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MicroRNA profile in understanding pathogenesis of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease caused by genetic and epigenetic alterations as well as environmental factors. SLE is a multisystem disease whose pathogenesis is poorly understood. Current therapies are based on nonspecific immunosuppression but till date no permanent cure has been documented for SLE. Recent advances in RNA interference have opened new directions for SLE pathogenesis, more significant being the discovery of microRNAs (miRNAs). miRNAs are small, evolutionary conserved, non-coding, RNA molecules. Emerging evidences show that miRNAs affect the stability and translational efficiency of messenger RNAs (mRNAs) by post transcriptionally regulating gene expression of the target mRNAs. miRNAs play a vital role in development, regulation and prevention of immune diseases. The potential of miRNAs as diagnostic biomarkers and therapeutic agents is extensively studied by researchers worldwide. In this article, various miRNAs associated with SLE pathogenesis and their associated mechanisms in cellular processes have been reviewed.

Keywords: Autoimmune disorder, microRNA (miRNA), systemic lupus erythematosus (SLE)

Introduction

RNA interference (RNAi) is a recently characterized gene silencing pathway by which specific mRNAs are either degraded or translationally suppressed. The present inventions are directed towards the methods and reagents useful in modulating gene expression in a variety of applications, including therapeutic, diagnostic, target validation and genomic discovery applications. These discoveries are specifically related to the synthetically and chemically modified small nucleic acid molecules, such as, short interfering nucleic acid (siNA), short interfering RNA (siRNA), double stranded RNA (dsRNA) and microRNA (miRNA) and short hairpin RNA (shRNA) molecules that are capable of mediating RNAi against target nucleic acid sequences[1].

The miRNAs are small, evolutionary conserved, ~ 22 nucleotide, noncoding, single stranded RNA molecules that funtion in the post transcriptional regulation of ~ 30% of messenger RNAs (mRNA) by binding to their 3′- untranslated region (3′-UTR); thus, targeting them for degradation or translational repression. To date, the miRNA sequence database lists over 1000 predicted miRNAs in the human genome (http://www.mirbase.org/cgi-bin/mirna_summary.pl?org=hsa). The function of these recently emerged modulators of gene expression is the control of protein production by target-mRNA degradation or translational repression[2-3]. Almost half of human miRNA genes are contained within the introns of protein-coding genes or in the exons of untranslated genes, while others reside apart from known genes in intergenic regions[4]. miRNAs have been significantly associated with the development, physiological functioning and disease process in autoimmune diseases (AID)[5]. miRNAs have been shown to play key roles in immune regulation including T cell selection in thymus, B cell affinity maturation and selection in germinal centers, and development of regulatory T cells (Tregs), suggesting that miRNA machinery may be crucial in the maintenance of immunological tolerance[6]. Till date numerous studies have been conducted to understand the role of miRNAs, miRNA targets, and mechanism and profiles of miRNAs in various diseases. However, role of miRNAs in immunology and diseases is beyond the scope of this article and, hence, the focus of this article is to present the various microRNAs and their associated mechanisms in classical autoimmune disease, systemic lupus erytheomatosus (SLE).

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microRNA Biogenesis

Biogenesis of miRNA in the genome is intiated by RNA polymerase II by transcribing primary miRNA transcripts (pri-miRNA)[7-8]. miRNA maturation is further achieved by ribonuclease III (RNase III) enzymes, Drosha and Dicer (Fig. 1). In the first step, Drosha along with the protein DiGeorge syndrome critical region 8 (DGCR8) processes the nuclear pri-miRNA into ~70 nucleotide precursor miRNA (pre-miRNA) molecule[9-13]. This pre-miRNA is transported from the nucleus to the cytoplasm by Exportin 5/RanGTP, which specifically recognizes the structure of pre-miRNA molecule[14-16]. Pre-miRNA is then cleaved by Dicer and protein trans-activator RNA binding protein (TRBP) into a ~21 nucleotide miRNA duplex. Out of the two strands, one strand is then incorporated into the RNA induced silencing complex (RISC)[17]. Some miRNA primary transcripts encode only a single mature miRNA (mir-203), while other loci contain clusters of miRNAs that appear to be produced from a single primary transcript (mir-17-92 cluster)[18.]

It has recently been discovered that miRNAs are differentially expressed in AID and miRNA regulation may impact its development or prevention[20]. miRNAs have the potential to regulate the endogeous gene expression in immune homeostasis. A few of the key components of the miRNA pathway are found to be known autoantibody targets[21]. Yu *et al*[22] have recently demonstrated the involvement of miRNA in a pathway regulating autoimmunity in T lymphocytes of Sanroque mouse. This group has also reported that miR-101 was needed for the Roquin-mediated degradation of inducible T cell costimulator (ICOS) mRNA. Introduced mutations into miR-101 binding sites in the 3′-UTR of ICOS mRNA disrupted the repression of Roquin. These findings revealed an interesting miRNA-mediated regulatory pathway that prevented AIDs and accumulation of lymphocytes[22]. This demonstrates an autoimmune response to key components of the RNAi/miRNA pathways, which indicate the involvement of the miRNA pathway in the induction and production of autoantibodies in AID.

