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REVIEW

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Abstract MicroRNAs (miRNAs) are about 20–22 nucleotide conserved non-coding RNA molecules that post-transcriptionally regulate gene expression by targeting the 3′-untranslated region of specific messenger RNAs (mRNAs) for degradation or translational repression. During the last two decades, miRNAs have emerged as criticalregulators of a range of biological processes including immune cell lineage commitment, differentiation, maturation, and immune signaling pathways. The endoribonucleases such as Dicer, which is required for miRNA biogenesis, has also been shown to play an important role in inflammatory response and autoimmunity. Thus, dysregulated miRNA expression patterns have been documented in a broad range of human diseases including inflammatory and autoimmune diseases. In this review, we will discuss recent advances in miRNAs mediated regulation of inflammatory responses and autoimmune pathogenesis. Specifically, we will discuss how miRNAs regulate autoimmunity through affecting the development, differentiation, and function of various cell types such as innate immune cells, adaptive immune cells and local resident cells. The identification of distinct miRNA expression patterns, and a comprehensive understanding of the roles of those dysregulated miRNAs in inflammatory autoimmune pathogenesis offers inspirations of not only potential molecular diagnostic markers but also novel therapeutic strategies for treating inflammatory autoimmune diseases.

Keywords MicroRNA · Immunity · Autoimmunity · Inflammation · Autoimmune disease

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Introduction

MicroRNA (miRNA) has emerged as a new and critical layer of gene expression regulation that affects many biological processes, including the mammalian development, differentiation, and function. MiRNAs are small non-coding RNA molecules that were first identified in the nematode *Caenorhabditis elegans* in 1993 [1, 2]. They were proved to regulate gene expression post-transcriptionally by binding to their target mRNAs, usually the 3′ untranslated region (UTR), to mediate translational repression or directly degradation of the target mRNAs. Currently, by using different experimental strategies such as deep sequencing and robust bioinformatics prediction, 25,141 mature miRNAs in 193 species (miRBase database, release 19, August 2012) have been identified, including 2,042 human mature miRNAs and 1,281 mouse mature miRNAs in the database. It is estimated that a single miRNA can regulate hundreds to thousands of target genes, and therefore, most of our human genes are possibly regulated by miRNA [3–5]. More than 100 miRNAs have been proved to have the potential to affect the molecular pathways in control of the development and function of innate and adaptive immune cells. Many miRNAs have also been observed dysregulated in different inflammatory autoimmune diseases and have anti-inflammatory or pro-inflammatory activities based on their specific target mRNAs [6]. Mutations or single nuclear polymorphism (SNP) in some miRNA genes or miRNA target sites are found associated with the onset and progression of certain inflammatory autoimmune diseases [7, 8]. In terms of these findings, miRNA

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expression profiles are wildly exploited for the diagnosis and prognosis of human autoimmune diseases. Here, we summarize our current understanding in regard to the role of miRNA in inflammatory autoimmune disease, with an emphasis on how miRNAs regulate autoimmunity or inflammation though their expression on different cell types.

MiRNA biogenesis and autoimmunity

Our understanding of miRNA biogenesis and mechanisms of post-transcriptional regulation of mRNA by miRNAs is deepening.

Identification of the processes is important for us to determine when, where, and how miRNAs are produced and to clarify the mechanisms how miRNAs mediate their physiological functions in immune cells. Based on the pioneer works in plants and nematodes [1, 9, 10], our understanding of miRNA biogenesis and cellular function of mammalian miRNA is expanding. As we know, miRNAs are encoded by genomic DNA and are commonly transcribed by RNA polymerase II. A single miRNA can also be produced by miRNA-containing primary transcripts such as miR-146a [11], whereas some other miRNAs were in the introns of genomic sequence with other transcripts encode proteins in their exons, such as miR-126 [12]. Alternatively, some miRNAs, such as the miR-17-92 family, which contained six different miRNAs, are located in clusters on a single transcript and are usually transcriptional together [12]. The primary transcripts of miRNA genes, known as the pri-miRNA, are usually cleaved by the RNAase III endonuclease Drosha in the nucleus, to produce a 60–70-nt stem-loop intermediate, called the pre-miRNA. The pre-miRNAs are subsequently exported from the nucleus by exportin 5 to the cytoplasm, where they are further cleaved by Dicer, which is a RNase III endonuclease, to release a double-stranded RNA duplex, which contains both the mature miRNA and its antisense strand. One strand of the duplex, called the guide strand, is then packaged into the RNA induced silencing complex (RISC), which consists of Argomaute (Ago) and other proteins. The miRNA then guides the RISC complex to the 3′ UTR site of its target mRNA which it recognizes through partial sequence complementarity. A major determinant in this recognition process depends on a perfect match between the seed region of 6–8 nt at the 5′ end of the miRNA. miRNA–mRNA interaction can promote the downregulation of protein expression by translational repression, mRNA cleavage, or mRNA decay by accelerated uncapping and deadenylation [13]. Recent proteomic experiments in mammalian cells have demonstrated that one miRNA can directly target multiple genes, increasing its biologic effect, although miRNA repression of individual target is typically mild. MiRNA-mediated repressive effect is mostly, but not always, due to both downregulation of mRNA levels and translation inhibition [14, 15]. Some evidence also revealed that miRNAs can also increase the translation of target mRNAs [16] or even directly target the promoter to interfere with gene transcription [17].

