Rheumatol Int (2016) 36:1617–1625
DOI 10.1007/s00296-016-3550-z

Rheumatology INTERNATIONAL

BIOMARKERS

The role of microRNA-31 and microRNA-21 as regulatory biomarkers in the activation of T lymphocytes of Egyptian lupus patients

Khalda Sayed Amr[1,6] · Faten S. Bayoumi[2,3] · Fatema T. Elgengehy[4] · Sanaa O. Abdallah[5] · Hanan H. Ahmed[4] · Eman Eissa[2]

Received: 30 April 2016 / Accepted: 3 August 2016 / Published online: 10 August 2016

Abstract Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by familial aggregation and genetic predisposition. MicroRNAs (MiRNAs) serve as critical biomarkers in lupus patients because of their aberrant expression in different SLE stages. The study aimed to investigate the correlation of miR-31 and miR-21 with IL-2 in SLE patients as regulatory biomarkers in the activation of T lymphocytes of Egyptian lupus patients. Quantitative RT-PCR is carried out to estimate the expressions of miR-31 and miR-21, and IL-2 levels were determined using ELISA in plasma of 40 patients with SLE, 20 of their first-degree relatives and 20 healthy controls. The study also determined the systemic lupus erythematosus disease activity index (SLEDAI) score and proteinuria in SLE patients. The results revealed that miR-31 was lower expressed, while miR-21 was high expressed in SLE patients compared to their first-degree relatives and controls. MiR-31 was negatively correlated with SLEDAI and proteinuria in lupus patients, while miR-21 showed positive correlation with them. Also we found that there is a significant positive correlation between miR-31 and IL-2 in SLE patients, while miR-21 was negatively correlated with IL-2 level in patients. In conclusion, the study disclosed a significant association between miR-31 and miR-21 expression with IL-2 level in SLE patients. The regulatory biomarkers of miR-31 and miR-21 might have an impact on regulating IL-2 pathway expression and in turn on the activation of T lymphocytes in SLE.

Keywords SLE · MiR-31 · MiR-21 · IL-2

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease in which auto-antibodies are generated toward self-antigens such as components of cell nucleus [1, 2]. It has been proposed that genes and

✉ Khalda Sayed Amr
khalda_nrc@yahoo.com

Faten S. Bayoumi
drfaten100.nrc@gmail.com

Fatema T. Elgengehy
fatematalaat1980@yahoo.com

Sanaa O. Abdallah
sanaa.osman.aa@yahoo.com

Hanan H. Ahmed
hananhuss11@yahoo.com

Eman Eissa
emanessa82@yahoo.com

[1] Head of Medical Molecular Genetics Department, National Research Centre, Cairo, Egypt

[2] Immunogenetics Department, National Research Centre, Cairo, Egypt

[3] Head of Microbiology and Immunology Department, MSA University, Cairo, Egypt

[4] Department of Rheumatology and Rehabilitation, Faculty of Medicine, Cairo University, Cairo, Egypt

[5] Faculty of Science, Cairo University, Giza, Egypt

[6] Head of Medical Molecular Genetics Department, National Research Centre, El Buhouth St., Cairo 12622, Dokki, Egypt

environments are required for lupus to develop and flare [3]. The first-degree relatives of lupus patients have been demonstrated to be affected with SLE much higher as compared to controls in studies of familial aggregation [4, 5]. The incidence of SLE was more common in females of child-bearing age, where the reported female/male ratio was 8–15:1 with peak age of onset between 15 and 40 years [6–8].

MicroRNAs (MiRNAs) are noncoding RNAs with 22 nucleotides in length and function as posttranscriptional modulators for the mRNA expression of their targets via suppressing their translation or enhancing their degradation [9–11]. It is strongly apparent that they have a significant impact on the immune system and can confer robustness to the development of immune cells under environmental stress [11, 12]. Further studies demonstrated that aberrant miRNA expression contributes to the pathogenesis of autoimmune diseases, such as SLE [2, 3, 13] and other related diseases [11, 14]. It was found that decreased miRNA-126 and miR-146a levels contribute to the initiation and development of SLE in an IFN signaling-dependent manner [15–17]. In lupus CD4+ T cells, the up-regulated miR-21 and miR-148a suppress the expression of DNA methyl transferase 1 and in turn enhance DNA hypomethylation [18]. Also, different studies demonstrated the role of MiR-31 and miR-21 in the pathogenesis of SLE [19, 20].

Moreover, interleukin-2 (IL-2) is an important cytokine generated by T cells and involved in the activation and proliferation of T cells [21]. IL-2 is required for the generation and survival of the Treg cells, which are necessary for normal immune homeostasis [22, 23]. Decreased IL-2 production strongly leads to the generation of T cell-dependent pathogenic antinuclear antibodies, loss of tolerance to self-antigens and the subsequent onset of SLE [24–26].

In the present study, we investigated the expression pattern of miR-31 and miR-21 and their correlations with SLEDAI and proteinuria in lupus patients. The study also disclosed the correlations between miR-31 and miR-21 with IL-2 in patients with SLE that evaluate the regulatory effect of miR-31 and miR-21 on the activation of T lymphocytes.

