

## Original Article

# Urinary TWEAK/Fn14 mRNA as a biomarker of lupus nephritis activity

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**Abstract:** This study aims to explore the clinical value of urine TWEAK/Fn14 mRNA in lupus nephritis (LN) patients. Thirty one SLE (Systemic Lupus Erythematosus) patients with or without active LN and another 10 healthy controls were recruited. LN activity was assessed by SLE Disease Activity Index. Urinary expression of TWEAK, Fn14 and MCP-1 at the mRNA level was measured by RT-PCR. Urinary expression of TWEAK, Fn14 and MCP-1 mRNA was significantly increased in LN patients ( $P < 0.05$ ). TWEAK, Fn14, and MCP-1 mRNA expression was positively correlated with 24-h urinary proteins, SLEDAI and serum anti-double stranded DNA antibodies ( $r = 0.632$ ,  $P = 0.0001$ ;  $r = 0.651$ ,  $P = 0.001$ ;  $r = 0.417$ ,  $P = 0.02$ ). TWEAK mRNA was positively associated with Fn14 and MCP-1 mRNA ( $r = 0.871$ ,  $P < 0.0001$ ;  $r = 0.561$ ,  $P < 0.0001$ ). In summary, TWEAK, Fn14 and MCP-1 mRNA are potential biomarkers of LN activity.

**Keywords:** Lupus nephritis, TWEAK/Fn14, MCP-1

## Introduction

Systemic lupus erythematosus (SLE) is a relapsing autoimmune disease with clinical manifestations that affect multiple organ systems [1]. Lupus nephritis (LN) is one of the most common and severe forms of SLE, accounting for at least 50% among SLE patients. Notably, LN is clinically characterized by the activation and inflammation of intra-renal lymphocytes [2]. A majority of the cytokines function in a paracrine fashion, thus detection of the expression levels of these cytokines at the site of pathology is of biological relevance. Although kidney biopsy has been widely employed to evaluate the histological severity of LN, it is invasive, has certain risk and is inapplicable for consecutive monitoring. In the past decade, extraction and quantification of messenger RNA (mRNA) from urinary sediment have emerged as a robust laboratory technique to assess the activity of SLE in a noninvasive pattern [3-8].

Tumor necrosis factor-related weak inducer of apoptosis (TWEAK) is a multifunctional cyto-

kine that regulates cell survival, growth, migration, inflammation and differentiation by its own known signaling receptor, fibroblast growth factor-inducible 14 (Fn14), thereby activating the NF- $\kappa$ B, MAPK, and potentially, other signaling pathways [9-11]. Fn14 is lowly expressed in normal tissues whereas up-regulated in diseased tissues, resulting in the activation of signaling pathway. Transient activation of TWEAK/Fn14 pathway is implicated in the repair and regeneration of physiologic tissues. Nevertheless, excessive or persistent activation of TWEAK/Fn14 pathway stimulates the responses of pathological tissue in the contexts of chronic injury and contributes to the progression and degeneration of tissue damage, indicating TWEAK/Fn14 pathway as a potential intervention target. In clinical practice, the urinary expression of TWEAK was significantly up-regulated in patients diagnosed with LN along with renal flares. Interestingly, intra-renal levels of TWEAK and Fn14 varied according to the histological classification of LN and were correlated with the activity index [12-16]. Taken together, both laboratory and clinical studies confirm that TWEAK/Fn14 interaction

plays a pivotal role in the pathogenesis of LN. This study was designed to evaluate the performance of urinary TWEAK/Fn14 mRNA in the diagnosis of LN.

## Materials and methods

### *Patient selection and clinical data*

Thirty one SLE patients from Zhong Da Hospital, Southeast University were divided into the active LN (n=15) and non-LN SLE groups (n=16), all of whom fulfilled the American College of Rheumatology diagnostic criteria for SLE [17]. Patients with systemic disease activity and renal involvement were enrolled in the active LN group (n=15) and those with systemic disease but no history of renal diseases were recruited in the non-LN SLE group. Active LN was defined as a SLEDAI score of 6 [18]. Ten healthy volunteers were allocated into the healthy control group. All studies were approved by the Ethical Committee of Southeast University. Written informed consents were obtained from all subjects to use their urine for research purpose.

