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Original Article

Prognostic and diagnostic value of HE4 expression in patients with chronic kidney diseases

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Abstract: *Background:* Chronic kidney diseases (CKD) is a progressive loss in renal function, of which the advanced stage is renal fibrosis caused by excessive accumulation of collagen I and other extracellular matrix. The HE4 gene encodes a putative serine protease inhibitor that is upregulated in human fibrotic kidneys and the serum of patients with kidney fibrosis. The expression and clinical significance of HE4 in patients with chronic kidney diseases are not clear. *Methods:* Here, we detected the expression and localization of HE4 in renal biopsies, correlated their immunostaining scores with clinical and histological parameters, and analyzed whether the HE4 protein level in the renal interstitium was correlated with renal survival. In addition, we detected serum HE4 concentrations in patients with CKD by enzyme-linked immunosorbent assay (ELISA). Receiver operating characteristic (ROC) curves and Cox analyses were used to evaluate its diagnostic and prognostic efficacy, respectively. *Results:* We found that HE4 was strongly expressed in the cytoplasm of tubular epithelial cells from kidneys of patients with CKDs. HE4 protein expression in the tubulointerstitium was inversely correlated with eGFR and positively correlated with the percent of tubulointerstitial fibrosis. Moreover, HE4 levels were higher in the serum of patients with CKD than in controls. The correlations between serum HE4 concentrations and tubulointerstitial fibrosis or eGFR were significant ($P < 0.05$). *Conclusions:* Our results suggested that HE4 was an available biomarker for the progression of renal fibrosis.

Keywords: HE4, chronic kidney diseases, renal survival, tubulointerstitial fibrosis

Introduction

Chronic kidney disease (CKD) is a long-period progressive loss in renal function. Renal fibrosis and even end-stage renal failure are inevitable when type I collagen and other extracellular matrix proteins were excessively accumulated during the progression of CKD [1, 2]. In clinic, CKD is usually diagnosed through screening of risk factors for kidney problems, such as those with high blood pressure or diabetes. Researchers have been trying to look for available earlier biomarkers for CKD.

The human epididymis protein 4 (*HE4*) gene encodes a WAP 4-disulphide core domain 2 (WFDC2) secreted protein [3]. WFDC2 is a member of the whey acidic protein (WAP) domain family of proteins, which was first identified in the epithelium of the distal epididymis and was suggested to be a putative protease inhibitor in sperm maturation [4]. HE4 was earlier found in the male reproductive system and a number of normal human tissues, and was also reported to be highly expressed in malignant adult tissues, such as carcinomas of the breast, transitional cells, and pancreas, and adenocarcinoma of the lung [5, 6]. It functioned as a protease inhibitor and conferred natural immunity through its 4 disulfide core domain repeats or WAP motifs, and play an important role in growth and differentiation [7]. Studies revealed that the *HE4* gene is dramatically upregulated in fibrotic kidneys of dogs and mice [8, 9]. A strong correlation between *HE4* transcript levels and estimated glomerular filtration rate (eGFR) has been shown in human kidney transplant biopsies [10]. These clues suggested an important role of HE4 in renal fibrosis. Additionally, expression of both HE4 mRNA and protein were dysregulated in fibrotic kidneys.

Table 1. Demographics and clinical characteristics of the research participants

Diagnosis	No. of cases	Gender M/F	Age (years)	Proteinuria (g/day)	SCR (mg/ml)	eGFR (ml/min)
Control	12	6/6	45.9±9.3	0 (0, 0.2)	0.71±0.23	101.62±19.52
DN	33	18/15	53.7±10.3	4.3 (3.8, 5.65)	1.22±0.49	74.66±30.04
FSGS	27	15/12	26.3±7.7	3.1 (2.3, 4.6)	1.17±0.38	88.28±44.24
IgAN	70	44/26	32.7±12.9	1.4 (0.8, 2.4)	1.22±0.48	82.23±33.52
HN	15	9/6	58.5±9.3	1.2 (0.9, 1.7)	1.05±0.37	80.29±31.62
TIN	11	6/5	42.0±12.2	1.2 (0.9, 2.1)	1.29±0.45	72.21±37.67

Abbreviations: DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; HN, hypertensive nephrosclerosis; IgAN, immunoglobulin A nephropathy; SCR, serum creatine; TIN, tubulointerstitial nephritis. Values are expressed as means ± s.d. for age, Scr and eGFR as median and interquartile range for proteinuria. eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula.

Table 2. Clinical characteristics of the research participants

Diagnosis	No. of glomeruli	Glomerulosclerosis (%)	Tubulointerstitial fibrosis (%)
Control	14.5	3 (0, 5)	5 (0, 9)
DN	15.6	39.0 (15, 62.5)	43 (17.5, 61)
FSGS	17.8	32 (11, 45)	20 (14, 38)
IgAN	15.3	23 (12, 47)	22 (16, 41)
HN	16.2	33 (16, 69)	31 (12, 54)
TIN	16.7	18 (13, 34)	37 (19, 57)

Values are expressed as median (interquartile range) for glomerulosclerosis and tubulointerstitial fibrosis.

