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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.
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 ±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 m²). Data collected concerning follow-up included duration of follow-up, serum creatinine, eGFR, rate of renal function decline (mL/min/1.73 m²/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 m². A composite renal outcome included 30% eGFR decline or ESRD, whichever occurred first. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic pressure ≥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 ± 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
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, MAF, 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.
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

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

| At biopsy | Variable  | Follow-up | Variable |
|-----------|-----------|-----------|----------|
| Age, years | 39.5 ± 13.0 | Duration of follow-up, months | 78 (69–91) |
| Males     | 64, 60.9 | Clinical outcome | 26, 24.7 |
| Hypertension, % | 31, 29.5 | Composite renal outcome | 24, 22.8 |
| MAP, mmHg, % | 95.2 ± 18.0 | 30% decline in eGFR | 14, 13.3 |
| 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 m²/year | –1.8 ± 6.7 |
| Serum uric acid, μmol/L | 356.3 ± 111.5 | Treatment | 86, 81.9 |
| Serum creatinine, μmol/L | 99.8 ± 51.2 | Median number of antihypertensive drugs | 1.8 (1.6–2.0) |
| eGFR, ml/min/1.73 m², % | 89.8 ± 37.2 | RAS inhibitor | 58, 55.2 |
| *CKD Stages 1–4, % | 39.3, 33.8, 23.1 and 4.8 | Immunosuppression, n (%) | 57, 54.3 |
| Proteinuria, g/day, % | 1.9 (0.7–3.7) | Prednisone, n (%) | 14, 13.3 |
| Other, n (%) |                  |                                     |  |

*Continuous variables are expressed as mean ± SD or median (interquartile ranges). Categorical variables are expressed as number (percent).

*CKD Stages 1–4 denote eGFR ≥ 90, 60–89, 30–59 and 15–29 ml/min/1.73 m², respectively, according to the Kidney Disease Outcomes Quality Initiative.
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 (all \( P < 0.05 \)) or 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

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**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.
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 m²/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 2. Comparison of characteristics of the subjects at biopsy according to tubular DcR2 expression

| Variablea | DcR2 <25% group (n = 72) | DcR2 ≥25% group (n = 33) | P-valueb |
|-----------|--------------------------|--------------------------|-----------|
| 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 m² | 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.

aContinuous variables are expressed as mean ± SD or median (interquartile ranges). Categorical variables are expressed as number (percent).

bP-values were calculated by an independent samples t test or chi-squared test or Mann–Whitney test.

![Figure 4](https://academic.oup.com/ckj/advance-article/doi/10.1093/ckj/sfaa257/6121202)
DcR2 as a predictor of prognosis in IgAN

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

| Variablea | Tubular DcR2 expression | | | |
| --- | --- | --- | --- |
| | DcR2 <25% group (n = 72) | DcR2 ≥25% group (n = 33) | P-valueb |
| 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 |

aContinuous variables are expressed as mean ± SD or median (interquartile ranges).
Categorical variables are expressed as number (percent).
bP-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

| Tubular DcR2 expression | Univariate slope, \( \text{mL/min}/1.73 \text{m}^2/\text{year} \) | Multivariate, \( \beta \) (SD) | |
| --- | --- | --- | --- |
| | Model Aa | Model Bb | Model Cc | Model Dd | Model Ee |
| DcR2 <25% group | \( -0.4 \pm 4.1 \) | \( -3.3 \) (1.4) | \( -2.9 \) (1.3) | \( -2.5 \) (1.2) | \( -2.1 \) (1.1) | \( -2.0 \) (1.0) |
| DcR2 ≥25% group | \( -3.4 \pm 7.2 \) | 0.000 | 0.000 | 0.000 | 0.000 | 0.008 |
| P-value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

aModel A was adjusted for age and sex.
bModel B was adjusted for hypertension, proteinuria and eGFR.
cModel C was adjusted for tubular atrophy/interstitial fibrosis (T1–T2 score).
dModel D was adjusted for hypertension, proteinuria, eGFR and T scores.
eModel 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

| Tubular DcR2 expression | Univariate HR (95% CI) | Multivariate HR (95% CI) |
|-------------------------|------------------------|--------------------------|
| DcR2 <5% group          | 1.0 (Reference)        | Model A<sup>a</sup> 0.000 |
| DcR2 ≥25% group         | 8.7 (3.6–9.2)          | Model B<sup>b</sup> 0.000 |
|                          | 0.000                  | Model C<sup>c</sup> 0.000 |
| P-value                  |                        | Model D<sup>d</sup> 0.000 |
|                         |                        | Model E<sup>e</sup> 0.005 |

<sup>a</sup>Model A was adjusted for age and sex.
<sup>b</sup>Model B was adjusted for hypertension, proteinuria and eGFR.
<sup>c</sup>Model C was adjusted for tubular atrophy/interstitial fibrosis (T1–T2 score).
<sup>d</sup>Model D was adjusted for hypertension, proteinuria, eGFR and T scores.
<sup>e</sup>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<sup>a</sup>           | 25.5  | 81.6            | 80.7            | 0.86 (0.73–0.96) | 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<sup>b</sup>| –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 |

<sup>a</sup>Representing the percentage of DcR2-positive RTECs.
<sup>b</sup>Comprising MAP, proteinuria and eGFR at biopsy.

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) or 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...
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 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/show proj.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.

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