### *Total RNA extraction from urine samples*

A whole-stream early-morning urine specimen was collected from each study participant at the first admission day [19]. Shortly after urine collection, the urine was centrifuged at 3,000×g for 30 minutes at 4°C. The urinary supernatant was discarded, and the remaining cell pellet was re-suspended in 1.5 ml DEPC-treated PBS and then centrifuged at 13,000×g for 5 minutes at 4°C. The pellet was then re-suspended in 1.0 ml RNAiso Plus (Takara, Dalian, China) and stored at -80°C until use. Total RNA was extracted according to the manufacturer's protocol (Takara, Dalian, China). All tubes and tips used for RNA extraction were treated with 0.1% DEPC to inhibit the RNase and total RNA was lysed in 10-30 ml 0.1% DEPC-treated ddH<sub>2</sub>O. The integrity of RNA was determined by running agarose gel, which was shown to be adequate for PCR. The RNA concentration and purity were confirmed using the relative absorbance ratio at 260/280 on a nano-drop 2000 (Thermo, Wilmington, USA). RNA samples with a ratio higher than 1.8 were used for RT-PCR.

### *Reverse transcription*

For reverse transcription, 2 µg of total RNA was mingled with 8 µl of 5X PrimeScript™ buffer, 2

µl of PrimeScript™ RT enzyme Mix1, 2 µl of Oligo dT primer (50 µM), 2 µl of Random 6 mers (100 µM), (Takara, Dalian, China), the solution was increased to a final volume of 40 µl with dH<sub>2</sub>O. Reverse transcription was performed at 37°C for 15 minutes, followed by an inactivation reaction at 85°C for 5 seconds. The resulting cDNA was stored at -20°C until use.

### *Real-time PCR*

Relative abundance of TWEAK, Fn14, and MCP-1 mRNA were quantitatively analyzed using the ABI Prism 7300 sequence detection system (Applied Biosystems, California, USA). Human b-actin was used as a reference house-keeping gene. The following oligonucleotide primer sequences were used: TWEAK: forward 5'-CCC TGC GCT GCC TGG AGG AA, reverse 5'-AGA CCA GGG CCC CTC AGT GA; Fn14: forward 5'-CCA AGC TCC TCC AAC CAC AA, reverse 5'-TGG GGC CTA GTG TCA AGT CT; MCP-1: forward 5'-AACACTCACTCCACAACCCAAG, reverse 5'-TGTGGTTCAAGAGGAAAAGCAAT; β-actin: forward 5'-TGGCACCCAGCACAATGAA, reverse 5'-CTAAGTCATAGTCCGCCTAGAAGCA (designed and synthesized by TAKARA). For real-time PCR, 2 µl of cDNA, 10 µl of SYBR Premix Ex Taq™, 0.4 µl of forward primer (10 µM), 0.4 µl of reverse primer (10 µM), 0.4 µl of ROX Reference dye (50X; all from TAKARA) and 6.8 µl of dH<sub>2</sub>O were mixed to make a 20 µl of reaction volume. All samples were run in duplicate. PCR technique was performed using a two-step process: 95°C for 30 s, 40 cycles at 95°C for 5 s and 60°C for 31 s. Then, dissociation curves (DC) and melting temperatures (T<sub>m</sub>) were recorded. The results were analyzed using sequence detection software version 1.4 (Applied Biosystems, California, USA). The relative gene expression of each target was quantitatively measured with a standard curve method. The pre-PCR product of each gene was used as standard, and the standard curve was established with a 10-fold serial dilution of the product. The standard curve was included in all PCR runs. The equation of target gene abundance/house-keeping gene abundance was used to evaluate the level of expression of each gene. Controls consisting of ddH<sub>2</sub>O were negative in all runs.