HE4 was demonstrated to suppress the activity of serine proteases that degrade type I collagen in patients with renal fibrosis. Thus HE4 was considered a potential biomarker and therapeutic target for the treatment of renal fibrosis [11]. However, the implication of HE4 level changes in serum and kidney samples in different kidney diseases remains largely unknown.

In this study, expression and localization of HE4 in kidney biopsies were detected, and then HE4 levels in serum samples from patients with CKDs were analyzed. We found that, compared with controls, patients with CKD displayed a higher serum level of HE4. The correlations between anti-HE4 antibodies and tubulointerstitial fibrosis or eGFR suggested that HE4 was an available biomarker for the progression of CKD.

Materials and methods

Ethical statement

The study was approved by the Hospital's Protection of Human Subjects Committee, and informed consent was obtained from all patients and volunteers.

Kidney biopsies and serum sample

Renal biopsy samples and serum samples were taken from patients with CKD at Xijing Hospital from January 2010 to August 2012. The renal biopsy with sufficient materials (at least 10 glomeruli) were selected for further study. The samples from patients with diabetic kidney disease were histologically diagnosed at the Pathology Department of Xijing Hospital, and corresponding clinical information was collected from patient records. Twelve tissue sections obtained from nephrectomy samples were used as controls. Patients with severe infection, immune deficiency, liver diseases and malignant tumors were excluded from the study.

Processing of samples

The renal biopsy samples were Formalin-fixed and paraffin-embedded (FFPE) stored at 4°C until use. Blood samples were collected by vena puncture from all subjects and centrifuged at 3000 rpm for 20 min at 4°C to eliminate any residual cells. All samples were stored at -80°C until use.

Immunohistochemistry

HE4 expression levels were evaluated with immunohistochemical staining using the avidin-biotin peroxidase complex method (Dako, Glostrup, Denmark). Immuno histochemistry was performed as previously described [12].

HE4 enzyme-linked immunosorbent assay (ELISA)

Twenty microliters sera from the patients or the healthy volunteers and recombinant twenty microliters HE4 protein standards with a final protein concentration of 5 mg/ml were mixed

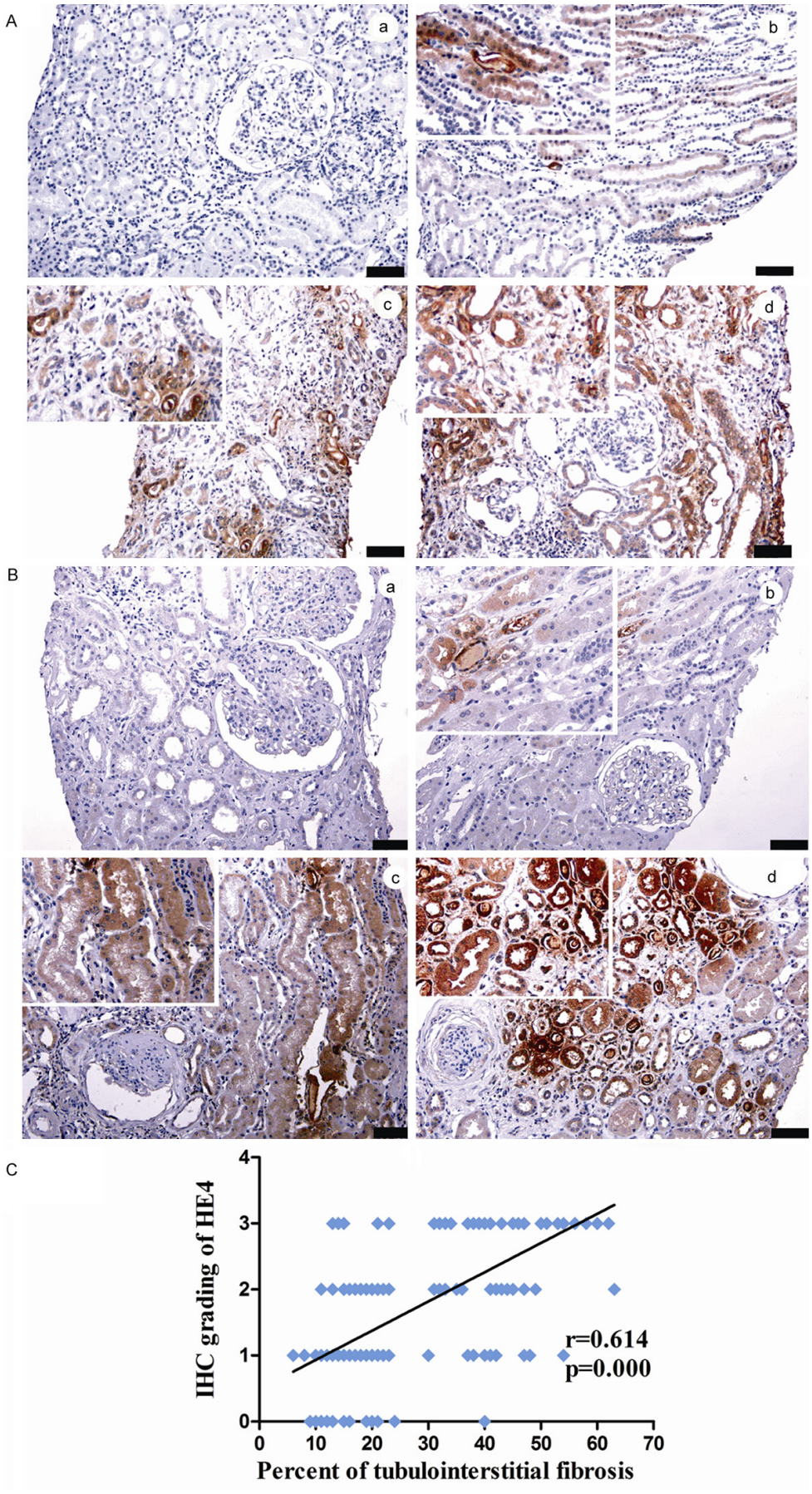
A
a
b
c
d
B
a
b
c
d
C
IHC grading of HE4
Percent of tubulointerstitial fibrosis
r=0.614
p=0.000
0
1
2
3
4
0
10
20
30
40
50
60
70

