CD71 mesangial IgA1 receptor and the progression of IgA nephropathy

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The transferrin receptor (CD71) is known as a receptor for IgA1 on mesangial cells, but the role of CD71 in IgA nephropathy (IgAN) is unknown. We studied clinical implication of mesangial CD71 in 282 patients with biopsy-proven IgAN (2005–2018). The transcript and protein expression of glomerular CD71 was determined by real-time polymerase chain reaction and immunohistochemistry. Ten subjects with microscopic hematuria only and no evidence of histologic abnormalities on kidney biopsy were considered as controls. Human mesangial cells (HMCs) were treated with sera from IgAN patients and expression levels of CD71 and inflammatory cytokine markers were compared according to disease status. Disease progression was defined as a ≥30% decline in estimated glomerular filtration rate from the baseline value. During a mean follow up of 53.5 (18.3–75.9) months, 80 (28.4%) patients developed disease progression. The mRNA expression of CD71 was significantly higher in progressors than in nonprogressors ($P = 0.001$). Among the Oxford classification scores, patients with M1 had significantly higher CD71 expression levels than those with M0. In a multivariable Cox model, elevated transcript levels of CD71 were significantly associated with 4.32-fold higher risk of disease progression ($P = 0.009$). Furthermore, CD71 expression levels independently predicted the increase in proteinuria of ≥50% from the baseline ($P = 0.03$). Finally, HMCs treated with sera from IgAN patients with the higher Oxford score (M1E1S1T0) more increased the mRNA expression of CD71 and inflammatory markers than those with sera from negative score (M0E0S0T0). However, silencing CD71 significantly reduced expression levels of the inflammatory cytokine genes. Our results show that mesangial CD71 is significantly associated with disease progression and may play a biologic role in IgAN. (Translational Research 2021; 230:34–43)

Abbreviations: BMI = body mass index; BP = blood pressure; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HMC = human mesangial cell; IgAN = IgA nephropathy; MCP-1 = monocyte chemoattractant protein-1; SBP = systolic blood pressure; sCD89 = soluble CD89; siRNA = short interfering RNA; TNF-α = tumor necrosis factor-alpha; UPCR = urine protein to creatinine ratio; YUHS = Yonsei University Health System

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Submitted for Publication August 3, 2020; received submitted October 12, 2020; accepted for publication October 21, 2020.

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1931-5244/$ - see front matter

https://doi.org/10.1016/j.trsl.2020.10.007

AT A GLANCE COMMENTARY

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Background

This study demonstrated that the mRNA expression level of glomerular CD71 was significantly higher in IgA nephropathy patients with disease progression. Glomerular CD71 expression predicted adverse renal outcomes such as estimated glomerular filtration rate decline and ≥50% increase in proteinuria from the baseline in patients with IgA nephropathy. Furthermore, human mesangial cells treated with sera from IgA nephropathy patients resulted in increased expression of CD71 and inflammatory cytokine genes, which was attenuated by silencing CD71.

Translational Significance

These findings suggest a substantial prognostic implication of mesangial IgA receptor as a triggering factor of glomerular injury in IgA nephropathy.

INTRODUCTION

IgA nephropathy (IgAN) is the most prevalent primary glomerular disease worldwide.[1-3] Most patients are asymptomatic with less aggressive clinical feature than other rapidly progressive glomerulonephritis and usually follow benign course. However, IgAN can progress to kidney failure and approximately 30% of patients eventually require dialysis or transplantation in the end within 20 years after diagnosis.[4-6]

The pathogenesis of IgAN is a complicated multistep process.[7] First, IgA1 with poorly galactosylated hinge region is produced and increased in the circulation. Then, IgG antibodies are generated against this misglycated IgA1 and drive the formation of a large immune-complex. Subsequently, the pathogenic immune-complexes are deposited within the mesangium via interaction with mesangial IgA receptors such as CD71. Upon binding to the receptors, the complement pathway is activated along with other sequential cascades that can damage mesangial cells and glomerular structures, which eventually lead to terminal fibrotic process.

To date, several IgA receptors and IgA-binding molecules have been identified.[8] These include IgA Fc receptor (FcαRI; CD89),[9] polymeric-Ig receptor (pIgR),[10] Fcα/μR,[11] asialoglycoprotein receptor,[12] β1,4-galactosyltransferase 1,[13] and transferrin receptor (CD71).[14] Among these, transferrin receptor was found in human IgAN and has been regarded to play a pathologic role in IgAN. In fact, CD71 is known as a multiligand receptor that binds transferrin, the hemochromatosis protein, and arenavirus.[15] Moreover, CD71 is expressed within the mesangium and functions as a mesangial IgA1 receptor. Its binding to undergalactosylated polymeric IgA1 and immune-complex containing poorly galactosylated IgA1 can induce the activation of mesangial cells and ignite the sequential pathologic process within glomeruli in IgAN.[14,16] In spite of the potential role of CD71, little is known about the association between the level of CD71 expression and adverse renal outcomes. To date, there has been only one study on CD71 in human IgAN. In this study, CD71 colocalized to IgA deposition within the glomeruli and were strongly associated with the proliferative lesion.[16] Notably, there was no relationship between the degree of CD71 expression and clinical or biologic findings. However, this was a cross-sectional study in only 16 patients.

