Evidence for microRNA-mediated regulation in rheumatic diseases

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ABSTRACT

MicroRNA (miRNA), a group of short non-coding RNA of approximately 20–22 nucleotides modulating the stability and translational efficiency of target messenger RNA, present an important new layer controlling gene expression. Hundreds to a thousand miRNA have been identified and are predicted to regulate at least one-third of protein-coding transcripts in the mammalian genome. This study reviews the recent advances reinforcing the awareness that miRNA are key players in rheumatic diseases by regulating major pathogenic molecules, such as tumour necrosis factor, central signal pathways, such as type I interferon pathway and critical immunoregulatory cells, such as regulatory T cells. In animals, blockade of miRNA maturation by the deletion of Dicer or Drasha, interference with miRNA function by the mutation of Roquin and the altered expression of individual miRNA (miR-146a) or miRNA cluster (miR-17–92) all lead to the development of autoimmune diseases. Growing evidence also reveals the differential expression of certain immunity-regulating miRNA in rheumatoid patients. The features of miRNA-mediated regulation, the direction of future miRNA study in rheumatic diseases and the application of miRNA in diagnosis, therapy and prognosis will also be briefly discussed.

MicroRNA (miRNA) are a group of short non-coding RNA of approximately 20–22 nucleotides (nt) in length primarily functioning as regulators of gene expression. MiRNA are produced from the stem-loop primary miRNA transcribed from an organism's own genome. Primary miRNA transcripts may encode individual miRNA, but in many situations they encode clusters of distinct miRNA.[1] The first miRNA, lin-4, was discovered in 1993 by Lee *et al*[2] when they found that a locus controlling developmental timing in *Caenorhabditis elegans* did not encode a protein but only a 22 nt transcript. It was not until 2000 that the second miRNA, let-7, was discovered, again in a study of the development of *C elegans*.[3] Soon after, let-7 was identified as playing orthologous roles in diverse metazoan species.[4] In 2001, this class of small regulatory RNA was named "microRNA".[5] The number of miRNA-related publications in Pubmed has almost doubled every year, from five5 in 2001 to 1731 in 2008, evincing a surge in the study of miRNA biology. Sanger's miRBase version 13.0[6] (released in March 2009), the most accepted miRNA registry, has a collection of 706 human miRNA genes, reported from various miRNA cloning strategies.[7]

Primary miRNA transcripts are processed sequentially by two members of the RNase III family, Drosha and Dicer, acting in concert with various cofactors. A typical animal primary miRNA consists of an imperfectly paired stem of approximately 33 bp, with a terminal loop and flanking segments. The first processing step, which occurs in the nucleus, excises the stem-loop from the remainder of the transcript to create a precursor miRNA product. The second processing step, which occurs in the cytoplasm, excises the terminal loop from the precursor miRNA stem to create a mature miRNA duplex of approximately 22 bp length. The mature miRNA duplex is rapidly unwound and a single strand thus acts as an adaptor for RNA-induced silencing complexes to specifically to recognise and regulate particular messenger RNA.[1]

The function of miRNA was elucidated from the evidence that miRNA could affect the expression of other genes and that miRNA have some sequence complementarity to their regulatory targets.[2 3] It is generally accepted that the recognition of its target by miRNA requires conserved Watson–Crick pairing of the 5′ region of the miRNA centred on nucleotides 2–7 (which is called the miRNA "seed") to the target mRNA, mainly in the 3′ untranslated region (3′UTR). Subsequently, catalytic or non-catalytic proteins facilitate the posttranscriptional repression of target protein expression by enhancing the cleavage of mRNA strand and/or promoting the inhibition of mRNA translation. A simplified model of miRNA biogenesis and function is depicted in fig 1. However, the efficacy of recognition and regulation is multifactorial, beyond just seed region pairing. Other factors such as complementarity beyond the seed region, positioning of the target site within the mRNA and proximity to other mRNA-regulating elements also play a role. The issue of miRNA target recognition is discussed in detail by Bartel,[5] whereas the mechanism of miRNA-mediated repression has been reviewed by Carthew and Sontheimer.[1]

The key functional study of miRNA is to identify their regulatory targets. Many algorithms have been developed to predict miRNA-mRNA recognition based on the pairing of the seed region of a miRNA to the target mRNA and the preferential evolutionary conservation of the seed region. Indeed, mature miRNA are conserved across species to a striking extent. The use of the conservation of miRNA target sequences within mRNA maintained under selective pressure for predicting miRNA targets significantly reduces the occurrence of false-positive prediction, which is otherwise problematical because of the short length of the seed sequence. TargetScan and other widely used algorithms have been summarised in another review.[5] Using proteomic methods to