### *Statistical analysis*

SPSS 16.0 was used for data analysis (SPSS Inc., Chicago, USA). All results were presented

**Table 1.** Baseline data of the patients between the active LN and non-LN SLE groups

Group	Active LN (n=15)	Non-LN SLE (n=16)	P-value
Female:male ratio	14:1	15:1	0.742
Age (year)	30.5±10.9	35.9±13.6	0.242
SLEDAI score*	13.4±4.1	6.1±1.5	0.0001
Serum creatinine (mmol/L)	97.9±57.5	72.7±29.8	0.145
Proteinuria (g/day)*	2.06±1.63	0.26±0.12	0.0002
Serum C3 (g/L)	0.55±0.26	0.75±0.29	0.063
Serum C4 (g/L)	0.13±0.16	0.12±0.04	0.875
Anti-dsDNA titre*	748.8±463.5	162.4±205.0	0.0001
ESR (mm/h)*	69.8±34.4	20.5±15.1	0.0001
CRP (mg/L)	9.7±16.3	13.6±20.4	0.574
WBC (10 <sup>9</sup> /L)*	4.25±1.15	5.64±1.67	0.015
HB (g/L)*	90.1±28.7	116.4±19.3	0.007
PLT (10 <sup>12</sup> /L)*	133.2±75.8	148.8±82.1	0.601

Note: \*Denotes statistical significance between active LN and non-LN SLE groups.

**Table 2.** Comparison of expression levels of target gene mRNA among three groups

Group	Active LN (n=15)	Non-LN SLE (n=16)	Healthy control (n=10)	P-value
TWEAK	0.034±0.027	0.0133±0.014	0.006±0.009	0.002
logTWEAK	-1.59±0.34	-2.26±0.48	-2.49±0.48	0.0001
Fn14	0.017±0.019	0.003±0.003	0.0007±0.0007	0.002
logFn14	-2.21±0.48	-2.96±0.60	-3.32±0.43	0.0001
MCP-1	0.011±0.010	0.002±0.002	0.003±0.007	0.001
LogMCP-1	-2.27±0.35	-2.80±0.39	-2.72±0.47	0.002

as mean ± SD unless otherwise specified. Baseline data were compared by a one-way analysis of variance (ANOVA) among three groups. Since gene expression levels were highly skewed, lg transformation was used prior to analysis. We used the β-actin normalized level as the dependent variable in a Kruskal-Wallis test to identify the differences among the three groups. The Mann-Whitney test was used for gene comparison between two groups. Correlation between gene expression and clinical parameters (SLEDAI, anti-dsDNA, serum creatinine, C3, C4, 24 h urinary proteins, blood test) were calculated using the Spearman rank-order correlation. If the difference between two groups was statistically significant, the receiver-operating-characteristic (ROC) curves were established and cutoff points that yielded the highest combined sensitivity and specificity were calculated. All *p*-values were two tailed,

and a value less than 0.05 was considered to be statistically significant.

## Results

### Clinical characteristics

The demographic and clinical characteristics of the patients were shown in **Table 1**. Age, sex and ethnicity did not significantly differ between two groups (all *P*>0.05). There was no significant difference in the level of serum C3, C4, creatinine, CRP and PLT between the active LN and non-LN SLE groups (all *P*>0.05).