With this background, we aimed to investigate the prognostic implication of CD71 in a longitudinal observation of IgAN cohort. Furthermore, we also studied whether mesangial CD71 plays a biologic role in the activation of IgAN.

MATERIALS AND METHODS

Study subjects. A total of 344 patients with biopsy-proven IgAN between 2005 and 2018 were eligible for this study. Demographic and laboratory data were obtained from the Glomerulonephritis Registry of Yonsei University Health System (YUHS). A flow diagram depicting the process of participant selection is presented in Supplementary Figure 1. We excluded 62 patients who met the following criteria: (1) aged <18 years, (2) inadequate sample with a number of glomeruli <7, (3) previous history of kidney transplantation, (4) diagnosed as end stage renal disease at the time of diagnosis, (5) estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m^2, and 6) Secondary conditions associated with IgAN such as infection-associated glomerulonephritis or combined with autoimmune disease including Henoch−Schönlein purpura, systemic lupus nephritis, rheumatoid arthritis, Sjogren's syndrome, IgG4-related disease, and ankylosing spondylitis. Thus, 282 patients with IgAN were included in the primary analysis. All subjects were enrolled in the study voluntarily, and informed consent was obtained in all cases. This study was carried out in accordance with the Declaration of Helsinki and was approved by the

institutional review board of the YUHS Clinical Trial Center (4-2019-0548).

Clinical, biochemical, and histologic data collection. Using the database from the Glomerulonephritis Registry of YUHS, demographic, clinical, and biochemical data at the time of renal biopsy including age, sex, blood pressure (BP), body mass index (BMI), past medical history, and medication history were retrieved and considered as baseline data. The eGFR was determined using the CKD Epidemiologic Collaboration equation.[17] Spot urine test for proteinuria including protein and creatinine were performed using an AU680 analyzer (Beckman Coulter Inc., CA, USA) and quantified as urine protein to creatinine ratio (UPCR). Follow-up data such as BP, UPCR, and eGFR were recorded at 3-month interval visits. All renal biopsy specimens were reassessed by one pathologist blinded to the patients' clinical data using the Oxford classification. For detailed methods, see Supplemental Material.

Preparation of mesangial cells and treatment with human sera from IgA nephropathy patients. Immortalized human mesangial cells (HMCs) were purchased from Applied Biological Materials Inc. (Richmond, BC, Canada). The cells were cultured in the RPMI 1640 medium containing 10% FBS (Gibco), 100 U/mL penicillin G (Sigma-Aldrich, UK), 2.5 μg/mL amphotericin B (Sigma-Aldrich, UK), and 20 ng/mL EGF (Sigma-Aldrich, UK). Sera were obtained from control (n = 3) and IgAN patients with the lower Oxford score (M0M0S0T0) (n = 3) and the higher score (M1E1S1T0) (n = 3). Serum samples were prepared for 100 μg/mL in RPMI medium following the methods as previously described and then given to HMCs.[18] The treated cells were harvested after 24 hours incubation. Then, we performed real-time polymerase chain reaction (PCR) to compare transcript levels of CD71, fibronectin, IL-6, tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1). The sequences of primers used in this study are presented in Supplementary Table 1. Furthermore, short interfering RNA (siRNA) against CD71 (ON_TARGETplus SMARTpool, Dharmacon, Inc., CO, USA) was used to further exam the changes of transcript levels of inflammatory and profibrotic marker genes after knockdown of CD71 gene. HMCs were transfected with Lipofectamine 2000 (Invitrogen Co., Carlsbad, CA, USA) for 24 hours. The cells were further incubated in media containing human sera for 24 hours and then harvested.

Study outcomes. The primary endpoint was the onset of a ≥30% decline in eGFR from the baseline during follow-up. This was defined as a sustained decrease in eGFR ≥30% for at least 2 consecutive measurements. The first of these consecutive measurements was retrospectively designated as the study endpoint. Accordingly, patients who reached this endpoint were as a disease progressor group. Moreover, the secondary endpoint was an increase in proteinuria ≥50% from the baseline.

