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*Original Article*

# Decreased expression of mucin 18 is associated with unfavorable postoperative prognosis in patients with clear cell renal cell carcinoma

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**Abstract:** Background: MUC18 is correlated with tumor progression and metastasis in types of malignancy. But the role of MUC18 in clear cell renal cell carcinoma remains unclear. In this study, we aimed to investigate the expression of MUC18 and its correlation with clinical outcomes in clear cell renal cell carcinoma. Patients and Methods: Immunohistochemical staining was performed in samples from 288 patients with clear cell renal cell carcinoma. We used Kaplan-Meier method and Cox proportional hazard models to value the association between MUC18 expression and clinical outcome. Nomogram was constructed to predict overall survival at 5 and 8 years after nephrectomy. Results: MUC18 expression was significantly decreased in tumor compared to non-tumor tissue ($P<0.001$). Lower MUC18 expression in tumor predicted a shorter survival time ($P=0.007$). By multivariate cox analysis, MUC18 was defined as an independent prognostic factor ($P=0.006$). The nomogram performed better in predicting 5- and 8-year overall survival than the TNM stage alone in clear cell renal cell carcinoma. Conclusion: MUC18 is an independent prognostic factor for clear cell renal cell carcinoma and could be incorporated with the other parameters to predict 5- and 8-year overall survival for clear cell renal cell carcinoma patients.

**Keywords:** Clear cell renal cell carcinoma, mucin 18, overall survival, disease free survival, nomogram

## Introduction

Renal cell carcinoma (RCC) accounts for 3-4% of the cases in adult malignant [1] and is the seventh most common cancer in men and the ninth in women [2]. The predominant histologic subtype is clear cell renal cell carcinoma (ccRCC) [3]. There estimated to be more than 209,000 new diagnosed individuals and 102,000 deaths caused by ccRCC per year worldwide [4]. The ccRCC is the kind of tumor with an unpredictable course, even tumors have the comparable or the same histological type, they could show a wide variation in biological behavior and clinical outcomes. In the past decades, the introduction of targeted agents has excitedly improved the overall survival for the patients with advanced ccRCC, but complete responses were reported in only 1-3% cases [5, 6]. The continuous exploration of the mechanisms involved in carcinogenesis of ccRCC and seeking novel approaches to the management of ccRCC still need to study.

MUC18, also named MCAM, Mel-CAM or CD146, belongs to the immunoglobulin superfamily (IgSF) [7]. It was known as a cell adhesion molecule, and first introduced as a biomarker of tumor progression and metastasis in melanoma [8]. MUC18 is expressed in types of normal tissue, including vascular endothelium, smooth muscle, and involved in cell-cell interactions, cell migration and angiogenesis [9, 10]. Thus, deregulation of MUC18 might be relevant to the altered cellular motility, invasiveness, and epithelial-mesenchymal transition (EMT) program in tumor [11]. Among the previous studies that focused on MUC18, it has always been reported as a marker for tumor progression in certain types of cancers including melanoma [12, 13], epithelial ovarian cancer [14], and prostate cancer [15]. However, MUC18

appears to act as a tumor suppressor in nasopharyngeal carcinomas [16] meanwhile its role in breast cancer remains controversial [11, 17].

Currently, whether MUC18 participated in ccRCC carcinogenesis and progression is still unclear. There are few studies about the correlation between MUC18 expression and the clinical outcomes in ccRCC. In the present study, we aimed to evaluate the prognostic significance of MUC18 and the correlation between MUC18 and the prognostic parameters in ccRCC. We integrated T stage, N stage, M stage, ECOG PS, and MUC18 into the nomogram, to predict 5- and 8-year overall survival in the patients with ccRCC.

