Original Article

External suppression causes the low expression of the Cosmc gene in IgA nephropathy

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Abstract

Objective. IgA₁ aberrant O-glycosylation is one of the main pathogeneses of IgA nephropathy (IgAN), and the core I β-3-Gal-T-specific molecular chaperone (Cosmc) mRNA expression of IgAN patients was significantly decreased. This study tried to clarify whether the down-regulation was a result of genetic disorders or external suppressions.

Method. Sixty-five IgAN patients, 23 non-IgAN glomerulonephritis patients and 21 normal controls were recruited. Genomic DNA was extracted and the Cosmc gene was PCR amplified and directly sequenced. Peripheral B lymphocytes of IgAN patients and normal controls were isolated, and cultured with RPMI-1640 alone or with lipopolysaccharide (LPS) for 72 h. The Cosmc mRNA expression levels at baseline, after RPMI culture or RPMI + LPS treatment were measured by real-time RT-PCR.

Results. (1) The whole coding frame region of the Cosmc gene was successfully amplified and directly sequenced. Four single nucleotide polymorphisms were detected in two IgAN patients. Two were missense mutations and the others were silent mutations. However, they are different from each other, and unrelated to expression levels; (2) the baseline Cosmc mRNA expression in IgAN patients was significantly lower than normal controls (CtCosmc/GAPDH 1.29 ± 0.08 versus 1.20 ± 0.01, 31% of normal controls); (3) the Cosmc mRNA expression level of IgAN patients was remarkably increased after the RPMI culture (1.22 ± 0.12 versus 1.29 ± 0.08, 219% of the baseline level), while not in normal controls and (4) treatment with LPS (culture with RPMI + LPS) could strongly inhibit the expression of Cosmc mRNA (1.25 ± 0.01 versus 1.22 ± 0.12, 61% of the RPMI treatment group).

Conclusion. No common Cosmc gene mutation was detected. Significantly increased Cosmc expression was observed in plasma-free culture, while LPS could significantly inhibit it, which suggested that it might not be genetic disorders but external suppression that causes the low Cosmc mRNA expression in IgAN.

Keywords: Cosmc; IgA nephropathy; LPS; mRNA expression; real-time RT-PCR

Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis in the world, accounting for >50% of biopsy-proven primary glomerulonephritis in Asia [1]. Approximately 20% of the patients progressed to end-stage renal disease (ESRD) within 20 years. However, the pathogenesis and mechanism of IgAN still remain to be determined.

Recent investigations indicated that abnormalities of IgA₁ O-glycosylation may be one of the key pathogeneses of IgAN [2–4]. The study showed that β1,3-galactosyltransferase synthesis (β1,3GT) activity was remarkably lower in IgAN patients [5] and the Cosmc (core I β-3-Gal-T-specific molecular chaperone) mRNA expression level in IgAN patients was significantly decreased and correlated with IgA glycosylation abnormality degree as well as clinical manifestations [6]. Yet, no study has been carried out to clarify the underlying basis of the expression suppression. Therefore, the current study was performed trying to shed light on this question: is it caused by a genetic disorder or external suppression?

In IgAN patients, bacterial infection (upper respiratory tract and intestinal) is often related to relapse and manifests as proteinuria, macroscopic haematuria and oedema [7,8]. Lipopolysaccharide (LPS) is the marker of G– bacterial infections [9], which could induce experimental IgAN [10] and stimulate the tonsillar lymphocyte to produce aberrant O-glycans [11,12]. LPS could also be detected during bacterial infections in the patients’ plasma and urine [13]. It is speculated that LPS may affect the expression of glycosylation-related genes such as Cosmc.

Therefore, this study aimed to clarify whether the down-regulation of Cosmc mRNA of IgAN patients was a result of genetic disorders or external suppressions,
and whether LPS is one of the underlying external suppressors of the Cosmc expression.

