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Review Series

Understanding the cell-intrinsic cues that permit self-reactivity in lymphocytes, and therefore autoimmunity, requires an understanding of the transcriptional and posttranscriptional regulation of gene expression in these cells. In this Review, we address seminal and recent research on microRNA (miRNA) regulation of central and peripheral tolerance. Human and mouse studies demonstrate that the PI3K pathway is a critical point of miRNA regulation of immune cell development and function that affects the development of autoimmunity. We also discuss how miRNA expression profiling in human autoimmune diseases has inspired mechanistic studies of miRNA function in the pathogenesis of multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, and asthma.

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MicroRNA regulation of lymphocyte tolerance and autoimmunity

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Understanding the cell-intrinsic cues that permit self-reactivity in lymphocytes, and therefore autoimmunity, requires an understanding of the transcriptional and posttranscriptional regulation of gene expression in these cells. In this Review, we address seminal and recent research on microRNA (miRNA) regulation of central and peripheral tolerance. Human and mouse studies demonstrate that the PI3K pathway is a critical point of miRNA regulation of immune cell development and function that affects the development of autoimmunity. We also discuss how miRNA expression profiling in human autoimmune diseases has inspired mechanistic studies of miRNA function in the pathogenesis of multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, and asthma.

Introduction

The immune system imposes extensive regulation on lymphocyte development to prevent the survival and activation of autoreactive lymphocytes. Dysregulation of cell fate checkpoints in developing and mature lymphocytes can result in autoimmunity. Developing thymocytes go through several checkpoints that depend on the strength of signaling received through the T cell receptor (TCR) (1). Similarly, the strength of B cell receptor (BCR) signaling determines whether developing B cells will survive and mature (2). In the periphery, prevention of autoreactive B and T cell responses continues to depend on properly tuned signaling pathways, cell death and survival factors, and transcriptional and epigenetic regulation of effector cell differentiation. In addition, Treg homeostasis and function are critical to restrain the activity of mature B cells and effector T cells such as Th1, Th2, or Th17 cells (3, 4). All of these tolerance mechanisms depend on tunable responses that are sensitive to minor perturbations in the expression of cascades of proteins. In particular, antigen receptor signaling can be quantitatively manipulated by minor changes in the expression of limiting regulators of downstream signaling pathways such as the PI3K and NF-κB pathways. This type of manipulation can be carried out by multiple epigenetic mechanisms, including regulation by microRNAs (miRNAs).

miRNAs are short, noncoding RNA molecules that are transcribed alone or in polycistronic clusters in the genome and sometimes appear within the introns or exons of coding genes (5). Their primary transcripts are sequentially processed by DROSHA/DGCR8 and Dicer to produce mature miRNAs that are loaded into the miRNA-induced silencing complex (miRISC) (6). The miRNA guides the miRISC to target mRNAs by complementary base pairing, usually in 3′ UTRs, resulting in translational repression and/or mRNA degradation (7). miRNAs that share a similar seed sequence (nucleotides 2-8 of the mature miRNA) are defined as a family and have substantial overlap in their mRNA targets. miRNAs regulate networks of target genes. Each miRNA can target hundreds of distinct mRNAs, and most mRNA transcripts are predicted targets of multiple miRNAs (7).

Although miRNA regulation of each target results in small changes in gene expression, the network activity of miRNAs targeting hundreds of genes simultaneously can effect dramatic changes in cell behavior. These changes can be easily observed in the immune system, where miRNAs modulate many cell fate decisions made by developing and mature lymphocytes (8–10). In this Review we discuss seminal work on miRNA regulation of lymphocyte development and function, which affects the prevention of autoimmunity. In addition, we highlight mechanistic studies that were guided by miRNA expression profiling in autoimmune diseases including multiple sclerosis (MS), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). Several other Reviews have more comprehensively discussed miRNA expression and function in particular autoimmune diseases (11–13). Here we focus on the principles of miRNA regulation of lymphocyte biology related to the establishment and maintenance of self-tolerance, and how that can inform future research in autoimmunity.

miRNA regulation of central tolerance

miRNAs are important nodes in the gene expression networks that govern lymphocyte development and the establishment of central tolerance. These processes operate through cell fate checkpoints that promote the maturation of cells that correctly recombine antigen receptor genes while eliminating those that form strongly self-reactive receptors by apoptosis. Because these checkpoints rely on proper cellular interpretation of antigen signal strength, dysregulated TCR or BCR signaling can increase the survival of autoreactive lymphocytes and contribute to the development of autoimmune disease. miRNAs that regulate cell survival, antigen receptor signaling, and the availability of self-antigens during lymphocyte development all play important roles in the development and selection of a repertoire of B and T lymphocytes bearing useful and safe antigen receptors.

