Elevated Endostatin Expression Is Regulated by the pIgA Immune Complex and Associated with Disease Severity of IgA Nephropathy

Yaling Zhai Xiaoqing Long Jingge Gao Xingchen Yao Xinnian Wang
Zhanzheng Zhao

Department of Nephrology, The First Affiliated Hospital of Zhengzhou University, The Renal Research Institution of Zhengzhou University, Zhengzhou, China

Keywords

IgA nephropathy · pIgA immune complex · Endostatin

Abstract

Background/Aims: Renal vascular injury accounts for the poor outcomes of patients with IgA nephropathy (IgAN). In this study, we investigated whether endostatin, a potent inhibitor of angiogenesis, is associated with IgAN. **Methods:** Serum endostatin levels were detected in patients with IgAN, disease controls, and healthy controls, and the correlation among endostatin and clinicopathologic manifestations, as well as prognosis in patients with IgAN, was analyzed. In addition, serum endostatin levels were compared in patients “before” and “after” treatment. Data on endostatin expression in the renal interstitium of patients with IgAN were downloaded and analyzed from the GSE35489 array in the GEO database. The poly-IgA1 (pIgA) immune complex is widely recognized as the “trigger” of IgAN initiation. pIgA in the plasma of patients was extracted and used to stimulate human glomerular endothelial cells (GECs). Endostatin, IL-6, and CXCL1 in the cell supernatant were detected by ELISA kits. **Results:** We found that serum endostatin levels were significantly increased in patients with IgAN, as

was endostatin expression in the renal interstitium. Patients with IgAN were divided into 2 groups according to the median value. The high endostatin expression group had significantly higher levels of serum creatinine and BUN and more severe tubular/interstitial damage. Moreover, patients with arteriolar injury and endothelial cell proliferation had higher serum endostatin levels. Patients with high serum endostatin levels had poor prognosis. According to the in vitro experiment, the GEC apoptosis rate and the supernatant levels of endostatin, IL-6, and CXCL1 were significantly increased following pIgA stimulation. **Conclusion:** Our study found that elevated endostatin expression was associated with disease severity and poor prognosis in patients with IgAN and can be upregulated by pIgA, but how it participates in the pathogenesis of IgAN deserves further exploration.

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Introduction

IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide [1]. The lack of effective treatment makes it an important disease leading to ESRD,

and it brings heavy economic and spiritual burdens to the society and the family. The pathogenesis of IgAN is very complex and still unclear. The deposition of poly-IgA1 (pIgA) immune complexes in the glomeruli is widely recognized as the “trigger”; pIgA can induce a series of “cascade reactions” and lead to renal tubular/interstitial fibrosis [2–4], a common result of renal dysfunction, indicating that circulating pIgA plays an important role in the initiation of injury in IgAN [5]. Endothelial cells are the first cells to be exposed to damage induced by pIgA. Recently, Kusano et al. [6] reported that the loss of endothelial cells may contribute to renal tubular atrophy/interstitial fibrosis in IgAN.

We found in previous studies that endothelial injury is very common in IgAN and is related to disease severity and disease prognosis [6–8]. Endothelial dysfunction, which is observed in a considerable proportion of patients with IgAN, is one of the various manifestations [9]. Our previous research found that soluble FMS-like tyrosine kinase (sFlt-1) is related to the disease severity of IgAN [10], proving that endothelial injury is related to IgAN. In addition to sFlt-1, other factors also participate in endothelial injury.

Endostatin is an endogenous angiogenesis inhibitor with a broad spectrum of antitumor activities [11–13]. It is a 20-kDa fragment of collagen XVIII, which is a ubiquitous component of basement membranes in renal glomeruli and peritubular capillaries [14, 15]. A recent study showed that endostatin was related to renal fibrosis in aged patients [16–18]. As renal tubular atrophy/interstitial fibrosis is also very important for IgAN, we aimed to explore whether endostatin contributes to the disease severity of IgAN and whether it is related to pIgA, a “trigger” of IgAN.