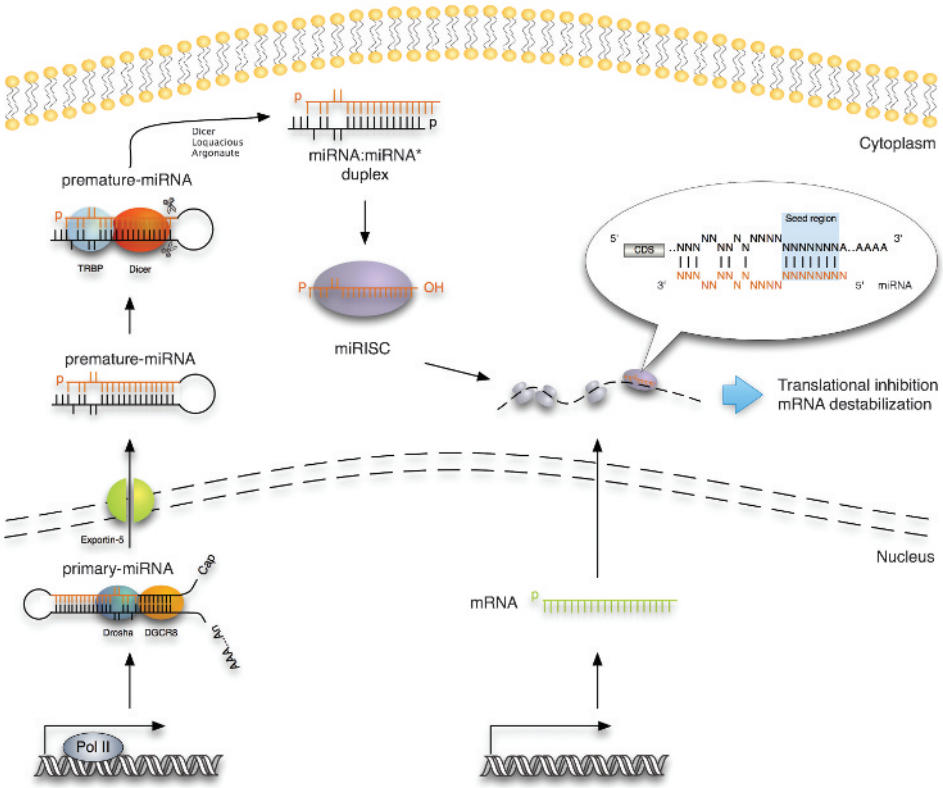

Figure 1 Mammalian miRNA biogenesis and function. In the mammalian genome, RNA polymerase II transcribes miRNA encoded genes into a long primary miRNA transcript. This primary miRNA is processed into a precursor miRNA by the nuclear RNase III enzyme Drosha partnered with DGCR8 (DiGeorge syndrome critical region gene 8). After being actively transported to the cytoplasm by exportin 5, the precursor miRNA is further processed by the RNase III enzyme Dicer and cofactor TRBP (TAR (HIV) RNA binding protein 2). The miRNA-induced silencing complex (miRNA-induced silencing complexes) recognises the miRNA duplex and loads the functional strand of the duplex (mature miRNA) onto the target mRNA. The base-pairing between miRNA and messenger RNA is often imperfect, allowing for several mismatches, although the so-called "seed" sequence of the miRNA (nucleotides 2–8 from 5′) usually matches perfectly. Once bound, the complex causes either inhibition of translation nor targets the messenger for degradation.

measure the impact of miRNA on global protein output, Baek *et al*[8] and Selbach *et al*[9] showed that individual miRNA repress hundreds of genes to a mostly modest degree. This fine-tuning feature distinguishes miRNA from other layers of gene regulation, such as chromatin remodelling and transcription factors, which tend to have a more profound change on gene expression. In total, it is estimated that miRNA may regulate 30–50% of protein-encoding genes.[5 10]