Important involvement of miRNA in immune systems has been explored by specific disruption of genes involved in miRNA biogenesis such as Dicer and Ago2. It has been reported that conditional knockout of Dicer in hematopoietic stem cells (HSCs) resulted in increased apoptosis and the loss of hematopoietic system reconstitution, and deletion of Ago2 in the hematopoietic system resulted in deficient B cell and erythroid differentiation [18]. In addition, conditional knockout of Dicer in T lymphocytes resulted in reduced T cells number in the thymus and periphery lymph tissues [19, 20]. Besides, deletion of Dicer in B lymphocytes has been reported to decrease B cell survival and the antibody repertoire [21]. These studies suggest critical functions of miRNAs in constitution of the immune system. The investigations on the mice with a conditional knockout of Dicer or Drosha in regulatory T (T_{Reg}) cells have indicated that the miRNA biogenesis pathway in Treg cells is critical for their suppressive fuctions [22, 23]. The knockout mice can develop a lethal autoimmune inflammatory disease due to impaired development or function of Treg cells. Also, the mice with intestinal epithelial cell (IEC)-specific deletion of Dicer1 displayed fewer goblet cells (mucus-secreting columnar epithelial cells) and a lower abundance of the goblet cell-specific Th2 effector RELMβ in the colon, accompanied by immunological defects that compromised parasite resistance [24]. In summary, these studies revealed the importance of miRNA in safeguarding hematopoietic or epithelial cells function to prevent autoimmunity (Fig. 1).

MiRNAs regulate autoimmunity through innate immune cells

Immunity is conventionally considered as two types, innate immunity and adaptive immunity. Innate immunity is the evolutionarily older and contains more widespread process, including immune organs and cells that respond to various pathogens by recognizing molecules on invading organisms. An established link associates innate immune response with human autoimmune diseases. Furthermore, experimental autoimmune diseases can be induced by autoantigens that are administered together with complete Freund's adjuvant, which contains killed *Mycobacterium tuberculosis*; in some cases, these bacteria can be replaced by individual pathogen-associated molecular patterns (PAMPs). Exogenous PAMPs and endogenous danger signals from necrotic cells bind to pattern recognition receptors (including Toll-like receptors) and activate signaling pathways in innate immune cells. This leads to pro-inflammatory cytokine

