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Original Article

Identification of mannose-binding lectin as a mechanism in progressive immunoglobulin A nephropathy

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Abstract: Immunoglobulin A nephropathy (IgAN), the pathogenesis of which remained still unclear is one of the leading courses of end-stage renal disease in approximately 50% affected patients. On the basis of several researches, the activation of complement mannose-binding lectin (MBL) pathway might be the underlying mechanism in disease progress. In order to investigate the relationship between MBL pathway and IgAN, we discussed the MBL gene polymorphism as well as its expressed level in serum, urine and renal parenchymal, with renal outcome in IgAN patients. The significantly down-regulated expression of MBL was discovered, which may serve as a potential urinary biomarker in progressive IgAN according to the results of difference in gel electrophoresis and matrix-assisted laser desorption/ionization time of flight mass spectrometry. The single nucleotide polymorphisms of MBL gene in promoter and exon region were found and confirmed relating with the poor prognosis of progressive IgAN patients. As a result, the deficient activation of MBL pathway caused by the mutation of MBL accompanied with low expressed level of MBL in serum might be the potential inspiring regulation in IgAN, and will attract a promising insight in remedy of IgAN to inhibit further progress.

Keywords: Immunoglobulin a nephropathy, mannose binding lectin, single nucleotide polymorphism

Introduction

Immunoglobulin A nephropathy (IgAN), first described by Heberden in 1801 was the most common glomerulonephritis all over the world and characterized by IgA antibody that deposited in the glomerulus [1]. The attack rate of IgAN increased rapidly in recent decades, and the presentation of IgAN in clinic was various including Henoch-Schönlein purpura (HSP), episodic hematuria, latent glomerulonephritis as well as rapidly progressive glomerulonephritis [2]. So far, the diagnosis of IgAN is depended on IgA accumulation observed by immunofluorescence of renal tissue. However, the differentiation of diseased renal tissue impeded the diagnosis of IgAN at early stage [3]. There is a slow progression of IgAN to chronic renal failure and 40%-50% cases will develop into end-stage renal disease (ESRD) during 20 years [4]. Since always occurred in people of 20-30 years old, IgAN is considered as the most common reason for the ESRD occurrence in young [5]. Therefore, diagnosing and intervening IgAN at early stage based on the investigation of progressive IgAN mechanism is necessary and will promote the prognosis of IgAN patients.

Although many researchers have put their efforts on its investigation, the underlying pathogenesis of progressed IgAN is still unclear. It is considered that the mucosal immune deficiency, decrease of the ability for immune complex removal, cytokines and genetic factor might be the potential mechanism of progressed IgAN, but with limited elucidation. Barratt J found the increase and the removal obstacle of structural abnormally IgA1 polymer in IgAN would stimulate the activation of complement system as well as the reaction of inflammatory response and might be the vital factor that led to the progressive IgAN.

Meanwhile, the activation of mannose-binding lectin (MBL) pathway in complement system might play important role in the mechanism of progressed IgAN [6].

MBL, as an oligomer for complementary activation was significant in the process of apoptosis and cell senescence [7]. Roos A. discovered the accumulation of MBL in renal tissue of IgAN patients and the amount of MBL related closely with the clinical presentation [8]. The study of genetics and molecular biology in recent years presented that the polymorphism of gene might participate into the progression of IgAN. And the polymorphism of MBL was considered as a potential regulator that influenced the IgA accumulation in IgAN patients confirmed by Gorgi Y [9]. According to the previous literatures, we indicated that the activation of MBL pathway might be the underlying mechanism of the progressive IgAN.

In current study, we detected the urine sample of progressive and non-progressive IgAN patients using difference in gel electrophoresis (DIGE) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to obtain the differentially expressed locus. And the biogenic marker of progressive IgAN was explored via MASCOT software analysis and NCBI database retrieval. Then, the polymorphism of MBL was discovered by direct sequencing and immunohistochemical method was applied to observe the accumulation of MBL in renal tissue. Finally, the enzyme-linked immunosorbent assay (ELISA) assay was used to analyze the expression level of MBL in progressive IgAN patients with different phenotype of MBL and further discussed the role of MBL polymorphism played in progressive IgAN.

