Clinical Rheumatology (2019) 38:1031–1038
https://doi.org/10.1007/s10067-018-4371-0

ORIGINAL ARTICLE

The potential role of Ets-1 and miR-326 in CD19$^+$B cells in the pathogenesis of patients with systemic lupus erythematosus

Li Jin[1] · Xuan Fang[1] · Chao Dai[1] · Nan Xiang[1] · Jinhui Tao[1] · Xiaoge Sun[1] · Farhana Azmi[2] · Qi Cao[2] · Xiaomei Li[1] · Yiping Wang[2] · Xiangpei Li[1]

Received: 31 August 2018 / Revised: 9 October 2018 / Accepted: 13 November 2018 / Published online: 19 November 2018

Abstract

Objectives The aim of this study was to investigate the B cell-associated transcription factors, Ets-1 and microRNA, miR-326 in systemic lupus erythematosus (SLE) patients, and their correlation with the pathogenesis of SLE.

Method A total of 44 SLE patients and 20 healthy controls were enrolled in this research, all patients fulfilled the American College of Rheumatology classification criteria for SLE. The mRNA expression of Ets-1 and miR-326 in CD19$^+$B cells from SLE patients were examined by qRT-PCR. The percentages of CD19$^+$CD138$^+$plasma cells were analyzed by Flow cytometry.

Results We found decreased expression of Ets-1 mRNA in SLE patients compared with the healthy controls ([0.228 (0.145, 0.507)] vs [0.583 (0.452, 0.763)], $p = 0.001$),while increased expression of miR-326 mRNA in CD19$^+$B cells SLE patients compared with the healthy controls([1.092 (0.457, 2.855)] vs [0.685 (0.274, 0.819)], $p = 0.008$). The percentage of CD19$^+$CD138$^+$plasma cells in SLE patients was higher than that of healthy controls ($0.55 \pm 0.21\%$ vs $0.36 \pm 0.21\%$, $p = 0.002$). Moreover, a negative correlation between expression of Ets-1 mRNA and miR-326 mRNA in CD19$^+$B cells was detected ($r = -0.334$, $p = 0.027$). A significant association between the occurrences of CD19$^+$CD138$^+$plasma cells and the levels of Ets-1 mRNA and miR-326 mRNA was observed ($r = -0.417$, $p = 0.005$ and $r = 0.482$, $p = 0.001$, respectively).

Conclusions Our results suggest that miR-326 might promote B cells differentiation by targeting Ets-1, a negative regulator of B cells differentiation and therefore participate in the pathogenesis of SLE.

Keywords B cells · Ets-1 · miR-326 · Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a complex, systemic, chronic autoimmune disease in which the human immune system becomes hyperactive with an uncertain etiology that can affect many different organ systems, including skin, kidney, and blood. SLE has many abnormal molecular pathways and several cell types can become dysregulated. B cells and autoantibodies have shown to play central roles in the progression of SLE. Furthermore, autoantibodies are critical for diagnosis and it arises years before any other evidence of immune dysregulation in patients [1].

Transcription factors (TFs) are a large class of regulators controlling gene expression by activating or repressing target genes at transcriptional level. Significant evidences indicated that TFs have important roles in the regulating networks of cell growth and developmental processes. Ets is the largest transcription factor family, which is known as E-twenty-six (ETS) DNA-binding domain to GGAA/T sequence motifs [2]. Among the Ets family, Ets-1 is one of the most important transcription factors, which controls a wide variety of cellular processes and plays a multifunctional role in autoimmune diseases. Recently, several studies that focused on the differentiation of B cells found that Ets-1 is vital in maintaining B cell identity, and Ets-1 deficiency can regulate B cell differentiation and lead to autoimmune disease in mice. [3] Ets-1 appeared to be a negative regulatory transcription factor for B

Li Jin and Xuan Fang contributed equally to this work.

✉ Xiangpei Li
lixiangpei55@126.com

[1] Department of Rheumatology and Immunology, Anhui Provincial Hospital Affiliated to Anhui Medical University, No.17 Lu Jiang Road, Hefei 230001, China

[2] Centre for Transplantation and Renal Research, Westmead Institute for Medical Research, The University of Sydney, Camperdown, NSW, Australia

cells and Th17 cells differentiation process [3, 4]. Particularly, Ets-1 is a susceptibility gene for SLE in Chinese populations. In addition, a decreased level of Ets-1mRNA is found in the peripheral blood mononuclear cell (PBMCs) of SLE patients [5, 6]. Despite of its important role in the development and function of human B cells, the molecular mechanisms that trigger and maintain Ets-1expression remains poorly understood.

