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Review

Therapeutic Approaches Targeting miRNA in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a potentially fatal systemic autoimmune disease, and its etiology involves both genetic and environmental factors such as sex hormone imbalance, genetic predisposition, epigenetic regulation, and immunological factors. Dysregulation of microRNA (miRNA) is suggested to be one of the epigenetic factors in SLE. miRNA is a 22-nucleotide single-stranded noncoding RNA that contributes to post-transcriptional modulation of gene expression. miRNA targeting therapy has been suggested to be useful for the treatment of cancers and other diseases. Gene knockout and miRNA targeting therapy have been demonstrated to improve SLE disease activity in mice. However, these approaches have not yet reached the level of clinical application. miRNA targeting therapy is limited by the fact that each miRNA has multiple targets. In addition, the expression of certain miRNAs may differ among cell tissues within a single SLE patient. This limitation can be overcome by targeted delivery and chemical modifications. In the future, further research into miRNA chemical modifications and delivery systems will help us develop novel therapeutic agents for SLE.

Key words: systemic lupus erythematosus, miRNA, miRNA targeting therapy

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by the production of multiple autoantibodies and the involvement of multisystemic organ damage [1]. The estimated incidence of 23.2 cases per 100,000 persons is the highest incidence reported in North America [2]. Due to treatment advances and earlier diagnosis, the mortality rate is now only 10% within 10 years, compared with 50% within 3 years in the 1960s [3]. Despite the use of corticosteroids, immunosuppressants, and biologic agents, some patients exhibit life-threatening organ damage by cardiovascular disease as a side effect of steroid therapy [4], renal failure with active lupus, and infections related to immune suppression [5]. New treatments are needed to overcome resistance to conventional therapy.

The pathophysiological mechanisms of SLE are incompletely understood but involve both genetic and environmental factors such as sex hormone imbalance, genetic predisposition, epigenetic regulation, immunological factors, and other, undefined factors [6].

A microRNA (miRNA) is a 22-nucleotide single-stranded noncoding RNA that contributes to post-transcriptional modulation of gene expression [7]. miRNAs control the immune system as epigenetic regulatory elements involved in the regulation of cellular develop-

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ment and differentiation [8]. miRNA dysregulation is implicated in the pathogenesis of SLE [9]. The exact mechanism by which miRNAs lead to SLE is still unknown, and anti-miRNA therapy for SLE remains in preclinical stages. However, the identification of miRNAs critically involved in SLE's pathogenesis will provide new therapeutic clues. This review focuses on the recent discoveries by which miRNAs can become promising therapeutic targets for the treatment of SLE.

miRNA Biology

Recent evidence suggests that 2,300 human mature miRNAs exist [10] and target approximately 20-30% of all human mRNAs [11]. miRNAs regulate gene expression by facilitating sequence-specific RNA interference and the induction of RNA degradation or the inhibition of its translation [12].

The synthesis of mature miRNAs begins with the transcription of nuclear genes into primary RNA transcripts (pri-miRNAs) and the cleavage into precursor miRNAs (pre-miRNAs) by the ribonuclease III (RNase III) enzyme Drosha and the protein DiGeorge syndrome critical region 8 (DGCR8) [13]. Exportin-5/Ran-GTP transports pre-miRNA to the cytoplasm [14]. Dicer and transactivation-response RNA-binding protein splice pre-miRNA into single-stranded mature miRNA. A functional miRNA strand is loaded into the RNA-induced silencing complex (RISC) with the Argonaut (AGO) protein [15]. The RISC complex binds to the 3′UTR of the target mRNA with the seed region of the miRNA (7-8 bases from the second 5′ end of the miRNA) and exerts translational suppression or target degradation [16, 17].

miRNA Targeting Therapy

Nucleic acid-based therapies include antisense oligonucleotides (ASOs), small interfering RNA (siRNA), anti-miRNA (antagomirs), miRNA mimics, aptamers, and unmethylated CpG-containing synthetic oligonucleotide [18].

Although 13 nucleic acid-based therapies, including 3 siRNA drugs, are approved by the U.S. Food and Drug Administration (FDA), no miRNA targeting therapies are on the market today [19]. The following oligonucleotide drugs are FDA approved: fomivirsen, an ASO for the treatment of cytomegalovirus infections [20]; pegaptanib, an aptamer for the treatment of ocular vascular disease [21]; mipomersen, a gapmer ASO for the treatment of homozygous and severe heterozygous familial hypercholesterolemia [22]; eteplirsen, a steric block ASO for the treatment of Duchenne muscular dystrophy [23]; nusinersen, an ASO for the treatment of spinal muscular atrophy [24]; CpG1018, an unmethylated CpG-containing synthetic oligonucleotide as an adjuvant for hepatitis B vaccines [25]; inotersen, a gapmer ASO for the the treatment of hereditary transthyretin amyloidosis [26]; patisiran, an siRNA lipid nanoparticle (LNP) formulation for the treatment of hereditary transthyretin-mediated amyloidosis [27]; givosiran, an siRNA (GalNAc conjugate) for the treatment of acute hepatic porphyria [28]; golodirsen, viltolarsen, and casimersen, ASOs for the treatment of Duchenne muscular dystrophy [29-31]; and lumasiran, an siRNA for the treatment of primary hyperoxaluria type 1 [32].