Statistical analysis. All statistical analyses were performed with IBM SPSS software for Windows version 23.0 (IBM Corporation, Armonk, NY, USA) and R software 3.3.1 (http://www.R-project.org). Continuous variables are expressed as mean ± standard deviation or median (interquartile range), and categorical variables as absolute numbers with percentages. Each variable was tested for normality before statistical analysis. Comparisons between the groups were made by using analysis of variance or Student's *t* test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. The Kolmogorov−Smirnov test was performed to determine the normality of the distribution of parameters. If the resulting data did not show a normal distribution, geometric mean ± standard deviation was reported; the Mann−Whitney U-test or Kruskal−Wallis test was used for multiple comparisons. The CD71 gene expression levels were compared between disease progressor and nonprogressor group. We further examined the expression levels of CD71 between proteinuria increased group vs nonincreased group. To assess the prognostic utility of CD71 expression levels for the progression of kidney disease, a multivariable Cox model was conducted to evaluate the association between eGFR reduction and baseline CD71 levels. Proportional hazards assumptions were confirmed using Schoenfeld residuals. Variables with *P* value <0.10 in univariable analysis or clinically important were included for adjustment. Patients who were lost to follow-up were censored at the date of the last examination. hazard ratios (HRs) for developing a ≥30% decrease in eGFR and the increment of proteinuria >50% from the baseline were assessed with CD71 expression levels as continuous variable. Because proteinuria and BP were highly variable during follow-up, these variables were modeled as time-varying exposures. For all analyses, a *P* value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics. Among 282 patients with IgAN, mean age was 40.4 ± 13.3 years, 55.7% were male, and mean eGFR was 89.9 ± 26.9 mL/min/1.73 m^2. Baseline characteristics were compared between progressor (n = 80, 28.4%) and nonprogressor (n = 202, 71.6%) group (Table 1). At the time of biopsy, the progressors were older, and more likely to be male and have hypertension than nonprogressors. Among Oxford classification components, the progressors had significantly higher M, E, and T scores than nonprogressors.

Table 1. Baseline characteristics

	Total (N = 282)	Nonprogressors (N = 202)	Progressors (N = 80)	*P*
Demographic data				
Age, years	40.4 ± 13.3	38.8 ± 13.4	44.7 ± 12.2	0.01
Male	157 (55.7)	105 (52.0)	52 (65.0)	0.03
BMI, kg/m^2	23.1 ± 3.2	22.9 ± 3.3	23.5 ± 3.2	0.26
SBP, mm Hg	125.7 ± 16.5	124.8 ± 16.5	127.8 ± 16.3	0.16
DBP, mm Hg	79.8 ± 11.8	79.4 ± 11.9	81.0 ± 11.5	0.31
Hypertension, n (%)	169 (59.9)	111 (55.0)	58 (72.5)	0.01
Oxford classification				
M1	35 (16.1)	13 (8.3)	22 (35.5)	<0.001
E1	65 (29.7)	42 (26.9)	23 (36.5)	0.11
S1	146 (66.7)	97 (62.2)	49 (77.8)	0.02
T1	14 (6.4)	7 (4.5)	7 (11.1)	<0.001
T2	5 (2.3)	0 (0.0)	5 (7.9)	
Laboratory data				
eGFR, mL/min/1.73 m^2	89.9 ± 26.9	96.4 ± 24.5	73.7 ± 26.0	<0.001
eGFR categories				<0.001
≥90 mL/min/1.73 m^2	160 (56.7)	135 (66.8)	25 (31.3)	
60–90 mL/min/1.73 m^2	73 (25.9)	45 (22.3)	28 (35.0)	
30–60 mL/min/1.73 m^2	45 (16.0)	22 (10.9)	23 (28.7)	
<30 mL/min/1.73 m^2	4 (1.4)	0 (0.0)	4 (5.0)	
UPCR, g/gCr	1.3 ± 1.9	1.0 ± 1.9	2.0 ± 1.7	<0.001
Hemoglobin, g/dL	13.0 ± 1.7	13.3 ± 1.6	12.1 ± 1.7	<0.001
Albumin, g/dL	4.0 ± 0.6	4.1 ± 0.6	3.8 ± 0.5	<0.001
Uric acid, mg/dL	5.5 ± 1.6	5.3 ± 1.5	5.8 ± 1.9	0.06
Fasting plasma glucose, mg/dL	96.4 ± 14.9	95.5 ± 13.8	98.8 ± 17.5	0.09
Total cholesterol, mg/dL	189.7 ± 46.8	189.5 ± 50.5	190.2 ± 36.2	0.90
hs-CRP, mg/dL	0.71 (0.34–1.37)	0.53 (0.23–0.96)	1.32 (0.71–3.14)	0.87
Serum IgA, mg/dL	327.5 ± 125.0	323.3 ± 121.1	131.8 ± 80.1	0.47
Medication usage, n (%)				
RAS blockades	216 (77.4)	141 (70.9)	75 (93.8)	<0.001
Statin	100 (35.8)	52 (26.1)	48 (60.0)	<0.001
Diuretics	30 (10.8)	11 (5.5)	19 (23.8)	<0.001
Corticosteroids	45 (16.1)	16 (8.0)	29 (36.3)	<0.001

Note: Data are presented as mean ± standard deviation, median (interquartile range) or number (%)
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; RAS, renin-angiotensin system; SBP, systolic blood pressure; UPCR, urine protein to creatinine ratio.