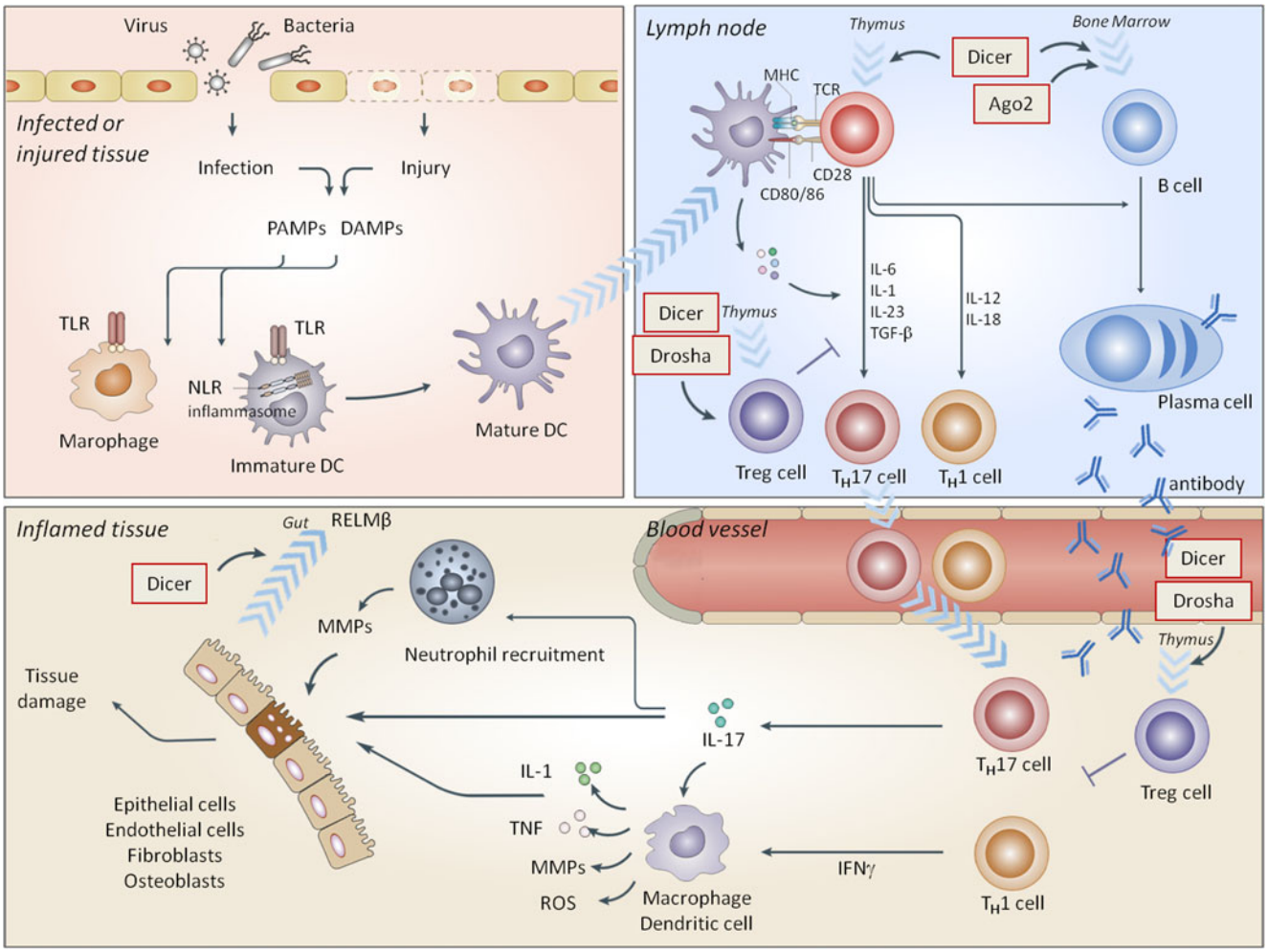

Fig. 1 The overall importance of miRNA in immune systems. The roles of enzymes required for miRNA biogenesis (Dicer/Ago2/Drosha) in immune response and immune cell development. When our tissue are infected or injured, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPS) bind to pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs) expressed in innate immune cells like macrophage and dendritic cells (DCs). This promotes macropahge activation, DC maturation, and migration to the lymph nodes. In the lymph nodes, mature DCs activate CD4+ T helper cells and release pro-inflammatory cytokines, to promote T cell differentiate to different subtype. T helper cells also help B cells activation and differentiation into plasma cells in lymph node. Dicer and Ago2 were reported to be critical for T cell and B cell development. Conditional knockout of Dicer in T lymphocytes resulted in fewer T cells in the thymus and periphery. Dicer deficiency in B lymphocytes led to diminished B cell survival and antibody repertoire, and deletion of Ago2 in the hematopoietic system resulted in impaired B cell and erythroid differentiation. Treg cells have been well recognized to repress effect T cell differentiation and function. Mice with a conditional deletion of Dicer or Drosha in Treg cells developed a lethal autoimmune inflammatory disease due to impaired development or function of Treg cells. Differentiated autoreactive T cells exit lymph nodes and migrate through the blood to inflamed tissues, where they are further activated by local antigen-presenting cells. In the meantime, cytokines produced by these T cells such as IL-17 and IFNγ activates macrophages, DCs and other cells, promoting the release of pro-inflammatory mediators, some of which further promote T cells function. IL-17 also induces the production of chemokines to recruit neutrophils to the site of inflammation. Together, all these cells and factors mediate the tissue damage. Also, gut specific deletion of Dicer1, resulted in lack of Th2 effector RELMβ in the colon, accompanied by immunological defects that compromised parasite resistance. *IFNγ* interferon-γ; *TCR* T cell receptor; *Treg* regulatory T cell. *Thymus* and *Bone marrow* in the figure indicate the sources of the different cells

production, and resident tissue inflammation, which are now considered to be critical factors in the development of autoimmunity (Fig. 1).

During the past years, a growing body of evidence has emerged to demonstrate that miRNAs are critical in the development of the immune system, as well as the function of both innate and adaptive immune system [6, 25–27]. We will discuss the recent findings regarding the roles of miRNAs in the regulation of innate immune cells development, function, and relevant autoimmune diseases in this section (Fig. 2). Innate immune cells, including monocytes (which can further differentiate into macrophages or myeloid-derived dendritic cells (DCs)), granulocytes, and natural killer (NK) cells, serve as an important first line of

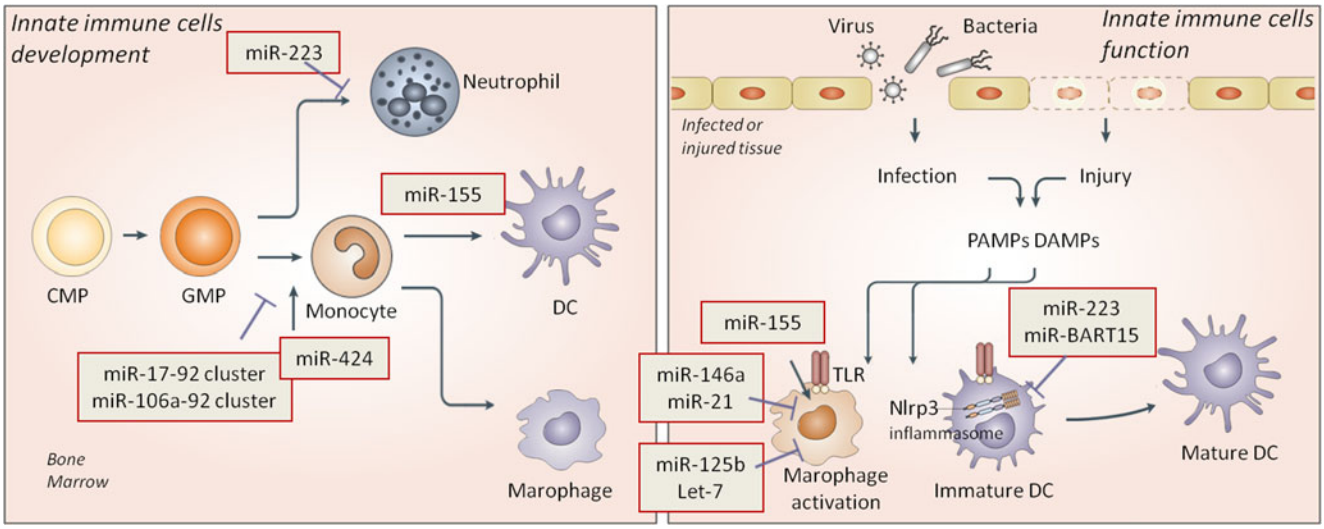

Fig. 2 MiRNAs regulate autoimmunity through innate immune cells. The roles of various miRNAs that have been reported to be involved in the development and function of innate immune cells, especially the myeloid lineage. Many miRNAs regulate the generation of different myeloid cell types by targeting relevant transcription factors. Common myeloid progenitors (CMPs) give rise to granulocyte monocyte progenitors (GMPs). GMPs produce both monocytes and neutrophils, and monocytes can further differentiate into macrophages or dendritic cells (DCs). The miR-17–92 cluster and miR-106a-92 cluster represses runt-related transcription factor 1 (Runx1) production, which regulates colony-stimulating factor receptor (CSFR) expression during monocytopoiesis. PU.1 is another transcription factor that is crucial for monocyte and macrophage differentiation and has been shown to upregulate miR-424 to promote monocytic differentiation by inhibiting the expression of nuclear factor I/A (NFIA). MiR-223 represses C/EBPβ and myeloid ELF1-like factor 2C (MEF2C) expression to regulate neutrophil differentiation and proliferation. MiR-155 represses the expression of dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) through directly suppressing PU.1 expression to affect antigen presentation by DCs. However, myeloid-derived DCs from miR-155 deficient mice show defects in antigen presentation to T cells. During Toll-like receptor activation by PAMPs, the expression of many miRNAs is regulated. The upregulated miRNAs include miR-155, miR-146, and miR-21 and the downregulated miRNAs include miR-125b and let-7. MiR-155 represses the mRNAs encoding SOCS1 and SHIP1, which are negative regulators of the TLR pathway; miR-146 represses the mRNAs encoding IRAK1, IRAK2, and TRAF6, which are positive regulators of TLR signaling. MiR-21 negatively regulates macrophage activation by repressing expression of programmed cell death 4 (PDCD4) and IL-12p35. MiR-125b targets TNF-α mRNA, whereas let-7 targets IL-6 mRNA, and these two miRNAs are downregulated by LPS, resulting in derepression of inflammatory responses. Recent studies also showed myeloid-specific miRNA miR-223 or EBV miRNA miR-BART15 which recognizes the same targeting site, directly target NLPR3 mRNA to suppress IL-1β maturation. *CMP* myeloid progenitor; *GMP* granulocyte–monocyte progenitor; *DC* dendritic cell; *TLR* toll-like receptor; *NLR* nod-like receptor

defense against invading pathogens and also function as the primary initiator of inflammatory response and autoimmune pathogenesis.

