

Original Article

Estrogen receptor alpha expression in renal tissue and its relationship with prognosis in immunoglobulin A nephropathy

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Abstract: Objective: Epidemiologic studies have shown that, compared to men, women have better prognoses in chronic renal diseases. This indicates that gender hormones may have some effect on renal function. The present study aimed to investigate the expression and relationship with prognosis of estrogen receptor alpha (ER α) in renal tissue of immunoglobulin A nephropathy (IgAN) patients. Methods: Expression of ER α was detected by immunohistochemistry. Specimens were collected from forty-five IgAN patients and five normal healthy participants. In addition to expression, the relationship of ER α with clinical data, prognosis, and the degree of glomerular damage were analyzed. Results: ER α was expressed in the glomeruli and renal tubules. The expression of ER α in the glomeruli of IgAN renal tissue decreased gradually ($P = 0.001$, $P < 0.05$) with the increasing severity of disease and its expression was associated with estimated glomerular filtration rate (eGFR), serum creatinine (Scr) and pathological grade ($r = 0.876$, -0.818 and -0.736 , $P < 0.05$). In addition, the expression of ER α was significantly decreased in hypertensive IgAN patients compared with the non-hypertensive group ($P = 0.011$). We found that ER α was of great significance for the prognosis of IgAN ($P = 0.01$, $P < 0.05$). Conclusions: The expression of ER α in the renal tissue of IgAN patients was reduced and there was a correlation with the degree of kidney damage and prognosis, suggesting that ER α may play a role in the progression of IgAN.

Keywords: Estrogen receptor alpha, immunoglobulin A nephropathy, immunohistochemistry, kidney disease, prognosis

Introduction

Epidemiological studies have shown that the incidence of chronic renal failure in male patients with kidney disease is higher than in females. However, the proportion of postmenopausal women has increased significantly [1, 2]. It has been estimated that the number of postmenopausal women with end-stage kidney disease (ESKD) is increasing by greater than 4% each year [3]. During the physiologic process, the glomerular filtration rate (GFR) declined faster in males than in females between 20 and 50 years of age [4]. In a mouse model of adenine-induced renal failure, there was a significantly greater reduction of ER α in male mice than in female mice, accompanied by renal impairment. However, modulating the ER α -mediated pathway with Tamoxifen in vitro

significantly ameliorated renal tubulointerstitial fibrosis [5, 6]. Addressing the crucial role of gender, both in humans and animals, for chronic disease, we investigated ER α expression in renal tissue and the relationship with the prognosis in IgAN, since IgAN is a common chronic primary glomerulonephritis and a regular cause of chronic renal failure.

Materials and methods

Specimen selection

Tissue samples from 45 patients with IgAN who were hospitalized at the Second Affiliated Hospital of Nanjing Medical University between January 2004 to December 2005 were selected for inclusion in the present study. Those with kidney disease caused by allergic purpura, sys-

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temic lupus erythematosus, or nodular vasculitis were excluded through clinicopathologic diagnosis. 5 normal, healthy cases were chosen from resected tissues adjacent to renal cell carcinoma.

Clinical data

Patient medical history, status, and laboratory results were recorded.

Pathologic changes of renal tissue and evaluation measures

The samples were divided into Lee's grades I (11 cases), II (10 cases), III (8 cases), IV (9 cases), and V (7 cases). The glomeruli and renal tubular areas were counted in each specimen. The cultured cells and positive cells were averaged from 15 non-overlapping fields, including 5 glomerular regions, 5 proximal tubule regions, and 5 distal tubule regions chosen randomly on microscopic slides. We evaluated the results by combining stain strength and percentage of positive cells. The samples were scored according to the degree of staining, referring to the literature, where 0 was no staining, 1 was weak staining, 2 was moderate staining and 3 was strong staining. Similarly, the percentage of positive cells was scored, where 0 referred to no positive cells, a score of 1 was < 1%, 2 included 1-10% positive cells, 3 from 10-30%, 4 from 30-66% and 5 above 66% [7, 8]. The result was based on the sum of these two scores. Pathologic readings were carried out by two experienced pathologists. In order to resolve incongruent scoring, the pathologists reached a consensus by using a double-headed microscope together.

