The clinical relevance of plasma CD147/Basigin in biopsy-proven kidney diseases

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Abstract

Background. Precise understanding of kidney disease activity is needed to design therapeutic strategies. CD147/basigin is involved in the pathogenesis of acute kidney injury and renal fibrosis through inflammatory cell infiltration. The present study examined the clinical relevance of CD147 in biopsy-proven kidney diseases that lead to the progression of chronic kidney disease.

Methods. Kidney biopsy specimens and plasma and urine samples were obtained from patients with kidney diseases, including IgA nephropathy (IgAN), Henoch-Schönlein purpura nephritis (HSPN), diabetic kidney disease (DKD), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy (MN), who underwent renal biopsy between 2011 and 2014. Plasma and urinary CD147 levels were measured and evaluated for their ability to reflect histological features. Disease activity of IgAN tissues was evaluated according to the Oxford classification and the Japanese histological grading system.

Results. In biopsy tissues, CD147 induction was detected in injured lesions representing renal inflammation. Plasma CD147 values correlated with eGFR in patients with inflammation-related kidney diseases such as IgAN, HSPN, and DKD. Particularly in IgAN patients, plasma CD147 levels were correlated with injured regions comprising more than 50% of glomeruli or with tubular atrophy/interstitial injury in biopsy tissues. Proteinuria showed a closer correlation with urinary values of CD147 and L-FABP. Of note, plasma and urinary CD147 levels showed a strong correlation with eGFR or proteinuria, respectively, only in DKD patients.

Conclusion. Evaluation of plasma and urinary CD147 levels might provide key insights for the understanding of the activity of various kidney diseases.

Introduction

Regardless of the primary process, persistent kidney injury provokes irreversible pathological changes such as glomerular obsolescence and interstitial fibrosis, eventually leading to the development of chronic kidney disease (CKD) with an occasionally sharp decline in kidney function [1, 2]. In this setting, progressive fibrosis is essentially due to the disruption of renal architecture in tissue remodeling processes that are accompanied by the marked accumulation of extracellular matrix proteins [3]. Therefore, appropriate intervention for at-risk patients would be extremely helpful to prevent CKD progression. Excessive filtration of lipid peroxide, glucose, and protein in the glomerulus causes tubular injuries and recruitment of inflammatory cells into the interstitium in a time-dependent manner [4]. There is often a vicious cycle of tubulointerstitial injury that spreads to the surrounding kidney tissues. It has been shown previously that reduction in albuminuria/proteinuria is closely correlated with the suppression of renal dysfunction [5, 6]. Accurate understanding of the process of kidney tissue damage and dysfunction should lead to the design of the ideal approach to develop appropriate therapeutics.

The glycosylated transmembrane protein CD147/basigin, also known as extracellular matrix metalloproteinase (MMP) inducer (EMMPRIN), contributes to cell survival, migration, proliferation, and apoptosis [7]. It is widely expressed in various types of cells, including hematopoietic, epithelial, and endothelial cells. In the normal kidney, CD147 is distributed in tubular epithelial cells (TECs), but to a lesser extent in glomerular components [8, 9]. In contrast, CD147 is strongly induced in injured glomeruli in patients with acute kidney injury (AKI), but not in injured tubules that represent atrophy. In a series of our prior studies, we showed that CD147 reflects renal

function in patients with AKI, as well as histological features representing acute inflammation in patients with lupus nephritis (LN) [9]. In addition, our basic research demonstrated that CD147 orchestrates renal fibrosis through the regulation of MMPs and macrophage infiltration with the responsiveness of transforming growth factor (TGF)-ß [10, 11]. Aberrant molecular mechanisms involving CD147-mediated tissue remodeling may be a crucial determinant of persistent kidney injury.

These findings enabled us to address the fundamental question of how persistent kidney injury that is caused by a vicious cycle between inflammation and fibrosis is regulated by CD147. To the best of our knowledge, the clinical relevance of CD147 in various kidney diseases that lead to CKD progression has not yet been elucidated. The present study examined whether CD147 can reflect the histological features and disease activity of persistent kidney injury that have the potential to induce CKD, compared to novel parameters such as the estimated glomerular filtration rate (eGFR) and L-fatty acid-binding protein (L-FABP).

