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Original Article

Expression of lncRNA NEAT1 in peripheral blood mononuclear cells of patients with systemic lupus erythematosus and its correlation with Th1/Th2 balance

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Abstract: Objective: This study explored and analyzed the expression of LncRNA NEAT1 in peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE) and its correlation with Th1/Th2 balance. Methods: We chose 97 SLE patients admitted in our hospital from Jun. 2016 to Feb. 2019 as SLE group, and randomly selected 50 healthy volunteers that underwent physical examination in our hospital during the same period as control group. We detected the expression of LncRNA NEAT1 in PBMCs of the two groups of subjects by qRT-PCR, the degree of Th1 and Th2 cells in both groups by flow cytometry, and the expression of TFN-γ and IL-4 in both groups by ELISA. Results: The relative expression of LncRNA NEAT1 in PBMCs of SLE group was higher than that of control group (*P<0.05*). The proportion of Th1 and the ratio of Th1/Th2 cells in PBMCs were markedly lower in the SLE group than the control group (*P<0.05*), while the proportion of Th2 was higher in the SLE group than the control group (*P<0.05*). IFN-γ level in SLE group was much lower than the control group (*P<0.05*), while IL-4 level was evidently higher in the SLE group than in controls (*P<0.05*). The expression of LncRNA NEAT1 in PBMCs of SLE group was notably negatively correlated with Th1 proportion and Th1/Th2 ratio *(P<0.05*), while positively correlated with Th2 proportion (*P<0.05*). Conclusion: LncRNA NEAT1 in PBMCs of SLE patients is abnormally highly expressed, and this expression is negatively correlated with Th1/Th2 balance. These two factors may interact and jointly affect the occurrence and progression of SLE.

Keywords: Systemic lupus erythematosus, peripheral blood mononuclear cells, LncRNA NEAT1, Th1/Th2 balance

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease featuring the production of extensive autoantibodies and the involvement of multiple organs. The disease generally occurs in women of childbearing age, and its clinical manifestations are complex, affecting major systems throughout the body, and posing a serious threat to human health [1]. Currently, the pathogenesis of SLE has not yet been completely clarified. A large number of studies have shown that disorder of the regulatory function in the immune system is tightly correlated with disease occurrence [2]. Long non-coding RNA (LncRNA) is associated with the occurrence of a variety of immune inflammatory diseases, and is expected to be a biological targets for the diagnosis and treatment of immune diseases [3, 4]. Long non-coding RNA (LncRNA) nuclear-enriched abundant transcript 1 (NEAT1) exerts a pivotal regulatory influence in immune regulation. Studies have shown that the expression of LncRNA NEAT1 is up-regulated in sepsis patients, owing to the increased risk of patients, disease progression, and intensifying inflammation. It can reduce the survival rate of patients, suggesting that it may perform a key part in the occurrence and progression of infectious immune system diseases [5, 6]. Currently, there is a view that LncRNA is related to the pathogenetic and regulatory mechanism of SLE [7]. In this study, we explored the expression of LncRNA NeAT1 in PBMCs of SLE patients and its association with Th1/Th2 balance, which is reported as follows.

Cases and methods

Research objects

We chose 97 SLE subjects admitted in our hospital from Jun. 2016 to Feb. 2019 as SLE group, and randomly selected 50 healthy volunteers that underwent physical examination in our hospital during the same period as control group. The experiment was carried out under the authorization of hospital Ethics Committee.

Inclusion and exclusion criteria

Inclusion criteria: (1) SLE patients who met the diagnostic criteria of SLE classification revised by American College of Rheumatology (ACR) in 1997 [8]; (2) Patients aged between 18-60 years old; (3) Both groups of subjects were voluntarily signed the informed consent.

Exclusion criteria: (1) Those with other autoimmune illness such as severe infection, Sjogren's syndrome or rheumatoid arthritis; (2) Patients with malignant tumor; (3) The control-group subjects had no autoimmune diseases, and their immediate relatives had not experienced any autoimmune diseases. In addition, no hormones or immunosuppressants were used in the past 1 month.