Much emerging evidence has revealed that miRNA play an instrumental role in various cellular processes, including cell growth, differentiation, proliferation and cell death. In addition, miRNA are also involved in the pathogenesis of cancer, cardiac diseases, neurodegenerative diseases, diabetes, autoimmune/inflammatory diseases and infection.[11] The finding of substantial phenotypes due to modulated miRNA expression introduces miRNA as new therapeutic targets to diagnose and treat common diseases.[12] In 2008, Santaris announced that it had commenced a clinical trial for a locked nuclear acid (LNA)-based antisense molecule targeting miR-122 for the treatment of hepatitis C virus infection, the first miRNA-based therapeutic agent to be trialled in humans.[13]

In this article, we will focus on the role of miRNA in rheumatic diseases. MiRNA have been shown to play an essential role in the development, homeostasis and function of both innate and adaptive immunity. Consequently, the dysregulation of these processes can induce autoimmunity and inflammation—phenomena central to the development of rheumatic diseases. Details of the general function of miRNA in immunity have been well reviewed elsewhere[14–16] and are therefore beyond the scope of this article. Our efforts here are directed towards summarising evidence of how miRNA regulate signal pathways and lymphocyte populations critical for the pathogenesis of rheumatic diseases and the differential expression of miRNA in rheumatoid patients. The hypothesis that miRNA act as rheostats and brakes to make fine-scale adjustments and provide feedback loops to control the intensity and duration of immune responses to prevent rheumatic diseases will also be discussed.

miRNAs REGULATE SIGNAL PATHWAYS CRITICAL FOR RHEUMATIC DISEASES

TNFα, miR-16, miR-369-3, miR-125b and miR-155

Pro-inflammatory cytokines represent critical mediators of rheumatic diseases. TNFα was among the first cytokines whose dysregulation was proposed to contribute to the pathogenesis of various autoimmune disorders, particularly rheumatoid arthritis (RA). The initiating role of TNFα in the pro-inflammatory cytokine cascade explains its promise as a therapeutic target, a promise fulfilled by extensive usage of TNF blockers in the treatment of RA and many other chronic inflammatory diseases.[17] The expression of TNFα is controlled transcriptionally and post-transcriptionally. The AU-rich element (ARE) within the 3′UTR of TNFα mRNA destabilises mRNA and represses mRNA translation.[18 19] Importantly, mice with the targeted genetic deletion of 69 bp TNFα ARE in the genome had chronic overproduction of TNFα and developed chronic inflammatory arthritis and Crohn's-like inflammatory bowel disease.[19]

ARE-mediated TNFα mRNA degradation was intriguingly shown to require miR-16, a miRNA containing a sequence complementary to the ARE sequence. Knockdown of endogenous miR-16 dramatically prolonged the mRNA half-life of a reporter gene containing the TNFα ARE.[20] The picture became even more complicated when miR-369-3, another miRNA binding to the TNFα ARE was demonstrated to control the translation of this mRNA. However, in contrast to the canonical miRNA role of repression, miR-369-3 enhanced the translation of TNFα mRNA when cells were under growth-arrest conditions.[21] In addition, miR-125b was recently shown

to repress TNFα through its 3′UTR, whereas miR-155 enhanced the production of TNFα via an unknown mechanism. When the regulatory role of miR-155 on TNFα production was tested in vivo, miR-155$^{-/-}$ B cells were deficient in TNFα production,[22] whereas mice overexpressing miR-155 in B cells produced higher levels of sera TNFα in response to lipopolysaccharide and were more sensitive to lipopolysaccharide-induced endotoxin shock.[23] Although it is still not clear how different miRNA and other RNA binding proteins synergise to regulate the stability and translation of TNFα mRNA, nor to what extent different elements contribute to the TNFα levels in health and disease, there is no doubt that this emerging new layer of regulation of TNFα is important and worthy of further investigation.

Type I interferon, TLR signalling and miR-146a

The past few years have seen growing evidence highlighting the central role of type I interferons (IFN) in the pathogenesis of systemic lupus erythematosus (SLE). SLE patients display elevated levels of type I IFN in their serum. Type I IFN, presumably produced largely by plasmacytoid dendritic cells, drives the maturation and activation of myeloid dendritic cells, which subsequently trigger the expansion and differentiation of autoreactive CD4+ and CD8+ T cells as well as the differentiation of autoreactive B cells into antibody-secreting cells. Increased release of autoantigens due to the damage caused autoreactive T cells and the elevated production of autoantibodies, by autoreactive antibody-secreting cells, encourages formation of immune complexes, which in turn activate plasmacytoid dendritic cells through Toll-like receptors (TLR) to produce more IFN-α, amplifying this pathogenic vicious cycle.[24] Using gene expression profiling, several groups have identified the expression pattern of IFN-inducible genes in peripheral blood samples from SLE patients, referred to as the IFN signature; this pattern has been correlated with disease activity.[25–27]