Materials and methods

Patients

The progressive IgAN group for proteomics study was defined as the patients whose eGFR was less than 60 ml/min/1.73 m^2 at beginning diagnosis and improved inconspicuously after four weeks therapy with Lee grads of IV-V, while the non- progressive IgAN group included the patients with eGFR > 90 ml/min/1.73 m^2 at the beginning of diagnosis and the content of urine protein decreased evidently after four weeks cure with Lee grads of II. For the investigation of MBL accumulation in IgAN, the 131 IgAN patients with the number of glomeruli ≥ 10 in renal biopsy were selected. The patients included 60 males and 71 females with the average age of 37 years ole. The primary IgAN patients (18-65 years old) diagnosed in our hospital in their first renal biopsy from January 2008 to December 2001 were chosen to participate into the study of MBL gene polymorphism in IgAN. The patients suffered secondary IgAN, diabetes, autoimmune diseases and malignancy were excluded. All the tested patients were signed up on informed consent form.

DIGE

The micro-protein was extracted from 20 mL urine added protease inhibitor (50:1 cocktail set 1 Calibiochem Merk) using ultra-filtration centrifuge tube (YM-3, molecular weight cut-off: 5000) centrifuged at 3000 r/min for 45 min. After desalted by pre-cold acetone, the protein was dissolved in protein lysis (9 mol/l urea and 4% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHPAS)) and quantified on the basis of Bradford method. For fluorescence labeling, 400 pmol of Cy5, Cy3 and Cy2 were used to stain 50 μg urine samples of progressive IgAN patients, non-progressive IgAN patients and internal standard substance, respectively. The labeled reaction was terminated by l μL lysine (10 mmol/l) and the labeled protein was mixed with isometric lysis (1% IPG (immobilized pH gradient) buffer, 2% DTT (DL-Dithiothreitol), 9 mol/l urea, 4% CHAPS) for isoelectric focusing and SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel) electrophoresis. The differentially expressed points after first dimensional gel electrophoresis were searched applying DeCyde 5.01 software and analyzed statistically using differences in gel (DIA) and bioklogical variation analysis (BVA). The two-dimensional gel electrophoresis was performed on the other 800 μg urine and the gel was stained by Coomassie brilliant blue after second-dimensional gel electrophoresis. After cut off the matched protein manually, the sample was digested in trypsin at 37°C for 24 h and mixed with the mixture of 60% acetonitrile and 0.1% three fluorhydric acid. The matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS, Shimadzu Biotch, Manchester, UK) was employed to obtain peptide mass fingerprint (PMF) and MASCOT software (http://www.matrixscience.com) accompanied with National Center for

Biotechnology information (NCBI) was introduced to match protein.

Immunohistochemical staining

The paraffin section was dewaxed and cultured in sodium citrate buffer solution (0.01 mol/l, pH = 6.0) at 95°C for 15 min. After cooled to room temperature (25°C), the section was digested using 0.5% pancreatin and the activity of endogenous peroxidase was eliminated using 3% H_2O_2. The section was then blocked and the primary antibody of anti-mannose-binding lectin (MBL) followed with secondary antibody of biotinylated anti-rabbit IgG was added. The section was developed using digital audio broadcasting (DAB, 0.04%) after streptavidin-biotin peroxidase complex (SABC) added and redyed using hematoxylin. The sections were grouped as MBL-positive and MBL-negative group according to the stained degree of glomerulus.

DNA extraction and SNP genotyping

Four mL of the peripheral venous blood of selected IgAN patients was extracted with 2 mL was stored in sodium citrate anticoagulation tubes at 4°C to extract peripheral blood (PB) DNA and another 2 mL was put into heparin anticoagulant tube to obtain supernate after centrifugation (3000 r/min, 15 min). The PBDNA was extract using blood DNA isolation Kit according to manual instruction. According to the nucleotide sequence of MBL gene, the codon 52, codon 54 as well as codon 57 in exon region and -221 as well as +4 in promoter region were selected. The corresponding PCR primers were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The sequence of positive primer was 5'-TTGCCAGTGGTTTTTGACTC-3', while negative primer was 5'-TCATATCCCCAGGCAGTTTC-3'. The semi-nested amplification was performed with 50 μL reaction system including 20 ng DNA, 5 μL 10× buffer, 5 pmol of upstream- or downstream-primer, 2 mmol/l dNTP, 2 mmol/l $MgCL_2$ and 0.25 μL Taq at 95°C for 5 min of initial denaturation, 30 s of denaturation and 30 s of anneal, then at 72°C for 10 min to extend, orderly. The amplified procedure was repeated 20 times and the products were stored at 10°C for further use. The capillary electrophoresis sequencing was performed using ABI 3730 automatic sequencer and the result of sequencing was compared using Clustal W software. Finally, the SNPs were determined and recorded for further analysis.

