported to regulate class switch recombination in activated B cells [50]. Expression of miR-181b in activated B cells impaired class switch recombination and resulted in the downregulation of activation-induced cytidine deaminase (AID) mRNA and protein levels [50]. These results provide evidence for a new regulatory mechanism that restricts AID activity and could be relevant to prevent B cell malignant transformation [50].

Other examples of miRNA-mediated regulation of immune cell development include miR-223 which was recently reported to regulate granulopoiesis [51,52] and miR-150 which has been shown to be critical for B cell differentiation [47,53].

5. Role of miRNA in autoimmunity

Given that certain miRNA play critical roles in the regulation of immune response and immune cell development, it is not surprising that recent studies have revealed links between miRNA function and autoimmunity (Table 2). In 2007, the involvement of miRNA in a new pathway regulating autoimmunity was discovered in T lymphocytes in the *sanroque* mouse [54]. The *sanroque* mouse was originally selected from screening mutant mice derived from the chemical mutagen N-ethyl-N-nitrosourea (ENU), and has been shown to result from a mutation in the gene Roquin that encodes a RING-type ubiquitin ligase. In normal T cells, Roquin regulates the expression of inducible T-cell co-stimulator (ICOS) by promoting the degradation of ICOS mRNA. In sanroque mice, however, the absence of this regulation leads to an accumulation of lymphocytes that is associated with a lupus-like autoimmune syndrome. Yu et al. reported that miR-101 is required for the Roquin-mediated degradation of ICOS mRNA [54]. Introducing mutations into the miR-101 binding sites in the 3′UTR of ICOS mRNA disrupted the repressive activity of Roquin [54]. These findings revealed a critical miRNA-mediated regulatory pathway that prevents lymphocyte accumulation and autoimmunity.

More recently, two studies revealed the importance of miRNA regulation in safeguarding regulatory T cell (T reg) function in the prevention of autoimmunity [55,56]. In these studies, mice with conditional Dicer knockout within the T reg cell lineage were developed and used to monitor T reg cell development and function in the absence of functional miRNA. Although thymic T reg cells developed normally in these miRNA-deficient mice, the cells exhibited altered differentiation and dysfunction in the periphery [56]. Specifically, the Dicer-deficient T reg cells failed to remain stable and altered expression of multiple genes and proteins associated with the T reg cell fingerprint, including FoxP3 [56]. In addition to their instability, Dicer-deficient T reg cells lost suppressor activity in vivo, and the mice rapidly developed fatal systemic autoimmune disease [56]. Interestingly, Liston et al. found that in disease-free *Foxp3*$^{Cre/wt}$*Dicer*$^{fl/fl}$ mice, Dicer-deficient T reg cells retained some suppressive activity, albeit reduced compared to wild-type mice [55]. However, in diseased *Foxp*^{Cre}-*Dicer*$^{fl/fl}$ mice exhibiting inflammatory conditions, Dicer-deficient T reg cells were completely devoid of any suppressor activity and instead showed a robust in vitro proliferative response leading to the progression of fatal early onset lymphoproliferative autoimmune syndrome indistinguishable from that observed in Foxp3 mutant mice devoid of T reg cells [55]. These data suggest that miRNAs preserve stable T reg cell function under inflammatory conditions.

6. Involvement of miRNA in autoimmune diseases

It is becoming increasingly clear from cell culture and animal studies that proper miRNA regulation is critical for the prevention of autoimmunity and normal immune functions. However, it is not yet well understood whether miRNA dysregulation could play a role in autoimmune disease pathogenesis in human patients. Several recent studies have uncovered possible roles for miRNA regulation in autoimmune diseases (Table 2), specifically rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

6.1. Rheumatoid arthritis

RA is a systemic autoimmune disorder characterized by chronic inflammation of synovial tissue that results in irreversible joint damage [57]. Inflammatory cytokines, especially TNF-α, IL-1β, and IL-6 are known to play an important role in RA pathogenesis, as inhibition of these cytokines can ameliorate disease in some patients [58,59]. Three studies have discovered altered miRNA expression in RA patients compared to controls [60–62].