In addition, eGFR was significantly lower in progressor group than nonprogressor group (73.7 ± 26.0 vs 96.4 ± 24.5 mL/min/1.73 m^2, P <0.001). The progressor group also had higher level of UPCR (2.0 ± 1.7 vs 1.0 ± 1.9 g/g Cr, P <0 .001), and lower hemoglobin (12.1 ± 1.7 vs 13.3 ± 1.6 g/dL, P <0.001) and serum albumin (3.8 ± 0.5 vs 4.1 ± 0.6 g/dL, P <0.001) levels than nonprogressor group. Furthermore, progressors were more likely to take medications including RAS blockades, statin, diuretics, and corticosteroids than nonprogressors. However, there were no significant differences in BMI, systolic and diastolic BPs (SBP and DBP), and laboratory findings such as uric acid, fasting plasma glucose, total cholesterol, hs-CRP, and serum IgA levels between 2 groups.

The mRNA and protein expression levels of CD71 according to disease progression. Next, we compared transcript level of CD71 according to disease progression status. For comparison, we also measured CD71 expression level in 10 subjects with microscopic hematuria only who had no histologic abnormalities and no evidence of other diseases such as Alport syndrome or thin basement membrane nephropathy on renal biopsy examination. These subjects were considered control group. Real-time PCR analysis revealed that the mRNA expression level of CD71 was significantly higher in progressor group than in nonprogressor group (P = 0.001). Interestingly, IgAN patients without progression had higher level of CD71 than control group (P = 0.01) (Fig 1).

CD71 expression was also confirmed at protein level. Immunohistochemical staining showed a similar pattern to the changes as observed in the quantitative PCR analysis. CD71 was mainly expressed within cytoplasm of mesangial cells. The intensity of CD71 expression was stronger in progressor group than in control and nonprogressor group (Fig 2, A–C).

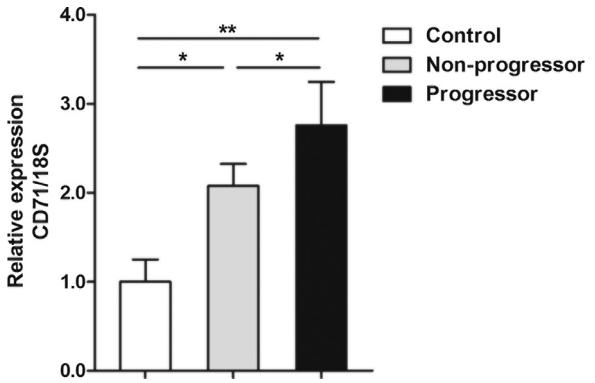

Fig 1. Comparison of CD71/18S mRNA expression levels among control, nonprogressor, and progressor groups. Real-time PCR revealed that CD71 mRNA expression normalized to 18S, which was expressed as -△△CT, was significantly higher in progressor group compared to nonprogressor, whereas nonprogressor group had higher level of CD71 than control group. (*P <0.05, **P <0.001).

Abbreviations: mRNA, messenger RNA; PCR, polymerase chain reaction.

CD71 expression according to the Oxford classification score. We then studied whether CD71 expression levels were different among the 4 Oxford-MEST lesions. The results showed that patients with M1 had a significantly higher transcript level of CD71 than those with M0 (0.34 ± 0.07 vs 0.20 ± 0.03, $P = 0.03$) (Supplementary Figure 2). For E, S, and T lesions, there were no differences in CD71 expression levels between the presence and the absence of each lesion.

CD71 as a prognostic marker of disease progression. We further evaluated prognostic value of CD71 levels in predicting the progression of kidney disease. During a mean follow up of 53.5 (18.3−75.9) months, 80 (28.4%) patients developed disease progression defined as a ≥30% decline in eGFR. Unadjusted HR for reaching the endpoint was 5.00 (95% confidence interval [CI], 1.94−12.83; $P = 0.001$). In a multivariable Cox model adjusted for age, sex, eGFR, UPCR, BMI, SBP, hemoglobin, serum albumin, total cholesterol, and the usage of medications including RAS blockade, statin, diuretics, and corticosteroid, the higher expression level of CD71 was significantly associated with a higher risk of disease progression (HR per 1 log (CD71/18S) increase, 4.32; 95% CI, 1.43−13.02; $P = 0.009$) (Table 2).