Methods

Study Population and Sample Collection

One hundred eighty patients with primary IgAN and 41 age- and sex-matched healthy controls, as well as 33 disease controls (9 with diabetic nephropathy, 9 with minimal change disease, 10 with focal segmental glomerular sclerosis, and 5 with membranous nephropathy), were recruited from the First Affiliated Hospital of Zhengzhou University between January 1, 2017, and December 1, 2017. Among the 180 patients with IgAN, 145 had been followed up for >1 month, and composite endpoint events were reached if any one of the following was met: (1) eGFR decreased >30% from baseline or eGFR <15 mL/min per 1.73 m²; (2) serum creatinine doubled; (3) renal replacement therapy was needed; or (4) death. Another 18 patients with primary IgAN whose serum was available “before” and “after” treatment were included in the study from the same hospital. They were divided into 2 groups: “with remission”

or “no remission.” Remission was defined as a decrease in 24-h proteinuria of 30% or more or reaching 0.15 g/day or less with stable serum Cr (no >25% increase from baseline). Patients who did not achieve remission were named the “no remission” group.

The diagnosis of primary IgAN was based on dominant IgA deposition in the mesangium by immunofluorescence and was confirmed by light microscopy and electronic microscopy at the same time. The exclusion criteria were as follows: (1) patients had been treated with a glucocorticoid or an immunosuppressor; (2) patients with secondary IgAN, such as Henoch-Schönlein purpura and liver cirrhosis, were excluded after careful examination; (3) patients with any kind of cancer or tumor; and (4) the number of glomeruli was <10. Clinical information, including 24-h urine protein excretion and hemoglobin, was collected from medical records. Oxford classification was used to score the pathological change by 2 pathologists [3, 4] who were both blinded to the clinical data.

After the consent form was signed, 10 mL of venous blood was collected in the morning of the day during which a renal biopsy was performed on the patients or on the day the healthy controls were recruited. The serum was separated from whole blood, divided into aliquots, and stored at –80°C for the subsequent measurement of endostatin.

The Medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University approved the study protocol, and informed written consent was obtained from each participant. All methods reported here were carried out in accordance with relevant guidelines and regulations of the First Affiliated Hospital of Zhengzhou University.

Detection of the Serum Endostatin Level

A commercial ELISA kit was used to detect the serum endostatin level in patients with IgAN and controls according to the manufacturer's specifications (R&D Systems, Minneapolis, MN, USA).

Tubular/Interstitial Expression of Endostatin

The mRNA expression of *COL18A1*, the endostatin-encoding gene, was extracted from the GSE35489 mRNA array, which was downloaded from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>).

Isolation of the pIgA Immune Complex

The pIgA immune complex was isolated from the mixed venous blood of 10 patients with primary IgAN using a previously described method [19]. In brief, total IgA1 was purified from the plasma with an agarose-bound jacalin affinity chromatography column (Pierce Chemical Co., Rockford, IL, USA) and then applied to a Sephacryl S-300 gel filtration chromatography column (GE Healthcare Life Sciences, Uppsala, Sweden). The fraction was pooled and concentrated at 280 nm, which corresponded to the peak representing the pIgA1 complex. After identification by Western blotting and ELISA, the pIgA immune complex was frozen at –80°C for the subsequent experiment.