In addition to transcription factors, microRNAs (miRNAs) are another class of important regulators of gene expression at the post-transcriptional level. They are endogenous single-stranded noncoding small RNA molecules composed of 20–24 nucleotides in length that can cause the degradation of mRNAs or repress translation by binding to the mRNAs of the target genes. Most of the miRNAs in organisms are conserved evolutionarily which are encoded by the gene families and are critically involved in a range of human diseases [7, 8]. Among them, miR326 is one of the miRNAs that is critical in regulating Th-17 differentiation, which is thought to be involved in some disease pathogenesis [9]. Our previous study has found the aberrant expression of miR-326 mRNA in SLE patients and MRL/lpr mice with SLE symptoms, which might have participated in the progression of the disease [10]. Here, we investigated the expression of miR-326 and Ets-1 mRNA in $CD19^+$B cells from SLE patients to study the role of miR-326, which may act through Ets-1 to regulate $CD19^+$B cell in SLE. These results will be the foundation for further elucidation of the association between Ets-1 and $CD19^+$ B in SLEs.

Materials and methods

Patients and healthy controls

According to the criteria of the American College of Rheumatology, 44 patients with SLE were recruited from the Rheumatology and Immunology Department, Anhui Provincial Hospital [11]. While individuals who had other rheumatic diseases, infections, or malignant tumors were excluded from this study. In the meantime, 20, sex- and age-matched healthy controls were also enrolled. Disease activity was evaluated/quantified by using the SLEDAI (systemic lupus erythematosus disease activity index). On the basis of SLEDAI scores, patients with inactive disease have SLEDAI score persistently ≤ 4 and had been taking prednisone 10 mg/day with stable condition for more than 1 year, but not taking immunosuppressant. However, if the score was ≥ 5, the disease was considered to be active.

Cell isolation

Peripheral blood mononuclear cells (PBMCs) from the patients and healthy controls were purified by density-gradient centrifugation on Ficoll-Hypaque centrifugation. PBMCs were further separated using $CD19^+$B Cell Isolation Kit (Miltenyi Biotec, Germany) according to the manufacturer's instructions. Isolated $CD19^+$B cells from SLE patients and healthy controls were used for the analysis of the mRNA expression of Ets-1 and miR-326.

Flow cytometry

PBMCs were stained in duplicate with Pecy5.5-anti CD19, FITC-anti-CD138, or isotype-matched control IgG (Beckton Dickinson, San Jose, USA) at 4 °C for 30 min, respectively. After being washed with PBS, the cells were acquired by FACS Calibur (Beckon Dickinson) and data were analyzed using FlowJo software (v5.7.2).

RNA extraction and preparation of cDNA

Total RNA containing mRNA and miRNA was extracted from $CD19^+$B cells immediately after isolation, using a Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. cDNA of mRNA and miRNA was generated by reverse transcription using PrimeScript RT reagent Kit (Takara Biotechnology, Japan) and Allin-One miRNA First-Strand cDNA Synthesis Kit (Gene Copoeia Inc., America) separately, according to the manufacturer's instructions.

Real-time quantitative PCR (qRT-PCR)

To quantify the mRNA and miRNA, the cDNA was amplified by qRT-PCR with SYBR Premix Ex Taq RT-PCR kit (Takara Biotechnology, Japan) and with All-in-One miRNA qRT-PCR Detection Kit. At the same time, the housekeeping gene β-actin and housekeeping gene U6 were used as the internal control. Samples were amplified in the ABI 7500 fast real-time PCR system (Applied Biosystems, CA, USA) which use a commercially available master mix, predesigned primer kits, and a default PCR cycle sequence. The relative expression level of miRNA was normalized to the internal control U6 and calculated using the $2^{-\Delta\Delta CT}$ (Livak) method. Also, the relative expression level of Ets-1 mRNA was normalized to the internal control β-actin and calculated using the $2^{-\Delta\Delta CT}$ (Livak) method.

Laboratory measurement

For all patients, serum antinuclear antibodies (ANA) were analyzed by indirect immunofluorescence assay on HEp-2 cell slides and ENA was measured by Europe-Mongolia immunity print method. Serum levels of immunoglobulin and complement were measured by immunoturbidimetry assay. Also, the quantity of white blood cell (WBC), red blood cell (RBC), platelet (PLT) in the whole blood, erythrocyte sedimentation rate (ESR), and so on were also tested.

Statistical analysis

SPSS software was used to analyze the data. Quantitative variables were described using mean ± SD, and the differences were analyzed by test. Nonparametric distribution data were expressed as median value and interquartile range (IQR). The differences of Ets-1 and miR-326 mRNA expression levels between subject groups were analyzed by Mann-Whitney *U* test. Correlations analysis was carried out using Spearman's rank test. Two tailed p values < 0.05 was considered statistically significant.

Results

The proportion of peripheral blood CD19$^+$CD138$^+$ plasma cells from SLE patients and healthy controls

In our study, we enrolled 22 active, 22 inactive SLE patients, and 20 healthy controls to detect the percentage of CD19$^+$CD138$^+$ plasma cells in CD19$^+$B cells in peripheral blood. The frequency of CD19$^+$CD138$^+$ plasma cells in SLE patients was significantly higher than that of the healthy controls (0.55 ± 0.21% vs 0.36 ± 0.21%, $p = 0.002$) (Fig. 1a), regardless being active or inactive patients ($p = 0.004$ and $p = 0.009$) (Fig. 1b). Furthermore, there is no apparent difference between active and inactive SLE patients (0.55 ± 0.24% vs 0.53 ± 0.18%, $p = 0.747$) (Fig. 1b).

