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Upregulated fractalkine levels in Chinese patients with lupus nephritis

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ABSTRACT

Objective: To investigate the expression levels of fractalkine (FKN) mRNA in peripheral blood mononuclear cells (PBMCs) and FKN protein in serum of patients with lupus nephritis (LN) from China, and to evaluate the associations between the expression of FKN and systemic lupus erythematosus disease activity index 2000 (SLEDAI-2 K), anti-double-stranded DNA and complement proteins in LN patients.

Methods: Real-time quantitative polymerase chain reaction and enzyme-linked immunosorbent assay were used to detect the expression levels of FKN mRNA in PBMCs and FKN protein in serum separately from 105 patients with LN and 52 healthy controls.

Results: Serum level and mRNA level of FKN were significantly increased in LN patients when compared to controls ($P < 0.001$). Higher FKN levels were found in active LN patients and LN patients with renal damage when compared with inactive LN patients and LN patients without renal damage ($P < 0.001$). Higher serum FKN levels were detected in inactive LN patients in comparison with healthy controls ($Z = -7.165$, $P < 0.001$). The FKN expression levels were positively correlated with SLEDAI-2 K, and was associated with the presence of autoantibodies and negatively correlated with complement proteins C3 and C4 in LN patients.

Conclusions: The results suggest that upregulation of FKN is associated with the pathogenesis and activity of LN in Chinese patients.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by deposition of antibody-antigen immune complexes in tissues. Lupus nephritis (LN) is one of the most common and severe complications of SLE, and its underlying causes are not fully understood [1]. A number of risk factors including immune reaction, genetics, infections, the environment and sex hormones have all been linked to LN, among which the immune reaction is assumed to be the leading cause of LN [2]. The pathogenesis of LN remains unknown. However, accumulated evidence indicates that dysregulation of cytokine and chemokine signaling could be involved in the initiation of LN as well as its evolution.

In humans, FKN is located on chromosome 16q13. It is a member of the CX3C chemokine superfamily [3] which participates in chemotaxis mediation, cell adhesion and regulation of cell growth [4] and it is involved in inflammatory immune responses. There are two types of FKN: a membrane-bound and a soluble form. Soluble FKN consists of a chemokine region and mucin-like stalk, whereas membrane-bound FKN is able to act as an adhesion molecule to promote adhesion of FKN receptors to leukocytes by resistance to the shear forces of its receptor, CX3CR1. Soluble FKN is chemotactic for various cells such as monocytes, T cells and natural killer cells and can be hydrolyzed by A disintegrin and metalloproteases (ADAMs) 10 and 17. Membrane-bound FKN provides integrin-independent adhesion early induction by tumor necrosis factor (TNF)-α, IL-1 and interferon (IFN)-γ in endothelial cells [5].

Expression of FKN in glomeruli is closely related to the pathology activity index, total number of $CD16^+$ monocytes/macrophages or $CD3^+$ T cells in LN patients. In patients who were biopsied before and

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after glucocorticoid and/or methylprednisolone therapy, a downward trend of FKN expression was clearly seen [6]. Our previous studies [7,8] showed that the expression of FKN was increased in lupus-prone MRL/lpr mice and HK-2 cells which were stimulated with lipopolysaccharide (LPS), the expression levels of FKN was significantly downregulated after methylprednisolone therapy. Yajima et al. [9] described a link between soluble FKN levels and SLE in Japanese patients and found the urinary FKN levels were elevated in a small number of children with various types of glomerulonephritis, including lupus nephritis [10]. These studies suggest that FKN seems to be a pathogenic factor and it may be involved in the progression of the disease in patients with LN. There are only a few studies that have focused on the impact of FKN in the progression of LN individuals.

In the present study, we investigated the expression of FKN mRNA in PBMCs and serum FKN levels from LN patients and healthy controls. The systemic lupus erythematosus disease activity index 2000 (SLEDAI-2K) was used to evaluate activity of the disease [11]. SLEDAI-2K allows for persistent activity in rash, mucous membranes, alopecia and proteinuria to be assessed and monitored [11]. Clinically SLEDAI-2K is a predictor for mortality and can be a measure of the global disease activity. The associations between the expression of FKN and SLEDAI-2K, auto-antibodies and complement proteins C3 and C4 in patients with LN were also evaluated.

2. Materials and methods

2.1. Subjects

The study was approved by the Affiliated Hospital Ethics Committee of Youjiang Medical University for Nationalities (Baise, Guangxi Province, P.R. China). Written informed consent was obtained from all patients. All participants were initially diagnosed with LN from May 2012 to May 2015 at the Affiliated Hospital of Youjiang Medical University for Nationalities.

One hundred and five newly diagnosed patients with LN and 52 healthy controls were recruited in this study. Patients with LN were aged 35 ± 11 years with mean proteinuria of 4.9 ± 2.2 g/24 h and a mean estimated GFR of 66 ± 32 ml/min/1.73 m^2.

