The Function of MicroRNAs in Autoimmune Diseases

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MicroRNAs (miRNAs) are a family of non-coding single-stranded RNAs with a length of around 18-25 nucleotides that play a critical role in the control of gene expression, cell proliferation, differentiation, and apoptosis, as well as biological growth and development and illness. Autoimmune diseases are a group of disorders characterized by tissue and organ damage and resulting dysfunction as a result of autoimmune responses. Numerous studies have discovered that aberrant expression of certain miRNAs may serve as biomarkers for early detection of these diseases or as therapeutic targets. The biological properties of miRNAs and their significance in autoimmune illnesses are discussed in this review.

Keywords: microRNAs; Autoimmune Diseases; Mechanisms; Diagnosis; Therapeutics

Introduction
miRNAs (miRNAs) are non-coding short RNAs found inside cells that are around 18-25 nucleotides in length. They control over 90% of protein-coding genes and are critical for the formation and maintenance of immunological homeostasis (1). The synthesis of miRNAs has been described in detail. In humans, miRNAs are encoded via coding genes’ introns and non-coding genes’ introns and exons. Their transcription and maturation occur in the nucleus, and they are subsequently regulated in the cytoplasm by proteins and enzymes (2). The genes for the majority of miRNAs are transcribed by RNA polymerase II into primary transcripts with lengths ranging from several hundred to several thousand nucleotides. These primary transcripts are then converted into pri-miRNAs (3). Pri-miRNAs feature a cap at the 5’ end and a polyadenylation tail hairpin structure at the 3’ end. Then, using the nuclear transport protein exportin-5, pri-miRNAs are identified by Drosha and DGCRI8 (DGGeorge Syndrome critical region gene 8) in RNase III and cleaved into pre-miRNAs with a length of around 70 nucleotides (4). Another RNase III Dicer cleaves the pre-miRNAs in the cytoplasm into double-stranded RNAs of around 22 nucleotides in length, and the leading strands connect with argonaute (Ago) to form the RNA-induced silencing complex (RISC) (5). miRNAs in their mature form can affect gene expression in two ways: block translation and initiate mRNA degradation. That is, RISC encourages mature miRNAs to bind to the 3’ untranslated region (3’ UTR) of target mRNAs via perfect or imperfect complementary pairing, hence inhibiting mRNA translation or destruction (6).

The Role of microRNAs in the Regulation of Immune Function
miRNAs have been implicated in the pathophysiology of a number of autoimmune illnesses, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren’s syndrome (SS), systemic sclerosis (SSc), and multiple sclerosis (MS).

Not only do miRNAs play a critical role in immune sys-
stem development, but they also play a role in the regulation of innate and adaptive immunological activities. Reduced expression of miR-17-5p, miR-20a, and miR-106a during monocyte production increased the expression of their target gene transcription factor AML-1 (acute myeloid leukemia-1) and boosted macrophage colony-stimulating factor receptor expression (M-CSFR) (7). Furthermore, miR-424 stimulates monocyte differentiation by inhibiting nuclear factor I A translation, consequently increasing M-CSFR expression (8).

Simultaneously, miR-223 regulated the production of granulocytes. Because aberrant activation of Toll-like receptors (TLRs) can result in an inflammatory response (9), the TLRs family may play a significant role in the onset and progression of autoimmune disorders. The inflammatory reaction induced by lipopolysaccharide (LPS) can result in increased production of miR-146a/b, miR-155, and miR-132; while miR-147 plays a negative regulatory function in the inflammatory response induced by TLR (10). miR-21 inhibits LPS-induced TLR4 signaling by targeting the tumor suppressor programmed cell death (PDCD) 4, ultimately inhibiting nuclear factor-xB (NF-xB) activation and IL-10 production (11).