Granulocytes and NK cells development

During granulopoiesis, the transcription factor, CCAAT enhancer-binding protein α (C/EBPα) highly induced miR-223 expression, subsequently led to the inhibition of transcription factors nuclear factor 1 A-type (NFI-A) and E2F1, and further resulted in increased granulocytes differentiation [28]. Also, the transcriptional factor growth factor independent 1 (Gfl1) was reported to bind to the promoter regions of pri-miR-21 and pri-miR-196b sequences and consequently repress miRNAs expression [29]. Furthermore, upregulation of miR-21 and miR-196 in bone marrow cells blocked the granulopoiesis in vitro, consistent with the phenotype observed in Gfi−/− mice [30]. MiR-155 was also shown to increase immature granulocyte numbers in vivo through its target SHIP1 [31]. Furthermore, miRNAs can also regulate the granulocyte function. MiR-223 was reported to negatively regulate both the proliferation and activation of neutrophils by targeting myeloid Elf1-like factor 2C (MEF2C) [32]. Deficiency of miR-223 in neutrophils rendered the cell with increased capacity to undergo oxidative burst and high efficiency to kill *Candida albicans* than that of wild-type cells [32] (Fig. 2). Two recent studies observed in the absence of Dicer, iNKT (invariant natural killer T) cells almost had a complete loss in the thymus and periphery [33, 34], suggesting miRNAs are also involved in the development of iNKT cells, which recognize glycolipid antigens presented by the major histocompatibility complex class I-related protein CD1 and then produce lots of immunoregulatory cytokines (Fig. 2).

Monocytes development and function

Both macrophages and myeloid-derived DCs play critical roles in the innate immunity. Several studies have suggested that some important transcription factors that involved in

monocytopoiesis can be regulated by specific miRNAs, which in turn, regulate these miRNAs themselves, indicating that miRNAs are invoved in monocyte development. During monocytopoiesis, the expression of miR-17-92 cluster and miR-106a-92 cluster miRNAs was decreased, consistent with the upregulation of their target genes, such as runt-related transcription factor 1 (RUNX1, also known as AML1) [35], in turn promoting the expression of M-CSFR critical for monocyte–macrophage differentiation and maturation. RUNX1 can also bind to the promoter regions of the miR-17-92 cluster and miR-106a-92 cluster and subsequently repress their expression, suggesting that these miRNAs and RUNX1 functioned in a negative feedback loop. Besides, PU.1 also known as an important transcription factor critical for monocyte and macrophage differentiation, has been demonstrated to upregulate miR-424 expression and further promote monocytic differentiation by suppressing the expression of nuclear factor I/A (NFIA) [36]. MiR-155 can inhibit the expression of dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) by directly suppressing PU.1 expression to interfere antigen presentation by DCs [37]. However, myeloid-derived DCs from miR-155-deficient mice show deficiency of antigen presentation to T cells, despite the normal expression of MHC class II and co-stimulatory molecules [38]. Thus, the exact function and underlying mechanism of miR-155 on antigen presentation still remain to be further explored.

The innate immune response is initiated largely by the signaling activities of pathogen recognition receptors including Toll-like receptors (TLRs), Rig-I-like receptors (RLRs), and Nod-like receptors (NLRs) that are expressed on macrophages and DCs and are responsive to PAMPs found on different classes of pathogens or host-derived danger signals (danger associated molecular patterns, DAMPs). TLR-mediated signaling can induce expression of many critical genes for host defense as well as inflammatory pathogenesis. TLR signaling also induces many miRNAs to regulate innate immune responses. The most characterized miRNA is miR-155, the expression of which is highly induced in mouse bone marrow-derived macrophages (BMM) after treatment of various TLR ligands or cytokines such as tumor necrosis factor (TNF) and interferon-β [39–41]. MiR-155 was shown to target and inhibit the negative regulators SHIP1 and SOCS1, in turn leading to the upregulated activation of AKT and IFN response genes [31, 42]. These pathways have been proven critical in mediating cell survival, growth, differentiation, and migration, as well as antiviral responses. miR-146a was also discovered earlier to play a negative role in TLR signaling [11]. Unlike miR-155, which promotes the immune response, miR-146a plays a negative role in the immune response, while both miRNAs are induced after TLR stimulation. MiR-146a targets and suppresses the expression of TRAF6 and IRAK1, two important proteins that mediate the transduction of TLR/IL-1 signaling for NF-κB activation [11]. During the activation of innate immune cells, miR-146a also inhibits the persistent production of pro-inflammatory cytokines such as IL-6 and TNF-α. Like miR-146a, TLR4 signaling can also induce miR-21 expression through NF-κB activation in a MyD88-dependent manner in macrophage. MiR-21 can further negatively regulate LPS-activated TLR4 signaling by targeting the tumor suppressor, PDCD4, in turn decreased NF-κB activation and resulted in anti-inflammatory cytokine IL-10 production [43]. Other miRNAs such as miR-125a and let-7 are also shown to be important in controlling innate immune responses. Both miR-125b and let-7 were downregulated by LPS stimulation in macrophages. MiR-125b targets TNF-αmRNA, whereas let-7 targets IL-6 mRNA [44, 45]. Taken together, these works show that the critical roles of miRNAs in both the regulation of innate immune cell development and controlling the innate immune responses through targeting either key intracellular signaling molecules or inflammatory cytokines to further suppress overwhelming inflammation in the body (Fig. 2). Although miRNA regulation of TLR signaling has been well defined, miRNA regulation of NLR-mediated signaling or inflammasome activation is less studied. Three recent studies showed myeloid-specific miRNA miR-223, and EBV miRNA miR-BART15 are critical regulators of NLRP3 inflammasome activity [46–48] (Fig. 2).