Materials and methods

Ethics

This study was approved by the ethics committee of National Research Center, Giza, Egypt, and all samples were obtained with the written informed consents of the subjects.

Study subjects

This study included 80 subjects with age ranging from 15 to 50 years. They were divided into 3 groups: *Group 1*: consisted of 40 SLE patients, all of them met at least 4 of the American College of Rheumatology criteria for SLE diagnosis [27]. *Group 2*: consisted of 20 of first-degree relatives of SLE patients. *Group 3*: consisted of 20 healthy subjects matched for age and gender, serve as a control group.

Patients were obtained from inpatient unit of Rheumatology and Rehabilitation Department, Kasr Al Ainy Hospital, Cairo, Egypt. Clinical manifestations and treatments of SLE patients are summarized in Table 1. The disease activity was determined using SLEDAI score [28].

RNA extraction and quantitative real-time PCR

MicroRNA was extracted and isolated from plasma of all subjects of the study populations using miRNeasy Mini kit of Qiagen (Germany) according to the manufacturer's instructions. For miRNA-specific reverse transcription, microRNA was reverse-transcribed to cDNA using TaqMan® MicroRNA

Table 1 Clinical characteristics of the study populations

Characteristic	SLE patients	First-degree relatives of SLE patients	Controls
No. of cases	40	20	20
Sex, no. of male/female	0/40	0/20	0/20
Age, mean ± SD (years)	30.3 ± 8.8	31.55 ± 8.36	34 ± 5.45
Disease duration, mean ± SD (years)	7.5 ± 4.2	0	0
SLEDAI, mean ± SD score	5.3 ± 4.5	0	0
Lupus nephritis, numbers of positive/negative	30/10	0	0
Proteinuria, numbers of positive/negative	30/10	0	0
Proteinuria (g/day), mean ± SD	1.5 ± 1.6	0	0
Medications (steroids/hydroxychloroquine)	40/40	0	0

Reverse Transcription Kit (Applied Biosystems) and specific primers according to the manufacturer's instructions. Reverse transcription was performed under the following conditions: 30 min at 16 °C, 30 min at 42 °C and 5 min at 85 °C; then, the resulting cDNA was kept at −80 °C until use.

A real-time quantitative PCR (qRT-PCR) was carried out to quantify the expression levels in triplicate of mature miR-31 and miR-21 using TaqMan® MicroRNA Assay kit (Applied Biosystems) and TaqMan® Universal Master Mix (Applied Biosystems) using step one real-time PCR system (Applied Biosystems) according to the manufacturer's instructions. RNU6B (Applied Biosystems) was used as endogenous control to normalize the expression levels of target miRs. Relative quantification (Rq) of miRNA expression was calculated using the $2^{-\Delta\Delta CT}$ threshold cycle method. ΔCt was determined by subtracting the Ct values for RUN6B from the Ct values for the gene of interest. qRT-PCR was performed under the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 50 cycles at 95 °C for 15 s and at 60 °C for 1 min.

Enzyme-linked immunosorbent assay (ELISA)

Plasma IL-2 levels of all study subjects were determined using Human IL-2 Quantikine ELISA kit (R&D Systems) according to the manufacturer's protocol.

Statistical analysis

Data were statistically analyzed using SPSS version 19.0 software (SPSS Inc., Chicago, Illinois, USA). Nonparametric *T* test (Kruskal–Wallis test) was used to compare gene expression levels between groups, and Spearman's rank correlation to test association between gene expression levels and clinical parameters. Data were presented as the mean ± SEM. A *P* value of less than 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve was constructed for each miRNA to evaluate the efficiency of miRNAs as biomarkers for lupus patients against controls. Area under curve (AUC) values and 95 % confidence intervals for each miRNA were calculated.

Results

Expression pattern of miR-31 in SLE patients

The study revealed that miR-31 expression level was significantly lower expressed in the SLE patients compared with normal controls and the first-degree relatives of SLE patients (Fig. 1). In SLE patients, the level of miR-31 expression was 7.75-fold lower compared with control group, and its expression in the first-degree relatives of SLE patients was 1.32-fold lower compared with normal controls. A linear correlation analysis demonstrated a significant negative correlation between miR-31 expression with SLEDAI ($r = -0.809$) and proteinuria ($r = -0.930$) of lupus patients at $p < 0.01$ (Fig. 1).

Expression pattern of miR-21 in SLE patients

The results of the study showed the higher expression levels of miR-21 among SLE patients than controls and the first-degree relatives (Fig. 2). Twenty-five-fold up-regulation of miR-21 in lupus patients and a 1.64-fold up-regulation in the first-degree relatives were detected in comparison with controls. In addition, miR-21 expression is positively correlated with SLEDAI ($r = 0.957$) and proteinuria ($r = 0.934$) of SLE patients at $p < 0.01$ (Fig. 2).

ROC curve of miR-31 and miR-21

ROC curve showed that miR-31 has an AUC value of 0.969 (95 % CI 0.919–1.000), while miR-21 has an AUC value of 0.900 (95 % CI 0.786–1.000) at $p < 0.001$ versus normal controls. These results revealed that both of miR-31 and miR-21 could serve as efficient biomarkers for SLE patients against healthy controls (Fig. 3).