Subjects and Methods

Patients and Procedures

In a cross-sectional study, plasma and spot urine samples were collected from 538 participants (male, n = 262; female, n = 276) with various kidney diseases who were diagnosed pathologically at Nagoya University Hospital and affiliated hospitals between January 2011 and August 2014 during renal biopsy for the differential diagnosis of various kidney diseases. Renal biopsies in Nagoya University hospital and affiliated hospitals were performed for essential cases, based on the relevant guidelines established by the Japanese Society of Nephrology. All were performed before medical

treatment such as anti-inflammatory and immunosuppressive reagents was started. In the same period, 6 patients with only microhematuria or a subtle amount of proteinuria were regarded as pathological controls. These manifestations have not been identified as kidney diseases from clinical and pathological aspects. Diagnosis was made pathologically using light, immunofluorescence, and electron microscopy. All diagnoses were performed in a blinded manner by five independent expert nephropathologists. Exclusion criteria included cases with malignant disorders, severe infectious diseases, drug-toxicity such as chemotherapeutic and antibiotic reagents, associated with the other kidney diseases or under 18 years of age. To further examine the application of CD147 values, 18 healthy individuals were enrolled as controls. Plasma and spot urine samples were collected before the biopsies. Plasma and spot urinary samples were centrifuged at 2000×*g* for 5 min to remove cellular components and debris, and then equal volumes of supernatants were stored at -80°C. Clinical parameters such as levels of eGFR, C-reactive protein (CRP), and albuminuria were measured, as described previously [12, 13].

Renal Histology

Kidney tissues were fixed in 10% formalin, embedded in paraffin, and then cut into 1-μm-thick sections for periodic acid-Schiff reagent (PAS) and Masson trichrome (MT) staining and 4-μm-thick sections for immunohistochemical staining. Sections were stained with mouse monoclonal anti-human CD147 antibody (Abcam, Cambridge, MA), followed by a second antibody of the Histofine Simple Stain Max PO kit (Nichirei, Tokyo, Japan) [9]. The staining was visualized with 3,3'-diaminobenzidine (Nichirei), producing a brown color.

Biomarker Measurements

All samples were tested using a dipstick before centrifugation and examination by microscopy. Plasma and urinary CD147 and urinary L-FABP values were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the respective instructions from the manufacturers (R&D Systems, Minneapolis, MN; Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Measured levels in urine were then normalized to urinary creatinine levels.

Morphological Assessment

The histologic findings of IgA nephropathy (IgAN) were classified according to the Oxford classification [14, 15], which scored four pathologic features in renal biopsy species. In brief, mesangial hypercellularity was scored according to whether more than 50% of the glomeruli had fewer (M0) or more (M1) than three cells per mesangial area. Segmental glomerulosclerosis was scored as absent (S0) or present (S1). Endocapillary hypercellularity was scored as absent (E0) or present (E1). The tubular atrophy/interstitial fibrosis score was based on the ratio of tubular atrophy to interstitial fibrosis in the total tubulointerstitium and was scored as: T0, 0–25%; T1, 26–50%; or T2, more than 51%. The Japanese histological grading of IgAN was used to further evaluate histological features reflective of an active lesion such as a cellular crescent, and of a chronic lesion including global and segmental glomerular sclerosis [16]. The observations of IgAN severity were categorized based on whether the injured regions comprised less than 25% of glomeruli (Grade I); 25-50% of glomeruli (Grade II); 50-75% of glomeruli (Grade III); or more than 75% of glomeruli (Grade IV). Patients

with isolated hematuria, Henoch-Schönlein purpura nephritis (HSPN), or co-existing conditions such as diabetes mellitus were excluded. All quantifications were performed in a blinded manner by five independent expert nephropathologists.