Human GEC Culture and Treatment

Human renal glomerular endothelial cells (GECs) (ScienCell, Carlsbad, CA, USA) were cultured according to the manufacturer's specifications in the endothelial cell medium supplemented with endothelial cell growth supplement, 5% fetal bovine serum,

Table 1. Baseline data of enrolled IgAN patients and healthy controls

Characters	Mean $\pm$ SD or median (25%, 75%) or <i>n</i> (%)	
	IgAN patients	healthy controls
<i>Clinical presentation</i>		
Male, <i>n</i> (%)	104 (57.78)	23 (56.10)
Age, mean $\pm$ SD	36.19 $\pm$ 13.30	33.98 $\pm$ 15.37
Hypertension, <i>n</i> (%)		
With hypertension	80 (44.44)	
24-h proteinuria, g/day	2.05 (0.96, 4.68)	
Serum creatinine, μ mol/L	93 (69.50, 157.50)	
BUN, mmol/L	6.50 (4.80, 9.30)	
Uric acid, mmol/L	376.97 $\pm$ 110.39	
TCHO, mmol/L	4.68 (3.85, 5.88)	
TG, mmol/L	1.58 (1.04, 2.24)	
Urine red blood cell, <i>n</i> /HP	37 (9, 140.50)	
<i>Pathological presentation</i>		
Oxford classification, <i>n</i> (%)		
M score (M1)	44 (24.44)	
E score (E1)	48 (26.67)	
S score (S1)	127 (70.55)	
T score (T0/T1/T2)	99 (55)/25 (13.89)/56 (31.11)	
C score (c0/c1/c2)	105 (58.33)/67 (37.22)/8 (4.45)	
Arteriolar injury, <i>n</i> (%)	124 (68.89)	
Ratio of global sclerosis	0.17 (0.03, 0.41)	
Ratio of segmental sclerosis	0.05 (0, 0.12)	

penicillin G (100 U/mL), and streptomycin (100 U/mL) at 37°C and 5% carbon dioxide. The cells were passaged for 3–7 generations before starting the treatment.

After 12 h of serum starvation, GECs were treated with 200 μ g/mL pIgA complex (after the concentrations of 50, 100, 200, and 400 μ g/mL were explored) from patients and healthy controls for 24 h. The supernatant was collected for endostatin, IL-6, and CXCL1 detection, and the cells were collected for proliferation activity detection.

Detection of GEC Proliferation Activity

A CCK8 kit (Genview, Beijing, China) was used for the detection of GEC proliferation activity according to the manufacturer's instructions.

Endostatin, IL-6, and CXCL1 Levels Were Detected in the Cell Culture Supernatant by ELISA

For the detection of endostatin, IL-6, and CXCL1 levels in the cell culture supernatant, standard sandwich ELISA assays were performed using commercial human endostatin, IL-6, and CXCL1 ELISA kits (all of them were purchased from R&D Systems, Minneapolis, MN, USA) according to the manufacturer's specifications.

Statistical Analysis

Statistical analyses were performed by SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA). Continuous variables were compared by the independent samples *t* test or 1-way ANOVA between groups or by the Mann-Whitney *U* test. Dichotomous and poly-

chromous data were analyzed by the χ^2 test. K-M curve and log-rank test were used for the prognosis analysis. The results are expressed as mean $\pm$ SD if the data were distributed normally, while other data are expressed as median (IQR). A *p* value <0.05 was considered statistically significant.

Results

Clinical and Pathological Manifestations of Patients with IgAN

Our study enrolled a total of 180 patients with IgAN. The primary clinical and pathological manifestations of the patients are summarized in Table 1. Within the 180 patients with IgAN, 104 patients were male and 76 patients were female. The average age at renal biopsy was 36.19 $\pm$ 13.30 years, and 80 (44.44%) patients presented with hypertension. The 24-h urine protein level was 2.05 (0.96, 4.68) g/day. The serum creatinine level in this group was 93 (69.50, 157.50) μ mol/L. The serum BUN level in this group was 6.50 (4.80, 9.30) mmol/L. The uric acid level in this group was 376.97 $\pm$ 110.39 mmol/L. The serum TCHO level in this group was 4.68 (3.85, 5.88) mmol/L. The serum TG level in this group was 1.58 (1.04, 2.24) mmol/L, and the urine red blood cell count

