Proteomic Analysis for Identification of Novel Urinary Biomarkers in Juvenile Systemic Lupus Erythematosus

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Abstract

**Background:** To identify new markers of juvenile systemic lupus erythematosus (JSLE) that facilitate patient stratification and prognosis is quite important. Therefore, our aim of the present study is to analyze alteration of protein expression and potential valuable biomarkers in juvenile systemic lupus erythematosus (JSLE) urine.

**Methods:** Based on this aim, proteomics assay analyzed the changes of urinary proteins in study groups consisting of 9 healthy controls, 9 inactive JSLE and 10 active JSLE patients. And the correlation between clinical characteristics of JSLE patients and new biomarkers discovered from proteomics assay was qualified.

**Results:** We have identified a group of 105 differentially expressed proteins with $\geq 1.3$ fold up-regulation or $\leq 0.77$ fold down-regulation in JSLE patients. In gene ontology and functional enrichment analysis, we discovered these proteins were involved in several important biological processes such as acute phase inflammatory responses, complement activation, hemostasis, and immune system regulation. Interestingly, we found that Ephrin type-A receptor 4 (EPHA4) and Vitronectin (VTN) were significantly reduced in the urine of both inactive and active JSLE patients. Especially, the urinary VTN was also linear correlated with clinical characteristics of JSLE.

**Conclusion:** In summary, this study provided a reliable proteomic reference profile for JSLE. Patients with active and inactive JSLE have differentially obvious metabolic proteins in the urine. VTN could be a specific diagnostic biomarker to distinguish inactive and active JSLE. These identified proteins as new biomarkers in this study can warrant some further exploiting in a larger prospective validation study.

**Background**

Systemic lupus erythematosus (SLE) is an autoimmune disease with inflammation that affects multiple organ systems, characterized by excessive production of antinuclear antibodies and high morbidity and low quality of life [1–3]. The precise pathophysiological mechanism of SLE is largely unknown. One of the most important mechanisms involved in SLE is alteration to immune reactions. This involves auto-antibodies that target the patient’s own tissues and subsequently lead to the inflammation [4]. Despite tremendous basic and clinical research progress regarding treatment [5], various cytokines and chemokines have emerged as the potential biomarkers of SLE disease activity [6]. However, due to the complexity of this disease, the clinical usefulness of these potential biomarkers in assessing disease in SLE is not well established, especially through assessing non-invasive and easy collected urine samples to diagnose renal activity of SLE.

The changes in humoral metabolomics including serum, plasma, urine and sputum can provide abundant information on the physiological and pathological states of individuals and useful clinical parameters. Consequently, to understand the enormous potential in terms of revealing disease conditions will also bring the promise of a revolution in disease diagnosis and therapeutic monitoring [7–10]. In a previous
study, 23 differential metabolites and 5 perturbed pathways including aminoacyl-tRNA biosynthesis, thiamine metabolism, nitrogen metabolism, tryptophan metabolism, and cyanoamino acid metabolism were identified between SLE patients and healthy controls [1]. Also, the studies exhibited that immunoglobulin binding protein [7], urinary vitamin D-binding protein and S100 calcium binding protein (S100) were the potential biomarkers in patients with lupus nephritis [11–12]. With significant advances in proteomic technologies, the comprehensive profiling of protein expression in biofluids from patients with a given disease prompts a deep exploration of disease and its underlying mechanisms. In attempt to reach greater understanding of the pathogenesis, based on advantage of proteomic technologies, we tried to seek potential information in urine to differentiate inactive juvenile SLE (JSLE) patients from active ones and healthy donors.