ELISA analysis

The serum sample or MBL standard solution was added into the micro-well plate embedded with anti-MBL and reacted at 37°C for 120 min. After washed and dried, 100 μL of primary antibody was added per well followed by enzyme conjugate added and cultured at 37°C for 30 min. Then, 100 μL of substrate was added and after 15 min incubation in dark, the reaction was terminated using 100 μL terminated solution. The optical density (OD) values were detected by microplate Reader (Wellscan MK3, Lab Systems Company, Helsinki, Finland) at 450 nm and analyzed using Microsoft Office Excel 2007 software. The corresponded MBL concentration in serum sample was evaluated on the basis of standard curve of OD vs. MBL concentrations.

Follow-up test

Set the confirmed date of renal biopsy for progressive IgAN as baseline and followed-up every 3-6 months. The index included blood pressure, weight, blood routine, urine routine, hepatorenal function, blood lipid, immunoglobulin etc. were detected and evaluated. The observed termination was defined as the development into ESRD and death.

Data analysis

All the data in current research were accessed using SPSS 13.0. The *P* values obtained using two-side test was considered statistically significant with $P < 0.05$. The scanned result of DIGE was analyzed using DeCyder 6.5 software. The origin data was processed using chi-square analysis followed with Fisher's method to calculate definite probability. The Spearman correlation method and polynomial regression were applied to perform the single-factor analysis and multi-factor analysis, respectively. The survival analysis was employed Kaplan-Meier and Cox regression analysis methods. All the experiments were repeated trice.

Results

Down-regulated urine protein in progressive IgAN

The basic information of selected IgAN patients for urine proteomics were shown in **Table 1**. Compared with non-progressive group, the pro-

Table 1. The basic information of selected IgAN patients for urine proteomics

Index	Progressed IgAN (n = 6)	Non-progressed IgAN (n = 6)	*P* value
Age	36.80 ± 7.19	37.83 ± 10.59	0.858
BMI (kg/m^2)	22.00 ± 0.71	24.17 ± 0.98	0.072
SBP (mmHg)	131.50 (120.00-142.50)	120.00 (110.00-130.00)	0.020
DBP (mmHg)	85.00 (75.00-88.00)	80.00 (72.00-84.50)	0.041
MAB (mmHg)	110.50 (105.50-120.50)	100.67 (91.50-110.00)	0.021
WBC (× 10^9/L)	6.45 ± 1.37	7.18 ± 1.72	0.120
Hb (g/dL)	125.43 ± 17.34	130.40 ± 15.75	0.973
PLT (× 10^9/L)	222.00 ± 53.44	211.70 ± 55.35	0.213
Urine RBC (Unit/HP)	22.30 (7.25-45.55)	20.40 (6.45-42.00)	0.567
24 hUTP (g)	2.85 (1.07-4.65)	0.88 (0.40-1.32)	0.038
ESR (mm/h)	21.00 (12.00-30.50)	20.00 (10.50-30.00)	0.393
Hs-CRP	1.85 (0.00-3.35)	0.75 (0.00-1.18)	0.160
sAlb (g/L)	34.97 ± 5.02	38.05 ± 6.90	0.830
Scr (μmol/L)	126.22 (90.44-145.10)	81.50 (60.85-100.85)	0.004
eGFR (ml/min/1.73 m^2)	33.53 ± 11.18	88.91 ± 17.46	0.022
UA (mmol/L)	476.56 ± 87.08	369.00 ± 91.67	0.015
sIgA (mmol/L)	3.02 ± 1.01	2.93 ± 0.95	0.810
C3 (g/L)	1.02 ± 0.14	1.08 ± 0.18	0.348
TC (mmol/L)	5.76 (5.04-6.71)	5.36 (4.41-5.83)	0.753
TG (mmol/L)	1.65 (0.88-2.38)	1.74 (1.25-2.93)	0.815
HDL (mmol/L)	1.50 ± 0.44	1.93 ± 0.41	0.128
LDL (mmol/L)	3.16 (2.88-3.97)	3.03 (2.54-3.61)	0.636
LP (a) (mmol/L)	353.00 (137.50-610.00)	288.00 (101.00-487.50)	0.435
GGS	0.67 ± 0.14	0.28 ± 0.13	0.012
TID	5.50 (3.50-6.25)	2.50 (2.00-4.00)	0.031

