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Original Article

High mobility group protein B1 (HMGB1) increased in kidney tissues of patients with lupus nephritis

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Abstract: High mobility group protein B1 (HMGB1) is both a nuclear and cytosolic protein that is increasingly recognized as an important pro-inflammatory mediator, actively secreted from monocytes and macrophages, and passively released from necrotic cells. Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease, characterized by in the appearance of varying circulating autoantibodies, immune complex deposition, and multisystem involvement. Lupus nephritis (LN) is considered one of the most serious manifestations of systemic lupus erythematosus. The aim of this study was to investigate the expression of high mobility group protein B1 (HMGB1) in the kidney tissue of patients with lupus nephritis. The study population consisted of 40 SLE patients. Among the renal biopsies of these patients, 3 showed Class III, 20 showed Class IV, 17 showed Class V LN. We carried out immunohistochemistry with HMGB1 antibodies and analyzed the expression and distribution of HMGB1 in all 40 renal biopsies. Clinical parameters were assessed according to routine procedure. We found that the expression of HMGB1 in Class III + IV and Class V LN groups in glomerular, tubular-interstitial biopsies was significantly higher than in the control group. In contrast to the normal control group, the positive expression of HMGB1 was not only within the nucleus, but also cytoplasmic and extracellular; the expression of HMGB1 in LN was positively correlated with SLE disease activity score (SLEDAI) and the activity index of kidney tissue. The expression of HMGB1 was significantly increased in the kidney tissue of patients with lupus nephritis and positively related to disease activity and renal pathological damage.

Keywords: High mobility group protein B1 (HMGB1), lupus nephritis (LN)

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease, characterized by a variety of circulating autoantibodies, immune complex deposition, and multisystem involvement [1]. Lupus nephritis (LN) is considered one of the most serious organ manifestations of systemic lupus erythematosus. Although the pathogenesis of LN has been well researched and with improved treatment programs implemented, LN is still one of the leading causes of death for patients with SLE [2].

High mobility group protein B1 (HMGB1) is ubiquitous in eukaryotes, which, primarily in combination with DNA in cells, is involved in DNA replication, transcription, translation and regulating several vital metabolic activities [3-6]; The molecular weight of human HMGB1 is approximately 30 kD, with genes of the protein located on chromosome 13q12 [7-12]. HMGB1 is released extracellularly both actively and passively. Following immune activation such as through monocyte/macrophage, dendritic and epithelial cell pathways, intracellular HMGB1 can be actively secreted into the extracellular space [13, 14]; whereas in the necrotic or damaged cells, cell membrane integrity has been disrupted, which leads to chromosome acetylation, HMGB1 segregation from the chromosome, and its passive release into the extracellular space [15, 16]. Extracellular HMGB1 is involved in various metabolic activities, such as cellular proliferation, differentiation, tumorneogenesis, angiogenesis, sepsis and the like. Currently, it has come our attention that HMGB, as a late inflammation mediator, participates in

Table 1. Scoring criteria of renal tissue activity index

	1 Point	2 Points	3 Points
Glomerular			
Cell quantity (cells/gcs)	120-150	151-230	>230
Leukocyte infiltration (cells/gcs)	<2	2-5	>5
Nuclear fragmentation (%)	<25	25-50	>50
Loop necrosis (%)*	<25	25-50	>50
Platinum ears (%)	<25	25-50	>50
Transparent thrombosis (%)	<25	25-50	>50
Cellular crescents (%)*	<25	25-50	>50
Interstitial cell infiltration	small	medium	large
Arterial necrosis or cell infiltration	If yes, 2 points		

Note: *Points ×2.

the response of heavy inflammation, and as a cytokine is involved in intracellular signal transduction [17-24].

Previous studies have confirmed that HMGB1 is involved in the pathogenesis of several autoimmune diseases including rheumatoid arthritis [25, 26], ANCA associated vasculitis [27], systemic lupus erythematosus [28-32] and other autoimmune diseases [33, 34]. Nevertheless, there is limited research with regards to high mobility group protein B1 (HMGB1) in lupus nephritis, particularly in kidney tissue expression of patients with untreated lupus nephritis, and the relationships between HMGB1 and LN. Thus, this study researched the expression of high mobility group protein B1 (HMGB1) in the kidney tissue of patients with lupus nephritis, and aimed to explore its significance in LN.