MiR-146a is induced in response to a variety of microbial components and pro-inflammatory cytokines, such as lipopolysaccharide, CpG, IFN-α and TNFα.[28 29] MiR-146a joined the type I IFN regulatory signal network when it was verified as a regulator of key signalling intermediates of the pro-inflammatory TLR-MyD88 pathway including IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6).[28] Very recently, the X chromosome-encoded gene *IRAK1* was identified as a disease susceptibility factor in SLE. Functional studies showed *IRAK1* deficiency abrogate all lupus-associated phenotypes in congenic mouse models bearing disease loci *Sle1* or *Sle3*.[30] The list of miR-146a targets has expanded since then. Another TLR-MyD88 pathway signalling molecule IFN regulatory factor 5 (IRF5), and the IFN-α receptor pathway signalling molecule signal transducer and activator of transcription 1 (STAT1) were added to the miR-146a target repertoire.[29] Because all miR-146 targets act in the same positive feedback loop between TLR and IFN-α signalling cascades, the cumulative negative effect of miR-146a on its target proteins' abundance would probably have a considerable effect. The induction of miR-146a might thus serve as a powerful regulator, by means of negative feedback. As expected, targeted deletion of miR-146a in mice led to an inflammatory phenotype (M Boldin, personal communication). Indeed, the levels of miR-146a expression are decreased in SLE patients[29] (see below).

TCR signalling, PTPN22 and miR-181a

Enormous receptor diversity of antigen-recognising receptors expressed on lymphocytes confers the adaptive immune protection against almost every possible pathogen. However, an estimated 20–50% of T-cell receptors (TCR) and B-cell receptors generated by V(D)J recombination recognise a self-antigen.[31] The elimination of these pathogenic clones largely relies on the central tolerance, mechanisms of negative selection during lymphocyte development. In the thymus, signals from TCR binding strongly to self-peptide–MHC complex trigger programmed cell death of thymocytes. Therefore, accurate determination of the TCR signalling threshold for selection is crucial in preventing autoimmune diseases. Many animal studies have shown systemic autoimmune diseases due to inefficient negative selection.[31]

Genetic studies on human autoimmune diseases revealed a robust association between *PTPN22* and RA, SLE, type I diabetes and many other human autoimmune conditions. Indeed, in terms of the strength and scope of association, *PTPN22* is second only to the major histocompatibility complex (MHC).[32] *PTPN22* endcodes Lyp, a lymphocyte intracellular tyrosine phosphatase. Lyp, when complexed with kinase Csk, inhibits Lck, a protein kinase critical for mediating TCR signalling.[33] The disease-predisposing allele encodes a variant with higher catalytic phosphatase activity and is a more potent negative regulator of TCR signalling.[34] A plausible explanation is that the augmented Lyp activity increases the threshold of TCR signalling required for the negative selection of autoreactive T cells in development, thus allowing autoreactive clones to appear in the periphery.[33] This mechanism has been exemplified before in a mouse model of autoimmune arthritis, whereby a mutation in TCR signalling molecule ZAP-70 reduced TCR signalling and impaired negative selection.[35]

An elegant study by Li and colleagues[36] showed that the expression of *PTPN22* is repressed by miR-181a. MiR-181a enhances TCR signalling by repressing many phosphatases, the negative regulators of TCR signalling including *PTPN22*. Therefore, higher miR-181a expression correlates with greater TCR sensitivity. MiR-181a expression is higher in immature T-cell populations but decreases as T cells mature, allowing selection for immature T-cell populations that recognise low-affinity self-antigens. Inhibiting miR-181a expression in the immature T cells reduces sensitivity and impairs both positive and negative selections, suggesting miR-181a acts as an intrinsic antigen sensitivity "rheostat" during T-cell development. Although the phenotypes of mice genetically modulated for miR-181a expression are under investigation (C-Z Chen, personal communication), miR-181a is differentially expressed in RA patients compared with healthy controls (Z-G Li, personal communication).