### Urinary mRNA expression levels of SLE groups and healthy controls

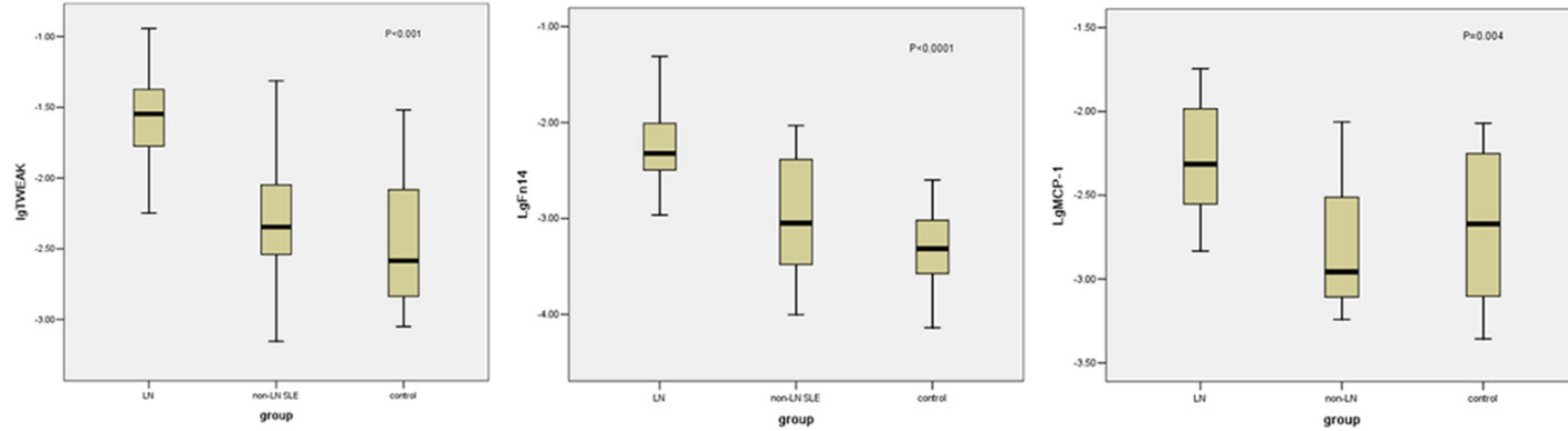
The levels of target gene expression, including TWEAK, Fn14, and MCP-1, among three groups were statistically compared, as illustrated in **Table 2**. The gene levels were compared by the log-transformed ratio of target gene mRNA to β-actin in urinary sediment cells, as illustrated in **Figure 1**. Comparative analysis revealed that urinary mRNA levels of all target genes in the active LN group were significantly higher compared with

those in the non-LN SLE and control groups (all *P*<0.05).