Fig. 1. Elevated serum endostatin levels in patients with IgAN. Serum endostatin levels were significantly increased in patients with IgAN compared with healthy controls (61.71 [53.42, 85.72] vs. 54.69 [52.23, 69.03] ng/mL, $p = 0.032$) and disease controls (61.71 [53.42, 85.72] vs. 56.21 [31.36, 77.73] ng/mL, $p = 0.032$). IgAN, IgA nephropathy.

in this group was 37 (9, 140.50)/HP. According to the Oxford classification, 44 (24.44%) patients were in grade M1, 48 (26.67%) were grade E1, 127 (70.55%) were grade S1, 99 (55%) were grade T1, 25 (13.89%) were grade T2, 105 (58.33%) were grade C1, and 67 (37.22%) were grade C2. A total of 124 (68.89%) patients had arteriolar injury in the renal interstitium. The mean ratio of global sclerosis was 0.17 (0.03, 0.41), and the mean ratio of segmental sclerosis was 0.05 (0.0, 0.12).

Serum Levels of Endostatin in Patients with IgAN

Serum endostatin levels were significantly increased in patients with IgAN compared with healthy controls (61.71 [53.42, 85.72] vs. 54.69 [52.23, 69.03] ng/mL, $p = 0.032$) and disease controls (61.71 [53.42, 85.72] vs. 56.21 [31.36, 77.73] ng/mL, $p = 0.032$), as shown in Figure 1.

Correlations between the Serum Levels of Endostatin and the Clinical as well as Pathological Characteristics of Patients with IgAN

The patients with IgAN were divided into 2 groups according to the median endostatin level (61.71 ng/mL), and we found significantly elevated serum creatinine and

BUN levels in the high endostatin group compared with those in the low endostatin group (serum creatinine: 115 [77, 207] vs. 82.50 [63, 117] $\mu\text{mol/L}$, $p = 0.001$; serum BUN: 7.39 [5.18, 11.45] vs. 6.20 [4.10, 8.60] $\mu\text{mol/L}$, $p = 0.024$; shown in Figure 2a, b). As tubular/interstitial damage was more severe, serum endostatin was higher (T0 vs. T1: 66.52 ± 26.63 vs. 70.28 ± 24.49 ng/mL, $p = 0.096$; T1 vs. T2: 70.28 ± 24.49 vs. 80.41 ± 36.33 ng/mL, $p = 0.474$; T0 vs. T2: 66.52 ± 26.63 vs. 80.41 ± 36.33 ng/mL, $p = 0.002$; shown in Fig. 2c). Furthermore, patients with endothelial cell proliferation and arteriolar injury had significantly elevated levels of serum endostatin (E1 vs. E0: 76.35 ± 30.21 vs. 69.55 ± 30.13 ng/mL, $p = 0.047$; A1 vs. A0: 74.59 ± 33.27 vs. 64.23 ± 20.49 ng/mL, $p = 0.026$; shown in Fig. 2d, e).

Trends in Serum Endostatin Levels after Treatment

After at least 3 months of treatment according to the KDIGO guidelines, 10 patients were divided into the “with remission” group and 8 into the “no remission” group. We detected the serum endostatin levels before treatment (on the day of renal biopsy) and after treatment and found that most patients with remission had a tendency toward a decrease although significance was not reached (Fig. 3a); however, serum endostatin levels in patients with “no remission” showed a significant increase (31.43 ± 13.6 vs. 63.71 ± 20.58 , $p = 0.029$; Fig. 3b).

Patients with High Serum Endostatin Levels Had Poorer Prognosis

A total of 145 of 180 patients with IgAN had been followed up for >1 month, and the median follow-up time was 16 (7.5–26.5) months. According to the mean value (71.36 ng/mL) of serum endostatin levels, patients were divided into the “high group” and the “low group,” and we found that patients with high endostatin levels had poorer prognosis (log-rank test, $p = 0.040$, Fig. 4).