gressive IgAN patients revealed higher values of blood pressure, 24 hUTP, Scr, GGS and TID, while the value of eGFR decreased significantly. Cy2, Cy3 and Cy5 were scanned by laser at different wave length of 448 nm, 532 nm and 633 nm, respectively. 36 protein points expressed differentially were obtained by matched the three sscannograms with interior label (**Figure 1A-C**). After matched with the results of DIGE analysis, 23 protein points were cut (**Figure 1D**). MALDI-TOF MS was applied to identified 23 differentiate proteins and the protein matched with the obtained peptide mass fingerprinting (PMF) (**Figure 1E**) was found via MASCOT software analysis and NCBI database retrieval. Compared with non-progressive IgAN, the five down-regulated proteins in 23 protein expressed differentially of IgAN patients were MBL, retinol binding protein (RBP), histone H2B, zinc-alpha-2-glycoprotein, chain B, carbamoyl-phosphate synthetase 1 (CPS-I), the other 18 protein, such as transthyretin, chain A and ATP synthetase etc. were up-regulated (**Table 2**). The significant down-regulation of MBL in progressive IgAN patients indicated that MBL might be an important regulator that participated into the development of IgAN.

Effect of MBL negative and positive accumulation on the prognosis of progressive IgAN patients

According to the basic information of 131 selected patients in this part, 60 males and 71 females (average age, 37.59) were graded on the basis of Lee grades and divided into four levels including II (3), III (42), IV (72) and V (14) (**Table 3**). After immunohistochemical staining, 27 patients revealed MBL positive while the others were MBL negative. In contrast with MBL positive group, the SBP, DBP, MABP, PLT and Scr of IgAN patients with MBL negative were higher significantly (**Table 4**). In current study, we followed up all the patients within 50 months. Four patients developed into end-stage renal disease (ESRD) and no death was

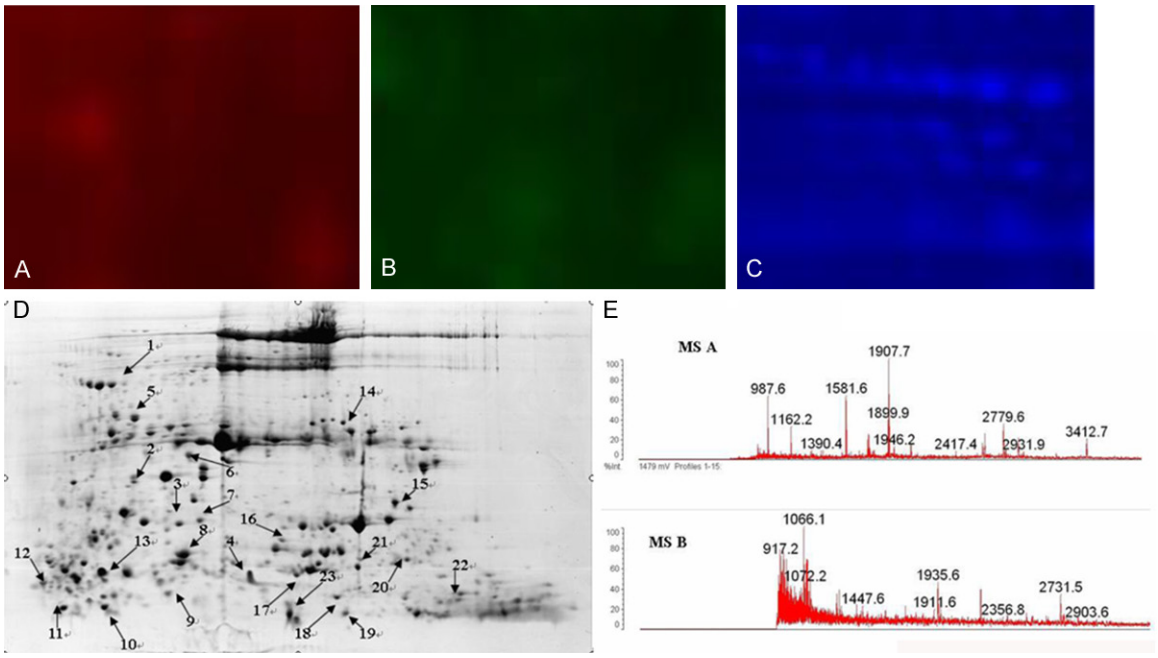

Figure 1. DIGE results of progressive and non-progressice IgAN patients. A. The urine sample of progressive IgAN patients labeled by Cy5. B. The urine sample of non-progressive IgAN patients labeled by Cy3. C. The interior label of Cy2. D. The protein expressed differentially of progressive and non-progressive IgAN patients. E. The PMF result obtained from MALDI-TOF analysis.

appeared among them. The result was presented in **Figure 2A**. The Scr increased significantly higher in MBL negative IgAN patients than MBL positive ones. Eight cases were found in twelve cases in which the patients reached at observed termination. The ratio of MBL negative accumulation in IgAN patients was proved high by chi-square test and the patients with negative MBL accumulation was confirmed in a worse condition of prognosis by Kaplan-Meier analysis.