Association of CD71 expression with change in proteinuria. In our previous study, we reported that only M1 lesion was independently associated with the development of persistent proteinuria among the Oxford-MEST lesions in IgAN.[19] Given a significantly higher level of CD71 in M1 lesion as described above, we further examined the relationship between CD71 and an increase in proteinuria. Patients were categorized into 2 groups, increased and nonincreased proteinuria group depending on the increase in proteinuria ≥50% from the baseline. Based on this cut-off value, proteinuria level increased in 46 (16.3%) patients during follow-up period. The CD71 mRNA expression levels were significantly higher in this group than in non-increased group (0.47 ± 0.26 vs 0.19 ± 0.02, $P = 0.02$) (Supplementary Figure 3). This association was further substantiated by Cox regression analysis. Unadjusted HR for a ≥50% increase in proteinuria was 3.99 (95% CI, 1.75−9.13, $P = 0.01$). In a multivariable model, the higher CD71 expression levels were significantly associated with higher risk of an increment of proteinuria (HR per 1 log (CD71/18S) increase, 3.04; 95% CI, 1.07−8.60; $P = 0.03$) (Supplementary Table 2).

The gene expression levels of CD71 and inflammatory cytokines in human mesangial cells treated with sera from patients with IgAN. Finally, we examined whether sera from patients with IgAN could increase CD71

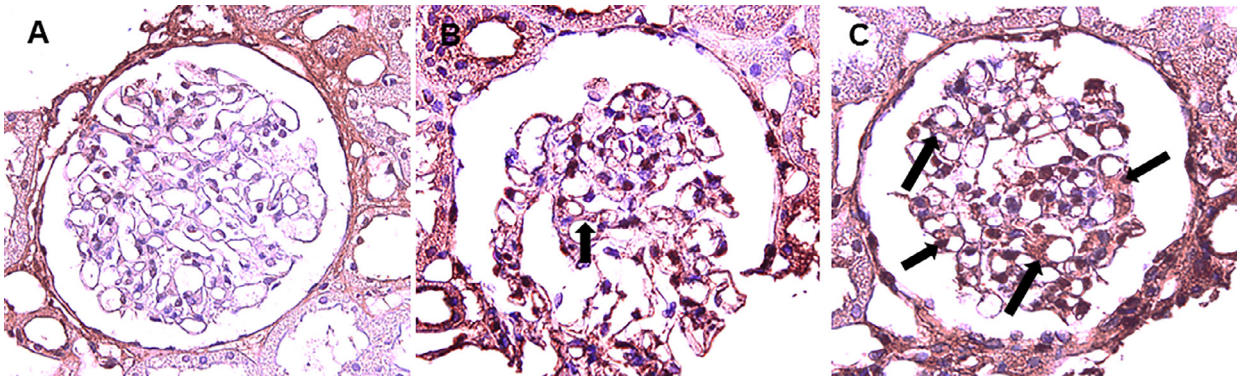

Fig 2. A representative immunohistochemical staining for CD71 protein in the (A) control, (B) nonprogressor, and (C) progressor groups. The staining for CD71 within glomeruli was strong in cells with the location and appearance of mesangium (arrow heads).

Table 2. Multivariable Cox models for 30% decline in eGFR according to CD71 mRNA levels

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
CD71, per 1 log (CD71/18S) increase	5.10 (1.94–13.42)	0.001	5.00 (1.94–12.83)	0.001	4.61 (1.64–12.90)	0.004	4.32 (1.43–13.02)	0.009

Note: CD71 represents the log-transformed expression level of CD71 mRNA normalized to 18S and treated as continuous variable.
Model 1: Unadjusted model.
Model 2: Adjusted for Model 1 + age and sex.
Model 3: Adjusted for Model 2 + BMI, SBP, eGFR, UPCR, hemoglobin, serum albumin, and total cholesterol.
Model 4: Adjusted for Model 3 + medication usage including RAS blockades, statin, diuretics, and corticosteroid.
Abbreviations: BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; RAS, renin-angiotensin system; SBP, systolic blood pressure; UPCR, urine protein to creatinine ratio.

expression levels in HMCs. As shown in Fig 3, HMCs treated with sera from patients with more inflammatory stage of IgAN (M1E1S1T0) resulted in higher mRNA expression of CD71 than those without inflammation (M0E0S0T0) (Fig 3A). We also found that the expression levels of fibronectin, IL-6, TNF-α, and MCP-1 concomitantly increased in HMCs in response to sera with the higher Oxford score (Fig 3, B–E). However, silencing CD71 with siRNA significantly reduced the expression levels of these inflammatory cytokine genes and profibrotic markers (Fig 4, A–E).