Materials and methods

Subjects

Experimental group: kidney tissue biopsy specimens were obtained from 40 cases of patients with LN in Sun Yat-sen Memorial Hospital between January 2007 and February 2013, All the patients fulfilled the classification criteria for SLE revised by ACR in 1997, and kidney damage was confirmed by biopsy or proteinuria (24 h), exceeding 0.5 g Renal biopsy by the ISN/RPS (International society of Nephrology/Renal Pathology Society) lupus nephritis classification criteria of 2003 [35], with 23 cases of Class III + IV group (3 cases of Class III, 20 cases of Class IV), 17 cases of Class V group. Of the patients, there were 4 males and 36 females, aged 13-61 years (mean 27.15 ± 11.31). The patients were not treated with glucocorticoids and (or) an immunosuppressant before admission.

Control group: 10 cases of normal kidney tissue, obtained distal to the tumor site during kidney tumor resection at this hospital.

This study was approved and monitored by the Ethics Committee of Sun Yat-sen Memorial Hospital.

Methods

Patient's laboratory data was collected: 24-hour urinary protein, BUN, creatinine, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complement C3, C4 and the SLE disease activity scores (SLEDAI) [36] according to patient history, combined with physical examination and laboratory test results.

Immunohistochemistry experiments on renal tissue HMGB1: Immunohistochemistry was performed on 4-μm sectioned paraffin-embedded renal biopsy specimens. After deparaffining with xylene and rehydrating with graded ethanol, sections were incubated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. After microwave antigen retrieval in a boiled water bath for 8 minutes in 1 mmol/L EDTA (pH = 8.0), the sections were incubated with rabbit anti-HMGB1 monoclonal antibody (1:200) (cell signaling technology, USA) for 16 hours at 4°C, After rinsing in PBS, according to the instructions, adding goat anti-rabbit IgG after horseradish peroxidase (HRP) link and incubated at room temperature for 30 min, and finally observed under a microscope with freshly prepared DAB color. The color of positive products showed brown.

Quantify: When the nucleus and (or) clear brown granules appear in the cytoplasm during immunohistochemical staining, it means they are positive cells. For quantifying positive expression, the expression of HMGB1 was assessed by counting the number of HMGB1 positive cells, respectively, in at least 10 glomerular profiles and 10 high-power (×400) fields of tubulointerstitium randomly chosen for each