Material and methods

Patients and normal controls

Sixty-five biopsy-proven IgAN patients were included in this study. Diagnosis criterion of IgAN was based on the manifestation of generalized glomerular mesangial proliferation with the presence of IgA as the sole or predominant immunoglobulin deposition in the mesangial area of glomeruli. Patients with a systemic disease such as Schönlein–Henoch purpura, systemic lupus erythematosus, Sjögren syndrome, rheumatoid arthritis, diabetes mellitus or liver cirrhosis were excluded. Twenty-three biopsy-proven non-IgAN glomerulonephritis patients were included as controls. Of them, there were seven cases of minimal change disease, two focal segmental glomerulosclerosis (FSGS), three membranous nephropathy, two of membranoproliferative glomerulonephritis and nine mesangial proliferative glomerulonephritis. The 1995 WHO classification criteria are applied in the diagnosis. Twenty-one healthy volunteers (unrelated to IgAN patients, without known disease and genetic disease family history) were selected as normal controls. Measurements of blood pressure (BP), urine routine and serum creatinine were performed in all patients.

Severity of proteinuria was classified according to 24-h quantification: >3.5 g/day was recognized as severe proteinuria. The WHO definition of hypertension was applied in the hypertension classification. Judgement of renal dysfunction was based on serum creatinine concentration; 133 µmol/L was applied as the upper normal limit. Measurements of BP, urine routine, serum creatinine and 24-h urinary protein quantification were performed in all patients.

Blood samples and B lymphocyte isolation

A twenty millilitre venous blood sample was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes (BD Vacutainer, USA). Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation on Lymphocyte-H lymphocyte isolation media (Cedarlane Laboratories Limited, Canada). PBMCs were washed three times with phosphate-buffered saline (PBS, Sigma, USA) and resuspended in PBS + 1% bovine serum albumin (BSA, Sigma, USA). Then, peripheral B lymphocytes were isolated using EasySep Human CD19 Selection Kit magnetic beads (Stem cell, USA) according to the manufacturer’s protocol. The purity of harvested cells was measured by flow cytometry, which indicated a B-cell population of >90%.

DNA extraction and gene sequencing

Genomic DNA was extracted from 200 µL EDTA anticoagulated peripheral venous whole blood using QIAamp® DNA Blood Mini Kits (Qiagen, Germany) according to the manufacturer’s protocol. Concentration and purity were determined by the Beckman DU-600 UV spectrometer (OD ratio of 260/280 nm).

The exon region of the Cosmc gene was amplified using the PTC220 thermocycler (MJ Research, USA). Primers (listed in Table 1) used were designed based on the sequence of Cosmc in the GeneBank database (NM_152692) [14,15] using Primer 5 software and synthesized by Takara, Japan. The PCR product was 1247 bps in length, which contains the whole region of the coding sequence. A 50 µL PCR reaction solution (10× Taq Buffer 5 µL; 25 mM MgCl2 5 µL; 10 mM dNTPs 1 µL; 10 µM primers 2 µL each; Ex-Taq DNA Polymerase 1 µL; DNA template 2 µL; ddH2O added to 50 µL, Ex-Taq DNA Polymerase kit, Takara, Japan) was applied. The thermocycles were as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, polymerization at 72°C for 60 s and then final polymerization at 72°C for 10 min.

The PCR products were purified using the Agarose Gel DNA Fragment Recovery Kit (Takara, Shiga, Japan) according to the protocol. The purified PCR products were directly sequenced by Invitrogen China (Shanghai, China). Sequencing results were analysed using Chromas 1.62 software (Technelysium Pty, Australia) and compared with the GeneBank sequence using NCBI Blast (Align two sequences, blast2seq; www.ncbi.nlm.nih.gov/BLAST/).