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Early research shows that the miRNA biogenesis pathway is critical for early B cell development, as ablation of *Dicer* leads to an almost complete block at the pro- to pre-B cell transition (14). A key function of pro-B cells is V(D)J recombination of BCR genes to generate a functional antigen receptor. *Dicer* deficiency does not alter the basic mechanism of V(D)J recombination, but it does alter the resulting BCR repertoire, suggesting that miRNAs play an important role in regulating the survival of potentially self-reactive B cells. Further analysis identified BIM (encoded by *Bcl2l11*) as a key miRNA target involved in the regulation of pro-B cell survival (14).

The work demonstrating the importance of the miRNA biogenesis pathway to B cell development was accompanied by further research that detailed the major role of miRNA-17~92 (miR-17~92) (15). The miR-17~92 cluster is composed of six miRNAs belonging to four families: miR-17, miR-18, miR-19, and miR-92. Additional members of these miRNA families are expressed from two related genomic clusters. The miR-17~92 cluster promotes the survival of developing B lymphocytes at least in part through its ability to regulate PI3K signaling and genes expressed downstream of this pathway (Figure 1A). BIM is induced by PI3K signaling and is directly targeted by several miR-17~92 cluster miRNAs (14, 15). The tumor suppressor PTEN, a potent negative regulator of the PI3K pathway, is also targeted by miR-17~92 miRNAs. When miR-17~92 is deleted from developing B cells, PTEN and BIM expression are elevated and cells undergo apoptosis, resulting in B cell deficiency. Conversely, miR-17~92 overexpression during B or T cell development leads to increased survival and promotes lymphoproliferative disease and autoimmunity (16).

In early stages of T cell development in the thymus, signaling through the newly formed TCR provides important survival signals. However, strong TCR signals in the thymus indicate autoreactivity and induce apoptosis. Therefore, miRNAs that alter the strength of TCR signaling can increase the survival of cells that normally would have been deleted from the repertoire. miR-181 acts as a "rheostat" of TCR signal strength by targeting several phosphatases (Figure 1B), including the phosphatases PTPN22, SHP-2, DUSP5, and DUSP6 (17), as well as PTEN (18). Early in development, double-negative (DN) thymocytes express high levels of miR-181, conferring high sensitivity to pre-TCR and TCR signals. miR-181 expression declines as thymocytes mature to the double-positive (DP) and single-positive (SP) stages, decreasing sensitivity to TCR ligation during positive and negative selection. Altering miR-181 expression dysregulates TCR signaling, and thymocytes that would normally be deleted from the repertoire survive instead (19). These findings suggested that miR-181 might be capable of regulating the onset and/or progression of T cell–mediated autoimmune disease. Indeed, thymic miR-181 expression is important for the elimination of self-reactive thymocytes responding to an endogenous positively selecting self ligand (19).

miRNAs also affect central tolerance through their activity in thymic antigen-presenting cells. Medullary thymic epithelial cells (mTECs) promiscuously express tissue-specific self-antigens and present them to developing thymocytes, eliminating cells bearing TCRs reactive against self-antigens. miRNA biogenesis in TECs is critical to the proper function of the thymus. *Dicer* (20, 21) or *Dgcr8* (22) deletion specifically in TECs dramatically disrupts thymic architecture with increased TEC apoptosis and severely reduced thymic cellularity, particularly in the mature mTEC population (21, 22). *Dicer* deletion in mTECs also leads to changes in promiscuous gene expression (21). In an inflammatory setting, miRNA-deficient mTECs do not prevent the maturation of self-reactive autoimmune uveitis–inducing interphotoreceptor retinoid-binding protein-specific T cells (22).

In addition, thymi with miRNA-deficient TECs undergo premature involution in response to IFN-α (20). Deletion of the miR-29a cluster, consisting of miR-29a and miR–29b-1, partially replicates the defects seen in Dicer deficiency. miR-29a is highly expressed in mTECs and targets *Ifnar1*, the mRNA that encodes the IFN-α receptor. Deletion of miR-29a increases IFNAR1 expression and downstream STAT1 phosphorylation, indicating that miR-29a regulates the strength of signaling through the IFN-α response pathway in mTECs (20).