Proliferation, apoptosis, Bim, PTEN and miR-17-92

The balance between proliferation and apoptosis controls the homeostasis of the immune system. For example, after stimulation, signals from antigen-recognising receptors and costimulatory receptors drive naive lymphocytes to proliferate and differentiate into effector populations. This process is coupled with the upregulation of pro-apoptosis molecules so that the immune responses will resolve when stimulation is sequestered and the balance is dominated by apoptosis. When lacking pro-apoptotic molecules, such as the bcl-2 interacting mediator of cell death (Bim), Fas or FasL, mice and humans develop severe systemic autoimmune lymphoproliferative syndromes with many characteristics similar to SLE.[37–39] Bim deficiency also leads to the exacerbation and prolongation of joint inflammation in experimental arthritis.[40] Phosphatase and tensin homolog (PTEN) is a tumour suppressor and is often

mutated in human lymphomas. PTEN is an inhibitor of the phosphatidylinositol-3-OH kinase pathway and controls cell proliferation and survival. The mutation of PTEN in humans and the deficiency in mice both lead to the development of autoimmune disease and lymphoma.[41] The defects of PTEN might also account for the invasive growth and destructive function of RA synovial fibroblasts (RASF).[42] Enhanced PTEN expression can also ameliorate collagen-induced arthritis in rats.[43] Both Bim and PTEN were recently identified as targets of miR-17-92.[44] Considering its role reported previously in inhibiting the translation of the pro-apoptotic transcription factor E2F1,[45] miR-17-92 holds a determination on the balance between proliferation and apoptosis by a synergetic effect from multiple targeting. Transgenic mice overexpressing miR-17-92 in lymphocytes developed lymphoproliferative disease and autoimmunity. The mice demonstrated splenomegaly, lymphadenopathy, increased levels of autoantibodies against single or double-stranded DNA and massive infiltration of lymphocytes into multiple organs, and consequently, died prematurely.[44]

miRNAs REGULATE T-CELL POPULATIONS INVOLVED IN RHEUMATIC DISEASES

Treg, Dicer and Drosha

Regulatory T (Treg) cells are a group of T cells dedicated to limiting the activation and expansion of leucocytes, predominantly T lymphocytes. Here, the term ''Treg cells'' will be used to indicate forkhead box P3$^+$ (Foxp3$^+$) CD4+ regulatory T cells but not other subsets, such as IL-10-secreting Tr1 cells, transforming growth factor (TGF) β-secreting T helper type 3 cells, CD8+ suppressor T cells and γδ T cells, which are less defined and not so essential as Foxp3$^+$ T cells according to current knowledge.[46] Treg-mediated suppression is the major dominant or cell-extrinsic strategy adopted by immune system to maintain tolerance. Several mechanisms including the secretion of immunosuppressive cytokines (such as IL-10, TGFβ and IL-35), the delivery of negative or killing signals by cell–cell contact (such as through CTLA-4, cyclic AMP (cAMP), granzyme B and perforin) and the consumption of immunostimulatory cytokines (such as IL-2) are proposed.[47] Studies on the relationship between Treg and rheumatic diseases are in lack of complete agreement, but most evidence suggests there is an impairment of Treg cells, quantitatively and/or qualitatively in patients. For example, decreased numbers of peripheral blood Treg cells have been reported in most studies of SLE patients with active disease, with some studies also showing functional defects.[48]

The profiling of miRNA expression in Treg cells revealed a distinct pattern compared with conventional CD4+CD25− cells, suggesting a regulatory role of miRNA in Treg cells.[49] In the same study, mice with CD4 cells specifically deficient in Dicer, the RNase III enzyme required to convert precursor miRNA to mature miRNA (fig 1), also showed a significant reduction of Treg cells generated naturally in the thymus or induced from peripheral conventional CD4+ T cells. In addition, these mice also developed splenomegaly, lymphadenopathy and inflammatory colitis as well as lymphocytic infiltration of multiple organs including the intestine, colon, lung, liver and skin, at age 3–4 months.[49] As natural Treg cells tend to develop from clones with TCR of high affinity to thymic MHC/self-peptide ligands and that are positively selected,[46] it is conceivable that blocking the generation of mature miRNA due to the deficiency of Dicer might affect TCR signalling, as discussed above using the example of miR-181a impairing the selection process. For a more specific study of the role of miRNA in the homeostasis and function of Treg cells, several groups investigated mice with Dicer or another miRNA maturation-required RNase III enzyme, Drosha conditionally deleted by Cre recombinase driven under the transcriptional regulatory elements of Foxp3.[50–52] In these mice, the maturation of miRNA was particularly blocked in Foxp3$^+$ Treg cells due to the lack of either Dicer or Drosha. Although the development of Treg cells in the thymus was hardly affected, they lost their suppressive function fundamentally, and were, surprisingly, able to be converted into effector cells. The homeostatic potential of Treg cells was also impaired. Taken together, the miRNA pathway as a whole is essential for Treg cells, not only for their development in the thymus but also in their maintenance of suppressive capability and homeostasis in the periphery. A very interesting observation demonstrated a striking downregulation of Dicer expression levels by type I IFN in various cell lines and primary tissues, which raises the question of whether the elevated type I IFN in SLE patients could impair Treg homeostasis by diminishing Dicer.[53] Although how individual miRNA function in Treg cells is still largely unknown, one miRNA, miR-155, originally identified as a candidate gene directly regulated by Foxp3,[54] was recently reported to target the suppressor of cytokine signalling 1 (SOCS1) to augment the IL-2 signal, thus maintaining Treg cell homeostasis.[55] Treg cells produce cAMP, which inhibits effector T-cell proliferation and IL-2 production. Adenylyl cyclase (AC) 9 is an enzyme for cAMP production but is usually repressed by miR-142-3p. Foxp3 suppresses miR-142-3p expression and by doing so, enhances the production of cAMP in Treg cells by maintaining AC 9 levels.[56]