Figure 1. A. HE4 in renal biopsies from patients with IgA nephropathy. HE4 immunostaining in renal biopsy tissues from patients with IgAN (original magnification, 200 ×). (a) Negative control. (b) Tissue from a IgAN kidney, both with 1+ staining (≤ 25% cells positive per visual field). (c) Representative photographs from IgAN kidneys with 2+ staining (25-50% cells stained positive per visual field). (d) Representative photographs from IgAN kidneys with 3+ staining (> 50% cells stained positive per visual field). Inserts in the upper left-hand corner of selected panels are higher magnification images of each corresponding panel. The number of biopsies with tubular staining (t) is shown in parentheses. –, absence of staining; +, 1%-25% of cells per visual field with positive staining; ++, > 25%-50%; +++, > 50% of cells with positive staining. B. HE4 in renal biopsies from patients with diabetic nephropathy. HE4 immunostaining in renal biopsy tissues from patients with DN (original magnification, 200 ×). (a) Negative control. (b) Tissue from a IgAN kidney, both with 1+ staining (≤ 25% cells positive per visual field). (c) Representative photographs from DN kidneys with 2+ staining (25-50% cells stained positive per visual field). (d) Representative photographs from DN kidneys with 3+ staining (> 50% cells stained positive per visual field). C. Correlation plots of serum concentration of HE4 protein concentration and tubulointerstitial fibrosis in renal biopsies. Scatter plot with fitted values intervals for concentration of HE4 and percent tubular of tubulointerstitial fibrosis.

Table 3. The IHC grading of HE4 in 156 CKD cases and 12 normal kidney tissues

Diagnosis	No. of cases	HE4 IHC results				*P* Value*
		Negative		Positive		
		0	1+	2+	3+	
Control	12	7	3	2	0	
CKD	156	33	36	44	43	0.009
DN	33	7	7	9	10	0.015
FSGS	27	6	7	6	8	0.039
IgAN	70	14	18	20	18	0.016
HN	15	4	2	5	4	0.047
TIN	11	2	2	4	3	0.036

Estimated by X^2 test; *Estimated by Fisher's Exact Test.

Table 4. Correlation of HE4 expression with clinical or histologic parameters

	r	*P* Value
Clinical parameters		
Gender	-0.014	0.852
Age	0.002	0.981
High BP	0.135	0.102
Urinary protein/24 h	-0.038	0.590
eGFR	-0.228	0.042
Histological parameters		
Glomerulosclerosis	0.201	0.155
Tubulointerstitial fibrosis	0.383	0.006

with 50 μl of 200 mM bicarbonate buffer (pH 9.5), and the mixture was dispensed into high affinity-binding 96-well ELISA plates in turn, and incubated overnight at 4°C. Then, washed the wells twice with PBS, One-hundred microliters of 1:4,500 dilution of horseradish peroxidase (HRP)-conjugated rabbit IgG antibody (Sigma, A0545) in 1% BSA and PBS was added per well for 30 min at room temperature. Washed twice with PBS and developed using TMB substrate for 15 min. The 1 M phosphoric acid used to stop the reaction, the absorbance values were read at of 450 nm.

Method of follow-up and definition of endpoint

The follow-up period was defined as the period between the date of the kidney biopsy and the endpoint. The renal endpoint was defined as doubling of serum creatinine or end-stage renal disease (ESRD).

Prognostic factors used in the study

Proteinuria: Proteinuria was measured quantitatively in all cases. The result was converted as follows: 1 ≤ 1 g/24 h, 2 = 1-3.5 g/24 h and 3 ≥ 3.5 g/24 h, All of the patients were divided into two groups: < 1 g/24 h and ≥ 1 g/24 h.