SNPs of MBL were found in IgAN patients

Table 5 presented the basic information of 93 patients including 49 males and 44 females in this part of study, the average age of them were 31. The SNPs were discovered in two observed points (promoter region -221 and exon region condon 54). The genotype of 93 selected IgAN including wild type HYP/HYP (71), heterozygote phenotype HYP/HXP (20), homozygote phenotype (2) in promoter region -221 and wild type GGC/GGC (65), heterozygote phenotype GGC/GAC (24), homozygote phenotype GAC/GAC (4) in exon region condon 54. The selected patients were proved representative via matched the chi-square testing results of gene polymorphism distribution with Hardy-Weinberg equilibrium assay.

Comparison of MBL content in serum of IgAN patients with different MBL genotype

In exon region, the MBL content in serum of IgAN with wild type GGC/GGC (916.63 ± 164.60 ng/mL) was higher than heterozygote phenotype GGC/GAC (233.10 ± 25.23 ng/mL) significantly (P = 0.001). The content of MBL was extremely low (less than 10 ng/mL) in serum of patients with homozygote phenotype GAC/GAC and decreased significantly compared with wild type and heterozygote phenotype ($P < 0.001$). In promoter region, the content of MBL in serum of patients with wild type HYP/HYP (696.25 ± 168.89 ng/mL) was higher than that with homozygote phenotype HXP/HXP (269.50 ± 54.50 ng/mL, P = 0.028) significantly, while in patients with heterozygote phenotype HYP/HXP, the content of MBL was 354.12 ± 81.23 ng/mL and discovered no differentiation statistically compared with the patients with wild type (P = 0.082) and homozygote phenotype (P = 0.425).

Different prognosis of IgAN patients with different MBL genotype

10 patients in 93 tested IgAN patients reached at observed termination during the followed-up

Table 2. The differentially expressed protein in urine of progressed IgAN

Protein	up/ down	Gi No.	MW (kDa)/PI	Sequence coverage (%)
Mannose-binding lectin	↓	168985178	3.095/4.44	100
Zinc-alpha-2-glycoprotein, chain B	↓	4699583	31.85/5.70	55
Retinol-binding protein	↓	4558179	20.75/4.94	74
Histone H2B	↓	28173554	13.91/10.32	48
Carbamoyl-phosphate synthetase 1, mitochondrial	↓	21361331	16.49/6.57	65
Zinc-alpha-2-glycoprotein, chain A	↑	4699583	31.85/5.70	49
Transthyretin Chain A	↑	55669575	12.83/5.33	81
ATP synthase, H+ transporting, mitochondrial F1 complex, beta subunit	↑	32189394	56.56/4.78	57
T-cell antigen receptor VJ junction beta chain	↑	631443	1.55/5.91	100
Immunoglobulin heavy chain	↑	112702583	11.17/7.83	75
Alpha-1-microglobulin/bikunin precursor	↑	4502067	39.00/5.95	72.3
Interferon-induced protein with etratricopeptide repeats 2	↑	34192824	56.97/6.32	31
T-cell receptor beta chain	↑	11527709	3.44/6.18	71
Serum transferin n-terminal lobe,chain A	↑	49258810	36.99/7.16	46
Chain A, solution structure of domain 3 from human serum albumin complexed to an anti-apoptotic ligand directed against bcl-Xl and bcl-2	↑	71042087	23.32/8.22	35
Transferrin	↑	339989	7.294/7.85	55
RNA polymerase II subunit	↑	717189	3.716/5.96	53
Ubiquitin-conjugating enzyme E2	↑	56203163	11.82/5.45	36
Suppression of tumor genicity	↑	73544648	4.754/8.94	57
MAP3K12 binding inhibitory protein 1	↑	119586268	32.44/6.28	38
Transcription factor HFK3	↑	1082852	13.13/9.81	45
T cell receptor alpha chain	↑	10304637	3.183/4.22	89
hypothetical protein	↑	169217323	1.71/10.39	85

