

IL-33 is associated with unfavorable postoperative survival of patients with clear-cell renal cell carcinoma

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Abstract The aim of this study was to evaluate the potential prognostic significance of interleukin-33 (IL-33) in patients with clear-cell renal cell carcinoma (ccRCC) after surgical resection. In this retrospective research, we enrolled 203 patients with ccRCC undergoing nephrectomy between 2003 and 2004 in a single institution. We recorded clinicopathologic features, overall survival (OS), and recurrence-free survival (RFS) and assessed IL-33 expression by immunohistochemical staining. On such bases, the correlations between IL-33 expression and clinicopathologic features and prognosis were evaluated. A high expression of IL-33 was significantly associated with advanced TNM stage and Fuhrman grade ($p=0.017$ and $p<0.001$, respectively) in patients with ccRCC. Moreover, multivariate analysis identified IL-33 as an independent prognostic factor of OS for patients with

ccRCC after surgery (hazard ratio=2.050; 95 % CI 1.223–3.447; $p=0.006$). The incorporation of IL-33 into the TNM stage and Fuhrman grade might help to refine individual risk stratification. The IL-33 expression may serve as an independent negative predictor of survival for patients with ccRCC after surgery.

Keywords Clear-cell renal cell carcinoma · Interleukin-33 · Prognostic biomarker · Overall survival

Introduction

Renal cell carcinoma (RCC) accounts for almost 90 % of all renal malignancies [1], and clear-cell renal cell carcinoma (ccRCC) comprises the major histological subtype of RCC [2]. Despite the advanced diagnostic technologies, such as the improved abdominal imaging, 20–30 % patients still present metastatic disease at diagnoses [3]. Besides, another 20 % of patients, having received surgical resection, are found during follow-up with relapses and metastatic RCC [1], which has an extremely poor prognosis. Currently, TNM stage, Fuhrman grade, and Eastern Cooperative Oncology Group performance status (ECOG-PS) have been proven to be independent prognostic factors for patients with ccRCC [4]. Based on these independent factors, several integrated prognostic systems have been established to predict patients' outcome, such as the University of California Integrated Staging System (UISS) [5] and Mayo Clinic stage, size, grade, and necrosis (SSIGN) score [6]. However, precise prediction of clinical outcomes remains difficult and improved predictors are still much needed. Previous studies have demonstrated that some specific RCC biomarkers have the

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potential to improve the prognostic ability when combined with traditional clinicopathologic variables [7].

IL-33, which is a member of IL-1 family, is constitutively expressed in endothelial and epithelial cells of mucous membranes, fibroblasts, and keratinocytes [8]. It can

lead to the activation of nuclear factor κ B (NF- κ B) and mitogen-activated protein kinase signaling pathways through binding to its receptor interleukin-1 receptor-like 1 (ST2) [9], which is expressed on most cells especially on mast cells and activated type 2 helper T (Th2) cells

Table 1 Clinical characteristics and correlations with the expression of IL-33

Variables	Total patients (n = 203)		IL-33 expression		p value ^a
	No.	%	Low (n = 151)	High (n = 52)	
Age (years)					0.617
Median (IQR)	54 (47–64)		55(47–65)	54 (44–61)	
Gender					0.451
Male	141	69.5	104	37	
Female	62	30.5	47	15	
Tumor size (cm)					0.108
Median (IQR)	4.0 (2.8–5.5)		4.0 (2.5–5.5)	5.0 (3.0–6.0)	
T stage					0.273
T1	128	63.1	98	30	
T2	19	9.4	16	3	
T3	52	25.5	35	17	
T4	4	2.0	2	2	
N stage					0.022
N0	199	98.0	150	49	
N1	4	2.0	1	3	
M stage					0.005
M0	196	96.6	149	47	
M1	7	3.4	2	5	
TNM stage					0.017
I	122	60.1	96	26	
II	18	8.9	16	2	
III	54	26.6	35	19	
IV	9	4.4	4	5	
Fuhrman grade					<0.001
1	32	15.8	30	2	
2	88	43.3	70	18	
3	54	26.6	39	15	
4	29	14.3	12	17	
Necrosis					0.003
Absent	152	74.9	121	31	
Present	51	25.1	30	21	
ECOG-PS					0.386
0	168	82.8	127	41	
≥1	35	17.2	24	11	
UISS					0.025
LR	68	33.5	58	10	
IR	115	56.6	81	34	
HR	20	9.9	12	8	

