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microRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases

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Abstract

microRNAs (miRNAs) are newly discovered, small, non-coding ribonucleic acids (RNAs) that play critical roles in the regulation of host genome expression at the post-transcriptional level. During last two decades, miRNAs have emerged as key regulators of various biological processes including immune cell lineage commitment, differentiation, maturation, and maintenance of immune homeostasis and normal function. Thus, it is not surprising that dysregulated miRNA expression patterns have now been documented in a broad range of diseases including cancer, inflammatory, and autoimmune diseases. This rapidly emerging field has revolutionized our understanding of normal immunoregulation and breakdown of self-tolerance. This review focuses on the current understanding of miRNA biogenesis, the role of miRNAs in the regulation of innate and adaptive immunity, and the association of miRNAs with autoimmune diseases. We have discussed miRNA dysregulation and the potential role of miRNAs in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS). Given that most autoimmune diseases are female-predominant, we have also discussed sex hormone regulation of miRNAs in inflammatory responses, with an emphasis on estrogen, which has now been shown to regulate miRNAs in the immune system. The field of miRNA regulation of mammalian genes has tremendous potential. Identification of specific miRNA expression patterns in autoimmune diseases and further a comprehensive understanding of the role of miRNA in disease pathogenesis offers promise of not only novel molecular diagnostic markers, but also new gene therapy strategies for treating SLE and other inflammatory autoimmune diseases.

1. Introduction

The recent discovery of the gene regulatory role of small non-coding RNAs, miRNAs has led to a paradigm shift in the understanding of expression and function of mammalian genome. The first miRNA, *lin-4* was identified in the nematode *C. elegans* at 1993.¹ However, the abundance and ubiquitous nature of miRNAs in different species was not explored until seven years later with the identification of the second miRNA, *let-7* in *C. elegans*.² which led to large scale screening and identification of new miRNAs in invertebrates and vertebrates.^{3–6} Currently, by employing a combination of different experimental strategies and robust computational prediction, a total of 17,341 mature

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miRNAs in 142 species (miRBase database, version 16.0, September 2010) have been identified.⁷ Thus far, 1,223 human miRNAs and 1,055 mouse miRNAs have been identified in the database, which validate the previous computation-based prediction that miRNA genes constitute about 3% of the human genome and that the total number of human miRNA genes may be in the range of thousands.^{8, 9} It is estimated that a single miRNA may regulate hundreds to over thousands of target genes, and therefore about 30%–92% of human genes are likely regulated by miRNA.^{10–13} The powerful gene regulatory role of miRNAs is now well recognized. The expression and function of miRNAs are essential for the development of diverse physiological systems and the maintenance of the cellular homeostasis and normal function.^{14–18} The field of miRNA research gained widespread attention with the recognition of aberrant expression and/or function of miRNAs in a broad range of human diseases including various types of cancer, metabolic, neurodegenerative, infectious, chronic inflammatory, and autoimmune diseases.^{19–23} This review will summarize the current knowledge regarding the role of miRNA in inflammatory autoimmune disease, with an emphasis on SLE.

2. miRNA biogenesis and action

Most mammalian miRNA genes (about 80%) have been identified in the intron region of either protein coding or non-protein coding transcripts. Only a small number of miRNA genes (20%) are located in the exon region of non-coding RNAs.^{24, 25} Some miRNA genes could be either exonic or intronic miRNAs depending on the alternative splicing pattern of host genes.^{24, 26} The miRNA biogenesis pathways and their regulation have been extensively studied in last decade and well reviewed in recent publications.^{6, 26–30} As depicted in figure 1, miRNA genes are mainly transcribed by RNA polymerase II into primary miRNA transcripts (pri-miRNAs), which are usually hundreds to thousands of nucleotides (nts) long and contain one or several hairpin loop structures.^{26, 27, 31} In the canonical miRNA biogenesis pathway, the pri-miRNAs are cleaved in the nucleus by a microprocessor complex that is composed of the nuclear RNase III enzyme, Drosha and the double-stranded-RNA-binding protein, DiGeorge syndrome critical region protein 8 (DGCR8) to produce precursor miRNAs (pre-miRNAs).^{32, 33} In an alternative, non-canonical miRNA biogenesis pathway, a new class of miRNA precursors, termed “mirtrons”, are produced independent of microprocessor processing.^{34–36} Unlike pri-miRNAs, the mirtrons do not have the lower stem-loop structure and the flanking single stranded segments that are crucial for the binding and processing by the DGCR8/Drosha microprocessor. Therefore, the mirtrons bypass Drosha processing and are spliced by spliceosome to yield branched pre-mirtrons, which then go through lariat-mediated debranching to generate pre-miRNAs (Figure1).^{30, 34, 35}

After being exported from the nucleus into cytoplasm by the nucleocytoplasmic shuttle protein Exportin-5, pre-miRNAs are further processed by the RNase III enzyme, Dicer to yield ~22nts long, imperfect matched miRNA/miRNA* duplexes, which are then loaded into the Argonaute (Ago) protein to generate RNA-induced silencing complex (RISC). The guided strand of the miRNA/miRNA* duplex remains in the RISC as a mature miRNA, and the complementary strand (the passenger strand miRNA*) is degraded.^{26, 28}

Once loaded onto RISC, the mature miRNA will interact with the 3' untranslated region (UTR) of its target messenger RNA (mRNA) to regulate gene expression. The 2–8 nts seed region of miRNA is crucial for target recognition. The complementary degree between the miRNA seed region and the target mRNA 3'UTR determines the mechanism of miRNA-mediated gene regulation: translation repression or miRNA cleavage and degradation.^{6, 11, 28} High-throughput proteomic analysis revealed that miRNA-regulated genes are mainly

subjected to translational inhibition, and those highly translationally repressed genes also display mRNA destabilization.^{37–39}

3. The role of miRNA in immune system development and normal function

During the last several years, mounting evidence has emerged to show that miRNAs are critical for not only the development of the immune system, but also for the function of both innate and adaptive arms of the immune system.^{18, 40–45} In the following sections, we will discuss the current relevant findings with regard to the roles of miRNAs in the regulation of innate immunity, adaptive immunity, autoimmunity, and inflammation.