The expression levels of Ets-1 and miR-326 mRNA in CD19$^+$B cells with SLE patients and healthy controls

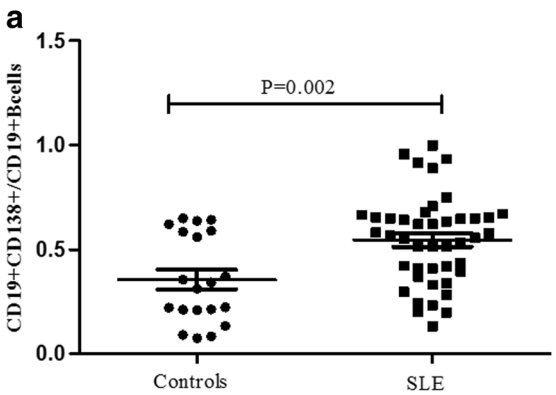
Ets-1 and miR-326 mRNA expression levels in CD19$^+$B cells from SLE and healthy controls were assessed by qRT-PCR. It was shown that Ets-1 mRNA expression level was decreased in CD19$^+$B cells with SLE patients (0.228 (0.145, 0.507)) than healthy controls (0.583 (0.452, 0.763)) ($p = 0.001$; Fig. 2a). Significant differences were also found in active SLE (0.155 (0.102, 0.201)) when compared with healthy controls (0.583 (0.452, 0.763)) and inactive SLE (0.454 (0.394, 0.752)) ($p < 0.05$). No significant difference was detected between inactive SLE groups and healthy controls ($p = 0.208$; Fig. 2b). In contrary, the expression levels of miR-326 mRNA was significantly higher in CD19$^+$B cells from SLE patients (1.092 (0.457, 2.855)) than that in healthy controls (0.685 (0.274, 0.819)) ($p = 0.008$; Fig. 2c). Meanwhile, when compared to active and inactive SLE and healthy controls, there is also an apparent increase in the expression levels of miR-326 mRNA in CD19$^+$B cells from active SLE patients (1.971(0.549, 4.369)). However, no significant difference was found between inactive SLE patients (0.771 (0.454, 1.573)) and healthy controls (0.685 (0.274, 0.819)) ($p = 0.227$; Fig. 2d).

Correlation between Ets-1 and miR-326 mRNA expression in CD19$^+$B cells of SLE patients

As it has been demonstrated that miR-326 suppressed the expression of Ets-1 protein in Treg cells [10, 11]; here, we explored the correlation between them in CD19$^+$B cells of SLE patients. As expected, we found negative correlation between miR-326 and Ets-1 mRNA expression in CD19$^+$B cells from SLE patients ($r = -0.334$, $p = 0.027$; Fig. 3). Although, no correlation was found between active and inactive SLE patients.

Relevance of the expression of Ets-1 and miR-326 mRNA with the percentage of CD19$^+$CD138$^+$ plasma cells in SLE patients

We also explore the relevance of the expression of Ets-1 and miR-326 mRNA with the percentage of CD19$^+$CD138$^+$ plasma cells in SLE patients. As shown in Fig. 4, negative correlation between Ets-1 mRNA expression and the proportion of CD19$^+$CDl38$^+$ plasma cells were found ($r = -0.417$, $p = 0.005$; Fig. 4a), whereas the level of miR-326 mRNA positively correlated with CDl9$^+$CDl38$^+$ plasma cells ($r = 0.482$, $p = 0.001$, Fig. 4b).



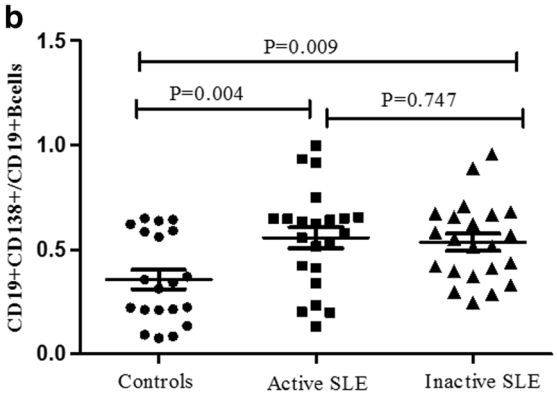


Fig. 1 The proportion of peripheral blood CD19$^+$CD138 $^+$plasma cells. **a** The percentage of expression of peripheral blood CD19$^+$CD138 $^+$plasma cells were compared between SLE and healthy controls. **b** The frequency of peripheral blood CD19$^+$CD138 $^+$plasma cells were compared with active SLE, inactive SLE, and healthy controls