IL-33 interleukin-33, ECOG-PS Eastern Cooperative Oncology Group performance status, UISS University of California Los Angeles Integrated Staging System, LR low-risk, IR intermediate-risk, HR high-risk, No. number of patients, IQR interquartile range

^a A P value <0.05 is considered statistically significant

[10]. IL-33 mainly promotes Th2 immune responses [11], and it is believed to have a close relationship with some chronic inflammatory diseases, such as asthma and rheumatoid arthritis [12]. Upon cell necrosis, IL-33 is released from the nucleus, and thus, it may serve as an endogenous danger signal to alert the immune system after endothelial or epithelial cell damage [13]. By far, the role of IL-33 in carcinogenesis has not been well understood, but there is increasing evidence that IL-33 can induce angiogenesis [14] and alternative macrophage activation [15], suggesting that it may play an important role in tumor growth and progression. Previous studies have demonstrated that abundant expression of IL-33 can be observed in human tumors [16], and it is of prognostic value in hepatocellular carcinoma [17], breast cancer [18], and lung cancer [19]. However, few studies have been designed to investigate the prognostic value of IL-33 in RCC, especially in ccRCC.

In this study, the expression of IL-33 was assessed by immunohistochemistry in ccRCC tumor tissues, and its associations with clinicopathologic features and prognosis were evaluated. Moreover, we explore the hypothesis that incorporation of TNM stage and UISS with IL-33 expression could refine these prognostic models.

Patients and methods

Patients

In this retrospective study, we enrolled 203 patients with ccRCC who received nephrectomy at Zhongshan Hospital of Fudan University (Shanghai, China) between 2003 and 2004. The inclusion criteria were as follows: no history of previous anticancer therapy, acceptance of radical or partial nephrectomy, and histopathologically proven ccRCC. Patients who suffered from other types of renal cancer, had tumors with necrosis >80 %, or died within the first month after surgery due to surgical complications were excluded. For each patient, the following clinicopathologic information was collected: age at the time of surgery, gender, tumor size, TNM stage, Fuhrman grade, presence of tumor necrosis, and ECOG-PS. All the patients were staged based on radiographic reports and postoperative pathologic data and were reassigned in accordance to the 2010 American Joint Committee on Cancer TNM classification [20]. A total of nine patients with N1- or M1-stage tumors were considered to have metastasis and were excluded while analyzed for RFS. Tumor size was recorded as the longest diameter described in pathologic reports. Tumor necrosis was defined as the presence of microscopic coagulative necrosis. The UISS predictive models were applied to classify patients into three risk

groups (low risk [LR], intermediate risk [IR], and high risk [HR]). We calculated OS and RFS from the date of surgery to the date of death and recurrence, respectively, or to the date of last follow-up. This study was approved by the hospital's Ethics Committee, and informed consent was obtained from each patient.

Tissue microarray and immunohistochemistry

Primary anti-IL-33 antibody (1:600 dilution, Abcam, Cambridge, MA, USA) was used for immunohistochemical staining on tissue microarray (TMA), which was established with formalin-fixed, paraffin-embedded tissue blocks of 203 patients. All the cases were stained at once. The detailed procedure was carried out as previously described [21]. The staining intensity of each specimen was scored independently by two pathologists without the knowledge of the patient's outcome. A semi-quantitative immunohistochemistry score for each specimen, ranging from 0 to 300, was calculated by multiplying the staining intensities (0, negative; 1, weak staining; 2, moderate staining; 3, strong staining) by the area distributions (0–100 %). Based on the association with the patients' OS, we selected the optimum cutoff score (205) for the expression of IL-33 through using X-tile software version 3.6.1 (Yale University School of Medicine, New Haven, CT, USA).