Identification of Elevated COL18A1 Expression in the Renal Interstitium of Patients with IgAN

Endostatin is a 20-kDa C-terminal fragment derived from type XVIII collagen (COL18A1) [20, 21], and its expression in the renal interstitium can be represented by the level of COL18A1 in the GSE35489 mRNA array. We found that COL18A1 expression was significantly different in patients with IgAN (IgAN vs. HC: GL96: 7.78 ± 0.36 vs. 7.30 ± 0.25 , $p = 0.007$; GL14663: 7.61 ± 0.32 vs. 7.18 ± 0.17 , $p = 0.001$), as shown in Figure 5a and b, respectively, further indicating the association between endostatin and IgAN.

Fig. 2. The association between serum endostatin levels and clinical and pathological manifestations. Serum creatinine (**a**) and BUN (**b**) levels in the high endostatin group were significantly elevated compared with those in the low endostatin group (serum creatinine: $115 [77, 207]$ vs. $82.50 [63, 117]$ $\mu\text{mol/L}$, $p = 0.001$; serum BUN: $7.39 [5.18, 11.45]$ vs. $6.20 [4.10, 8.60]$ $\mu\text{mol/L}$, $p = 0.024$). **c** As the tubular/interstitial damage was more severe, serum endostatin was higher (T0 vs. T1: 66.52 ± 26.63 vs. 70.28 ± 24.49 ng/mL, $p = 0.096$; T1 vs. T2: 70.28 ± 24.49 vs. 80.41 ± 36.33 ng/mL, $p = 0.474$; T0 vs. T2: 66.52 ± 26.63 vs. 80.41 ± 36.33 ng/mL, $p = 0.002$). Furthermore, patients with endothelial cell proliferation (**d**) and arteriolar injury (**e**) had significantly elevated levels of serum endostatin (E1 vs. E0: 76.35 ± 30.21 vs. 69.55 ± 30.13 ng/mL, $p = 0.047$; A1 vs. A0: 74.59 ± 33.27 vs. 64.23 ± 20.49 ng/mL, $p = 0.026$). A1, with arteriolar injury; A0, without arteriolar injury; E1, with endothelial cell proliferation; E0, without endothelial cell proliferation.

Fig. 3. Trends in serum endostatin levels after treatment. **a** Most patients with IgAN in the “with remission” group had a tendency toward a decrease although significance was not reached. **b** However, serum endostatin levels in patients with “no remission” showed a significant increase (31.43 ± 13.6 vs. 63.71 ± 20.58 , $p = 0.029$). IgAN, IgA nephropathy.

Fig. 4. Patients with high serum endostatin levels had poorer prognosis. Patients with high endostatin levels had poorer prognosis (log-rank test, $p = 0.040$).

Increased Endostatin Expression in the Supernatant after GECs Were Stimulated with the pIgA Immune Complex

After stimulation with the pIgA immune complex, we found that endostatin expression in the supernatant was significantly increased compared with that in the control group, which was stimulated with PBS instead (pIgA vs. control: 11.75 ± 0.66 vs. 8.61 ± 0.85 ng/mL, $p = 0.007$; shown in Fig. 6a).

Decreased Cell Proliferation Activity and Increased Inflammatory Factor Secretion after GECs Were Stimulated with the pIgA Immune Complex

To reveal more clues about endostatin and IgAN, we also detected the GEC proliferation activity and inflammatory factors following pIgA immune complex stimulation, and our results showed that GEC proliferation activity significantly decreased (pIgA vs. control: 0.59 ± 0.16 vs. 1.06 ± 0.13 , $p = 0.017$; shown in Fig. 6b) and that supernatant IL-6 and CXCL1 levels significantly increased (IL-6: pIgA vs. control: $1,362.19 \pm 65.29$ vs. $1,034.54 \pm 61.69$ pg/mL, $p = 0.003$; CXCL1: pIgA vs. control: $160,833.57 \pm 8,772.20$ vs. $12,273.40 \pm 2,245.10$ pg/mL, $p = 0.001$; shown in Fig. 6c, d).

