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Original Article

ORIGINAL ARTICLE

Tubular decoy receptor 2 as a predictor of prognosis in patients with immunoglobulin A nephropathy

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ABSTRACT

Background. Accelerated senescence of renal tubular epithelial cells (RTECs) might contribute to immunoglobulin A nephropathy (IgAN) progression. This study aimed to determine whether the RTEC senescence marker, decoy receptor 2 (DcR2), could predict prognosis in IgAN.

Methods. We included a retrospective cohort of 105 patients with biopsy-proven IgAN. Tubular DcR2 expression was assessed at renal biopsy and the Oxford histological MEST-C score [mesangial hypercellularity (M), endocapillary proliferation (E), segmental sclerosis (S), interstitial fibrosis/tubular atrophy (T) and crescents (C)] defined disease severity. IgAN progression was defined as a composite of end-stage renal disease or a 30% decline in the estimated glomerular filtration rate (eGFR), analyzed using Kaplan–Meier and Cox regression analyses.

Results. Tubular DcR2 was overexpressed in IgAN. Numbers of DcR2 and p16 double-positive RTECs increased with increasing severity of tubular atrophy/interstitial fibrosis (T lesion). Patients with ≥25% tubular DcR2 expression experienced worse proteinuria, T lesions and a lower eGFR. Cumulative renal survival was significantly lower in patients with ≥25% DcR2 positivity. Multivariate regression analyses showed that ≥25% tubular DcR2 expression was significantly associated with worse eGFR slopes (the rate of renal function decline; $P = 0.003$) and the incidence of the composite outcome ($P = 0.001$) in IgAN. The addition of tubular DcR2 to a model with clinical data at biopsy (mean arterial pressure, proteinuria and eGFR) or MEST-C score significantly improved the 5-year risk prediction of IgAN progression, as confirmed by receiver operating characteristic curve analyses.

Conclusions. Tubular DcR2 expression detected at biopsy was a strong independent predictor for IgAN progression and might have prognostic value in addition to established risk markers.

Keywords: DcR2, IgA nephropathy, predictor, renal outcome, senescence

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis and a highly heterogeneous disease with a variable risk (<10% and 60%) of disease progression to end-stage renal disease (ESRD) after 10 years [1]. Therefore, it is challenging to accurately identify patients at high risk of disease progression.

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Cellular senescence is an irreversible fate of damaged cells, featuring growth arrest, DNA damage and enhanced expression of senescence-related proteins such as p16 and p21. Emerging evidence suggests that accumulation of chronic senescent cells could promote pathologies and shorten healthy lifespan [2]. Senescent cells not only lose their growth and repair abilities, but also secrete senescence-associated secretory phenotype (SASP) components, including proinflammatory cytokines and growth factors, which attract inflammatory cells, affect neighboring cells, leading to inflammation, fibrosis and organ dysfunction [3]. Chronic kidney disease (CKD) has been considered as a clinical model of premature aging or accelerated senescence [4, 5]. Our previous results indicated that renal tubular epithelial cells (RTECs) in patients with IgAN exhibit upregulated expression of senescence-associated markers, such as senescence-associated beta-galactosidase (SA-β-gal) and p16, which increased gradually in patients with Grades I–V (Lee's grading system) IgAN [6]. Moreover, increased levels of senescence-associated markers were closely associated with tubulointerstitial injury and renal dysfunction severity, suggesting that accelerated RTECs senescence might contribute to IgAN progression [6].

Decoy receptor 2 (DcR2), a DcR of tumor necrosis factor-related apoptosis-inducing ligand, is a cellular senescence marker found in senescent tumor cells and fibroblasts [7–10]. After sorting DcR2-positive cells from various senescent models using magnetic-activated cell sorting, we observed significantly higher expression of senescence markers and SASP in DcR2-positive RTECs than in the pre-sorted model cells [11]. Our previous study demonstrated that DcR2 is primarily expressed in renal proximal tubules, and tubular DcR2 expression was significantly higher in patients with diabetic nephropathy compared with that in healthy controls and is co-expressed with senescent markers (p16, p21 and SA-β-gal) and fibrotic markers (smooth muscle actin alpha, Collagen I and Collagen IV) [12]. Moreover, the levels of urinary DcR2 could identify the severity of tubulointerstitial injury and were negatively associated with the estimated glomerular filtration rate (eGFR) in patients with IgAN [13]. However, whether DcR2 in this context is simply associated with tissue damage or contributes to disease progression remains to be determined. Moreover, whether increased DcR2 expression at biopsy predicts progression and functional decline better than existing markers remains untested.

This study aimed to investigate whether the tubular DcR2 expression at biopsy is associated with disease progression and predicts renal outcome in IgAN. Furthermore, we studied whether DcR2 adds prognostic value over established IgAN risk markers including mean arterial pressure (MAP), proteinuria, eGFR and the Oxford classification [MEST-C; mesangial hypercellularity (M), endocapillary proliferation (E), segmental sclerosis (S), interstitial fibrosis/tubular atrophy (T) and crescents (C)].

MATERIALS AND METHODS

Design

This was a retrospective cohort study of 105 patients with biopsy-proven IgAN followed in Daping Hospital of the Army Medical University.

Study population

Renal pathology archives from 751 patients diagnosed with IgAN between October 2006 and December 2012 were reviewed. Figure 1 shows a flowchart for patient recruitment. Ultimately, 105 patients were recruited. In addition, 15 normal renal tissue specimens displaying insignificant alterations biopsied from patients with renal carcinoma were used as controls. The research was approved by the Ethical and Protocol Review Committee of the Army Medical University. Informed consent was obtained from all enrolled individuals.

Clinical data set

Clinical and laboratory data at biopsy (within $\pm$5 days) were defined as the baseline including: sex, age at biopsy (years), systolic and diastolic blood pressure, hemoglobin (g/L), serum albumin (g/L), serum uric acid (μmol/L), serum creatinine (μmol/L), proteinuria (g/day) and eGFR (mL/min/1.73 m^2). Data collected concerning follow-up included duration of follow-up, serum creatinine, eGFR, rate of renal function decline (mL/min/1.73 m^2/year) and treatment modalities. Recorded treatment modalities included immunosuppressive agents and a number of antihypertensive medications, including renin–angiotensin system (RAS) inhibitors.

Pathological data set

IgAN was confirmed as the predominant or codominant immunoglobulin in the mesangial deposits. All renal biopsy specimens were re-assessed independently by two experienced pathologists who were blinded to patients' outcomes. Renal histologic lesions were graded according to MEST-C score [14].

Immunohistochemistry and immunofluorescence double staining

Assay was performed as previously described [12].