Statistical analysis

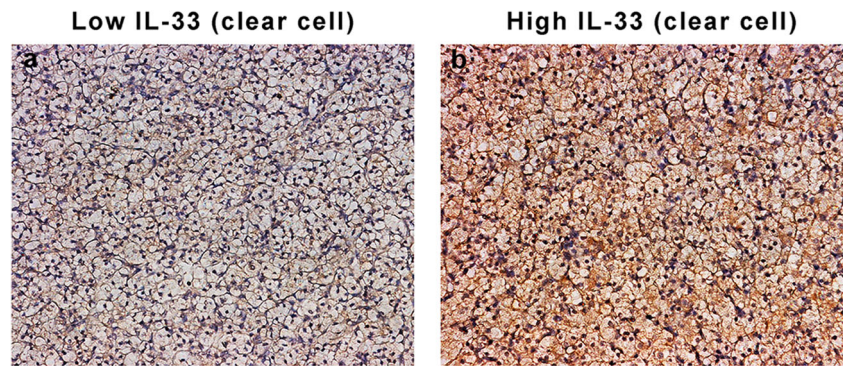
SPSS 21.0 (IBM Corporation, Armonk, NY, USA), R software version 3.0.2 and the "rms" package (R Foundation for Statistical Computing, Vienna, Austria) were applied for statistical analysis. Categorical variables were compared by Pearson χ^2 test or Fisher's exact test while continuous variables were analyzed by Wilcoxon rank-sum test or Kruskal–Wallis test. Survival curves were established using the Kaplan–Meier method, and the log-rank test was applied to compare the difference between curves. The Cox proportional hazards regression model was used to perform univariate and multivariate analyses. The Harrell's Concordance index (C-index) was used to quantify the predictive accuracy of multivariate models. All statistical tests were two-sided and were performed at a significance level of 0.05.

Results

Correlations between patients' characteristics and IL-33 expression

A total of 203 patients with ccRCC were included, and 69.5 % of them were men. As it is summarized in Table 1, the median age at surgery and tumor size were 54 years

Fig. 1 Interleukin-33 (IL-33) expression in tumor tissues from clear-cell renal cell carcinoma (ccRCC) patients. Representative microphotographs of tumor tissues with high intratumoral IL-33 expression (a) and low IL-33 expression (b). Original magnification ($\times 200$)



and 4.0 cm, respectively. Positive staining of IL-33 was mainly observed in cytoplasm and membrane (Fig. 1), and 74.4 % (151/203) of the tumor tissues were considered to have low IL-33 expression. As shown in Table 1, a high IL-33 expression was statistically associated with advanced TNM stage ($P=0.017$), high Fuhrman grade ($P<0.001$), presence of tumor necrosis ($P=0.003$), and high UISS risk-level ($P=0.025$).

Kaplan–Meier survival analysis and subgroup analysis

We applied Kaplan–Meier survival analysis to compare OS and RFS, respectively, in order to investigate the relations between IL-33 expression and patients' outcomes. Patients with synchronous nodal (N1) or distant metastasis (M1) were excluded during the analyzing of RFS. Patients with high IL-33 expression tended to have poorer