Compared with siRNA drugs, only 10 miRNA targeting therapies have entered clinical trials and none has progressed to phase III [33]. In contrast to siRNA, miRNA targeting therapy can influence not only a single gene but also cellular pathways or processes [16]. The usefulness of miRNA targeting therapy has been suggested for the treatment of cancers and other diseases [34]. Major companies have active programs focused on developing novel miRNA targeting therapies for cancer and other diseases [35].

The therapeutic application of miRNAs involves three main strategies: first, through antisense-mediated inhibition of overexpressed miRNAs with ASOs, miRNA antagomirs, and locked nucleic acid (LNA)-modified oligonucleotides, such as miR-122 [36, 37]; second, through the replacement of underexpressed miRNAs with either miRNA mimics or viral vector-encoded miRNAs such as miR-34a, which targets SIRT3 in prostate cancer [38]; third, miRNA manipulation to enhance a patient's response to standard therapies, such as miR-34a antagomirs to radio-sensitize breast cancer cells [39, 40].

The first anti-miRNA drug to enter clinical trials was miravirsen, which is an ASO for the treatment of chronic hepatitis C virus (HCV) infection by targeting the liver-specific miR-122 [37]. Currently, 5 miRNA targeting therapies are undergoing clinical trial and in development: RG-012 for Alport nephropathy by targeting miR-21, RG-125 for nonalcoholic fatty liver

disease by targeting miR-103/107, Cobomarsen for cutaneous T-cell lymphoma by targeting miR-155, Remlarsen for keloids by targeting miR-29, and MRG-110 for skin excisional wounds by targeting miR-92a [33]. However, 6 anti-miRNA drugs were terminated or suspended despite beginning clinical trials: miravirsen and RG-101 for HCV infection by targeting miR-122, MesomiR 1 for malignant pleural mesothelioma and non-small-cell lung cancer by targeting miR-16, pSil-miR200c and PMIS miR200a for tooth extraction status NOS by targeting miR-200a/c, MRX34 for melanoma, primary liver cancer, and hematologic malignancies by targeting miR-34a, and RGLS4326 for polycystic kidney disease by targeting miR-17 [33]. The differences between siRNA and miRNA are the target sequences, the sequence complementarity, and the number of target genes [33]. miRNAs exhibit 20-90% complementarity to the 3′ untranslated region of mRNA [41] and the targets of the anti-miRNA drug ranged from 30 to 250 in number [33]. siRNAs exhibit 100% complementarity to the coding region of mRNA [42] and a single siRNA targets 1 to 3 genes [33]. For example, MRX34 (a miR-34a mimic) was discontinued during a phase I clinical trial by serious immune-related adverse events. Dysregulation of immune pathways including cytokine signaling was predicted by using KOBAS (a web server for the annotation and identification of enriched pathways and diseases) [33, 43]. The important challenge in the progression of miRNA targeting therapy is to overcome such therapy's limitation, which is that each miRNA has multiple targets. This limitation may be overcome by targeted delivery and chemical modifications.

Chemical modifications and delivery systems of miRNA in *in vivo* application can enhance the efficiency of miRNA by wrapping the unstable state of naked nucleotides. Commonly used delivery vehicles include adenoviral vector, poly (lactide-co-glycolide) (PGLA), EnGeneIC Delivery Vehicle (EDV) nanocells, and polyethylenimine (PEI) molecules [44]. Safety issues as well as tumor-specific delivery systems are still tested in animal models and clinical trials [34]. For example, tiny LNAs are highly chemically modified anti-miRNA antisense oligonucleotides with high activity and specificity. N-acetylgalactosamine (GalNAc)-conjugated miR-122–targeting tiny LNA is 300-500 times more potent than the original, unconjugated tiny LNA in *in vivo* activity and is expected to become a clinically useful anti-miRNA therapy [45].

Moreover, miRNAs derived from plants may become potential miRNA therapies, because they affect only genes of a pathogen and do not interfere with host genes [41, 46]. The effect of miRNA may be stronger in stressed or diseased conditions than in healthy ones, cell/tissue-type-specific miRNA expression may influence gene expression profiles in different cell types, and utilizing the synergistic effects of targeting multiple miRNAs may be an effective therapeutic approach [47]. Therefore, miRNA targeting therapy is worthy of further investigation and development.