3.1 miRNAs in the regulation of innate immunity

The cells of the innate immune system include granulocytes, monocyte-derived macrophages and dendritic cells. Of the granulocytes, neutrophils constitute the largest percentage of peripheral white blood cells and are regarded as the first cellular line of defense against invading pathogens. The Toll-like receptors (TLRs) on the membrane of monocytes recognize and bind to specific microbial products called pathogen-associated molecular patterns (PAMPs) and then trigger downstream signaling pathway to initiate inflammatory responses.^{46, 47} The TLR signaling is tightly controlled *in vivo* by different classes of negative regulators to prevent overwhelming inflammation.⁴⁸ Recent studies revealed that miRNAs not only regulate innate immune cell development, but also fine-tune the innate immune response, thereby adding a new layer of negative feedback regulation of TLR signaling (Figure 2).^{40, 42, 43}

During granulopoiesis, miR-223 was highly induced by the transcription factor, CCAAT enhancer binding protein α (C/EBP α), which in turn resulted in the repression of transcription factors nuclear factor 1 A-type (NFI-A) and E2F1, and led to enhanced granulocytes differentiation.^{49, 50} Nevertheless, an *in vivo* study with miR-223 knockout mice revealed that miR-223 acted as a negative regulator of granulocyte differentiation by targeting another transcription factor, myocyte-specific enhancer factor 2C (*mef2c*).⁵¹ The miR-223 knockout mice surprisingly had increased number of granulocyte progenitors, which led to increased expansion of granulocytes. Furthermore, the miR-223 knockout mice spontaneously develop inflammatory lung pathology due to the hyperactivity of neutrophils. These data suggested that although the role of miR-223 in neutrophils development and function is apparent, further work is warranted to understand the precise function of miR-223 in the regulation of granulopoiesis and granulocyte function at different differentiation stages.

Monocyte-derived macrophages play critical roles in innate immune responses. Monocytopoiesis is controlled by a circuitry loop consisting of miR-17-5p, miR-20a, miR-106a, acute myeloid leukaemia-1 (AML-1; also known as Runt-related transcription factor 1, Runx1), and macrophage colony-stimulating factor receptor (M-CSFR).⁵² During monocytopoiesis, the expression of miR-17-5p, miR-20a, and miR-106a was decreased. In parallel, their target gene, AML-1, was upregulated to promote the expression of M-CSFR, which plays a pivotal role in monocyte–macrophage differentiation and maturation. On the other hand, AML-1 also negatively regulated the expression of miR-17-5p-20a-106a by binding to the promoter of the miR-17-92 and miR-106a-92 cluster, which suggests a mutual negative feedback regulation loop in monocytopoiesis.⁵² In addition to the above miRNAs, miR-424 was shown to enhance monocyte differentiation by translational repression of NFI-A, which also led to the activation of M-CSFR.⁵³

The importance of miRNA in the regulation of innate immune responses is evident in a series of studies in which various miRNAs were shown to alter dramatically in response to

PAMPs or inflammatory cytokines stimulation.^{42, 54–57} In a pioneer study, Taganov et al. have shown that miR-146 was rapidly upregulated in response to lipopolysaccharide (LPS) stimulation in human monocytic cells, and acted as a negative feedback regulator of TLR signaling by targeting tumor necrosis factor receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1).⁵⁷ The upregulation of miR-146a was also observed in monocytes in response to the activation of TLR4, TLR2, and TLR5 signaling pathways or inflammatory cytokine such as tumor necrosis factor (TNF) α and IL-1 β stimulation.⁵⁷ In addition to miR-146a, other miRNAs were identified as negative regulator of TLR signaling. miR-155 was induced in macrophages in response to both bacterial and viral derived antigens that activate TLR4, TLR2, TLR3, or TLR9.^{54, 55, 57} In a different study, miR-155 was shown to downregulate inflammation by targeting myeloid differentiation primary response protein 88 (MyD88), a key adaptor protein in the TLR signaling pathway.⁵⁸ miR-21 was found to negatively regulate LPS- activated TLR4 signaling by targeting the tumor suppressor, PDCD4, which in turn decreased nuclear factor- κ B (NF- κ B) activation and IL-10 production.⁵⁹ miR-147 was induced following the activation of TLR2, TLR3, or TLR4, and acted as a negative regulator to prevent excessive inflammatory responses in macrophages in the lungs of mice.⁶⁰ miR-9 was induced by LPS in both neutrophils and macrophages and negatively regulated NF- κ B dependent inflammatory responses by fine-tuning the expression of NF- κ B.⁶¹ Some miRNAs such as *Let7i* and miR-125b were decreased during innate immune responses.^{55, 56} LPS stimulation and parasite *Cryptosporidium parvum* infection of cultured human cholangiocytes decreased *let7i* expression in a MyD88 and NF- κ B- dependent manner, which led to the upregulation of TLR4 protein and enhanced immune responses. Accordingly, there was decreased parasite infection in *let7i* antisense inhibitor transfected cells and increased parasite burden in *let7i* precursor-transfected cells when compared to control.⁵⁶ Decreased miR-125b expression in response to LPS may contribute to elevated TNF α level in activated macrophages as miR-125b was shown to target TNF α mRNA.⁵⁵

Taken together, these reports clearly demonstrate the importance of miRNA in the regulation of innate immune cell development and in fine-tuning innate immune responses to prevent overwhelming inflammation in the body.