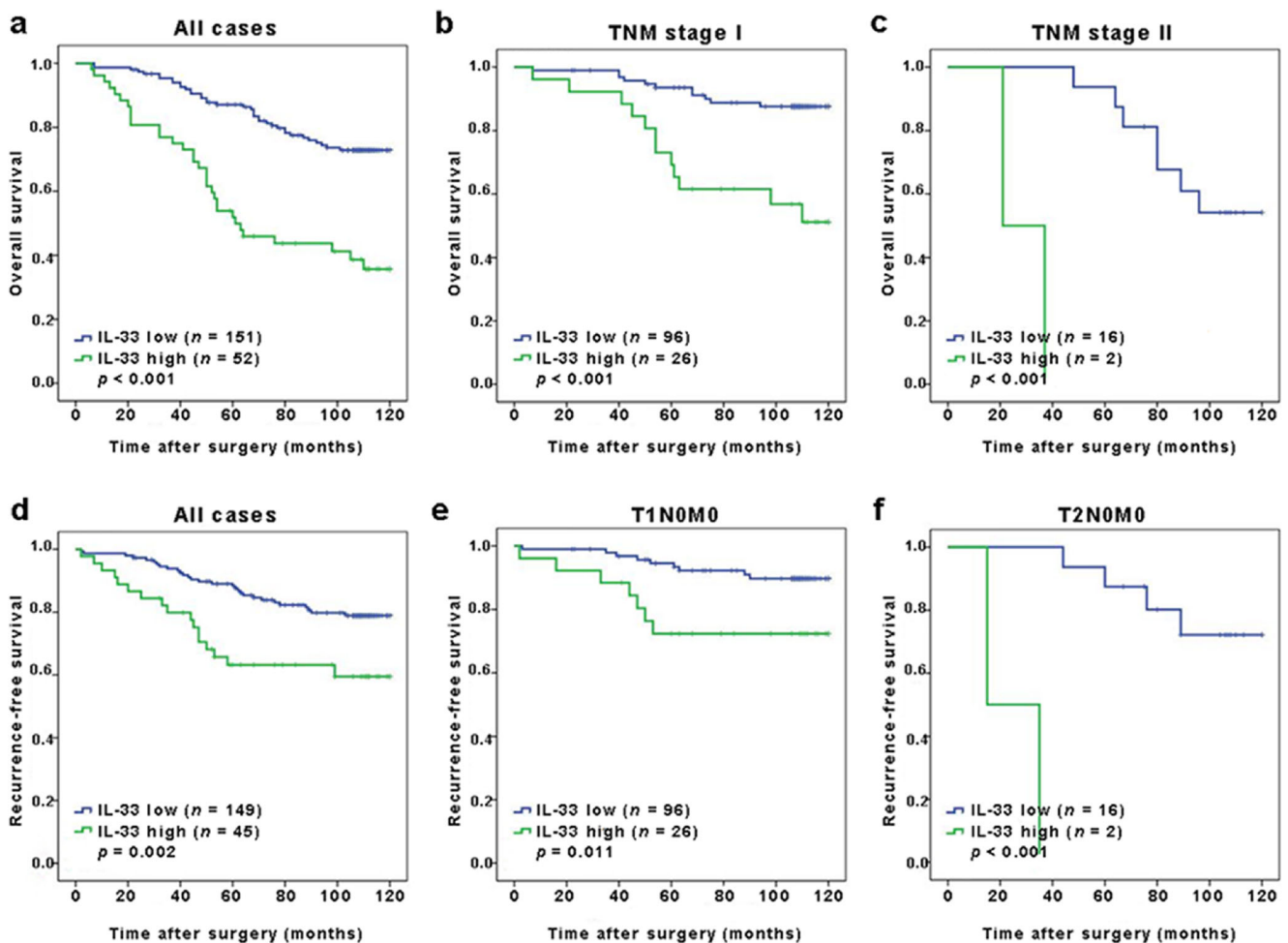


Fig. 2 Kaplan–Meier analysis of overall survival (OS) and recurrence-free survival (RFS) based on the expression of IL-33. Kaplan–Meier analysis of OS in all cases (a), patients with TNM stage I (b), and stage

II (c). Kaplan–Meier analysis of RFS in all cases (d), patients with T1N0M0 (e), and T2N0M0 (f). P value was calculated by log-rank test

OS and RFS ($P < 0.001$ and $P = 0.002$, respectively; Fig. 2a, d).