Expression of miR-31 and miR-21 in correlation with IL-2 concentration in lupus patients

The results clarified that the production of IL-2 was decreased in patients with SLE compared to control group and the first-degree relatives (Fig. 4). A linear correlation analysis was performed to examine the relationship between miR-31, miR-21 and IL-2 expressions in SLE patients. A positive correlation was detected between miR-31 expression and IL-2 concentration ($r = 0.893$) (Fig. 4). MiR-21 expression level was found to be negatively correlated with IL-2 concentration ($r = -0.861$) at $p < 0.01$ (Fig. 4).

Discussion

SLE is characterized by disturbance of immune system components, including the innate immune system, altered mechanisms of immune tolerance, increased activation of T and B cells, reduced clearance ability of immune complexes and apoptotic cells, and disturbances of immune regulators [29, 30]. SLE affects various organ systems such as kidney, skin, lung, joints and heart [2, 31]. MiRNAs serve as critical biomarkers in lupus patients because of their aberrant

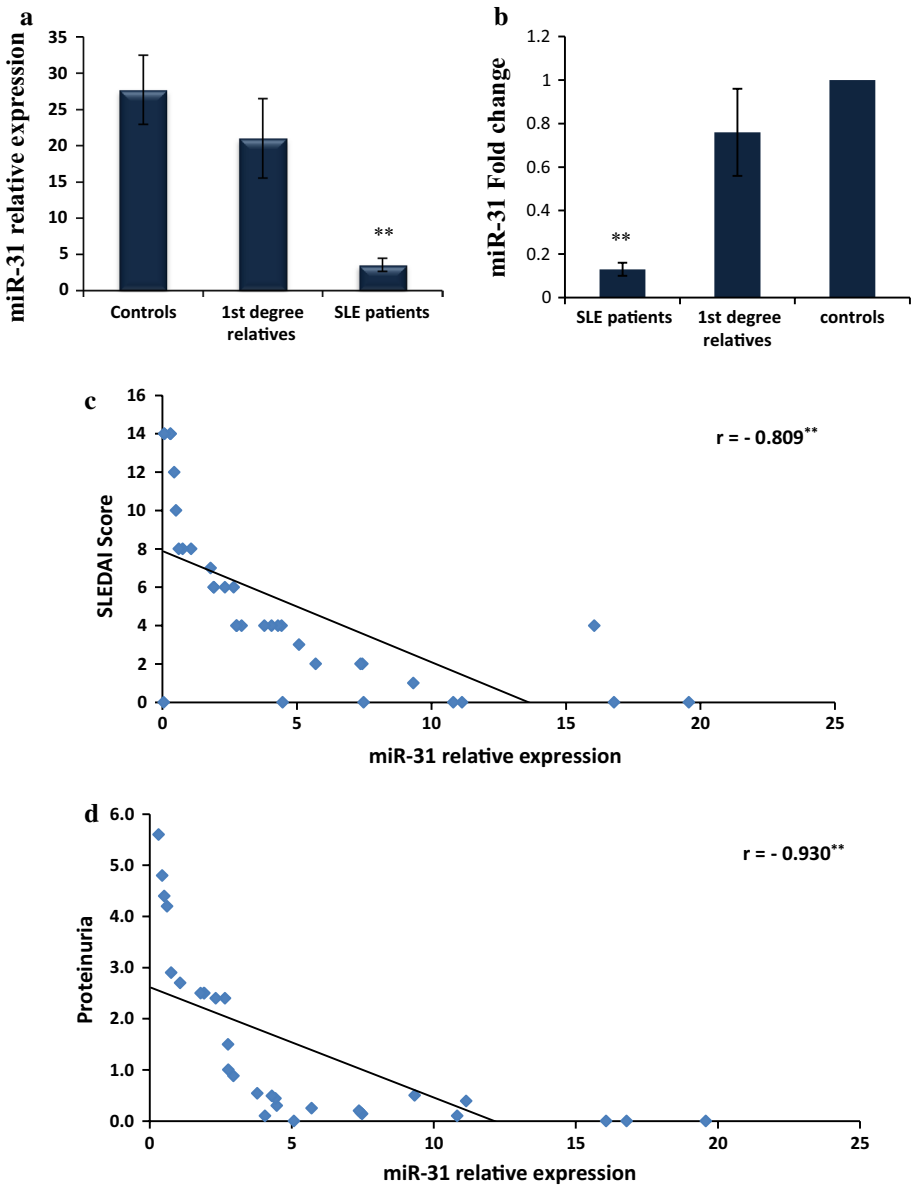

Fig. 1 **a** *Bars* show miR-31 expression in PBMCs from 40 SLE patients, 20 of their first-degree relatives and 20 normal controls examined by qRT-PCR. Results are represented as mean ± SEM relative expression. *Error bars* represent the standard error of the mean. *P* value between the groups was determined by nonparametric *T* test (Kruskal–Wallis test). $^{**}p < 0.01$ versus controls. **b** Fold change in miR-31 expression of lupus patients and their first-degree relatives is shown compared to controls. *Bar graph* represents the mean ± SEM fold change. $^{**}p < 0.01$ versus controls (by Kruskal–Wallis test). **c** A linear correlation analysis shows a negative correlation between miR-31 expression and SLEDAI in lupus patients. **d** A significant negative correlation between miR-31 expression and proteinuria in patients with SLE is shown by a correlation analysis. *r* Spearman correlation's rank. ** Correlation is significant at the 0.01 level

expression in different SLE stages [12, 13, 32]. They can be used in monitoring SLE disease severity function as novel potential targets for lupus treatment [2, 12, 13, 32].

