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microRNA-126 expression and its mechanism of action in patients with systemic lupus erythematosus

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Abstract. – OBJECTIVE: The aim of the present study is to investigate the expression level of microRNA-126 (miRNA-126) in the plasma of patients with systemic lupus erythematosus (SLE).

PATIENTS AND METHODS: Thirty SLE patients admitted at our institution were recruited in study group and 30 normal subjects seeking health check-up at our institution during the same period were included in control group. Plasma levels of miRNA-126 were determined using fluorescent quantitative RT-PCR. Plasma levels of IFN-α were determined and compared between two groups using ELISA. RT-PCR was performed to measure the levels of ISG56 mRNA, an IFN-inducible gene (IFNG), in the peripheral blood mononuclear cells (PBMC) of both groups. miRNA-126 expression was interfered in PBMC of SLE patients by introducing the mimic and inhibitor of miRNA-126. Levels of ISG56 mRNA in transfected PBMCs were determined using RT-PCR.

RESULTS: Levels of miRNA-126 were significantly lower in the plasma of SLE patients than normal controls ($p < 0.05$). Plasma levels of IFN-α were significantly higher in SLE patients than normal population ($p < 0.05$). ISG56 mRNA in PBMC was significantly higher in SLE patients than controls ($p < 0.05$). As for SLE patients, levels of IFN-α and ISG56 mRNA were significantly decreased in PBMCs with high expression of miRNA-126 but were significantly increased in PBMCs with low expression of miRNA-126 ($p < 0.05$).

CONCLUSIONS: miRNA-126 expression is reduced in SLE patients. miRNA-126 may be involved in the initiation and development of SLE by inhibiting the production of IFN.

Key Words:
microRNA-126, Systemic lupus erythematosus, Interferon.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple organs with complex clinical features, which is characterized by the disorder of the immune system. The pathogenesis of SLE involves a variety of factors, mainly including hormones, environmental and genetic factors. In recent years, the interferon (IFN) family and its signaling pathway have been shown to play critical roles in the pathogenesis of SLE[1]. Studies have demonstrated the elevated plasma levels of INF-α in SLE patients and increased expression of type I IFN-induced MxA gene[2]. However, to date, the exact pathogenesis and regulatory mechanisms of SLE have not been entirely elucidated.

MicroRNA (miRNA) is a class of non-coding, endogenous, 22- nucleotide long RNA involved in post-transcriptional gene regulation. They have critical functions across various biological processes, such as regulation of the proliferation, differentiation, metabolism and apoptosis of cells, by suppressing mRNA translation. Recent studies have shown that aberrant miRNA expression is associated with the pathogenesis of many diseases, such as tumors[3-6], inflammatory diseases[7] and autoimmune diseases[8]. Of these miRNAs, miRNA-126 exhibits an anti-atherosclerotic effect by restoring the epithelial cells[9]. In addition, down-regulated expression of miRNA-126 in autoimmune diseases (such as diabetes mellitus) suggests its role as a biomarker for diabetes[10]. However, to date, few studies have been reported on the expression and the mechanism of action of miRNA-126 in SLE patients. To this end, in the present study, the expressions of miRNA-126 in the plasma of SLE patients were compared with those in normal population. Moreover, the effect of miRNA-126 in the IFN signaling pathways was investigated, thereby providing the experimental evidence for clinical research on the treatment of SLE.

Patients and Methods

Patients

Between April 2011 and September 2014, lupus patients admitted to the Department of

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Rheumatology and Immunology of our institution were included in this study. Of these patients, 30 patients (all females) with an age range of 48-72 years and mean age of (51.43 ± 12.25) years were included in the study group. All lupus patients met the classification and diagnosis criteria of SLE revised by 1997 American College of Rheumatology (ACR). Severe tuberculosis, fungal infection and other severe infections were not observed in these patients upon treatment. The control group included 30 female subjects (age range 51-71 years, mean age 50.46 ± 11.47 years) seeking health check-up during the same period. No significant differences were observed in the age, gender and clinical manifestations between two groups ($p > 0.05$).