To investigate if IL-33 expression could stratify patients with different TNM stage, we performed a subgroup analysis by TNM stage. And, we found that IL-33 expression showed a strong relevance with OS in patients with TNM stage I and stage II ($p < 0.001$ for both; Fig. 2b, c) but not for patients with TNM stage III and TNM stage IV ($P = 0.083$ and $P = 0.127$, respectively; Fig. S1a, b). And, for nonmetastatic ccRCC, IL-33 expression could also be a prognostic factor for recurrence in patients with T1 stage and T2 stage ($P = 0.011$ and $P < 0.001$, respectively; Fig. 2d, e) whereas it failed in patients with T3–4 stage ($P = 0.472$; Fig. S1c)

We further performed a subgroup analysis by UISS groups. In UISS low-risk group, IL-33 expression was of prognostic value for both of OS and RFS ($P = 0.002$ for both; Fig. 3a, c). However, in UISS intermediate/high-risk group, IL-33 expression could only stratify patients for OS ($P = 0.021$; Fig. 3b), while it failed in terms of RFS ($P = 0.074$; Fig. 3d).

Next, we used univariate and multivariate Cox regression analysis to evaluate the prognostic value of IL-33. As shown in Table 2, IL-33 had strong relevance with OS and RFS

($P < 0.001$ and $P = 0.003$, respectively). Based on multivariate analysis, IL-33 remained an independent prognostic factor for OS (HR 2.050, 95 % CI 1.223–3.447, $P = 0.006$), as well as TNM stage ($P < 0.001$), Fuhrman grade ($P = 0.001$), and ECOG-PS ($P = 0.026$). However, IL-33 failed to be proven as an independent prognosticator of RFS ($P = 0.159$) (Table 2).

Extension of prognostic models with IL-33 expression

We combined IL-33 expression with TNM stage, UISS, and SSIGN scores, respectively, to assess whether the predictive accuracy could be improved for OS. The C-indices of the TNM stage, UISS, and SSIGN scores were originally 0.692, 0.666, and 0.732, respectively, and increased to 0.749, 0.730, and 0.760 when IL-33 expression was incorporated (Table 3).

Predictive nomogram for OS

Depending on the proven independent indicators consisting of TNM stage, Fuhrman grade, ECOG-PS, and IL-33 expression, we constructed a nomogram to further predict the

Fig. 3 Subgroup analysis to identify the prognostic value of IL-33 by UISS in ccRCC patients. Kaplan–Meier analysis of OS in patients classified into UISS low-risk group (a) and UISS intermediate/high-risk group (b), Kaplan–Meier analysis of RFS in patients classified into UISS low-risk group (c) and UISS intermediate/high-risk group (d). P value was calculated by log-rank test