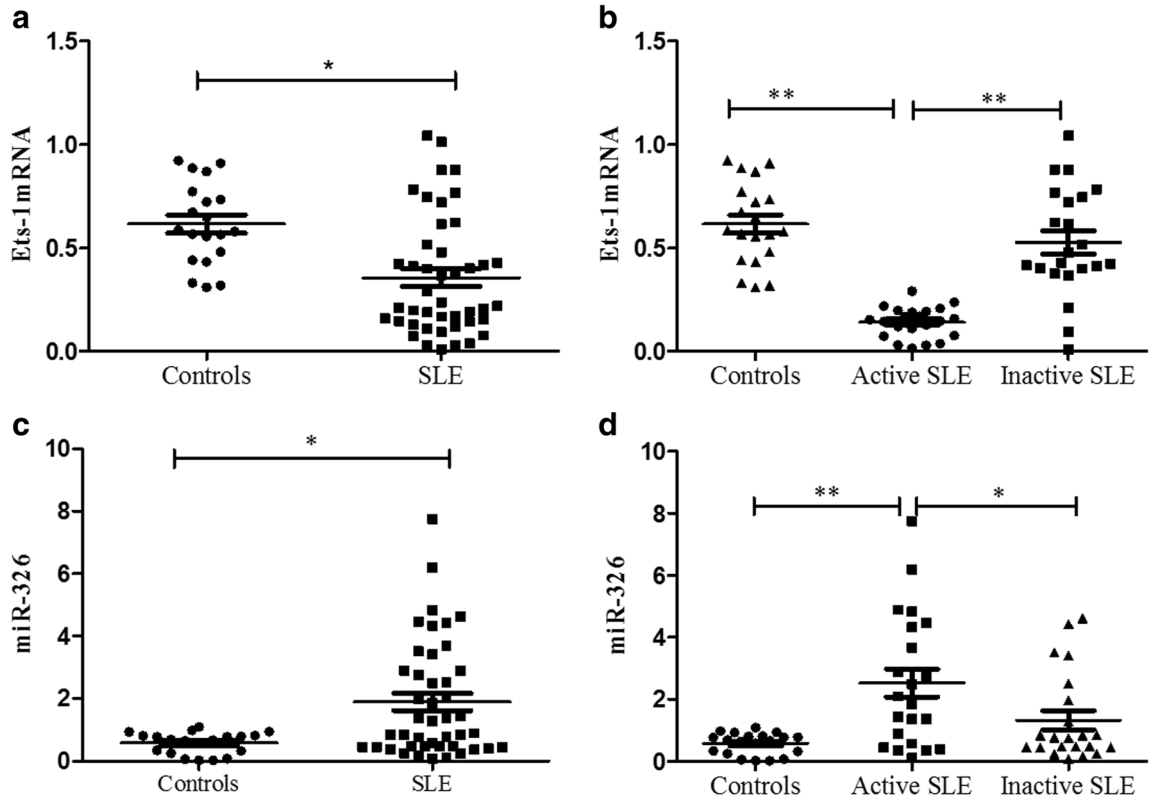


Fig. 2 The expression levels of Ets-1 and miR-326 mRNA in CD19$^+$B cells **a**, **c** Ets-1 and miR-326 mRNA expression levels in CD19$^+$B cells were compared between SLE and healthy controls. **b**, **d** The expression levels of Ets-1 and miR-326 mRNA in CD19$^+$B cells were compared with active SLE, inactive SLE, and healthy controls (*$p < 0.05$, **$p < 0.001$)

Relationship between Ets-1 and miR-326 mRNA levels in CD19$^+$B cells and clinical manifestations from SLE patients

It is well known that SLE is a heterogeneous disease that has a lot of different clinical manifestations. So, we compared Ets-1 and miR-326 mRNA expression levels in CD19$^+$B cells between 22 active SLE patients with or without some major clinical features, including butterfly erythema, alopecia, arthritis, oral ulcer, Raynaud phenomenon, fever, hematological abnormalities, nephritis, and serositis. We found that the levels of Ets-1 mRNA decreased in active patients with Butterfly erythema, alopecia, arthritis, whereas the levels of miR-326 mRNA increased. However, there is no significant difference between the comparisons. All the results were shown in Table 1. Further analysis was undertaken between the expression level of Ets-1, miR-326 mRNA in CD19$^+$B cells and SLEDAI in the group of SLE. Ets-1 mRNA expression levels were negatively correlated with SLEDAI scores in all SLE patients ($r = -0.581$, $p < 0.001$; Fig. 5a); in the contrary, miR-326 mRNA expression levels were positively correlated with SLEDAI ($r = 0.486$, $p = 0.001$; Fig. 5b). However, no association of the levels of Ets-1 or miR-326mRNA in CD19$^+$B cells with SLEDAI was found in active or inactive SLE patients.