B lymphocyte culture and treatment

Four groups were established in this study, i.e. Group A (n = 20): B lymphocytes from normal controls cultured with the RPMI-1640 medium (Gibco, USA) alone; Group B (n = 20): B lymphocytes from normal controls cultured with RPMI-1640 + LPS; Group C (n = 22): B lymphocytes from IgAN patients cultured with the RPMI-1640 medium and Group D (n = 22): B lymphocytes from IgAN patients cultured with RPMI-1640 + LPS. In Groups A and C, isolated B lymphocytes (10⁶ cells per well) were cultured in the RPMI-1640 medium (with 15% fetal bull serum, Gibco, USA) at 37°C in a humidified atmosphere of 5% CO₂ in air. The RPMI-1640 medium (with 15% fetal bull serum, Gibco, USA) was applied as the upper normal limit.

Table 1. Primers and fluorescence probes

| Internal suppression causes the low expression of the Cosmc gene in IgA nephropathy | External suppression causes the low expression of the Cosmc gene in IgA nephropathy | and whether LPS is one of the underlying external suppressors of the Cosmc expression. | Material and methods | Patients and normal controls | Sixty-five biopsy-proven IgAN patients were included in this study. Diagnosis criterion of IgAN was based on the manifestation of generalized glomerular mesangial proliferation with the presence of IgA as the sole or predominant immunoglobulin deposition in the mesangial area of glomeruli. Patients with a systemic disease such as Schönlein–Henoch purpura, systemic lupus erythematosus, Sjögren syndrome, rheumatoid arthritis, diabetes mellitus or liver cirrhosis were excluded. Twenty-three biopsy-proven non-IgAN glomerulonephritis patients were included as controls. Of them, there were seven cases of minimal change disease, two focal segmental glomerulosclerosis (FSGS), three membranous nephropathy, two of membranoproliferative glomerulonephritis and nine mesangial proliferative glomerulonephritis. The 1995 WHO classification criteria are applied in the diagnosis. Twenty-one healthy volunteers (unrelated to IgAN patients, without known disease and genetic disease family history) were selected as normal controls. Measurements of blood pressure (BP), urine routine and serum creatinine were performed in all patients. Severity of proteinuria was classified according to 24-h quantification: >3.5 g/day was recognized as severe proteinuria. The WHO definition of hypertension was applied in the hypertension classification. Judgement of renal dysfunction was based on serum creatinine concentration; 133 µmol/L was applied as the upper normal limit. Measurements of BP, urine routine, serum creatinine and 24-h urinary protein quantification were performed in all patients. | DNA extraction and gene sequencing | Genomic DNA was extracted from 200 µL EDTA anticoagulated peripheral venous whole blood using QIAamp® DNA Blood Mini Kits (Qiagen, Germany) according to the manufacturer’s protocol. Concentration and purity were determined by the Beckman DU-600 UV spectrometer (OD ratio of 260/280 nm). The exon region of the Cosmc gene was amplified using the PTC220 thermocycler (MJ Research, USA). Primers (listed in Table 1) used were designed based on the sequence of Cosmc in the GeneBank database (NM_152692) [14,15] using Primer 5 software and synthesized by Takara, Japan. The PCR product was 1247 bps in length, which contains the whole region of the coding sequence. A 50 µL PCR reaction solution (10× Taq Buffer 5 µL; 25 mM MgCl2 5 µL; 10 mM dNTPs 1 µL; 10 µM primers 2 µL each; Ex-Taq DNA Polymerase 1 µL; DNA template 2 µL; ddH2O added to 50 µL, Ex-Taq DNA Polymerase kit, Takara, Japan) was applied. The thermocycles were as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, polymerization at 72°C for 60 s and then final polymerization at 72°C for 10 min. The PCR products were purified using the Agarose Gel DNA Fragment Recovery Kit (Takara, Shiga, Japan) according to the protocol. The purified PCR products were directly sequenced by Invitrogen China (Shanghai, China). Sequencing results were analysed using Chromas 1.62 software (Technelysium Pty, Australia) and compared with the GeneBank sequence using NCBI Blast (Align two sequences, blast2seq; www.ncbi.nlm.nih.gov/BLAST/). | B lymphocyte culture and treatment | Four groups were established in this study, i.e. Group A (n = 20): B lymphocytes from normal controls cultured with the RPMI-1640 medium (Gibco, USA) alone; Group B (n = 20): B lymphocytes from normal controls cultured with RPMI-1640 + LPS; Group C (n = 22): B lymphocytes from IgAN patients cultured with the RPMI-1640 medium and Group D (n = 22): B lymphocytes from IgAN patients cultured with RPMI-1640 + LPS. In Groups A and C, isolated B lymphocytes (10⁶ cells per well) were cultured in the RPMI-1640 medium (with 15% fetal bull serum, Gibco, USA) at 37°C in a humidified atmosphere of 5% CO₂ in air. The RPMI-1640 medium (with 15% fetal bull serum, Gibco, USA) was applied as the upper normal limit. | 1. Primers and fluorescence probes