Hypertension: We defined hypertension as measured blood pressure (BP) ≥ 140/90 mm Hg.

Estimated GFR (eGFR): eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula. We stratified the eGFR date of patients into two groups: ≥ 60, < 60 ml/min/1.73 m^2.

Age: Patients were stratified according to age < 35 vs. ≥ 35 years.

Image analysis

This image analysis was carried out according to the procedures described previously [12].

Statistical analysis

Statistical analysis was performed as our described previously [12].

Results

Patient characteristics and renal histology

The demographic and clinical characteristics of the research participants at the time of the kidney biopsy are summarized in **Tables 1** and **2**.

Table 5. Univariate analysis of risk factors affecting the renal survival

	Total patients/≥ 100% increase or ESRD	*P* Value
Clinical parameters		
Gender		0.027
Male	92/29	
Female	64/30	
Age		0.690
≥ 35 years	73/33	
< 35	83/26	
Bp		0.000
≥ 140	59/36	
	97/23	
Urinary protein/24 h		0.000
≥ 3.5 g	49/29	
1-3.5 g	67/24	
< 1 g	40/6	
eGFR		0.000
≥ 60 mL/min	109/20	
< 60	49/39	
Histological parameters		
Glomerulosclerosis		0.000
≥ 20%	75/40	
< 20%	81/19	
Tubulointerstitial fibrosis		0.000
≥ 30%	67/41	
< 30%	89/18	
HE4+		0.000
≥ 2+	87/48	
< 2+	69/11	

Table 6. Multivariate analysis of risk factors affecting the renal survival

	Relative ratio	95% CI	*P* Value
Clinical parameters			
Gender (male)	0.626	0.338, 1.159	0.136
Age (≥ 35)	1.251	0.736, 2.126	0.408
High Bp	0.593	0.295, 1.193	0.143
Urinary protein/24 h (≥ 1.0 g)	1.755	1.144, 2.694	0.010
eGFR (< 60 mL/min)	7.092	3.215, 15.625	0.000
Histological parameters			
Glomerulosclerosis (≥ 20%)	1.076	0.309, 3.741	0.909
Tubulointerstitial fibrosis (≥ 30%)	0.786	0.366, 1.687	0.537
HE4 positive	9.759	2.707, 35.181	0.000

There was an over-representation of men (58.97%), and all cases presented with proteinuria (i.e., baseline of 1.2 [0.90-2.15] g/24 h).

Expression of HE4 in renal tissue samples from patients with CKD

The relationship between HE4 expression and clinicopathological features was shown in **Figure 1**, HE4 was predominantly located in the cytoplasm of renal tubular epithelial cells from IgAN and DN patients, Despite the infrequent positive staining for HE4 in the renal tubules of normal kidneys (i.e., HE4 scores of ≤ 1 and absence in glomeruli; **Figure 1A**, **1B**), prominent HE4 staining (> 25% of all cells positive) was found in 87 of 156 patients with CKD (HE4 score of ≥ 2+, $P = 0.009$ compared with normal controls when grouped by scores of ≥ 2+ or < 2+; **Table 3**). When classified by disease, prominent HE4 staining (> 25% of all cells positive) was found in 18 of 33 patients with DN, 38 of 70 fibrotic areas of renal tissues from patients with IgAN, 14 of 27 patients with FSGS, 9 of 15 patients with HN, and 7 of 11 patients with TIN (each *p*-value < 0.05; **Table 3**). These results indicated that HE4 was overexpressed in a wide range of renal diseases.

Correlation of HE4 with clinical or histological parameters

To clarify the potential role and involvement of HE4 in the progression of CKD, we examined the relationships between HE4 staining and various clinical and histological parameters. Among the clinical parameters tested (**Table 4**), eGFR was inversely correlated with HE4 expression in the tubulointerstitium ($r = -0.228$, $P = 0.042$). There were no significant correlations between HE4 expression levels and other clinicopathological factors, including the amount of proteinuria, hypertension, serum creatinine, gender, and age (all $P > 0.05$).

Next, we studied whether there were correlations between HE4 protein expression levels and histological parameters. The immunostain-