### Correlation between expression of target gene mRNA and clinical parameters

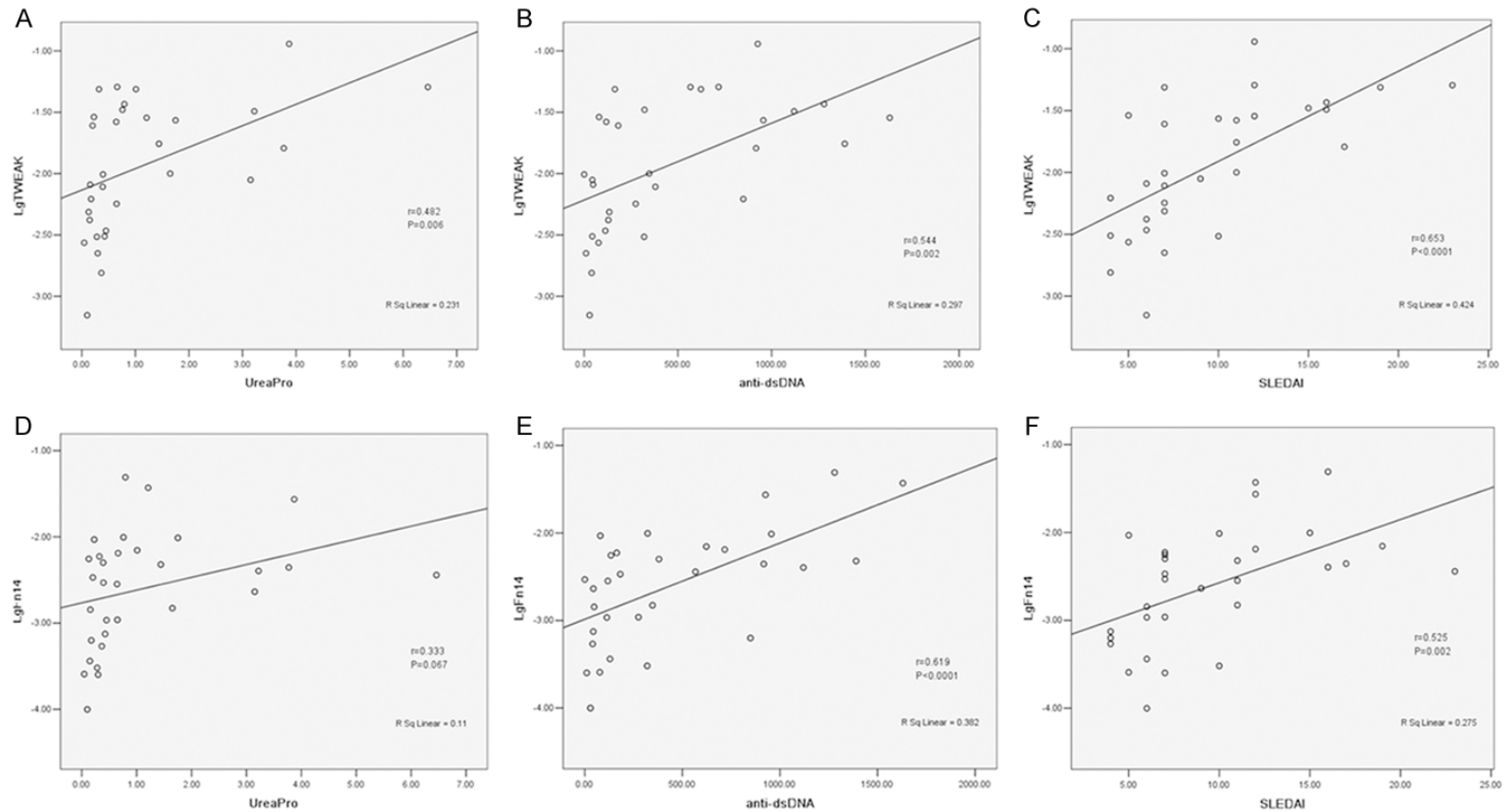
The expression levels of target gene mRNA were positively correlated with 24 h urinary proteins, SLEDAI and serum anti-dsDNA antibodies. The expression of TWEAK, Fn14, and MCP-1 mRNA was correlated with 24 h urinary proteins (*r*=0.622, *P*=0.0001; *r*=0.5289, *P*=0.002; *r*=0.442, *P*=0.013), as shown in **Figure 2A** and **2D**. The expression of TWEAK, Fn14, and MCP-1 mRNA correlated with SLEDAI (*r*=0.719, *P*=0.0001; *r*=0.612, *P*=0.001; *r*=0.568, *P*=0.001), as revealed in **Figure 2C** and **2F**. The expression of TWEAK, Fn14, and MCP-1 mRNA correlated with anti-dsDNA (*r*=0.632, *P*=0.0001; *r*=0.651, *P*=0.001; *r*=0.417,

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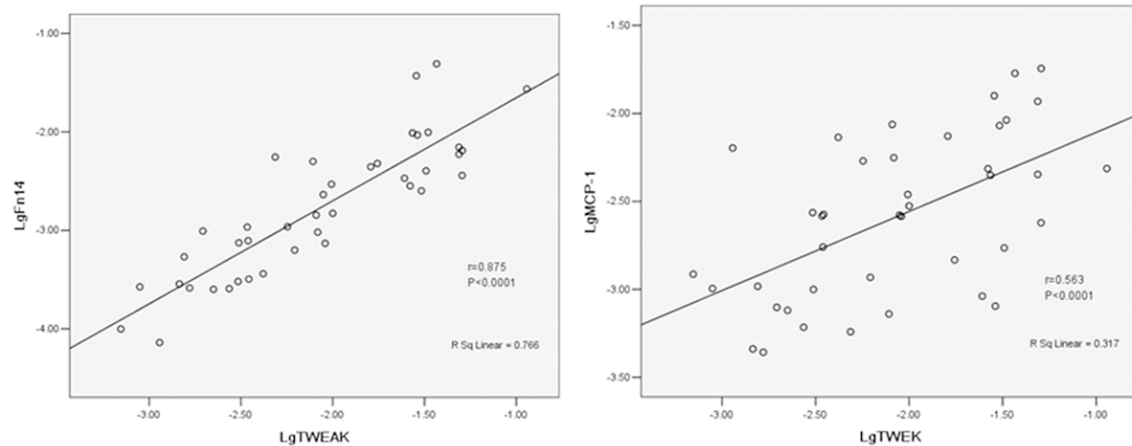
**Figure 1.** Comparison of expression levels (log) of target gene mRNA among three groups.