Expression of LncRNA NEAT1 detected by qRT-PCR

We collected early morning fasting venous blood from two groups of subjects, placed in heparin-free anticoagulant tubes, and separated PBMCs by human Ficoll lymphocyte separator (purchased from Beijing Solarbio Science & Technology Co., Ltd.). We applied Trizol reagent (purchased from Invitrogen, USA) to extract the total RNA of PMBCs, determined the concentration and purity of RNA by spectrophotometer, and selected those that have high purity and were without DNA contamination or degradation for cDNA synthesis. The whole extracting procedures were strictly handled in accordance with the reagent instructions. Subsequently, by using the one-step method with One Step RT-PCR Master Mix Kit (purchased from Beijing Biolabo Technology Co., Ltd.), we transcribed and synthesized the cDNA. Using cDNA as template, the reaction system was prepared on the basis of 2 × SYBR Green PCR Master Mix, and PCR amplification and detection were performed by qRT-PCR instrument. The primers were synthesized by Shanghai Shenggong Biological Engineering Co., Ltd. with sequence as follows: The forward and reverse primers of LncRNA NEAT1 is 5'-TGGCTAGCTCAGGGCTTTCAG-3' and 5'-TCTCCTTGCCAAGCTTCCTTC-3'; and those of GAPDH are 5'-TGAACGGGAAGCTCACTGG-3' and 5'-TCCACCACCCTGTTGCTGTA-3', respectively. By using GAPDH as the reference gene, we calculated the relative expression level of LncRNA NeAT1 by $2^{-\Delta\Delta CT}$ method.

Detection of Th1 and Th2 cell degree by flow cytometry

We collected the early morning fasting venous blood in two groups of objects for the isolation of PBMCs, detected the Th1 and Th2 cell degree by flow cytometry (purchased from Beckman, USA), and calculated the Th1/Th2 ratio. The specific operation method was given previously [9].

Detection of IFN-γ and IL-4 levels by ELISA

We collected the early morning fasting venous blood in two groups of objects, separated the blood serum, and detected the levels of IFN-γ and IL-4 with ELISA method. The operation was carried out in strict accordance with kit instructions. IFN-γ Kit (Shanghai Biyuntian Biotechnology Co., Ltd. No: PI511), IL-4 Kit (Shanghai Biyuntian Biotechnology Co., Ltd. No: PI618).

Statistical analysis

Data processing and analysis was conducted by SPSS 23.0. The measurement data was compared by *t*-test, the enumeration data was compared by χ^2 test, and the correlation between the parameter was performed by Pearson correlation analysis. A statistically significant difference was set at $P<0.05$. The graphic software was Graphpad prism 9.0.

Results

Clinical data

There was no significant difference in gender, age and BMI between the two groups ($P>0.05$) (**Table 1**).

Expression of LncRNA NEAT1 in PBMCs of both groups

The relative expression of LncRNA NEAT1 in SLE group was critically higher than the control group ($P<0.05$) (**Table 2**).

Table 1. Comparison of clinical data between the two groups of subjects

Group	Number of cases	Gender		Age (yd, $\bar{x} \pm s$)	BMI (kg/m^2, $\bar{x} \pm s$)
		Male	Female		
SLE group	97	69	28	35.42±6.30	23.17±3.28
Control group	50	31	19	36.01±8.36	23.64±3.45
t/χ^2	-	1.266		0.480	0.809
P	-	0.261		0.632	0.420

Table 2. Expression of LncRNA NEAT1 in PBMCs of both groups ($\bar{x} \pm s$)

Group	Number of cases	Relative expression of LncRNA NEAT1	t	P
SLE group	97	1.731±0.289	16.203	<0.05
Control group	50	1.028±0.142		

Table 3. Comparison of Th1 and Th2 cell percent between two groups of subjects ($\bar{x} \pm s$)

Group	Number of cases	Th1 cells (%)	Th2 cells (%)	Th1/Th2
SLE group	97	8.29±2.11	8.13±1.92	1.05±0.33
Control group	50	12.32±3.16	5.42±1.55	2.21±0.78
t	-	9.206	8.631	12.644
P	-	<0.05	<0.05	<0.05

Comparison of Th1 and Th2 cell degree between two groups

The proportion of Th1 and the ratio of Th1/Th2 cells in the SLE group were markedly lower than in the control group (*P<0.05*), while the proportion of Th2 was higher in the SLE group than in controls (*P<0.05*) (**Table 3** and **Figure 1**).