Therapeutic Approaches Targeting miRNA in SLE

miRNAs play an important role in the pathogenesis of SLE, represented by the breakdown of self-tolerance (Table 1). Innate and adaptive immune aberrant responses against self-antigens induce the production of autoantibodies, and the deposition of immune complexes in tissues leads to the activation of complement, the accumulation of neutrophils and monocytes, and the development of self-reactive lymphocytes [48]. Specifically, the rate of apoptotic cells increases despite the reduction of its clearance in SLE. This leads to the exposure of its nuclear antigens to the innate immune system and induces endogenous type I interferon (IFN) production through the activation of the Toll-like receptor (TLR) family [49]. Moreover, IFN-α can promote the transformation of monocytes into dendritic cells (DCs), improve the antigen presentation ability of DCs, and continuously produce IFN-α [50]. The involvement of let-7c [51], miR-155 [52], and miR-150 [53] in regulating the functions of DCs in response to TLR stimulation has been reported recently. As target genes, suppressors of cytokine signaling-1 (SOCS1), CD40, and TREM-1 are identified by bioinformatics prediction and validation by reporter gene assays and/or Western blotting (Table 1).

The loss of central and peripheral tolerance of B cells is also a characteristic of SLE patients. Autoantibodies are produced by self-reactive B cells. In addition, aberrant B cells mediate the presentation of antigen to T cells, co-stimulatory functions through the expression of accessory molecules engaging stimulatory receptors on T cells, and the production of cytokines such as IL-6, IL-10, IFNγ, and TNF [54]. The over-reactivity of B cells in SLE contributes to the Janus kinase/signal

Table 1 The roles of miRNAs in the pathogenesis of SLE

miRNA	Change in miRNA expression	Target genes	Change in target gene expression	Sample	SLE mouse model or human	Associated pathway (biological function)	Reference
let-7a	↑	IL-6	↑	mesangial cells	New Zealand Black/White (NZB/W) mice	Enhancement of IL-6 production	[41]
let-7c	↑	suppressor of cytokine signaling-1 (SOCS1)	↓	dendritic cells	DC Blimp1ko mice	Enhancement of IL-6 production from DC	[51]
let-7a and let-7e	↑	TNF alpha induced protein 3 (TNFAIP3)	↓	kidney tissues	SLE patients (with lupus nephritis)	Enhancement of NF-κB activity	[87]
miR-7	↑	PTEN	↓	B cells	$MRL^{lpr/lpr}$ mice	Promotion of B-cell differentiation into plasmablasts/plasma cells and spontaneous germinal center formation through downregulation of PTEN/AKT signaling	[56]
miR 10a	↑	IL-8	↓	$CD19^+$ cells	SLE patients	Block the generation of autoreactive antibodies by B cells	[63]
miR 10a-3p	↓	regenerating islet-derived 3 α (REG3A)	↑	PBMC	SLE patients (with lupus nephritis)	Increment of Th17/Treg ratio in $CD4^+$ T cells and promotion of JAK2/STAT3 pathway activation	[68]
miR-15a	↑			splenic cell and plasma	(NZB × NZW) F1 or B/W mice	Reduction of IL-10–producing $CD1^{dhi}CD5^+$ B cells (B10 cells) and increase in dsDNA autoantibody production	[61]
miR-15b	↓	cyclinD3 (CCND3)	↑	human $CD19^+$ B cells and spleen B cells	SLE patients and $B6\text{-}Fas^{lpr}$ mice	Abnormal activation of Toll-like receptor 7 (TLR7) signaling pathway in SLE B cells	[64]
miR-16	↓	differentially expressed in chondrocytes 2 (DEC2)	↑	kidney tissues	Fcγ receptor II-b-deficient ($Fcgr2b^{-/-}$) mice	Activation of the TLR4 signaling pathway	[88]
miR-21	↑	RASGRP1	↓	$CD4^+$ cells	$MRL^{lpr/lpr}$ mice	Suppression of Ras-MAPK pathway signaling and downregulaton of DNMT1	[80]
miR-21	↑	programmed cell death 4 (PDCD4)	↓	$CD4^+$ cells	SLE patients	Regulation of aberrant T-cell responses	[69]
miR-21	↑	PDCD4	↓	$CD4^+$ T cells	B6.Sle123 mice	Regulation of aberrant T-cell responses	[70]
miR-21	↑	3-hydroxy butyrate dehydrogenase 2 (BDH2)	↓	$CD4^+$ T cells	SLE patients	DNA demethylation and self-reactive T cells by dysregulation of iron homeostasis in $CD4^+$ T cells	[81]
miR-23b	↓	TGF-β-activated kinase 1/MAP3K7 binding protein 2 (TAB2), TAB3, and inhibitor of nuclear factor κ-B kinase subunit α (IKK-α)	↑	kidney tissues	SLE patients and $MRL^{lpr/lpr}$ mice	Enhancement of IL-17-, tumor necrosis factor α (TNF-α)-, or IL-1β-induced NF-κB activation and inflammatory cytokine expression	[89]
miR-26a and miR-30b	↓	human epidermal growth factor receptor 2 (HER-2)	↑	kidney tissues	SLE patients (with lupus nephritis) and lupus-prone NZM2410 mice	Activation of the type I IFN pathway	[90]
miR-29b	↑	sp1	↓	$CD4^+$ T cells	SLE patients	Reduction of DNMT1 levels and DNA hypomethylation	[82]
miR-30a	↑	Lyn	↓	B cells	SLE patients	Promotion of B-cell proliferation and the production of IgG	[60]
miR-31	↓	forkhead box P3 (FOXP3)	↑	$CD4^+CD25^-$ T cells	SLE patients	Negative regulation of Treg cell development	[71]
miR-31	↓	RhoA	↑	$CD3^+$ T cells	SLE patients	Reduction of IL-2 production	[72]
miR-34a	↑	FOXP3	↓	Treg	SLE patients	Disruption of Treg/Th17 balance	[73]
miR-98	↓	IL-6	↑	PBMC	SLE patients	Amelioration of STAT3-mediated cell proliferation and inflammatory cytokine production	[107]
miR-124	↓	TRAF6	↑	serum and human renal mesangial cells	SLE patients (with active lupus nephritis)	Activation of the growth and inflammation of renal mesangial cells	[91]