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**Figure 2.** Correlation analysis between expression levels of TWEAK, Fn14 mRNA and clinical parameters.

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**Figure 3.** Correlation analysis between expression of TWEAK mRNA and Fn14 and MCP-1 in urinary sediment.

$P=0.02$ ), as illustrated in **Figure 2C** and **2E**. In addition, TWEAK mRNA was found to be positively correlated with Fn14 and MCP-1 mRNA ( $r=0.871$ ,  $P<0.0001$ ;  $r=0.561$ ,  $P<0.0001$ ), as shown in **Figure 3**.

### ROC curve analysis

ROC curves were calculated to assess the diagnostic power of each target gene (TWEAK, Fn14 and MCP-1) in terms of AUCs when SLE patients and controls were compared. All target genes could be used to discriminate LN patients and healthy controls, with an AUC above 0.5. The AUC of TWEAK mRNA level was 0.896 (95% confidence interval, 0.801 to 0.991), 0.882 for Fn14 mRNA level (95% confidence interval, 0.782 to 0.982) and 0.810 for MCP-1 mRNA level (95% confidence interval, 0.681 to 0.940).

### Discussion

LN is one of the most common complications of SLE. Recent studies have demonstrated that a disorder of the regulation of apoptosis, such as TWEAK, serves as a pivotal factor during the progression of SLE [20]. TWEAK combined with its receptor Fn14 could activate the expression of Ik-B kinase (IKK), resulting in the degradation and activation of Ik-B. Subsequently, Ik-B is transferred into cell nucleus and up-regulates the expression levels of MCP-1, RANTES and IL-6, leading to inflammatory cell infiltration. Recent findings have linked TWEAK signaling pathway to renal inflammation in an animal model of SLE. Moreover, TWEAK and Fn14 mRNA are up-regulated locally in both glomeru-

lar and tubular compartments in patients diagnosed with LN. Fn14 can be expressed by various resident cells within the kidney, including murine and human mesangial cells, podocytes and tubular cells [14, 21], and potentially on infiltrating macrophages as well [22, 23]. It is the most likely to generate TWEAK by infiltrating leukocytes including macrophages. Therefore, we hypothesized that urinary levels of TWEAK and Fn14 mRNA may reflect the presence and activity level of LN in SLE patients.

The results of our present study demonstrated that urinary levels of TWEAK, Fn14 and MCP-1 mRNA in the active LN group were significantly higher compared with those in the non-LN group and healthy controls, which is consistent with previous findings [24, 25]. However, Wen et al proposed a different idea in another cross-sectional study that TWEAK was merely detected in the urine samples of 6 among 51 LN patients [26]. Urinary mRNA levels of all target genes were positively correlated with 24h urinary proteins, SLEDAI and serum anti-dsDNA antibodies, indicating that high urinary levels of TWEAK, Fn14 and MCP-1 mRNA are a relatively unique feature of LN patients and neither due to the systemic inflammatory process nor the renal disease in isolation. Previous studies conducted by our group revealed that urinary levels of TWEAK, Fn14 and MCP-1 mRNA have the potential of being developed as non-invasive biomarkers for active LN. Cytokines could be measured in the urine at the protein level, which represents the sum of local production and passive glomerular filtration from the sys-



temic circulation. On the other hand, most cytokines are produced locally, exert effects within the renal parenchyma and may not be excreted in the urine to any appreciable extent.