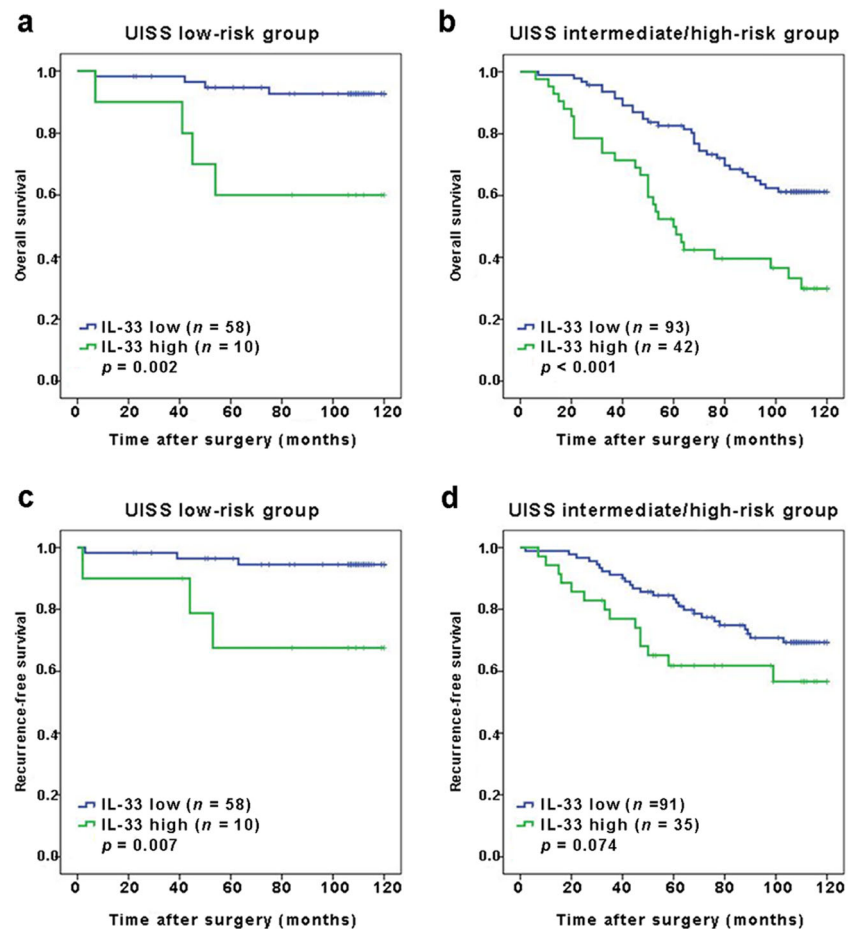


Table 2 Univariate and multivariate Cox regression analyses for overall survival and recurrence-free survival

Variables	Univariate		Multivariate	
	HR (95 % CI)	<i>p</i> value ^a	HR (95 % CI)	<i>p</i> value ^a
Overall survival (<i>n</i> = 203)				
Age (years) ^b	1.011 (0.990–1.033)	0.310		
Gender (male vs. female)	0.980 (0.588–1.643)	0.938		
Tumor size (≥5 vs. <5 cm)	2.160 (1.341–3.478)	0.002	1.421 (0.853–2.367)	0.177
Necrosis (present vs. absent)	2.194 (1.322–3.494)	0.002	1.219 (0.719–2.067)	0.462
TNM stage (III + IV vs. I + II)	3.810 (2.374–6.116)	<0.001	2.890 (1.763–4.737)	<0.001
Fuhrman grade (3 + 4 vs. 1 + 2)	2.827 (1.746–4.579)	<0.001	2.184 (1.307–3.649)	0.001
ECOG-PS (≥1 vs. 0)	2.573 (1.541–4.299)	<0.001	1.824 (1.076–3.090)	0.026
IL-33 (high vs. low)	3.439 (2.137–5.503)	<0.001	2.050 (1.223–3.447)	0.006
Recurrence-free survival (<i>n</i> = 194)				
Age (years) ^b	1.012 (0.985–1.309)	0.392		
Gender (male vs. female)	0.728 (0.400–1.325)	0.298		
Tumor size (≥5 vs. <5 cm)	1.703 (0.955–3.037)	0.073		
Necrosis (present vs. absent)	2.143 (1.177–3.192)	0.013	1.433 (0.760–2.698)	0.266
T stage (3 + 4 vs. 1 + 2)	3.606 (2.018–6.446)	<0.001	2.911 (1.593–5.322)	0.001
Fuhrman grade (3 + 4 vs. 1 + 2)	3.306 (1.667–5.528)	<0.001	2.550 (1.366–4.760)	0.003
ECOG-PS (≥1 vs. 0)	2.801 (1.498–5.274)	0.001	2.295 (1.213–4.245)	0.011
IL-33 (high vs. low)	2.461 (1.350–4.488)	0.003	1.584 (0.836–3.001)	0.159

IL-33 interleukin 33, ECOG-PS Eastern Cooperative Oncology Group performance status, CI confidence interval, HR hazard ratio

^a *p* value <0.05 is considered statistically significant

^b Factors were treated as continuous variables

outcome of the patients (Fig. 4a). The calibration plots are shown for 5- and 10-year survival (Fig. 4b, c). The C-index of the prognostic model based on TNM stage, Fuhrman stage, and ECOG-PS was 0.755, and it improved to 0.787 when the IL-33 expression was incorporated.