T and B lymphocytes are the primary adaptive immune cells. MiR-17-92, miR-155, miR-181a, and miR-326 are all miRNAs associated with adaptive immunity. MiR-17-92 expression was increased in pre-T and B cells but decreased in mature T and B cells, and increased miR-17-92 expression in progenitor lymphocytes encouraged lymphocyte proliferation (12). After cells are stimulated, their expression is increased, and it regulates several genes to maintain lymphocyte homeostasis and proper immunological function. In T cells, miR-181a controls the inhibitory factor phosphatase SHP-2, PTPN-22, and DUSP5/6 of the TCR signaling pathway. It is involved in both negative and positive selection throughout development (13).

To preserve immunological homeostasis and tolerance to self-antigens, regulatory T cells (Tregs) suppress the activity of reactive T cells. miRNAs have been shown to be critical in the formation and function of Tregs. Foxp3 affects Treg formation and activity by modulating miR-155, which in turn modulates the IL-2 and Signal transduction and activation of trion (TAT) 5 signaling pathways by direct interaction with suppressor of cytokine signaling (SOCS) 1 (14). The IL-2/STAT5 pathway is critical for Treg homeostasis and function. MiR-155 knockout mice had fewer Tregs in the thymus and peripheral lymphoid tissue, although miR-155 knockout animals retained an inhibitory function (15). Simultaneously, miR-155 can alter CD+4 T cell sensitivity and boost the inhibitory function of Tregs (16). Not just lymphocytes, but also some cytokines, such as IL-17, are regulated by miRNAs. IL-17 is a Th17-secreted cytokine that can drive local tissue cells to create chemokines, attract neutrophils, and contribute to the development of autoimmune disorders (17). miR-326 and miR-155 promote the differentiation of Th17 cells and the production of IL-17 (18).

The Relationship between miRNAs and Autoimmune Diseases

miRNAs are crucial regulators of immune responses and autoimmunity, miRNAs have been implicated in the onset and progression of a range of autoimmune illnesses, regulating DNA methylation pathways, TLR receptors, plasma cell differentiation and antigen presentation, Treg activity, pro-inflammatory cytokines, and autoantibody synthesis. miRNAs persist in serum indefinitely, and their expression is constant across individuals of the same species. Because studies have established that miRNAs are abnormally expressed in a variety of autoimmune disorders, miRNAs are predicted to serve as biomarkers and therapeutic targets for the early detection of autoimmune diseases.

miRNAs and Systemic Lupus Erythematosus

SLE is a kind of systemic autoimmune disease that affects several organs, including the skin, joints, kidneys, lungs, and nervous system. It manifests clinically in a variety of ways and is impacted by pathogenic variables including genes, hormones, and the environment. SLE patients have distinct miRNA expression features when compared to healthy controls or patients with other illnesses, and aberrant miRNA expression is strongly associated with disease progression and affected organs.

Microarray study and miRNA sequencing methods have revealed that the expression of hundreds of miRNAs, including miR-21, miR-126, miR-142, miR-146a, miR-148, and miR-155, is changed in SLE patients. miR-21 has a role in the onset and progression of several autoimmune disorders by controlling the balance of Th1 and Th2 cells, as well as Th17 cells (19). miR-21 overexpression in the plasma, peripheral blood mononuclear cells (PBMCs), and CD+4 T cells of SLE patients inhibits the selective protein translation inhibitor PDCD4 and acts on RAS guanyl releasing protein 1 (RASGRP1), thereby downregulating DNA methyltransferase (DNMT1) and resulting in hypomethylation of CD+4 T cells (20).

Moreover, overexpression of miR-21 boosted AP-1 and IL-2 production, all of which resulted in aberrant T cell activation and increased B cell activity (21). Another study discovered that suppressing miR-21 expression in lupus-like animals alleviated splenomegaly symptoms (22). All of the aforementioned data suggests that miR-21 plays a significant role in the pathophysiology of SLE.

miRNA-126 expression was significantly reduced in the plasma of SLE patients, despite increased interferon-α (IFN-α) and IFN-stimulated gene 56 (ISG56) mRNA levels, indicating that miR-126 may block IFN-α (23). IFN-secretion and expression are both related to the incidence of SLE. By binding to the 3’-UTR of DNMT1, miR-126 suppresses its expression, resulting in hypomethylation of CD+4 T cells and activation of autoreactive T and B cells (24).

miR-146a functions as a negative regulator of TLRs and adversely controls the type I IFN pathway in innate immunity by targeting Interferon Regulatory Factor 5 (IRF-5), STAT, IRAK1, and TRAF6 (25). Reduced miR-146a expression in PBMCs of SLE patients enhances type I IFN secretion, regulates TLR and IL-1 signaling pathways, activates the NF-xB signaling pathway, and releases pro-inflammatory factors (26).