Reagents

Trizol RNA isolation kit was purchased from Gibco, (Grand Island, NY, USA). RT-PCR kit was purchased from Thermo Fisher (Waltham, MA, USA). The mimic and inhibitor of miRNA-126 as well as the endogenous reference gene U6, were designed and synthesized by Guangzhou RiboBio Co., Ltd., China.

RNA Isolation and RT-PCR

In the morning, 5mL of fasting peripheral venous blood sample was collected from each SLE patient as well as from the healthy subjects. Blood samples were anticoagulated with EDTA followed by centrifugation at 4000 g for 5 min at 4°C, and the plasma at the upper layer was collected and stored at –80°C. A sample of ~200 µl plasma was homogenized in 1 mL Trizol reagent and total RNAs were isolated from these samples using Trizol RNA isolation kit. The extracted RNAs were dissolved in 30 µl diethylpyrocarbonate (DEPC) treated water and the concentration and purity of RNAs were analyzed using UV spectrophotometer (Shanghai AuCy Technology Instrument Co., LTD., China). Subsequently, RT-PCR was performed using ABI 7300 RT-PCR system (ABI, Foster City, CA, USA) and the generated cDNAs were stored at –20°C. RT-PCR reaction conditions were as follows: denaturation at 95°C for 20s, followed by 50 cycles of 60°C for 20s and 70°C for 1s. The primers for miRNA-126 were as follows: F: 5 -TATAAGATCTGAGGATAGGTGGGTTCCCGAGAACT-3 ; R: 5 -ATATGAATTCTCTCAGGGCTATGCCGCCTAAGTAC-3 . The primers for endogenous reference U6 were F: F:5 -AGCACAGAGCCTCGCCTTTG-3 R:5 -ACATGCCGGAGCCGTTGT-3 F: 5 -CTTGAGCCTCCTTGGGTTCG-3 ; R:5 -GCTGATATCTGGGTGCCTAAGG-3 . Relative quantitative analysis of RNA was performed using $2^{-\Delta\Delta Ct}$ method.

Isolation of Peripheral Blood Mononuclear Cells (PBMC)

A total of 10 mL whole blood was transferred in a 50ml centrifuge tube and diluted in 10 ml phosphate buffered saline (PBS). Five milliliters of Ficoll separation solution (Qcbio Science & Technologies Co, Ltd, China) was placed respectively in two 15 ml centrifugation tubes. Subsequently, 10 ml of diluted blood samples were placed on top of Ficoll solution respectively and centrifuged at 2000 rpm for 20 min. Peripheral blood mononuclear cells (PBMCs) that were aggregated in the white cell layer were collected with a pipette and stored in a 15 ml centrifuge tube for further analysis.

Cell Transfection

The expression of miRNA-126 was interfered by introducing the inhibitor and mimic of miRNA-126 into the PBMCs of SLE patients using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The efficiency of interference was evaluated at 48h after cell transfection.

ELISA

A total of 5 mL fasting peripheral venous blood sample was collected in the morning and anticoagulated with EDTA. Blood samples were centrifuged at 4000 g for 20 min at 4°C within 2h and the plasma at the upper layer was collected and stored for further analysis. The plasma levels of interferon-α (IFN-α) were measured using ELISA kit (BioLegend, San Diego, CA, USA) strictly following the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA) and graphs were generated using GraphPad Prism software version 5.0. Differences between two groups were analysed using Student's *t*-test. $p < 0.05$ was considered statistically significant.

Results

Plasma Levels of miRNA-126 mRNA in SLE Patients

Compared with normal population, the expression of miRNA-126 was significantly decreased in the plasma of SLE patients ($p < 0.05$) (Table I).

Table I. Serum levels of miRNA-126 mRNA in SLE patients and normal controls.

Group	microRNA-126	*t/p*
Normal controls (30)	1.31 ± 0.43	
SLE patients (30)	0.51 ± 0.28•	8.54/0.000

•*vs*. the serum of normal controls, $t = 8.54$, $p < 0.05$.

Plasma levels of IFN-α in SLE Patients

The result of ELISA showed that plasma levels of IFN-α were significantly elevated compared to those of normal population ($p < 0.05$) (Table II).

Levels of ISG56 mRNA in the PBMCs of SLE Patients

The levels of interferon-inducible gene, ISG56 mRNA were significantly higher than those of normal population ($p < 0.05$) (Figure 1).