Table 3. The basic information of IgAN patients for immunohistochemistry

Index	Number
Male/Female	60/71
Age	37.59 ± 11.60
BMI (kg/m^2)	24.03 ± 0.55
SBP (mmHg)	120 (110-130)
DBP (mmHg)	80 (70-82)
MABP (mmHg)	106.67 (96.67-115.00)
WBC (× 10^9/L)	6.78 ± 0.27
Hb (g/dL)	134.85 ± 2.25
PLT (× 10^9/L)	204.15 ± 9.52
Urine RBC (Unit/HP)	27.45 (9.9-41.65)
24 hUTP (g/d)	1.60 (1.12-2.98)
ESR (mm/h)	20.00 (8.00-35.00)
Hs-CRP (mg/L)	0.00 (0.00-1.15)
sAlb (g/L)	38.36 ± 0.65
SCr (μmol/L)	78.85 (69.60-103.30)
eGFR (ml/min/1.73m^2)	85.14 ± 13.92
UA (mmol/L)	369.35 ± 12.77
sIgA (mmol/L)	3.09 ± 0.14
C3 (g/L)	1.05 ± 0.03
TC (mmol/L)	5.25 (4.55-5.82)
TG (mmol/L)	1.59 (1.12-2.27)
HDL (mmol/L)	1.30 ± 0.05
LDL (mmol/L)	3.16 (2.79-3.68)
LP (a) (mmol/L)	192.00 (87.00-512.50)
Lee grades	n (%)
II	3 (2.29)
III	42 (32.06)
IV	72 (54.96)
V	14 (10.69)
GGS	0.40 ± 0.03
TID	4.5 (3.0-6.0)

period of 31 month. 2 of them developed into ESRD, the other 8 experienced an increase (more than 50%) in the level of Scr. Compared with normal patients, the Scr during followed-up (9.75 (6.65-13.75) vs. 8.95 (-0.15-16.65) μmol/l/year, P = 0.05) and at observed termination (86.75 (68.90-120.80) vs. 80.95 (74.10-104.55) μmol/l, P = 0.022) were higher obviously in patients with SNP in exon region condon 54. While in promoter region -221, no evident change of Scr was discovered. Among 10 IgAN who reached at observed termination, the numbers of patients with wild type GGC/GGC, heterozygote GGC/GAC and homozygote GAC/GAC in exon region condon 54 were 6, 4 and 0, respectively. While in promoter region -221, the distribution of these 10 patients was 9 for wild type of HYP/HYP and 1 for heterozygote of HYP/HXP. According to the result of chi-squared test, the occurrence rate of terminated cases in patients with SNP of exon region condon 54 (P = 0.009) was higher significantly than that of promoter region -221 (P = 0.087). The IgAN patient with SNP in exon region condon 54 revealed a worse prognosis (P = 0.005) by a further analysis of Kaplan-Meier (**Figure 2B**). Cox regression analysis demonstrated that the SNP in exon region condon 54 (β = 2.460, P = 0.033) accompanied with initial Scr (β = 0.966, P = 0.04), DBP (β = 1.272, P = 0.045) and TID (β = 0.202, P = 0.048) was the risk of the prediction for the renal prognosis of IgAN patients.

Discussion

The occurrence of IgAN-the most common glomerulonephritis worldwide-was reported in creasing rapidly in recent decades with 40%-50% of cases developed into ESRD during 20 years. Since progressive IgAN would lead to a high occurrence of ESRD in young, the investigation of its underlying mechanism was a promising insight for promoting the prognosis of IgAN patients [10].

DIGE was a technique of gel electrophoresis that was able to label three different protein samples simultaneously and applied cy2 as interior label, cy3 as well as cy5 as marker to mark the sample on different gel [11]. Although employed in several researches of renal disease, the usage of DIGE in the study of progressive IgAN was limited. In current study, we associated DIGE with MALDI-TOF-MS technology to detect the urine of progressive and non- progressive IgAN patients and found 23 differentiated proteins that matched with PMF results. Among them, 18 up-regulations and 5 down-regulations of proteins demonstrated that the progression of IgAN was related with the expression of specific protein. Meanwhile, the expression of MBL revealed a significant down-regulation in progressive IgAN. It was indicated that the complement lectin pathway might be an underlying mechanism of IgAN progression.