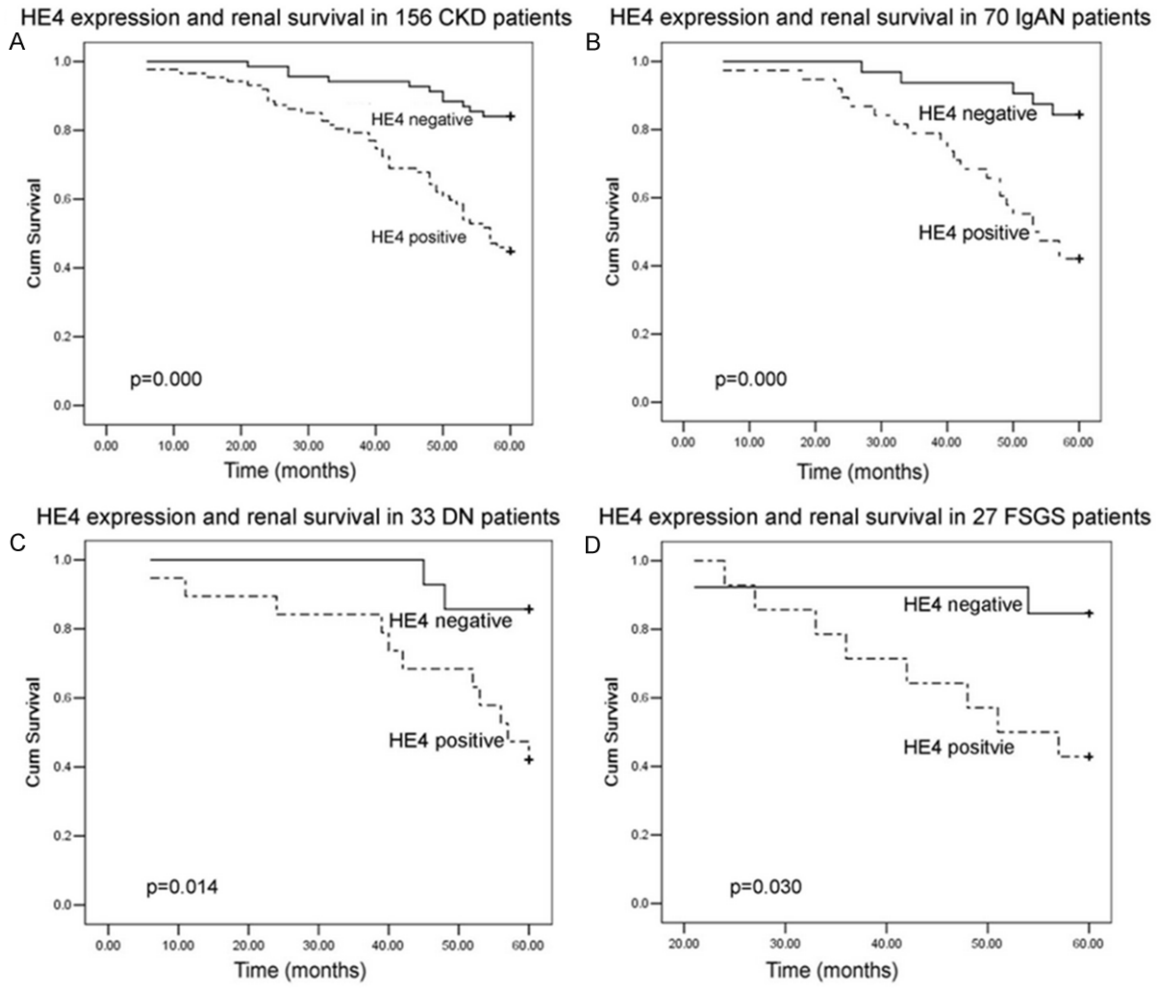

Figure 2. Kaplan-Meier analysis of cumulative rates of renal survival stratified into three groups on the basis of HE4 immunostaining scores. Renal survival estimated on the basis of an increase in serum creatinine to > 100% above baseline levels. Strong HE4 immunostaining were associated with diminished renal survival among those patients with chronic kidney diseases (A), IgA nephropathy (B), Diabetic nephropathy (C) and FSGS (D).

ing score of HE4 in the tubulointerstitium was correlated with the percent of tubulointerstitial fibrosis (r = 0.383, *P* = 0.006; **Table 4**). Linear regression analyses showed that the percentage of HE4-positive cells in the tubulointerstitium was closely correlated with the percentage of tubulointerstitial fibrosis (r = 0.614, *P* < 0.001; **Figure 1C**). These results indicated that the expression of HE4 correlated with the degree of tubulointerstitial fibrosis in CKD patients.

Upregulation of HE4 was correlated with poor outcomes in CKD patients

To determine whether staining for HE4 correlated with the long-term prognosis of patients with CKD, we prospectively analyzed 156 CKD patients. The follow-up period ranged from 5 to 60 months (averaged 36.5 months), during which a total of 59 patients (37.8%) reached the endpoint (i.e., a doubling in baseline serum creatinine). Eighteen patients (11.53%) progressed to end-stage renal disease (ESRD). The significance of each factor affecting renal survival rate is presented in **Table 6**. Among the clinical parameters, female sex, urinary protein excretion (≥ 1.0 g/day), hypertension, high serum creatinine (≥ 1.0 mg/dL), and low eGFR (< 60 mL/min) were significant risk factors for renal function deterioration, whereas age was not (**Table 5**). Using multivariate analyses, urinary protein excretion and eGFR were found to