Immunohistochemical detection of ER α

For pathologic analysis, 4 μ m-thick renal sections were cut from the paraffin blocks. DAB immunohistochemistry staining was performed using the ZSGB-BIO System (ZLI9018, Beijing, China) for renal immunohistochemical assays. The slides were incubated with a primary antibody against ER α (1:200; Abcam, Cambridge, UK).

Follow-up

Patients were monitored for 5 years until ESRD (eGFR < 15 mL/min per 1.73 m² or renal

replacement therapy (RRT) for at least 3 months) or until death.

Statistical analysis

Data were reported as mean \pm standard deviation or median (quartile spacing). Statistical analysis of data was performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Comparisons among groups were made with analysis of variance (ANOVA) or Rank-Sum test. Spearman correlation was used for the degree and correlation of these variables. Counted data were expressed as a percentage and the chi-square (X^2) test was used for comparisons among groups. The survival rate based on kidney disease was estimated by Kaplan-Meier method. The log-rank test was used to compare the survival curves. The Cox proportional hazards model was established to analyze the risk factors affecting the prognosis of the kidney. $P < 0.05$ was considered significant.

Results

Distribution of ER α in normal renal tissue

Immunohistochemical staining of human renal biopsies showed ER α protein in both the glomeruli and renal tubules (**Figure 1A**).

Distribution of ER α in IgAN renal tissue

The expression of ER α was markedly reduced in IgAN renal tissue, particularly in the glomeruli. In grade I lesions, ER α levels were similar to normal ($P > 0.05$). With the increasing severity of the disease, glomerular ER α gradually lessened ($P = 0.001$) (**Figure 1B-D**; **Table 1**).

Clinical data

Of the 45 patients with IgAN, 13 were male, 32 were female and the average age was 37.93 \pm 11.53 years. The cases of IgAN were divided into Lee's grades I (n = 11), II (n = 10), III (n = 8), IV (n = 9), V (n = 7). There were differences in hemoglobin, urea, creatinine, and the glomerular filtration rates in the patients with different histologic grades at the time of diagnosis. However, there were no differences in urinary protein, urinary erythrocyte count, or serum albumin among the different histologic grades. As the lesion progressed, glomerular filtration rate decreased significantly (**Table 2**).