Statistical Analysis

Continuous variables are presented as means ± standard deviation (SD), and categorical variables are presented as numbers and ratios (%). Continuous variables were compared using Student's *t*-test, the non-parametric Mann-Whitney *U*-test, or the Kruskal-Wallis test followed by Steel-Dwass post hoc multiple comparisons, as appropriate. Spearman correlation coefficients were used to examine the strength of association between two variables. Univariate and multivariate logistic regression analyses were performed to assess the independent predictive abilities of biomarkers. The predictive ability of combinations of biomarkers was also examined using multivariate logistic regression models. Receiver-operating characteristic (ROC) curves were constructed to assess the diagnostic accuracy of each biomarker, and diagnostic performance was quantified as the area under the curve (AUC). Two-tailed P-values < 0.05 were considered significant. Statistical analysis was performed using SPSS and STATA version 9, commercial software.

Results

Clinicopathological characteristics of the patients in the cross-sectional study

Table 1 summarizes the clinical characteristics of the 538 participants enrolled in the cross-sectional study. All participants were Japanese, with a mean age of 48.8 ± 17.3 years, and 51.3% were females. The number of patients with kidney diseases stratified

by disease categories was as follows: IgAN, 289 patients; HSPN, 33; diabetic kidney disease (DKD), 28; focal segmental glomerulosclerosis (FSGS), 60; and membranous nephropathy (MN), 128 (Table 1). Patients with DKD showed more renal dysfunction in terms of parameters such as proteinuria and eGFR levels than patients with other kidney diseases. At the time of renal biopsy, no patients with nephrotic syndrome were treated with glucocorticoids and/or immunosuppressive therapies. In about 80% of IgAN patients, the histological features indicated injured regions comprising less than 50% of glomeruli (Grade I or II) according to the Japanese histological grading of IgAN (Table 2). The pathological control group had a mean age of 32.4 ± 16.6 years and was 60.6% female. No obvious abnormalities were found in healthy individuals (data not shown).

CD147 expressions in various kidney diseases

In the biopsy tissues of the patients with the kidney diseases, including IgAN, HSPN, DKD, FSGS, and MN, CD147 expression was markedly induced in injured glomeruli, including in tuft adhesions to Bowman's capsule and in cellular crescents, as well as in infiltrating cells such as inflammatory cells and fibroblasts in injured interstitial areas (Figure 1). Marked CD147 expression was not found in segmental sclerosis, mesangial areas, and glomerular basement membranes in injured glomeruli. Compared to the CD147 expression that was observed in normal kidneys, CD147 expression decreased greatly in the tubules as renal tubular damage became more severe in kidney diseases. These CD147 expression profiles were similar in various kidney diseases regardless of the primary cause of the kidney disease.

Plasma and urinary CD147 levels in the kidney diseases

No significant differences in plasma or urinary CD147 values were apparent between control patients (plasma, 3067 ± 622 pg/ml; urine, 10.5 ± 3.92 μg/gCr) and healthy individuals (plasma, 3034 ± 859 pg/ml; urine, 13.39 ± 6.00 μg/gCr). The values of both plasma and urinary CD147 levels in IgAN patients were widely distributed, and they showed no significant differences from those of control patients (Supplemental figure). Patients with DKD or MN showed higher levels of plasma and urinary CD147. The profiles of both values in FSGS patients were opposite.

To verify the clinical characteristics of CD147 in each kidney disease, the relationships with clinical indicators, including eGFR, proteinuria, and L-FABP, were examined next. In general, urinary values of L-FABP secreted from injured TECs are known to be a reliable diagnostic tool in the early stage of tubulointerstitial injury [17]. Plasma CD147 levels of patients with IgAN, HSPN, and DKD correlated strongly with eGFR, whereas those of patients with FSGS or MN did not (Figure 2A). In each kidney disease, urinary values of CD147 showed a significant association with proteinuria (Figure 2B). The correlations between urinary L-FABP levels and proteinuria were remarkable. Of note, plasma or urinary values of CD147 were associated with urinary L-FABP and markers of renal injury in DKD patients. In participants with DKD, FSGS, or MN, a marked association was evident between plasma and urinary CD147 values (Figure 2C). Urinary levels of CD147 were closely correlated with those of L-FABP, but plasma CD147 levels were not. The correlations of urinary L-FABP with eGFR or proteinuria were similar to those of urinary CD147. Plasma CD147 was not correlated with fractional excretion of CD147 (FECD147), whereas urinary CD147 showed a strong association with FECD147 (data not shown).