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USA) for 72 h alone. In Groups B and D, 12.5 μg/mL LPS (Sigma, USA) were added to mimic the infectious process during external pathogen attack. Cell morphology and viability were monitored using a phase contrast microscope (OLYMPUS, Japan) and trypan blue (Sigma, USA) stain. After culture, B lymphocytes were collected through centrifuge.

**RT-PCR and real-time RT-PCR**

Total RNA was extracted from isolated B lymphocytes using the RNeasy Mini RNA extracting kit (QIAGEN, USA) according to the manufacturer’s protocol; concentration and purity were determined by the Beckman DU-600 UV spectrometer (OD ratio of 260/280 nm). The cDNA was synthesized using 500 ng total RNA with the ExScript RT Reagent Kit (Perfect Real Time, Takara, Japan). In order to accurately quantify the expression level of Cosmc, real-time RT-PCR with the Taqman probe technique was applied. The Cosmc gene of all 109 subjects included was successfully sequenced. The sequencing-PCR product was directly sequenced using the bi-direction method. Four single nucleotide polymorphisms (SNPs) were observed in the coding region of the Cosmc gene in two patients (one male and one female) after comparing with the GeneBank recorded data.

Two of these SNPs were missense mutations; the other two were silent mutations. A C/T mutation at 623 bp caused a stop codon at 626 bp, which resulted in a truncated protein. A C/T mutation at 1324 bp caused a stop codon at 1327 bp, which resulted in a truncated protein.

**Statistical analysis**

In order to exclude the bias induced by sample volume, statistical analysis was carried out using the Ct ratio (CtCosmc/CtGAPDH). According to the mathematic model of real-time PCR, the higher Ct value indicates the lower mRNA level; therefore, the higher the Ct ratio, the lower the expression level is. ANOVA and paired t-test analyses were carried out using SPSS 12.0 software. The two-sided P-value of 0.05 was taken as the level of statistical significance. Pfaffl’s method [16], the most accepted method of qPCR analysis that could calculate the relative level of expression levels, was applied during the analysis of real-time PCR results.

**Results**

**General features of subjects included**

No significant difference was observed in the age, sex and ethnological background of IgAN patients, non-IgAN patients and normal controls included. In IgAN patients enrolled, the hypertension rate, severe proteinuria rate, renal dysfunction rate and macroscopic haematuria rate are 15.4, 35, 17 and 48%, respectively, which were similar to those of non-IgAN patients (Table 2, P > 0.05).

**Cosme gene sequencing**

The Cosmic gene of all 109 subjects included was successfully sequenced. The sequencing-PCR product was 1247 bps in length; Agarose-gel electrophoresis showed clear strips (Figure 1A). Purified PCR products were directly sequenced using the bi-direction method. Four single nucleotide polymorphisms (SNPs) were observed in the coding region of the Cosmic gene in two patients (one male and one female) after comparing with the GeneBank registered sequence. No mutation was observed in non-IgAN patients and normal controls.