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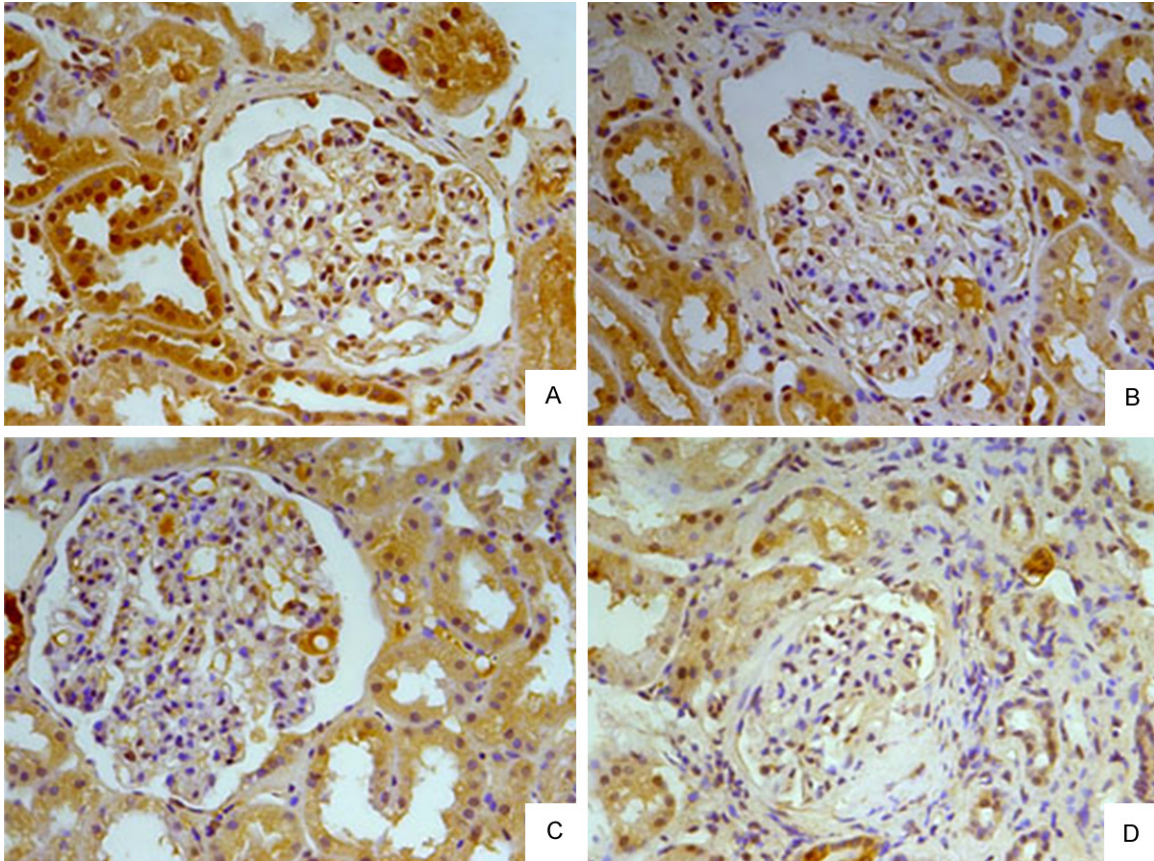


Figure 1. Distribution of ER α in renal tissue. A. Normal renal tissue. B. IgAN renal tissue of Lee's grade II. C. IgAN renal tissue of Lee's grade III. D. IgAN renal tissue of Lee's grade V.

Table 1. ER α expression in different pathologic types of kidney (score, $\bar{x} \pm s$)

Kidney pathology type	Numbers	Glomerular ER α	Tubule ER α
Normal group	5	6.40 \pm 0.55	6.60 \pm 0.55
I grade	11	6.18 \pm 1.40	6.27 \pm 1.19
II grade	10	5.60 \pm 1.26	5.50 \pm 1.08
III grade	8	4.88 \pm 0.64	6.38 \pm 1.30
IV grade	9	3.89 \pm 1.36	6.33 \pm 1.66
V grade	7	2.14 \pm 0.38	4.57 \pm 1.51

Relationship between ER α and clinical data

Expression of ER α in the glomeruli was positively correlated with the estimated glomerular filtration rate (eGFR) ($r = 0.876$, $P < 0.01$) and negatively related to Scr and the pathologic grade of IgAN ($r = -0.818$, -0.736 ; $P < 0.01$). Glomerular ER α was decreased significantly in IgAN patients with hypertension ($P = 0.011$, $P < 0.01$; **Table 3**). ER α expression and pathologic grade of IgAN had no relevance to urine protein

($r = 0.091$, 0.172 ; $P > 0.05$). The pathologic grade of IgA nephropathy was positively correlated with Scr and negatively correlated with eGFR ($r = 0.757$, -0.781 ; $P < 0.05$).

Survival analysis

The forty-five IgAN patients were followed up for 12-60 months (median 60 months). A total of 8 (17.8%) patients were admitted to ESRD or died and 2 patients ceased participation in the follow-up examinations. Because of the small number of cases, we divided them into two groups of Lee's grade I-III and IV-V for survival analysis, which showed a significant difference in renal survival between the two groups ($P < 0.01$) (**Figure 2**).