Assessment of the ability of CD147 to reflect pathological features in patients with IgAN

Since IgAN is one of the most causative primary diseases of CKD progression, whether CD147 reflects various histopathological features such as glomerular damage, tubular atrophy, and interstitial fibrosis was evaluated. The Oxford classification of IgAN has been confirmed as an international evaluation method for IgAN, both in the original cohort and in a number of validation studies [18-20]. As described earlier, the values of both plasma and urinary CD147 were relatively widely distributed in IgAN (Supplemental figure). No significant differences in plasma or urinary levels of CD147 were found between different grades of mesangial hypercellularity (M) (Figure 3A) or between different grades in the development of segmental glomerulosclerosis (S) (Figure 3C) Whereas no differences in plasma levels of CD147 were found between different grades of endocapillary hypercellularity (E), urinary CD147 levels were significantly higher in E1 than in E0 (Figure 3B). Both plasma and urinary CD147 values increased significantly with each step of injury as the injured categories of tubular atrophy/interstitial fibrosis (T) increased from T0 to T2 (Figure 3D).

Since CD147 has been suggested to be a potential driver of renal inflammation, further evaluation of histological features reflective of an active glomerular lesion such as a cellular crescent and of a chronic lesion including segmental sclerosis was performed using the Japanese histological grading of IgAN [16]. Plasma CD147 values showed a significant increase in Grade III/IV, which are categorized as injured regions comprising more than 50% of glomeruli, compared to the lower grades (Figure 4A). Although urinary CD147 levels were significantly higher in grade III versus Grade I, no significant difference was observed between the levels in Grade II and IV and the level in Grade I (Figure 4B). Of note, plasma CD147 levels (4776 ± 1308 pg/ml) in Grade

III/IV category IgAN patients were at a similar level to those in DKD (6122 ± 2443 pg/ml) and in MN (5005 ± 1987 pg/ml) patients (Figures 4A, Supplemental figure).

The ability of urinary concentrations of CD147 or L-FABP or plasma CD147 levels to function as a novel indicator of the pathological features of IgAN patients according to the Oxford classification (Table 3) or to the Japanese histological grading of IgAN was determined next (Table 4). The candidate biomarkers were assessed by multivariate logistic regression analysis. Such prediction using a single molecule might be very difficult since persistent kidney diseases such as IgAN are caused by multiple complex pathogeneses. In this study, plasma CD147 levels showed AUC scores similar to urinary L-FABP and superior to eGFR with regards to reflection of tubular atrophy/interstitial fibrosis in the Oxford classification and glomerular injuries in the Japanese histological grading of IgAN.

Discussion

The present study examined the clinical relevance of CD147 for various kidney diseases that lead to the progression of CKD. Plasma CD147 levels in patients with inflammation-related kidney diseases such as IgAN and HSPN showed a strong correlation with eGFR, whereas they did not show a close association with urinary CD147 values. Other investigators have reported enhanced expression of cell surface CD147 on these cells relative to inactivated leukocytes [21]. In accordance with previous reports and our basic research, circulating bioactive CD147 derived from activated leukocytes may be detected as plasma CD147. Particularly in IgAN patients, induction of CD147 in serum might occur in the active phase of glomerular injuries before the appearance of segmental sclerosis and fibrous crescent formation. T scores in

the Oxford classification independently predict the development of end-stage renal diseases [18, 22]. Since plasma and urinary CD147 levels increased with each step of the T score, they may become promising predictors of the progression of CKD.