Several biomarkers, such as SLEDAI, proteinuria, serum complement and anti-dsDNA antibody levels have been commonly used to evaluate the severity of LN. However, test sensitivity and specificity of these parameters are relatively low in terms of assessing the severity of LN especially for renal flare and response [27]. Since LN is clinically characterized by serious intra-renal lymphocyte activation and inflammatory cell infiltration, measurement of the expression levels of inflammatory cytokines in urinary sediment can be utilized to assess the extent of the renal damage, which is subsequently validated by pathological examination according to the WHO-defined classification of IIIb, IVb and IVc [28]. Hence, detection of TWEAK/Fn14 signaling genes expressed in the inflammatory and renal cells secreted from the urinary tract can reflect the pathological changes of the kidney.

In addition, positive correlation was observed between the expression of TWEAK/Fn14 mRNA and several clinical parameters, such as SLEDAI, anti-dsDNA, 24 h urinary protein and C3, could be utilized to evaluate the severity of renal involvement, serving as promising novel biomarkers for evaluating LN severity and monitoring the changes in the progression of LN.

Taken together, the mRNA profiles of TWEAK, Fn14, and MCP-1 in the urine sampling from LN patients were found to significantly increase in patients diagnosed with active. The expression levels of TWEAK, Fn14 mRNA were demonstrated to be significantly positively correlated with levels of MCP-1 mRNA, indicating its potential role as a novel biomarker of evaluating the severity of active LN.

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#### Disclosure of conflict of interest

None.

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#### References

- [1] Lisnevskaja L, Murphy G, Isenberg D. Systemic lupus erythematosus. *Lancet* 2014; 384: 1878-1888.
- [2] Yung S, Chan TM. Mechanisms of Kidney Injury in Lupus Nephritis-the Role of Anti-dsDNA Antibodies. *Front Immunol* 2015; 6: 475.
- [3] Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 2001; 344: 947-954.
- [4] Muthukumar T, Dadhania D, Ding R, Snopkowski C, Naqvi R, Lee JB, Hartono C, Li B, Sharma VK, Seshan SV, Kapur S, Hancock WW, Schwartz JE, Suthanthiran M. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N Engl J Med* 2005; 353: 2342-2351.
- [5] Chan RW, Tam LS, Li EK, Lai FM, Chow KM, Lai KB, Li PK, Szeto CC. Inflammatory cytokine gene expression in the urinary sediment of patients with lupus nephritis. *Arthritis Rheum* 2003; 48: 1326-1331.
- [6] Chan RW, Lai FM, Li EK, Tam LS, Chow KM, Li PK, Szeto CC. Messenger RNA expression of RANTES in the urinary sediment of patients with lupus nephritis. *Nephrology (Carlton)* 2006; 11: 219-225.
- [7] Avihingsanon Y, Phumesin P, Benjachat T, Akkasilpa S, Kittikowit V, Praditpornsilpa K, Wongpiyabavorn J, Eiam-Ong S, Hemachudha T, Tungsanga K, Hirankarn N. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. *Kidney Int* 2006; 69: 747-753.
- [8] Szeto CC, Tam LS, Kwan BC, Lai KB, Wang G, Li EK, Chow KM, Lai FM, Li PK. Monitoring of urinary messenger RNA levels for the prediction of flare in systemic lupus erythematosus. *Clin Chim Acta* 2012; 413: 448-455.
- [9] Chicheston Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, Garcia I, Browning JL. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *J Biol Chem* 1997; 272: 32401-32410.
- [10] He F, Dang W, Saito K, Watanabe S, Kobayashi N, Güntert P, Kigawa T, Tanaka A, Muto Y, Yokoyama S. Solution structure of the cysteine-rich domain in Fn14, a member of the tumor