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miRNA	Change in miRNA expression	Target genes	Change in target gene expression	Sample	SLE mouse model or human	Associated pathway (biological function)	Reference
miR-125a	↓	Stat3, Ifng, and Il13	↑	$CD4^+$ T cells	SLE patients	Shifting of the balance from immune suppression to inflammation	[74]
miR-125a	↓	KLF13	↑	$CD3^+$ T cells	SLE patients	Elevated expression of chemokine RANTES level	[75]
miR-126	↑	DNA methyltransferase 1 (DNMT1)	↓	$CD4^+$ T cells	SLE patients	Demethylation and upregulation of genes encoding CD11a and CD70, thereby causing T-cell and B-cell hyperactivity	[83]
miR-130b	↓	IFN regulatory factor 1 (IRF-1)	↑	kidney tissues	SLE patients (with lupus nephritis) and (NZB × NZW) F1 lupus-prone mice	Activation of the type I IFN pathway	[92]
miR-130b	↑	phosphatase and tensin homolog (PTEN)	↓	kidney tissues	SLE patients (with lupus nephritis)	Interference with the viability and apoptosis of mesangial cells	[108]
miR-133	↓	Lim and SH3 protein 1 (LASP1)	↑	kidney tissues	SLE patients (with lupus nephritis)	Suppression of proliferation and promotion of apoptosis	[93]
miR-142-3p/5p	↓	signaling lymphocytic activation molecule–associated protein (SAP), CD84, and interleukin-10 (IL-10)	↑	$CD4^+$ T cells	SLE patients	Overactivation of T cells and hyperstimulation of B cells	[76]
miR-145	↓	signal transducer and activator of transcription-1 (STAT-1)	↑	$CD3^+$ T cells	SLE patients	Association with lupus nephritis	[77]
miR-146a	↓	IFN regulatory factor 5 and STAT-1	↑	PBMCs	SLE patients	Abnormal activation of the Type I interferon pathway	[109]
miR-146a	↓	TRAF6	↑	PBMCs	SLE patients (with lupus nephritis)	Promotion of NF-κB pathway (e.g., IL-1β, IL-6, IL-8, and TNF-α) in lupus nephritis	[110]
miR-146a	↓			PBMCs, lung, spleen, and kidney tissues	lupus-prone BXSB mouse	Enhancement of the production of autoantibodies and SLE progression in lupus-prone mice	[111]
miR-148a	↑	DNA methyltransferase 1 (DNMT1)	↓	$CD4^+$ T cells	$MRL^{lpr/lpr}$ mice	Contribution to DNA hypomethylation and T-cell hyperactivity	[80]
miR-148a	↑	BACH1, BACH2, and PAX5	↓	B cells	SLE patients (with multiple relapses of lupus nephritis)	Association with development of multiple relapses in patients with lupus nephritis	[65]
miR-148a-3p	↑	phosphatase and tensin homology deleted on chromosome ten (PTEN)	↓	serum and kidney tissues	$MRL^{lpr/lpr}$ mice and SLE patients (with lupus nephritis)	Enhancement of glomerular cell proliferation	[94]
miR-150	↓	triggering receptor expressed on myeloid cells 1 (TREM-1)	↑	splenic conventional dendritic cells	$MRLL^{lpr/lpr}$ mice	Enhancement of inflammation responses in splenic cDCs	[53]
miR-150	↑	suppressor of cytokine signaling 1 (SOCS1)	↓	proximal tubular and mesangial cells from kidney biopsies	SLE patients	Promotion of renal fibrosis by increasing profibrotic molecules through downregulation of SOCS1	[105]
miR-152-3p	↑	Kruppel-like factor 5 (KLF5)	↓	B cells	SLE patients	Increment of BAFF expression	[66]
miR-152	↓	macrophage migration inhibitory factor (MIF)	↑	kidney tissues	SLE patients (with lupus nephritis)	Increment of COL1A1 expression	[95]
miR-155	↑	CD40	↑	bone marrow derived plasmacytoid dendritic cell	Lupus-prone NZB/W F1 mice	Hyperactivation of TLR7-mediated cytokine modulation	[52]
miR-155	↑	CD1d	↓	B cells	$MRL^{lpr/lpr}$ mice	Impairment of antigen presentation to iNKT cells	[57]
miR-155	↑	SH2 domain-containing inositol 5′-phosphatase 1 (SHIP-1)	↓	B cells	$B6\text{-}Fas^{lpr}$ mice	Increment of serum IgG anti-dsDNA antibodies and kidney inflammation	[58]
miR-155	↓	PU.1, TNF-α	↑	PBMC, B cells	SLE patients	Enhancement of TNF-a/BAFF/CD19 signaling pathway	[59]