3.2 miRNAs in the regulation of adaptive immunity

T and B lymphocytes are major cellular components of adaptive immunity. The overall significance of miRNA in the regulation of adaptive immunity is clearly evident in studies in which the disruption of miRNA biogenesis in lymphocyte progenitors was found to impair the development of T and B lymphocytes. Disruption of miRNA synthesis by conditional depletion of Dicer in early stages of T lymphocytes impaired T cell development with reduced T cell numbers in thymus and peripheral lymphoid organs, and caused aberrant T helper (Th) cell differentiation and cytokine production.^{62, 63} Depletion of Dicer in early B cell progenitors resulted in a complete developmental block of B cells at pro- to pre-B transition and affected antibody diversity, which was partially due to the loss of miR-17-92 mediated repression of the proapoptotic protein, *Bim*.⁶⁴

The distinct role of individual miRNA in the regulation of lymphocyte development and function is also documented in recent studies (Figure 3). miR-155 is upregulated in both T and B cells upon activation and is required for maintaining lymphocyte homeostasis and normal immune function by targeting different genes including *c-maf*, Pu.1, and activation-induced cytidine deaminase (AID).^{65–68} Although miR-155 knockout mice apparently possess normal lymphocyte development, they have defective T and B immunity including abnormal Th1/Th2 differentiation ratio with increased Th2 polarization, increased Th2 cytokine production, a reduced number of Treg cell, decreased germinal center responses, and low numbers of IgG class-switched plasma cells and memory cells.^{65–67, 69, 70}

miR-181a also plays an important role in the regulation of both of T and B cell development. While ectopic expression of miR-181a in hematopoietic progenitor cells *in vitro* resulted in an increase of B-lineage cells without affecting T-lineage cells, transfer of bone marrow cells overexpressing miR-181 into irradiated host mice led to a substantial increase of CD19⁺ B cells accompanied by a significant decrease in T cells.⁷¹ miR-181a was found to target phosphatases SHP-2, PTPN-22, and DUSP5/6, negative regulators of TCR signaling, and therefore plays a critical role in T cell maturation by regulating T cell receptor (TCR) signaling strength and then tuning T cells sensitivity to positive and negative selection.⁷² Inhibiting of miR-181a expression in immature T cells impaired both positive and negative selection during T cell development.⁷² miR-150 is selectively expressed in mature resting B and T cells but not in their progenitors.^{73, 74} Overexpression of miR-150 prematurely in hematopoietic stem cells blocked the B cell development at pro- to pre-B stages without affecting T cells, granulocytes and macrophage maturation.^{74, 75} Further, Xiao et al. demonstrated that miR-150 controls B cell development by targeting transcription factor, *c-myb*, in a dose dependent manner. Mice with miR-150 deficiency have increased number of B1 cells in spleen and peritoneal cavity and enhanced humoral responses.⁷⁴ In contrast to miR-150, miR-17-92 is highly expressed in T and B precursors and decreased following maturation. Overexpression of miR-17-92 in lymphocyte progenitors promoted T and B lymphocyte proliferation and resulted in the development of lymphoma by targeting proapoptotic proteins, *Bim* and phosphatase and tensin homolog (PTEN). On the other hand, ablation of miR-17-92 inhibited B cell development at the pro-B to pre-B transition, which is attributed to apoptotic death of Pro-B cells due to augmented expression of proapoptotic protein *Bim* that is targeted by miR-17-92.^{76, 77}

Regulatory T cells (Tregs) suppress the activation of effector T cells to maintain immune system homeostasis and tolerance to self-antigens. Various miRNAs have been shown to play critical role in the regulation of Treg development and/or function. miR-155 knockout mice have a reduced number of Tregs in both thymus and peripheral lymphoid tissues. However, the miR-155 deficient Tregs have competent suppressive activity *in vivo*.⁷⁰ This suggests that miR-155 is critical for Tregs development, but not essential for Treg suppressive functions. In a different study, miR-155 was shown to play a role in Treg-mediated suppression activity by regulating the sensitivity of effector CD4⁺ Th cells. miR-155 inhibition in CD4⁺ Th cells resulted in enhanced natural Treg (nTreg) mediated suppression.⁷⁸ miR-146a has been shown to be upregulated in Th1 cells and abolished in Th2 cells during T cell differentiation.⁷³ A recent study revealed that miR-146a is highly expressed in Tregs and selectively controls Treg-mediated suppression of interferon (IFN) γ -dependent Th1 responses and inflammation by targeting signal transducer and activator of transcription (STAT)-1.⁷⁹ The mice with miR-146a ablation in Tregs have elevated production of Th1 cytokine IFN γ and develop severe Th1-mediate lesions in a variety of organs leading to conjunctivitis, blepharitis, and dermatitis.⁷⁹ In addition, miR-31 and miR-21 have also been shown to regulate Treg development by negatively and positively, respectively, regulating the expression of Foxp3, a critical transcription factor in Treg development.⁸⁰

Many recent studies have shown that IL-17, a powerful neutrophilic inflammation-inducing cytokine, is involved in autoimmune diseases.⁸¹ Two miRNAs, miR-326 and miR-155, have now been shown to be associated with the Th17 differentiation and induction of IL-17. miR-326 was shown to target Ets, a transcription factor that negatively regulates Th17 development, which consequently led to the promotion of Th17 differentiation and IL-17 production.⁸² miR-155 was required for the production of Th17-promoting cytokines. When immunized with experimental allergic encephalitis (EAE)-inducing myelin oligodendrocyte glycoprotein₃₅₋₅₅ (MOG₃₅₋₅₅), miR-155 knockout mice had a marked decrease in number

of Th-17 cells compared to wild type mice, and displayed remarkably resistance to (MOG₃₅₋₅₅)-induced EAE.⁸³

In summary, the above emerging data clearly show that proper expression and function of miRNAs is essential for T and B cell development, differentiation, and function. It is therefore important that the expression of miRNA must be tightly regulated for the maintenance of immune homeostasis. Conceivably, dysregulation of miRNA can promote the induction of autoimmunity.

4. miRNAs in the development and prevention of autoimmunity

To maintain tolerance, there are many checkpoints at both central and peripheral lymphoid organ levels. These checkpoints ensure that autoreactive T and B cells that are routinely and randomly generated during lymphogenesis are either deleted or silenced.^{84, 85} However, in some circumstances, the self-reactive lymphocytes escape the checkpoints and survive in peripheral lymphoid tissues. Once being activated, these autoreactive cells mount a devastating attack on self-tissues, leading to the induction of autoimmune diseases. With the increased recognition that miRNAs are stringently regulated in the cells of the normal immune system to maintain immune homeostasis and normal immune function, it is conceivable that dysregulated miRNA expression will lead to the immune tolerance breakdown and the development of autoimmune diseases. Disruption of miRNA synthesis by depleting Droscha or Dicer in Tregs led to the complete loss of immune regulatory function of Tregs, which resulted in the development of inflammatory disease and fatal autoimmunity.⁸⁶⁻⁸⁸ Selective ablation of miR-146a in Treg cells resulted in the breakdown of immune tolerance and the development of autoimmune diseases in mice. This was mainly due to a selective loss of Treg mediated suppression of Th1 inflammatory responses in mice with miR-146a deficient Tregs.⁷⁹ Overexpression of miR-17-92 in lymphocytes induced lymphoproliferative disease and autoimmunity in miR-17-92 transgenic mice as the result of miR-17-92-mediated repression of *Bim* and PTEN, which play critical roles in immune tolerance mechanism.^{76, 84} Moreover, the unique dysregulated miRNA expression patterns have been identified in human patients with SLE, RA, and MS. Selected disease-associated miRNAs have been shown to play critical pathogenic roles in the development of autoimmune diseases (Table 1).