## Patients and methods

### *Patients*

We searched the patients that received nephrectomy at Zhongshan Hospital Fudan University, Shanghai, China, during 2005 to 2007. We eventually recruited 288 cases who had no history of previous anticancer therapy or other malignancies, and of which the histopathological type proved to be ccRCC. Cases that could not be classified as pure clear cell carcinomas either because they were mostly necrosis or because they demonstrated morphologic features of both clear cell type and other RCC types were excluded from the present study. All the clinicopathological and baseline demographic characteristics were collected retrospectively, including age, gender, tumor size, Fuhrman grade, necrosis and tumor stage. Patients' TNM stage were reassessed according to the 2010 AJCC TNM classification [18] by two independent pathologists. All patients underwent regular follow up every 6 months or earlier for the first 2 years right after the nephrectomy and every 12 months thereafter. The median follow up time was 99 months, ranging from 2.63 to 120.47 months. This study was approved by the Zhongshan Hospital ethics committee. Informed consent was obtained from all the individual participants in the study.

### *Immunohistochemical staining*

For each patient, sections were prepared from areas of tumor representing the dominant focus of ccRCC. Primary antibody was rabbit polyclonal anti-MUC18 antibody (ab174326, Abcam, Cambridge, MA, USA). The images of stained tissue were captured by the computerized image system composed of an Olympus CCD camera connected to a Nikon eclipse Ti-s microscope. The immunohistochemistry samples were scanned at high-power magnification (×200) and photographed by NIS-Elements F3.2 software. We identified three independent microscopic fields with the strongest staining of each slide to ensure representativeness and homogeneity, and all the photos were took under the same circumstance.

Integrated optical density (IOD), calculated by Image-Pro Plus version 6.0 software (Media Cybernetics Inc., Bethesda, MD, USA) was used as the parameter of the immunostaining density of MUC18. The mean IOD of three selected areas was regarded as the expression intensity of MUC18 in the corresponding tissue. The cut point of high expression or low expression was determined by X-tile software (Version 3.6.1).

### *Statistic analysis*

The purpose of the study is to determine the association between MUC18 expression and clinical outcomes in ccRCC patients who received nephrectomy. The overall survival (OS) was defined from date of surgery to date of death or last follow-up, and the disease-free survival (DFS) was defined from the date of surgery to the date of local or distant progression, death as a result of any cause, or the date of last follow-up. Paired t-test is used to compare the MUC18 expression between tumor and non-tumor tissue. The association between MUC18 expression and clinical characteristics were compared by Fisher's exact test or $\chi^2$ test or t test. Survival curves for OS and DFS were constructed using Kaplan-Meier method. OS and DFS for each group with low and high stained density were compared using the log-rank test. Cox proportional hazard models were used for univariate and multivariate analysis to estimate and test demographic characteristics and clinical features for their associations with overall survival (OS). Hazard ratio (HR) and 95% CI for survival outcomes were calculated. In the multivariate Cox model, variables with $P<0.05$ from the univariate model were included. All statistical analyses above were performed