miRNA	Change in miRNA expression	Target genes	Change in target gene expression	Sample	SLE mouse model or human	Associated pathway (biological function)	Reference
miR-155	↑	sphingosine-1-phosphate receptor 1 (S1PR1)	↓	splenocyte and PBMC	$MRL^{lpr/lpr}$ mice and SLE patients	Enhancement of autoimmune inflammation of systemic lupus erythematosus	[112]
miR-155	↑	PPARα	↓	lung tissues	pristine-induced lupus mouse model (C57BL/6 mice)	Enhancement of the progression of diffuse alveolar hemorrhage	[113]
miR-181b	↓	activation-induced cytidine deaminase (AID) and interferon-α (IFN-α)	↑	PBMC	SLE patients	Impairment of negative regulation to IFN-α	[114]
miR-182-5p	↑	forkhead Box O1 (Foxo1)	↓	kidney tissues and blood samples	$MRL^{lpr/lpr}$ mice and SLE patients	Promotion of development of lupus nephritis	[96]
miR-183	↓	mammalian target of rapamycin (mTOR)	↑	kidney tissues	$MRL^{lpr/lpr}$ mice and SLE patients (with lupus nephritis)	Enhancement of mTOR pathway	[97]
miR-183	↓	transforming growth factor beta receptor 1 (Tgfbr1)	↑	kidney tissues	$MRL^{lpr/lpr}$ mice	Activation of TGF-β/Smad/TLR3 pathway and renal fibrosis	[115]
miR-198	↑	phosphatase and tensin homology deleted on chromosome ten (PTEN)	↓	kidney tissues	SLE patients	Promotion of glomeruli cell growth and proliferation in LN	[98]
miR-199a	↑	Klotho	↓	kidney tissues	pristine-induced lupus mouse model (BALB/c mice)	Promotion of LPS-induced NF-κB activation and the secretion of TNF-α and IL-1β	[99]
miR-223	↓	S1pr1	↑	$CD4^+$ T cells	$MRL^{lpr/lpr}$ mice	Stimulation of $CD4^+$ T-cell infiltration into the kidney tissue	[78]
miR 224	↑	interferon regulatory factor 4 (IRF4)		$CD4^+$ cells	SLE patients	B-cell hyperresponsiveness	[63]
miR-224	↑	apoptosis inhibitory protein 5 (API5)	↓	$CD3^+$ T cells	SLE patients	Acceleration of T-cell activation-induced cell death	[77]
miR-302d	↓	interferon regulatory factor (IRF)-9	↑	$CD14^+$ monocyte	SLE patients	Elevated expression of interferon-stimulated genes (ISGs) including MX1 and OAS1	[116]
miR 345	↑	interferon regulatory factor 8 (IRF8)	↓	$CD19^+$ cells	SLE patients	Regulation of B-cell differentiation	[63]
miR-371-5p	↓	hypoxia inducible factor 1α (HIF-1α)	↑	kidney tissues	SLE patients	Promotion of mesangial cell proliferation and inhibition of apoptosis	[100]
miR-410	↓	Stat3	↑	$CD3^+$ T cells	SLE patients	Reduction of IL-10 expression levels	[79]
miR-410	↓	IL-6	↑	kidney tissues	$MRL^{lpr/lpr}$ mice	Promotion of fibrosis through upregulation of TGF-β1	[101]
miR-422a	↑	kallikrein-related peptidase 4 (KLK4)	↓	kidney tissues	SLE patients (with lupus nephritis) and NZB/W F1 mice	Inhibition of renoprotective properties	[102]
miRNA-451a	↑	IFN regulatory factor (IRF) 8	↓	spleen and thymus	$B6\text{-}Fas^{lpr}$ mice	Enlargement of the spleen and increment of the proteinuria and immune complex deposits	[117]
miR-654	↓	macrophage migration inhibitory factor (MIF)	↑	PBMC	SLE patients	Enhancement of the phosphorylation of ERK and AKT and upregulation of downstream inflammatory cytokine production of MIF	[118]
miR-663a/ miR-423-5p	↑	TNIP2	↓	kidney tissues	SLE patients	Increment of IL-1β, IL-6, and TNF-α secretion	[103]
miR-873	↑	forkhead box O1 (Foxo1)	↓	PBMC	SLE patients	Promotion of Th17 cell differentiation	[119]
miR-1246	↓	early B-cell factor 1 (EBF1)	↑	B cells	SLE patients	Activation of the AKT signaling pathway	[62]