In contrast, no close correlation between plasma CD147 levels and eGFR was observed in patients with FSGS and MN. A significant correlation existed between plasma and urinary CD147 levels in kidney diseases with moderate and severe proteinuria, including FSGS and MN. Proteinuria, an independent prognostic factor for CKD mortality and morbidity, showed a closer correlation with urinary levels of CD147 and L-FABP than plasma CD147 values. Urinary CD147 may be partly filtered from circulating blood by failure of the glomerular filtration barrier. The fractional excretion of CD147 showed a strong correlation with urinary CD147, but not with plasma CD147 (data not shown). Most urinary CD147 might be derived from injured TECs. These findings led us to speculate that tubular reabsorption of excess protein filtered through the glomeruli might cause urinary CD147 induction before the appearance of pathological features. No obvious differences in urinary CD147 values of FSGS patients were found, perhaps because their degrees of proteinuria did not reach nephrotic status in the current study. Despite the fact that there were no or few pathological changes, of note, plasma and urinary CD147 levels in patients with minimal change nephrotic syndrome and MN were twice those of pathological control patients and healthy individuals (Supplemental figure B, data not shown). Unlike patients with IgA, AKI, and LN, plasma CD147 values might not provide an accurate understanding of the pathological features in these diseases. Increased levels of CD147 were present in pre-eclamptic woman with proteinuria [23]. The increases of plasma CD147 may be due to the breakdown of crosstalk between podocytes and endothelial dysfunction. The

kinetics of CD147 would not be explained by a single mechanism, and further basic research is needed to evaluate the mechanisms involving CD147 in the respective kidney diseases.

Particularly in DKD patients, plasma and urinary CD147 values showed a strong correlation with eGFR and proteinuria, respectively. Diverse factors such as inflammation and proteinuria may be involved in the progression of DKD. Patients who were undergoing appropriate treatment for DKD showed a marked reduction in plasma CD147 levels (data not shown). Even if some effective treatments have already been given, especially in DKD patients, marked CD147 inductions were found in active kidney diseases requiring renal biopsies. Although such mechanisms need to be examined from a variety of perspectives, the kinetics of CD147 may become an indicator of therapeutic effect.

Identification of biomarkers that accurately reflect the disease activity of CKD is of increasing importance. The molecular mechanism underlying CD147 activities involves a variety of cytokines and growth factors, including MCP-1, TGF-ß, E-selectin, interleukin-17, and MMPs. The expression profile of CD147 would be dependent on the pathogenesis of the respective kidney diseases. Evaluation of plasma and urinary CD147 levels might provide key insights for the understanding of the disease activity of various kidney diseases that have the potential to induce CKD, and it might allow initiation of appropriate medication.

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Statements

The authors declare no conflicts of interests. This study was conducted according to the principles of the Declaration of Helsinki, the Japanese National Ethical Guidelines, and the institutional review boards of Nagoya University Hospital and affiliated hospitals (approval number, 1135). All patients provided written, informed consent to participate in the study.

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Table 1. Clinicopathological characteristics of the study patients