Innate immune cells and autoimmunity

In human autoimmune disease, miR-146a, as a negative regulator of TLR signaling, was found to be significantly decreased in peripheral blood of patients with systemic lupus erythematosus (SLE) versus healthy controls. Importantly, the expression of miR-146a correlated inversely with clinical disease activity and interferon scores in patients with SLE [49]. MiR-146a was further found to negatively regulate the type I IFN pathway by targeting two critical signal mediators in IFN type I pathway, signal tranducers IFN regulatory factor-5 (IRF5), and STAT-1. The overexpression of miR-146a in normal peripheral blood mononuclear cell (PBMC) decreased while the inhibition of endogenous miR-146a expression increased the production of IFNα/β in response to TLR-7 activation [49]. Thus, decreased expression of miR-146a in PBMC can contribute to the enhanced type I IFN production in human lupus. A genetic variant (rs57095329) in the promoter region of miR-146a was also found associated with SLE. This risk allele resulted in decreased binding activity of miR-146a promoter to transcription factor Ets-1, in turn leading to reduced levels of miR-146a in SLE patients [8]. Another study showed that expression of miR-146a was significantly increased in Sjogren's syndrome (SjS) patients compared with healthy

controls. MiR-146a was also upregulated in the salivary glands and PBMCs of the SjS-prone mouse. Moreover, miR-146 has also been shown to play a critical role in increasing phagocytic activity and repressing inflammatory cytokine production in human monocytic THP-1 cells [50]. Taken together, these studies suggest that dysregulation of microRNAs in innate immunity can contribute to the initiation and progression of autoimmune diseases.

MiRNAs regulate autoimmunity through adaptive immune cells

T and B lymphocytes are two major components of adaptive immunity. They might play more prominent roles in chronic inflammatory situations or in secondary response to an inflammatory trigger. The overall implication of miRNA in the regulation of adaptive immunity has been clearly shown in studies in which the deficiency of miRNA biogenesis in lymphocyte progenitors blocked the normal development of T and B lymphocytes. Conditional depletion of Dicer in lymphocytes resulted in abnormal T cell development with decreased T cell numbers in thymus and peripheral lymphoid organs, severe blockage of peripheral $CD8^+$ T cell development, and loss of proliferation of T helper cells, while these helper cells were skewed to preferentially express interferon-gamma [19, 20]. Depletion of Dicer in early B cell progenitors led to a complete developmental blockage of B cells at the pro- to pre-B transition stage and consequently affected antibody diversity [21].

T cell development and function

Recent studies showed that specific miRNAs are involved in T cell development, differentiation, effector, and regulatory functions. During thymic development, immature T lymphocytes undergo positive and negative selection, further differentiate into mature cells, and exit into the peripheral lymph tissues. T cell will be eliminated during negative selection if the antigen binds with its TCR with inappropriate affinity. Recent studies have suggested that miR-181a has a significant effect on the positive and negative selection processes by controlling the strength of TCR signaling during T cell thymic development through downregulation of multiple phosphatases, which led to increased steady-state levels of phosphorylated mediators and a reduction of the T cell receptor signaling threshold [51, 52]. Apoptosis is another tolerance mechanism during T cell development to remove unwanted T cell clones, and miRNAs have been clearly linked to this process. In T cells, miRNAs of the miR-17-92 cluster are responsible for managing cell survival by repressing BCl-2-interacting mediator of cell death (BIM) and phosphatase and tensin homologue (PTEN), both of which promote cell death [53].

Once mature T cells sense their cognate antigens in the periphery, the naïve state will turn into distinct subtypes (Th1, Th2, Th17 cells, and so on) with the capacity to elicit unique inflammatory outcomes. Several reports have demonstrated that specific miRNAs can control T cell differentiation by targeting specific transcription factors that affect these decisions. Deficiency of miR-155 in T cells, rendered the activated CD4+T cells to exhibit a Th2 bias under neutral conditions in vitro [38, 54]. It is suggested that miR-155 promotes differentiation into Th1 cells through repressing Th2 transcriptional factor c-Maf [38]. MiR-29 was also shown to regulate Th1 differentiation by targeting transcription factors such as T-bet and Eomes or cytokine such as IFNγ [55–57]. Th17 cells have been identified as important mediators of inflammatory diseases. A recent study found that miR-326 promotes TH17 cell development and autoimmune pathogenesis by targeting ETS1, which is a negative regulator of Th17 differentiation [58]. T follicular helper (Tfh) cells are reported to support B cells in germinal centers and facilitate antibody affinity maturation and class switching [59, 60]. miR-17-92 cluster has been shown to be involved in the development and effector function of Tfh cells. A recent study also showed a TGF induced miR-10a can attenuate the phenotypic conversion of inducible Treg cells into follicular helper T cells by simultaneously targeting the transcriptional repressor Bcl-6 and the corepressor Ncor2 [61]. When a T cell recognizes its cognate antigen and proper costimulation, it undergoes activation, clonal expansion, thus greatly increases its numbers. Thus, it can be seen that the T cell must reduce the expression of limited factors that control cell division. A recent report found that miR-182, which is upregulated in T cells in response to IL-2, plays an important role during clonal expansion by inhibiting Foxo1 [62]. MiR-214, which is also upregulated during costimulation, promotes T cell expansion by repressing PTEN [63].

Regulatory T cells (Tregs) which are responsible for repressing inflammatory responses, also produced in the thymus. Interestingly, deletion of miRNAs specifically in Tregs by conditional knockout of Dicer in $Foxp3^+$ cells resulted in a systemic and lethal inflammatory condition due to a failure of Tregs generation [22, 23, 64]. MiR-146a-deficient mice also succumbed to a chronic inflammatory disorder [65], and the absence of miR-146a led to the increased expression of one of its targets, STAT1, critical for skewing Th1 response, suggesting its role in Treg function [66]. Several studies reported other two foxp3 regulated miRNAs. miR-155 was elevated by foxp3 and was required for maintaining Treg cell proliferative activity [67], while miR-142-3p was shown to be repressed by Foxp3, leading to increased production of cyclic AMP and enhanced suppressive function of Treg cells [68] (Fig. 3).