Definitions

eGFR was estimated using the CKD Epidemiology Collaboration equation [15]. ESRD was defined as initiation of dialysis, kidney transplantation or reaching an eGFR $<$15 mL/min/1.73 m^2. A composite renal outcome included 30% eGFR decline or ESRD, whichever occurred first. Hypertension was defined as a systolic blood pressure $\geq$140 mmHg and/or a diastolic pressure $\geq$90 mmHg or taking antihypertensive medications to prevent hypertension. MAP was defined as diastolic pressure plus one-third of pulse pressure. Immunosuppressive treatment is reported as intent to treat regardless of the type or duration of therapy. 'RAS inhibitors' indicates any exposure to either angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, or both.

Statistical analyses

Quantitative variables are presented as the means $\pm$ standard deviation (SD) (for normally distributed data) or medians with interquartile range (for non-normally distributed data). Categorical data are summarized as absolute frequencies and percentages. For continuous variables, an independent sample t-test was used if the data were normally distributed; if not, Mann–Whitney tests were performed. Categorical variables were compared using the Chi-squared test. Correlation analyses

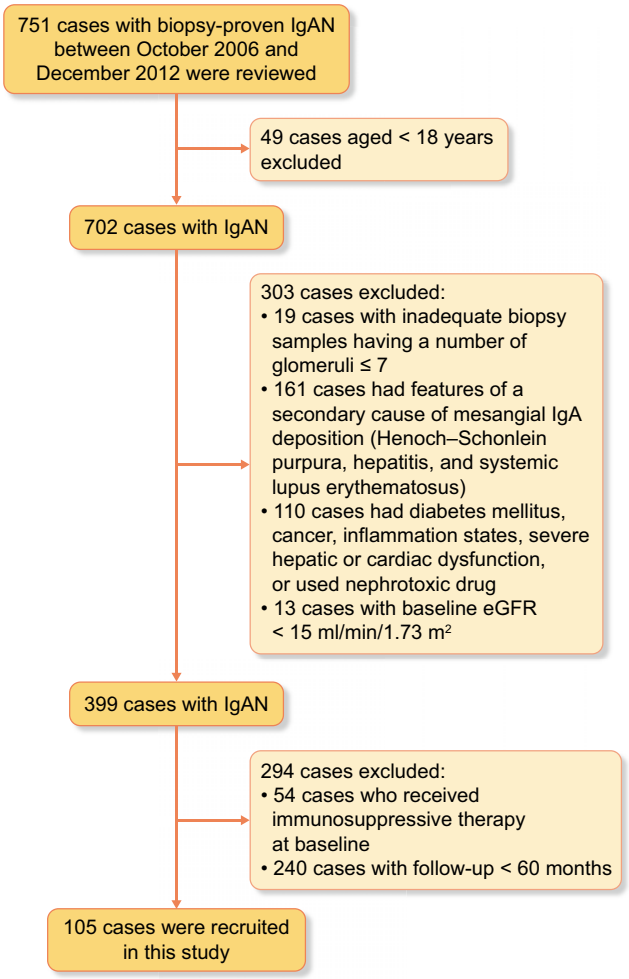

FIGURE 1: Flowchart of patient recruitment.

between the percentage of DcR2-positive RTECs and renal tubulointerstitial injury were performed using Pearson's correlation.

A follow-up duration of ≥5 years was chosen as clinically relevant and was available in all subjects. Renal survival curves for the composite renal outcome and its individual components were derived using the Kaplan–Meier method, and differences between curves were analyzed using a log-rank test. Two clinical outcomes were considered to evaluate the predictive value of DcR2: the rate of renal function decline (slope of eGFR) and the incidence of a composite renal outcome. The rate of renal function decline was determined by fitting a straight line through available data for eGFR using the principle of least squares. This was plotted and visually examined in each patient. Obvious outliers were censored. To determine an independent association between DcR2 expression and the slope of eGFR, univariate and multivariate linear regression analyses were used. Univariate analysis followed by multivariate Cox regression was performed to test the association between DcR2 expression and the composite renal outcome. Lastly, areas under the receiver operating characteristic (ROC) curves were calculated to investigate the performance of DcR2, MAP, proteinuria, eGFR and their combination as prognostic markers for the composite renal outcome. Statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, USA). For all analyses, $P < 0.05$ (two-tailed) was considered significant.

RESULTS

Clinical features and clinical outcomes of the cohort

Table 1 summarizes the main characteristics of the 105 recruited patients with IgAN (64 males; mean age 39.5 ± 13.0 years) at renal biopsy and during follow-up.

Table 1. Clinical characteristics of 105 patients with IgAN at biopsy and follow-up

At biopsy	Variable[a]	Follow-up	Variable
Age, years	39.5 ± 13.0	Duration of follow-up, months	78 (69–91)
Males	64, 60.9	Clinical outcome	
Hypertension, %	31, 29.5	Composite renal outcome	26, 24.7
MAP, mmHg, %	95.2 ± 18.0	30% decline in eGFR	24, 22.8
Hemoglobin, g/L, %	126.9 ± 17.2	ESRD	14, 13.3
Serum albumin, g/L	33.1 ± 9.9	Rate of renal function decline, mL/min/1.73 m^2/year	−1.8 ± 6.7
Serum uric acid, μmol/L	356.3 ± 111.5	Treatment	
Serum creatinine, μmol/L	99.8 ± 51.2	Median number of antihypertensive drugs	1.8 (1.6–2.0)
eGFR, mL/min/1.73 m^2, %	89.8 ± 37.2	RAS inhibitor	86, 81.9
[b]CKD Stages 1–4, %	39.3, 33.8, 23.1 and 4.8	Immunosuppression, *n* (%)	58, 55.2
Proteinuria, g/day, %	1.9 (0.7–3.7)	Prednisone, *n* (%)	57, 54.3
		Other, *n* (%)	14, 13.3

[a]Continuous variables are expressed as mean ± SD or median (interquartile ranges). Categorical variables are expressed as number (percent).
[b]CKD Stages 1–4 denote eGFR ≥ 90, 60–89, 30–59 and 15–29 mL/min/1.73 m^2, respectively, according to the Kidney Disease Outcomes Quality Initiative.