Vitronectin (VTN), a multifunctional glycoprotein, found abundantly in the serum, extracellular matrix and platelets [13], can regulate cell adhesion, coagulation, fibrinolysis, complement activation, and apoptosis by interacting with avβ3, PAI-1 and urokinase plasminogen activator (uPAR) [14, 15]. In addition, it has been reported that VTN was associated with inflammation in several biological processes, such as acute lung injury, burns, and sepsis, which were involved in the neutrophil, autoreactive T and B lymphocytes activation and tissue injury [16–19]. Interestingly, a study showed that the ratios of neutrophil to lymphocyte and platelet to lymphocyte could reflect the inflammatory response and disease activity in SLE patients [20]. These findings implied that VTN might have a critical role in SLE disease. In addition to VTN, Ephrin type-A receptor 4 (EPHA4), belonged to the ephrin receptor subfamily of the protein-tyrosine kinase family, has been implicated in mediating developmental events, particularly in the nerve system [21]. One study showed that Ephrin-A1-EphA4 signaling was negatively regulated with myelination in the central nerve system [21]. Up to now, some proteomic changes of urine have been described in SLE patients. However, the proteomics of urinary specific biomarkers that distinguish the inactive JSLE and active JSLE remained deserve to be further investigated.

In this study, we showed that the VTN and EPHA4 from 105 differentially expressed proteins among healthy donors, inactive and active JSLE patients were considered as the novel biomarkers associated with the autoimmune inflammation. Therefore, the alteration of VTN and EPHA4 proteins could be a potential therapeutic strategy in the discovery of JSLE cellular therapies.

Materials And Methods

Patients and study design

Total of 9 healthy controls (Group1/G1) and 19 SLE patients (Group2/G2: 9 inactive JSLE patients; Group3/G3: 10 active JSLE patients) who satisfied the American College of Rheumatology criteria were diagnosed in the Guangzhou Women and Children's Medical Center. Patients were excluded from the study if they were pregnant, ≥ 18 years of age, or reluctant to give valid consent. The written informed consent was obtained from all subjects according to the Declaration of Helsinki. This protocol was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center. None of the
patients had other underlying primary diseases. The clean morning urine samples from all included subjects were collected and centrifuged at 3000 g, at 4 °C for 15 min. The supernatant was stored at -80 °C for further proteomics analysis.

**Disease activity assessment**

Disease activity assessment was performed using the modified SLE Disease Activity Index 2000 (SLEDAI-2K) as described in the previous study [22]. The modified SLEDAI-2K excludes 2 immunological items (the complement levels and anti-double-stranded DNA/dsDNA) from the original SLEDAI-2K, while maintaining the 16 clinical manifestations and 4 laboratory tests (white blood cell count, platelets count, urinalysis and 24-hour proteinuria). Since the British Isles Lupus Assessment Group 2004 (BILAG-2004) index provided detailed assessments including severity for affected organs and system manifestations [23], we have modified total BILAG-2004 index based on the scorings of A = 12, B = 8, C = 1 and D/E = 0. The inactive disease of JSLE patients were defined as above modified BILAG-2004 index C/D/E or modified SLEDAI-2K scores < 5. The active JSLE patients were determined by modified BILAG-2004 index A/B or modified SLEDAI-2K scores ≥ 5. Several clinical manifestations of healthy controls were also demonstrated in the comparison to JSLE patients. Therefore, the correlation between the differentiated protein levels and the severity of certain organ/system manifestations of JSLE patients were also determined.

**Sample preparation, mass spectrometry (MS), and MS interpretation**

All collected midstream urine samples from JSLE patients (n = 19) and healthy controls (n = 9) were prepared for proteomics assay with following preparations. Protein concentration in the urine was determined using a BCA kit (Beyotime, P0009) according to the manufacturer's instructions. For digestion, dithiothreitol was used to concentrate the total protein solution for 30 min at 56 °C, and then alkylation of protein with 11 mmol/L iodoacetamide for 15 min at room temperature in the dark. After trypsin digestion, the peptides were combined and dried, then subjected to an nitrogen solubility index source followed by tandem mass spectrometry (MS/MS) in Q-Exactive (Thermo Fisher Scientific, San Jose, CA, USA) coupled online to an ultra-performance liquid chromatography system. The resulting MS/MS data were processed using MaxQuant with integrated Andromeda search engine (v.1.5.2.8). Gene Ontology (GO) was a major bioinformatics initiative to unify the representation of gene and gene product attributes across all species. The GO annotation proteome was derived from the UniProt-GOA database (http://www.ebi.ac.uk/GOA). The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to connect known information on molecular interaction networks. These pathways were classified into hierarchical categories according to the KEGG website.