These studies demonstrate that miRNAs are essential regulators of lymphocyte development and selection. They regulate cell survival and critical signaling pathways in multiple key cell types, tuning response thresholds and developmental checkpoints. Further research will undoubtedly uncover additional miRNAs and target networks that contribute to the robust imposition of central tolerance.

miRNA regulation of peripheral tolerance and lymphocyte function

Mature autoreactive lymphocytes that have escaped central tolerance can become effector cells that drive autoimmune disease. Peripheral tolerance mechanisms restrict autoantibody production by B cells and tissue inflammation and destruction caused by T cells. These mechanisms are subject to miRNA regulation, and altering miRNAs that regulate peripheral lymphocyte fate decisions can lead to autoimmunity. Understanding how miRNAs regulate B and T cell activation, survival, proliferation, differentiation, and effector functions can help to identify pathways that are dysregulated in autoimmune disease or that could be targeted to prevent or dampen autoimmune pathology.

Immature B cells leave the BM to complete their development and selection in the periphery. As in the BM, the fate of immature B cells depends on BCR signals, and dysregulation of BCR signal strength can skew the B cell repertoire and allow maturation of autoreactive cells (23). Deletional tolerance and receptor editing eliminate BCRs that react strongly to peripheral self-antigens. Moderate BCR signals promote the generation of marginal zone (MZ) or transitional B cells, while weaker BCR signals promote the generation of follicular (FO) B cells (24). Ablation of *Dicer* late in B cell development leads to a marked decrease in the number of FO B cells but relatively normal numbers of MZ and transitional B cells (25). miR-185 and several other miRNAs are more abundant in FO B cells compared with MZ or transitional B cells. Overexpression of miR-185 in FO B cells decreases expression of its target BTK, a kinase that transduces signals downstream of the BCR (25). In addition to effects on B cell fate, *Dicer* ablation in peripheral B cells also alters the BCR repertoire through increased class-switch recombination and usage of certain J_H elements and positively charged amino acids in the CDR3 implicated in autoreactivity. Female mice lacking *Dicer* in mature B cells spontaneously develop

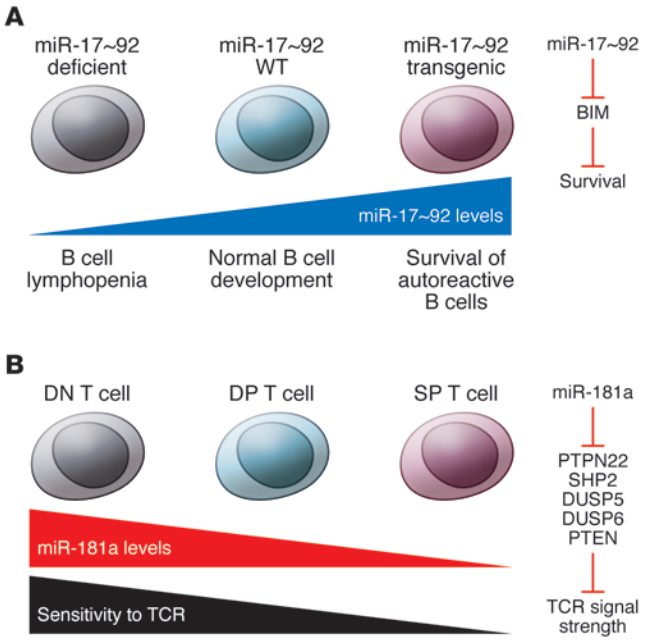

Figure 1. miRNAs set thresholds for lymphocyte development. (**A**) miR-17~92 sets a threshold for B cell survival during development. miR-17~92 targets several genes in the PI3K pathway, including the proapoptotic molecule BIM. Mice deficient in miR-17~92 in the B cell lineage develop B cell lymphopenia. Mice with overexpression of miR-17~92 in the B cell lineage develop self-reactive B cells. (**B**) miR-181 sets a threshold for TCR signal strength during T cell development in the thymus. miR-181 expression decreases as thymocytes differentiate from DN to DP to SP thymocytes. miR-181 expression confers greater sensitivity to TCR stimulation by modulating the targets PTPN22, SHP2, DUSP5, DUSP6, and PTEN.

serum autoantibodies and deposition of immune complexes in the kidneys (25). Thus, miRNA regulation of BCR signal strength is necessary to prevent maturation of a self-reactive B cell repertoire and the onset of autoimmunity.