The clinical and pathologic data of patients were analyzed by single-factor Cox regression, showing that low hemoglobin levels (Hb < 90 g/L), eGFR < 60 ml/min, pathologic grade

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Table 2. Analysis of clinical data in different pathologic types

	Lee I (n = 11)	Lee II (n = 10)	Lee III (n = 8)	Lee IV (n = 9)	Lee V (n = 7)	P
Age (year)	31.82 \pm 8.40	30.60 \pm 7.40	40.63 \pm 9.77	46.33 \pm 12.81	44.14 \pm 11.25	0.003
male, n (%)	3 (27.27)	4 (40)	1 (12.5)	3 (33.33)	2 (28.57)	0.782
Upro (g/24 h)	0.9 (0.13-10.2)	0.54 (0.08-4.6)	0.3 (0.08-10)	0.8 (0.07-11)	2.3 (0.1-5.36)	0.471
RBC (million/ml)	80 (3-1012)	21.5 (0-95)	100 (0-220)	43 (6-560)	14 (5-1200)	0.450
ALB (g/L)	40 (14.8-47)	42 (22.2-48)	43.35 (25.3-50.4)	38.5 (19-47)	35.9 (18.7-44.4)	0.134
Hb (g/L)	124.82 \pm 16.63	137.90 \pm 20.48	135.75 \pm 13.37	125.78 \pm 14.54	101.14 \pm 18.69	0.001
BUN (mmol/L)	4.38 \pm 1.11	5.38 \pm 0.92	5.18 \pm 1.60	6.88 \pm 2.78	9.48 \pm 2.09	0.000
Scr (μ mol/L)	70 (44.7-82)	81.6 (62.3-105.6)	89 (42.8-102.9)	98.5 (66-155)	209 (140-309)	0.000
eGFR (ml/min)	113.58 \pm 44.32	86.32 \pm 9.69	76.69 \pm 28.37	65.41 \pm 23.41	31.93 \pm 12.55	0.000
hypertension, n (%)	2 (18.18)	5 (50)	5 (62.5)	3 (33.33)	4 (57.14)	0.274

RBC: urine sediment red cell blood count; ALB: albumin; Hb: hemoglobin; BUN: urea nitrogen.

Table 3. Relationship between ER α expression and hypertension (score, $\bar{x} \pm s$)

	Numbers	Glomerular ER α
hypertensive group	21	4.0 \pm 1.82
non-hypertensive group	24	5.38 \pm 1.47

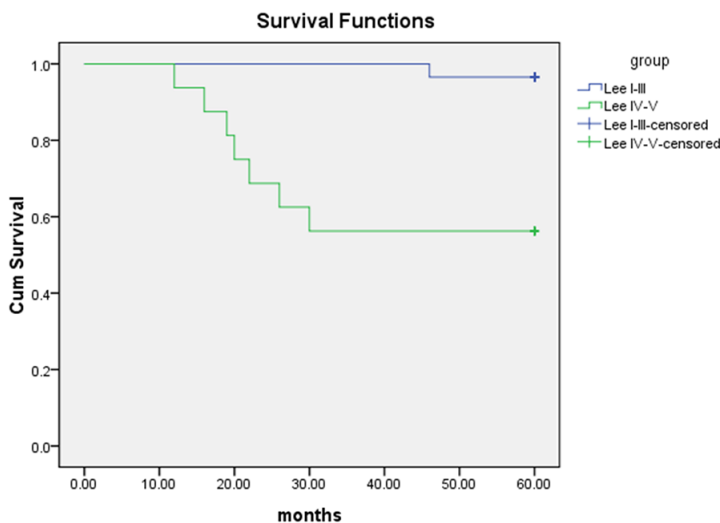


Figure 2. Survival curve of patients with IgAN.

(Lee's grade I-III and IV-V groups), glomerular ER α (score 1-4 and 5-8 groups) and tubule ER α (score 1-4 and 5-8 groups) correlated with renal survival (**Table 4**). Multivariate Cox regression analysis showed that pathologic grade (HR 12.690, $P < 0.05$) and glomerular ER α expression (HR 0.059, $P < 0.05$) were independent factors affecting the prognosis of patients with IgAN.

Discussion

While clinical and epidemiologic studies have provided growing evidence for a substantial gender difference in the progression of chronic glomerular diseases, the current report suggests a protective effect of ER on IgAN.