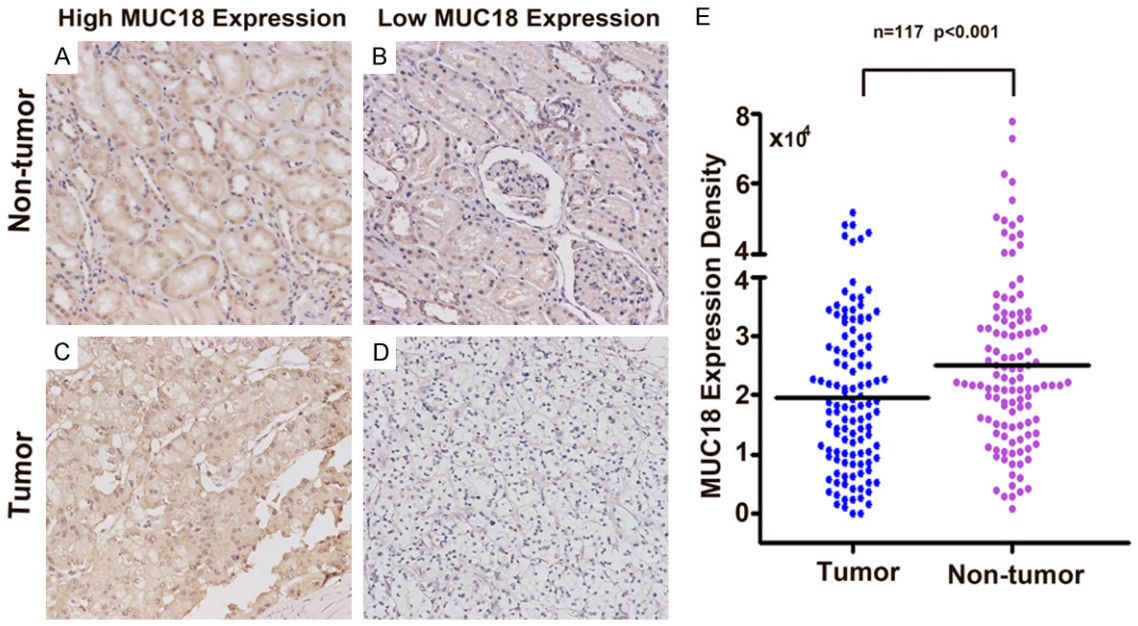


**Figure 1.** Immunohistochemical analysis of MUC18 expression in ccRCC. A: High MUC18 expression in non-tumor tissue; B: Low MUC18 expression in non-tumor tissue; C: High MUC18 expression in tumor tissue; D: Low MUC18 expression in tumor tissue; E: IOD score of MUC18 expression in tumor and non-tumor tissue. *P* value, was calculated by paired t-test, <0.05 was regarded as statistically significant. Magnification 200×.

using SPSS version 19.0 (SPSS Inc., IL, Chicago, USA). The Nomogram was constructed by R software with "rms" package (R Foundation for Statistical Computing, Vienna, Austria). Calibration plot was used to examine the accuracy of the predictive nomogram. The Harrell's concordance indices (c-indices) were used to measure the prognostic efficiency. All results were considered significant at two-sided P<0.05 level.

## Results

### *MUC18 expression in human ccRCC*

The immunohistochemical staining of MUC18 was predominantly located on the cell membrane and/or in the cytoplasm in tumor tissue and nephric tubule in normal kidney tissue. The representative images for high or low immunostaining of MUC18 were exhibited in **Figure 1A-D**. We paired up 117 tumor and non-tumor tissues in all, and the immunostaining density revealed a significantly decreased level between tumor and non-tumor tissues (*P*<0.001). The mean IOD was 19712.10 (rang 34.01-51847.90) in tumor and 24962.04 (rang 728.45-77788.66) in non-tumor tissue (**Figure 1E**).

### *Correlation between MUC18 expression and clinicopathological characteristics*

We analyzed a total of 288 patients in the present study. As **Table 1** presented, the mean (SD) age was 55.6 (13.1) years old and the median follow up was 99 (range 2.63-120.47) months. Fifteen patients had metastasis at time of surgery. There were significantly associations between MUC18 expression status with age (*P*=0.013) and gender (*P*=0.015).

### *Prognostic value of MUC18 expression in ccRCC*

The survival outcomes of the patients classified into two subgroups according to MUC18 expression were illustrated in **Figure 2**. Overall survival rate (**Figure 2A**) was significantly different in two subgroups (P=0.007). We further analyzed the OS in TNM I+II and TNM III+IV patients; both showed an unfavorable OS in low MUC18 expression subgroup (*P*=0.032, *P*=0.177) (**Figure 2C** and **2E**), although the difference was not statistically significant in TNM III+IV patients (*P*=0.177). The disease free survival analysis was not statistically significant either in all (**Figure 2B**), TNM I+II (**Figure 2D**) or