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A proline/leucine (P208L) change in amino acid, and the T/A mutation at 742 bp led to serine/threonine (S248T) switch (Figure 2). However, T/G at 720 bp and C/G at 807 bp would not change the protein sequence.

**Cell culture**

B lymphocytes were successfully cultured in the RPMI medium as well as the LPS + RPMI medium. During the culture process, no significant morphological change was noticed and the cell viability was higher than the 90% measured by trypan blue stain.

**RT-PCR**

Clear fragments of PCR products could be seen after RT-PCR Agarose-gel electrophoretic analysis. The PCR product of Cosmc was 90 bp; GAPDH was 109 bp, as shown in Figure 1. Analysis with NCBI blast-2-sequence software showed that the amplified fragment was in accordance with the GeneBank recorded data.

**Real-time RT-PCR analysis**

Standard curves were established to examine the efficiency of real-time PCR reaction. Figure 3 indicates that the efficiency and correlation index ($R^2$) were >0.99. The slope of Cosmc and GAPDH PCR reaction was $-3.377$ and $-3.581$, respectively, as shown in Figure 3.

$Ct$ values of baseline mRNA expression levels as well as post-culture mRNA expression levels are listed in Table 3. ANOVA analysis indicated that the baseline Cosmc gene mRNA expression level was significantly lower in IgAN patients compared with normal control (1.29 ± 0.08 versus 1.20 ± 0.01, $P < 0.01$). After RPMI-1640 medium culture (which removed the influence of the patients’ plasma on gene expression), the Cosmc gene mRNA level was remarkably increased (1.22 ± 0.12 versus 1.29 ± 0.08, $P = 0.001$). However, co-culture with LPS could obviously inhibit the up-regulated expression of the Cosmc gene (1.25 ± 0.11 versus 1.22 ± 0.12, $P = 0.003$). In contrast, RPMI or LPS treatment could not induce apparent changes of the Cosmc gene expression in normal control B lymphocytes (1.20 ± 0.01 versus 1.19 ± 0.01 versus 1.20 ± 0.02, $P > 0.05$).

Moreover, in order to compare the Cosmc mRNA expression level before and after the RPMI culture as well as LPS treatment, Pfaffl’s method was applied. Using this method, relative quantification in real-time RT-PCR of a target gene transcript was measured in comparison to a reference gene transcript. It was found that the baseline Cosmc gene expression level in IgAN patients was only 31.3% of the normal control, which was similar to previous findings [6]. Seventy-two-hour culture with the RMPI-1640
medium increased the Cosmc level for 219% and reached near to the normal level (68.4%). However, LPS could partly inhibit this increase, as the analysis indicated that the Cosmc level was significantly decreased after the LPS treatment compared with the RPMI culture alone (61.3%).

Furthermore, we determined the baseline Cosmc gene expression level of patients with SNPs and effects of RPMI or LPS on them; however, similar results were noticed comparing with those without mutations.

**Discussion**

IgAN is the most common glomerulonephritis in the world, especially in Asian countries. Abnormality of the hinge-region O-glycosylation of IgA1 molecule may be one of the key causes of IgAN. O-linked glycans were significantly decreased in sera, mesangial deposited and tonsil secreted IgA1 molecules in IgAN patients [3,4] and the glycosylation aberrance was closely associated with pathologic phenotypes [17]. IgA1 hinge-region O-glycans are formed under the catalysis of core 1β1,3-galactosyltransferases (C1GALT1) [18] assisted by its special functional chaperone Cosmc [14]. It was reported that the β1,3GT activity was remarkably lower in peripheral B lymphocytes of IgAN patients [5] and that the Cosmc gene mRNA expression level in peripheral B lymphocytes of IgAN patients is significantly decreased compared with non-IgAN patients and normal controls, which is also negatively correlated to the IgA glycosylation degree and clinical features. However, no significant changes were noticed in the C1GALT1 gene itself [6]. Therefore, it was speculated that suppression of the Cosmc gene mRNA expression rather than the C1GALT1 gene itself may be the underlying pathogenesis of IgAN. It was well known that gene expression abnormalities could be the result of (1) genetic disorders such as mutations caused by transcription disorder, (2) external suppression caused by transcription suppression or (3) other factors influencing the transcription process, such as DNA-methylation or histone acetylation disorders. However, up till now no study was performed to clarify the exact cause of the down-regulation observed in IgAN.