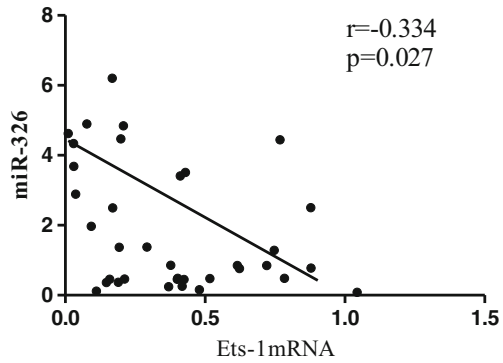


Fig. 3 Correlation between Ets-1 and miR-326 mRNA expression in CD19$^+$B Cells of SLE patients

Association of miR-326 and Ets-1 mRNA level in CD19$^+$B cells with laboratory assessments of SLE patients

We used Spearman's correlation to evaluate the association of Ets-1 and miR-326 mRNA expression level in CD19$^+$B cells from SLE patients with major laboratory parameters (WBC, RBC, PLT, ANA(D), IgM, IgG, IgA, C3, C4, CRP, ESR, anti-dsDNA, Anti C1q antibody, AnuA (anti-nucleosome antibodies)). Subsequently, we found positive association between Ets-1 mRNA expression level and WBC, RBC, HGB, C3,

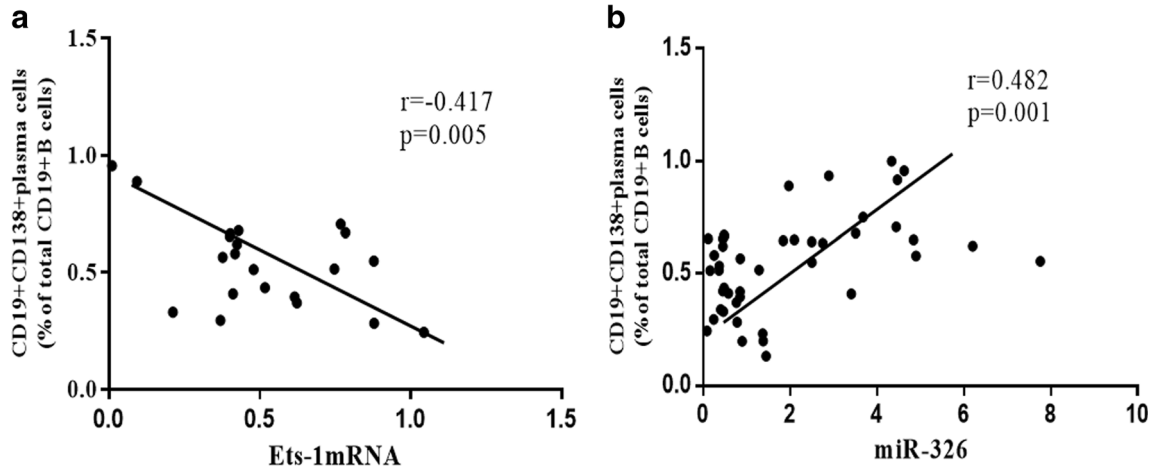


Fig. 4 Relevance of the expression miR-326 and Ets-1 mRNA with the percentage of $CD19^{+}CD138^{+}$ plasma cells in SLE patients. **a** The negative correlation between Ets-1 mRNA expression and the proportion of $CD19^{+}CDl38^{+}$ plasma cells. **b** The level of miR-326 mRNA positively correlated with $CDl9^{+}CDl38^{+}$ plasma cells

and C4, but negative correlation with ESR and ANA(D) (Table 2). At the same time, significant negative association with the expression level of miR-326 mRNA and C4 was also detected, ($r = -0.425$, $p = 0.004$). No significant association was found between other laboratory assessments (Table 2).

Discussion

Both B and T cells play essential roles in the pathogenesis of SLE. Abnormal activation of B cells produced mass of the autoantibodies (such as anti-dsDNA antibodies and antinucleosome antibodies) and cytokines involved in SLEs [12]. Therefore, the regulators of B cells differentiation are considered to have potential for clinical applications in the diagnosis or treatment of SLE patients. Ets-1 and PRDM1 (also known as Bimp1) are two molecules that can regulate B cells to differentiate into plasma cells to generate antibodies (Abs), a process relevant to both normal immune responses and autoimmune diseases.

