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Expression and clinical significance of NF− κ B, CTGF and OPN in mononuclear cells in peripheral blood as well as renal tissues in patients with IgA nephropathy

Cheng−Luo Hao*, Chang−Bin Liao

Department of Nephrology, Zigong Third People's Hospital, Zigong Sichuan, 643020, China

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ABSTRACT

Objective: To study the expression and clinical significance of NF- κ B, CTGF and OPN in mononuclear cells in peripheral blood as well as renal tissues in patients with IgA nephropathy. **Methods:** A total of 25 nephropathy patients diagnosed with IgA nephropathy and 25 patients receiving nephrectomy due to trauma or tumor in our hospital were studied. Peripheral blood and kidney tissues were collected to test NF- κ B, CTGF, OPN, T-bet, GATA-3, ROR γ T and Foxp3 expressions. **Results:** CTGF and OPN percentages in peripheral blood mononuclear cells and kidney tissues of nephropathy patients were higher than those of the control group. NF- κ B, CTGF and OPN expressions were significantly higher in M1, E1, S1 group patients' peripheral blood mononuclear cells and renal tissues than those in M0, E1 and S1 group. T-bet, GATA-3 and ROR γ T expressions in nephropathy patients' peripheral blood were significantly higher than those in the control group, and were positively correlated with NF- κ B, CTGF and OPN expressions. The expression of Foxp3 was significantly lower than that of control group, and was negatively correlated with NF- κ B, CTGF and OPN expressions. **Conclusions:** The expression of NF- κ B, CTGF and OPN in peripheral blood mononuclear cells and renal tissue in patients with IgA nephropathy is abnormally high and can evaluate the prognosis of the disease and the differentiation of $CD4^+$T cells.

1. Introduction

IgA nephropathy is the most common primary glomerulus disease in the world, accounting for 1/3 of all renal biopsy cases[1,2]. Epidemiological data shows that more than 30% of IgA nephropathy will eventually develop into end-stage renal disease[3]. Currently, renal biopsy is the gold standard for the diagnosis of IgA nephropathy and can judge the severity of the disease. But as an invasive examination, it cannot be used for long-term follow-up and evaluation of the disease. Therefore, to explore the key molecules in the occurrence and development of IgA nephropathy can provide basis for the discovery of new indicators of disease assessment. Glomerular sclerosis, renal tubular injury, renal interstitial fibrosis and extracellular matrix accumulation are important links in the progression of IgA nephropathy, and a variety of molecules including Nuclear transcription factor (NF- κ B), connective tissue growth factor (CTGF) and osteopontin (OPN) participate in and mediate the process[4,5]. In the following studies, we analyzed the expressions and clinical significance of NF- κ b, CTGF and OPN in peripheral blood mononuclear cells and renal tissues in patients with IgA nephropathy.

2. Materials and methods

2.1. Objects

A total of 25 patients with IgA nephropathy diagnosed in our hospital from April 2009 to June 2014 and 25 patients receiving renal resection due to trauma or tumor were selected. All patients received informed consent. Nephropathy patients included 18 male cases and 7 female cases, whose age was (35±5) years old. Control

*Corresponding author: Cheng-Luo Hao, Department of Nephrology, Zigong Third People's Hospital, Zigong Sichuan, 643020, China.
Tel: 0813-3302033, 13990079811
E-mail: Haochengluo@qq.com
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patients included 20 male cases and 5 female cases, whose age was (37±4) years old. There was no difference in the general data of patients between nephropathy patients and control patients.

2.2. Methods

2.2.1. Specimen collection method

Nephropathy patients and control patients' peripheral blood was collected from peripheral vein and then mononuclear cells were separated for subsequent detection. Nephropathy patients' renal tissues were obtained by percutaneous renal puncture. Control patients' normal renal tissues were isolated in surgery of kidney resection away from lesions.

2.2.2. Indexes detection methods

Peripheral blood mononuclear cells were separated, and after incubating NF- κ B, CTGF, OPN, T cell-expressed T box (T-bet), GATA junction protein 3 (GATA-3), retinoic acid related orphan receptor γ t (ROR γ t) and forkhead protein 3 (Foxp3) monoclonal antibodies, the proportion of positive expressions of these molecules was detected by flow cytometry. Kidney tissues were taken and homogenized for the determination of the contents of NF- κ B, CTGF and OPN by enzyme linked immunosorbent assay kit.

