The expression and significance of miR-17-92 cluster miRs in CD4+ T cells from patients with systemic lupus erythematosus

Sirs,

Systemic lupus erythematosus (SLE) is a chronic, potentially fatal systemic autoimmune disease that affects multiple organs. Although the etiology of SLE remains unclear, auto-reactive CD4+ T cells have been reported to contribute to the pathogenesis of SLE. The auto-reactive CD4+ T cells in SLE patients provided co-stimulatory signals and cytokines such as IL-21 to the B cells which stimulated B cell self-activation and autoantibodies production (1, 2). However, the mechanism of CD4+ T cells aberrant activity is incompletely elucidated.

MicroRNAs (miRNAs, miRs) are small noncoding, single-stranded RNA molecules that regulate gene expression at the posttranscriptional level by degrading or blocking translation of messenger RNA (mRNA) which involved in diverse biological processes. Dysregulation of miRNAs has also been described in various diseases including SLE (3). The miR-17-92 cluster, precursor for 7 mature miRs (miR-17, miR-17a, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a), is a regulator of immune system. Recent studies have shown that miR-17-92 play pivotal roles in T lymphocyte immunity. Higher miR-17-92 expression in transgenic mice could cause accumulation of CD4+ T cells which further leads to a breakdown of T cell tolerance, B-cell activation and autoantibodies generation (4). In addition, miR-17-92 cluster (except miR-92a) were upregulated in splenic T cells from MRL-lpr mice (5). Furthermore, transgenic mice overexpressing miR-17-92 in lymphocytes developed lymphoproliferative disease and autoimmunity (6). These suggested that the elevated miR-17-92 cluster miRs might be an important cause of aberrant CD4+ T cells reactivity. However, the role of miR-17-92 on CD4+ T cell has not been evaluated in SLE patients. In this study, we investigate the expression and significance of miR-17-92 cluster miRs in CD4+ T cells from SLE patients.

Thirty SLE patients who fulfilled the 1997 ACR revised criteria for classification of SLE were enrolled in this study (7). Disease activity was assessed using the SLE Disease Activity Index (SLEDAI). 18 age-and sex-matched healthy controls were recruited voluntarily. Relevant information regarding the study subjects is shown in Table I.

Table I. Clinical and laboratory characteristics of the subjects.

Characteristics	SLE	Controls
No. of cases	30	18
Male: Female	6:24	3:15
Age, yrs	31.7 ± 10.4	32.4 ± 10.9
C3, g/l	0.50 ± 0.27	—
C4, g/l	0.09 ± 0.03	—
dsDNA, IU/ml	522.8 ± 363.8	—
Red cell count, $\times 10^{12}$/l	3.74 ± 0.58	—
Lymphocyte count, $\times 10^{9}$/l	1.06 ± 0.56	—
Platelet count, $\times 10^{9}$/l	186.7 ± 57.6	—
No. of incipient patients	11	—
No. of active patients (SLEDAI ≥5)	17	—
No. of inactive patients (SLEDAI <5)	13	—
No. of Pred. treatment	22	—
No. of Pred. +CTX treatment	2	—
No. of Pred. +AZA treatment	2	—
No. of Pred. +HCQ treatment	3	—
No. of Pred. +MTX treatment	1	—

Values are mean ± SD; SLEDAI: SLE Disease Activity Index; Pred: Prednisone; CTX: cyclophosphamide; AZA: azathioprine; MTX: methotrexate; HCQ: hydroxychloroquine.

Using CD4 magnetic beads (Miltenyi Biotec, Germany), we obtained CD4+ T cells (purity>94%) from all subjects and extracted total RNA. Quantitative real-time RT-PCR assays were then performed in the rotor-gene Q (Qiagen, Germany) using All-in-One™ miRNA qRT-PCR Detection Kit (genecopoiea, USA). The independent-samples *t*-test for equality of means was used to compare values. The Pearson correlation test was used to examine the relationship between two continuous variables. A *p*-value less than 0.05 was considered statistically significant.