4.1 miRNA in SLE

SLE is a classical autoimmune disease that is characterized by the production of autoantibodies against a wide-range of nuclear and phospholipid antigens and multi systemic organ injuries. Numerous immunological abnormalities including dysregulated B and T lymphocyte functions, aberrant TLR, type I IFN, NF- κ B signaling pathways, and abnormal interactions among immune cells have been identified in human and murine lupus.⁸⁹⁻⁹² In addition to the existence of genetic loci with SLE predisposition, epigenetic regulatory defects such as abnormal DNA methylation, histone modification have also been shown to play important role in lupus pathogenesis.⁹³⁻⁹⁵ Recent studies have now revealed a potential role of miRNA in SLE, thereby providing new insights into lupus pathogenesis. A computational prediction analysis of 72 lupus susceptibility genes revealed that most lupus-related genes (71 out of 72 genes) contain at least one miRNA target site for over 140 miRNAs.⁹⁶ Interestingly, four lupus susceptibility genes (Roquin, B-cell lymphoma 2 (Bcl2), *Bim*, and PTEN), which are involved in the regulation of T cell co-stimulation, cell survival, apoptosis and/or tolerance control, were each predicted to contain over 50 miRNA target sites. This study emphasized the potential contribution of miRNA in the regulation of autoimmune genes and self-tolerance control in autoimmune lupus.⁹⁶

Dysregulated miRNA expression pattern in the peripheral blood mononuclear cells (PBMCs) from patients with SLE was first reported by Dai et al. at 2007.⁹⁷ They identified 16 lupus-related miRNAs that were altered specifically in patients with lupus, but not in patients with idiopathic thrombocytopenic purpura (ITP, an organ specific autoimmune disease).⁹⁷ Since then, several reports have been published to show dysregulated miRNA expression profiles in PBMC, CD4⁺ T cells, kidney biopsy, and Epstein-Barr virus (EBV)-transformed B cell lines from human patient with lupus.^{98–102}

Using Taqman PCR array, Tang et al. identified 42 miRNAs that were differentially expressed in PBMC from human patients with SLE when compared to healthy controls.¹⁰⁰ Among them, miR-146a, a negative regulator of TLR signaling, was profoundly decreased (more than 6-fold) in patients with SLE versus healthy controls. Further, the authors reported that miR-146a negatively regulated the type I IFN pathway by targeting interferon regulatory factor (IRF)-5 and STAT-1. Overexpression of miR-146a in normal PBMC greatly reduced the induction of IFN α/β , and inhibition of endogenous miR-146a increased the production of IFN α/β in response to the activation of TLR-7. Therefore, decreased expression of miR-146a in PBMC may contribute to the enhanced type I IFN production in human lupus.¹⁰⁰ In a separate study from the same group, Pan et al. identified that miR-21 and miR-148a were upregulated in CD4⁺ T cells from both MRL-lpr mice and human patients with lupus.⁹⁹ A previous report has shown that miR-148a targets DNA (cytosine-5-)-methyltransferase 3 beta (DNMT3B),¹⁰³ which led to the discovery of the role of miR-148a and miR-21 in the regulation of DNA methylation in lupus T cells. Pan et al. confirmed that miR-148a targeted DNA methyltransferase 1 (DNMT1) directly by binding to the protein coding region, and that miR-21 indirectly downregulated DNMT1 by targeting its upstream regulator, RAS guanyl nucleotide-releasing protein 1 (RASGRP1). Overexpression of miR-148a and miR-21 in CD4⁺ T cells led to DNA hypomethylation and then increased expression of autoimmune-associated methylation-sensitive genes, CD70 and lymphocyte function-associated antigen 1 (LFA-1).⁹⁹ In a more recent report, Zhao et al. reported that miR-125a was reduced in PBMCs from patients with lupus when compared to healthy controls. Further experiments revealed that miR-125a, which is mainly expressed in T cells, targeted Kruppel-like factor 13 (KLF13) gene, a critical transcription factor in the regulation of the chemokine RANTES, in a dose dependent manner. Decreased expression of miR-125a resulted in the upregulation of KLF13, which in turn contributes to the elevation of the inflammatory chemokine RANTES in lupus T cells.¹⁰¹ Together, the above three studies have shown that dysregulated miRNAs in lupus contribute to disease pathogenesis by regulating Type I interferon signaling pathway,¹⁰⁰ DNA methylation,⁹⁹ and RANTES expression,¹⁰¹ respectively.

So far, numerous dysregulated miRNAs were identified in human patients with lupus.^{97–100},¹⁰² Nevertheless, many dysregulated miRNAs in lupus that were determined by microarray assay need further validation by Real-time RT-PCR or Northern blotting. Interestingly, it seems that the dysregulated miRNAs in patients with lupus that were determined in one study were not well reproduced in other studies. For example, the decrease of miR-146a in PBMC from patients with lupus was observed in Tang et al.'s report,¹⁰⁰ but not in other studies.^{97, 98, 102} The discrepancy of the data generated in different human lupus miRNA studies may be due to differences in the type of samples, sensitivity of detection methods, and also the diversity in the medical history, disease severity, length of disease, and race of human patients with lupus.