Discussion

To our knowledge, this is the first study to investigate the prognostic value of IL-33 expression in tumor tissues for patients with ccRCC after surgery. High level of IL-33 expression is associated with shortened survival time and increased risk of recurrence, especially for patients categorized into UISS low-risk group and TNM stage I and II. Furthermore, high IL-33 expression is proven to be an independent negative prognostic factor of OS. In addition, the incorporation of intratumoral IL-33 expression into TNM staging system, UISS, and SSIGN scores would refine the predictive accuracy of these models for OS. Thus, we consider the IL-33 expression as a potential prognostic biomarker for better individual risk stratification to select patients who need to receive adjuvant therapy and intense follow-up after surgery, even if they have a low-risk according to the traditional clinicopathologic analysis. However, IL-33 fails to demonstrate a statistically

significant association with RFS after multivariate analysis in our study. Thus, it needs to be further investigated whether IL-33 was an independent prognostic biomarker for patients' RFS in larger cohorts.

Table 3 Comparison of the prognostic accuracies of models for overall survival

Models	Overall survival (<i>n</i> = 203)	
	C-index	AIC
IL-33	0.639	685.371
TNM stage	0.692	673.067
TNM stage + IL-33	0.749	657.452
UISS	0.666	671.289
UISS + IL-33	0.730	660.541
SSIGN score	0.732	655.843
SSIGN score + IL-33	0.760	650.636
TNM + ECOG-PS + Fuhrman grade	0.755	647.552
TNM + ECOG-PS + Fuhrman grade + IL-33	0.787	642.115

An elevated C-index or a decreased AIC score means a better prognosis
C-index Harrell's concordance index, AIC Akaike information criterion, IL-33 interleukin-33, UISS University of California Los Angeles Integrated Staging System, ECOG-PS Eastern Cooperative Oncology Group performance status, SSIGN stage, size, grade, and necrosis