As shown in Figure 1A, The expression levels of the cluster miRs (except miR-92a) were significantly increased in CD4+ T cells

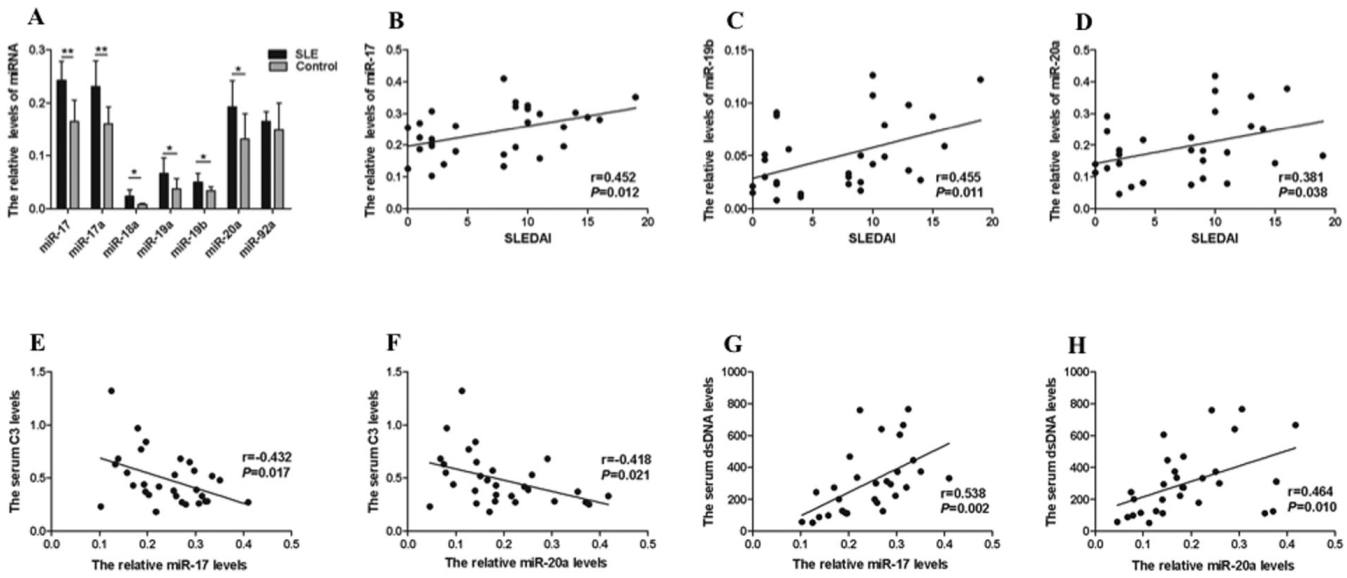

Fig. 1. Aberrant expression of miR-17-92 cluster miRs and correlations between the miR-17-92 cluster miRs and SLEDAI, C3, dsDNA in CD4+ T cells from SLE patients. A, Quantitative PCR analysis of miR-17-92 cluster miRs expression in CD4+ T cells from SLE patients and normal controls. The relative expression levels were normalised to the expression of U6 and the data were shown as mean±SD. B-D, correlations between SLEDAI score and miR-17 (B), miR-19b (C), miR-20a (D) in CD4+ T cells from SLE patients. E-H, correlations between C3 and miR-17 (E), miR-20a (F) and between dsDNA and miR-17 (G), miR-20a (H) in in CD4+ T cells from SLE patients. $^{*}p<0.05$; $^{**}p<0.01$.

from SLE patients than that from controls (miR-17: $p<0.001$; miR-17a: $p=0.001$; miR-18a: $p=0.02$; miR-19a: $p=0.04$; miR-19b: $p=0.031$; miR-20a: $p=0.02$; miR-92a: $p=0.36$). The findings were compatible with a previous study in MRL-lpr mice which showed that the cluster miRs (except miR-92a) were upregulated in splenic T cells (5). However, the expression levels of each miRNA in the cluster were different. The reason for the discrepancy on the each miRNA expression profiling between patients and normal controls remains unclear. Each miRNA within the cluster possesses individual or even antagonising functions may actually be one cause (8-10). We further analysed the correlation between the cluster miRs levels and SLEDAI, laboratory parameters in patients with SLE and observed positive correlations between SLEDAI and miR-17 (r=0.452, $p=0.012$; Fig. 1B), miR-19b (r=0.455, $p=0.011$; Fig. 1C), miR-20a (r=0.381, $p=0.038$; Fig. 1D) while positive correlations between dsDNA titres and miR-17 (r=0.538, $p=0.002$; Fig. 1G), miR-20a (r=0.464, $p=0.01$; Fig. 1H) and negative correlations between C3 levels and miR-17 (r=-0.432, $p=0.017$; Fig.1E), miR-20a (r=-0.418, $p=0.021$; Fig. 1F). These results suggested that the miR-17-92 cluster miRs might play pivotal roles in the pathogenesis of SLE. Abnormal expression levels of the miRs might be a useful indicator for disease activity and severity. Further investigation on the targets of cluster miRs in $CD4^+$ T cells would give us a deeper understanding of the pathogenesis of SLE.

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This research was supported by National Natural Science Foundation of China (grant no. 30972656) and Construction Program on Medicine-Phase ш of 211 Project (grant no. 211Med--XZZD03).

Competing interests: none declared.

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