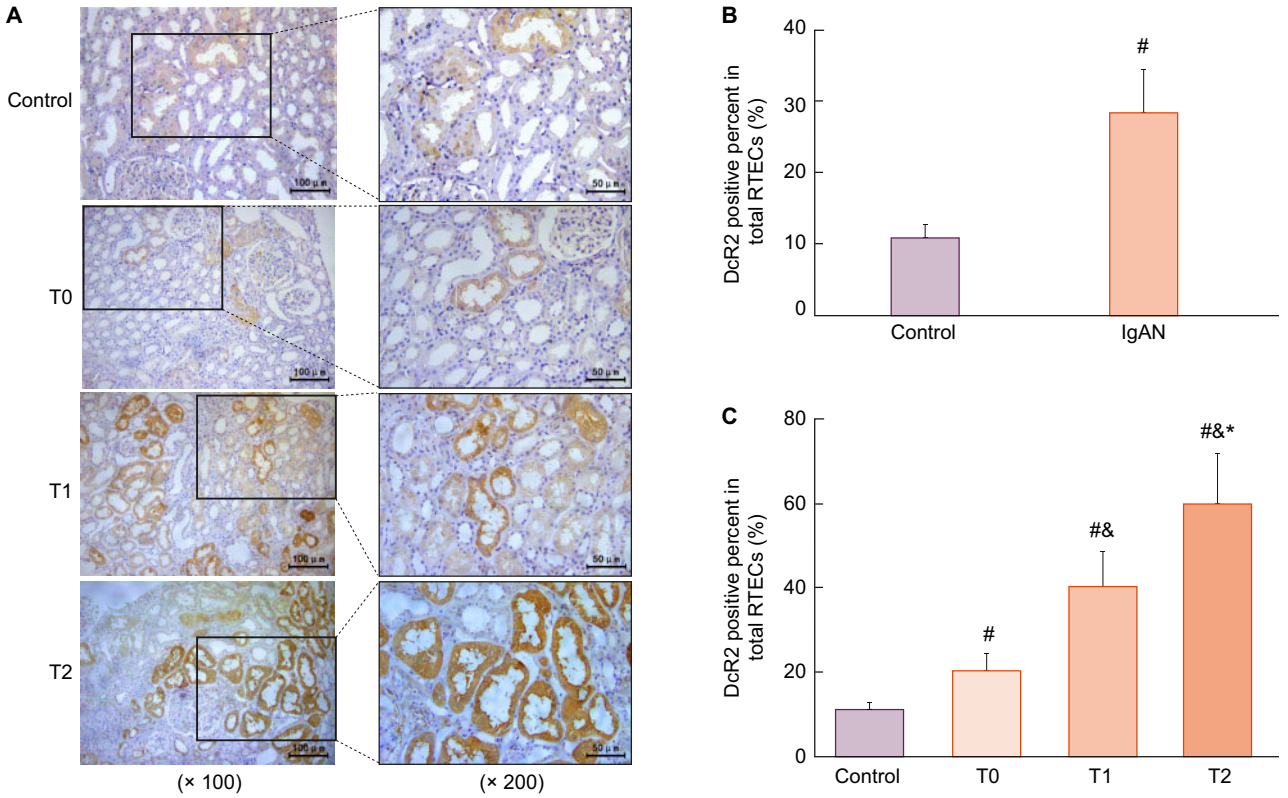

FIGURE 2: DcR2 immunostaining in renal biopsy tissues from controls and patients with IgAN. (**A**) Representative micrographs showing immunohistochemical staining of DcR2 expression in IgAN ($n = 105$) and controls ($n = 15$). (**B** and **C**) Quantification of DcR2 expression in RTECs. #P < 0.05 versus control; [IN/AI]P < 0.05 versus T0; *P < 0.05 versus T1.

Expression of DcR2 in renal tissue samples from patients with IgAN

First, we assessed DcR2 expression levels and cellular locations in renal biopsy samples from the normal kidney control tissues and those from patients with IgAN using immunohistochemistry. DcR2 was predominantly located in the cytoplasm and cytomembrane of proximal RTECs, but not in the distal renal tubule, glomeruli and tubulointerstitial space (Figure 2A). The percentage of tubular DcR2 expression was significantly higher in IgAN samples than in normal controls (28.2 ± 6.2% versus 10.8 ± 2.1%; P = 0.009; Figure 2B) and increased progressively with higher T scores (all P < 0.05; Figure 2C). These results suggested that DcR2 was overexpressed in IgAN and increased with the severity of renal tubulointerstitial injury.

Co-expression of DcR2 and p16 in the renal tissue of patients with IgAN

Next, we evaluated the correlation between DcR2 and p16 expression, a senescence-associated marker, in patients with IgAN. The percentage of DcR2+p16 positive-RTECs increased gradually with the higher T score in patients with IgAN (Figure 3A, B and D). Double-labeling immunofluorescence