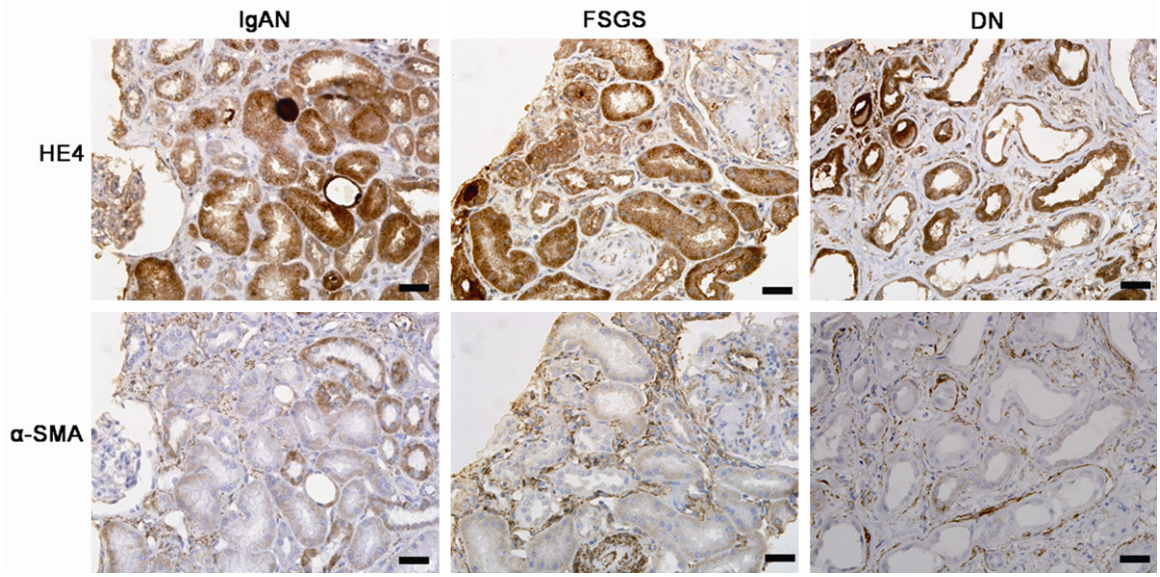

Figure 3. HE4 and α-SMA in renal biopsies from patients with IgAN, FSGS and DN. HE4 and α-SMA were immunohistochemically stained in renal biopsy tissues from patients with IgA nephropathy, focal segmental glomerulosclerosis and diabetic nephrosclerosis (original magnification, 200 ×).

Table 7. Correlation of sHE4 autoantibodies with clinical or histologic parameters

	r	*P* Value
Clinical parameters		
Gender	0.011	0.884
Age	0.019	0.792
High BP	0.019	0.817
Urinary protein/24 h	0.054	0.434
eGFR	-0.226	0.037
Histological parameters		
Glomerulosclerosis	0.092	0.466
Tubulointerstitial fibrosis	0.334	0.003

be significantly associated with renal function deterioration (**Table 6**).

In the histological parameters test, high incidence of tubulointerstitial fibrosis (≥ 30% of total specimen area), tubulointerstitial fibrosis (≥ 20% of total specimen area), and HE4 immunostaining score of ≥ 2 were all risk factors for renal function deterioration (**Table 5**). Besides, HE4 levels were inversely associated with the renal survival (**Table 6**). Kaplan-Meier curves revealed that increased expression of HE4 (relative risk [RR] 9.759; 95% confidence interval [CI], 2.707-35.181; $P = 0.006$) was associated with reduced renal survival (**Figure 2**). The follow-up period was defined as the period between the date of the kidney biopsy and the endpoint. Our findings above demonstrated that upregulation of HE4 was associated with poor histological parameters in CKD patients.

We further examined the prognostic value of HE4 expression in different subgroups of CKD patients stratified according to kidney disease etiology. A significant inverse correlation between HE4 expression and overall survival was found. Individuals with high HE4 expression had significantly shorter overall survival than those with low expression in IgAN (n = 70, $P < 0.001$), FSGS (n = 27, $P = 0.030$), and DN subgroups (n = 33, $P = 0.014$; **Figure 3**). Moreover, IHC staining showed an abundant expression of HE4 and its upstream regulator α-SMA in the tissues from patients with IgAN, FSGS and DN (**Figure 3**). These data indicated that HE4 may be a available prognostic marker for CKD in all disease etiologies.

Serum HE4 levels

Next, we assessed the expression levels of HE4 in serum using HE4 ELISAs to determine the relationship between HE4 expression and clinicopathological features. As shown in **Table 7**, there was an inverse correlation between serum HE4 concentrations and eGFR ($r = -0.226$, $P = 0.037$). However, HE4 levels were