Discussion

IgAN is a common, complex disease with variable manifestations. Endothelial dysfunction is commonly observed in patients with IgAN [8, 19, 20]. Our study indicated that elevated serum endostatin levels were associated with the disease severity and prognosis of IgAN and could be upregulated by the pIgA immune complex.

Endostatin is a natural C-terminal fragment of 20 kDa and is encoded by *COL18A1* [21, 22]. It is reported to be a potent antiangiogenic protein that is able to inhibit angiogenesis and tumor growth [23]. However, a recent study revealed that in the elderly, it was associated with kidney interstitial fibrosis [16], which always occurs in

Fig. 5. *COL18A1* expression in the renal interstitium of patients with IgAN in the GSE35489 mRNA array. *COL18A1* expression was significantly different in the renal interstitium of patients with IgAN (IgAN vs. HC: GL96: 7.78 ± 0.36 vs. 7.30 ± 0.25 , $p = 0.007$ (a); GL14663: 7.61 ± 0.32 vs. 7.18 ± 0.17 , $p = 0.001$ (b)). IgAN, IgA nephropathy.

Fig. 6. The increased endostatin expression and cell apoptosis rate as well as inflammatory factor levels of GECs after pIgA stimulation. **a** After stimulation with the pIgA immune complex, endostatin expression in the supernatant was significantly increased compared with that of the control group, which was treated with PBS instead (pIgA vs. control: 11.75 ± 0.66 vs. 8.61 ± 0.85 ng/mL, $p = 0.007$). **b** At the same time, GEC proliferation activity was significantly decreased (pIgA vs. control: 0.59 ± 0.16 vs. 1.06 ± 0.13 , $p = 0.017$), and supernatant IL-6 (**c**) and CXCL1 (**d**) levels were significantly increased (IL-6: pIgA vs. control: $1,362.19 \pm 65.29$ vs. $1,034.54 \pm 61.69$ pg/mL, $p = 0.003$; CXCL1: pIgA vs. control: $160,833.57 \pm 8,772.20$ vs. $12,273.40 \pm 2,245.10$ pg/mL, $p = 0.001$). GEC, glomerular endothelial cell; pIgA, poly-IgA.

CKD and indicates a poor prognosis. As an antiangiogenesis factor, there is no report about whether endostatin is associated with IgAN. We found in our study that the serum endostatin level in patients with IgAN was significantly higher than that in patients with CKD in another study [24]. The mean level of endostatin in healthy controls was comparable with 2 large cohorts [18], but endostatin levels in patients with IgAN were significantly lower than those in patients undergoing hemodialysis (254.3 ng/mL) [22] and slightly higher than those in renal cell carcinoma and systemic lupus erythematosus [25, 26], possibly because vascular injury was more severe in patients with hemodialysis and different ELISA kits were used in different races in the studies. Moreover, serum endostatin levels in patients with IgAN were significantly increased compared with disease controls (diabetic nephropathy, minimal change disease, focal segmental glomerular sclerosis, and membranous nephropathy were included) in this study, and there was no significant difference between disease controls and healthy controls, indicating that vascular injury in patients with IgAN was more severe than most kinds of glomerular disease. We also compared urine endostatin levels in patients with IgAN with healthy controls and found that it increased in patients although a significant difference was not reached ($p = 0.074$).

Furthermore, we explored the relationship between elevated endostatin expression and the clinical and pathological data in patients with IgAN (e.g., creatinine level, BUN level, proteinuria, and Oxford classification score). According to the results, serum creatinine and BUN levels were higher in patients with high endostatin expression, and more severe renal tubule/interstitial damage, renal arteriole injury, and GEC proliferation were also noted in these patients. This discovery indicates that endostatin was associated with the disease severity of IgAN. However, compared with a nonsignificant change in serum endostatin levels in patients with effective treatment, a significant increase was observed in patients with no response to treatment, indicating that a persistent increase in serum endostatin levels may be a biomarker for poor response to treatment. We also found that the expression of the endostatin-encoding gene *COL18A1* was increased significantly in the renal interstitium of 2 independent cohorts of IgAN in the mRNA array, which further suggests that endostatin may play a certain role in patients with IgAN, which was proven by our result that patients with IgAN with high serum endostatin levels had a poorer prognosis; unfortunately, endostatin was not an independent risk factor after adjustment.