Recent work in leukemia revealed miRNA regulation of PI3K signaling downstream of BCR stimulation relevant to autoimmune disease. miR-150 expression is inversely correlated with severity of chronic lymphocytic leukemia (CLL) (26). miR-150 targets *Gab1*, which recruits PI3K to the plasma membrane upon BCR stimulation, and *Foxp1*, a negative feedback regulator of PI3K signaling. miR-34a also targets *Foxp1,* suppresses B cell development, and is a candidate gene in the Idd9.3 locus that protects mice from type 1 diabetes (T1D) (27, 28).

PTEN has proven to be a multipotent miRNA target, having effects in many different immune cell types (18, 29–32). A subset of mature B cells implicated in CLL progression expresses increased miR-22, which targets PTEN, leading to increased PI3K activity and proliferation (33). *Pten*$^{+/-}$ mice develop splenomegaly and lymphadenopathy, have increased serum IgG and anti-ssDNA antibodies, and develop lung and kidney inflammation (34). Interestingly, PTEN expression is decreased in SLE patient B cells, and this correlates with increased expression of miR-22 and two other miRNAs, miR-7, and miR-21, that also target PTEN (35).

While B cells mature in the spleen and lymph node into antibody-secreting cells, T cells differentiate into several lineages of effector cells such as CD4$^+$ T helper cells (Th1, Th2, Th17) and cytotoxic CD8$^+$ T lymphocytes. Genetic factors as well as miRNAs are involved in the differentiation of these lineages (8). Recent work has focused particularly on the Th17 lineage, thought to be involved in the pathogenesis of MS and other autoimmune diseases (9, 10, 36). Mice lacking miR-155 in T cells do not develop severe experimental autoimmune encephalomyelitis (EAE) and have a marked decrease in the production of IL-17A, a critical cytokine for EAE pathogenesis (37). miR-155–deficient mice are also resistant to collagen-induced arthritis and have impaired Th17 differentiation (38). More recently, the mechanism behind miR-155 induction of Th17 differentiation was more clearly defined by its targeting of *Jarid2*, a component of the polycomb complex that mediates gene repression (39). While miR-155 encourages Th17 differentiation, miR-210 restrains the production of IL-17 by Th17 cells and contributes to autoimmune pathogenesis in a mouse model of inflammatory bowel disease (40). miR-210 also restricts autoantibody development in aged mice, and overexpression of miR-210 decreases class switch recombination, suggesting that miR-210 limits autoimmune responses in both B and T cells (41).

The miR-17~92 cluster is also involved in Th17 differentiation and function by targeting *Pten* and *Ikzf4*. Deletion of the miR-17~92 cluster decreases Th17 differentiation and reduces EAE pathogenesis (42). However, the miR-17 family member miR-20b suppresses IL-17 production in Th17-polarizing cultures by targeting *Rorc* and *Stat3* (43). Overexpression of miR-17~92 in T cells induces anti-dsDNA autoantibodies, multiorgan inflammation, splenomegaly, and lymphadenopathy (32). This may be related to miR-17~92 promotion of FO Th (Tfh) cell differentiation, mediated in part by targeting of *Pten* and the PKB phosphatase *Phlpp2* (30, 32).

miRNA regulation of Treg function

miRNAs are necessary to maintain peripheral tolerance through their essential roles in Treg homeostasis and function. Genetic ablation of mouse *Dicer* or *Drosha* in FOXP3-expressing cells leads to fatal systemic autoimmune disease. Despite normal thymic Treg development in these mice, miRNA-deficient Tregs exhibit diminished homeostatic potential and are unable to adequately perform their suppressive functions (44–46). Some of these defects can now be at least partly attributed to specific miRNAs expressed in Tregs.

miR-155 is highly expressed in Tregs in a FOXP3-dependent manner, and miR-155 deficiency impairs Treg development and homeostasis (47, 48). Tregs lacking miR-155 have increased expres-