In this study, we estimate the regulatory effect of miR-31 and miR-21 on the activation of T lymphocytes and their correlations with IL-2 in Egyptian patients with SLE.

The study demonstrated that miR-31 was significantly down-regulated in SLE patients compared to healthy controls and the first-degree relatives. In addition, the expression levels of miR-31 are inversely proportional to SLEDAI score and proteinuria of SLE patients. Consistent with these results, several studies reported the significant down-regulation of miR-31 in lupus patients [16, 19, 33]. Tang et al. [16] showed that 7 miRNAs (miR-31, miR-95, miR-99a, miR-130b, miR-10a, miR-134 and miR-146a) were under-expressed more than six-fold in SLE patients in comparison with normal controls. Another study done by Fan et al. [33] found that miR-31 expression was negatively correlated with SLEDAI and renal SLEDAI score in lupus patients, concluding that miR-31 expression may be related to disease activity and the degree of renal injury.

The study results demonstrated that IL-2 levels are significantly decreased in our SLE patients. For more than 30 years ago, it has been reported that the expression of IL-2 is decreased in lupus patients and in animal models of

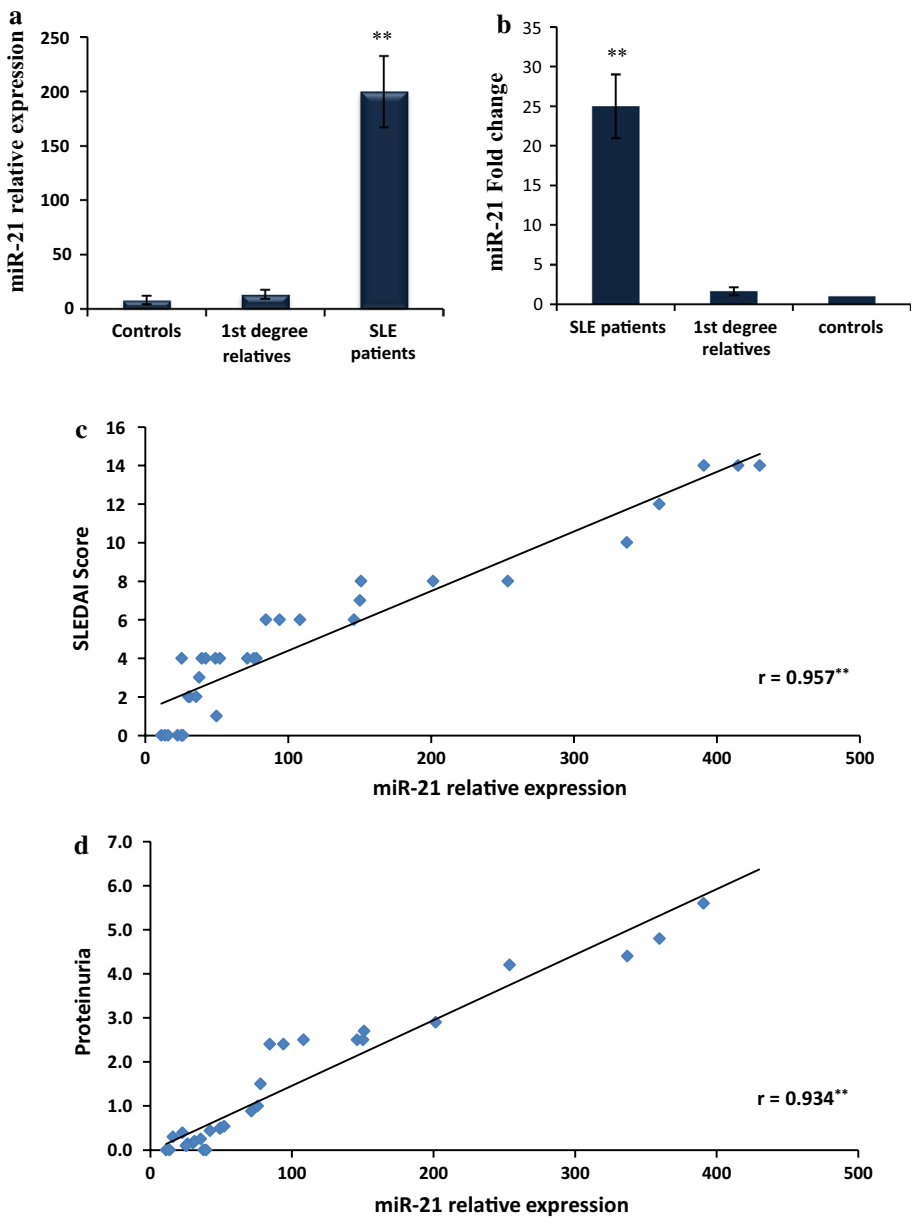

Fig. 2 **a** Relative miR-21 expressions quantified in PBMCs among the entire study sample using qRT-PCR are represented as mean ± SEM relative expression. $^{**}p < 0.01$ versus controls. *Error bars* represent the standard error of the mean. *P* value between the groups was determined by non-parametric *T* test (Kruskal–Wallis test). **b** MiR-21 fold change in lupus patients and their first-degree relatives is determined in relation to controls. *Bars* show the mean ± SEM fold change. $^{**}p < 0.01$ versus controls (by Kruskal–Wallis test). **c** MiR-21 expression is positively correlated with SLEDAI in patients with SLE (a linear correlation analysis). **d** A significant positive correlation is shown between expression of miR-21 and proteinuria in lupus patients (a linear correlation analysis). *r* Spearman correlation's rank. ** Correlation is significant at the 0.01 level