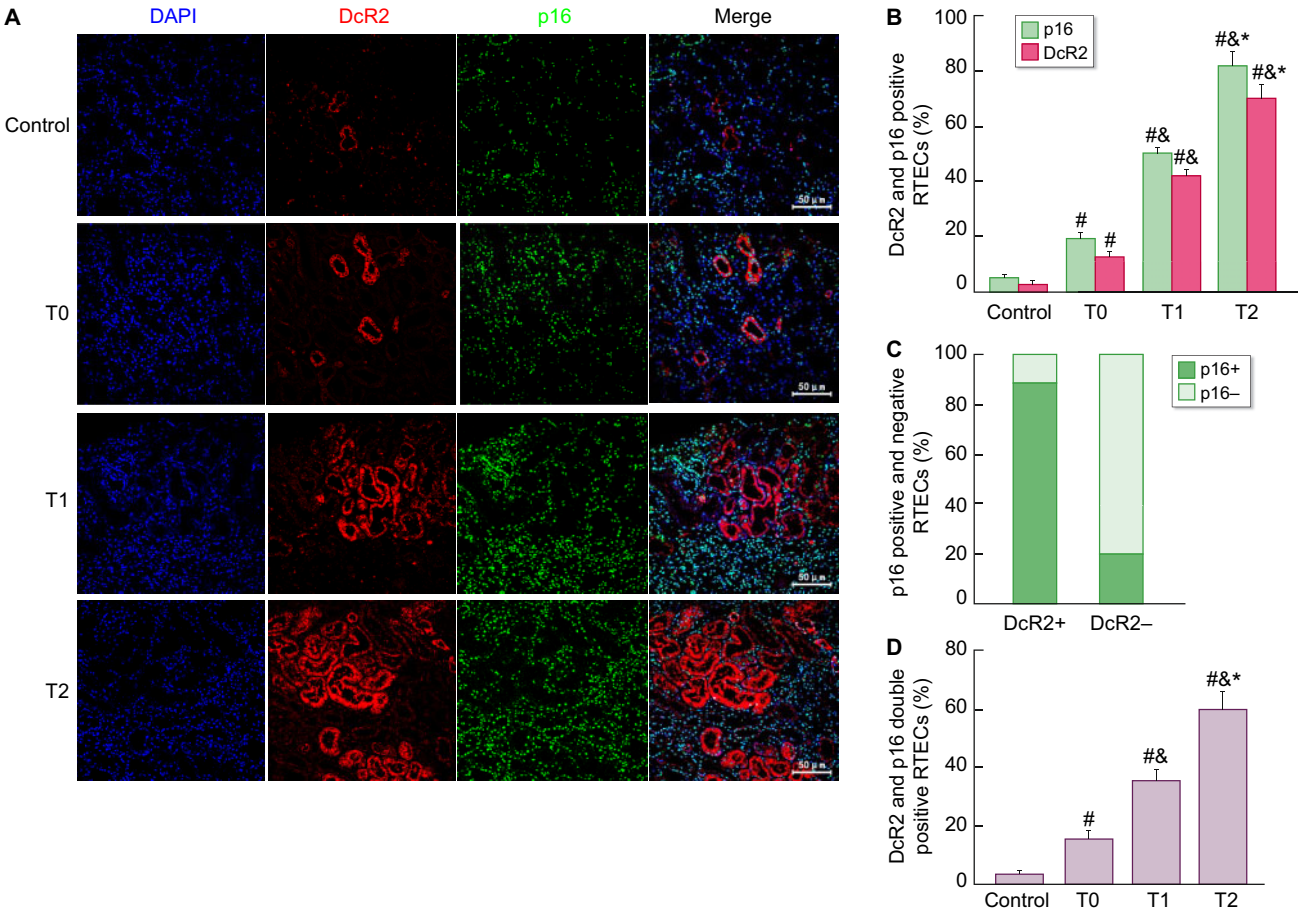

FIGURE 3: Co-expression analysis of DcR2 with the senescence marker p16 in renal biopsy tissues from controls and patients with IgAN. (**A**) Tubular DcR2 (red) co-expressed with p16 (green). (**B**) Quantification of the percentages of p16 and DcR2 expression in RTECs. (**C**) Distribution of p16 positive RTECs among DcR2 positive and DcR2 negative RTECs. (**D**) Quantification of the percentages of co-expression of DcR2 and p16 in RTECs. $^{\#}P < 0.05$ versus control; $^{\&}P < 0.05$ versus T0; $^{*}P < 0.05$ versus T1.

showed that 88.3% of DcR2-positive RTECs co-expressed p16 compared with 20.1% among DcR2-negative RTECs (Figure 3C). These results indicated that DcR2-positive RTECs might possess a senescent phenotype and accelerated renal tubulointerstitial injury.

Correlation of DcR2 with clinical and pathological features at baseline

To test whether DcR2 expression at biopsy was associated with disease severity in IgAN, we examined the relationships between clinical and histological parameters and the percentage of DcR2-expressing RTECs. We divided the patients into three groups according to the percentage of DcR2-positive RTECs: <25% DcR2-positive; 25–50% DcR2-positive; and >50% DcR2-positive group. The number of patients in the >50% DcR2-positive group was relatively small (seven cases); therefore, we merged the 25–50% and >50% groups into a DcR2-high group to maximize the statistical power. Age, serum uric acid, serum creatinine and proteinuria were significantly higher in the DcR2-high group versus the <25% DcR2-positive group (DcR2-low) (all $P < 0.05$). Conversely, hemoglobin and eGFR were much lower in the DcR2-high group versus the DcR2-low group ($P < 0.05$ for all; Table 2). We next analyzed the correlation between tubular DcR2 expression and histological score: tubular DcR2 expression was associated with the T score only. The T1 and T2 prevalence in the DcR2-high group was significantly higher than that in the DcR2-low group (T1, 63.64% versus 18.06%, $P = 0.000$; T2, 12.12% versus 0%, $P = 0.007$; Table 2). Linear regression analyses showed that the percentage of DcR2-positive cells in the tubulointerstitium correlated closely with the percentage of tubular atrophy/interstitial fibrosis ($R = 0.632$, $P = 0.000$; Figure 4A) and interstitial inflammation ($R = 0.296$, $P = 0.018$; Figure 4B). These results indicated that DcR2 expression correlates with the severity of renal tubulointerstitial injury in patients with IgAN.

DcR2 is associated with worse renal outcome in patients with IgAN

To determine whether DcR2 expression at biopsy was associated with disease outcome and IgAN progression, tubular DcR2 expression was used to predict composite renal outcome, and the rate of renal function decline (slope of eGFR) was verified. In the DcR2-low group, four patients (5.5%) reached the composite renal outcome of 30% decline in eGFR ($n = 4$) or ESRD ($n = 2$). In the DcR2-high group, composite renal outcome occurred in 22

Table 2. Comparison of characteristics of the subjects at biopsy according to tubular DcR2 expression

	Tubular DcR2 expression		
Variable[a]	DcR2 <25% group ($n=72$)	DcR2 ≥25% group ($n=33$)	P-value[b]
Age, years	37.2 ± 10.26	45.5 ± 9.3	0.03
Male	29 (40.2%)	12 (36.3%)	0.551
Hypertension, %	19 (26.3)	8 (24.2)	0.593
Hemoglobin, g/L	132 ± 16	114 ± 12	0.000
Serum albumin, g/L	34 ± 10.6	31 ± 7.2	0.303
Serum uric acid, μmol/L	329 ± 107	413 ± 99	0.000
Serum creatinine, μmol/L	80 ± 42	144 ± 74	0.000
eGFR, mL/min/1.73 m^2	103 ± 31	56 ± 27	0.000
Proteinuria, g/24 h	1.3 (0.8, 1.8)	2.4 (1.6, 3.1)	0.017
Oxford MEST-C, *n* (%)			
M1	69 (95.8)	31 (93.9)	0.237
E1	46 (63.8)	23 (69.6)	0.191
S1	30 (41.6)	18 (54.5)	0.085
T1	13 (18.06)	21 (63.64)	0.000
T2	0 (0)	4 (12.12)	0.007
C1	7 (9.7)	3 (9.0)	0.621
C2	1 (1.3)	3 (9.0)	0.056

MEST-C, histologic score based on mesangial hypercellularity, the presence of endocapillary proliferation, segmental glomerulosclerosis/adhesion and severity of tubular atrophy/interstitial fibrosis and crescents formation.
[a]Continuous variables are expressed as mean ± SD or median (interquartile ranges). Categorical variables are expressed as number (percent).
[b]P-values were calculated by an independent samples *t* test or chi-squared test or Mann–Whitney test.