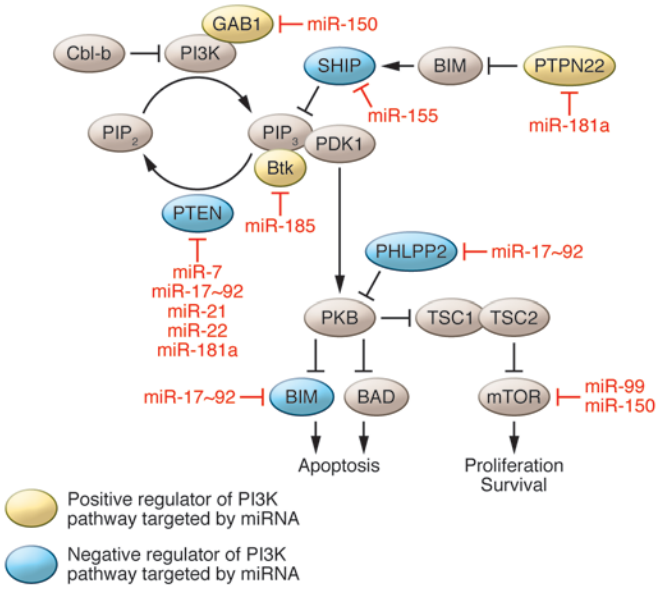

Figure 2. miRNA regulation of the PI3K pathway. PI3K inhibitors are currently in clinical trials for acute and advanced leukemia (ClinicalTrials.gov identifiers NCT01756118 and NCT01396499), B and T cell lymphoma (NCT02049541, NCT02017613, NCT01693614, NCT01282424, NCT01796470, NCT02367040, NCT01306643, NCT01719250, NCT01088048, NCT02258555, NCT01300026, and NCT01705847), asthma (NCT01653756), and allergic rhinitis (NCT01066611 and NCT00836914), and preclinical data support their therapeutic potential in autoimmune diseases, as well (95). The immune system is highly sensitive to manipulation of PI3K signaling, and this pathway is subject to several layers of regulation that permit fine-tuning of signal output. Just a two-fold change in PI3K signal activity through Akt is sufficient to alter lymphocyte homeostasis and induce autoimmunity in mice (34). This degree of fine-tuning falls well within the range of regulation mediated by miRNAs. The PI3K pathway is strictly regulated by the phosphatases PTEN and SHIP, which dephosphorylate PI3K products and/or limit substrate availability. PTEN is a prominent target of many miRNAs (miR-17~92 and miR-181 family members) in many different immune cell types (Th1, Th2, Th17, Tfh, CD8 T cells, and B cells). SHIP is a prominent target of miR-155 and appears to play an important role in its proinflammatory functions in several cell lineages. miRNAs also modulate PI3K signaling by targeting downstream signaling mediators and inhibitors. Understanding which miRNAs are expressed in specific cell types, as well as understanding the varying limiting roles played by their targets, will lead to a more comprehensive understanding of miRNA regulation of cell fates and behaviors.

sion of the miR-155 target suppressor of cytokine signaling 1 (*Socs1*) and reduced responses to IL-2, a critical regulator of Treg homeostasis. However, miR-155 may be dispensable for Treg suppressive functions, since miR-155–deficient Tregs are capable of preventing autoimmune disease in mice (47, 48).

The miR-17~92 cluster limits induced Treg (iTreg) differentiation in vitro through the combined activity of miR-17 and miR-19 (49). Although miR-17~92 is dispensable for thymic Treg development and homeostasis in vivo, it is required to support IL-10 production by antigen-specific Tregs in EAE (50).

miR-10a is expressed in Tregs but not in conventional effector T cells (51). This expression is reinforced by retinoic acid signaling and correlates inversely with autoimmune disease susceptibility in mouse strains, with highly susceptible mice having the lowest expression of miR-10a and resistant mice having the highest (52). miR-10a stabilizes the Treg gene expression program through repression of non-Treg genes such as *Bcl6* and *Ncor2* (51, 52). However, genetic ablation of miR-10a does not induce Treg defects or autoimmunity (52), suggesting redundancy with other miRNAs such as miR-10b or perhaps miRNAs of the related miR-99/100 family. Like miR-10a, miR-99a expression is induced in Tregs by retinoic acid, and its overexpression promotes iTreg differentiation in vitro (53). Surprisingly, miR-99a cooperates with miR-150 through an as yet unknown mechanism to repress mTOR.