Considering that genetic factors contribute significantly to lupus etiology and miRNA expression, we have recently identified a common lupus disease-associated miRNA expression pattern in three murine autoimmune-prone models (MRL-lpr, C57BL/6-lpr, and NZB/W) with different genetic background.¹⁰⁴ By a combination of miRNA microarray and

Real-time RT-PCR analysis, we identified that the miR-182-96-183 cluster, miR-31, and miR-155 are commonly upregulated in splenocytes from three strains of lupus prone mice when compared to their respective controls. It is interesting to note that the upregulation of these common lupus-related miRNAs was evident in NZB/W mice only at an age when lupus disease is manifested, which suggested that the dysregulation of these miRNAs are lupus associated. In addition, by Real-time RT-PCR, we identified that miR-146a, miR-17-5p, and miR-101a, which have been shown to be dysregulated in human lupus or implicated the development of autoimmunity,^{23, 76, 99, 100, 105, 106} were also dysregulated in splenocytes and/or purified splenic lymphocytes subsets from MRL-lpr mice when compared to control MRL mice.¹⁰⁴ While the direct contribution of these common lupus disease-associated miRNAs to the pathogenesis of murine lupus is as yet not determined, the identified targets and immune regulatory function of miR-155, miR-182-96-183 and miR-31 strongly suggest the potential contribution of these miRNAs to immune tolerance breakdown and altered B and T lymphocyte function in autoimmune-prone mice (Figure 4).¹⁰⁴ miR-155 plays a critical role in the regulation of B cell and T cell immunity,^{65–67, 83} therefore, the upregulation of miR-155 in lupus B and T lymphocytes may lead to abnormal B cell activation, enhanced germinal center responses, and abnormal inflammatory T cell development and cytokine production in human and murine lupus. The upregulation of miR-31 in T cells may correlate with the deficiency of Treg cell development/function in lupus since miR-31 targets Foxp3, which is critical for Treg cell development.⁸⁰ The miR-182-96-183 cluster is primarily expressed in the retina, and rarely detected in the spleen, thymus, and other tissues from normal B6 mice.¹⁰⁷ Importantly, miR-182 and miR-96 target forkhead transcription factor O (Foxo)1 and Foxo3a, which are newly emerged regulator of T lymphocyte homeostasis and tolerance, and T cell immune responses.^{108–113} T cell-specific deletion of Foxo1 gene in mice led to spontaneous T cell activation, an increased number of IFN γ , IL-17 secreting CD4⁺ T cells, anti-dsDNA autoantibody production, and tissue inflammation.¹¹⁰ Inactivation of Foxo3a in mice led to NF- κ B activation, hyperactivation of Th cell with increased Th1/Th2 cytokine production and autoimmune inflammation in salivary gland, lung and kidney.¹⁰⁹ The direct involvement of miR-182 in immune regulation is further supported by a recent report in which miR-182 was shown to be induced by IL-2 to suppress the activity of Foxo1. This in turn promoted clonal expansion of activated Th cells and IL-2 driven Th mediated immune responses.¹¹⁴ Extrapolation of these findings, it is therefore conceivable that the upregulation of miR-96-182-83 in lupus lymphocytes could potentially cause the decrease of Foxo1/3a, which in turn may lead to T lymphocyte hyperactivation, self-tolerance breakdown, autoantibody production, and development of autoimmunity. This view is supported by a finding of decreased Foxo1 transcript in human SLE that has been reported.¹¹⁵ Further, we have also observed that Foxo1 and Foxo3a protein levels are decreased in splenocytes from MRL-lpr mice when compared MRL mice (unpublished lab data).

The current studies have revealed that various miRNAs are dysregulated in human and murine lupus. However, the functional significance of most of lupus-related miRNAs remains elusive, which impedes our comprehensive understanding of the role of miRNA in lupus pathogenesis.

4.2 miRNA in RA

RA is an autoimmune disease that causes chronic inflammation primarily in the joints, as well as in the tissue around the joints and other organs in the body. In reports from different research groups, miR-146a and miR-155 were found consistently to be upregulated in synovial fibroblasts (RASFs),¹¹⁶ PBMC,¹⁰⁶ synovial fluid,¹¹⁷ PBMC-derived CD4⁺ T cells,¹¹⁸ and Th-17 cells from patients with RA when compared to healthy control or patients with osteoarthritis (OA).¹¹⁹ Stanczyk et al. confirmed that miR-155 targets matrix

metallopeptidase (MMP)3 and MMP1. This suggests that elevated miR-155 in RA may have a protective function in RASFs-mediated tissue damages by inhibition of MMP1 and MMP3.¹¹⁶ miR-146a has been shown to negatively regulate inflammatory immune responses by targeting TRAF6 and IRAK-1.⁵⁷ Although miR-146a was upregulated in active RA disease, its target genes, TRAF6 and IRAK-1, had no changes in RA when compared to healthy controls. It is plausible that the increased level of miR-146a in RA does not function effectively as a negative regulator of inflammation, thereby resulting in constitutive production of inflammatory TNF α in RA.¹⁰⁶ Recent reports revealed that miR-146a was increased in CD4⁺ T cells and correlated with the levels of the inflammatory cytokines TNF α and IL-17, and that elevated miR-146a expression may contribute to RA pathogenesis by suppressing T cell apoptosis and enhancing the differentiation of IL-17 cells, respectively.^{118, 119} By comparing to patients with OA, Nakamachi Y et al. found that miR-124a was significantly decreased in RA synoviocytes.¹²⁰ Further investigation revealed that miR-124a targeted cyclin-dependent kinase 2 (CDK-2). Overexpression of miR-124a in RA synoviocytes significantly repressed cell proliferation and arrested the cell cycle at G1 phase. In addition, miR-124a was also shown to target monocyte chemoattractant protein 1 (MCP-1), which is elevated in RA synovial fluid and tissues and has been implicated in the RA pathogenesis by recruiting mononuclear phagocytes into joint. Taken together, decreased miR-124a expression may contribute to RA pathogenesis by increasing cell proliferation and MCP-1 production.¹²⁰

In addition, several other miRNAs including miR-223, miR-16, and miR-132 were found to be dysregulated in human patients with RA.^{106, 117, 119, 121} However, the potential contribution of these miRNAs in RA inflammation and pathogenesis is not known yet, therefore, further investigation is warranted.