Ets-1 is one of the transcriptional factors of the Ets family which acts as a negative regulator in the differentiation of B cells and Th17 cells [13]. Genome-wide association studies (GWAS) in Asian SLE patients identified genetic variants in Ets-1 which is associated with SLE [14, 15]. Also, the study

Table 1 Comparisons of Ets-1 and miR-326 mRNA level in $CD19^{+}$B cells with clinical parameters of active SLE patients

Group	+/−	*N*	Ets-1			miR-326		
			mRNA	*Z*	*p*	mRNA	*Z*	*p*
Butterfly erythema	+	14	0.149 (0.763, 0.201)	− 0.683	0.495	2.625 (1.179, 4.560)	− 1.502	0.133
	−	8	0.174 (0.119, 0.212)			1.134 (0.389, 2.528)		
Alopecia	+	6	0.122 (0.030, 0.199)	− 0.958	0.338	4.009 (1.427, 11.01)	− 1.769	0.077
	−	16	0.155 (0.122, 0.205)			1.610 (0.417, 2.855)		
Arthritis	+	12	0.145 (0.055, 0.216)	− 0.462	0.644	2.167 (0.528, 4.714)	− 0.198	0.843
	−	10	0.164 (0.115, 0.194)			1.970 (0.549, 3.181)		
Oral ulcer	+	6	0.183 (0.146, 0.223)	− 1.401	0.161	3.609 (1.175, 6.591)	− 1.180	0.238
	−	16	0.140 (0.074, 0.191)			1.610 (485, 3.482)		
Raynaud phenomenon	+	8	0.178 (0.059, 0.205)	− 0.401	0.682	3.284 (1.134, 4747)	− 1.160	0.246
	−	14	0.149 (0.102, 0.199)			1.412 (0.441, 3.149)		
Fever	+	10	0.149 (0.098, 0.208)	− 0.330	0.742	2.425 (1.135, 4.369)	− 0.528	0.598
	−	12	0.164 (0.085, 0.204)			1.408 (0.485, 4.352)		
Serositis	+	6	0.163 (0.138, 0.204)	− 0.590	0.555	2.061 (0.441, 6.591)	− 0.221	0.825
	−	16	0.149 (0.074, 0.205)			1.970 (0.660, 4.173)		
Nephritis	+	14	0.168 (0.121, 0.198)	− 0.599	0.549	2.098 (0.581, 4.337)	− 0.247	0.805
	−	8	0.128 (0.077, 0.208)			1.842 (0.364, 4.840)		

All values are median value (interquartile range)

+/−, with/without

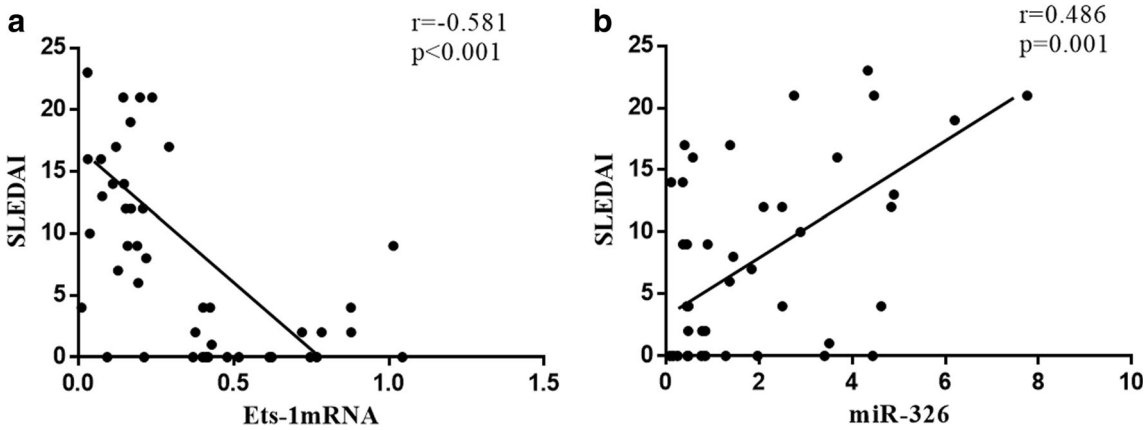


Fig. 5 The relationship between the expression of Ets-1mRNA and miR-326 in CD19$^+$B cells and SLEDAI with SLE patients. **a** The negative correlation between Ets-1 mRNA expression and SLEDAI. **b** The levels of miR-326 mRNA positively correlated with SLEDAI

detected that Ets-1 mRNA level was declined in PBMCs of SLE patients compared to healthy controls [16]. Our previous study similarly found that expression of the Ets-1 mRNA in Treg cells decreased in SLE patients [10, 17], while the expression of the Ets-1 mRNA in B cells is still unclear. Our data shows that the expression of the Ets-1 mRNA was lower in SLE patients when compared with healthy controls; moreover, the negative correlation between the percentage of CD19$^+$CD138$^+$ plasma cells and the level of the Ets-1 mRNA in B cells was shown. The data supports that decreased expression of Ets-1 mRNA may hinder the repressive activity of B cells differentiation in SLE patients.