The studies described above have provided insight into the roles of specific miRNAs in Treg functions, but the question of how miRNAs affect T cell signaling pathways in Tregs remains unclear. The PI3K pathway in particular is influenced by miRNA regulation (Figure 2) and is a central node in regulating the differentiation of a $CD4^+$ T cell into either an effector Th cell or a Treg. Dicer ablation in $CD4^+$ T cells increases mTOR activity and sensitivity to TCR stimulation (54). mTOR is activated by the PI3K pathway and is a key integrator of signals from antigen receptors, cytokine receptors, and TLRs (55). Increased PI3K signaling in mice leads to an expansion of non-Treg effector $CD4^+$ T cells (56). In fact, increased PI3K signaling blocks FOXP3 expression in Tregs (57), and key negative regulators of the PI3K pathway, such as Cbl-b (58) and PTEN (59), are essential to proper FOXP3 expression (60). Premature termination of PI3K signaling confers FOXP3 expression and Treg behavior (59), indicating that manipulation of the time period of PI3K signaling can lead to alterations in the balance between Tregs and effector T cells. Mice deficient for the E3 ubiquitin ligase Cbl-b develop spontaneous T cell activation and are predisposed to autoimmune disease (61). Cbl-b is induced by NF-κB in response to TCR stimulation, and it negatively regulates not only the PI3K pathway but also the NF-κB and MAPK pathways (58). Since negative regulation of these pathways promotes Treg gene expression programs, future research will likely uncover additional miRNAs that affect Treg generation and function by regulating PI3K, NF-κB, or MAPK signaling.

While miRNAs generally function in a cell-autonomous manner by posttranscriptional regulation of gene expression programs, recent work has highlighted a non–cell-autonomous role of miRNAs. Many cells, including T cells and other immune cells, release miRNAs into the extracellular space within exosomes. These vesicles can transfer miRNAs into recipient cells, enabling RNA-mediated intercellular communication. Recent evidence suggests that this process may operate as a mechanism of immune suppression by Tregs and other immune suppressive lymphocytes (62, 63). Exosomes released from Tregs in culture can transfer suppressive miRNAs into recipient Th1 cells (62). Tregs lacking both Rab27a and Rab27b are unable to secrete exosomes and also fail to suppress effector T cell responses in in vitro coculture and in vivo colitis experiments. Further work is needed to clarify the direct role of transferred miRNAs in enforcing immune tolerance and to determine whether other Treg suppressive functions remain intact in Rab27-deficient Tregs. Extracellular miRNAs released from CD8 T cells also suppressed T cell responses in a mouse model of high-dose antigen tolerization to contact sensitivity (63).