MicroRNA Profile in SLE

SLE is a complex AID caused by genetic and epigenetic alterations and characterized by chronic immune activation and multiple immunologic phenotypes. According to a comparative study between active and inactive SLE patients, eight miRNAs were downregulated, whereas no miRNA was upregulated in the active disease group[23-24].

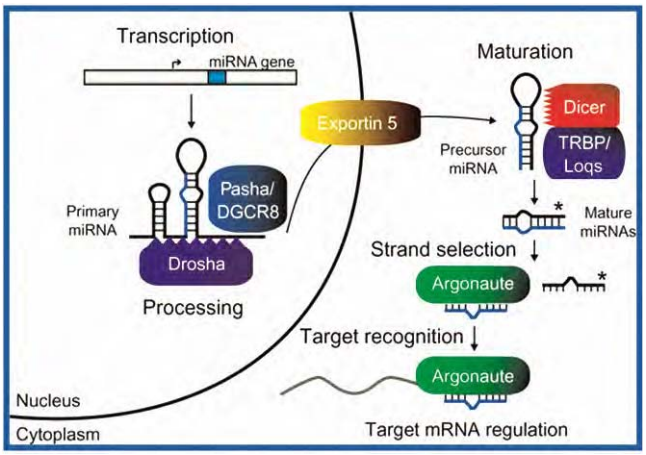

Fig. 1—Biogenesis of mammalian microRNA[19]

DNA methylation abnormalities play an important role in SLE disease processes. Though miRNAs can regulate DNA methylation by targeting the DNA methylation machinery, the role of miRNAs in aberrant $CD4^+$ T cell DNA hypomethylation of lupus is unclear. In a study involving high throughput microRNA profiling, Pan *et al*[25] identified two microRNAs, namely, miR-21 and miR-148a, that were over expressed in $CD4^+$ T cells from lupus patients, which promoted cell hypomethylation by repressing DNA methyltransferase1 (DNMT1) expression. This in turn led to the over expression of autoimmune-associated methylation-sensitive genes, such as, CD70 and LFA-1, *via* promoter demethylation. It was further understood that miR-148 directly down regulated DNMT1 expression by targeting the protein coding region of its transcript, while miR-21 indirectly down regulated the same by targeting the gene RAS guanyl releasing protein1 (RASGRP1). This study leads to the possibility that inhibition of miR-21 and miR-148a expression in $CD4^+$ T cells from patients with lupus could increase DNMT1 expression and attenuate DNA hypomethylation[25].

miRNAs, such as, miR-21, miR-25, miR-106b and miR-148b are reported to be highly correlated with SLE disease activity (r2>0.85), with miR-21 displaying the strongest correlation. The function of programmed cell death 4 (PDCD4) in SLE is not yet understood, however, it is likely that PDCD4 controls apoptotic or cell proliferation pathways. It is also likely that PDCD4, *via* miR-21, regulates cell-

signalling pathways participating in the orchestration of the cross talk between hyperactive B and T cells in lupus. Studies have shown that SLE T lymphocytes increase the basal and activation-induced miR-21 expression, in correlation with decreased expression of its gene target PDCD4. However, this effect was reversed by silencing miR-21, resulting in increased PDCD4 expression. Aberrant T cell responses in active SLE and T cell-driven B cell functions in human lupus were connected to the function of miR-21. This new evidence supports a central role for miR21/PDCD4-controlled pathways in mouse and human lupus. Further, elucidation of these pathways with studies in mouse models and human cells will shed light on the molecular complexity of this prototype autoimmune disease[26].

Along with miR-21, 8 other miRNAs, namely, miR-189, miR-61, miR-78, miR-142-3p, miR-198, miR-342, miR-299-3p and miR-298, were found to be up regulated, whereas seven miRNAs, namely, miR-196a, miR-17-5p, miR-409-3p, miR-141, miR-383, miR-112 and miR-184, were down regulated in peripheral blood mononuclear cells (PBMCs) of SLE patients. It was also notified that miR-31, miR-95, miR-99a, miR-130b, miR-10 and miR-134 were 6-fold down regulated in SLE patients as compared to controls[24]. A study conducted by Zaho *et al*[27] verified reduced expression of miR-125a and elevated levels of its predicted target Krupple-like factor (KLF13). This study also supported that miR-125a negatively regulated RANTES expression by targeting KLF13 in activated T cells and this under expression contributed to the elevated expression of RANTES in lupus[27].