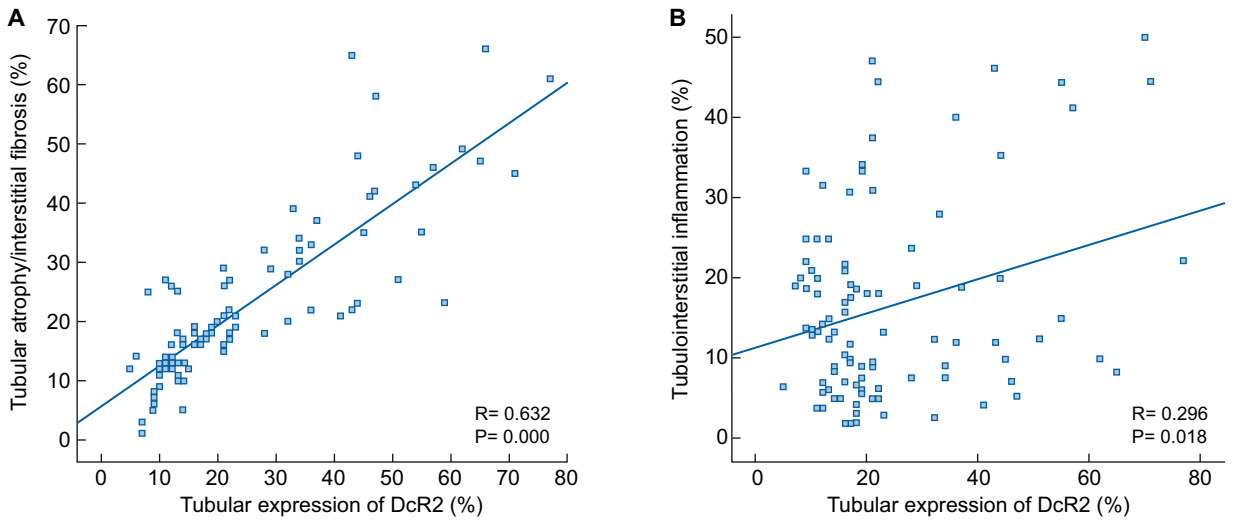

FIGURE 4: Associations between tubular DcR2 expression and renal tubulointerstitial injury. (**A**) Scatter plot with fitted value intervals for percent tubular expression of DcR2 and tubular atrophy/interstitial fibrosis. (**B**) Scatter plot with fitted value intervals for percent tubular expression of DcR2 and tubulointerstitial inflammation.

patients (66.6%); a 30% decline in eGFR occurred in 20 (60.6%) and ESRD in 12 (36.3%; Table 3). No participant died during the study period. The median number receiving antihypertensive drugs was higher in the DcR2-high group than that in the DcR2-low group. However, the rate of drug use, including RAS inhibitors and immunosuppressive agents, did not differ significantly between the patients in the two groups (Table 3). Kaplan–Meier survival analysis revealed that renal survival for the composite outcome (P = 0.000), as well as the individual components 30% decline in eGFR (P = 0.000) or ESRD (P = 0.000), was significantly lower in the DcR2-high group than in the DcR2-low group (Figure 5). Correspondingly, patients in the DcR2-high group showed worse eGFR slopes than those in the DcR2-low group (−3.4 ± 7.2 versus −0.4 ± 4.1 mL/min/1.73 m^2/year; P = 0.000; Table 4). Univariate Cox regression also showed that ≥25% tubular DcR2 expression was a significant predictor for the composite renal outcome [hazard ratio (HR) = 8.7; P = 0.000; Table 5].

In a disease as complex as IgAN, multiple risk factors are involved in progression. Therefore, we used multiple linear regression and Cox proportional hazards models to predict IgAN prognosis. Five models of multivariate analysis were calculated. Models A, B, C and D were designed to address whether tubular DcR2 expression levels predicted long-term outcome independently of age and sex or the baseline data comprising the clinical and pathological variables. Model E was designed to address whether tubular DcR2 expression levels were an independent predictor of outcome even when follow-up data for treatment were considered; this model included clinical data, pathological

Table 3. Comparison of characteristics of the subjects during follow-up according to tubular DcR2 expression

Variable[a]	Tubular DcR2 expression		P-value[b]
	DcR2 <25% group ($n = 72$)	DcR2 ≥25% group ($n = 33$)	
Duration of follow-up, months	79 (76–83)	83 (77–88)	0.294
Clinical outcome, %			
Composite renal outcome	4 (5.5)	22 (66.6)	0.000
30% decline in eGFR	4 (5.5)	20 (60.6)	0.000
ESRD	2 (2.7)	12 (36.3)	0.000
Treatment, %			
Median number of antihypertensive drugs	1.2 ± 0.9	2.0 ± 1.1	0.000
RAS inhibitor	60 (83.3)	26 (78.7)	0.474
Immunosuppression	38 (52.7)	20 (60.6)	0.170
Prednisone	36 (50.0)	20 (60.6)	0.127
Other	6 (8.3)	8 (22.2)	0.021

[a]Continuous variables are expressed as mean ± SD or median (interquartile ranges).
Categorical variables are expressed as number (percent).
[b]P-values were calculated by an independent samples *t*-test or chi-squared test or Mann–Whitney test.

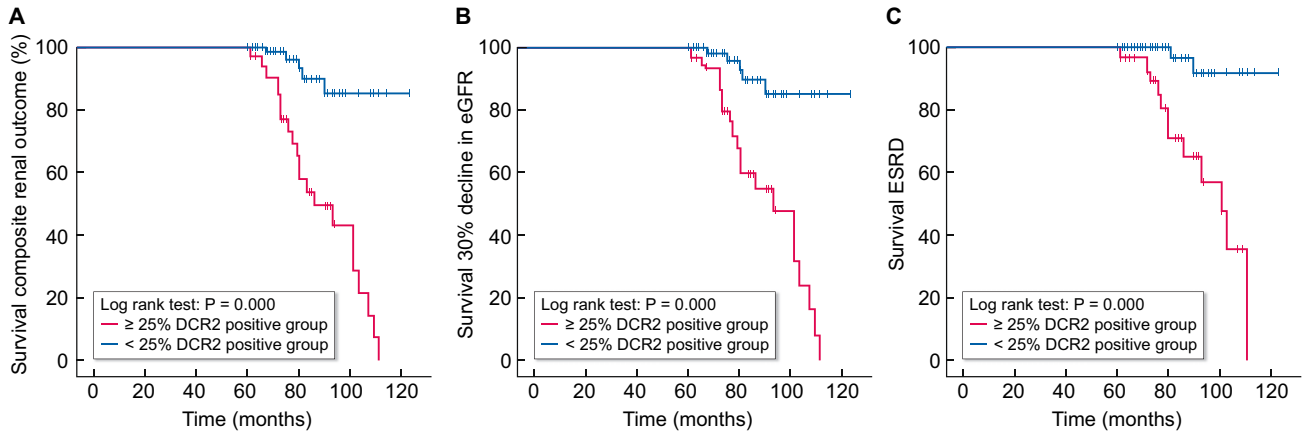

FIGURE 5: Kaplan–Meier survival curves for tubular DcR2 expression. Displayed are the incidence of the composite renal outcome (**A**) and the individual components that define 30% decline in eGFR (**B**) or ESRD (**C**).