transducer and activator of transcription (JAK-STAT), B-cell receptor/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and TLRs [48]. B-cell activating factor (BAFF), which is involved in interaction between T cells and B cells, is overexpressed, promotes the proliferation of B cells, and prolongs the survival time of self-reactive B cells in SLE [55]. Aberrant expression in the B cells of SLE patient has been reported for miRNAs including miR-7 [56], miR-155 [57-59], miR-30a [60], miR-15a [61], miR-1246 [62], miR-10a [63], miR-15b [64], miR-148a [65], miR-152-3p [66], and miR-345 [63]. Their target genes are phosphatase and tensin homolog (PTEN), CD1d, SH2 domain-containing inositol 5′-phosphatase 1 (SHIP-1), PU.1, TNF-α, Lck/Yes novel tyrosine kinase (Lyn), Early B cell factor 1 (EBF1), IL-8, CyclinD3 (CCND3), BACH1, BACH2, PAX5, Kruppel-like factor 5 (KLF5), and interferon regulatory factor 8 (IRF8) (Table 1).

Aberrant activation, differentiation, and function of $CD4^+$ T cells are also characteristics of SLE patients. It can initiate and amplify the inflammatory process through the activation of B cells and DCs in lymphoid organs, secrete pro-inflammatory cytokines, and induce abnormal cell signal transduction such as in the T-cell receptor (TCR)-CD3, CD44-Rock-ERM, and PI3K-Akt-mTOR signaling pathways [50, 67]. In addition, naive $CD4^+$ T cells can differentiate into various effector T-cell subsets, including Th1, Th17, Th2, and follicular helper T (Tfh) cells. Imbalances of Th1/Th2, Th17/regulatory T (Treg) cells, and enhanced Tfh-cell response are recognized in SLE patients [50]. Aberrant expression of miRNAs in $CD4^+$ T cells and $CD3^+$ T cells has been reported in miR-10a-3p [68], miR-21 [69, 70], miR-31 [71, 72], miR-34a [73], miR-125a [74, 75], miR-142-3p/5p [76], miR-145 [77], miR-223 [78], miR-224 [63, 77], and miR-410 [79]. Their target genes are regenerating islet-derived 3 α (REG3A), programmed cell death 4 (PDCD4), forkhead box P3 (FOXP3), RhoA, Stat3, IFNγ, IL-13, KLF13, signaling lymphocytic activation molecule–associated protein (SAP), CD84, IL-10, signal transducer and activator of transcription-1 (STAT-1), sphingosine-1-phosphate receptor (S1pr1), interferon regulatory factor 4 (IRF4), apoptosis inhibitory protein 5 (API5), and Stat3 (Table 1).

A part of the aberrant expression of miRNAs in $CD4^+$ T cells of SLE has associated to DNA methylation, such as miR-21 [80, 81], miR-29b [82] miR-126 [83], and miR-148a [80]. Target genes are RASGRP1, 3-hydroxy butyrate dehydrogenase 2 (BDH2), sp1, and DNA methyltransferase 1 (DNMT1) (Table 1). Global DNA methylation levels are reduced by 15-20% in the $CD4^+$ T cells of patients with active SLE by genome-wide analysis [84]. As DNA methylation is usually repressive, hypomethylation typically induces overexpression of genes, such as ITGAL, CD40LG, CD70 and PPP2CA in SLE. In SLE patients, DNMT1, which maintains the methylation status of genes in proliferating cells [85], was significantly lower than in healthy subjects [48]. The change in expression of miRNAs was involved in hypomethylation in the $CD4^+$ T cells of SLE patients and led to aberrant activation and differentiation of them.