It was once suspected that mutations genetically inherited or occurring randomly in the precursor stem cell might be associated with decreased β1,3GT activity in B cells responsible for IgA1 production [19]. It was reported that the lack of C1GALT activity in some cell lines was due to mutations in the Cosmc gene: in the LSC line there is a T insertion that leads to termination of translation, and in Jurkat cells there is a missense C–T mutation and a T deletion that lead to termination of translation [14,15]. Recently, Ju reported that a chaperone mutation caused the abnormal glycosylation in Tn syndrome, which is also characterized
by aberrant expression of bare GalNAc O-linked glycans on red blood cells (RBC) and platelets [20]. Considering that IgAN is similar to Tn syndrome in glycosylation disorders as well as genetic features, we focused on the gene sequence structure of the Cosmc gene. In this study, the whole coding region of the Cosmc gene was PCR amplified and directly sequenced in order to find out whether there is a genetic disorder. However, few SNP mutations were noticed in only two subjects included (65 IgAN patients, 23 non-IgAN patients and 21 normal controls). Moreover, the mutations found were different from each other, and half of them were even silent mutations. In addition, the mutations observed in LSC, Jurkat cell lines and Tn syndrome patients were not detected in Chinese Han IgAN patients. This result is in accordance with Nie’s recent finding in the Chinese population: they tried to screen a somatic mutation of the Cosmc gene in the B lymphocytes from 27 IgAN patients; however this resulted in negative findings [21]. Furthermore, q-PCR analysis indicated that the Cosmc gene mRNA expression levels in individuals with mutations were similar to those without. Therefore, the significance of the mutations found was doubtful; they may just be some individual differences unrelated to the disease. Regarding the fact that disease-related common mutations could not be found in the Cosmc gene coding frame region, it was speculated that external expression-affecting factors might play a more important role in the down-regulation of the Cosmc gene expression observed.

We turned our focus onto the external suppression hypothesis. It was reported that treatment with 5-azacytidine and NaB could reactivate the suppressed β1,3GT activity in Tn syndrome, which indicates that external suppression rather than genetic disorders was the basis of the Tn phenotype [22]. Therefore, it was suspected that there might be some suppressive factors in the patients’ plasma which could inhibit the expression of Cosmc and removal of them may increase the expression. Just as expected, study results showed that the blank culture (use only the RPMI-1640 medium) could significantly up-regulate the Cosmc gene mRNA expression compared with the baseline level (ΔCt(COSMC/CtGAPDH 1.22 ± 0.12 versus 1.29 ± 0.08, P = 0.001) in a short time (only 72 h), and the expression level was increased 2.19-fold. Along with the negative findings of the Cosmc gene sequence, these results confirmed the external suppressive hypothesis. When the plasma was exchanged by the blank culture medium (RPMI-1640), the suppression was removed; therefore, significant recovery of the Cosmc gene mRNA expression level was observed in a considerably short time.