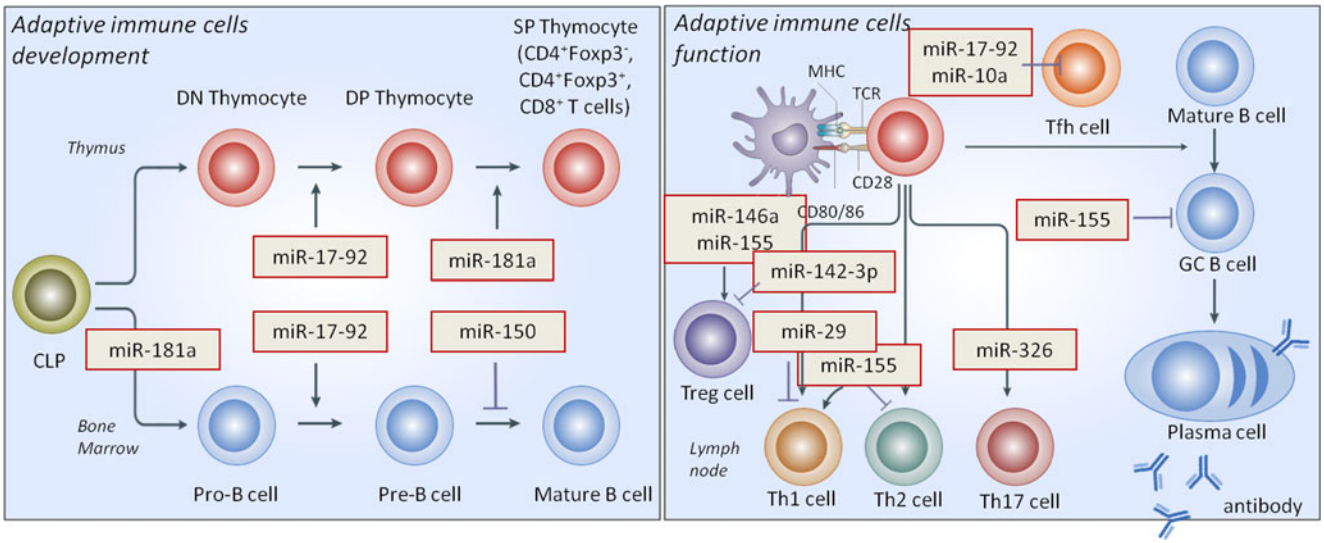

Fig. 3 MiRNAs regulate autoimmunity through adaptive immune cells. The roles of various miRNAs that have been reported to be involved in the development and function of adaptive immune cells. T cells or B cell go through a stepwise developmental program in the thymus or bone marrow, respectively. During T cell development, T cell survival and selection is influenced by the miR-17–92 cluster which targets the mRNAs encoding BIM and PTEN; and miR-181a which targets mRNAs encoding several phosphatases including DUSP5, DUSP6, SHP2, and PTPN22. During B cell development, miR-181 causes a skewing of hematopoiesis towards the development of B cells, and miR-150 and the miR-17–92 cluster influence early development through repression of Myb and BIM expression, respectively. Mice with miR-150 deficiency also showed an increased number of B1 cells. In the periphery, T cell differentiation is regulated by miRNAs including miR-155 which promotes skewing towards T helper 1 (Th1) cells through repressing Th2 transcriptional factor c-Maf and miR-326 which promotes skewing towards Th17 cells by targeting the a negative regulator ETS1. Regulatory T (Treg) cells also require miRNAs to maintain immune tolerance, thereby preventing autoimmunity. The absence of miR-146a in Treg leads to elevated expression of one of its targets, STAT1, and an unchecked Th1 response. MiR-155 and miR-142-3p were reported to be regulated by Foxp3. MiR-155 mediated repression of SOCS1 expression has been implicated in Treg cell survival. MiR-142-3p targets cyclic AMP to enhance suppressive function of Treg cells. T follicular helper (Tfh) cells are dedicated to supporting B cells in germinal centers and facilitating antibody affinity maturation and class switching. The core transcriptional factor bcl-6 represses the expression of many miRNAs to control the Tfh cell signature, including miR-17-92 which repressed CXCR5 expression. MiR-10a attenuated the phenotypic conversion of inducible Treg cells into follicular helper T cells by simultaneously targeting the transcriptional repressor Bcl-6 and the corepressor Ncor2. Further development of mature B cells is regulated by miR-155 which targets mRNAs encoding activation-induced cytidine deaminase (AID) and PU.1 and in turn inhibits antibody class switching and antibody production. *CLP* common lymphoid progenitor; *DN* $CD4^{-}CD8^{-}$ double negative; *DP* $CD4^{+}CD8^{+}$ double positive; *SP* single positive; *Tfh* follicular T cell; *GC* germinal center

B cell development and function

In the course of developing specific, long-lasting immunity against pathogens, B cells exert its roles by secreting highly specific immunoglobulins. B cells develop in the bone marrow and acquire a surface immunoglobulin receptor by V(D)J recombination, then are subject to activation (class switch recombination, somatic hypermutation and antibody secretion) in peripheral lymphoid organs during inflammatory responses by interacting with Tfh cells and follicular DCs. Both of these two steps are dynamically regulated by miRNAs. [69, 70]. Overexpression of miR-181 rendered hematopoiesis towards the development of B cells, resulting in a two- to threefold elevation of B cell numbers. Notably, the gene expression pattern of Dicer-deficient B cell precursors is similar to that of B cells deficient of the miR-17-92 family [71], which has been associated with pathological lymphoproliferative conditions in humans and mice [53]. So, the function of conditional knockout of Dicer in B cells could be mainly attributed to the absence of these crucial miRNAs in early B cells. MiR-150 is selectively induced in mature B and T cells while not in their progenitors. Overexpression of miR-150 prematurely in hematopoietic stem cells disrupted the B cell development at the pro- to pre-B stage while not affecting T cells, granulocytes, and macrophage maturation [70, 72]. Furthermore, an in vivo study showed that miR-150 can control B cell development by targeting a critical transcription factor c-myb in a dose-dependent manner [70]. MiR-155 has also been shown to be induced in B cells stimulated by BCR, CD40, or TLR agonists and play an important role in regulating T cell-dependent germinal center response for optimal antibody production. MiR-155 deficient mice also showed marked defects in T cell-dependent antibody response and class switch recombination in response to immunization [54, 73], due to miR-155-mediated repression of more than 60 target genes, including Pu.1, SHIP1, and AID [73] (Fig. 3).