A study conducted by Lee *et al*[8] on patients with SLE indicated that miR-146a was intrinsically under expressed and miR-146a expression levels correlated negatively with disease activity and interferon score. miR-146a was said to be associated with the type I IFN regulatory signal network after it was verified as a regulator of key signaling intermediates of the pro-inflammatory TLR-MyD88 pathway including IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6)[28]. It was also documented that by targeting critical proteins, such as, interferon regulatory factor 5 (IRF-5) and signal transducers and activators of transcription protein 1 (STAT-1), miR-146a reduced the expression of IFN inducible genes and regulates type I IFN pathway[29]. The level of miR-146a expression was positively correlated with levels of tumor necrosis factor-alpha (TNF-α) and *in vitro* studies showed the TNF-α up regulated miR-146a expression in T cells. Moreover, miR-146a over expression was found to suppress Jurkat T cell apoptosis. Tang *et al*[30] reported an over expression of miR-146a in primary PBMCs, resulting in inhibition of TLR-7 mediated IFN-α and IFN-β production. This study also reported that transfection with synthetic miR-146a hairpin inhibitor decreased the endogenous mRNA expression, leading to increased type IFN production[30]. It is hypothesized that manipulation of miR-146a levels might provide a potential beneficial therapy for SLE.

MiRNA and Cellular Mechanisms

Much emerging evidence has revealed that miRNA played an instrumental role in various cellular processes, including cell growth, differentiation, proliferation and cell death. miRNA profiling in Treg cells revealed a distinct pattern with conventional $CD4^+CD25^+$ T cells, thus suggesting an important role in the development of Tregs in thymus[31]. However, the involvement of miRNAs after Treg lineage commitment was less clear because effortor T cells (Teff) with dysregulated miRNAs were functionally impaired or even prone to autoimmunity through a mechanism involving miRNA-mediated mRNA decay[32]. Research studies are being conducted in profiling miRNAs in T cells and B cells, which are derived from the lupus-prone tri-congenic mouse model. It is believed that changes in the expression of miRNAs and a defected link in the molecular chain that each of these pathways represent will serve as a marker to trace these pathways, offering new insights into the pathogenesis of lupus[33]. Recently, Stagakis *et al*[26] hve studied miRNAs silencing to assess the effect of miRNAs on anti-CD3/anti-CD28-induced T cell proliferation and cytokine production. miRNA profiling in Treg cells displayed a unique profile of these cells when compared to naive T cells. Increased expression of certain miRNAs, such as, miR-21, miR-146a miR-223, miR-214, miR-125a and miR-155, as well as decreased expression of miR-150 and miR-142-5p were reported[31]. Studies by Li *et al*[34] have suggested that miR-181a acted as an intrinsic antigen sensitivity 'rheostat' during T-cell development and repressed the expression of PTPN22. miR-181a represses many phosphates that are negative regulators of TCR signaling including the PTPN22, thus enhancing the TCR signaling[34]. Finally, transcriptome analysis of miR-146a over expression in T cells has identified Fas associated factor 1

(FAF1) as a miR-146a regulated gene that was critically involved in modulating T cell apoptosis, revealing the significant role of miRNAs in cellular mechanisms in AID[14].

miRNA as Biomarkers

MiRNAs are attractive potential biomarkers because their expression pattern is reflective of underlying pathophysiologic processes and they are specific to various disease states. Moreover, miRNAs can be detected in a variety of sources, including tissue, blood and body fluids. They are reasonably stable and appear to be resistant to differences in sample handling, which increases their appeal as practical biomarkers. There is accumulating evidence that miRNAs have an important role as biomarkers in systemic rheumatic diseases as these diseases at different stages are associated with distinct miRNA expression profiles[29].

Future Applications

Further studies on miRNAs as crucial regulators in autoimmune response and diseases will be helpful in selecting interesting target miRNA for the development of new therapeutics. One such possible approach can be manipulation of miRNA expression levels that could help in designing new therapeutic interventions. As discussed earlier, it is also possible that some miRNAs could serve as biomarkers with clinical applications for the diagnosis or assessment of disease activity in SLE. Involvement of miRNA in induction and production of autoantibodies has been studied. It can, therefore, be speculated that the aberrant expression of certain miRNA may change the dynamics of the macro-molecular complex having a catalytic enzyme function. Gene silencing with antisense oligonucleotides can exploit the catalytic action of RNAse, allowing a single trigger to inherent the translation of a number of mRNA sequence. This approach can be gainfully utilized in designing catalytic antibodies as target therapies to treat AIDs as there is no evidence of autoantibodies directed toward miRNA as a group or toward individual miRNA. It is also believed that miRNA silencing could help in treatment of AIDs; however, in depth research in this direction is awaited.

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