Table 4. Univariate followed by multiple linear regression model testing associations of tubular DcR2 expression with the rate of renal function decline

Rate of renal function decline (linear regression)						
Tubular DcR2 expression	Univariate slope, mL/min/1.73 m^2/year	Multivariate, β (SD)				
		Model A[a]	Model B[b]	Model C[c]	Model D[d]	Model E[e]
DcR2 <25% group	−0.4 ± 4.1	−3.3 (1.4)	−2.9 (1.3)	−2.5 (1.2)	−2.1 (1.1)	−2.0 (1.0)
DcR2 ≥25% group	−3.4 ± 7.2					
P-value	0.000	0.000	0.000	0.000	0.000	0.008

[a]Model A was adjusted for age and sex.
[b]Model B was adjusted for hypertension, proteinuria and eGFR.
[c]Model C was adjusted for tubular atrophy/interstitial fibrosis (T1–T2 score).
[d]Model D was adjusted for hypertension, proteinuria, eGFR and T scores.
[e]Model E was adjusted for hypertension, proteinuria, eGFR and T scores and follow-up immunosuppressive therapy.

data and therapeutic follow-up data. The DcR2-high group remained a strong predictor of worse eGFR slopes compared with the DcR2-low group in the slope analysis (Model A, $\beta = -3.3$, P = 0.000; Model B, $\beta = -2.9$, P = 0.000; Model C, $\beta = -2.5$, P = 0.000; Model D, $\beta = -2.1$, P = 0.000; Model E, $\beta = -2.0$, P = 0.008; Table 4). When the composite endpoint of 30% decline in eGFR or ESRD was considered as the outcome, multivariate Cox regression analysis showed for the three models, patients in the DcR2-high group had significantly worse renal outcomes versus those in the DcR2-low group (Model A, HR = 7.9,

Table 5. Univariate and multivariate cox regression models testing associations of tubular DcR2 expression with incidence of the composite renal outcome

30% decline in eGFR or ESRD (Cox regression)

Tubular DcR2 expression	Univariate HR (95% CI)	Multivariate HR (95% CI)				
		Model A[a]	Model B[b]	Model C[c]	Model D[d]	Model E[e]
DcR2 < 5% group	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
DcR2 ≥25% group	8.7 (3.6–9.2)	7.9 (3.0–14.2)	4.4 (2.2–9.5)	2.9 (1.2–7.7)	2.1 (1.1–6.2)	1.8 (1.0–5.1)
P-value	0.000	0.000	0.000	0.000	0.000	0.005

[a]Model A was adjusted for age and sex.
[b]Model B was adjusted for hypertension, proteinuria and eGFR.
[c]Model C was adjusted for tubular atrophy/interstitial fibrosis (T1–T2 score).
[d]Model D was adjusted for hypertension, proteinuria, eGFR and T scores.
[e]Model E was adjusted for hypertension, proteinuria, eGFR and T scores and follow-up immunosuppressive therapy.

Table 6. Area under the ROC curve for various variables for the composite renal outcome

Variable	Value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P-value
Tubular DcR2[a]	25.5	81.6	80.7	0.86 (0.79–0.96)	0.000
MAP	83.2	83.0	40.0	0.61 (0.49–0.73)	0.086
Proteinuria	1.2	84.6	63.3	0.75 (0.75)	0.000
eGFR	66.0	70.5	81.6	0.81 (0.72–0.90)	0.000
MEST-C	2.5	70.0	79.6	0.77 (0.65–0.88)	0.000
Tubular DcR2+MAP	−1.12	82.1	72.4	0.82 (0.72–0.91)	0.000
Tubular DcR2+proteinuria	−1.21	81.7	74.7	0.83 (0.73–0.92)	0.000
Tubular DcR2+eGFR	−1.33	76.8	80.8	0.84 (0.75–0.94)	0.000
Tubular DcR2+MEST-C	−0.77	80.0	80.4	0.85 (0.76–0.95)	0.000
Clinical data at biopsy[b]	−1.26	76.9	75.0	0.79 (0.69–0.88)	0.000
Tubular DcR2+clinical data at biopsy	−1.41	78.0	81.1	0.84 (0.76–0.95)	0.000
Clinical data at biopsy+MEST-C	−0.813	80.9	80.8	0.85 (0.77–0.96)	0.000
Tubular DcR2+clinical data at biopsy+MEST-C	−1.6	84.6	81.6	0.88 (0.80–0.96)	0.000

[a]Representing the percentage of DcR2-positive RTECs.
[b]Comprising MAP, proteinuria and eGFR at biopsy.

P = 0.000; Model B, HR = 4.4, P = 0.000; Model C, HR = 2.9, P = 0.000; Model D, HR = 2.1, P = 0.000; Model E, HR = 1.8, P = 0.005; Table 5).

The areas under the ROC curve for the incidence of composite renal outcome confirmed that tubular DcR2 expression, proteinuria, eGFR and MEST-C score were significantly associated with the composite renal outcome (Table 6). Tubular DcR2 expression showed the highest area under the curve (AUC) of 0.86 at a cut-off value of 25.5% tubular DcR2-positive expression (Table 6), which corresponded with the threshold predicting worse renal outcome in the linear/Cox regression models. Adding tubular DcR2 expression to each of the other prognostic markers under investigation increased the AUC values significantly (Table 6). When tubular DcR2 expression was added to the clinical data at biopsy (MAP, proteinuria and eGFR) or MEST-C score, the predictive performance improved significantly [DcR2+clinical data: AUC = 0.84; 95% confidence interval (CI) 0.76–0.95; DcR2+MEST-C score: AUC = 0.85; 95% CI, 0.76–0.95] compared with those of the clinical model alone (AUC = 0.79; 95% CI 0.69–0.88) or MEST-C score alone (AUC = 0.77; 95% CI 0.65–0.88). Furthermore, combining tubular DcR2 expression with the clinical data at biopsy and MEST-C score yielded better performance to predict the 5-year risk of composite renal outcome (AUC = 0.88; 95% CI 0.80–0.96) versus the clinical data and MEST-C score (AUC = 0.85; 95% CI 0.77–0.96; Table 6 and Figure 6).