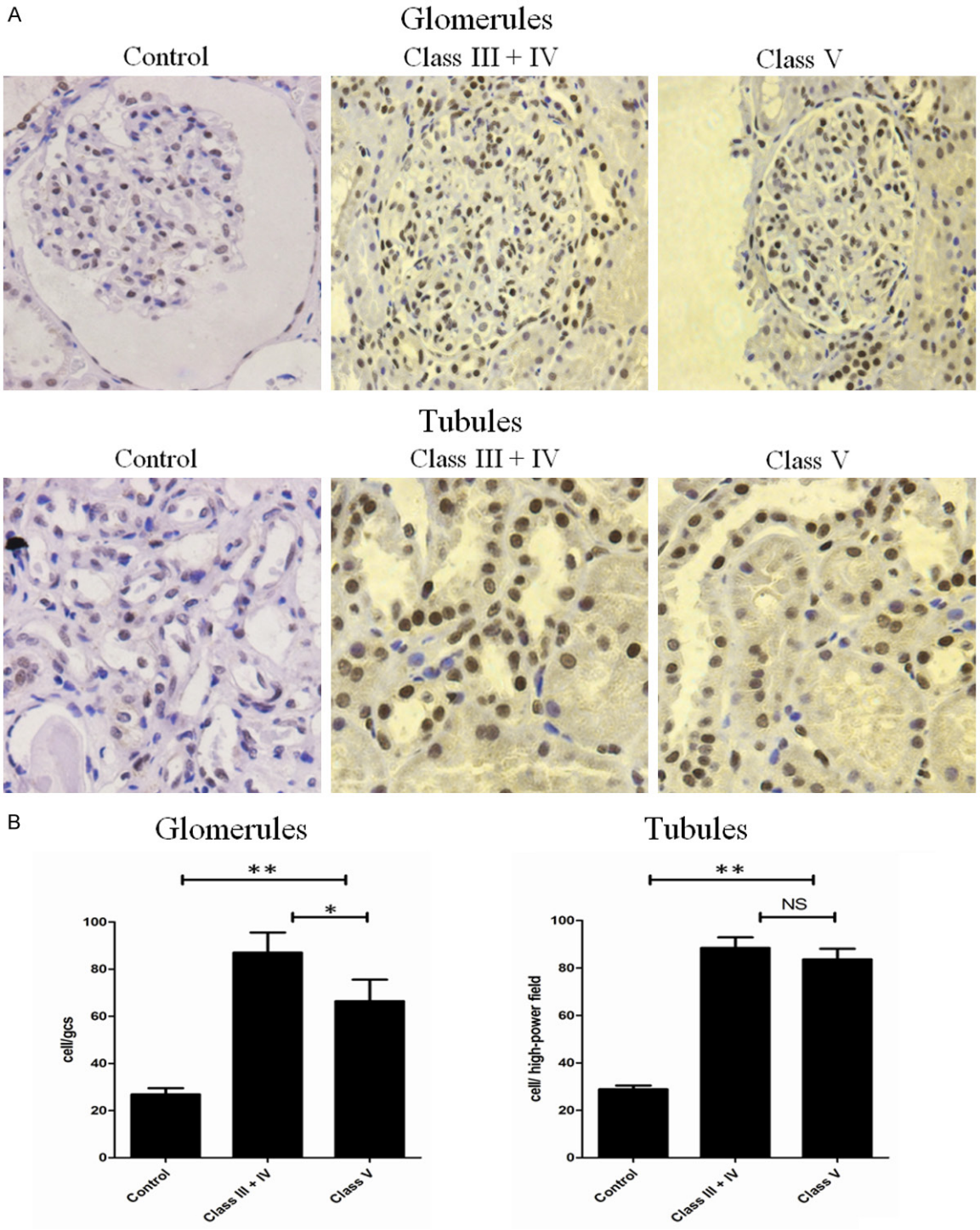

Figure 1. The expression of HMGB1 was significantly increased in the kidney tissue of patients with lupus nephritis. (A) The expressions of HMGB1 in Class III + IV and Class V LN group were significantly higher than that within the control group ($P<0.01$) in glomeruli and tubularinterstitial areas. (B) Compared with Class V LN group, the expression of HMGB1 in Class III + IV group's glomeruli areas was statistically significant increase ($P<0.05$). There is no statistical difference between the expression of HMGB1 in tubularinterstitial areas of Class III + IV group and Class V LN group.

kidney. The mean number of positive cells in one glomerulus and per high-power area of tubulointerstitium was calculated.

The extent of proliferation of glomerular cells have also been classified: Semi-quantitative classifications to assess the degree of prolifer-

Table 2. Clinical data of control and LN patients

	Control (n = 10)	Class III + IV (n = 23)	Class IV (n = 17)
BUN ((mmol/L))	3.83 ± 1.04	9.89 ± 6.22	7.78 ± 5.22
Serum creatinine ((umol/L))	99.00 ± 19.44	166.61 ± 66.1	128.06 ± 58.99
24-hour urinary protein (g)	-	2.79 ± 2.37	2.47 ± 1.95
ESR ((mm/h))	-	82.92 ± 8.77	61.50 ± 10.25
CRP (mg/L)	-	7.37 ± 3.74	4.68 ± 2.13
Complement C3 (mg/L)	-	550.67 ± 92.09	738.88 ± 112.70
Complement C4 (mg/L)	-	92.83 ± 20.46	121.94 ± 27.93
SLEDAI	-	15.17 ± 1.17	13.69 ± 1.20

ation of renal glomerular tissue cells was conducted [37].

Renal tissue activity index of LN patients (active index, AI) [38]: Indicators of renal tissue activity index (**Table 1**) include:

Statistical analysis

Computer-based statistical analysis was performed by using SPSS 13.0 software. All data is presented as means ± SD. Differences between groups was evaluated by one-way ANOVA, Correlation analysis was assessed by Pearson or Spearman rank correlations. Statistical significance was defined as $P<0.05$.