**Protein–protein interaction (PPI) network construction and module analysis**
PPI analysis is important for the interpretation of molecular mechanisms of the key cellular activities in JSLE. In our study, the Search Tool for the Retrieval of Interacting Genes (STRING) online database (https://string-db.org/cgi/input.pl) tool was employed to construct the PPI network.

**Statistical analysis**

Statistical analysis of *in vivo* data was described in the section of each assay. Results were expressed as mean ± standard deviation (SD) for normally distributed data. Mann-Whitney U-tests were tested the continuous variables. Comparison of different groups was made with Kruskal-Wallis analysis of variance (ANOVA), followed by Dunn's post-test for comparing the differences and calculating a probability (P) value for each pair of comparison. All hypotheses were two-tailed, and P-values < 0.05 were considered significant. Spearman's rank correlation test was used to assess the lineal correlations among the urinary VTN expression with clinical parameters of JSLE patients. r = correlation coefficient. Data were analyzed using GraphPad Prism (version 8.0 for Windows; GraphPad Software, La Jolla, CA, USA).

**Results**

**Patients and clinical characteristics**

A total of 28 early morning urine samples were collected from 9 healthy controls, 9 patients with inactive JSLE, and 10 patients with active JSLE as summarized in Table 1. In the comparisons of clinical parameters, we found the mean of creatinine in healthy control is 22.87 ± 4.37 µmol/L is lower than inactive JSLE patients (61.13 ± 15.99 µmol/L). Whereas, the mean ratio of aspartate amino transferase (AST)/ alanine amino transferase (ALT) is higher in healthy controls (2.54 ± 0.79 U/L), when compared with inactive (1.47 ± 0.62 U/L) and active (1.36 ± 0.72 U/L) JSLE patients. In addition, higher hematuria and lower concentration of hemoglobin (HB) in active JSLE patients (96.78 ± 25.92 g/L) were observed, when compared to that in healthy controls (126.10 ± 31.90 g/L) and inactive JSLE patients (116.00 ± 21.33 g/L). Moreover, when compared between active and inactive JSLE patients, there were significant higher SLEDAI (11.50 ± 7.29), ANA (417.60 ± 201.80 g/L) and dsDNA (443.10 ± 309.70 g/L), but lower erythrocyte sedimentation rate (ESR) (6.50 ± 6.76 mm/h), Complement C3 (0.38 ± 0.39 g/L) and C4 (0.06 ± 0.05 g/L) in active JSLE patients. However, no statistical differences were obtained in analysis of SLICC, proteinuria, white blood cell (WBC), blood platelet (PLT), and C-reactive protein (CRP). The detailed statistical differences in clinical characteristics between JSLE patients and healthy controls were demonstrated in Table 1.

**Prolife of proteome in the urine of JSLE patients**

Proteomics analysis was then performed on the urine samples. As shown in Fig. 1A, heatmap results showed that 105 differentiated proteins have been identified among groups (Group1/G1: Healthy controls; Group2/G2: inactive JSLE patients; Group3/G3: active JSLE patients). All identified differentiated proteins were quantified through liquid chromatography (LC)-mass spectrometry (MS). Further bioinformatics analysis 16 downregulated proteins (≤ 0.77 fold downregulation), and 9
upregulated proteins (≥ 1.3 fold upregulation) were quantified in these 105 differentiated proteins between JSLE patients (G2 + G3) and healthy controls (Fig. 1A-B). While these proteins including 3 increased (≥ 1.3 fold upregulation) and 34 decreased (≤ 0.77 fold downregulation) proteins were quantified between G2 and G1 (Fig. 1C). In addition, a number of 21 increased (≥ 1.3 fold upregulation) and 16 decreased (≤ 0.77 downregulation) proteins were observed between G3 and G1 (Fig. 1D). However, Compared to the G3, 2 decreased proteins and 47 increased proteins were found in the urine of inactive JSLE patients (Fig. 1E).