Two of these studies examined miRNA expression in RA synovial tissue and fibroblasts. Stanczyk et al. reported increased miR-155 and miR-146a expression in RA synovial fibroblasts compared to

Table 2
MicroRNAs in autoimmune disorders[a].

miRNA	Function	Disease	References
miR-101	Required for Roquin-mediated degradation of ICOS mRNA	Lupus-like autoimmune disease	[54]
Specific miRNA not determined	T reg cell stability and function	Fatal systemic autoimmunity	[55,56]
miR-146a	Targets TRAF6/IRAK-1, regulate inflammatory response	Rheumatoid arthritis	[60,61]
miR-155	Targets MMP-3, regulates inflammatory response	Rheumatoid arthritis	[61,62]
miR-132	Not yet determined	Rheumatoid arthritis	[61]
miR-16	Function in rheumatoid arthritis not yet determined	Rheumatoid arthritis	[61]
Numerous miRNAs	Differential expression in lupus, specific function not yet determined	Systemic lupus erythematosus	[65,66]

[a] Data obtained from animal model and human studies.

those in osteoarthritis (OA) patients [62]. Furthermore, miR-155 expression was higher in RA synovial tissue compared to OA synovial tissue [62]. Interestingly, miR-155 expression was higher in RA synovial fluid monocytes compared to RA peripheral blood monocytes [62]. Enforced expression of miR-155 in RA synovial fibroblasts revealed matrix metalloproteinase 3 (MMP-3) as a potential target of miR-155, suggesting that miR-155 may modulate downstream tissue damage [62].

Nakasa et al. reported that miR-146a was highly expressed in RA synovial tissue compared to OA and normal synovial tissue [60]. In situ hybridization studies revealed that miR-146a expression can be detected in RA synovial tissue primarily in CD68+ macrophages, but also in some CD3+ T cell subsets and CD79a+ B cells [60].

Our group implemented a different approach to examine miRNA expression in RA patients compared to healthy controls [61]. Peripheral blood mononuclear cells (PBMCs) were obtained from sixteen RA patients, nine healthy donors, and four disease controls, and total RNA was isolated for miRNA expression analysis [61]. RA PBMCs displayed between 1.8-fold and 2.6-fold increases in miR-146a, miR-155, miR-132, and miR-16 expression, whereas miRNA let-7a expression was not significantly different compared with healthy controls [61]. Interestingly, increased miR-146a and miR-16 expression correlated with active disease in RA patients; however, there was no correlation between the observed increased miRNA expression and patient age, race, or medications [61]. Two known targets of miR-146a, TRAF6 and IRAK-1, were examined and despite increased miR-146a expression in RA patients, there was no significant difference in mRNA or protein levels of TRAF6 or IRAK-1 between RA patients and healthy controls [61]. In vitro studies revealed that repression of TRAF6 and/or IRAK-1 in THP-1 human monocytes resulted in up to an 86% reduction in TNF-α production, implicating that normal miR-146a function could be critical for the regulation of TNF-α production [61]. Given that prolonged TNF-α production is known to play a role in RA pathogenesis, our data suggest a possible mechanism contributing to RA pathogenesis where miR-146a is upregulated, but unable to properly regulate TRAF6/IRAK-1 leading to prolonged TNF-α production in RA patients. Further studies are needed to elucidate this mechanism and whether or not miRNAs contribute to RA pathogenesis, or if miRNAs could serve as useful disease markers or therapeutic targets.

6.2. Systemic lupus erythematosus

SLE is a systemic inflammatory autoimmune disease characterized by the presence of autoantibodies against numerous self-antigens including chromatin, ribonucleoproteins, and phospholipids. Clinical manifestations are diverse and include malar rash, photosensitivity, arthritis, glomerulonephritis, and neurological disorders [63,64]. In 2007, Dai et al. used microarray analysis to examine miRNA expression in PBMCs of 23 SLE patients compared to 10 healthy controls [65]. In these SLE patients, seven miRNAs (miR-196a, miR-17-5p, miR-409-3p, miR-141, miR-383, miR-112, and miR-184) were downregulated and nine miRNAs (miR-189, miR-61, miR-78, miR-21, miR-142-3p, miR-342, miR-299-3p, miR-198, and miR-298) were upregulated compared to healthy controls [65]. In 2008, Dai et al. reported the miRNA profile of kidney biopsies from lupus nephritis patients and healthy controls and found 66 differentially expressed miRNAs (36 upregulated and 30 downregulated) in lupus nephritis [66]. These data suggest a possible role for miRNA as diagnostic markers and as factors involved in SLE pathogenesis. Further studies examining larger patient cohorts and different patient populations are needed to determine if the differential expression of these miRNA in SLE are reproducible.