lupus [34, 35], which in turn had an impact on the decrease in Tregs and the elevation of cells producing IL-17 [36, 37]. IL-2 therapy at low doses has been suggested for reversing clinical manifestations of lupus [38–41]. Mizui et al. [41] showed that the inflammation of organs by the inflammatory cells is reduced by IL-2 administration. In addition, the treatment with IL-2 restored the function of Tregs and reduced tissue damage in SLE patients and in mice models of lupus [38–41].

The statistical results revealed that there was a linear positive correlation between miR-31 expression levels and IL-2 concentrations in SLE patients. These findings are in line with results of Fan et al. [19] who found that expression levels of miR-31 are positively correlated with the expression levels of IL-2 in activated lupus T cells. Moreover, IL-2 mRNA and protein levels were increased in human primary T cells upon transfection with miR-31, while these levels were decreased via silencing of the endogenous miR-31 by the inhibitory oligonucleotide antagomir-31 [19, 42]. The increased production of IL-2 through the miR-31 over-expression in T cells upon stimulation of TCR is mediated by altering the expression of NF-AT, targeting KSR2 and enhancing the activity of IL-2 promoter [9, 42]. In addition, over-expression of RhoA,

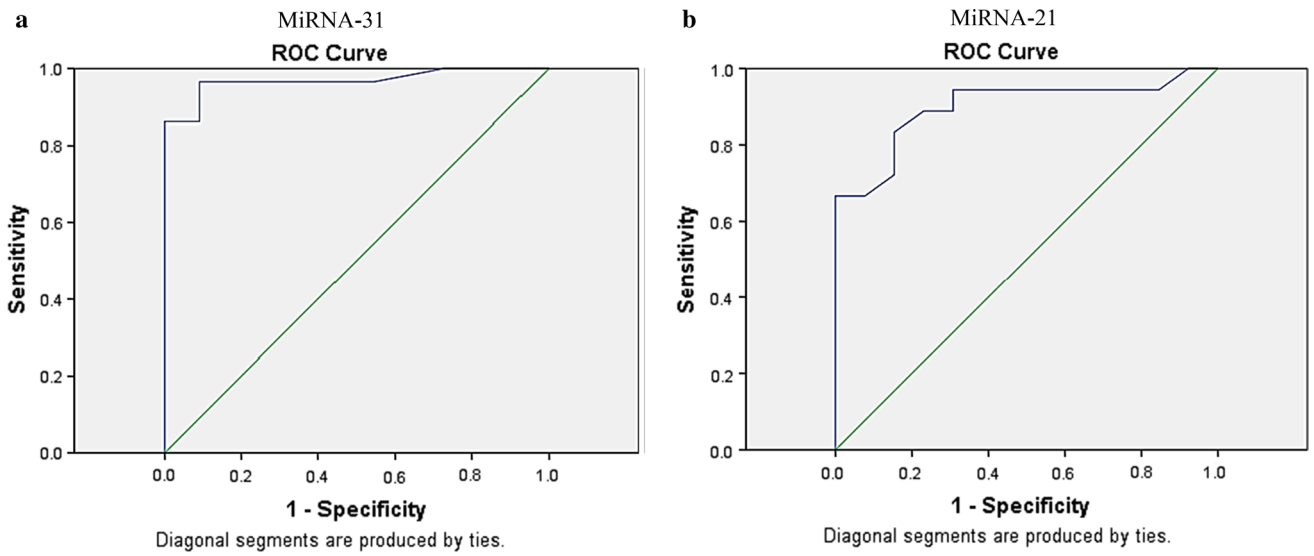

Fig. 3 **a** ROC curve of miR-31 for SLE patients versus normal controls (AUC value 0.969). **b** ROC curve of miR-21 for SLE patients versus normal controls (AUC value 0.900)

a target of miR-31, may result in impaired IL-2 promoter activity, which causes reduction in IL-2 expression in lupus T cells in a mechanism that depended on NF-AT [19, 43, 44]. The transcription factor NF-AT is essential for IL-2 expression by binding to IL-2 promoter and regulating its activity in Jurkat T cells [45–47].

On the other hand, the study results showed that miR-21 was significantly up-regulated in SLE patients compared to healthy controls and the first-degree relatives, and the expression levels of miRNA-21 are positively correlated with SLEDAI score and proteinuria of SLE patients. These results are similar to that of previous studies that reported that miR-21 expression was up-regulated in B and T lymphocytes of mouse lupus model B6.Sle123 as compared to control age-matched B6 mice [48] and in human lupus patients [49]. Furthermore, its expression was positively correlated with lupus disease activity [48, 49]. MiR-21 up-regulation in normal T cells resulted in exhibiting an activated phenotype, while T cells from lupus patients lose their activated phenotype upon miR-21 silencing. Therefore, miR-21 regulated aberrant responses of T cells of human SLE [49].