**Table 1.** Associations between MUC18 expression and clinicopathological characteristics in patients with ccRCC

| Characteristics | All patients | MUC18 Expression | | |
|---|---|---|---|---|
| | | High | Low | P* |
| Age (years) | | | | 0.013 |
| Mean ± SD | 55.6±13.1 | 53.6±12.7 | 57.4±13.1 | |
| Gender | | | | 0.015 |
| Female | 87 | 31 | 56 | |
| Male | 201 | 103 | 99 | |
| T stage | | | | 0.278 |
| T1 | 183 | 89 | 94 | |
| T2 | 25 | 8 | 17 | |
| T3 | 76 | 34 | 42 | |
| T4 | 4 | 3 | 1 | |
| N stage | | | | 1.000 |
| N0 | 286 | 133 | 153 | |
| N1 | 2 | 1 | 1 | |
| M stage | | | | 0.113 |
| M0 | 273 | 130 | 143 | |
| M1 | 15 | 4 | 11 | |
| TNM stage | | | | 0.378 |
| I | 177 | 87 | 90 | |
| II | 21 | 8 | 13 | |
| III | 73 | 34 | 39 | |
| IV | 17 | 5 | 12 | |
| Fuhrman nuclear grade | | | | 0.251 |
| 1 | 30 | 17 | 13 | |
| 2 | 215 | 95 | 120 | |
| 3 | 41 | 20 | 21 | |
| 4 | 2 | 2 | 0 | |
| Necrosis | | | | 0.726 |
| Absent | 267 | 125 | 142 | |
| Present | 21 | 9 | 12 | |
| ECOG PS | | | | 0.408 |
| 0 | 206 | 90 | 115 | |
| 1 | 52 | 27 | 26 | |
| 2 | 25 | 15 | 10 | |
| 3 | 5 | 2 | 3 | |

Abbreviations: ECOG PS: Eastern Cooperative Oncology Group performance status; CI: confidence interval. *$\chi^2$ test or t test was performed. *P*<0.05 was regard as statistically significant.

TNM III+IV (**Figure 2F**) patients, the *P* values were 0.165, 0.204 and 0.586, respectively.

We also explored the prognostic impact of MUC18 in ccRCC, **Table 2** presents the results of the univariate and multivariate survival analyses that included gender, Fuhrman nuclear grade, necrosis, T stage, N stage, M stage, ECOG PS and MUC18 expression. In the univariate analysis, Fuhrman nuclear grade (*P*=0.007), necrosis (*P*=0.010), T stage (*P*<0.001), M stage (*P*<0.001), ECOG PS (*P*<0.001), and MUC18 expression (*P*=0.008) showed a significant correlation with risk of death. We included variables with *P*<0.05 from univariate analysis in the multivariate cox model, and the multivariate model confirmed that T stage (*P*=0.001), M stage (*P*=0.006), ECOG PS (*P*=0.010) and MUC18 expression (*P*=0.012) were independent prognostic factors for OS in ccRCC.

*Nomogram for predicting overall survival in ccRCC*

Furthermore, we constructed a nomogram to predict OS at 5 and 8 years after nephrectomy (**Figure 3A**). The prognostic factors incorporated in the nomogram were T stage, N stage, M stage, ECOG PS and MUC18 expression. Total points were calculated to evaluate the clinical outcomes, with higher point indicating more adverse outcomes probability. Calibration plots were used to examine the accuracy of the predictive nomogram and the nomogram performed well with the ideal model (**Figure 3B**, **3C**). The Harrell's c-index for the nomogram, which indicated the efficiency of the prognostic model, was 0.722 (95% CI 0.668-0.775) higher than that of the TMN stage (0.656, 95% CI 0.600-0.712), which suggest a better performance of nomogram in predicting OS in ccRCC.

## Discussion

In the present study, we revealed the relation between MUC18 expression with the clinical