MBL, as an activator to activate lectin pathway was reported as a potential regulator in various diseases, including ischemia/reperfusion renal

Table 4. The relationship between renal MBL precipitation and the clinical and biochemical index of IgAN patients

Index	MBL negative (n = 27)	MBL positive (n = 104)	*P* value
Age	36.42 ± 12.08	38.36 ± 13.00	0.128
BMI (kg/m^2)	24.22 ± 3.76	24.38 ± 4.65	0.550
SBP (mmHg)	120.00 (110.00-130.00)	110.00 (107.50-120.00)	0.028
DBP (mmHg)	80.00 (70.00-85.00)	70.00 (70.00-82.00)	0.017
MABP (mmHg)	106.67 (98.33-117.00)	96.67 (95.00-115.67)	0.020
Blood WBC (× 10^9/L)	6.83 ± 1.88	7.00 ± 1.74	0.718
Hb (g/dL)	134.15 ± 18.67	133.15 ± 14.54	0.815
PLT (× 10^9/L)	218.96 ± 67.72	184.90 ± 53.33	0.033
Urine RBC (Unit/HP)	28.55 (13.70-41.65)	29.10 (9.90-92.70)	0.250
24 hUTP (g)	1.64 (1.08-2.93)	1.91 (1.26-3.02)	0.768
ESR (mm/h)	18.50 (7.00-36.50)	24.00 (14.00-26.50)	0.557
Hs-CRP	0 (0.00-1.21)	0 (0.00-0.75)	0.385
sAlb (g/L)	37.26 ± 6.97	36.49 ± 6.06	0.642
Scr (μmol/L)	86.1 (72.45-122.05)	71.00 (66.50-109.00)	0.010
GFR (mL/min/1.73 m^2)	81.84 ± 27.09	87.56 ± 33.44	0.549
UA (mmol/L)	400.61 ± 109.79	357.48 ± 103.38	0.120
sIgA (mmol/L)	3.01 ± 1.08	3.04 ± 0.93	0.924
C3 (g/L)	1.10 ± 0.21	1.06 ± 0.29	0.605
TC (mmol/L)	5.28 (4.55-5.82)	5.56 (5.07-6.44)	0.654
TG (mmol/L)	1.69 (1.09-2.27)	1.89 (1.34-2.52)	0.622
HDL (mmol/L)	1.31 ± 0.45	1.29 ± 0.35	0.863
LDL (mmol/L)	3.20 (2.79-3.75)	3.30 (2.84-3.79)	0.680
LP (a) (mmol/L)	256.50 (121.00-529.15)	177.50 (80.00-557.50)	0.413

injury [12], diabetic nephropathy [13], systemic lupus erythenlatosus nephritis (SLE) [14], rheumatoid arthritis [15], disease of respiratory system [16] and atherosis [17]. The positive immumohistochemical staining of MBL in IgAN was first discovered by Endo M [18] and the accumulation of MBL was discovered in IgAN patients with more severe clinical presentation [19]. Roos A found the lectin pathway related factors of MBL, L-ficolin, MBL associated serine protease (MASP), C4d and C4d binding protein were positive in IgAN patients via immumohistochemistry and indicated that the complement activated by MBL pathway might lead to the injury of kidney. In present study, immumohistochemistry was carried out in 131 progressed IgAN patients and 20.61% of their renal tissues were discovered with positive MBL accumulation while 79.39% negative. The progressive IgAN patients with negative MBL presented a significantly high level of SBP, DBP, MABP, PLT and Scr as well as a worse prognosis compared with positive ones. It was suggested that the insufficient of MBL activation might play important role in IgAN progression.

The mutation of MBL was considered influencing immunological function and the content of MBL was found lower in immunodeficiency patients than healthy people. Sumiya M discovered the mutation of MBL at No. 54 codon in immunodeficiency patients with lower content of MBL [20] and the same phenomena was found by Garred P as well [21]. So far, the mutation of MBL accompanied with low content of MBL in serum was reported as the potential mechanism of several diseases, including SLE [22], cystic fiber lesions [23], rheumatoid arthritis [24]. However, the report concerned with the deficient activation of MBL in progressed IgAN was limited. In current study, we detected genotype of MBL in 93 progressed IgAN patients and found SNP in promoter region -221 and exon region 54. The ratios of wild type, heterozygote phenotype and homozygote phenotype were 74.34% (HYP/HYP), 21.51% (HYP/HXP) as well as 2.15% (HXP/HXP), respectively in promoter region and 69.89% (GGC/GGC), 25.81% (GGC/GAC) as well as 4.30% (GAC/GAC), respectively in exon region. The differentiation of MBL content in serum of patients with different