Based on these findings, to further explore the aberrant differentially expressed genes (DEGs) among three groups, we overlapped the downregulated proteins that reflected gene expressions, and we achieved two downregulated proteins in JSLE patients compared with healthy controls (VTN and EPHA4, shown in Fig. 2A), whereas none were obtained by overlapping the upregulated proteins (Fig. 2B). All of these DEGs were listed in Fig. 2C.

**Functional enrichment analysis**

The enrichment analysis was analyzed by the GO and KEGG terms, including molecular function, cellular component, and biological process that were significantly enriched to indicate the nature of the differentially expressed proteins in urine between JSLE patients and healthy controls using the Database for Annotation, Visualization and Integrated Discovery online tool. As for the DEGs in analysis of inactive JSLE patients and healthy controls, multiple enriched GO terms and KEGG pathways were demonstrated. The top 20 enrich GO terms and KEGG pathways were selected and shown in Fig. 3. Figure 3B showed that the main enrich GO terms were majorly associated with cell adhesion, biological adhesion, peptidyl-tyrosine phosphorylation, peptidyl-tyrosine modification and cell-cell adhesion via plasma-membrane adhesion molecules. When compared with healthy controls and inactive JSLE patients, the differentiated KEGG pathways were listed in Fig. 3A. Also, the involvement of salivary secretion, PI3K-Akt signaling pathway and human papillomavirus infection as well as focal adhesion were observed, while biological function were related to post-translational protein modification, protein modification, cell adhesion, and cellular protein modification process in the comparison of active JSLE patients and healthy controls (Fig. 3C-D). In addition, from the comparison results of inactive and active JSLE patients, we also found DEGs participated in multiple biological pathways, such as necroptosis, focal adhesion and PI3K-Akt signaling that involved in the regulation of mitotic nuclear division and nuclear division (Fig. 3E-F).

**The protein-protein interaction analysis of differential genes**

In order to clearly understand the interaction of downregulated DEGs, PPI network of these DEGs were generated to identify the key genes and their interactions in JSLE utilizing the STRING online database. As shown in Fig. 4, these results showed that VTN is formed a core molecular of PPI network among three groups, at least interacting with other genes in the interatomic networks, including collagen type XVIII alpha 1 (COL18A1), FBLN5, Aggrecan (ACAN), F11R and prion protein (PRNP). As for the active and inactive JSLE, VTN, COL18A1 and ACAN were also significant hub genes in the interatomic networks (Fig. 4C).
Correlation between urine VTN levels and lupus clinical parameters

In this study, upon the quantitative results, we tried to seek the clinical values for a detection of urinary VTN in JSLE patients. We found that VTN was negatively associated with serum dsDNA (inactive JSLE: r=-0.6328, p < 0.05, Fig. 5A; active JSLE: r=-0.7002, p < 0.05, Fig. 5B), and the similar relationship was obtained in the correlation of ANA and VTN (inactive JSLE: r=-0.743, p < 0.05, Fig. 5C; active JSLE: r=-0.5603, p < 0.05, Fig. 5D). In addition, Complement C3 and C4 were positively associated with VTN in both inactive JSLE (C3: r = 0.6440, p < 0.05, Fig. 5E; and C4: r = 0.8343, p < 0.01, Fig. 5G) and active JSLE (C3: r = 0.7156, p < 0.05, Fig. 5F; and C4: r = 0.8192, p < 0.01, Fig. 5H).