SLE was diagnosed according to the revised criteria of the American Rheumatism Association in 1997. Patients were clinically diagnosed as LN by kidney biopsies according to the International Society of Nephrology (ISN)/Renal Pathology Society classification. All patients with LN were divided into two subgroups as active LN (score ≥ 10) and inactive LN (score < 10) according to SLEDAI-2 K or for LN with renal damage (GFR < 60 ml/min) and without renal damage (GFR ≥ 60 ml/min) according to patients' renal function. Patients with active LN were divided into two subgroups with respect to high and low FKN levels as measured by the FKN mRNA level in PMBCs (values ≥ 6 and < 6 respectively) or serum FKN levels (values ≥ 50 ng/mL and < 50 ng/mL respectively).

The definition of leukopenia was that the white blood cell count was $< 3.5 \times 10^9$/L. The definition of thrombocytopenia was that the blood platelet count was $< 100 \times 10^9$/L. Erythrocyte sedimentation rate (ESR) of more than 26 mm/h and C-reactive protein (CRP) of more than 3 mg/L were defined as elevated. Serum complement C3 of < 580 mg/L and complement C4 of < 70 mg/L were regarded as decreased.

2.2. Reagents

Commercially available human FKN ELISA kit (Ray Biotech), Trizol Reagent, M-MLV reverse transcriptase, recombinant ribonuclease inhibitor, Oligo dT 18 (Invitrogen), and SYBR Green PCR kits (Qiagen) were used for measurements. Human FKN (NM_002996.3) and PGK1 primers (NM_000291.3) were synthesized by Dalianbao Biology Company with PAGE purification. Rabbit polyclonal anti-FKN antibody (ab25088) and horseradish peroxidase-conjugated goat anti-rabbit polyclonal secondary antibody (ab6721) were purchased from the Abcam Company.

2.3. Blood collection and analysis

Peripheral blood was sampled from patients with LN before they received the immunosuppressive treatment which included glucocorticoid and immunosuppressant to avoid the influence of therapy on FKN expression.

Laboratory abnormalities were recorded including the presence of anti-double-stranded DNA (anti-dsDNA), anti-Smith (anti-Sm), anti-SSA/Ro52KD, anti-SSA/Ro60KD antibodies, white blood cell count, blood platelet count, ESR and CRP. Serum complement proteins C3 and C4 were also assessed.

2.4. Preparation of PBMCs and RT-qPCR

Five milliliters of peripheral blood were collected in evacuated tubes containing EDTA as the anticoagulant. PBMCs were purified from peripheral blood by centrifugation. The One-step Trizol-based procedure was used for total RNA extraction from freshly isolated PBMCs. RNA was reverse transcribed into cDNA after the purity and concentration was checked. The FKN primer sequence was: forward primer 5′-TTGCTCATCCACTATCAACAGAACC-3′, reverse primer 5′-CCTGTGC TGTCTCGTCTCCAA -3′ with a product size of 75 bp. The internal reference PGK1 primer sequence was: forward primer 5′-GAAGTGGAGA AAGCCTGTGC-3′, reverse primer 5′-CCCTTCTTCCTCCACATGAA-3′ with a product size of 84 bp. The PCR reaction system consisted of SYBR Green Mix, forward primer and reverse primer, cDNA and deionized RNAase-free water. PCR was initially denatured at 95 °C for 30 s followed by 95 °C for 10 s and 65 °C for 30 s for 40 cycles and 81 cycles at 55–95 °C for 10 s for melting curve analysis. After the cycle threshold (Ct) was determined, the expression of FKN mRNA was quantified according to the reference gene and analyzed by using the $2^{-\triangle\triangle Ct}$ method [12].

2.5. Measurement of serum FKN levels by ELISA

Serum FKN levels were detected using a commercial ELISA assay kit according to the manufacturer's instruction.

2.6. Statistical analysis

SPSS 18.0 software was used for statistical analysis. Quantitative variables were described by mean ± SD. Nonparametric distribution data were expressed as median value and interquartile range (IQR), and the data unpaired samples were tested by Mann-Whitney *U* test. Correlation analysis between the FKN expression levels and SLEDAI-2K was performed using Spearman's rank correlation coefficient. Correlation analysis of the FKN expression levels with laboratory parameters in active LN patients was performed using Chi square test. All tests were two-tailed, and a *P* value of < 0.05 was considered to be statistically significant.

3. Results

3.1. The expression levels of FKN mRNA in PBMCs from healthy controls and patients with LN

FKN mRNA expression levels were significantly increased in patients with LN and active LN when compared to age-matched healthy controls ($P \leq 0.01$) (Table 1 and Fig. 1). LN patients with renal damage presented with higher values of FKN mRNA levels when compared to those patients without renal damage ($P \leq 0.01$) (Table 1). FKN mRNA levels in active LN patients were significantly higher than in inactive LN