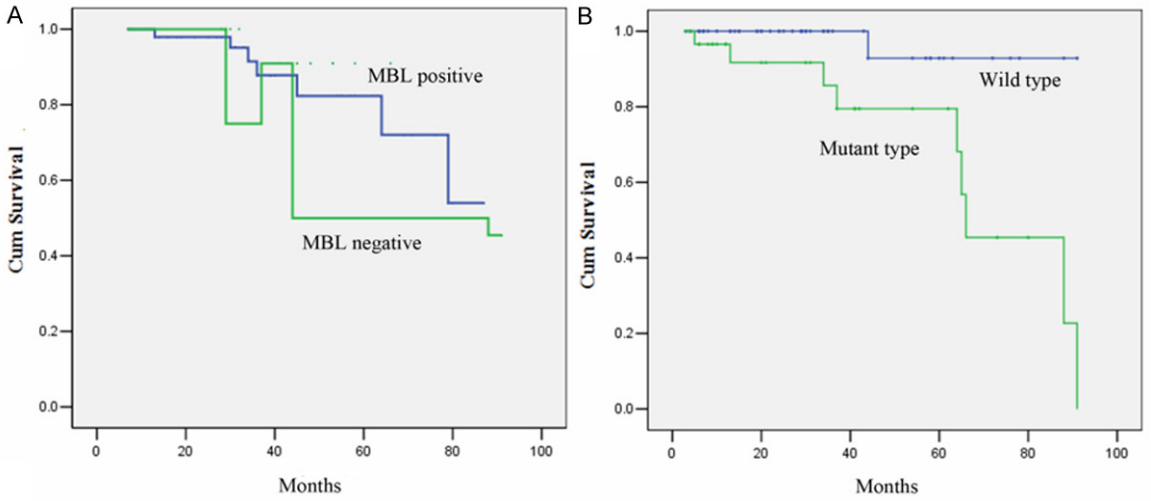

Figure 2. A. Kaplan-Meier analysis revealed that the prognosis of progressive IgAN with negative MBL accumulation in renal tissues was worse significantly. B. Kaplan-Meier analysis revealed a significantly worse prognosis of progressive IgAN patients with SNP in condon 54 of exon region.

Table 5. The basic information of IgAN patients for MBL gene sequencing analysis

Index	Number
Male/Female	49/44
Age	31.00 ± 6.11
BMI (kg/m^2)	26.54 ± 2.22
SBP (mmHg)	118 (108-130)
DBP (mmHg)	82 (70-85)
MABP (mmHg)	108.35 (97.40-116.00)
WBC (× 10^9/L)	6.33 ± 0.99
Hb (g/dL)	125.00 ± 8.02
PLT (× 10^9/L)	222.33 ± 23.67
Urine RBC (Unit/HP)	28.70 (19.30-93.90)
24 hUTP (g/d)	1.56 (1.52-2.28)
ESR (mm/h)	30.00 (25.00-50.00)
Hs-CRP (mg/L)	0.80 (0.00-1.67)
sAlb (g/L)	41.73 ± 0.95
SCr (μmol/L)	84.70 (59.50-141.30)
eGFR (ml/min)	80.64 ± 30.28
UA (mmol/L)	317.90 ± 39.29
sIgA (mmol/L)	2.12 ± 0.12
C3 (g/L)	1.02 ± 0.07
TC (mmol/L)	5.30 (4.99-5.88)
TG (mmol/L)	2.01 (1.67-2.22)
HDL (mmol/L)	1.20 ± 0.12
LDL (mmol/L)	3.71 (3.38-3.90)
LP (a) (mmol/L)	111.00 (80.50-296.50)
GGS	0.29 ± 0.11
TID	3.0 (3.0-3.75)
Lee grades	n (%)
II	2 (2.66)
III	21 (28.00)
IV	40 (53.34)
V	12 (16.00)

genotype was confirmed using ELISA method. The SNP in promoter region was proved impacting the MBL content in progressive IgAN patients potently which indicated that the mutation in promoter region affected the protein synthesis of MBL. Besides, the poor prognosis of progressed IgAN patients with SNP in promoter region codon 54 of MBL further illustrated the important role of this mutation in the regulation of IgAN progression.

In conclusion, the deficient activation of MBL- a vital activator of complement lectin pathway activation-which caused by the mutation of MBL in promoter region condon 54 might be the underlying mechanism of progressive IgAN and provided a promising insight for the management and amelioration of progressive IgAN.

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Disclosure of conflict of interest

None.

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