ERs are members of the nuclear receptor (NR) superfamily. They have been identified as two subtypes, ER α and ER β . Studies have shown that ERs are significantly expressed in glomerular podocytes, mesangial cells, and renal tubular epithelial cells [9-11]. Research in cultured mouse podocytes showed that ER α existed both in the cytoplasm and in the nucleus. Short-term (24 h) stimulation of estrogen induced an increase of nuclear ER α , while long-term (> 48 h) stimulation increased cytoplasmic expression [12]. Our previous study showed that Tamoxifen, an agonist of

estrogen receptor, inhibited the expression of α -smooth muscle actin and ameliorated renal interstitial fibrosis [13].

In human renal biopsies, immunohistochemical staining showed the ER α protein had cytoplasmic and nuclear localization. The expression of ER α in IgAN renal tissue was significantly decreased, especially in the glomeruli. With

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Table 4. Factors affecting the prognosis of patients with IgAN

	Univariate Cox regression		Multivariate Cox regression	
	X ²	P	HR (95% CI)	P
hypertension	0.2	0.655		
Low Hb (< 90 g/L)	13.949	0.000		
Low ALB (< 35 g/L)	0.190	0.633		
eGFR < 60 ml/min	19.40	0.000		
Pathologic grade	12.751	0.000	12.690 (1.762-91.387)	0.012
Glomerular ER α	11.206	0.001	0.059 (0.007-0.511)	0.01
Tubule ER α	17.787	0.000		

In the univariate Cox regression, pathologic grade was divided into two groups of Lee's grade I-III and IV-V; Glomerular ER α and tubule ER α were divided into two groups of score 1-4 and 5-8.

increasing severity of the lesion, glomerular ER α gradually decreased, and our further analysis showed a certain correlation between the expression of ER α and pathologic grade of IgAN together with eGFR. Based on the above results, it could be concluded that ER α played a role in the development of IgAN, but the specific mechanism remains to be explored further.

Increasingly, recent studies have shown that estrogen regulates blood pressure by promoting vasodilation, reducing the inflammatory response, regulating oxidative stress, and regulating the activity of the renin-angiotensin-aldosterone system [14-16]. Our study also showed that, compared with IgA nephropathy patients without hypertension, those with hypertension often had significantly reduced glomerular ER α expression, suggesting that ER α may be involved in the regulation of blood pressure.

Through Cox risk analysis, we found that renal pathology and ER α expression were of great significance in the prognosis of IgAN. From a single-center study in Korean adults, the author found that when IgAN patients were categorized as Lee's classification grade I/II/III and grade IV/V, grade IV/V was associated with a significantly increased risk of ESRD. Histopathologic classifications should be developed to predict renal outcome [17]. By utilizing a refined version of H. S. Lee's glomerular grading system in IgAN, research indicated that glomerular grade was an independent prognostic factor for progressive renal disease [18]. Because of the small sample size, we also divided the pathologic classification into two categories, grade I/II/III and grade IV/V, for survival analysis. Compared to grade I/II/III, the

prognosis of grade IV/V patients was poor. This was consistent with previous results.

Though the complexity of the physiologic and clinical interaction between female sex hormones and kidney function remains poorly understood, evidence suggests that estrogen has renoprotective effects. For example, ovariectomized glomerulosclerosis-prone ROP Os/p mice developed more severe glomerular lesions and renal dysfunction than did age-matched female controls [19]. Several findings in humans suggested that postmenopausal hormone therapy and selective estrogen receptor modulators may play beneficial roles with respect to kidney function. The selective estrogen receptor modulator raloxifene has been associated with renoprotection in women with kidney disease [20, 21]. Other animal model data also reported a renoprotective role for estrogen [22-26]. In summary, we infer that ER has renal protective effects both in people and in animals, which also supports our result that ER expression was associated with the degree and prognosis of IgAN. The specific mechanism of ER in IgAN requires further exploration, in multicenter studies with a sample size larger than ours, to verify our results.

Disclosure of conflict of interest

None.

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