Adaptive immune cells and autoimmunity

In human patients of multiple sclerosis (MS), miR-326 has been identified highly expressed in PBMC of a cohort of patients with MS. Studies of mouse model showed that miR-326 could promote disease severity in the context of experimental autoimmune encephalomyelitis (EAE) by enhancing the development of Th17 cells [58], which are important T cell subset in mediating the tissue damage during antigen-specific inflammation. Another miRNA expression study with MS patients identified that miR-18b and miR-599 were related to relapse stage and miR-96 was involved in remission stage. In addition, differentially expressed miRNAs have also been identified in different T lymphocyte subsets including $CD4^+$ $CD25^-$ T cells, $CD8^+$ T cells, $CD4^+CD25^+$ Treg cells, as well as B lymphocytes from patients with MS [74–76]. MiRNAs are also shown dysregulated in human patients with rheumatoid arthritis (RA). T cell-isolated RA patients showed upregulation of miR-146a, miR-155, and miR-16 [77]. Mouse models of arthritis have further demonstrated the functional relevance of miR-155 and miR-182 in regulating B and T cell function during disease pathogenesis [62, 78]. MiR-21 and miR-148a were also found upregulated in $CD4^+$ T cells from both human patients with lupus and the relevant autoimmue MRL–lpr mice. MiR-148a targeted DNA methyltransferase 1 (DNMT1) by directly binding to the protein-coding region, whereas miR-21 indirectly downregulated DNMT1 by targeting its upstream regulator, RASGRP1. Overexpression of miR-148a or miR-21 in $CD4^+$ T cells led to DNA hypomethylation and subsequent increase of the expression of autoimmune-associated methylation-sensitive genes [79]. In addition, downregulated expression of miR-125a in PBMCs from patients with lupus led to elevation of its target Kruppel-like factor 13 (KLF13), a critical transcription factor in the regulation of the chemokine RANTES [80], and the upregulation of miR-31 in T cells of lupus patients correlated well with the deficiency of Treg cell development in lupus because miR-31 directly targets Foxp3 which is critical for Treg cell development [81]. Links between miRNAs and other autoimmune diseases, including type I diabetes, inflammatory bowel disease (IBD), Sjogren's syndrome, atopic dermatitis, etc., are emerging [82–85].

MiRNAs regulate autoimmunity through local resident cells

Evidence has accumulated that miRNAs are involved in diverse aspects of tissue inflammation by affecting not only immune cells, which initiate antigen-specific responses and further produce large inflammatory cytokines, but also resident tissue cells, which amplify the inflammatory response through mechanisms such as chemokine production to recruit granulocytes. For example, one recent study, through miRNA screening and functional analyses of human and mouse resident tissues or primary cells from several interleukin-17 (IL-17)-related autoimmune diseases, demonstrates that miRNA-23b is a critical regulator of inflammation in resident tissue cells during autoimmune pathogenesis. MiR-23b was downregulated in inflamed tissues of EAE (a mouse model of MS), RA, CIA (a mouse model of RA), SLE, and MRL/*lpr* (a lupus-prone mouse strain), and the loss of miR-23b led to uncontrolled inflammation in the mouse model of EAE, CIA, and MRL/*lpr* [86]. The elevated cytokine IL-17 is responsible for miR-23b downregulation during autoimmune pathogenesis. MiR-23b targets signaling molecules TAB2, TAB3, and IKKα to suppress pro-inflammatory cytokines' (TNF, IL-1β, and IL-17) mediated signaling and consequently inflammatory pathogenesis. Another study showed that miR-124 was proven to be downregulated in resident microglia in the brain, leading to microglia activation during EAE development. MiR-124 targets the transcription factor CCAAT/enhancer-binding protein-α (C/EBP-α) to render the microglia from activated state to quiescent one [87]. Some other miRNAs were also observed dysregulated in autoimmune tissues or primary cells while the function of those miRNAs expressed in local resident cells still remains to be explored during autoimmune pathogenesis. MiR-34a, miR-155, and miR-326 were found upregulated in active MS lesions and might contribute to MS pathogenesis by targeting CD47 to promote phagocytosis of myelin [88]. Synovial tissue samples from patients with RA showed some changes in the expression of miR-155 and miR-146a compared with control samples [77]. MiR-155 was shown to targets matrix metallopeptidase 3 (MMP3) and MMP1 in RA synovial fibroblast (RASF), suggesting that elevated expression of miR-155 in RA might have a protective function in RASF-mediated tissue damages [89]. However, miR-155 deficient mice did not show enhanced CIA, but instead showed a protective role in K/BxN serum-transfer arthritis model which only depends on innate effector mechanisms [90]. Administration of miR-146a has been reported to suppress joint destruction in CIA, while it is not clear whether miR-146a mediates its suppressive effect through local resident cells [91]. Several miRNAs were found upregulated or downregulated in rheumatoid fibroblast-like synoviocytes treated with LPS, and, among these, miR-346 was shown to modulate IL-18 production [92]. When compared with patients with osteoarthritis (OA), miR-124a was found markedly decreased in RA synoviocytes [93]. Further investigation suggested that miR-124a targets cyclin-dependent kinase 2 (CDK-2) to repressed RA synoviocyte cell proliferation and arrested the cell cycle at G1 phase. Moreover, miR-124a was also shown to target monocyte chemoattractant protein 1 (MCP-1) to recruit mononuclear phagocytes into joint. As

local resident cells also express TLR, those miRNAs involved in innate immune response and expressed in those cells might also have a role in resident cellular function. While recent studies have demonstrated that some miRNAs are dysregulated in the tissues of autoimmune patients, the in vivo function of these miRNAs during autoimmune pathogenesis still remains to be further explored (Fig. 4).