Comparison of IFN-γ and IL-4 levels between two groups of subjects

The IFN-γ level in SLE group was markedly lower than in controls (*P<0.05*), while the IL-4 level was higher in the SLE group than in the control group (*P<0.05*), as shown in **Table 4**.

Correlation analysis

The expression of LncRNA NEAT1 in the SLE group was notably negatively correlated with Th1 proportion and Th1/Th2 ratio *(P<0.05*), while positively correlated with Th2 proportion (*P<0.05*) (**Table 5**; **Figure 2**).

Discussion

Many pathogenic antibodies against their own tissue components are produced during the occurrence and development of systemic lupus erythematosus (SLE). These antibodies cause damage to target tissues, and lead to the involvement of multiple organ systems [10]. Due to the poor sensitivity and specificity of the current SLE biomarkers, new biomarkers need to be developed [11]. In recent years, the study of genetics, especially the function of non-coding RNA in rheumatic diseases, has become a focus of researchers. Studies have shown that miR-196a and miR-21 are differentially expressed in SLE patients, and are closely associated with the activity of SLE [12, 13]. The non-coding RNA-lncRNA, that has the same regulatory function as miRNA, has also gradually attracted attention [15, 16]. According to related studies, a large number of LncRNAs can be regulated by miRNA or interact between each other [17]. Since LncRNA itself can regulate and control the biologic functions of the required cells, it has exerted a regulatory role in growth, progression, differentiation, and apoptosis of cells [18, 19].

LncRNA is a eukaryotic RNA without coding function, and its length exceeds 200 nucleotides [20]. Compared with short-stranded non-coding RNA, LncRNA is prone to mutations in its sequence, and its position on the chromosome is stable, so its function is highly conserved [21]. The correlation between LncRNA and SLE has been confirmed with the continuous development of genome-wide association analysis, and studies have also shown that multiple gene loci are associated with the pathogenesis of SLE [22]. LncRNA is = expressed in the nucleus, which is not only involved in the occurrence and progression of tumors, but also participates in immune regulation and imposes crucial regulatory function over the immune response [23]. Studies have found [24] that the LncRNA NEAT1 expression level is sub-