Furthermore, miR-148a was discovered to be overexpressed in CD+4 T cells from SLE patients and MRL-lpr mice, and its interaction with the DNMT1 3’ UTR led to hypomethylation of CD+4 T cells, but autoimmune-related methylation was responsive to miR-148a (27). The promoter
region of the CD70 and lymphocyte function related antigen-1 (LFA-1) genes is hypomethylated, which leads to the immunological activation of autoreactive T and B cells (28). miR-155 expression was increased in Treg cells of MRL-lpr mice but reduced in serum and urine of SLE patients, and that the increased expression of miR-155 was associated with increased PP2Ac expression and IL-2 secretion promotion (29).

Along with the miRNAs mentioned above, there are many miRNAs related with SLE. Among these, miR-17-5p, miR-112, miR-141, miR-184, miR-196a, miR-383, and miR-409-3p were down-regulated in PBMC cells of SLE patients compared to healthy controls; whereas miR-61, miR-78, miR-142-3p, miR-189, miR-198, miR-298, miR-29-3p, and miR-342 were up-regulated (30). Similarly, alterations in the miRNAs associated with type II lupus nephritis were seen in the kidney tissue of patients with the disease (31). As a result, these miRNAs may have a role in the pathogenesis of SLE and are likely to serve as diagnostic markers for the disease.

**miRNAs and Rheumatoid Arthritis**

RA is a prevalent inflammatory illness that affects approximately three times as many women as males, peaking between the ages of 40 and 60. The joint is the major organ impacted, which can result in chronic inflammation of the joint and surrounding tissues, as well as other organs of the body. Stimulation of innate immune cells and cells found in certain organs, such as synoviocyte-like fibroblasts, can result in rheumatoid arthritis and autoimmunity.

Studies indicated that miR-146a and miR-155 expression are increased in RA patients’ PBMCs (32), synovial fluid (33), synovial fibroblasts, CD+4 T cells (34), and Th17 cells (35). Pro-inflammatory substances (IL-1, TNF) and TLR ligands (poly I:C, LPS) had an effect on the production of miR-155 in synovial fibroblasts and that miR-155 was upregulated in synovial fibroblasts (36). It inhibits the specific production of matrix metallopeptidase (MMP) 1 and MMP3, as well as the activation of both by cytokines and TLRs. Thus, miR-155 may protect against synovial fibroblast-mediated tissue damage by blocking certain MMPs, hence averting the development of rheumatoid arthritis (37).

When rheumatoid arthritis arises, pro-inflammatory molecules such as TNF-α, IL-1, and TLR ligands further enhance miR-155 production in synovial fibroblasts. Additionally, aberrant miRNA-146a expression is strongly associated with the onset and progression of rheumatoid arthritis. Along with increased expression in synovial tissue and other cells, miR-146a expression was lowered in serum. According to the findings, miR-146a inhibits inflammatory immune responses via IRAK1 and TRAF6 targeting. It is worth mentioning that whereas miR-146a is overexpressed in rheumatoid arthritis, there is no substantial change in IRAK1 or TRAF6, indicating that miR-146a cannot limit the inflammatory response in rheumatoid arthritis by targeting IRAK1 or TRAF6 (38). Certain inflammatory mediators, such as IL-1, enhance miR-146a production in synovial fibroblasts during rheumatoid arthritis.