Table 2 Association of miR-326 and Ets-1 mRNA level in CD19$^+$B cells with laboratory assessments of SLE patients

Laboratory assessments	Ets-1		miR-326	
	r	*p* value	*r*	*p* value
WBC	0.356	0.019*	−0.163	0.295
RBC	0.447	0.003*	−0.234	0.131
HGB	0.438	0.003*	−0.294	0.056
PLT	0.039	0.801	0.050	0.752
IgA	−0.078	0.625	0.119	0.453
IgG	0.008	0.961	0.275	0.078
IgM	−0.146	0.476	0.214	0.179
C3	0.512	0.001*	−0.295	0.055
C4	0.405	0.007*	−0.425	0.004*
ESR	−0.406	0.010*	0.312	0.053
CRP	−0.099	0.639	0.211	0.312
ANA(D)	−0.391	0.01*	0.226	0.151
Anti-ds-DNA(D)	0.036	0.907	−0.119	0.697
anti-C1q antibody	0.211	0.345	0.150	0.506
AnuA	0.017	0.940	0.181	0.421

ANA(D) Antinuclear antibodies titers, *AnuA* Anti-nucleosome antibodies
*$p < 0.05$

It is demonstrated that miRNAs are involved in crucial cellular processes and their dysregulation has been reported in a number of autoimmune diseases [18]. Liu et al have reported that miR-155 can directly regulate the expression of CD1d, which maybe one of the characteristics of dysfunctional B cells in patients with SLE [19]. We used the software miRanda, TargetScan, and PicTar, which on the basis of the bioinformatics methods, selected the miRNAs which had common target gene Ets-1. miR-326 was included among those miRNAs. Many studies have revealed that miR-326 affected the functions of Th17 and Treg cells, therefore modulating autoimmune pathogenesis [10, 11]. In our previous study, we found that, in the MRL/lpr mice, miR-326 overexpression led to B cell hyperactivity and also involved in renal pathology in SLE [8, 20]. Subsequently, in this research, we also confirmed the increased expression of miR-326 mRNA in CD19$^+$B cells, and a positive co-relationship between expression of miR-326 mRNA and the frequency of CD19$^+$CD138$^+$ plasma cells. Similarly, the levels of miR-326 mRNA were positively correlated with SLEDAI from SLE patients.

Complement activation is considered to be involved in tissue damage associated with SLE flare [21]. Our data also demonstrated that the levels of complement was declined in active SLE patients compared to inactive SLE patients, in spite of the levels of C3 or C4 (data was not shown). DJ Birmingham et al have reported that C4 activation is important in initiating renal flare while C3 activation is involved in the actual tissue damage, and these effects are influenced by genetic variability in complement activation and regulation [22]. Our current study established a positive correlation between the levels of C3, C4, and Ets-1 mRNA expression in CD19$^+$B cells in SLE patients, and negative correlation between the C4 levels and the expression of miR-326 mRNA in CD19$^+$B cells from SLE patients. Moreover, we also found that the expression of Ets-1 mRNA was correlated with more laboratory parameters, such as WBC, RBC, and HGB. Hence, it can be

confirmed that Ets-1 is particularly associated with disease activity in SLE patients.

Evidence showed that miR-326 mRNA expression significantly correlates with multiple sclerosis severity and onset of new SLE patients [7, 10]. Consistent with previous studies, we found positive correlation between the levels of miR-326 mRNA in $CD19^+$B cells and SLEDAI from patients with SLE. In contrast, the expression of Ets-1 mRNA in $CD19^+$B cells negatively correlated with SLEDAI in SLE patients. However, there is no relationship between active or inactive patients. It may be due to the smaller number of samples, resulting in poor statistical analysis of test efficiency.

Our preceding research identified that miR-326 could directly inhibit the expression of Ets-1 in B cells in the MRL/lpr mice. In the patients with SLE, we also found negative correlation between the levels of Ets-1 mRNA and miR-326 mRNA in $CD19^+$B cells. Further studies are necessary to explore the regulation mechanism of Ets-1 in B cells by gene transfection and functional research, which could reveal the role of miR-326 acting on Ets-1 in the pathogenesis of SLE. The molecular mechanisms of transcription and maturation of B cells regulated by miR-326 need to be confirmed as well.

In conclusion, we report quantifying the mRNA levels of Ets-1 and miR-326 in $CD19^+$B cells from SLE patients and healthy controls. For the first time, our group investigated and demonstrated an association between Ets-1 and miR-326 expression in $CD19^+$B cells in SLE patients. Our results suggested that the Ets-1 mRNA expression in $CD19^+$B cells decreased, while miR-326 expression increased in SLE patients. There is a negative correlation between Ets-1 mRNA and miR-326 mRNA expression, indicating that miR-326 may inhibit Ets-1 in $CD19^+$B cells. As a consequence, plasma cells differentiated from aberrant B cells will generate multiple of autoantibodies which might participate in the pathogenesis of SLE. Hence, miR-326 may become a new target of B cell regulation in the treatment of SLE patients.

Acknowledgements We thank all the members of our laboratory for their technical assistance.

Funding This work was supported by the National Natural Science Foundation of China (grant number 81373186).

Compliance with ethical standards

Disclosures None.