Impact of miRNA-126 on INF Pathway

In SLE patients, levels of miRNA-126 mRNA were significantly increased in the PBMCs transfected with the mimic of miRNA-126, whereas the levels of IFN-α proteins and ISG56 mRNA were significantly decreased. Conversely, after introducing the inhibitor of miRNA-126 into PBMCs of SLE patients, miRNA-126 mRNAs were significantly decreased, whereas the levels of IFN-α proteins and ISG56 mRNA were significantly increased ($p < 0.05$) (Figure 2).

Discussion

SLE is an autoimmune disease that affects multiple systems and organs by multiple autoantibodies. SLE occurs more frequently in women and is featured by insidious or abrupt onset with severe and relapsing course, commonly manifested by skin lesions, including discoid erythema, butterfly-shaped rash, annular erythema and multiform erythema[11]. SLE, as a typical autoimmune disease, the development of SLE involves the disorders of nearly the entire immune system. Recent studies have revealed that miRNA plays crucial roles in the disorder of the immune system.

Table II. Serum levels of IFN-α in SLE patients and normal controls (pg/ml).

Group	IFN-α	*t/p*
Normal controls (30)	3.51 ± 0.91	
SLE patients (30)	5.98 ± 1.15•	9.23/0.000

•*vs*. the serum of normal controls, $t = 9.23$, $p < 0.05$.

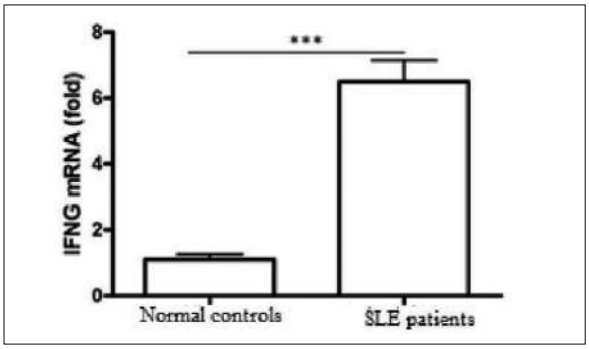

Figure 1. Levels of ISG56 mRNA in PBMCs from two groups. ***vs*. normal controls, $p < 0.001$.

miRNAs are a group of newly discovered non-coding RNAs of 22 nucleotides that are widely present in tissues, plasma, plasma or other body fluids. They can cause the degradation of mRNA or suppress gene translation by interacting with the 3'UTR of target mRNA. Recent studies have shown that alterations in the expression of miRNAs is closely associated with autoimmune disease by demonstrating that aberrant expression of miRNA-21 is closely associated with the initiation and development of type 1 diabetes and multiple sclerosis[12]. Moreover, miRNA146a has been shown to play regulatory roles in both rheumatoid arthritis and Sjogren's syndrome[13]. Furthermore, studies have demonstrated low expression of miRNA-126 in the epithelial cells of patients with type 2 diabetes and also showed that miRNA126 is involved in the initiation and development of diabetes by promoting the proliferation, migration and apoptosis of the epithelial cells[14]. However, to date, the expression of miRNA-126 and its function in other autoimmune diseases, such as SLE, have rarely been reported.

The results of the present work revealed that levels of miRNA-126 in the plasma of SLE patients were lower than those of normal population, indicating that reduced expression of miRNA-126 plays a critical role in the initiation and development of SLE. Liu et al[9] have shown that miRNA-126 expression is decreased in the plasma of patients with type 2 diabetes, further sup-