Another study discovered that miR-146a expression was raised in CD+4 T cells from rheumatoid arthritis patients, was favorably linked with the amount of TNF-α, and prevented T cell death via regulation of Fas pathway-related factor 1 (39). Additionally, increased miR-146a regulates STAT1 to create a pro-inflammatory phenotype in Tregs, all of which are associated with the pathophysiology of rheumatoid arthritis (40). miR-21 has a role in a variety of autoimmune illnesses by balancing Th1/Th2 and Th17 cells (41). miR-21 enhances Th2 cell development by suppressing Th1 cell differentiation and IL-12 release, and is implicated in the pathophysiology of airway inflammatory disorders. In a study of miRNAs and rheumatoid arthritis, it was shown that miR-21 stimulates the STAT3 signaling pathway and decreases Foxp3 transcriptional activity, resulting in a decrease in Treg inhibitory activity, which enhances pro-inflammatory factor production (42). When the equilibrium between Th17 and Treg is disrupted, Th17 secretes pro-inflammatory factors (43).

Furthermore, several studies have identified numerous other miRNAs associated with rheumatoid arthritis. In RA patients’ PBMCs, serum, synovial tissue T cells, and macrophages, miR-223 expression is increased in comparison to the normal population, and the highly expressed miR-223 may alter the release of IL-10 in vivo, resulting in pro-inflammatory cells (44). miR-301a-3p was overexpressed in RA patients’ PBMCs, RORα and STAT3 expression were considerably enhanced in Th17 cells, and miR-301a-3p expression was positively linked with the frequency of Th17 cells (45).

miR-16 and miR-132 were upregulated in synovial fluid and plasma, but miR-188-5p was downregulated in synovial tissue and fibroblasts, and regulated related genes in fibroblasts, including hyalin protease binding protein and collagen knot gene (46). These proteins are involved in the development and degradation of the extracellular matrix during the course of rheumatoid arthritis. miR-124a expression was considerably decreased in the synovial cells of RA patients and that it was controlled by cyclin-dependent kinase-2 (CDK-2) and monocyte chemoattractant protein 1 (MCP-1) (47). Recruitment of mononuclear phagocytes to membrane tissue may contribute to the development of rheumatoid arthritis.

**miRNAs and Multiple Sclerosis**

MS is an autoimmune illness that is characterized by central nervous system white matter inflammatory demyelinating lesions. It is a chronic inflammatory disease of the central nervous system. When MS strikes, CD+4 T lymphocytes specialized for myelin in the central nervous system are activated in the peripheral immune system and subsequently cross the blood-brain barrier, causing neuronal damage (48). At the moment, the involvement of miRNAs in the pathophysiology of multiple sclerosis is receiving considerable interest. miR-21, miR-146a, miR-146b, and miR-155 were significantly overexpressed in the PBMCs of relapsing-remitting MS patients compared to normal controls (49). Experimental autoimmune encephalomyelitis (EAE) is a well-established animal model of MS. According to several studies, animals lacking miR-21 exhibit aberrant Th17 cell development, block Th17 cell-mediated autoimmunity, and nearly totally avoid developing EAE (50). Not only were miR-155 and miR-326 upregulated in PBMCs of MS patients, but they were also overexpressed in CD+4 T cells (51). Recent investigations have revealed a link between miR-155 expression
and the severity of MS and EAE. MiR-155 overexpression stimulates the proliferation of Th1 and Th2 cells (52), as well as the development of Th17 cells (53). miR-326 enhances the course of EAE during relapse and remission by regulating Ets-1, resulting in the differentiation of Th17 cells. In EAE mice, silencing miR-326 prevented the development of Th17 cells and decreased the severity of the disease (54).