References

1. Arbuckle MR, Mcclain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB (2003) Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 349(16):1526–1533
2. Seuter S, Neme A, Carlberg C (2018) ETS transcription factor family member GABPA contributes to vitamin D receptor target gene regulation. J Steroid Biochem Mol Biol 177:46–52
3. Wang D, John SA, Clements JL, Percy DH, Barton KP, Garrettsinha LA (2005) Ets-1 deficiency leads to altered B cell differentiation, hyperresponsiveness to TLR9 and autoimmune disease. Int Immunol 17(9):1179–1191
4. Eyquem S, Chemin K, Fasseu M, Chopin M, Sigaux F, Cumano A, Bories JC (2004) The development of early and mature B cells is impaired in mice deficient for the Ets-1 transcription factor. Eur J Immunol 34(11):3187–3196
5. Wen Z, Xu L, Chen X, Xu W, Yin Z, Gao X, Xiong S (2013) Autoantibody induction by DNA-containing immune complexes requires HMGB1 with the TLR2/microRNA-155 pathway. J Immunol 190(11):5411–5422
6. Zhong H, Li XL, Li M, Hao LX, Chen RW, Xiang K, Qi XB, Ma RZ, Su B (2011) Replicated associations of TNFAIP3 , TNIP1 and ETS1 with systemic lupus erythematosus in a southwestern Chinese population. Arthritis Res Ther 13(6):R186
7. Aigner A (2011) MicroRNAs (miRNAs) in cancer invasion and metastasis: therapeutic approaches based on metastasis-related miRNAs. J Mol Med 89(5):445–457. https://doi.org/10.1007/s00109-010-0716-0
8. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136(2):215–233
9. Du C, Liu C, Kang J, Zhao G, Ye Z, Huang S, Li Z, Wu Z, Pei G (2009) MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol 10(12):1252–1259
10. Sun XG, Tao JH, Xiang N, Li XM, Wang GS, Fang X, Dai C, Zhang M, Chen Z, Li XP (2016) Negative correlation between miR-326 and Ets-1 in regulatory T cells from new-onset SLE patients. Inflammation 39(2):822–829
11. Du C, Liu C, Kang J, Zhao G, Ye Z, Huang S, Li Z, Wu Z, Pei G (2009) Corrigendum: MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol 10(12):1252–1259
12. Vaughn SE, Kottyan LC, Munroe ME, Harley JB (2012) Genetic susceptibility to lupus: the biological basis of genetic risk found in B cell signaling pathways. J Leukoc Biol 92(3):577–591
13. Moisan J, Grenningloh R, Bettelli E, Oukka M, Ho I (2007) Ets-1 is a negative regulator of Th17 differentiation. J Exp Med 204(12):2825–2835
14. Han JW, Zheng HF, Cui Y, Sun LD, Ye DQ, Hu Z, Xu JH, Cai ZM, Huang W, Zhao GP (2009) Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat Genet 41(11):1234–1237
15. Yang W, Shen N, Ye DQ, Liu Q, Zhang Y, Qian XX, Hirankarn N, Ying D, Pan HF, Chi CM (2010) Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. PLoS Genet 6(2):e1000841
16. Li Y, Sun LD, Lu WS, Hu WL, Gao JP, Cheng YL, Yu ZY, Yao S, He CF, Liu JL (2010) Expression analysis of ETS1 gene in peripheral blood mononuclear cells with systemic lupus erythematosus by real-time reverse transcription PCR. Chin Med J(Engl) 123(16):2287–2288
17. Xiang N, Li XP, Li XM, Wang GS, Tao JH, Pan HF, Fang X, Ma Q, Yu N (2014) Expression of Ets-1 and FOXP3 mRNA in CD4(+)CD25 (+) T regulatory cells from patients with systemic lupus erythematosus. Clin Exp Med 14(4):375–381

18. Wang S, Lu S, Geng S, Ma S, Liang Z, Jiao B (2013) Expression and clinical significance of microRNA-326 in human glioma miR-326 expression in glioma. Med Oncol 30(1):1–6
19. Liu F, Fan H, Ren D, Dong G, Hu E, Ji J, Hou Y (2015) TLR9-induced miR-155 and Ets-1 decrease expression of CD1d on B cells in SLE. Eur J Immunol 45(7):1934–1945
20. Xia Y, Tao JH, Fang X, Xiang N, Dai XJ, Jin L, Li XM, Wang YP, Li XP (2018) MicroRNA-326 upregulates B cell activity and auto-antibody production in lupus disease of MRL/lpr mice. Mol Ther Nucleic Acids 11:284–291. https://doi.org/10.1016/j.omtn.2018.02.010
21. Bao L, Quigg RJ (2007) Complement in lupus nephritis: the good, the bad, and the unknown. Semin Nephrol 27(1):69–80
22. Birmingham DJ, Irshaid F, Nagaraja HN, Zou X, Tsao BP, Wu H, Yu CY, Hebert LA, Rovin BH (2010) The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. Lupus 19(11):1272–1280