Correlation between urine VTN levels and different lymphocyte subsets

SLE is characterized by the loss of tolerance to self-antigens, downstream activation and expansion of autoreactive T and B lymphocytes [19]. In this study, upon the quantitative results, we found that urine VTN level was negatively associated with %CD19 + B cells (r=-0.8978, p < 0.01, Fig. 6A) and %CD3 + CD4-CD8- T cells (r=-0.8313, p < 0.05, Fig. 6B) in peripheral blood mononuclear cell (PBMC), as well as positively associated with %CD16 + CD56 + NK cells (r = 0.6724, p < 0.05, Fig. 6C) in whole blood of inactive JSLE. The similar relationship was obtained in an analysis for the correlations of VTN with the ratio of %CD3 + T cells/ %CD45 + lymphocytes (r = 0.6257, p < 0.05, Fig. 6D), %CD3 + CD4 + helper T cells (Th) (r=-0.8716, p < 0.01, Fig. 6E), %CD3 + CD8 + suppressor T cells (Ts) (r = 0.8385, p < 0.01, Fig. 6F), the ratio of %CD4 + Th/ %CD8 + Ts (r=-0.6881, p < 0.05, Fig. 6G), %CD16 + CD56 + NK cells (r=-0.7663, p < 0.01, Fig. 6H), and absolute number of NK cells (r=-0.6813, p < 0.01, Fig. 6I) in PBMC of active JSLE. Moreover, no significant associations were observed between VTN and other index (data not shown).

Discussion

The development of SLE is closely associated with the alteration in molecular functions, biological processes and signaling pathways. However, the molecular characteristics and the associated molecular functions, pathways, and interactions in different stage of SLE are not well understood. To explore whether distinct molecular and biological functions were enriched in active JSLE, in the currently study, multiple molecular and biological function as well as pathways were observed in the common DEGs, implying that these factors were involved the development of JSLE. Since the non-invasive urine samples can be easily collected and diagnose, in this study, we proposed to reflect specific and sensitive of renal activity by using JSLE urine samples, and revealed that 105 different proteins in the comparison between JSLE patients and healthy controls based on proteomic assay. The results showed that these specific DEGs had different molecular functions and associated with different biological pathways. In addition, these common and unique DEGs formed complex interactome networks. The most important discovery of our study is that we have found both urine EPHA4 and VTN levels are reduced in inactive and active JSLE patients when compared to healthy controls. Further bioinformatics analysis implied us the EPHA4
and VTN in urine might have a potential possibility to be biomarkers of JSLE patients. Furthermore, VTN in the urine of JSLE patients, including inactive and active JSLE, was inversely correlated with the serum dsDNA and ANA. And VTN appeared to be selectively distinguished in inactive JSLE from active JSLE, suggesting that it might serve as a useful biomarker for clinical prognosis.

Coincidentally, in some previous studies, the presence of soluble membrane attack complex (MAC) has been well documented in the renal pathological process along with immune deposits containing circulating immune complexes (CIC) and C3 [24]. In addition, it is reported that in SLE patients, the elevated urinary MAC has been used as a biomarker for disease flare [25]. Furthermore, in another study, the high levels of VTN are associated with the CIC-MAC in SLE patients with active nephritis [26]. Further expanded studies are required to establish the value of VTN binds to CIC as a biomarker of active nephritis. Interestingly, in our study, we found a significant decreased level of urinary VTN and lower expression of serum C3 and C4 in inactive JSLE, and also a positive association between VTN and C3/C4, which may derive from the metabolic dissociation of CIC-MAC in JSLE glomerulus. These findings of identifying a novel mechanism may contribute to the JSLE nephritis.

Besides, it has been demonstrated that EPHA4 performs an important role in a lot of cellular processes, like promoting cell proliferation and cell adhesion-mediated drug resistance via the Akt signaling pathway [27]. While COL18A1 interacted with VTN and EPHA4, plays a crucial role in response of acute liver injury through binding α1β1 integrin on hepatocytes [28]. In addition, the PI3K/Akt and Wnt/β-catenin signaling pathways as well as ERK1/2, the downstream of EPHA4 receptor activation, play an important role in the regulation of events related with the epithelial-mesenchymal transition development [29]. These findings in line with the analysis of these pathways are shown in Fig. 4D-E, including PI3K-Akt, cell adhesion molecules and focal adhesion. Specifically, EPHA4 is the most abundant ephrin receptor, which interacted with almost all ephrin ligands, ranging from effects on inflammatory responses to axonal degeneration and regeneration in the central nervous system, especially in neurological functional recovery by regulating various processes, such as neuroinflammation, angiogenesis, neurogenesis, axonal reorganization and synaptic plasticity [20, 30]. Although update to now, there is no available evidence proved the function of EPHA4 in the pathogenesis of SLE, EPHA4 as a possible inflammatory mediator may contribute to the immunopathological disease like multiple sclerosis and neuropsychiatric disorder in SLE [31–36]. Occasionally, in our study, we have found a significant decreased protein level of EPHA4 in the urine of JSLE patients, which may imply a potential role of EPHA4 in neurodevelopment of JSLE patients via PI3K-Akt signaling pathway. However, because the expressions of EPHA4 in some JSLE patients are below the detection limit, we cannot obtain the valid comparison of urinary EPHA4 between JSLE patients and healthy controls. The correlation between EPHA4 and clinical parameters will be further addressed in future study with a large number of JSLE patients.