DISCUSSION

In this study, we showed that the mRNA and protein expression level of glomerular CD71 was significantly higher in IgAN patients with disease progression. In addition, among 4 components of the Oxford classification, only M1 score was significantly associated with a higher level of glomerular CD71 expression. Furthermore, glomerular CD71 expression predicted adverse renal outcomes such as eGFR decline and ≥50% increase in proteinuria from the baseline in patients with IgAN.

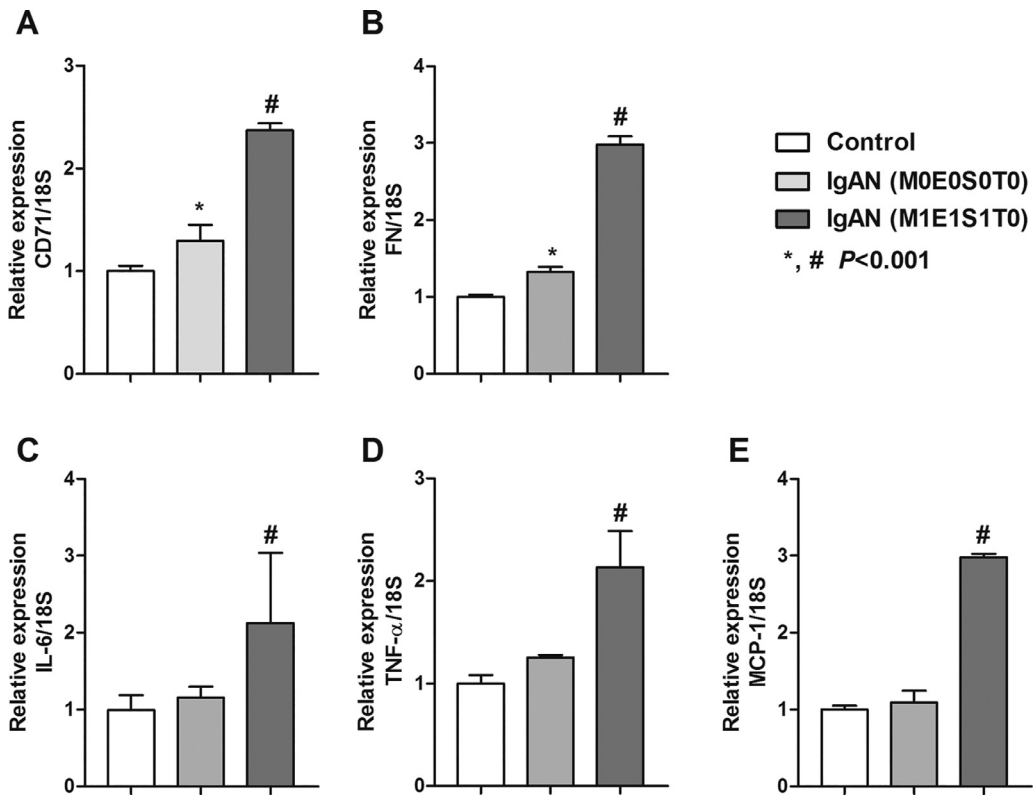

Fig 3. Comparison of mRNA expression of (A) CD71 and (B) profibrotic and (C, D, and E) inflammatory markers in human mesangial cells treated with the sera from control and IgAN patients with the lower Oxford score (M0E0S0T0) and the higher score (M1E1S1T0). (*P <0.001). *Abbreviation*: IgAN, IgA nephropathy; FN, fibronectin; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1