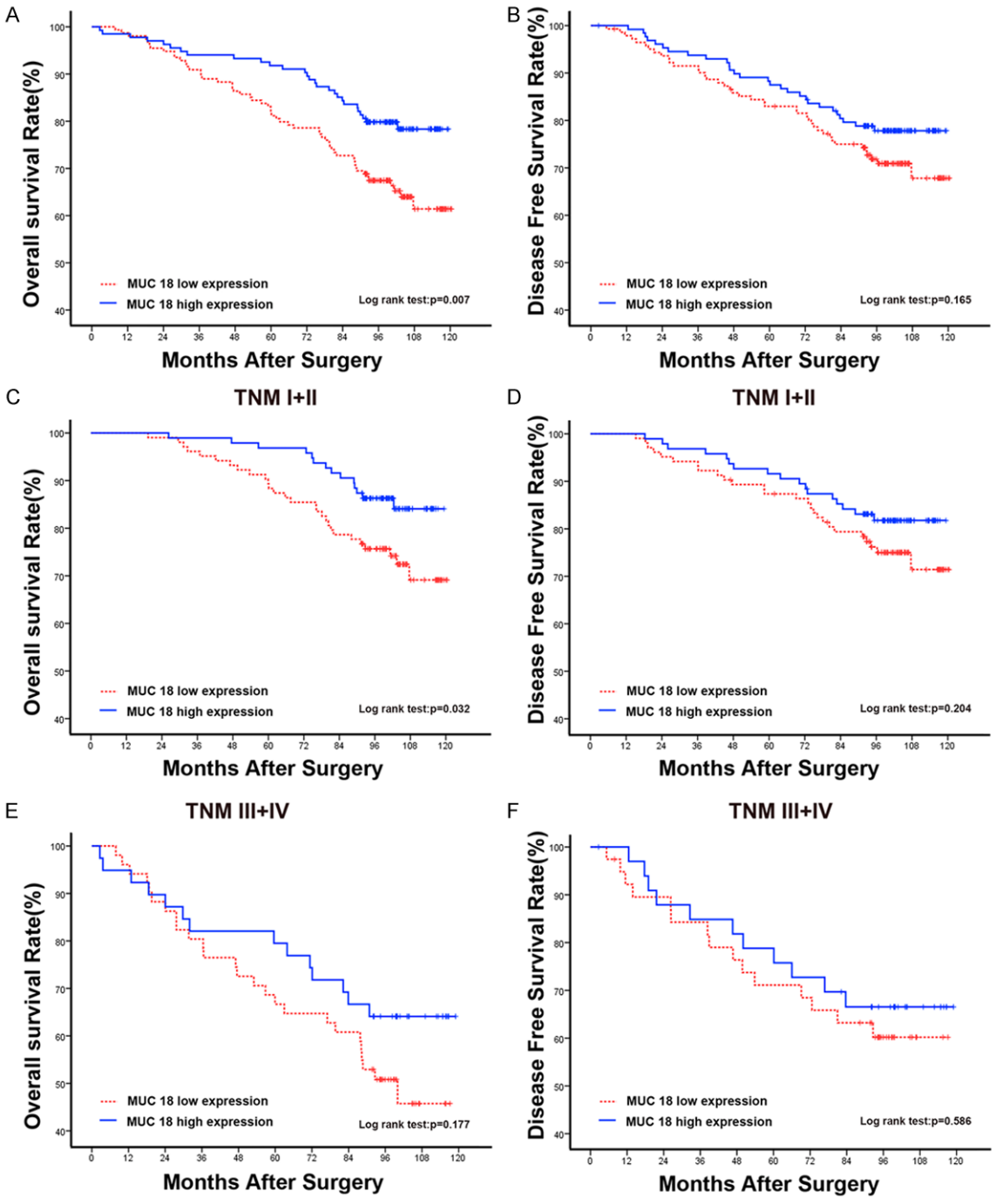


**Figure 2.** Kaplan-Meier analysis for overall survival and disease free survival of patients with ccRCC according to MUC18 expression. Overall survival according to tumor MUC18 expression in all (A), TNM I+II (C) and TNM III+IV (E) patients; Disease free survival according to tumor MUC18 expression in all (B), TNM I+II (D) and TNM III+IV (F) patients. *P* value, calculated by Log rank test, <0.05 was regarded as statistically significant.

outcomes in ccRCC for the first time. Our results have clearly demonstrated the value of MUC18 as a prognostic predictor in patients with ccRCC, especially those with low-stage disease. It proved to be an independent prognostic factor as compared in a multivariate analysis to the different classic predictive histologic characteristics of the tumor. The nomogram we