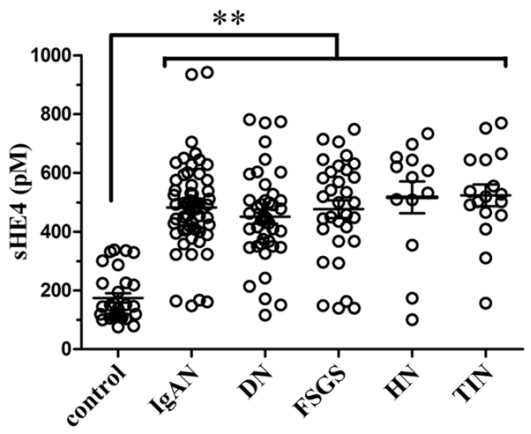

Figure 4. Serum HE4 was upregulated in patients with chronic kidney disease. The scatter plot shows the serum HE4 (sHE4) concentrations from healthy control and patients with chronic kidney disease, which shows sHE4 levels are significantly increased in CKD patients as compared to controls. $^{**}P < 0.05$ vs control determined by two-tailed *t* test.

not correlated with other clinicopathological factors, including proteinuria, hypertension, serum creatinine, gender, and age.

Serum HE4 concentration was significantly higher in CKD patients with biopsy-confirmed fibrosis compared to controls ($P < 0.05$, **Figure 4**; **Table 8**). As shown in **Table 7**, when classified by disease type, HE4 auto-antibodies were detected in CKD patients and healthy individual (each *p*-value < 0.05). These results indicated that HE4 auto-antibodies were induced in a wide range of renal diseases and that the levels of serum HE4 in CKD patients was significantly higher than those in controls.

Finally, we found that serum HE4 concentration was correlated with the percent of tubulointerstitial fibrosis ($r = 0.334$, $P = 0.003$; **Table 8**). Linear regression analyses showed that there was a significant correlation between the concentrations of serum anti-HE4 antibody and the percentage of tubulointerstitial fibrosis ($r = 0.578$, $P < 0.001$; **Figure 5**).

In ROC analyses, the area under the ROC curve AUC values at serum HE4 concentrations was 0.771 (95% CI, 0.693-0.849) (**Figure 6**). When the cut-off value was set to the optimal point, 367.4 pg/ml, the sensitivity and specificity was 91.5% and 72.2%, respectively.

Discussion

HE4 (WFDC2) was originally found to be expressed in epithelial cells of the epididymal duct [3]. Recently, LeBleu et al generated a new transgenic mouse model expressing a red fluorescent protein under the control of the α-SMA promoter and identified HE4 as the most highly upregulated gene by gene expression profiling from cultured myofibroblasts [13, 14]. It was proven that recombinant HE4 could bind to and inhibit a number of known proteases and the neutralization of HE4 alleviated kidney fibrosis in murine disease models, i.e. 5/6 nephrectomy, unilateral ureteral obstruction (UUO), and nephrotoxic serum-induced nephritis [11, 15]. However, in human HE4 expression and localization in fibrotic kidney has not yet been elucidated. In the present investigation, the rate of high HE4 expression was 55.8% in all types of CKD suggested that overexpression of HE4 may represent a common downstream pathway for CKD and renal fibrosis. HE4 expression in the tubules showed a strongest association with tubulointerstitial fibrosis that was a sign of poor renal function.

We also verified that proteinuria, hypertension, and eGFR were univariate risk factors for renal survival. Proteinuria (> 1 g/24 h), hypertension, and eGFR (< 60 mL/min) were also multivariate predictors of renal function deterioration. Similarly, several previous reports also demonstrated that proteinuria, hypertension, kidney disease etiology, and eGFR are risk factors for the progression to ESRD in CKD patients [16, 17]. In the present study, we confirmed that a high incidence of tubulointerstitial fibrosis (≥ 30% of total specimen area) and glomerulosclerosis were univariate predictors of renal function deterioration. HE4 activation in the tubulointerstitium was a univariate predictor of renal function deterioration. Interestingly, our results also showed that strong staining for HE4 in epithelial cells was a multivariate predictor of renal function deterioration. In fact, strong staining for HE4 (≥ 2+) was indicative of a 9.759-fold increase in the risk of progression to renal function deterioration. Furthermore, The Kaplan-Meier curves revealed that high expression of HE4 was correlated with decreased renal function. The ROC show that HE4 can be characterized as a fair prognostic mark. These results support the hypothesis that HE4

Table 8. The levels of HE4 in serum samples

Diagnosis	No. of cases	HE4 levels pM			*P* Value*
		Mean	SD	Medain (range)	
control	29	174.4±15.87	85.46	144.9 (75.90-337.5)	
DN	42	451.5±23.98	155.4	443.7 (116.1-781.7)	0.0014
FSGS	32	476.4±30.17	170.7	499.3 (139.5-748.4)	0.0004
IgAN	59	482.0±19.47	149.5	490.3 (147.8- 941.9)	0.0018
HN	13	516.7±54.05	194.9	585.0 (100.5-733.3)	0.0003
TIN	17	523.8±36.71	151.3	519.2 (156.5-769.8)	0.0079

*p-value < 0.05 compared with control determined by two-tailed t test.