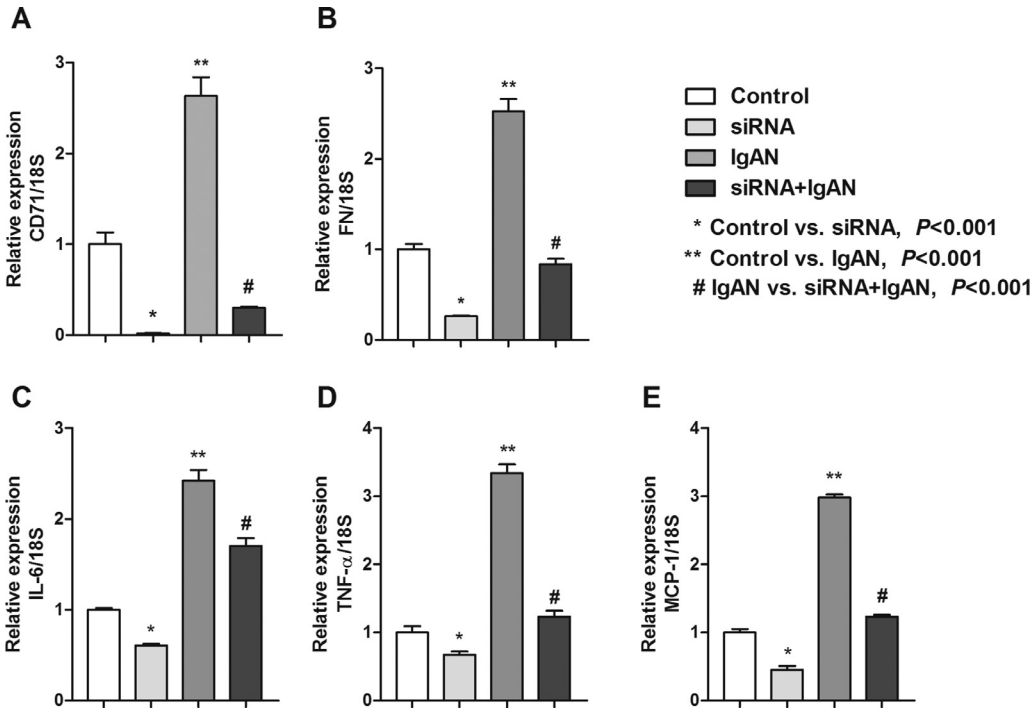

Fig 4. Silencing CD71 significantly reduced expression levels of (A) CD71, (B) profibrotic and (C, D, and E) inflammatory markers in human mesangial cells treated with sera from IgAN. (*P <0.001).
Abbreviation: IgAN, IgA nephropathy; siRNA, short interfering RNA; FN,fibronectin; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1

Finally, HMCs treated with sera from IgAN patients resulted in increased expression of CD71 and inflammatory cytokine genes, which was attenuated by silencing CD71. These findings suggest a substantial prognostic implication of mesangial IgA receptor as a triggering factor of glomerular injury in IgAN.

IgAN is featured by multistep immune processes. Misglycated IgA1-containing immune-complex is formed in the circulation and then deposits in the glomerular mesangium. This sequentially induces mesangial cell proliferation, mesangial matrix expansion, and infiltration of intraglomerular or interstitial inflammatory cells.[20] Several studies have tried to stop these pathogenic processes in experimental models of IgAN.[21-23] However, to date, there have been no clinically available drugs that specifically target each step and nonspecific immunosuppression such as corticosteroids and cyclophosphamide has been used in clinical practice with conflicting results and many side effects. Therefore, it would be interesting to know clinical implications of initial pathogenic step at the receptor level in the glomeruli because it is a starting point where the actual glomerular injury begins.

Receptors for IgA have been highlighted due to their important role in pathogenesis of IgAN. These include IgA Fc receptor (FcαRI; CD89) expressed on myeloid cells,[9] polymeric-Ig receptor (pIgR) on mucosal epithelial cells,[10] Fcα/μR on B cells and macrophages[11] asialoglycoprotein receptor on hepatocytes,[12] and transferrin receptor (CD71).[14] Several groups have studied whether these receptors have an impact on disease progression of IgAN. However, there has been much controversy on this issue. For example, Berthelot et al[24] suggested CD89 as a predictive marker for recurrent IgAN among kidney transplant patients. In this study, disease recurrence was associated with increased serum IgA-soluble CD89 (sCD89) complex level and mesangial sCD89 deposits. However, other studies failed to demonstrate its prognostic value as the circulating complex level did not correlate with any clinical parameters and did not differ between patients with IgAN, those with other glomerulonephritis, and healthy controls.[25,26] Tissandie et al showed that patients with alcoholic liver cirrhosis had elevated sCD89-IgA complex levels, but no glomerular abnormalities were observed in these patients.[27] These findings corroborate our recent study, showing that IgA-CD89 complex level was not associated with progression of IgAN.[28] Thus, we surmise that there are other factors that can play more important role in the pathogenesis, although sCD89 can help IgG autoantibodies to recognize IgA1. In addition, IgG autoantibodies can

independently form complexes with poorly galactosylated IgA1 without the help of sCD89 and elevated levels of autoantibodies were reported to be associated with disease progression.[29,30] Furthermore, circulating levels of these factors should be interpreted with caution because even elevated levels of sCD89-IgA complex levels or galactose-deficient IgA does not always lead to disease progression and this requires multistep process to evolve the disease.[31,32]

On the other hand, Haddad et al[16] identified CD71 as a major receptor for IgA on mesangial cells. In fact, CD71 colocalized with IgA deposits in the kidney biopsies of IgAN patients.[14,33] CD71 well correlated with proliferative lesion, but no relationship was found between glomerular CD71 expression level and clinical parameters such as kidney function or proteinuria. However, due to the cross-sectional design and small sample size of only 16 patients, the findings of this study cannot be extrapolated to whole patients with IgAN. Unfortunately, no follow-up studies have been conducted to clarify clinical implication of this mesangial IgA receptor thereafter. In this context, our findings deserve attention because we first found a prognostic significance of CD71 in longitudinal follow-up observation.