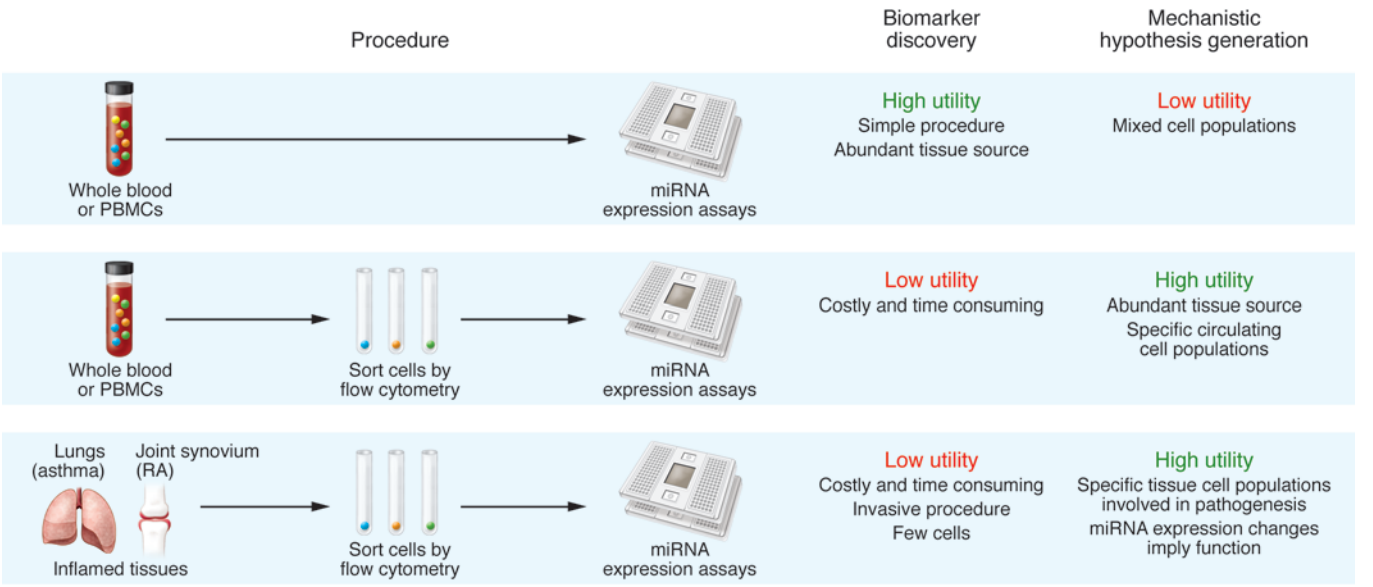

Figure 3. miRNAs as biomarkers or indicators of pathogenic processes. miRNA expression analysis can be a useful tool to identify biomarkers of disease or to generate mechanistic hypotheses about disease pathogenesis. Useful biomarkers are identified from abundant biospecimens like blood with little sample processing before miRNA expression analysis. To generate mechanistic hypotheses, more sample processing is necessary to determine the cell type specificity of miRNA expression changes. Even stronger support for the role of a particular miRNA in disease pathogenesis can be obtained using tissue-infiltrating immune cells at the sites of autoimmune or allergic inflammation. The invasiveness of these procedures limits their utility for biomarker identification.

miRNAs as biomarkers and active players in autoimmunity

The emergence of miRNAs as useful diagnostic and prognostic biomarkers in cancer has inspired extensive efforts to profile miRNA expression in human autoimmune disease (64). Depending on how these studies are conducted, they also have the potential to provide insight into disease pathogenesis and the roles of specific miRNAs (Figure 3). As described in the previous sections, most of our current understanding of miRNA function in immunity was established by combining mouse genetics with cell culture methods and disease models. The conservation of many (but not all) miRNAs and their target networks make these approaches productive and valuable. However, it is also important to study miRNAs in the context of human disease, and blended approaches have been successful at connecting disease-associated miRNA expression patterns with clear disease-associated functions.

Many studies have determined miRNA expression in human diseases using blood, an abundant and noninvasive biospecimen that consists of mixed populations of hematopoietic cells. This biospecimen is particularly useful for systemic autoimmune diseases such as SLE, in which multiple organs are affected. One study found that miR-155 expression is decreased in peripheral blood mononuclear cells (PBMCs) of juvenile patients with SLE (65). Transfection of miR-155 into PBMCs from juvenile patients with SLE rescued IL-2 secretion, suggesting a functional role of miR-155. However, which cell type requires miR-155 for IL-2 secretion remains unclear. miR-146a is also downregulated in PBMCs from SLE patients, and its expression is inversely correlated with disease activity (66). Overexpression of miR-146a by transfection of an expression vector in PBMCs from SLE patients reduced the excessive production of IFN-α and IFN-β. A recent meta-analysis of genome-wide association studies (GWAS) found miRNA polymorphisms that confer risk for SLE (miR-146a rs57095329) and RA (miR-499 rs3746444) (67). The polymorphism associated with SLE risk at the promoter for miR-146a decreased the binding of the transcription factor ETS1 to the promoter, thus reducing miR-146a expression (68).

miRNA expression profiling in PBMCs (69, 70), whole blood (71, 72), and serum (73) revealed miRNAs that may be involved in MS. miR-326 is highly expressed in PBMCs from patients with relapsing MS compared with patients with remitting MS or healthy subjects (74). miR-326 expression correlates with disease severity and IL-17 production, suggesting a role for miR-326 in Th17 cell function. In mice, overexpression of miR-326 during EAE leads to increased Th17 cell differentiation. Interestingly, miR-326 was also found to be upregulated in PBMCs from autoantibody-positive T1D patients (75), suggesting a more general role for miR-326 in autoimmunity.

Differential expression of a miRNA in complex specimens such as whole blood may reflect changes in cellular composition as well as changes in miRNA expression within one or more cell types (Figure 3). Importantly, the high expression of miR-326 in MS was limited to the $CD4^+$ T cell compartment and especially in CCR6-expressing Th17 cells (74). Identifying the cell type harboring high miR-326 expression as the Th17 cells that are involved in the autoimmune process provided stronger evidence for miRNA function in MS pathogenesis. In another study, circulating $CD4^+$ T cells from MS patients expressed more miR–17-5p and lower levels of miR-17 targets in the PI3K pathway (76).