- necrosis factor receptor superfamily. *Protein Sci* 2009; 18: 650-656.
- [11] Brown SA, Richards CM, Hanscom HN, Feng SL, Winkles JA. The Fn14 cytoplasmic tail binds tumour-necrosis-factor-receptor-associated factors 1, 2, 3 and 5 and mediates nuclear factor-kappaB activation. *Biochem J* 2003; 371: 395-403.
- [12] Sanz AB, Sanchez-Nino MD, Ortiz A. TWEAK, a multifunctional cytokine in kidney injury. *Kidney Int* 2011; 80: 708-718.
- [13] Justo P, Sanz AB, Sanchez-Nino MD, Winkles JA, Lorz C, Egido J, Ortiz A. Cytokine cooperation in renal tubular cell injury: the role of TWEAK. *Kidney Int* 2006; 70: 1750-1758.
- [14] Gao HX, Campbell SR, Burkly LC, Jakubowski A, Jarchum I, Banas B, Saleem MA, Mathieson PW, Berman JW, Michaelson JS, Putterman C. TNF-like weak inducer of apoptosis(TWEAK) induces inflammatory and proliferative effects in human kidney cells. *Cytokine* 2009; 46: 24-35.
- [15] Xia Y, Campbell SR, Broder A, Herlitz L, Abadi M, Wu P, Michaelson JS, Burkly LC, Putterman C. Inhibition of the TWEAK/Fn14 pathway attenuates renal disease in nephrotoxic serum nephritis. *Clin Immunol* 2012; 145: 108-121.
- [16] Xia Y, Herlitz LC, Gindea S, Wen J, Pawar RD, Misharin A, Perlman H, Wu L, Wu P, Michaelson JS, Burkly LC, Putterman C. Deficiency of fibroblast growth factor-inducible 14 (Fn14) preserves the filtration barrier and ameliorates lupus nephritis. *J Am Soc Nephrol* 2015; 26: 1053-1070.
- [17] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
- [18] Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-640.
- [19] Zheng M, Lv LL, Ni J, Ni HF, Li Q, Ma KL, Liu BC. Urinary podocyte-associated mRNA profile in various stages of diabetic nephropathy. *PLoS One* 2011; 6: e20431.
- [20] Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nature Reviews Drug Discovery* 2008; 7: 411-425.
- [21] Campbell S, Burkly LC, Gao HX, Berman JW, Su L, Browning B, Zheng T, Schiffer L, Michaelson JS, Putterman C. Proinflammatory effects of TWEAK/fn14 interactions in glomerular mesangial cells. *J Immunol* 2006; 176: 1889-1898.
- [22] Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, Park YB, Kwon BS, Park JE, Lee WH. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. *Circ J* 2004; 68: 396-399.
- [23] Molano A, Lakhani P, Aran A, Burkly LC, Michaelson JS, Putterman C. TWEAK stimulation of kidney resident cells in the pathogenesis of graft versus host induced lupus nephritis. *Immunol Lett* 2009; 125: 119-128.
- [24] Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, Hojaili B, Mackay M, Aranow C, Stohl W, Rovin BH, Michaelson JS, Putterman C. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. *Arthritis Res Ther* 2009; 11: R143.
- [25] Schwartz N, Su L, Burkly LC, Mackay M, Aranow C, Kollaros M, Michaelson JS, Rovin B, Putterman C. Urinary TWEAK and the activity of lupus nephritis. *J Autoimmun* 2006; 27: 242-50.
- [26] Wen XH, Zheng Y, Chen SS, et al. Clinical significance of neutrophil gelatinase-associated lipocalin, monocyte chemoattractant protein-1 and tumor necrosis factor-like apoptosis weak inducer in patients with systemic lupus erythematosus. *Chin J Rheumatol* 2013; 17: 611-615.
- [27] Austin HA. Clinical evaluation and monitoring of lupus kidney disease. *Lupus* 1998; 7: 618-621.
- [28] Wada T, Yokoyama H, Su SB, Mukaida N, Iwano M, Dohi K, Takahashi Y, Sasaki T, Furuichi K, Segawa C, Hisada Y, Ohta S, Takasawa K, Kobayashi K, Matsushima K. Monitoring urinary levels of monocyte chemotactic and activating factor reflects disease activity of lupus nephritis. *Kidney Int* 1996; 49: 761-767.