2.2.3. Statistical methods

SPSS21.0 software was used to input and analyze data. *t*-test was applied for the analysis between two groups. $P<0.05$ was set as statistical significant differences between groups.

3. Results

3.1. Expression of NF- κ B, CTGF and OPN in peripheral blood mononuclear cells and renal tissues

NF- κ B, CTGF and OPN percentage were higher in nephropathy patients' peripheral blood mononuclear cells than those in the control patients. NF- κ B, CTGF and OPN percentage were higher in nephropathy patients' renal tissues than those in the control patients, shown in Table 1.

3.2. NF- κ B, CTGF and OPN expressions in patients with different severity of IgA nephropathy

NF- κ B, CTGF and OPN expressions were significantly higher in M1, E1 and S1 group patients' peripheral blood mononuclear cells and renal tissues than those in M0, E0 and S0 group, shown in Table 2.

3.3. Expressions of T-bet, GATA-3, ROR γ t and Foxp3 in peripheral blood

T-bet, GATA-3 and ROR γ t expressions in nephropathy patients' peripheral blood were significantly higher than those in the control group, and was positively correlated with NF- κ B, CTGF and OPN expressions. The expression of Foxp3 was significantly lower than that in control group, and was negatively correlated with NF- κ B, CTGF and OPN expressions, as shown in Table 3.

Table 1

Expressions of NF- κ B, CTGF and OPN in peripheral blood mononuclear cells and renal tissues.

Groups	Peripheral blood mononuclear cells			Kidney tissues		
	NF- κ B(%)	CTGF(%)	OPN(%)	NF- κ B(ng/mL)	CTGF(pg/mL)	OPN(ng/mL)
Nephropathy patients	10.39±1.28	6.58±0.73	4.95±0.55	65.85±7.87	177.87±22.34	128.58±14.58
Control patients	6.27±0.86	4.03±0.57	2.77±0.32	40.39±4.96	97.86±10.34	74.75±8.97
t	8.686	7.384	9.119	6.897	8.784	7.915
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 2

Expressions of NF- κ B, CTGF and OPN in patients with IgA nephropathy with different severity of disease.

Groups	Peripheral blood mononuclear cells			Kidney tissues		
	NF- κ B(%)	CTGF(%)	OPN(%)	NF- κ B(ng/mL)	CTGF(pg/mL)	OPN(ng/mL)
Membrane cell proliferation						
M0	7.92±0.93	5.11±0.67	3.37±0.42	50.25±6.74	136.85±15.86	101.42±13.04
M1	12.57±1.28	7.94±0.89	6.11±0.76	73.85±9.48	209.38±25.91	155.46±16.58
Endothelial cell proliferation						
E0	7.75±0.89	4.88±0.58	3.58±0.39	52.33±6.29	140.23±16.14	98.37±12.27
E1	12.91±1.43	8.15±0.94	6.38±0.79	70.12±9.97	201.52±23.77	161.28±18.10
Segmental glomerular sclerosis						
S0	8.03±0.96	4.69±0.54	3.82±0.46	55.47±6.91	143.49±16.76	104.65±12.96
S1	12.11±1.18	8.41±0.99	5.99±0.65	67.86±8.57	196.76±23.07	150.38±17.19

Table 3

Expressions of T-bet, GATA-3, ROR γ t and Foxp3 in peripheral blood.

Groups	T-bet(%)	GATA-3(%)	ROR γ t(%)	Foxp3(%)
Nephropathy patients	5.58±0.68	3.91±0.44	3.28±0.36	1.96±0.22
Control patients	3.14±0.45	2.27±0.26	1.42±0.18	3.04±0.33
t	7.181	6.685	14.282	6.118
P	< 0.05	< 0.05	< 0.05	< 0.05

4. Discussion

NF- κ B is a class of transcription factor regulating immunity and inflammation-related gene transcription, which can activate the transcription of a variety of immune and inflammatory cytokines, increase the infiltration of a variety of immune cells and inflammatory cells as well as the production of related cytokines in peripheral blood and lesion tissue, and cause glomerular sclerosis, renal interstitial fibrosis, tubular atrophy and other pathological changes[6]. *In vitro* studies have confirmed that NF- κ κ B can regulate the synthesis and secretion of many cytokines in mesangial cells, and participate in the development of IgA nephropathy[7]. The study analyzed expressions of NF- κ B in peripheral blood mononuclear cells and kidney tissues. Results showed that: NF- κ B percentage was higher in nephropathy patients' peripheral blood mononuclear cells than that in the control patients. NF- κ B content was higher in nephropathy patients' renal tissues than that in the control patients. This showed that the high expression of NF- κ B in peripheral blood and kidney tissue was closely related to the occurrence of IgA nephropathy.