DISCUSSION

In this retrospective cohort study, we demonstrated that tubular DcR2 expression detected at biopsy was an independent and strong predictor of IgAN progression. Multivariate regression analyses showed that ≥25% tubular DcR2 expression was significantly associated with eGFR decline and the incidence of composite renal outcome (a 30% decline in eGFR or ESRD) in IgAN after multiple adjustments. More importantly, the addition of tubular DcR2 expression significantly improved risk prediction compared with the clinical model incorporating major clinical risk factors at biopsy (MAP, proteinuria and eGFR) and MEST-C score, as confirmed by ROC curve analyses. This newly identified relationship between senescent cell burden at biopsy and long-term renal outcome in IgAN suggested that DcR2 could further improve risk prediction of IgAN progression over the predictive model based on clinical data and the MEST-C score.

IgAN exhibits a widely varying clinical course, from asymptomatic urinary abnormalities to rapidly progressive renal failure [1]. Determining the prognosis based on parameters obtained at biopsy is important in clinical decision making to inform patients of their prognosis and balance the benefits of immunosuppression against the anticipated risks. Proteinuria, eGFR, blood pressure and the MEST-C score are the most readily available risk factors to predict renal prognosis in IgAN [16, 17]; however, they do not explain most of the variability in outcome of patients with IgAN, and lack sensitivity to detect progression

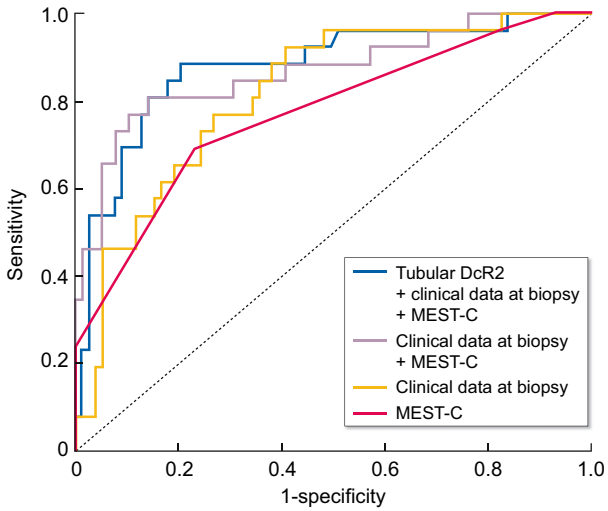

FIGURE 6: ROC curve analysis of various variables for the composite renal outcome in patients with IgAN.

of early irreversible fibrotic changes. Therefore, a combination of additional biomarkers of disease prognosis and activity at biopsy would help to manage patients with IgAN.

Senescent cell accumulation has been detected in early clinical nephropathy, when patients have mild proteinuria and normal GFR, suggesting that cell senescence is an early event in CKD [18, 19]. RTECs are frequently implicated in renal senescence, although other cell types can exhibit positive staining for markers of cell-cycle arrest [5, 20, 21]. Previously, we demonstrated that increased p16 and p21 protein expression was confined to RTECs in patients with IgAN [6]. In this study, we confirmed that DcR2, a marker of senescence, was highly specifically overexpressed in IgAN RTECs. Moreover, nearly all DcR2-positive RTECs co-expressed the senescence marker p16. The percentage of DcR2-positive senescent RTECs was elevated with the increasing severity of tubulointerstitial fibrosis (TIF; T0–T2) in IgAN. Tubulointerstitial lesions have the most powerful association with the renal prognosis of IgAN, independent of clinical features [22, 23]. Tubular DcR2 expression was closely associated with both tubular atrophy/interstitial fibrosis and interstitial inflammation. Among all MEST-C lesions, only the prevalence of T was significantly higher in patients with DcR2-high RTECs compared with those without. Furthermore, we have evidence that DcR2 interacts with peroxiredoxin 1 and aggravates renal fibrosis by mediating RTECs senescence *in vivo* and *in vitro* [24]. The clinical and experimental model results suggested that DcR2, which is involved in TIF, could play a critical role in the progression of IgAN.

In this study, the requirement for study entry was at least 5 years of follow-up, because IgAN progresses slowly. Longer periods of observation up to 5 years after renal biopsy are necessary to more accurately predict the rate of renal function decline [22, 25, 26]. In addition, the patients in this study belonged mostly to Stages 1, 2 and 3 CKD (39.3, 33.8 and 23.1%, respectively); therefore, these patients needed longer follow-up to reach the study outcome. Studies have shown that time-averaged proteinuria and blood pressure, which require the entire period of observation for each patient, are better predictors of disease progression compared with those at biopsy [27, 28]. However, risk stratification approaches that require prolonged periods of observation have limited utility in clinical practice, which requires the identification of high-risk patients near the time of biopsy to guide treatment decisions. Additionally, immunosuppressive therapy might be a significant confounding factor. Therefore, we used two widely accepted clinical outcomes in the models to assess the independent relevance of DcR2, baseline variables and follow-up therapy. They include an outcome using a continuous variable (slope of eGFR) and a renal endpoint (ESRD or 30% reduction from baseline of eGFR) to provide reassurance that we could correctly identify a poor prognostic cohort. High (≥25%) tubular DcR2 expression was determined as a strong, independent predictor of IgAN progression (worse eGFR slopes and worse renal outcome) at 5 years after biopsy after multiple adjustments. Furthermore, adding tubular DcR2 expression to the risk model (clinical data at biopsy and MEST-C score) achieved the largest improvement in predicting and reclassifying the risk of IgAN progression at 5 years after biopsy.

This was the first study to investigate the impact of cellular senescence on the long-term renal survival of IgAN. We emphasized improving earlier risk stratification at the time of diagnosis and compared the predictive performance of tubular DcR2 expression with a clinical model of IgAN. We also evaluated the effect of combining tubular DcR2 expression, clinical data and MEST-C score on risk reclassification, which has not been tested previously.

Our study had some limitations. First, this was a retrospective observational study. These results require validation on an independent prospective cohort. Second, the numbers of patients with moderate (25–50%) and marked (>50%) tubular DcR2 expression were small. Although both groups were combined to verify the correlation of renal outcome with percentage of tubular DcR2 expression, more patients with moderate and marked tubular DcR2 expression are needed to validate these findings. Third, because of the retrospective design of our study, there were insufficient data on urinary DcR2 levels to support our hypothesis. The extracellular section of DcR2, as a 42 kDa transmembrane protein, can be sheared away and filtered freely by glomeruli, allowing it to be detected in urine [12, 13, 29]. Unfortunately, because of the retrospective design of our study,

there were insufficient data on urinary DcR2 levels to support our hypothesis. Although only preliminary data, we observed a positive correlation between tubular DcR2 expression and urinary DcR2/creatinine (uDcR2/Cr) levels ($R = 0.763$, $P = 0.000$; Supplementary data, Figure S1) from 20 of the 105 patients included in this study. In addition, our previous cross-sectional study [13] found that levels of uDcR2/Cr were significantly higher in patients with IgAN and in those with more severe TIF, compared with healthy controls and uDcR2/Cr was independently associated with TIF. Further studies are needed to confirm the utility of urinary DcR2 levels to evaluate prognosis in patients with IgAN as a non-invasive biomarker. We have registered a prospective study named 'The study of urinary DcR2 in the progression and prognosis of IgA nephropathy (IgAN)' in the Chinese Clinical Trial Registry (http://www.chictr.org.cn/showproj.aspx? proj=27127; Registration number: ChiCTR1800016254). Last, we lacked data for the doses of RAS inhibitors or immunosuppressive agents and thus could not perform dose–response analysis.