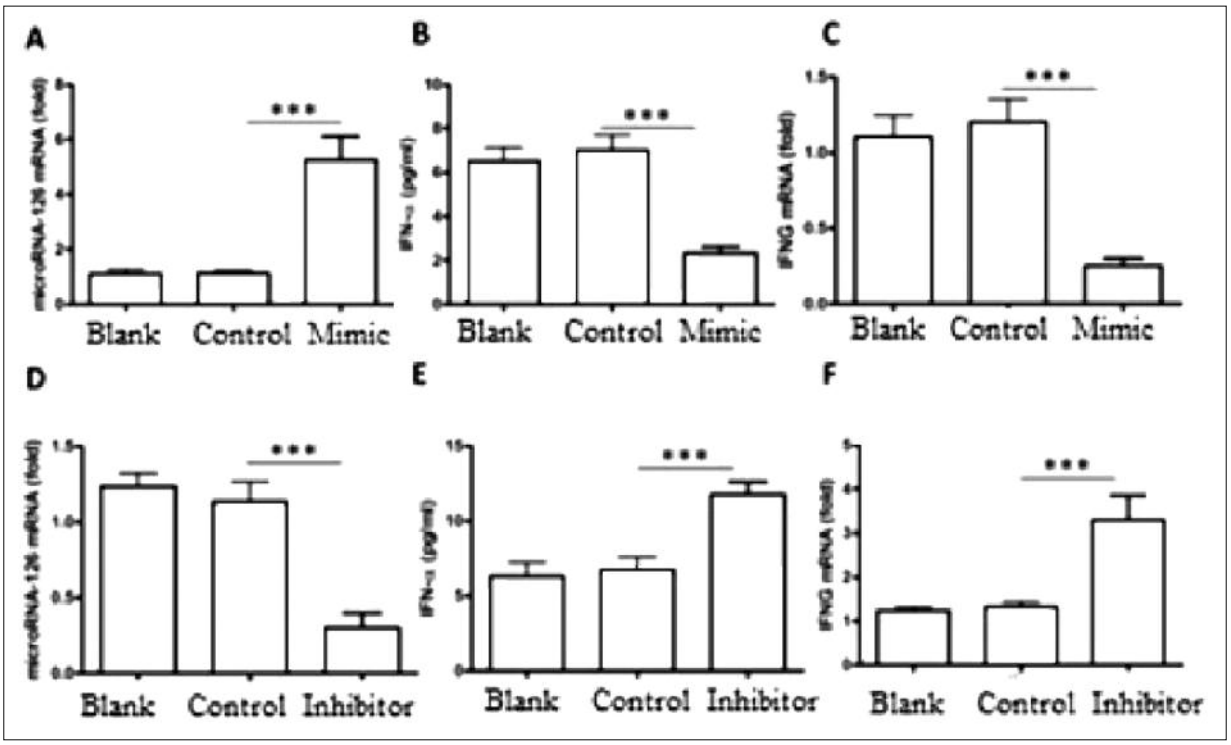

Figure 2. Impact of interfered miRNA-126 expression on IFN signaling pathway in PBMCs from SLE patients. ***vs*. normal controls, $p < 0.000$.

porting the findings that low expression of miRNA-126 plays regulatory roles in autoimmune diseases.

Inappropriate activation of type 1 IFN has been shown to be closely associated with the pathogenesis of SLE[15]. Some studies have shown that levels of IFN-α in SLE patients are significantly correlated with the titer of anti-dsDNA antibodies as well as with SLE activity scores[16]. The present study showed that plasma levels of IFN-α and ISG56 mRNA in the PBMC of SLE patients were significantly higher than those of normal controls, thereby, further suggesting that aberrant expression of genes in IFN pathway is closely associated with SLE. In addition, cell transfection with the mimic and inhibitor of miRNA-126 was performed to further investigate the impact of miRNA-126 on IFN signaling pathway in SLE patients. The results demonstrated that in SLE patients, levels of IFN-α and ISG56 mRNA were significantly decreased in the PBMC with high expression of miRNA-126. Conversely, levels of IFN-α and ISG56 mRNA were significantly increased in the PBMC with low expression of miRNA-126. These findings indicated that miRNA-126 can regulate the progression of SLE by suppressing IFN signaling pathway in PBMC of SLE patients. Narwal et al[17] have shown that clinical symptoms of SLE are significantly suppressed by interfering IFN-α with neutralizing antibodies. The present study suggested that miRNA-126 can serve as a therapeutic target in the treatment of SLE.

Conclusions

Low level of miRNA-126 plays an important role in the initiation and development of SLE. miRNA-126 can suppress the initiation and development of SLE by inhibiting IFN signaling pathway in PBMCs of SLE patients. In addition, the detection of miRNA-126 in the plasma of SLE patients is of certain value in the diagnosis, treatment and prognostic evaluation of SLE.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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