	IgAN n=289	HSPN n=33	MN n=128	FSGS n=60	DKD n=28	Pathological control n=6
Sex (%) female	59.5	54.0	39.7	46.9	25.0	66.6
Age (years)	40.1±14.1 (18-79)	51.4 ±16.72 (18-77)	65.9 ±10.4 (35-89)	52.8 ±16.3 (19-89)	58.5 ±12.3 (29-78)	32.4 ±16.6 (19-63)
BMI	22.6 ± 3.64 (15.5-36.1)	23.3 ± 4.2 (14.3-33.5)	23.5 ± 3.4 (16.5-36.0)	23.9 ± 4.8 (15.8-42.6	25.3 ± 3.1 (20.1-32.8)	19.1 ± 2.0 (16.7-22.2)
Mean BP (mm Hg)	93.4 ± 12.5 (66.3-136.6)	94.9 ± 10.9 (72.3-117.3)	98.5 ± 12.6 (67.3-132.3)	97.6 ± 12.4 (75.6-131.0)	104.9 ± 15.6 (80.6-150.0)	83.3 ± 13.2 (63.3-100.0)
eGFR (ml/min)	73.4 ± 22.1 (129.2-21.6)	78.5 ± 27.2 (140.2-25.1)	47.6 ± 16.7 (19.0-81.5)	72.1 ± 20.0 (25.1-130.7)	70.7 ± 17.9 (28.6-133.1)	86.4 ± 21.3 (58.3-113.6)
Proteinuria (g/gCr)	0.91± 0.92 (0-4.7)	2.34± 2.22 (0.23-9.65)	2.77 ± 3.97 (0-15.44)	2.68 ± 2.58 (0-9.09)	4.55 ± 3.79 (0.05-15.7)	0 ± 0.054 (0.02-0.03)
Hb (g/dl)	13.3 ± 1.7 (7.3-18.0)	12.9± 2.0 (8.4-17.9)	13.5 ± 1.88 (8.2-17.7)	13.5 ± 2.0 (8.2-18.4)	12.1 ± 2.2 (7.1-18.3)	13.6 ± 1.7 (11.4-16.9)
HbA1c (%)	5.2 ± 0.3 (4.4-7.9)	5.4 ± 0.6 (4.2-7.1)	5.5 ± 0.5 (4.4-7.8)	5.4 ± 0.5 (4.6-7.7)	6.6 ± 1.0 (4.4-8.8)	5.1 ± 0.3 (4.7-5.7)
CRP (mg/dl)	0.2 ± 0.7 (0.0-7.5)	1.2 ± 2.2 (0.0-9.2)	0.3 ± 0.8 (0.0-6.8)	0.1 ± 0.2 (0.0-1.5)	0.2 ± 0.3 (0.0-1.2)	0.06 ± 0.08 (0.0-0.18)

Data are expressed as means ± SD (range).

IgAN, IgA nephropathy

HSPN, Henoch-Schönlein purpura nephritis

MN, membranous glomerulonephritis

FSGS, focal segmental glomerulosclerosis

DKD, diabetic kidney disease

BMI, body mass index

BP, blood pressure

eGFR, Estimated glomerular filtrating ratio

Cr, creatinine

Hb, hemoglobin

CRP, C-reactive protein.

Table 2. Histological features of patients with IgAN

		n (%of N)
Japanese histological grading of IgAN	Grade I	146 (50.5%)
	Grade II	94 (32.5%)
	Grade III	42 (14.5%)
	Grade IV	7 (2.4%)
Oxford classification of IgAN	M0	187 (64.7%)
	M1	102 (35.2%)
	E0	194 (67.1%)
	E1	95 (32.8%)
	S0	294 (70.1%)
	S1	85 (29.4%)
	T0	204 (70.5%)
	T1	55 (19.0%)
	T2	15 (5.1%)

IgAN, IgA nephropathy

M, score of mesangial hypercellularity

E, score of endocapillary hypercellularity

S, score of segmental glomerulosclerosis

T, score of tubular atrophy/interstitial fibrosis

Table 3. Comparison of predictive biomarker candidates in patients with IgA nephropathy according to the Oxford classification

	predictor variables	**model outcome variable**				
		M1	E1	S1	T1+T2	T2
AUC (95% CI)	pCD147	0.52 (0.45-0.59)	0.50 (0.43-0.57)	0.56 (0.49-0.63)	0.74 (0.67-0.80)	0.76 (0.66-0.86)
	U-CD147	0.50 (0.43-0.57)	0.61 (0.54-0.68)	0.50 (0.42-0.57)	0.65 (0.57-0.73)	0.73 (0.64-0.82)
	U-Alb	0.57 (0.50-0.64)	0.57 (0.50-0.64)	0.69 (0.62-0.75)	0.64 (0.57-0.72)	0.79 (0.67-0.91)
	L-FABP	0.59 (0.52-0.65)	0.57 (0.50-0.64)	0.61 (0.55-0.68)	0.60 (0.52-0.66)	0.73 (0.60-0.87)
	eGFR	0.51 (0.45-0.57)	0.52 (0.45-0.58)	0.52 (0.45-0.58)	0.55 (0.48-0.62)	0.61 (0.49-0.73)