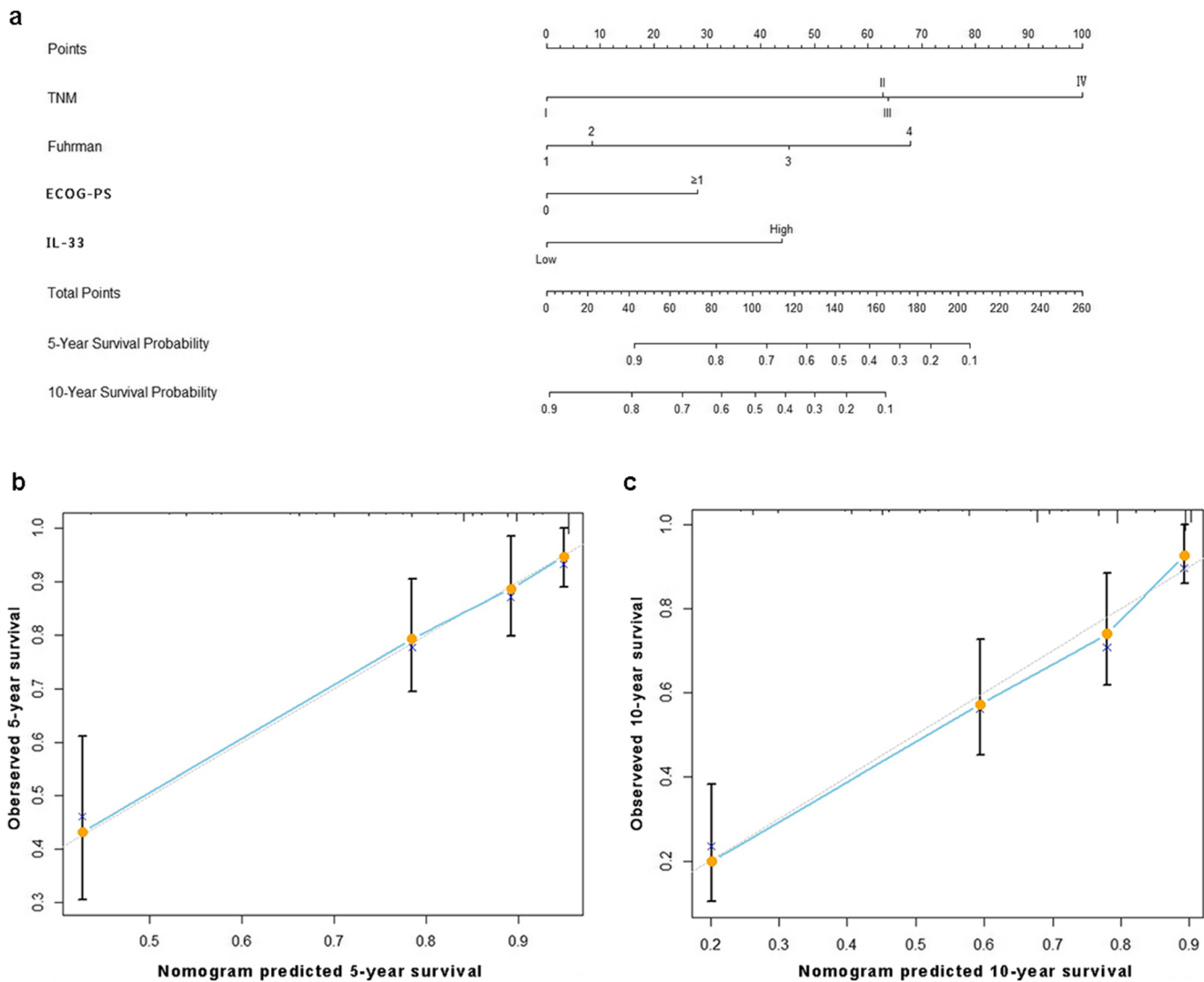


Fig. 4 Nomogram and calibration plots for the prediction of outcome in ccRCC patients. Nomogram for predicting 5- and 10-year OS of ccRCC patients after nephrectomy (a). Calibration plots of nomogram for 5-year (b) and 10-year (c) survival

Recent studies have demonstrated that IL-33 could be involved in the development and progression of many tumor types, such as breast cancer [18], myeloproliferative neoplasms [22], and gastric cancer [23]. In BALB/c mice, repeated administration of IL-33 enhanced mammary tumors progression and metastases to lungs and livers could be observed [18]. Besides, serum IL-33 may serve as a diagnostic and prognostic marker in nonsmall cell lung cancer [19]. In line with these findings, our data shows that high levels of IL-33 were remarkably correlated with advanced tumor stages and poor clinical outcomes.

One of the possible mechanisms for these phenomena may be the ability of IL-33 to induce angiogenesis and vascular permeability, which is indispensable for the growth and metastatic potential of all solid tumors. In addition, IL-33 can lead to the activation of NF- κ B by binding to its receptor ST2 [24]. An early report has revealed that NF- κ B can be an activator of anti-apoptotic

genes and induce proinflammatory cytokines which have negative effects on tumor development [25]. In this way, the downstream transduction of IL-33 signal pathway may promote certain kinds of malignancies. Moreover, IL-33 is believed to have the ability to promote Th2 immune response, which in turn can facilitate intratumoral accumulation of immunosuppressive cells, thus creating a micro-environment beneficial for tumor development and progression [18].

In addition to prognostic power, biomarkers have the potential to become therapeutic targets. One study demonstrated that the deletion of IL-33 receptor, ST2, could significantly attenuate mammary tumor growth and metastasis in the murine model [26], suggesting that targeting IL-33 signaling pathway with antagonist might be a novel and efficient anticancer therapy in the future. The potential roles of IL-33 and its antagonist in malignancies are worthy to be fully investigated.

We acknowledge that there were a few limitations in our study. First, due to the retrospective nature and no external validation, the prognostic significance of IL-33 in ccRCC patients remains to be investigated prospectively in other populations and larger cohorts. Second, the TMA technique only displays a small piece of the original tumor, and the information contained may not be typical. Third, blood samples in our study are not available, and we hope to evaluate the prognostic value of serum IL-33 in the future.

In conclusion, our study is the first to demonstrate the intratumoral IL-33 expression as an independent prognostic factor in ccRCC. High expression of IL-33 is associated with shortened survival, especially in patients with early stage of disease. When incorporated with TNM stage, UISS, and SSIGN, IL-33 could improve the prognostic accuracy. In addition, IL-33 signaling pathway might be a promising target for individualized ccRCC therapeutic strategy.””

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Compliance with ethical standards

Conflicts of interest None

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