Splenomegaly, one of the autoimmunity manifestations of B6.Sle123 mice and cGVHD-induced mice, was reversed by miR-21 silencing in vivo [20, 48]. In addition, the splenic CD4+-to-CD8+ T cell ratio was significantly reduced and the number of splenic B cells expressing Fas receptor was decreased after knockdown of miR-21 [48].

Moreover, autoantibody titers and proteinuria were significantly decreased in miR-21 deficient hosts as compared to WT controls [20]. Collectively, these experiments in a model of lupus indicated that miR-21 deficiency in the host was sufficient to prevent lupus-like autoimmunity [20, 48].

In this study, we revealed that miRNA-21 expression levels are negatively correlated with IL-2 concentrations. This agrees with the results of Rouas et al. [50] that demonstrated that the expression of miR-21 can serve as an important positive regulator for FOXP3 expression. They also indicated that the expression of FOXP3 was negatively regulated by the effect of miR-31. FOXP3 is a transcription factor expressed on the surface of regulatory T cells, and it negatively regulates IL-2 gene expression by binding to its promoter region [51].

In addition, Murugaiyan et al. [52] revealed that the activation under Th17 conditions increased the expression of miR-21 specifically in T cells and there is a disturbance in the differentiation of Th17 with high IL-2 production in Mir21-/- mice as compared to control mice. They also demonstrated large amounts of IL-17 and other Th17-related cytokines and low IL-2 level upon inhibition of SMAD-7, a target of miR-21, in CD4+ T cells. Together, these observations demonstrated that miR-21 limited the production of IL-2 to promote Th17 differentiation. During Th17 differentiation, miR-21 plays a significant role in IL-2 under-expression via promoting the SMAD-mediated transcriptional down-regulation of IL-2 [52]. In autoimmune diseases, administration of IL-2 inhibits IL-17-producing CD4−CD8-double-negative T cells and activates Treg cells [41].

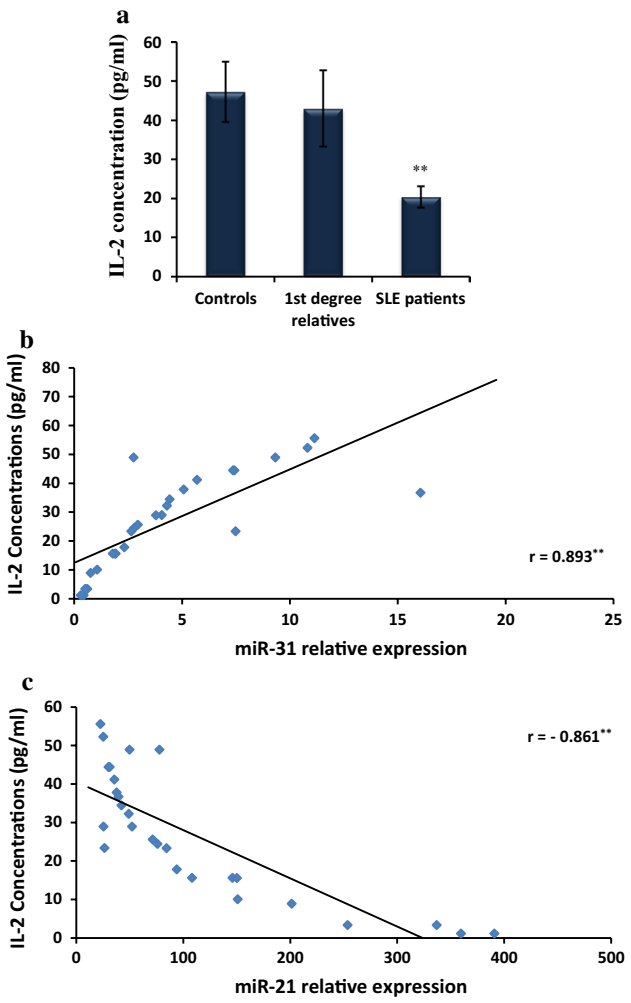

Fig. 4 **a** IL-2 concentrations determined in PBMCs from 40 SLE patients, 20 first-degree relatives and 20 normal controls using ELISA are represented. Bars show results as the mean ± SEM. *Error bars* represent the standard error of the mean. *P* value between the groups was determined by nonparametric *T* test (Kruskal–Wallis test). $^{**}p < 0.01$ versus controls. **b** A significant positive correlation between miR-31 expression and IL-2 concentration in lupus patients (a linear correlation analysis). **c** A significant negative correlation was detected between miR-21 expression and IL-2 concentration in SLE patients using a linear correlation analysis. *r* Spearman correlation's rank. ** Correlation is significant at the 0.01 level

Conclusion

Our study showed the correlations of miR-31 and miR-21 expressions with IL-2 levels in lupus patients. Our results indicated that both of miR-31 and miR-21 could serve as regulatory biomarkers in the activation of T lymphocytes in patients with SLE. We suggested that modulation of miR-31 and miR-21 expressions could restore the function of Treg cells and in turn reverse the clinical manifestations of SLE.