Tfh and *Roquin*

Production of autoantibodies is the hallmark of many rheumatic diseases, as exemplified by RA with anti-citrullinated protein antibodies and SLE with antinuclear antibodies. Although the pathogenic role of autoantibodies in these diseases is not fully understood, currently favoured mechanisms include either direct interference with normal cellular functions or the induction of disease through the formation of immune complexes, such as Fc-mediated activation of the complement system and recruitment of inflammatory cells.[57] The majority of self-reactive antibody-secreting cells secrete high-affinity and somatically mutated antibodies, suggesting that they are derived from germinal centres (GC), GC-like lymphoid structures or possibly, extra-GC sites in some cases.[57] A subset of CD4+ T cells was recently identified localising in B-cell follicles and with the specialised function of helping B cells to produce high-affinity antibodies and generate B-cell memory. This subset, named follicular B helper T (Tfh) cells, expresses high levels of the chemokine receptor CXCR5, the cytokine IL-21 and the costimulatory receptors programmed death-1 (PD-1) and inducible T-cell costimulator (ICOS)—thus, it is distinguished from other CD4+ helper T-cell subsets by homing preference and helper function.[58 59] In 2005, several years after the identification of Tfh cells, Vinuesa and colleagues[60] provided a fundamental piece of evidence for the role of Tfh cells in the pathogenesis of autoimmune diseases. They investigated the mutant mouse strain *sanroque*, which develops lupus-like systemic autoimmune disease and is susceptible to autoimmune arthritis. In this strain, they found excessive generation of Tfh cells and spontaneous formation of GC. Importantly, the follow-up studies showed that the pathology in *sanroque* mice was GC-dependent and driven by Tfh cells. Selective reduction of Tfh cells in *sanroque* mice abrogated the formation of GC, autoantibody production and renal pathology.[61] Although the

role of Tfh cells in human autoimmune diseases needs far more investigation, this study in mice highlights the importance of the regulation of Tfh population in preventing autoimmunity.

The aberrant differentiation of Tfh cells in *sanroque* mice is due to a missense mutation in the *Roquin* gene.[60] ICOS is a costimulatory receptor of the CD28 family and is required for the development of Tfh cells and the formation of GC.[58] The overexpression of ICOS in *sanroque* mice significantly contributes to the autoimmune pathology.[62] Importantly, ICOS is regulated by *Roquin* through its mRNA 3′UTR via miRNA-mediated mRNA decay, whereas the regulation is impaired due to *Roquin* mutation. Our new studies have indicated that many other Tfh-associated functional molecules are regulated by miRNA in a similar mechanism (unpublished data). Therefore, differentiation towards Tfh cells is restricted by miRNA-mediated repression of many molecules. This differentiation can only be initiated when the restriction is removed by differentiating signals, presumably through the downregulation of many miRNA. The mutation of *Roquin* leads to impairment of miRNA-mediated repression and releases cells from the restriction of Tfh differentiation, leading to excessive generation of Tfh cells.[62] It is therefore necessary to investigate whether there is an abnormality in the miRNA–functional molecule–Tfh–GC–autoantibody axis in rheumatic diseases, particularly in those shown to be more autoantibody-dependent, such as SLE and Sjogren's syndrome.