In addition to the EPHA4, we found that dsDNA and ANA had a negative correlation with urine VTN level, which was similar with many other studies like circulating S100 and serum triggering receptor expressed on myeloid cell-1 [37–38]. Otherwise, VTN was reported to involve in promoting the expression of inflammatory factors, such as IL-6 and leukemia inhibitory factor via integrin-focal adhesion kinase and
uPAR signaling pathways [39], and to promote neurogenesis [40]. Another study also found an increased co-localization of MAC, VTN and VTN receptor (avβ3 integrin), both of which are within and around the subepithelial deposits in membranous nephropathy [41]. What's more, prevention of autoimmune diseases depends on immune homeostasis, which results from the balances of different T-lymphocyte subsets. Therefore, in this study, we have found that urine VTN level was significantly negative associated with %CD19 + B cells in PBMC and %CD3 + CD4-CD8- T cells in the whole blood of inactive JSLE (Fig. 6A-B). The similar relationship was found in an analysis for the correlations of VTN with %CD3 + CD8 + suppressor T cells in PBMC of active JSLE (Fig. 6F). These results imply that due to the higher expression of VTN in active JSLE and lower expression of VTN in inactive JSLE, the corresponding increased %CD3 + CD8 + suppressor T cells in active JSLE and higher %CD19 + B cells in inactive JSLE may involve into the auto-inflammation and accumulation of MAC. This reminds us the urine VTN level may become a novel biomarker for the diagnostics of JSLE progression. However, due to the limited number of JSLE patients and healthy controls in this investigation, several concerns may be not clarified precisely. For example, what kind of relationship between EPHA4 and VTN, and whether EPHA4 is a novel receptor of VTN still need to be further justified.

Conclusions

In summary, the present study demonstrated the differential abundance of proteins in the urine of JSLE patients, which provided a new insight into the prognosis and development of JSLE. Particularly, the urinary EPHA4 and VTN were considered to be the potential biomarkers which deserved to be further studied before their utility for JSLE diagnosis.

List Of Abbreviations

**ALS**: Amyotrophic lateral sclerosis;

**ALT**: Alanine amino transferase;

**ANA**: Antinuclear antibody;

**AST**: Aspartate amino transferase;

**BILAG**: British Isles Lupus Assessment Group Index;

**CIC**: Circulating immune complexes;

**COL18A1**: Collagen type XVIII alpha 1;

**CRP**: C-reactive protein;

**DEGs**: Differentially expressed genes;
**dsDNA**: Double-stranded DNA;

**EPHA4**: Ephrin type-A receptor 4;

**ESR**: Erythrocyte sedimentation rate

**GO**: Gene Ontology;

**HB**: Hemoglobin;

**KEGG**: Kyoto Encyclopedia of Genes and Genomes;

**LC**: Liquid chromatography;

**MAC**: Membrane attack complex;

**MS**: Mass spectrometry;

**PBMC**: Peripheral blood mononuclear cell;

**PLT**: Blood platelet;

**PPI**: Protein-protein interaction;

**PRNP**: Prion protein;

**JSLE**: Juvenile systemic lupus erythematosus;

**SLEDAI**: SLE disease activity index;

**SLICC**: SLE international collaborating clinics

**STRING**: Search Tool for the Retrieval of Interacting Genes;

**S100**: S100 calcium binding protein;

**uPAR**: urokinase plasminogen activator;

**VTN**: Vitronectin;

**WBC**: White blood cell;

**Declarations**

**Ethics approval and consent to participate**
The written informed consent was obtained from all subjects according to the Declaration of Helsinki. This protocol was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center.