4.3 miRNA in MS

MS is an autoimmune disease with chronic inflammation of myelin sheaths in the central nervous system (CNS). The functional significance of miRNAs in MS pathogenesis was highlighted in a recent study where miR-326 was shown to play a critical role in the pathogenesis of MS by upregulating the differentiation of damaging Th-17 cell.⁸² By Real-time RT-PCR analysis of miRNA expression in peripheral blood leukocytes from patients with relapsing-remitting MS, neuromyelitis optica (a CNS inflammatory disease with similar signs and symptoms as MS) and also healthy controls, Du, et al. identified that miR-326 was significantly upregulated in patients with relapsing-remitting MS, but not in patients with neuromyelitis optica when compared to healthy controls. The potential role of miR-326 in MS was reinforced in studies with EAE mice, a classical murine model of MS. The authors have shown that while silencing miR-326 *in vivo* in EAE mice inhibited Th-17 cell production and led to mild EAE disease symptoms, overexpression of miR-326 *in vivo* led to increased Th-17 cell number and severe EAE symptoms.⁸² In addition, miR-34a, miR-155, and miR-326 were found upregulated in active MS lesions and contributed to MS pathogenesis by targeting CD47 to release macrophages from the inhibitory control signal, which in turn cause increased phagocytosis of myelin.¹²² A very recent report also revealed that miR-155 may contribute to EAE by promoting the development of inflammatory Th1 and Th17 cells.⁸³

By using Taqman RT-PCR array and miRNA microarray assay, Otaegui et al. and Kelly, et al. have identified over one hundred miRNAs that were differentially expressed in PBMC from patients with MS when compared to healthy controls.^{123, 124} Specifically, the expression of miR-18b and miR-599 was related to relapse and miR-96 might be involved in remission.¹²³ In addition, differentially expressed miRNAs have also been identified in different lymphocyte subsets including CD4⁺ T cells, CD8⁺ T cells, B cells, and CD4⁺CD25⁺ Treg cells from patients with MS.¹²⁵⁻¹²⁷ Intriguingly, while Lindberg et al.

demonstrated that miR-17-5p was upregulated in CD4⁺ T cells,¹²⁵ Cox et al. found that miR-17-5p was underexpressed in whole blood cells from patients with MS when compared to healthy controls.¹²⁶ Although the potential function of the MS-associated miRNAs is suggested by the correlation of the miRNA expression level and their candidate genes in some studies, the direct involvement and contribution of dysregulated miRNAs in MS has largely remained unknown and needs further investigation.

5. Estrogen, miRNA and autoimmune diseases

A striking common feature of many autoimmune diseases in both humans and experimental animal models is that females are more susceptible to autoimmune conditions than males.^{128–130} More than 85 percent of patients with thyroiditis, scleroderma, lupus, and Sjögren's syndrome are women.¹³¹ The female to male ratios for SLE, RA, and MS are 9-13:1, 2-4:1, and 2:1, respectively.^{128, 130} In addition to genetic factors such as X-chromosome abnormalities, sex hormones such as estrogens and androgens are believed to play a significant role in the sex-based susceptibility to many autoimmune diseases. The studies with murine lupus models, especially with NZB/W mice, revealed that estrogen exacerbated lupus development and that androgens inhibited autoantibody production and exerted protective effect on lupus.^{132, 133} Moreover, estrogen-treatment can break B cell tolerance even in normal non-autoimmune mice to induce several lupus-related autoantibodies including those against dsDNA, cardiolipin, and other phospholipids.^{134–137} While in murine lupus models, estrogen has been shown to promote lupus, the role of estrogen in promotion of human lupus is thus far equivocal.^{138–140} The Nurses' cohort studies indicated that the use of oral contraceptive pills (OCP) and hormone replacement therapy (HRT) increased the risk for developing SLE.^{141–143} However, the studies of the Safety of Estrogen in Lupus: National Assessment (SELENA) trials of using OCP and HRT revealed that the use of OCP has no significant effect on global lupus disease activity and incidence of flares, and that the use of HRT is only associated with a small risk for mild and moderate lupus flare, but not severe flare.^{144–146} In addition to estrogen, other sex hormones such as prolactin, androgen, and testosterone should be considered in the gender bias of SLE and the disease course changes during pregnancy and menopause.¹³⁹

It is clear that sex hormones have profound influence on immune system development and function. Depending on the cell types, animal models, dose, administration methods and duration, estrogen has been shown to exert both anti-inflammatory pro-inflammatory immune responses in different studies.^{147–151} We, and others, have reported that *in vivo* estrogen (17 β -estradiol) treatment of non-autoimmune mice promoted the secretion of IFN γ , a major inflammatory cytokine that is implicated in autoimmune diseases.^{152, 153} In addition, we have recently shown that estrogen upregulated a powerful inflammatory cytokine, IL-17, in non-autoimmune C57BL/6 mice.¹⁵⁴ We found that estrogen not only modulated key transcription factors involved in inflammation,^{155–157} but also regulated miRNA expression to promote inflammatory responses in activated splenocytes from estrogen-treated mice.¹⁵⁸ Through the combination of high throughput microarray assay and Real-time RT-PCR confirmation, we identified that miR-451, miR-486, miR-223, miR-148a, miR-18a, and miR-708 were upregulated, and that miR-146a, miR-125a, miR-125b, miR-143, miR-145, *let-7e*, miR-126, and miR-181a were downregulated in splenocytes from estrogen-treated orchietomized male C57BL/6 mice when compared to placebo controls. Further, we demonstrated that the downregulation of miR-146a and upregulation of miR-223 contributed to enhanced IFN γ production in LPS activated splenocytes from estrogen-treated mice, which suggested a novel mechanism of immune modulation by estrogen.¹⁵⁸ Of relevance, the decreased level of miR-146a and miR-125a and increased level of 148a have been identified in human patients with lupus and reported

to contribute to lupus pathogenesis by regulating type I IFN pathway, the inflammatory chemokine RANTES and DNA methylation, respectively.^{99–101}

Recent studies revealed that estrogen receptor (ER) α , rather than ER β plays a critical role in the regulation estrogen-mediated promotion of inflammatory responses,¹⁵⁹ autoimmunity in NZB/W mice,¹⁶⁰ and also miRNA induction in MCF-7 cell line.^{161–163} Interestingly, a subset of estrogen-induced miRNAs also target ER α mRNA or ER α signaling components, therefore forming a negative feedback loop to coordinate and fine-tune cellular responses to estrogen.¹⁶²

Androgens such as 5- α -dihydrotestosterone and testosterone have been shown to suppress immune responses and inflammation and exert protective effect on a broad range of autoimmune diseases in experimental models of these diseases.^{133, 164–167} Experiments have shown that androgens regulate miRNA expression in non-lymphoid organs including prostate, muscle and liver, and that the mutual interaction between miRNA and androgen receptor signaling is implicated in prostate cancer progression or suppression.^{168–171} Although there are no reports so far of androgen regulation of miRNAs in the immune system or in autoimmune diseases, it is conceivable that miRNA may also be regulated by androgen in lymphoid organs and plays a role in androgen-mediated immune suppressive function.