In conclusion, we identified tubular DcR2 expression detected at biopsy as an independent and strong predictor for IgAN progression. The addition of tubular DcR2 expression to the clinical data at the time of biopsy or MEST-C score significantly improved the prediction of the 5-year risk of disease progression in IgAN.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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AUTHORS' CONTRIBUTIONS

H.D. and W.H. performed the experiments and analyzed the data; J.C. and L.L. interpreted the results of the experiments; L.W. prepared the tables and figures; W.H. and L.L. collected the data; Y.H. and J.C. conceived and designed the study; H.D. edited the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Lai KN, Tang SC, Schena FP *et al.* IgA nephropathy. *Nat Rev Dis Primers* 2016; 2: 20
2. Sturmlechner I, Durik M, Sieben CJ *et al.* Cellular senescence in renal ageing and disease. *Nat Rev Nephrol* 2017; 13: 77–89
3. Munoz-Espin D, Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Biol* 2014; 15: 482–496
4. Stenvinkel P, Larsson TE. Chronic kidney disease: a clinical model of premature aging. *Am J Kidney Dis* 2013; 62: 339–351
5. Docherty MH, O'Sullivan ED, Bonventre JV *et al.* Cellular senescence in the kidney. *J Am Soc Nephrol* 2019; 30: 726–736
6. Liu J, Yang JR, He YN *et al.* Accelerated senescence of renal tubular epithelial cells is associated with disease progression of patients with immunoglobulin A (IgA) nephropathy. *Transl Res* 2012; 159: 454–463
7. Collado M, Gil J, Efeyan A *et al.* Tumour biology: senescence in premalignant tumours. *Nature* 2005; 436: 642
8. Xue W, Zender L, Miething C *et al.* Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 2007; 445: 656–660
9. Flyvbjerg A. Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily. *Nat Rev Endocrinol* 2010; 6: 94–101
10. Sagiv A, Biran A, Yon M *et al.* Granule exocytosis mediates immune surveillance of senescent cells. *Oncogene* 2013; 32: 1971–1977
11. Chen J, Chen KH, Fu BQ *et al.* Isolation and identification of senescent renal tubular epithelial cells using immunomagnetic beads based on DcR2. *Exp Gerontol* 2017; 95: 116–127
12. Chen J, Zhang WW, Chen KH *et al.* Urinary DcR2 is a novel biomarker for tubulointerstitial injury in patients with diabetic nephropathy. *Am J Physiol Renal Physiol* 2017; 313: F273–F281
13. Chen J, Hu W, Xiao F *et al.* DCR2, a cellular senescent molecule, is a novel marker for assessing tubulointerstitial fibrosis in patients with immunoglobulin A nephropathy. *Kidney Blood Press Res* 2019; 44: 1063–1074
14. Trimarchi H, Barratt J, Cattran DC *et al.* Oxford classification of IgA nephropathy 2016: an update from the IgA nephropathy classification working group. *Kidney Int* 2017; 91: 1014–1021
15. Levey AS, Stevens LA, Schmid CH *et al.*; for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604–612
16. Barbour S, Reich H. An update on predicting renal progression in IgA nephropathy. *Curr Opin Nephrol Hypertens* 2018; 27: 214–220
17. Barbour SJ, Coppo R, Zhang H *et al.*; for the International IgA Nephropathy Network. Evaluating a new international risk-prediction tool in IgA nephropathy. *JAMA Intern Med* 2019; 179: 942–952
18. Verzola D, Gandolfo MT, Gaetani G *et al.* Accelerated senescence in the kidneys of patients with type 2 diabetic nephropathy. *Am J Physiol Renal Physiol* 2008; 295: 1563–1573
19. Zhu K, Kakehi T, Matsumoto M *et al.* NADPH oxidase NOX1 is involved in activation of protein kinase C and premature senescence in early stage diabetic kidney. *Free Radic Biol Med* 2015; 83: 21–30
20. Melk A, Schmidt BM, Vongwiwatana A *et al.* Increased expression of senescence-associated cell cycle inhibitor p16INK4a in deteriorating renal transplants and diseased native kidney. *Am J Transplant* 2005; 5: 1375–1382
21. Sis B, Tasanarong A, Khoshjou F *et al.* Accelerated expression of senescence associated cell cycle inhibitor p16INK4A in kidneys with glomerular disease. *Kidney Int* 2007; 71: 218–226
22. Cattran DC, Coppo R, Cook HT *et al.*; A Working Group of the International IgA Nephropathy Network and the Renal Pathology Society. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int* 2009; 76: 534–545

23. Zeng CH, Le W, Ni Z *et al.* A multicenter application and evaluation of the oxford classification of IgA nephropathy in adult Chinese patients. *Am J Kidney Dis* 2012; 60: 812–820
24. Jia C, Ke-Hong C, Fei X *et al.* Decoy receptor 2 mediation of the senescent phenotype of tubular cells by interacting with peroxiredoxin 1 presents a novel mechanism of renal fibrosis in diabetic nephropathy. *Kidney Int* 2020; 98: 645–662
25. Chen T, Li X, Li Y *et al.* Prediction and risk stratification of kidney outcomes in IgA nephropathy. *Am J Kidney Dis* 2019; 74: 300–309
26. Pozzi C, Bolasco PG, Fogazzi GB *et al.* Corticosteroids in IgA nephropathy: a randomised controlled trial. *Lancet* 1999; 353: 883–887
27. Bartosik LP, Lajoie G, Sugar L *et al.* Predicting progression in IgA nephropathy. *Am J Kidney Dis* 2001; 38: 728–735
28. Reich HN, Troyanov S, Scholey JW *et al.* Remission of proteinuria improves prognosis in IgA nephropathy. *J Am Soc Nephrol* 2007; 18: 3177–3183
29. Kimberley FC, Screaton GR. Following a TRAIL: update on a ligand and its five receptors. *Cell Res* 2004; 14: 359–372