DIFFERENTIAL EXPRESSION OF miRNAs IN PATIENTS WITH RHEUMATIC DISEASES

Given the growing body of evidence indicating the involvement of miRNA in rheumatic diseases, as discussed above, the identification of dysregulated miRNA and characterisation of their roles in disease pathogenesis is emerging as an exciting field of study. Studies of human patients have begun to reveal the relevance of miRNA genes in the biological and clinical behaviour of rheumatic diseases, mainly in SLE and RA.

Using miRNA microarry to examine peripheral blood mononuclear cells (PBMC), the first effort to profile miRNA expression in SLE patients was reported in 2007.[63] In comparison with healthy and diseased (idiopathic thrombocytic purpura) controls, seven miRNA were found to be downregulated and nine miRNA upregulated in SLE patients. Subsequently, the same research team profiled miRNA of kidney biopsy specimens from patients with grade II lupus nephritis against healthy controls and detected 66 differentially expressed miRNA in disease lesion samples.[64] However, these 66 miRNA included no miRNA dysregulated in PBMC from SLE patients reported in the previous study, suggesting organ-specific or cell type-restricted expression of miRNA in particular physical or disease states.

The Taqman miRNA assay is a more specific and sensitive method for high-throughput miRNA profiling.[65] Using this method, we performed a more comprehensive profiling in SLE patients, and identified 42 differentially expressed miRNA out of 156 miRNA tested, including the downregulation of miR-146a in SLE patients compared with healthy controls.[29] The underexpression of miR-146a was subsequently confirmed in a larger number of samples. Importantly, there was a negative association between miR-146a levels and clinical disease activity, as assessed by the SLE disease activity index (SLEDAI) and renal-SLEDAI. In addition, a negative correlation was also revealed between miR-146a levels and the activation of the type I IFN pathway in lupus patients. This observation promoted us to continue the functional study and validate two new miR-146a targets, IRF5 and STAT1, intermediator molecules involved in type I IFN signalling, as discussed above. The overexpression of miR-146a greatly reduced, while the inhibition of endogenous miR-146a increased, the induction of IFN-α and IFN-β. Furthermore, miR-146a directly repressed the transactivation downstream of type I IFN, as determined by the inhibitory effect of miR-146a on the activity of a reporter gene embedded with IFN-stimulated response elements or on the induction of IFN-inducible genes in PBMC. MiR-146a thus intrinsically modulates the onset and activation of the type I IFN pathway by targeting multiple signalling proteins, each with an essential role for full activation. The underexpression of miR-146a in SLE patients would therefore impair the function of a master negative regulation, leading to the excessive expression of several signalling molecules and sustained overproduction of type I IFN and their downstream activation. In conclusion, miR-146a underexpression is one causal factor in the abnormal activation of the type I IFN pathway in SLE. Interestingly, rescuing miR-146a expression in PBMC from five SLE patients by introducing a miR-146a-expressing plasmid significantly reduced the coordinated activation of the type I IFN pathway. All together, miR-146a is a good candidate as both a diagnostic biomarker and a therapeutic target in SLE.

Interestingly, according to several studies in RA, miR-146a expression was increased in synovial fibroblasts, synovial tissue and PBMC in RA patients compared with controls.[66–68] In-situ hybridisation studies revealed that primary miR-146 expression could be detected in RA synovial tissue, primarily in macrophages, but also in some T cells and B cells.[66] In addition, increased miR-146 expression in PBMC correlated with disease activity in RA patients. Given that RA and SLE are both systemic rheumatic diseases, it is somehow surprising that independent studies demonstrated decreased levels of miR-146 in SLE patients but increased levels in RA patients compared with healthy controls. A possible explanation is that the discrepancy might actually reflect the different aetiologies of the two diseases: type I IFN play a dominant role in SLE, whereas TNF and IL-6 are essential for RA.[69]