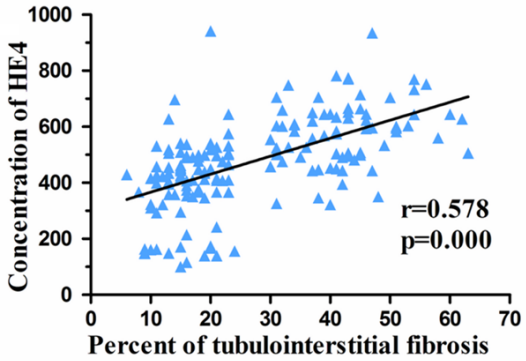

Figure 5. Correlation plots of serum concentration of HE4 autoantibodies and tubulointerstitial fibrosis. Scatter plot with fitted values intervals for concentration of HE4 and percent tubular of tubulointerstitial fibrosis.

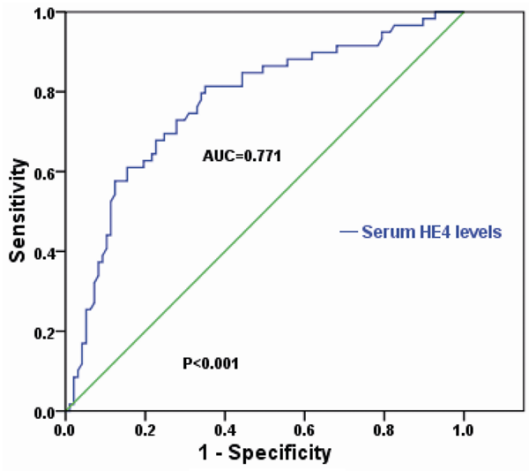

Figure 6. Receiver operator characteristic (ROC) curves comparing the serum concentration and serum creatinine, respectively. The upper curve represents the predictive accuracy with HE4 as explanatory variable where P < 0.001 with c statistic 0.771.

is a sensitive biomarker for renal function deterioration and can be applied, together with histopathological parameters, to evaluate CKD progression.

CKD is a major public health problem due to its high prevalence and association with high mortality, high morbidity, and low quality of life [18-21]. Renal fibrosis, particularly tubulointerstitial fibrosis, is the common consequence of a wide variety of progressive CKD, whatever the initial causes [1, 18, 22]. It is urgent to identified available earlier biomarkers for renal fibrosis, which is expected to be critical to earlier diagnosis, prevention, and therapy of CKD. Recently, several studies have shown that a variety of proteins, including Snail, zinc finger E-box binding homeobox 1 (Zeb1), connective-tissue growth factor (CTGF), and α smooth muscle actin (α-SMA), could be used as markers for renal fibrosis [23-26]. However, it is still a lot to be improved in diagnosis, prognosis and therapeutics for patients with CDK. Here, we presented evidence that overexpression of HE4 in the tubulointerstitium occurs in a wide range of CKD and that high expression of HE4 in the tubulointerstitium is correlated with poor renal histological parameters. Moreover, our data showed that serum HE4 levels in CKD patients were significantly higher than those in healthy controls. Additionally, serum HE4 was correlated with the proportion of tubulointerstitial fibrosis and the tubulointerstitial HE4 expression. These results indicated that HE4 protein may represent a promising biomarker for the progression of renal fibrosis and may facilitate the CKD diagnosis and therapeutic evaluation.

HE4 was originally identified as a target downstream of α-SMA in cultured myofibroblasts from fibrotic renal tissues by LeBleu and colleagues. They confirmed that HE4 was localized and robustly expressed in myofibroblasts in the UUO animal model [11, 27, 28]. Therefore, it is interesting that HE4 was largely localized in the cytoplasm of tubular epithelial cells in fibrotic renal tissues from patients with CKD; this was similar to the sublocalization of HE4 in tumor cells (HE4 was also localized in myofibroblasts in human fibrotic renal tissues, but this result will need to be further confirmed) [29]. However, our results suggested that HE4 overexpression in the tubulointerstitium occurred in a wide

range of chronic kidney diseases, indicated that HE4 expression was not consistent between fibrotic animal models and clinical specimens.

There was an inverse correlation between serum HE4 concentrations and eGFR and a positive correlation between serum HE4 concentrations and renal interstitial fibrosis. We proposed firstly that HE4 may have a role in prognosis of patients with CKD.

This study has several limitations. First, this was a retrospective, single-center study with a small study population. Second, the follow-up time was relatively short to observe renal outcomes. Third, CKD patients with various clinical presentations and pathologic features received different therapies, and outcomes of the therapy might affect renal outcomes. Finally, we detected the expression of HE4 in the serum, but we did not analyze the relationship between serum HE4 expression and renal outcomes due to the lack of patient information.

In conclusion, our present experimental data showed that serum HE4 could be used as a new marker of renal fibrosis because of the positive correlation between serum HE4 concentration and the degree of renal tubulointerstitial fibrosis, which suggested that HE4 may be secreted by the renal tubulointerstitial tissue during early fibrotic stages. This study provides a basis for the early diagnosis of renal fibrosis using serum HE4 concentrations and supports further studies investigating the use of HE4 as a target for treatment.

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Disclosure of conflict of interest

None.

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