Together, these data suggest that sex hormones such as estrogen may contribute to the pathogenesis of lupus and other gender biased autoimmune disease via the regulation of miRNA expression.

6. Other potential causes of miRNA dysfunction in SLE

In addition to the hormonal factors we discussed above, genetic and environmental factors, two key factors that are involved in autoimmune disease etiology, may also contribute to lupus pathogenesis by altering the expression and/or function of miRNAs (Figure 4).^{96, 172} It has been shown that the genetic polymorphism in miRNA genes and the 3' UTR sequences of its target genes, and translocation, gene amplification or deletion in host genome all contribute to dysregulated miRNA expression and/or function in diseases.^{96, 173–176} For example, a single-nucleotide polymorphism (SNP) in pre-miR-146a decreased the expression of mature miR-146a, which has been shown to relate to the genetic predisposition to papillary thyroid carcinoma.¹⁷⁷ So far, there is only one study to explore the association of genetic variants of miRNA with SLE susceptibility.¹⁷⁸ Although this study did not reveal any significant relationship between the SNP in miR-146a and miR-449 and SLE, it suggested the importance of investigation of miRNA polymorphism in SLE susceptibility. Environmental factor such as EBV viral infections have been implicated in SLE pathogenesis. EBV infection induced the expression of miR-155 and miR-146a in a NF- κ B dependent manner.^{179–181} Although miR-146a was decreased in PBMC from human patients with lupus,¹⁰⁰ both of miR-146a and miR-155 were found to be upregulated in CD4⁺ T cells, PBMC, RASFs from human patient with RA,^{106, 116, 118} and splenic T cells from murine lupus.¹⁰⁴ These data suggest the potential role of these two miRNAs in EBV-mediated autoimmune pathogenesis.

7. Conclusion and future direction

Even though that miRNAs were recognized as key regulators of mammalian genome only a few short years ago, there has been a stunning pace of publications documenting the physiological and pathological roles of miRNA in the immune system. A single miRNA may exert diverse immunoregulatory function, which may be specific to developmental stages and the microenvironment of different types of immune cells. miR-146a and miR-155

are two well studied miRNAs, which have been shown to play critical role in the regulation of lymphocyte development, differentiation, function, and also in the control of both innate and adaptive immune responses.^{18, 40, 43, 54, 57, 79, 83, 182} Conceivably, dysregulated miR-146a and miR-155 expression and/or function has been associated with various autoimmune diseases including SLE, RA, and MS (table1).^{100, 106, 116, 122} Although miR-146a was decreased in PBMC from human patients with lupus,¹⁰⁰ it was increased in PBMC and RASFs from human patients with RA and in murine lupus T cells.^{104, 106} Thus, the unique role of individual miRNA must be recognized based on the cell type or disease type and not generalized.

The identification of unique, signature autoimmune disease-related miRNAs provides the foundation for the next phase of studies: 1) to mechanistically understand the pathogenic contribution of these disease-related miRNAs, 2) to determine the cause and mechanism underlying miRNA dysregulation in autoimmune diseases, and 3) to ultimately develop novel, miRNA-based biomarkers for diagnosis and therapeutic strategies for curing and preventing autoimmune diseases. To address these central questions, relevant animal models of autoimmune diseases will be instrumental. Figure 4 summarizes the potential causes of miRNA dysregulation in SLE, and also documents potential pathogenic contribution of selected miRNAs in human and murine lupus. While a single miRNA may target hundreds of genes, the effect of miRNA on individual target protein synthesis is mild and moderate.^{38, 39} Given that a specific lupus-related gene may contain targeting sites for different miRNAs,⁹⁶ it is plausible that multiple lupus-associated miRNAs, rather than a single miRNA, synergistically act together to disrupt the tolerance regulatory mechanism and promote lupus. Current studies have indicated a huge potential of using miRNAs as gene therapy targets *in vivo* to treat cancer.^{183, 184} We anticipate that in the near future, to treat autoimmune diseases, novel effective miRNA-based gene therapies will be developed to replace the traditional immune suppressive therapies, which are usually life-long with undesirable side effects.

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Abbreviations

AID	activation-induced cytidine deaminase
AML-1	acute myeloid leukaemia-1
Ago	Argonaute
C/EBPα	CCAAT enhancer binding protein α
CNS	central nervous system
DGCR8	DiGeorge syndrome critical region protein 8
DNMT	DNA methyltransferase
EAE	experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus

ER	estrogen receptor
Foxo	forkhead transcription factor O
HRT	hormone replacement therapy
IFN	interferon
IRAK-1	interleukin-1 receptor-associated kinase 1
IRF	interferon regulatory factor
KLF13	Kruppel-like factor 13
LPS	lipopolysaccharide
MCP-1	monocyte chemotactic protein-1
M-CSFR	macrophage colony-stimulating factor
Mef2c	myocyte-specific enhancer factor 2C
miRNA	microRNA
MMP	matrix metalloproteinase
MOG₃₅₋₅₅	myelin oligodendrocyte glycoprotein ₃₅₋₅₅
mRNA	messenger RNA
MS	multiple sclerosis
MyD88	myeloid differentiation primary response protein 88
NFI-A	nuclear factor 1 A-type
NF-κ B	nuclear factor- κ B
OA	osteoarthritis
OCP	oral contraceptive pills
PAMPs	pathogen-associated molecular patterns
PBMC	peripheral blood mononuclear cell
pri-miRNA	primary miRNA transcripts
pre-miRNA	precursor miRNA
PTEN	phosphatase and tensin homolog
RA	rheumatoid arthritis
RASF	synovial fibroblasts
RASGRP1	RAS guanyl nucleotide-releasing protein 1
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
SLE	systemic lupus erythematosus
SNP	single-nucleotide polymorphism
STAT	signal transducers and activators of transcription
TCR	T cell receptor
Th	T helper