Acknowledgments This study was funded by National Research Centre, Cairo, Egypt.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study which involves human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

References

1. Ramos PS, Brown EE, Kimberly RP, Langefeld CD (2010) Genetic factors predisposing to systemic lupus erythematosus and lupus nephritis. Semin Nephrol 30(2):164–176
2. Qu B, Shen N (2015) MiRNAs in the pathogenesis of systemic lupus erythematosus. Int J Mol Sci 16(5):9557–9572
3. Xiao G, Zuo X (2016) Epigenetics in systemic lupus erythematosus. Biomed Rep 4(2):135–139
4. Hochberg MC (1987) The application of genetic epidemiology to systemic lupus erythematosus. J Rheumatol 14:867–869
5. Alarcón-Segovia D, Alarcón-Riquelme ME, Cardiel MH, Caeiro F, Massardo L, Villa AR, Pons-Estel BA, GrupoLatinoamericano de Estudio del Lupus Eritematoso (GLADEL) (2005) Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. Arthritis Rheum 52(4):1138–1147
6. Masi AT, Kaslow RA (1978) Sex effects in systemic lupus erythematosus: a clue to pathogenesis. Arthritis Rheum 21:480–484
7. Lu LJ, Wallace DJ, Ishimori ML, Scofield RH, Weisman MH (2010) Male systemic lupus erythematosus: a review of sex disparities in this disease. Lupus 19:119–129
8. Murphy G, Isenberg D (2013) Effect of gender on clinical presentation in systemic lupus erythematosus. Rheumatology 52:2108–2115
9. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297
10. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP (2008) The impact of microRNAs on protein output. Nature 455:64–71
11. Mehta A, Baltimore D (2016) MicroRNAs as regulatory elements in immune system logic. Nat Rev Immunol 16(5):279–294
12. Stypińska B, Paradowska-Gorycka A (2015) Cytokines and MicroRNAs as candidate biomarkers for systemic lupus erythematosus. Int J Mol Sci 16(10):24194–24218
13. Husakova M (2016) MicroRNAs in the key events of systemic lupus erythematosus pathogenesis. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. doi:10.5507/bp.2016.004
14. Martínez-Ramos R, García-Lozano JR, Lucena JM, Castillo-Palma MJ, García-Hernández F, Rodríguez MC, Núñez-Roldán A, González-Escribano MF (2014) Differential expression pattern of microRNAs in CD4+ and CD19+ cells from asymptomatic patients with systemic lupus erythematosus. Lupus 23(4):353–359
15. Liu YJ, Fan WJ, Bai JZ (2015) MicroRNA-126 expression and its mechanism of action in patients with systemic lupus erythematosus. Eur Rev Med Pharmacol Sci 19(20):3838–3842
16. Tang Y, Luo X, Cui H, Ni Z, Yuan M, Guo Y et al (2009) MicroRNA-146A contributes to abnormal activation of the type I