CTGF was first found in human umbilical vein endothelial cells. Recent studies have confirmed that CTGF has stimulative effect on crescent formation, mesangial proliferation and balloon adhesions, and also can promote hyperplasia and accumulation of extracellular matrix, infiltration of inflammatory cells and glomerular sclerosis[8,9]. OPN is powerful molecule of macrophage adhesion and chemotaxis. Macrophage surface highly expresses receptor with OPN high affinity, and OPN, after binding with corresponding membrane receptor, can promote macrophages migration and adhesion to the lesion tissue. That a large number of macrophages enter kidney tissue will aggravate renal mesenchyma injury and fibrosis[10]. Our analysis of CTGF, OPN expressions in peripheral blood mononuclear cells and renal tissues showed that the percentage of CTGF and OPN in nephropathy patients' peripheral blood mononuclear cells were higher than those in the control patients. CTGF and OPN contents were significantly higher in the kidney tissues of patients with nephropathy than those in control kidney tissue. This showed that the high expressions of CTGF and OPN in peripheral blood and kidney tissue were closely related to the occurrence of IgA nephropathy.

Biopsy is the gold standard for the diagnosis of IgA nephropathy, which not only can diagnose the disease accurately, but also can effectively assess the disease condition[11]. According to the 2009 IgA nephropathy Oxford classification standard, the proliferation of mesangial cells (M), glomerular capillary endothelial cell proliferation (E) and segmental glomerulosclerosis (S) are independent risk factors causing poor prognosis of IgA nephropathy[12]. In order to clarify NF- κ B, CTGF and OPN expressions in peripheral blood mononuclear cell and kidney tissues of patients with IgA nephropathy and their relationship with severity of the disease and prognosis, we analyzed the different membrane cell proliferation, glomerular capillary endothelial cell proliferation, section of renal glomerular sclerosis situation of IgA nephropathy patients' NF- κ B, CTGF and OPN expressions. Results were as follows: NF- κ B, CTGF and OPN expressions in M1, E1 and S1 group patients' peripheral blood mononuclear cells and renal tissues were significantly higher than those in M0, E0 and S0 group patients. This showed that the expressions of NF- κ B, CTGF and OPN in patients with IgA nephropathy were directly related to the severity of the disease and the prognosis.

Immunological injury and inflammatory injury are important links of renal function injury in IgA nephropathy patients. $CD4^+$T cell is the key cell to regulate the immune inflammatory response in vivo. It belongs to helper T cells, which has a regulatory effect on the initiation of immune response and the amplification of the inflammatory response[13]. When stimulated by external antigens, $CD4^+$T cells can differentiate into different subgroups, including Th1, Th2, Th17, and Treg[14]. The above mentioned Th cells' differentiation and maturation are mediated by specific transcription factors T-bet, GATA-3, ROR γ t and Foxp3 respectively. Polarized differentiations of Th1 and Th2 cells are able to regulate bone marrow-derived B cells and normal B cells to enhance the humoral immune response and produce too much IgA[15]. Th17 can secrete IL-17 and cause glomerular injury while Treg has an inhibitory effect on Th17 activation[16]. Our analysis of Th cell regulatory molecule expressions in peripheral blood showed that T-bet, GATA-3 and ROR γ t expressions in peripheral blood of patients with IgA nephropathy were significantly higher than those of the control group, and were positively correlated with the NF- κ B, CTGF and OPN expressions. Foxp3 was significantly lower than that of control group and was negatively correlated with the NF- κ B, CTGF and OPN expressions. This showed that the excessive activation of Th1, Th2 and Th17 as well as weakened Treg function were related to the over expressions of NF- κ κ B, CTGF and OPN.

Based on the above analysis, we draw the conclusions that NF- κ B, CTGF, OPN are highly expressed in peripheral blood mononuclear cells and renal tissues of IgA nephropathy patients. The expressions of NF- κ B, CTGF and OPN can assess disease prognosis and $CD4^+$T cells' differentiation.

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