Several inflammatory cytokines such as TNFα and IL-1β produced by infiltrating immune cells and RASF are considered to play an important role in the inflammation of synovial tissue and lead to joint damage in RA.[70] By screening using miRNA microarrays, miR-155 was found to be prominently upregulated in RASF after ex-vivo TNFα stimulation.[68] The induction of miR-155 in RASF could also be achieved by other pro-inflammatory mediators, such as IL-1β, lipopolysaccharide and poly(I-C). In vivo, RA synovia, compared with non-inflamed synovia from patients with osteoarthritis, demonstrated a significant increase in miR-155 levels. In addition, miR-155 expression was higher in synovial fluid monocytes than in peripheral blood monocytes in RA patients. Functional studies showed that enforced expression of miR-155 in RASF could repress the levels of matrix metalloproteinase (MMP) 3 and reduce the induction of MMP-3 and MMP-1 by TLR ligands and pro-inflammatory cytokines.[68] The authors speculated that miR-155, induced by pro-inflammatory stimuli, might function as a protective miRNA to downregulate the expression of certain MMP, thereby controlling excessive tissue damage due to inflammation in arthritic joints. Indeed, aged mice deficiency of miR-155 displayed significant remodelling of lung airways, with increased bronchiolar subepithelial collagen deposition and increased cell mass of subbronchiolar myofibroblasts.[71] However, considering miR-155 enhances TNFα production,[22 23] the role of miR-155 in the pathogenesis of RA might be double-faced, depending on cell types, and needs careful interpretation.

PERSPECTIVES

Rheumatic diseases are caused by an interactive combination of genetic and environmental factors. Each individual disease appears to have a unique combination of these factors so the aetiology is distinct from others. However, the fundamental abnormality across all diseases is the exaggerated immune response against self. Although the trigger of an immune response against self-antigens is essential for the initiation of an autoimmune cascade, the intensity and duration of autoimmune responses determine the severity of diseases. Immune responses are controlled by gene expression via multiple layers, including chromatin remodelling, transcription factors, alternative splicing, miRNA and so forth. We propose miRNA-mediated control occupies a special regulatory niche on the intensity and duration of immune responses. Compared with other layers, miRNA tend to change gene expression to a modest level (see discussion on miR-181a above). The fine-tuning of miRNA-mediated regulation thus most likely results in a quantitative effect on immune responses and controls the intensity. In addition, the majority of miRNA negatively regulate their targets. The nature of repressive regulation allows miRNA readily to play a role in negative feedbacks to attenuate or even stop immune responses, particularly when stimuli are removed (see discussion on miR-146a above). We, therefore, believe that miRNA act as rheostats and brakes for immune responses and play an essential role in preventing rheumatic diseases.

We present here substantial evidence for miRNA-mediated regulation in many aspects of rheumatic diseases. The key question is the relation of aberrant miRNA levels to disease pathogenesis: are they secondary to the disease development or contributing factors for clinical manifestation? To address the issue, functional studies of a given miRNA or miRNA cluster on signalling and cell fate determination with canonical postulate for the pathogenesis of rheumatic diseases (genetic predisposition, infection and hormones) should be performed.

Genetic variations in miRNA genes or their binding site(s) on target genes affecting miRNA expression or their reorganisation have been exemplified in other diseases as disease predisposition. For example, a common polymorphism within the precursor miR-146a sequence reduces the amount of precursor and mature miR-146a and predispose to papillary thyroid carcinoma,[72] whereas a hypertension-associated single-nucleotide polymorphism within the 3′UTR of the human type 1 angiotensin II receptor (*AGTR1*) gene abrogates the regulation by miR-155, thereby elevating *AGTR1* levels.[73] Strikingly, a point mutation in the seed region of miR-96, with a strong impact on miR-96 biogenesis and a significant reduction in mRNA targeting, even resulted in inheritable autosomal dominant, progressive hearing loss in Spanish families.[74]

Interestingly, miR-146a expression has been shown to be decreased in freshly isolated splenic lymphocytes from oestrogen-treated mice compared with placebo controls, which suggests a possible explanation for the contribution of oestrogen to SLE pathogenesis.[75] In addition, Epstein–Barr virus infection, a process implicated in several major rheumatic diseases,[76] also affect human cellular miRNA expression.[77 78]

Although miRNA-based therapy is underway, it faces the same challenge of in-vivo delivery as conventional gene therapy does. Compared with mRNA, miRNA has the advantage of better stability in vitro due to the short length. In addition, the small number of total miRNA compared with protein-code genes makes miRNA profiling and analysis more efficient. The miRNA-based diagnosis thus holds a great promise. Hopefully, miRNA expression patterns unique to a given rheumatic disease are to be disclosed in the near future. However, due to inconsistency among various studies, such as the quantification method, disease activity and medication of different patient cohorts, as well as sample types (whole blood, fractionised cell subsets and lesion tissues), discrepancies are expected, which might postpone the use of miRNA as disease biomarker. The inclusion of serial samples at various disease stages and paralleled comparison of miRNA expression between peripheral blood and target organs will help to aid the solution.

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