Taken together, these human studies demonstrate that miRNA expression is altered in the autoimmune diseases RA and SLE. However, the next critical steps are to identify the targets of these miRNA and determine the mechanisms by which miRNA regulation/dysregulation contribute to the pathogenesis of these diseases. While some progress has been made, for example it is known that miR-155 can target MMP-3 which could potentially modulate tissue damage [62] and miR-146a can target TRAF6/IRAK-1 which should suppress inflammatory cytokine production [61], more studies focusing on these miRNA/target relationships and dissecting the pathways involved are clearly needed.

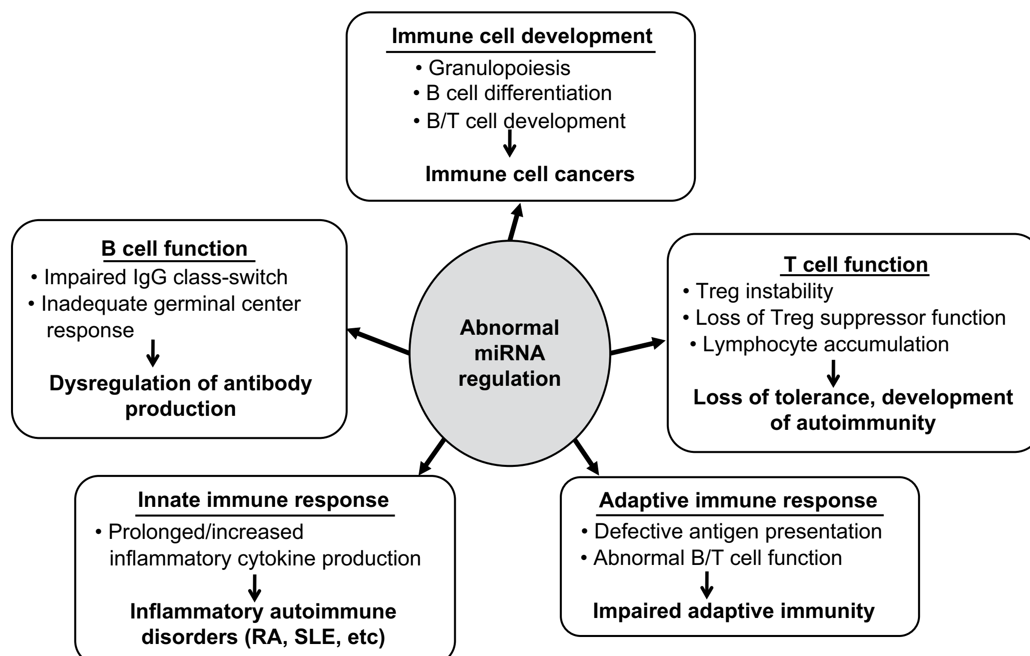

Fig. 1. Summary of potential consequences of abnormal miRNA regulation in immune functions. See text for detailed explanations.

6.3. Future of miRNA as therapeutic tools/targets

The detailed roles that miRNA regulation/dysregulation play in the development or prevention of autoimmunity and autoimmune diseases are still coming to light. Fig. 1 summarizes the potential consequences of abnormal miRNA regulation in immune functions. It is clear that miRNAs are emerging as potential targets or tools for new therapeutic strategies in the treatment/prevention of autoimmune disorders. miRNA therapies could involve administration of a specific miRNA to downregulate specific target genes or the blocking of certain miRNA to increase expression of target genes. However, in either case, the immediate priority and challenge lies in identifying the gene targets of miRNAs, a task complicated by the fact that a single miRNA can have multiple gene targets and vice versa. Another challenge is the risk of triggering a cellular immune response with RNA therapy. However, a recent study by Bauer et al. has demonstrated that modifying a short hairpin RNA (shRNA) construct by implementing features of a naturally occurring pre-miRNA can avoid triggering an immune response in primary cell cultures [67]. This is an important step for shRNA use in future gene therapies. To date, no miRNA therapies have been employed in vivo for the treatment of autoimmune disorders.

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