Then, the question of what caused the suppression in the plasma of IgAN patients needed to be answered. Clinically, bacterial infection (i.e. upper respiratory tract infection) can induce a relapse in IgAN with symptoms such as proteinuria, macroscopic haematuria and oedema [7,8]. LPS is one of the most potent stimulators of the innate immune system, which could induce gene expression in monocytes, especially the cytokine and chemokines [23]. It was reported that in IgAN patients, bacterial antigens (such as LPS) could stimulate the tonsillar lymphocyte and produce aberrant O-glycans [10–12]. It is believed that after the initial pathogenic immunoglobulin deposition in the glomeruli, the mesangial responsiveness to a common systemic infection might affect the progress of IgAN. In this study we found that LPS could partly inhibit the effect of plasma-free culture; when 12.5 μg/mL LPS was added to mimic the infection condition, the peripheral B lymphocyte Cosmc mRNA expression level was remarkably decreased compared with the plasma-free culture group (ΔCt(COSMC/CtGAPDH 1.25 ± 0.11 versus 1.22 ± 0.12, P = 0.003, 0.61 times). From our findings, it is speculated that during the bacterial infection, an endotoxin (such as LPS) could suppress the mRNA expression of the Cosmc gene, which leads to the O-glycosylation aberrant level of IgA1.

Although external inhibition of Cosmc gene expression in IgAN patients was confirmed in this study, the underlying mechanism of this inhibition is still unknown. As the study indicated that the Cosmc mRNA expression level of peripheral B lymphocytes from normal controls was not affected by RPMI-1640 and LPS treatment, it could be speculated that the LPS signalling pathway in IgAN patients may be different from normal controls. DNA methylation is an important gene-expression regulation, which could regulate the target gene expression in spite of the gene sequence itself [24,25], and LPS could enhance the methylation of certain genes [26–28]. It was reported that DNA methylation modification could reverse the suppressed β1,3GT activity in the Tn syndrome, which indicated that a hyper-level of DNA methylation might be the potential mechanism of the aberrant glycosylation observed [29]. Therefore, we think that, in IgAN patients, the glycosylation disorder may also be caused by the methylation abnormality, and external depressors such as LPS might increase the methylation level of the Cosmc gene and down-regulate its expression; therefore, down-regulate DNA methylation regulatory substance, such as 5-AZA, may be a potential treatment of these glycosylation aberrant-associated diseases. However, further studies are needed for clarification.

Although we found some interesting results from this study, limitations were still clear in the current study. First, because the antibodies needed to study the protein levels are unavailable at present, only mRNA levels were measured in the present study. Although normally there is a good correlation between protein and mRNA levels, further studies were needed to elucidate the protein expression level when conditions allowed. Second, immunoglobulin is secreted by plasma cells; therefore, it is most proper to carry out this study in plasma cells. However, it is technically impossible to collect enough plasma cells from IgAN patients to perform the study. Considering that the peripheral B lymphocyte is the precursor of the plasma cell and studies using B cells showed a strong correlation between β1,3GT activity and IgA1 glycosylation level, it was acceptable to use B lymphocytes in the study. However, further studies were needed, if technically practicable, to clarify the potential error brought forward by this. Third, the introns of the Cosmc gene were not sequenced in this study. Although the expression-adjusting effects of blank culture and LPS treatment indicated the correctness of the Cosmc gene sequence in IgAN patients, further sequencing studies of the intron region should be carried out to confirm this directly.
Conclusion

The decreased Cosmc gene expression level in peripheral B lymphocytes is the basis of IgA1 hinge-region O-glycosylation aberration of IgAN patients. The negative finding of the Cosmc gene sequencing suggested that genetic mutation of Cosmc gene may not be the reason of the disease. The significantly increased Cosmc gene expression level observed in plasma-removed blank culture as well as LPS’ inhibition effect on the expression strongly indicated that external suppressors such as LPS may be the reason for the Cosmc gene down-regulation observed in IgAN patients, and may be the pathogenic basis of acute relapse of IgAN.

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Conflict of interest statement. None declared.

References

2. Coppo R, Amore A. Aberrant glycosylation in IgA nephropathy (IgAN). *Kidney Int* 2004; 65: 1544–1547

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