The dysregulation of miRNA in kidney samples from lupus nephritis (LN) patients and SLE mouse models leads to abnormal renal cell proliferation, inflammation, and kidney fibrosis in LN [86]. Aberrant expression of miRNAs in kidney tissues has been reported in let-7a and let-7e [87], miR-16 [88], miR-23b [89], miR-26a/miR-30b [90], miR-124 [91], miR-130b [92], miR-133 [93], miR-148a-3p [94], miR-152 [95], miR-182-5p [96], miR-183 [88, 97], miR-198 [98], miR-199a [99], miR-371-5p [100], miR-410 [101], miR-422a [102], and miR-663a/miR-423-5p [103]. Their target genes are TNF alpha induced protein 3 (TNFAIP3), differentially expressed in chondrocytes 2 (DEC2), TGF-β-activated kinase 1/MAP3K7 binding protein 2 (TAB2), TAB3 and inhibitor of nuclear factor κ-B kinase subunit α (IKK-α), human epidermal growth factor receptor 2 (HER-2), TRAF6, IFN regulatory factor 1 (IRF-1), Lim and SH3 protein 1 (LASP1), phosphatase and tensin homology deleted on chromosome ten (PTEN), macrophage migration inhibitory factor (MIF), forkhead box O1 (Foxo1), mammalian target of rapamycin (mTOR), transforming growth factor beta receptor 1 (Tgfbr1), PTEN, Klotho, hypoxia-inducible factor 1α (HIF-1α), IL-6, kallikrein-related peptidase 4 (KLK4), and TNIP2 (Table 1). Aberrant expression of miRNAs has also been reported with let-7a, which targets IL-6 in mesangial cells [104], and miR150, which targets SOCS1 in proximal tubular and mesangial cells from kidney biopsies [105].

miRNA targeting therapy and gene knockout (KO) have been demonstrated to improve disease activity of SLE in mice (Tables 2 and 3). The following SLE mouse models are mainly used to verify *in vivo* therapeutic

Table 2 miRNA targeting drugs for SLE

miRNA targeting drug	Chemical modifications and delivery system	Target gene/organ	Change in target gene expression	SLE mouse model or human	Associated pathway (biological function)	Reference
miR-7 atagomirs	–	PTEN	↑	$MRL^{lpr/lpr}$ mice	Downregulation of PTEN/AKT signaling promoted B-cell differentiation into plasmablasts/plasma cells and spontaneous germinal center (GC) formation	[56]
miR-16 agomirs	–	differentially expressed in chondrocytes 2 (DEC2)	↓	Fcγ receptor II-b-deficient ($Fcgr2b^{-/-}$) mice	Inhibition of mesangial cell proliferation and inactivation of the TLR4 signaling pathway	[88]
adenovirus encoding miR-23b	adenovirus encoding			$MRL^{lpr/lpr}$ mice	Suppression of the severity of renal lesions, including the proliferation of glomerular cells and the infiltration of periglomerular, perivascular, and interstitial mononuclear cells	[89]
PEAL (miR-125a)	monomethoxy (polyethylene glycol)-poly(d,l-lactide-co-glycolide)-poly(l-lysine) (mPEG-PLGA-PLL) nanoparticles (PEAL)	splenic T cells		$MRL^{lpr/lpr}$ mice	Alleviates SLE disease progression by reversing the imbalance of effector/regulatory T cells	[120]
miR-130b agomir	–	IRF-1	↓	(NZB × NZW) F1 lupus-prone mice	Amelioration of IFNα accelerated lupus nephritis	[92]
miR-146a mimic	–	RELA, IRAK1, interleukin-1β (IL1β), and IL-10 in kidney tissues	↓	$MRL^{lpr/lpr}$ mice	Inhibition of classical and nonclassical NF-κB signaling pathways	[121]
MS2 VLP-based delivery of miR-146a	bacteriophage MS2 virus-like particles (VLPs)			lupus-prone BXSB mice	Inhibition of the production of autoantibodies and SLE progression in lupus-prone mice	[111]
miR-146a mimic	-			pristine-induced lupus mouse model (C57BL/6 (B6) mice)	Suppression of the pristine-induced pulmonary hemorrhage through type I IFN pathway inactivation	[122]
Anti-miR-148a-3p adenovirus	adenovirus	phosphatase and tensin homology deleted on chromosome ten (PTEN)	↑	$MRL^{lpr/lpr}$ mice	Inhibition of glomerular cell proliferation	[94]
LNA-anti-miR-150	locked nucleic acid (LNA)	kidney		Fcγ receptor II-b-deficient ($Fcgr2b^{-/-}$) mice	Anti-fibrosis and anti-inflammation as well as reduction of the infiltrated kidney resident macrophages	[123]
miR-155 antagomir	–	PPARα	↑	pristine-induced lupus mouse model (C57BL/6 mice)	Inactivation of NF-κB pathways and reduction of the progression of diffuse alveolar hemorrhage	[113]
miR-182-5p antagomir		forkhead Box O1 (Foxo1)	↑	$MRL^{lpr/lpr}$ mice	Amelioration of renal structure and functional impairments associated with LN	[96]
miR-183 mimic	–	mammalian target of rapamycin (mTOR)	↓	$MRL^{lpr/lpr}$ mice	Inhibition of mTOR pathway	[97]
miR-654 mimic	–	macrophage migration inhibitory factor (MIF)	↓	pristine-induced lupus mouse model (BALB/c mice)	Suppression of the phosphorylation of ERK and AKT and reduction of downstream inflammatory cytokine production of MIF	[118]
LV-anti-miR-873	Lentivirus-encoding	forkhead box O1 (Foxo1)	↑	$MRL^{lpr/lpr}$ mice	Downregulation of the levels of anti-dsDNA, anti-Sm/RNP autoantibodies and proteinuria and IL-17A production	[119]