According to a previous study, the mainstream doctrine of the IgAN mechanism research is the “four-hit theory” [27]. Circulating pIgA deposition in the mesangial area and endocapillary wall is the key step leading to subsequent damage, such as endocapillary cell proliferation, arteriolar injury, and tubular atrophy/interstitial fibrosis. To further investigate the relationship between the pIgA immune complex and endostatin in patients with IgAN, we carried out an in vitro experiment and found that pIgA could stimulate GECs to secrete more endostatin, and the GEC apoptosis rate increased at the same time, indicating that the balance of angiogenesis was disturbed. IL-6 and CXCL1, well-known proinflammatory factors [28], were upregulated in GECs after pIgA stimulation in our study. According to a previous study, monocyte-macrophage infiltration is responsible for renal interstitial fibrosis, and the inflammatory factors (such as IL-6 and CXCL1) secreted by these cells could induce renal tubular epithelial cell transdifferentiation, which is the key step in renal interstitial fibrosis [29]. These inflammatory factors could be the “messenger molecules” that promote the “cross-talk” between different renal cells and may finally lead to renal dysfunction [30]. However, it is important to note that IL-6 and CXCL1 were secreted by the injured GECs in our study, which was consistent with a recent report that endothelial cells could produce potent proinflammatory cytokines, such as IL-6, in an in vitro model of lupus nephritis [31]. A study by Tanabe et al. [32] showed that endostatin could inhibit monocyte/macrophage accumulation, decrease the number of CD31(+) blood vessels, and alleviate peritoneal fibrosis in a peritoneal dialysis mouse model. Additionally, another study proved that higher cathepsin S, which modulates the antiangiogenic activities of human endostatin [33], was associated with higher CRP and IL-6 levels [34], providing us with a bold speculation – whether the possibility exists that IL-6 and CXCL1 secretion by GECs may be regulated by endostatin, which was upregulated by pIgA.

Notably, our present study had disadvantages. First, it was a retrospective study. Second, we did not extract pIgA separately from each patient because of its very small amount in human serum, and the extraction process is very complex. Third, our study only observed a relationship between endostatin and IgAN; however, how endostatin participates in the pathogenesis of IgAN is still unclear.

A prospective design would have probably helped to understand more about the pathogenetic role of endostatin and could have provided more exact information on the effect of the therapy on endostatin. In addition,

exploration of the pathogenesis of endostatin involvement in IgAN needs to be conducted.

In conclusion, the present study found that elevated endostatin expression in patients with IgAN was correlated with clinical and pathological manifestations, as well as prognosis, which provides the possibility of endostatin serving as a prognostic biomarker. Endostatin expression in GECs could be regulated by pIgA, which may provide some clues about the pathogenesis of IgAN.

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Statement of Ethics

The Medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University approved the study protocol, and informed written consent was obtained from each participant. All methods reported here were carried out in accordance with relevant guidelines and regulations of the First Affiliated Hospital of Zhengzhou University.

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Conflict of Interest Statement

The authors declare no competing interests.

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Author Contributions

Yaling Zhai and Zhazheng Zhao conceived and designed the experiments. Xiaoqing Long, Xingchen Yao, and Xinnian Wang performed experiments. Jingge Gao analyzed the data. Yaling Zhai and Zhazheng Zhao contributed reagents/materials/analysis tools. Yaling Zhai wrote the paper. All authors read and approved the final manuscript.

Availability of Data and Material

Raw data used during the current study are available from the corresponding author on reasonable request for noncommercial use.

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