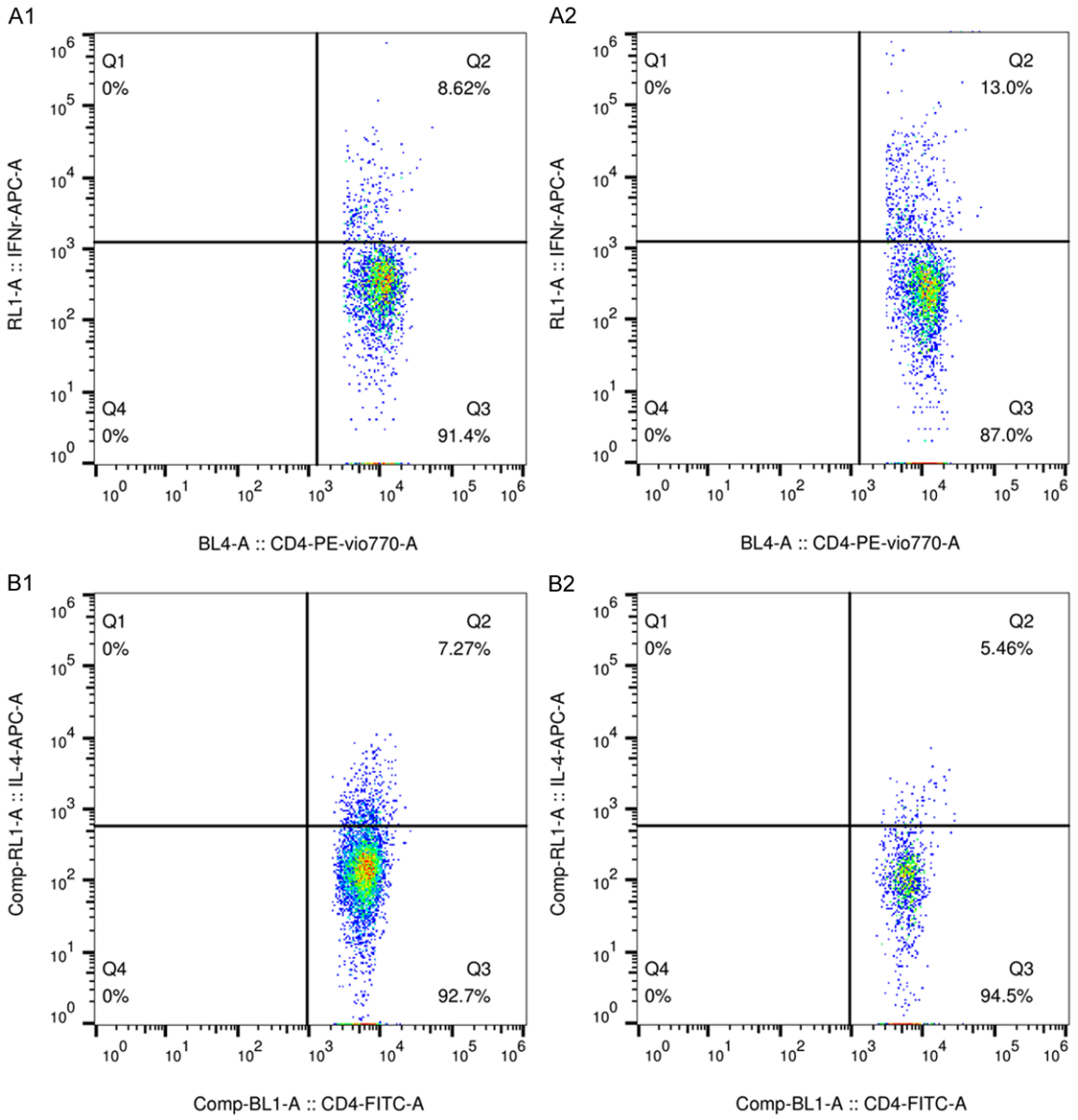

Figure 1. Percentages of Th1 and Th2 cells were detected by flow cytometry. Note: A1: Th1 cells in SLE group; A2: Th1 cells in control group; B1: Th2 cells in SLE group; B2: Th2 cells in control group.

Table 4. Comparison of IFN-γ and IL-4 levels between two groups of subjects ($\bar{x} \pm s$)

Group	Number of cases	IFN-γ (μg/L)	IL-4 (ng/L)
SLE group	97	31.68±4.29	65.48±21.52
Control group	50	59.82±13.39	20.19±4.20
t	-	18.947	14.714
P	-	<0.05	<0.05

stantially increased during tuberculosis infection with Mycobacterium tuberculosis, and is related to the progression and outcome of tuberculosis. It is considered that LncRNA NEAT1 might be involved in the immune regulation of tuberculosis, and contribute to its diagnosis and treatment. In addition, reports have shown [25] that LncRNA NEAT1 is overexpressed in the PBMCs of patients with HIV-1. It may interact and jointly affect the progression of the disease, thus it making it a possible biomarker of HIV-1 infection. The above studies have confirmed that LncRNA NEAT1 may produce a marked regulatory effect in immune-

Table 5. Correlation between LncRNA NEAT1 expression and Th1/Th2 balance in SLE patients

Index	Statistical index	Th1 cells	Th2 cells	Th1/Th2
LncRNA NEAT1	r	-0.488	0.444	-0.604
	P	<0.05	<0.05	<0.05