Table 3 miRNA knockout lupus mouse models

miRNA	Target gene/organ	Change in target gene expression	SLE mouse model	Associated pathway (biological function)	Reference
miR-21	3-hydroxy butyrate dehydrogenase 2 (BDH2)	↑	miR-21$^{-/-}$CD4Cre conditional knockout mice	Enhancement of global DNA methylation and the reduction of global DNA hydroxymethylation and intracellular iron concentration	[81]
miR-155	SH2 domain-containing inositol 5′-phosphatase 1 (SHIP-1)	↑	miR-155$^{-/-}$Fas$^{lpr/lpr}$ mice	Repression of serum IgG anti-dsDNA antibodies and kidney inflammation	[58]
miR-155			pristine-induced miR155$^{-/-}$ mice	Reduction of autoantibody levels and severity of nephritis and pneumonitis	[124]
miR-155	PPARα	↑	pristine-induced miR155$^{-/-}$ mice	Inactivation of NF-κB pathways and reduction of the progression of diffuse alveolar hemorrhage	[113]
miR-155	Sphingosine-1-phosphate receptor 1 (S1PR1)	↑	miR-155$^{-/-}$Fas$^{lpr/lpr}$ mice	Amelioration of autoimmune inflammation of systemic lupus erythematosus	[112]
miR-223	S1PR1	↑	miR-223$^{-/-}$Fas$^{lpr/lpr}$ mice	Inhibition of CD4+ T-cell infiltration into the kidney tissue	[78]
miR-451a	IFN regulatory factor (IRF) 8	↑	miR-451a$^{-/-}$Fas$^{lpr/lpr}$ mice	Repression of enlargement of the spleen and reduction of the urine protein content and immune complex deposits	[117]

effects of miRNA targeting agents: MRL$^{lpr/lpr}$ mice, Fcγ receptor II-b-deficient (Fcgr2b$^{-/-}$) mice, (NZB×NZW) F1 lupus-prone mice, lupus-prone BXSB mice, and a pristine-induced lupus mouse model (C57BL/6 or BALB/c mice). Parts of agomir, mimic, and antagomir are modified by viral encoding or the use of monomethoxy (polyethylene glycol)-poly(d,l-lactide-co-glycolide)-poly(l-lysine) (mPEG-PLGA-PLL) and nanoparticles (NPs) as a delivery system. In particular, treatment with miR-125a-loaded mPEG-PLGA-PLL (PEAL(miR-125a)) NPs shows excellent therapeutic efficacy and safety. By delivering miR-125a directly into splenic T cells with NPs, the imbalance of effector/regulatory T cells is improved [41]. The reported miRNA KO mice were conventional except miR-21$^{-/-}$CD4-Cre conditional (Table 3). Although the disease activities of lupus models were improved by miRNA targeting therapy and KO, these approaches have not yet reached the level of clinical application. One reason for this is the still-insufficient analysis of adverse events such as MRX34 (an miR-34a mimic), as mentioned above.

As for new miRNA-targeting drugs for the treatment of SLE, an oral miR-155 inhibitor was identified by using a drug discovery platform based on iterative fragment-based screening by nuclear magnetic resonance and machine learning to identify ligands of pre-miR-155. This oral miR-155 inhibitor reduced not only miR-155 but also TNFα in a mouse model [106]. Low molecular weight drugs, including those used in miRNA targeting therapy are an attractive alternative to biologics such as belimumab (Benlysta®, GlaxoSmithKline), because they can be developed and produced quickly and with low cost, and because they can be administered orally. Chemical modifications and delivery systems of miRNA, which can expand the range of target tissues that ASOs reach, are important for the development of SLE therapeutic agents, because the expression of a specific miRNA differs among cells and tissues within a single SLE patient, and one miRNA targets various mRNAs.

Conclusions and Perspectives

Investigations into miRNAs involved in SLE could clarify the complex pathogenesis of this disease and lead to the development of new therapeutic agents for SLE. Although miRNA targeting therapies for the treatment of a variety of diseases have been in development, they have not yet reached the clinical level in SLE and in other diseases. In the future, further research into chemical modifications and delivery systems of miRNA will help us develop novel therapeutic agents for SLE.

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