TNF	tumor necrosis factor
TLR	toll-like receptor
TRAF-6	tumor necrosis factor receptor associated factor 6
TRBP	TAR RNA binding protein
Treg	regulatory T cell
UTR	untranslated region

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Figure 1. miRNA biogenesis and action in animal cells

Mature miRNAs are short (with average about 22 nts long), single stranded RNA molecules derived from long primary transcripts, pri-miRNAs through sequential processing in both the nucleus and cytoplasm. In the canonical miRNA biogenesis pathway, the pri-miRNA is processed by DGCR8/Drosha microprocessor to generate pre-miRNA. The 33nts lower stem loop structure, the terminal loop, and the two single stranded segments flanking the hairpin loop in pri-miRNA are together required for the binding of pri-miRNA with DGCR8 and Drosha cleavage at the accurate site. In non-canonical miRNA biogenesis pathway, pre-miRNA is generated from small intronic miRNA, mirtron, through spliceosome splicing and then lariat-mediated debranching. Exportin 5 transports the pre-miRNA that derived from either canonical miRNA or mirtron from the nucleus into the cytoplasm. In the cytoplasm, the pre-miRNA is further processed by Dicer to generate miRNA/miRNA* duplex, which is then loaded into Argonaute protein and forms RISC together with Dicer and TAR RNA binding protein (TRBP). Although both strands of miRNA/miRNA* duplex have the

potential to be functional miRNA, only guided strand remains in the RISC as a mature miRNA. Depending on the complementary degree between miRNA and the 3'UTR sequence of target gene, miRNA regulates the expression its target genes through either translation inhibition or mRNA degradation/cleavage. The imperfect sequence complementary between miRNA and target mRNA usually leads to translational inhibition of target gene without affecting the amount of mRNA. While, the perfect complementary match between miRNA and target mRNA usually results in the repression of target genes by mRNA decay or cleavage.

Figure 2. miRNAs in the regulation of innate immunity

Illustrates the potential role of various miRNAs that have been reported to be involved in the development and function of neutrophils and monocyte/macrophage (Mφ). Please see the text for a detailed explanation. CMP, myeloid progenitor; GMP, granulocyte-monocyte progenitor; PAMPs, pathogen-associated molecular patterns.

Figure 3. miRNAs in the regulation of adaptive immunity

This figure depicts various miRNAs that have been reported in the development and differentiation of T cells (upper part of the figure), and B cells (lower part of the figure). Please see the text for a detail explanation. CLP, common lymphoid progenitor; DN, CD4⁻CD8⁻ double negative; DP, CD4⁺CD8⁺ double positive; Treg, regulatory T cell.

Figure 4. An overview of dysregulated miRNAs and the potential pathogenic contribution of dysregulated miRNAs in SLE

Multiple factors including genetic, hormonal, and environmental factors may contribute to the miRNA dysregulation in SLE. The published reports have shown that miR-146a, miR-125a, miR-21, and miR-148a contribute to lupus pathogenesis by regulating the type I interferon pathway, inflammatory chemokine RANTES expression, and DNA methylation, respectively. The potential pathogenic contribution of miR-155, miR-182-96-183 and miR-31, which are commonly upregulated in three murine lupus models, is suggested by either their well-defined function in immunity or the role of their target genes in the control of immune tolerance to self-antigens. The targets of these specific miRNAs are indicated. Please see the text for a detail explanation.

Table 1

Diseases	miRNAs	Pathogenic contribution	Reference
Systemic lupus erythematosus	miR146a, ↓PBMC	Targets STAT-1 and IRF-5, negative regulator of Type I interferon pathway	100
	miR-148a and miR-21 ↑CD4 ⁺ T cells	Target DNA methyltransferase 1 (DNMT1) directly and indirectly, respectively, induces DNA hypomethylation and the expression autoimmune-associated genes.	99
	miR-125a ↓ T cells	Targets Kruppel-like factor 13 (KLF13), Negative regulator of inflammatory chemokine RANTES	101
	Other miRNAs dysregulated in SLE including miR-223, miR-31, miR-371-5p, miR-473-5p.	Unknown	97, 98, 100, 102
Rheumatoid Arthritis	miR-146a, ↑PBMC, CD4T cells, Th-17 cells, synovial fibroblasts (RASFs) and/or synovial tissues	Targets FAF1, Negative regulator of T cell apoptosis	106, 116– 119
	miR-155, ↑PBMC, Th-17 cell, RASFs and synovial tissues	Targets Matrix metalloproteinase (MMP)-3/1 in RASFs, Regulation of inflammation and potentially involved in RASFs mediated tissue damages	106, 116, 117
	miR-124a, ↓ synoviocytes	Targets cyclin-dependent kinase 2 (CDK-2) and chemokine MCP-1, Negative regulator of cell proliferation and MCP-1 secretion	120
	Other miRNAs dysregulated in RA including miR-223, miR-132, and miR-16.	Unknown	106, 117, 119, 121
Multiple sclerosis	miR-326, ↑ CD4 T cells	Targets Ets-1, Promotes Th-17 cell differentiation	82
	miR-17-5P, ↓ whole blood and ↑CD4 T cells	Potentially involved in the regulation of T cell activation	125, 126
	miR-20a, ↓ whole blood	Potentially involved in the regulation of T cell activation	126
	miR-34a, miR-155 and miR-326, ↑ MS lesion	Targets CD47, promotes phagocytosis of myelin by releasing macrophage from inhibitory signaling.	122
	Other miRNAs dysregulated in MS including miR-25, miR-18b, and miR-599.	Unknown	123, 124, 127

miRNAs in human inflammatory autoimmune diseases