Results

The expression of HMGB1 was significantly increased in the kidney tissue of patients with lupus nephritis

In normal kidney tissue, a small amount of HMGB1-positive cells may be observed, distributed in glomerular mesangial cells, podocytes and tubular epithelial cell nuclei. The expressions of HMGB1 in Class III + IV and Class V LN group were significantly higher than that within the control group ($P<0.01$) in glomeruli and tubulointerstitial areas, the HMGB1 positive signals of LN group was distributed not only in the nucleus, but also within the cytoplasm and outside of cells (**Figure 1A**). Compared with Class V LN group, the expression of HMGB1 in Class III + IV group's glomeruli areas was statistically significant increase ($P<0.05$). There is no statistical difference between the expression of HMGB1 in tubulointerstitial areas of Class III + IV group and Class V LN group (**Figure 1B**).

The HMGB1 positive cells in glomeruli and tubulointerstitial areas were positively correlated with disease activity

The clinical laboratory data of patients (**Table 2**), among which the HMGB1 positive cells in glomeruli ($r^2 = 0.602$, $P<0.01$) and tubulointerstitial ($r^2 = 0.713$, $P<0.01$) were positively correlated with SLEDAI. However, the amount of HMGB1 positive cells in the glomeruli or tubulointerstitial areas were not positively correlated with 24-hour urinary protein excretion, blood urea nitrogen, creatinine, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), or complement C3, C4 (**Figure 2**).

HMGB1 positive cells in glomeruli was positively correlated with renal pathological damage

In this study, we found the HMGB1 positive cells in glomeruli was positively correlated with the degree of proliferation of glomerular cells and the renal tissue activity index ($r^2 = 0.556$, $P<0.05$) (**Figure 3**).

Discussion

SLE (systemic lupus erythematosus) is an autoimmune connective tissue disease, of which lupus nephritis (LN) is one of the most common serious complications and the main causes of death. Dysregulation of the immune system plays a dominant role in the pathogenesis of SLE and LN, especially the role of cytokines concerned in the process of lesions and progression. HMGB1, as a late mediator and a novel cytokine, plays a dominant role in the autoimmune disease. Previous studies have confirmed that HMGB1 is associated with the pathogenesis of rheumatoid arthritis, ANCA associated vasculitis, Sjogren's syndrome, systemic lupus erythematosus and other autoimmune diseases.

In this present study, we focused on the expressions of HMGB1 in kidney tissues of patients with lupus nephritis by using immunohistochemical staining to detect HMGB1's localization and expression. Results show that in the

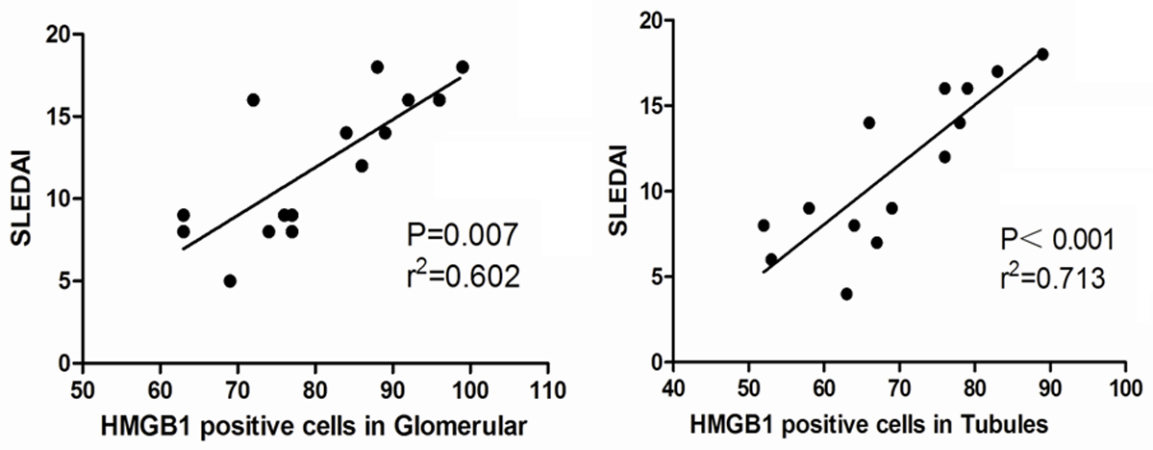

Figure 2. *The HMGB1 positive cells in glomeruli and tubularinterstitial areas were positively correlated with disease activity*. The HMGB1 positive cells in glomeruli (r^2 = 0.602, P<0.01) and tubulointerstitial (r^2 = 0.713, P<0.01) were positively correlated with SLEDAI.