miRNA expressed in stoma cells could also regulate their contraction with hematopoietic cells. A recent study showed that miR-29a expressed in thymic epithelial cells elevates the threshold for infection-associated thymic involution though suppression of the IFNα receptor [94]. It is also becoming clear that miRNAs can be found in extracellular compartments. A study which analyzed the patients with IgA nephropathy-inflammation of the glomeruli in the kidney associated with deposition of IgA in the kidney found increased levels of intrarenal and urinary miR-155 and miR-146a [95], and another study detected miRNA expression in human peripheral blood microvesicles from multiple sclerosis patients [96]. This finding suggests that miRNA expression levels in different body fluids might serve as an important diagnostic purpose.

Conclusions and perspectives

As described in this review, recent studies indicate that autoimmune inflammation is temporally and spatially regulated by miRNAs through affecting distinct cell types and signaling pathways in the inflammatory process. These include the activation of antigen-presenting cells (for example, miR-155 [39], miR-146a [11], and miR-124 [93]), antigen receptor signaling (miR-181a [51]), cellular differentiation (miR-155 [97]) and miR-326 [98]), cytokine production (miR-29 [57], miR-125b [44] and let-7 [45]), and signaling events downstream of cytokine receptors (miR-23b [86] and miR-146a [11]). Accordingly, therapeutic approaches can be developed to target individual miRNA to block a specific stage in these processes or to modulate multiple miRNAs simultaneously in an effort to achieve a more potent effect on tissue inflammation.

Autoimmunity is generally defined as the failure of an organism to recognize its own constituent parts as "self", which allows an immune response against its own cells and tissues. It is a stubborn and expanding problem for the biomedical community and a serious burden for society. Thus, improved approaches to conquer these debilitating conditions are definitely needed. Targeting miRNAs to treat human diseases has shown some promise in not only preclinical models of various diseases but also some human clinical trials, such as inhibition of miR-122 emerging as an efficient treatment for hepatitis C virus infections [99]. Although miRNA therapy shares many limitations of siRNA therapy, such as delivery limitations, instability in vivo, and potential non-specific targeting; and in most cases, the repression of protein expression by miRNAs is mild; as we continue to explore the functions of the distinct miRNAs that are involved in various pathological tissue inflammation, such as miR-155,

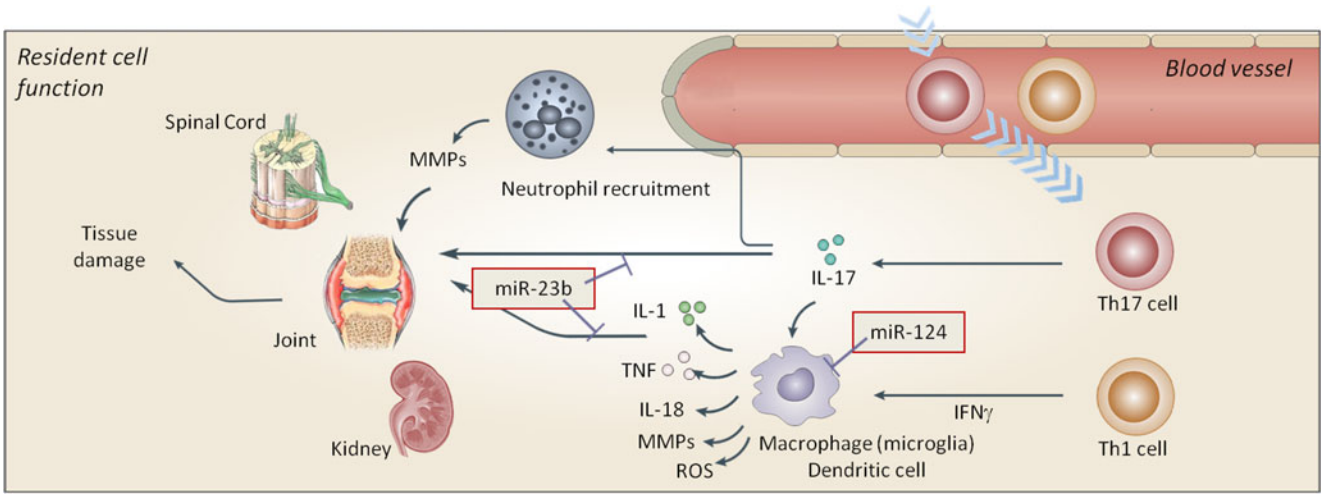

Fig. 4 MiRNAs regulate autoimmunity through local resident cells. The roles of two miRNAs that have been reported to regulate the function of resident cells during autoimmune pathogenesis. MiR-23b is downregulated in inflamed tissues of human autoimmue patients (RA or SLE) and relevant mouse models (EAE CIA or MRL/lpr) and the increased IL-17 is responsible for miR-23 downregulation in local resident cells. MiR-23b targets the signaling mediators (TAB3, IKKα, TAB2) in inflammatory cytokines (TNFα, IL-1β, and IL-17) signaling and consequently suppresses inflammatory pathogenesis in the mouse model of EAE, CIA, and spontaneous lupus. MiR-124 is expressed in microglia but not in peripheral monocytes or macrophages. When overexpressed in macrophages, miR-124 directly targeted C/EBP-α and inhibited its expression as well as the expression of its downstream target PU.1, resulting in transformation of these cells from an activated phenotype into a quiescent one resembling resting microglia. During EAE, miR-124 was downregulated in activated microglia. Peripheral administration of miR-124 in EAE mice caused marked suppression of the disease. *MMP* matrix metalloproteinases

miR-146a, and miR-23b; and potentiate new technologies for delivering miRNA mimics or inhibitors to specific cell types in vivo; there would be a possibility to exploitate miRNAs as therapeutic entities for treating human inflammatory autoimmune diseases.

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