Additionally, investigations have revealed that several miRNAs express abnormally at various phases of MS (i.e., onset, remission, and relapse). In comparison to remission and healthy controls, miR-27a expression was increased in PBMCs of MS patients during the relapsing phase (55); in contrast, miR-214 expression was decreased during the relapsing phase of MS (56). Compared to miR-21, miR-146a/b, miR-155, and others were up-regulated in PBMCs with the beginning of MS, but miR-140-5p was considerably reduced in PBMCs (57). Some researchers discovered that when PBMCs were transfected with miR-140-5p, STAT1 activity was decreased and encephalitic Th1 cells were quickly reduced, indicating that miR-140-5p may have a role in the pathogenesis of MS via the STAT signaling pathway (58). Unlike the majority of miRNAs implicated in the pathogenesis of MS, certain miRNAs, such as miR-96, miR-18b, and miR-599, are also engaged in the relapse phase of MS, whereas miR-193a rises during the remission period (59). Furthermore, the dysregulation of the same miRNA varies by region. miR-17-5p expression levels were increased in CD4+ T cells but decreased in blood cells (60).

miRNAs and Other Autoimmune Diseases

Researchers have increasingly focused on the function of miRNAs in the etiology and pathophysiology of autoimmune disorders, demonstrating that dysregulation of miRNA expression is strongly associated with autoimmune illnesses. Along with the above-mentioned SLE, RA, and MS, there is a range of other autoimmune disorders related to altered miRNA expression.

Sjögren’s syndrome (SS) is an autoimmune inflammatory illness that progresses over time. Because the primary organs affected are exocrine glands such as salivary and lacrimal glands, common clinical signs include dry mouth and dry eyes, which can have a systemic effect. The autoantigens Ro/SSA and La/SSB are increased in SS patients, and studies have discovered that several miRNAs, including let-7b, miR-16, miR-181a, miR-200b-3p, miR-200b-5p, miR-223, and miR-483-5p, can target the miRNAs of Ro/SSA and La/SSB (61). Apart from miR-200b-5p, which was discovered to be down-regulated in mucosa-associated lymphoid tissue in SS patients, numerous other miRNAs were found to be significantly expressed. The expression is enhanced in a variety of tissues, organs, and cells (62), miR-146a/b and miR-181a expression was increased in PBMCs from SS patients (63). Through the TGF-β and TLR/NF-κB signaling pathways, increased expression of certain miRNAs, such as miR-34b-3p and miR-300, enhances the incidence of SS (64).

Systemic sclerosis (SSc) is another reasonably frequent autoimmune illness that is often characterized by increased skin and organ fibrosis and tissue destruction. MiR-15b, miR-16, miR-27a, miR-27b, miR-132, miR-150, and miR-335 have been reported to stimulate fibroblast proliferation and prevent apoptosis (65), whereas miR-21, miR-27a, miR-92a, miR-133, miR-142-3p, miR-200a/b, and miR-590 may be involved in the process of reducing fibrosis (66). Around 60 miRNAs are over-expressed in people with psoriasis. PBMCs up-regulate miR-142-3p, miR-146a, and miR-155, whereas miR-125b and miR-181a are down-regulated (67). Meanwhile, miR-223 expression was decreased in Th17 cells while miR-125b expression was decreased in Treg cells (68).

Concluding Remarks

Autoimmune disorders occur when autoimmune tolerance is compromised due to genetic and environmental causes or improper control of autoimmune cells, and the immune system mounts a persistent immunological response to self-antigens, eventually causing damage or malfunction of self-tissue cells. Antinuclear antibody positivity in serum serves as the gold standard and biomarker for autoimmune disease detection. At the moment, the role of miRNAs in the immune system is gaining increasing attention, and a vast amount of research has established a link between aberrant miRNA expression and autoimmune disorders.

However, these studies discovered that the kinds of miRNAs, their up- or down-regulation, and the regulation of the onset and progression of autoimmune disorders vary among autoimmune diseases. The miRNAs expressed in various organs or cells, such as lymphocytes in the spleen, PBMCs, and plasma, are also distinct in the same autoimmune illness. Second, miRNA expression in autoimmune illnesses is also influenced by genetic background, and miRNA modifications in various species may be distinct. Thus, by further elucidating the changes in miRNA associated with autoimmune illnesses, it may be employed as a biological marker or therapeutic target for the early detection of autoimmune diseases, thereby introducing a novel concept for the research of autoimmune diseases.

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