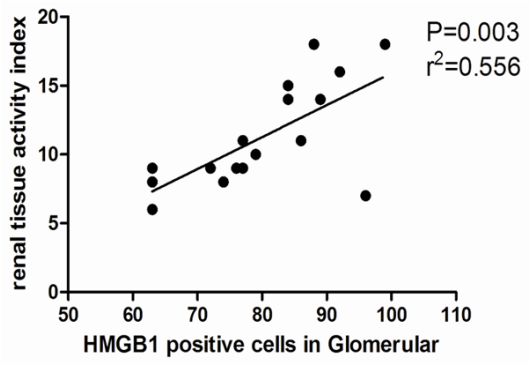

Figure 3. HMGB1 positive cells in glomeruli was positively correlated with renal pathological damage. The HMGB1 positive cells in glomeruli was positively correlated with the degree of proliferation of glomerular cells and the renal tissue activity index (r^2 = 0.556, P<0.05).

normal control group, there is only a small amount HMGB1 expression in glomerular and tubulointerstitial epithelial cells, which indicates that in renal tissue under normal physiological conditions, HMGB1 is a typical chromosome-binding protein, combined with DNA molecules non-specificity, participating in DNA replication, transcription, translation, DNA repair and a series of important metabolic activities. HMGB1 protein expression in kidney tissues of patients with lupus nephritis is significantly higher than that in the control group, and is expressed not only in the nucleus of glomerular and tubular cells, but also in the cytoplasm and outside of the cell, indicating that the pathogenesis of lupus nephritis is associated with increased HMGB1 expression. HMGB1 is likely to play a role of inflammatory mediators and (or) cytokines, participating in the high inflammatory response.

We have found that HMGB1-positive cells in glomeruli and tubular endoplasm was positively correlates with SLE disease activity score (SLEDAI), consistent with the results of Li's research on serum HMGB1 levels and disease activity of patients with SLE [30]. We have also detected HMGB1 expression is strongly positive in the glomeruli with significant cell proliferation, the amount of HMGB1-positive cells in glomeruli is positively correlated with renal tissue activity index, indicating that the increased expression of HMGB1 may be involved in glomerular cell proliferation, inflammatory cell infiltration, etc. It is unclear yet whether the highly expressed HMGB1 in glomeruli is released by the peripheral immune cells or immune cells infiltrated in renal tissue synthesis, or whether it is synthesized and shifted by mesangial cells and endothelial cells. However, what we are clear on is that the HMGB1 expression levels in renal tissue of LN patients have positive correlations with clinical laboratory data and with renal pathology, which indicates that HMGB1 is closely related to the disease activity of lupus nephritis.

HMGB1 needs to combine with its' respective receptors when it plays a role in lupus nephritis. Receptor for advanced glycoslyation end products (RAGE) is the most important receptor of HMGB1 [39]. Yamamoto and others transferred

human's RAGE genes into mice, forcing expression of RAGE, and found that renal damage expanded, glomerular cells proliferated, and proteinuria increased in the transgenic mice [40]. RAGE combined with HMGB1 can be expressed on the cell surfaces of monocytes/macrophages, vascular smooth muscle cells, neurons, endothelial cells, and mesangial cells of glomeruli, etc. The combination of HMGB1 and RAGE can activate a variety of intracellular signal transduction mechanisms, and stimulate inflammation. Currently, there are two signaling pathways, named mitogen-activated protein kinase (MAPK) and the shift-mediated inflammatory responses for nuclear factor NF-kB. The members of Toll-like family of receptors (TLRs) are also involved in signal transduction of HMGB1, regulating transcription factor NF-kB. Thus NF-kB plays a role in stimulating the maturation of immune cells and biological secretion of cytokines [41]. The effect of HMGB1 on lupus nephritis may be achieved by binding to its receptor RAGE or TLR2, TLR4, TLR9, etc, and activating these signals.

In conclusion, the expression of HMGB1 was significantly increased in the kidney tissue of patients with lupus nephritis and positively related to disease activity and renal pathological damage, which may play an important role in the pathogenesis of LN.

Disclosure of conflict of interest

None.

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