It is uncertain on molecular mechanism for CD71-induced glomerular injury. One potential explanation is an upregulation or activation of CD71 by interaction with abnormally glycosylated IgA1 as suggested by Haddad et al.[16] In pathogenic process of IgAN, the primary event in the glomeruli is deposition of IgA1 on mesangial cells and binding to its receptor such as CD71. It can be presumed that this interaction can induce an upregulation of CD71 expression on the cell surface either by redistribution of cytoplasmic CD71 or by induction of CD71 synthesis within the cells. The upregulation of CD71 can further trigger an increase of IgA1 deposition on mesangial cells. Interestingly, in an *in vitro* study by Tamouza et al, polymeric IgA1 induced activation and proliferation of human mesangial cells and mesangial cells in turn secreted proinflammatory cytokine.[34] Such sequential events were abolished by blocking CD71, suggesting that this response was dependent on CD71. Furthermore, role of CD71 in abnormally proliferative status was also reported in other types of cells.[34,35] The results of these experimental studies fits well with clinical findings that mesangial CD71 expression level strongly correlated with the intensity of cellular proliferation in IgAN.[16] In agreement with such evidence, we showed that sera from patients with more severe IgAN significantly increased expression levels of CD71, fibronectin, IL-6, TNF-α, and MCP-1. These findings together suggest that CD71 is indeed an important predictor of the future adverse outcomes.

Another interesting finding in our study is that higher CD71 mRNA transcript level was significantly associated with an increase in proteinuria. Furthermore, among 4 components of Oxford-MEST lesions CD71 expression level was higher only in the presence of M1. In our previous study, we found that M1 conferred a significantly increased risk of the development of persistent proteinuria in early stage IgAN, while other lesions did not.[19] This is in line with many other studies indicating that the presence of M1 predicted adverse renal outcomes regardless of clinical factors, whereas E or S score yielded conflicting results.[36-40] Given mesangial event as an initiating point in the evolution of IgAN, CD71 can be an important mechanistic link between mesangial pathology and disease progression.

Our study has several limitations. First, because of the observational study design, we could not fully establish potential mechanisms underneath CD71-associated disease progression of IgAN. In addition, we did not measure serum level of undergalactosylated IgA1, which was not able to be adjusted in multivariable Cox proportional hazard model. Thus, residual confounding could not be completely excluded. Second, although we demonstrated that the treatment of sera from IgAN patients resulted in mRNA expression of CD71 and increased expression of inflammatory cytokine and profibrotic markers in HMCs, we were unable to study IgA1 isolated from patients' sera. Thus, clear mechanism how undergalactosylated IgA1 activates CD71 cannot be found in this study. However, isolation of undergalgactosylated IgA1 has not yet been standardized and IgA1 alone is not sufficient to induce disease. Thus, we used patient's serum containing immune-complexes, complements, and inflammatory cytokines to mimic similar environment to IgAN.[41] Third, this is a single center study including only one ethnic group. Therefore, our findings should be interpreted within caution. In fact, the prevalence, biopsy practice pattern, treatment, and renal survival rate vary depending on regions.[42-44] Given such geographic variability and ethnic differences, the clinical role of CD71 may differ among different ethnic population. Fourth, it is possible that other IgA1 receptors exist because CD71-specific antibodies partially blocked the binding of IgA1 to HMCs in previous studies.[14,34,45] Interestingly, β1,4-galactosyltransferase 1 has recently been reported to be expressed in HMCs and play a role in the initial activation of IgAN.[13] In depth comparative analysis between CD71 and β1,4-galactosyltransferase 1 is beyond the scope of our study. Future studies should address this issue to clarify the differential role of these mesangial IgA receptors.

In conclusion, this study showed that glomerular CD71 expression is significantly associated with kidney function

decline and proteinuria development in patients with IgAN. In addition, we provided experimental evidence that CD71 is involved in the activation of mesangial cells. Our findings contribute to a better understanding on clinical and biological role of mesangial CD71 in IgAN.

AUTHOR CONTRIBUTIONS

Research idea and study design: JHJ, SHH; data acquisition: JHJ, BYN, and HYK; data analysis/interpretation: JHJ and BYN; statistical analysis: JHJ; supervision or mentorship: JTP, TIC, EWK, THY, SWK, and SHH. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors have read the journal's authorship agreement and that the manuscript has been reviewed by and approved by all named authors. All authors have read the journal's policy on disclosure of potential conflicts of interest and declare no conflict of interest.

ACKNOWLEDGMENTS

This research was supported by Research of Korea Centers for Disease Control and Prevention (2019ER690101). This work has also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. NRF-2019R1C1C1003077).

Conflicts of interest: All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.trsl.2020.10.007.

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