**Table 2.** Univariate and multivariate cox regression analyses for overall survival in ccRCC patients

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P* | HR | 95% CI | P |
| Gender | | | 0.793 | | | |
| Female | 1 | reference | | | | |
| Male | 1.066 | 0.662-1.715 | | | | |
| Fuhrman nuclear grade | | | 0.007 | | | 0.381 |
| 1 | 1 | reference | | 1 | reference | |
| 2 | 3.058 | 0.959-9.756 | 0.059 | 2.081 | 0.644-6.726 | 0.221 |
| 3 | 6.032 | 1.783-20.400 | 0.004 | 2.979 | 0.815-10.895 | 0.099 |
| 4 | 8.336 | 0.866-80.287 | 0.067 | 1.878 | 0.155-22.760 | 0.621 |
| Necrosis | | | 0.010 | | | 0.310 |
| Absent | 1 | reference | | 1 | reference | |
| Present | 2.306 | 1.220-4.357 | | 1.504 | 0.685 -3.302 | |
| T stage | | | <0.001 | | | 0.001 |
| T1 | 1 | reference | | 1 | reference | |
| T2 | 4.931 | 2.392-8.060 | <0.001 | 3.428 | 1.836-6.401 | <0.001 |
| T3 | 2.344 | 1.432-3.837 | 0.001 | 1.885 | 1.111-3.196 | 0.019 |
| T4 | 11.111 | 3.400-36.304 | <0.001 | 3.694 | 0.741-18.408 | 0.111 |
| N stage | | | 0.590 | | | |
| N0 | 1 | reference | | | | |
| N1 | 1.720 | 0.239-12.366 | | | | |
| M stage | | | <0.001 | | | 0.006 |
| M0 | 1 | reference | | 1 | reference | |
| M1 | 4.848 | 2.564-9.164 | | 2.938 | 1.372-6.294 | |
| ECOG PS | | | <0.001 | | | 0.010 |
| 0 | 1 | reference | | 1 | reference | |
| 1 | 1.340 | 0.773-2.325 | 0.297 | 1.286 | 0.699-2.367 | 0.418 |
| 2 | 2.036 | 1.060-3.912 | 0.033 | 2.177 | 1.094-4.330 | 0.027 |
| 3 | 7.741 | 2.777-21.578 | <0.001 | 4.527 | 1.556-13.171 | 0.006 |
| MUC18 expression | | | 0.008 | | | 0.012 |
| Low | 1 | reference | | 1 | reference | |
| High | 0.541 | 0.343-0.854 | | 0.529 | 0.322-0.867 | |

Abbreviations: ECOG PS: Eastern Cooperative Oncology Group performance status; CI: confidence interval; HR: hazard ratio.
*Data obtained from the Cox proportional hazards model; $P<0.05$ was regard as statistically significant.

constructed showed a more efficient value in predicting clinical outcomes than the TNM stage alone. While the reversal of the T2 and T3 stage cannot be ignored, which might due to the relatively larger tumor size and burden in T2 stage compared with T3 in ccRCC patients.

The ccRCC is characterized by VHL gene inactivation and subsequent abnormal production of tumor derived VEGF [19], and an important feature of ccRCC is its increased vascularity. It is well acknowledged that the wealthy of tumor angiogenesis is indispensable for solid tumor growth and metastasis. There is plenty of evidence show that higher microvessel density (MVD) in various solid malignant tumors indicates a higher risk of developing metastases, as well as a worse overall survival [20]. The abundance of tumor microvasculature provides the malignant cell access to distant dissemination, which is known as metastasis [21]. However, case may be different in ccRCC, which is in contrast with other tumors where MVD is strongly associated with more aggressive behaviors [22-24]. In ccRCC, higher tumor MVD was reported to be associated with lower tumor grade and improved patient survival [25]. Esin Yildi's study also showed a higher MVD was cor-