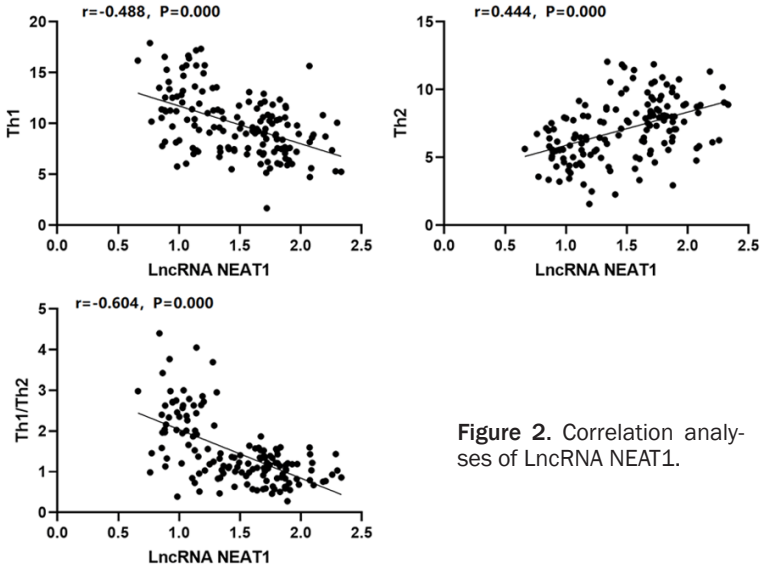

Figure 2. Correlation analyses of LncRNA NEAT1.

related diseases, and is likely to be a target for diagnosis and treatment of immune diseases. This study explored and discussed the correlation between LncRNA NEAT1 expression and Th1/Th2 balance in PBMCs of SLE patients. The results elaborated that the relative expression level of LncRNA NEAT1 in PBMCs of SLE group was critically higher than in the control group, suggesting that LncRNA NEAT1 is overexpressed in the progression of SLE.

Th1/Th2 keeps a balanced state in the body under normal physiologic conditions, and once the secondary balance is broken, it can trigger an abnormal immune response to force the body into apathological state [26]. Studies have reported that the function of auxiliary T lymphocyte subsets is extremely active in SLE patients, while the inhibitory T lymphocyte function is reduced. As a result, patients have an imbalance Th1/Th2, and such imbalance is closely connected with the occurrence and progression of SLE [27]. This clinical view generally considers that a shift of the Th1/Th2 cell balance to Th2 is one of the key processes in the pathogenesis of SLE [28]. This research results showed that Th1 proportion and Th1/Th2 ratio in PBMCs of the SLE group were markedly lower than in the control group (*P<0.05*), while the Th2 proportion was higher in the SLE group than in controls. The IFN-γ level in the SLE group was markedly lower than in the control group, while the IL-4 level was evidently higher in the SLE group than in controls. IFN-γ, which is primarily secreted by Th1 cells, stimulates antigen presentation, and activates $CD4^+$ T lymphocytes to enable monocyte macrophages exerting an immune response. IL-4, secreted by Th2 cells and natural killer cells, exerts a crucial pro-inflammatory effect in the inflammatory response [29]. This study's conclusions are in line with related reports [30], that an imbalance of Th1/Th2 exists in the pathogenesis of SLE. In addition, the correlation analysis showed that the expression of LncRNA NEAT1 in PBMCs of the SLE group was notably negatively correlated with Th1 proportion and Th1/Th2 ratio, while positively correlated with the Th2 proportion. This suggests that that LncRNA NEAT1 may exert an influence in progression of SLE through its balanced interaction with Th1/Th2.

Studies [31] have revealed that LncRNA NEAT1 exerts a crucial regulatory role in immune-related diseases. This study further confirmed a connection between the mechanism of LncRNA NEAT1 in SLE and the Th1/Th2 balance, which may be an important mechanism of LncRNA NeAT1 that affects SLE. To conclude, LncRNA NEAT1 in PBMCs of SLE patients is abnormally highly expressed, and this expression is negatively correlated with the Th1/Th2 balance. These two factors may interact and jointly affect the occurrence and progression of SLE.

Disclosure of conflict of interest

None.

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