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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**Authors' contributions**

All authors made substantial contributions to study design, analysis and interpretation of data, drafting the manuscript and editing for important intellectual content of this article. Study conception and design: Zhe Cai and Huasong Zeng. Performed the experiments: Song Zhang, Zhe Cai, Cheng Zhi and Ping Wu. Acquisition of data: Song Zhang and Yanhao Lin. Contributed reagents/materials/analysis tools: Qi Ren, Ping Wei, Rui Chen, Feng Li, Ying Xie, Yu Feng, Zhe Cai, Chun Kwok Wong, Hong Tang and Huasong Zeng. Wrote the paper: Song Zhang and Zhe Cai.

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**References**


Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures
Figure 1

Profile of urinary proteins. (A) Heatmap shows the differentially expressed genes (DEGs) reflected by differentially expressed matching proteins in the indicated groups (Group1/G1: healthy controls, Group2/G2: inactive JSLE patients, Group3/G3: active JSLE patients). Red and green colors indicate higher and lower expression, respectively. (B-E) Volcano dot plot presents the mass spectrometry data of proteome as -log10 (p value) plotted against the log2 (fold change/FC). The -log10 (p value) and log2
(FC) analysis thresholds are indicated by dotted lines (≥ 1.3 fold up-regulation or ≤ 0.77 fold down-regulation, p<0.05). Green dots represent the downregulated proteins, while red dots represent the upregulated proteins, and gray dots represent no significant difference in proteins. The symbol of differentially expressed proteins labeled with relevant color lists beside each graph.

**Figure 2**

Identification of aberrant differentially expressed proteins including downregulated and upregulated proteins. (A) Two aberrantly downregulated proteins EPHA4 and VTN, (B) but no aberrantly upregulated proteins are obtained by Venn diagram among the indicated groups (G1: healthy controls, G2: inactive JSLE patients, G3: active JSLE patients). (C) The aberrant DEGs reflected by their matching proteins are listed in the indicated groups.
Figure 3

Functional enrichment analysis of signaling pathways and biological process of DEGs. A comparison of top 20 Gene Ontology terms of signaling pathways (A, C, E) and biological process (B, D, F) is indicated among groups (Group1: healthy controls, Group2: inactive JSLE patients, Group3: active JSLE patients).
Figure 4

The protein-protein interaction (PPI) networks of common DEGs. (A-C) The comparisons of PPI networks of DEGs are shown among the indicated groups (Group1: healthy controls, Group2: inactive JSLE patients, Group3: active JSLE patients) by using the STRING 1 online database. The top 20 enrich KEGG pathways are selected and classified into hierarchical categories according to the comparison between groups.
Clinical correlation between VTN with dsDNA, ANA, Complement C3 and C4. The dot-plot shows the correlation between urinary VTN expression and (A) serum dsDNA level, (C) serum ANA level, (E) serum Complement C3 level, and (G) serum Complement C4 level in inactive JSLE patients; as well as the correlation between urinary VTN expression and (B) serum dsDNA level, (D) serum ANA level, (F) serum
Complement C3 level, and (H) serum Complement C4 level in active JSLE patients (Group2: inactive JSLE patients, Group3: active JSLE patients).

Figure 6

Clinical correlation between VTN and lymphocyte subsets. The dot-plot shows the correlation between urinary VTN expression with (A) %CD19+ B cells, (C) %CD16+CD56+ NK cells in PBMC, and (B) %CD3+CD4+CD8- T cells in whole blood of inactive JSLE patients; as well as the correlation between urinary VTN expression with (D) the ratio of %CD3+ T cells/ %CD45+ lymphocytes, (E) %CD3+CD4+ helper T (Th) cells, (F) %CD3+CD8+ suppressor T (Ts) cells, (G) %CD4+ Th/ %CD8+ Ts, (H) %CD16+CD56+ NK cells, (I) Absolute number of NK cells in PBMC of active JSLE patients (Group2: inactive JSLE patients, Group3: 21 active JSLE patients).
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.JPG