Several studies have identified miRNA expression changes in specific cell types from blood in human disease. miRNA expression analysis of circulating $CD4^+$ T cells from SLE patients revealed

elevated miR-21 and miR-148a expression, which contribute to DNA hypomethylation by targeting the cytosine methyltransferase x*Dnmt1* and *Rasgrp1*, which mediate the RAS/MAPK pathway upstream of DNMT1 (77). miR-223 is upregulated in circulating T cells from RA patients (78, 79). miR-223 expression is highest in naive RA $CD4^+$ T cells but nearly undetectable in non-naive RA $CD4^+$ T cells and in naive $CD4^+$ T cells from healthy subjects (78). miR-223 targets *Igf1r*, resulting in decreased IL-10 secretion in response to IGF-1 stimulation (79). Another study found upregulation of miR-143 and miR-223 in $CD4^+$ T cells as well as upregulation of miR-10a and miR-345 in CD19 B cells in patients with SLE (80).

Treg dysfunction plays a critical role in autoimmune disease pathogenesis, and a better understanding of the cellular processes leading to their dysfunction will aid the development of Treg-based therapies. In MS, miRNA expression profiling of circulating Tregs revealed differential expression of some members of the miR-106b~25 cluster (81). In RA, miRNA expression profiling of circulating naive and memory Tregs and effector T cells defined a miRNA signature of naive and memory Tregs (82). This paper did a very careful analysis of specific cell types and left many open questions about the role of these miRNAs in Treg function (82). In patients with T1D, miR-510 is upregulated and miR-342 is downregulated in circulating Tregs compared with effector T cells (83). miR-146a is upregulated in Tregs in both T1D patients and healthy subjects, suggesting that miR-146a has a role in Tregs in both healthy and disease states.

Isolating cells from the inflammatory setting, such as the lungs in asthma or the joint synovium in RA, provides even more direct evidence connecting miRNA expression changes to their role in disease pathogenesis. Obtaining specific cell types involved in disease pathogenesis often involves invasive procedures and complex downstream cell separation protocols. The ability to profile miRNA expression in very small cell numbers is often essential. For example, in our own work we profiled miRNA expression in airway-infiltrating T cells sorted by flow cytometry from research bronchoscopy specimens in observational clinical studies of asthma (31, 84). miR-19a is significantly upregulated ($P = 0.0199$) in $CD4^+$ T cells from asthmatic airways compared with $CD4^+$ T cells from healthy airways. Mouse miR-17~92 conditional-KO $CD4^+$ T cells produce significantly less ($P < 0.05$) Th2 cytokines compared with WT cells, and this defect was rescued when miR-19a was added back to the cells. In a mouse model of ovalbumin-induced airway inflammation, $CD4^+$ T cells lacking the miR-17~92 cluster do not induce eosinophilia or mucus secretion in the airway. This suggests that blocking miR-19a might ameliorate the pathogenic inflammation observed in asthma (31).

Very few studies have looked for functionally relevant miRNA expression differences in immune cells in sites of autoimmune inflammation. miRNA expression profiling of $CD4^+$ T cells in the synovial fluid of a small number of RA patients revealed upregulation of miR-146a (85). The upregulation of miR-146a may contribute to the resistance of RA synovial fluid $CD4^+$ T cells to apoptosis, at least in part through targeting Fas-associated factor 1 z(*Faf1*). Overexpression of miR-146a in mice induces symptoms similar to human autoimmune lymphoproliferative syndrome (ALPS) (86). In fact, the upregulation of miR-146a has been reproduced in several studies and highlights the importance of this miRNA in autoimmune disease (87). miRNA expression changes in structural cells such as synoviocytes in RA and lung epithelial cells in asthma can also contribute to disease pathogenesis, but these studies do not contribute to our understanding of lymphocyte involvement in autoimmunity (88–90).

Additional studies are needed to understand how miRNAs function in pathogenic lymphocytes in these sites of inflammation. Such studies would advance our understanding of gene regulation in the immune system and may also point the way to novel therapies for autoimmune diseases. miRNAs are viable drug targets, and sequence-specific miRNA inhibitors are both easy to design and proven to work in patients (91). Although it has so far been challenging to deliver these molecules to immune cells, some preclinical studies have indicated that local or systemic miRNA inhibitor administration may be a viable approach to treating autoimmune and allergic diseases (92–94).

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