AUC, the area under the receiver operating characteristic (ROC) curve; 95% CI, 95% confidence interval

pCD147, plasma CD147

u-CD147, urinary CD147

U-Alb, urinary albumin

L-FABP, urinary L-fatty acid-binding protein

eGFR, Estimated glomerular filtrating ratio

M, score of mesangial hypercellularity

E, score of endocapillary hypercellularity

S, score of segmental glomerulosclerosis

T, score of tubular atrophy/interstitial fibrosis

Table 4. Comparison of predictive biomarker candidates in patients with IgA nephropathy according to the Japanese histological grading system

	predictor variables	**model outcome variable**		
		Grade II, III, IV	Grade III, IV	Grade IV
AUC (95% CI)	pCD147	0.68 (0.62-0.74)	0.73 (0.65-0.80)	0.75 (0.64-0.86)
	U-CD147	0.58 (0.52-0.65)	0.62 (0.53-0.71)	0.52 (0.27-0.77)
	U-Alb	0.67 (0.61-0.74)	0.68 (0.60-0.76)	0.71 (0.48-0.94)
	L-FABP	0.57 (0.51-0.63)	0.62 (0.54-0.71)	0.66 (0.40-0.92)
	eGFR	0.51 (0.45-0.58)	0.52 (0.44-0.60)	0.62 (0.45-0.79)

AUC, the area under the receiver operating characteristic (ROC) curve; 95% CI, 95% confidence interval

pCD147, plasma CD147

u-CD147, urinary CD147

U-Alb, urinary albumin

L-FABP, urinary L-fatty acid-binding protein

eGFR, Estimated glomerular filtrating ratio

Figure legends

Figure 1. Renal histology in the patients with various kidney diseases

A: Representative Periodic acid-Schiff reagent (PAS) staining (upper panel) and immunohistochemical staining of CD147 expression (lower panel) in the glomeruli of patients with the indicated kidney disease and in the pathological control. Bar, 100 μm. Black arrowheads, cellular crescent; white arrowheads, nodular lesion. **B:** Representative photos of Masson trichrome (MT) staining (upper panel) and immunohistochemical staining of CD147 expression (lower panel) in the tubulointerstitium of patients with the indicated kidney disease and in the pathological control. Bar, 100 μm. **C:** High magnification of the immunohistochemical staining for CD147 expression in the indicated patients. Injured tubules show a marked reduction in CD147 expression compared to non-injured tubules. Migration of various cells is seen around atrophic damaged tubules. Black arrowheads, infiltrating cells. Bar, 100 μm.

Figure 2. Correlations between the levels of various parameters in biopsy-proven kidney diseases

A: Correlations between estimated glomerular filtration rate (eGFR) levels and plasma CD147, urinary CD147, and urinary L-fatty acid-binding protein (L-FABP) levels. **B:** Correlations between proteinuria and plasma CD147, urinary CD147, and urinary L-FABP levels. **C:** Correlations between urinary CD147 levels and plasma CD147 and urinary L-FABP levels.

Figure 3. Comparison of plasma and urinary CD147 values in patients with IgAN classified according to the Oxford classification

Box and whisker plots of plasma and urinary CD147 values in IgAN patients with different grades of **A:** Mesangial hypercellularity (M), **B:** Endocapillary hypercellularity (M), **C:** Segmental glomerulosclerosis (S), or **D:** Tubular atrophy/interstitial fibrosis (T). Data are expressed as means ± SD. *, $p < 0.05$; **, $p < 0.01$.

Figure 4. Plasma and urinary CD147 levels in IgAN patients graded according to the Japanese histological grading system.

Box and whisker plots of **A:** Plasma CD147 levels and **B:** Urinary CD147 levels in patients with the indicated grade of IgAN. Data are expressed as means ± SD. *, $p < 0.05$; **, $p < 0.01$.

Supplemental figure legends

Supplemental figure. Plasma and urinary values of CD147 in the patients with the kidney diseases.

Box and whisker plots of **A:** Plasma CD147 levels (pg/mL) and **B:** Urinary CD147 values (μg/gCr) in the indicated patients and controls. Data are expressed as means ± standard deviations (SD). *, $p < 0.05$; **, $p < 0.01$ versus controls.