interferon pathway in human lupus by targeting the key signaling proteins. Arthritis Rheum 60(4):1065–1075
17. Hai-yan W, Yang L, Mei-hong C, Hui Z (2011) Expression of MicroRNA-146a in peripheral blood mononuclear cells in patients with systemic lupus erythematosus. Zhongguo Yi XueKeXue Yuan XueBao 33(2):185–188
18. Pan W, Zhu S, Yuan M, Cui H, Wang L, Luo X, Li J, Zhou H, Tang Y, Shen N (2010) MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyl transferase 1. J Immunol 184(12):6773–6781
19. Fan W, Liang D, Tang Y, Qu B, Cui H, Luo X et al (2012) Identification of microRNA-31 as a novel regulator contributing to impaired interleukin-2 production in T cells from patients with systemic lupus erythematosus. Arthritis Rheum 64(11):3715–3725
20. Garchow B, Kiriakidou M (2016) MicroRNA-21 deficiency protects from lupus-like autoimmunity in the chronic graft-versus-host disease model of systemic lupus erythematosus. Clin Immunol 162:100–106
21. Lieberman LA, Tsokos GC (2010) The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity. J Biomed Biotechnol 2010:740619
22. Burchill MA, Yang J, Vogtenhuber C, Blazar BR, Farrar MA (2007) IL-2 receptor and β-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells. J Immunol 178:280–290
23. Liao W, Lin JX, Leonard WJ (2013) Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. Immunity 38:13–25
24. Schorle H, Holtschke T, Hünig T, Schimpl A, Horak I (1991) Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature 352(6336):621–624
25. Horak I (1995) Immunodeficiency in IL-2-knockout mice. Clin Immunol Immunopathol 76(3 Pt 2):S172–S173
26. Horwitz DA (2010) The clinical significance of decreased T cell interleukin-2 production in systemic lupus erythematosus: connecting historical dots. Arthritis Rheum 62(8):2185–2187
27. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25:1271–1277
28. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang DH, The Committee on Prognosis Studies in SLE (1992) Derivation of the SLEDAI: a disease activity index for lupus patients. Arthritis Rheum 35:630–640
29. Firestein GS (2008) Kelley's textbook of rheumatology. W. B Saunders, Philadelphia
30. Chafin CB, Reilly CM (2013) MicroRNAs implicated in the immunopathogenesis of lupus nephritis. Clin Dev Immunol 2013:430239
31. Yan S, Yim LY, Lu L, Lau CS, Chan VS (2014) MicroRNA regulation in systemic lupus erythematosus pathogenesis. Immune Netw 14(3):138–148
32. Miao CG, Yang YY, He X, Huang C, Huang Y, Zhang L, Lv XW, Jin Y, Li J (2013) The emerging role of microRNAs in the pathogenesis of systemic lupus erythematosus. Cell Signal 25(9):1828–1836
33. Fan W, Liang D, Tang Y, Qu B, Cui H, Luo X, Huang X, Chen S, Shen N (2011) Expression of miR-31 in peripheral blood of patients with systemic lupus erythematosus. J Shanghai Jiaotong Univ 31:39–42
34. Song YC, Tang SJ, Lee TP, Hung WC, Lin SC, Tsai CY, Ng WV, Wu MF, Sun KH (2010) Reversing interleukin-2 inhibition by anti–double-stranded DNA autoantibody ameliorates glomerulonephritis in MRL-lpr/lprmice. Arthritis Rheum 62:2401–2411
35. Tsokos GC (2011) Disease pathogenesis: systemic lupus erythematosus. N Engl J Med 365:2110–2121
36. Mellor-Pita S, Citores MJ, Castejon R, Tutor-Ureta P, Yebra-Bango M, Andreu JL, Vargas JA (2006) Decrease of regulatory T cells in patients with systemic lupus erythematosus. Ann Rheum Dis 65:553–554
37. Wong CK, Lit LC, Tam LS, Li EK, Wong PT, Lam CW (2008) Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in autoimmunity. Clin Immunol 127:385–393
38. Webster KE, Walters S, Kohler R, Mrkvan T, Boyman O, Surh CD, Grey ST, Sprent J (2009) In vivo expansion of Treg cells with IL-2 mAb complexes; induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. J Exp Med 206:751–760
39. Sgouroudis E, Kornete M, Piccirillo CA (2011) IL-2 production by dendritic cells promotes Foxp3 + regulatory T-cell expansion in autoimmune resistant NOD congenic mice. Autoimmunity 44:406–414
40. Matsuoka K, Koreth J, Kim HT, Bascug G, McDonough S, Kawano Y et al (2013) Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. Sci Transl Med 5:179ra43
41. Mizui M, Koga T, Lieberman LA, Beltran J, Yoshida N, Johnson MC, Tisch R, Tsokos GC (2014) Interleukin-2 protects lupus-prone mice from multiple end organ damage by limiting CD4−CD8—Interleukin-17-producing T cells. J Immunol 193:2168–2177
42. Xue F, Li H, Zhang J, Lu J, Xia Y, Xia Q (2013) MiR-31 regulates interleukin 2 and kinase suppressor of ras 2 during T cell activation. Genes Immun 14(2):127–131
43. Helms WS, Jeffrey JL, Holmes DA, Townsend MB, Clipstone NA, Su L (2007) Modulation of NFAT-dependent gene expression by the RhoA signaling pathway in T cells. J Leukoc Biol 82:361–369
44. Crispin JC, Tsokos GC (2009) Transcriptional regulation of IL-2 in health and autoimmunity. Autoimmun Rev 8(3):190–195
45. Shaw JP, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR (1988) Identification of a putative regulator of early T cell activation genes. Science 241:202–205
46. Rooney JW, Sun YL, Glimcher LH, Hoey T (1995) Novel NFAT sites that mediate activation of the interleukin-2 promoter in response to T-cell receptor stimulation. Mol Cell Biol 15:6299–6310
47. Chow CW, Rincon M, Davis RJ (1999) Requirement for transcription factor NFAT in interleukin-2 expression. Mol Cell Biol 19:2300–2307
48. Garchow BG, BartulosEncinas O, Leung YT, Tsao PY, Eisenberg RA, Caricchio R, Obad S, Petri A, Kauppinen S, Kiriakidou M (2011) Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. EMBO Mol Med 3(10):605–615
49. Stagakis E, Bertsias G, Verginis P, Nakou M, Hatziapostolou M, Kritikos H, Iliopoulos D, Boumpas DT (2011) Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. Ann Rheum Dis 70(8):1496–1506
50. Rouas R, Fayyad-Kazan H, El Zein N, Lewalle P, Rothé F, Simion A et al (2009) Human natural Treg microRNA signature: role of microRNA-31 and microRNA-21 in FOXP3 expression. Eur J Immunol 39(6):1608–1618

51. Chen C, Rowell EA, Thomas RM, Hancock WW, Wells AD (2006) Transcriptional regulation by Foxp3 is associated with direct promoter occupancy and modulation of histone acetylation. J Biol Chem 281:36828–36834

52. Murugaiyan G, da Cunha AP, Ajay AK, Joller N, Garo LP, Kumaradevan S, Yosef N, Vaidya VS, Weiner HL (2015) MicroRNA-21 promotes Th17 differentiation and mediates experimental autoimmune encephalomyelitis. J Clin Invest. 125(3):1069–1080