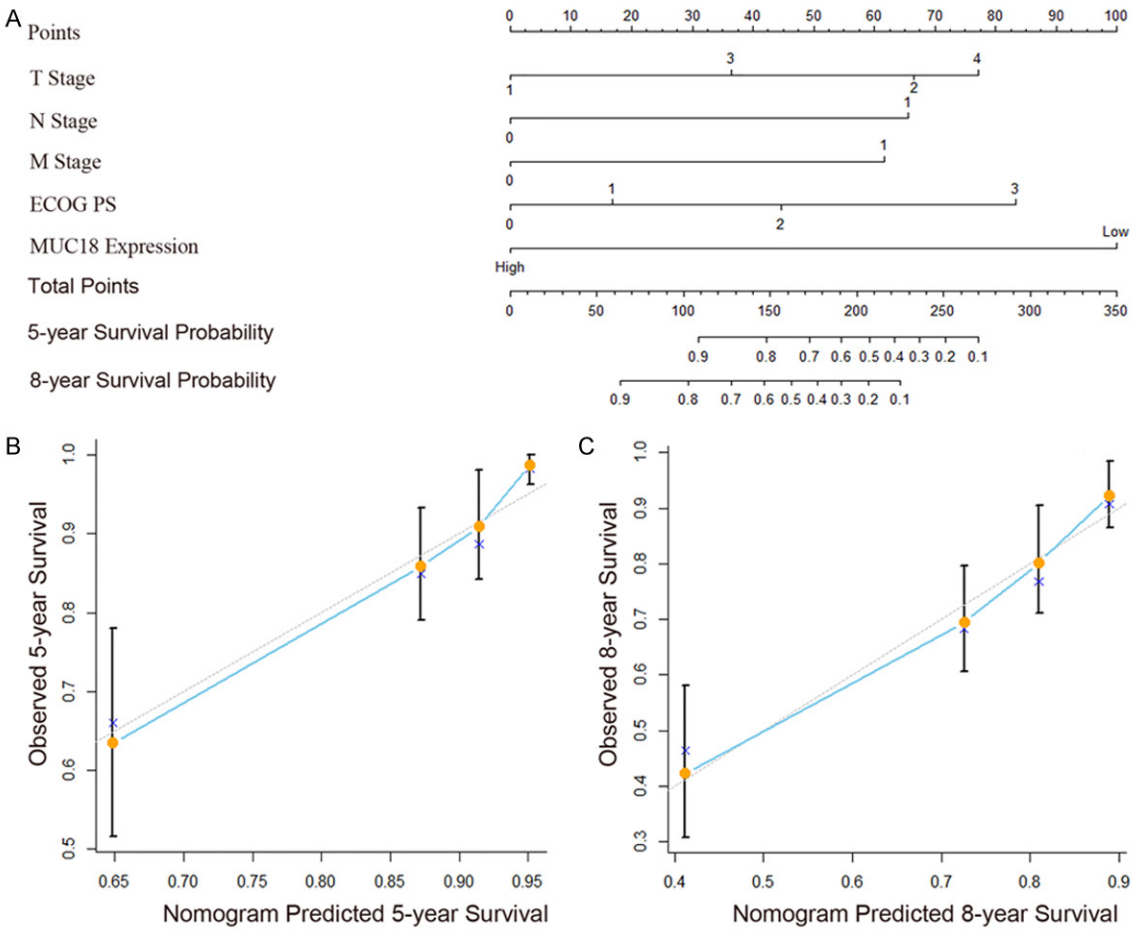


**Figure 3.** Nomogram for predicting 5- and 8-year overall survival in patients with ccRCC. A: Nomogram for predicting clinical outcomes integrated with T stage (1/2/3/4), N stage (0/1), M stage (0/1), ECOS PS (1/2/3), and MUC18 expression level (Low/High); B: Calibration plot for nomogram predicted and observed 5-year survival rate; C: Calibration plot for nomogram predicted and observed 8-year survival rate. Line of dashes: ideal model; vertical bars, 95% confident interval. A higher total point indicated a more adverse outcome probability.

related with lower risk clinicopathological characteristics in RCC [26].

The reason why abundance of vascularity in ccRCC indicated a favor prognosis is still unclear. It is something of a paradox that tumor progression would destroy the very blood supply supporting the continuous growth. On the one hand, angiogenesis is the key step for the growth of tumor, and the agents targeted on the angiogenesis progress have shown a certain effect in ccRCC. On the other hand, with impaired blood supply, tumor cells suffered insufficient nutrient and metabolite exchange causing the necrotic of tumor cells, which is regarded as histological poor prognostic factors for ccRCC [27]. Rakesh K. Jain hypothesized that tumors might evade the immune system, strengthen the invasive and metastatic potential due to the impaired blood supply [28], which was coincident with the notion of hypoxia's linkage with the tumor metastasis and poor clinical outcomes [29]. Hypoxia might induce tumor cells experience natural selection in the circumstance of hypoxia, by which cells with higher malignancy survive hypoxia, become more aggressive and adapt to the stressful oxygen-deficient growing conditions. In a well vascularize condition, tumor cells just proliferate slowly as a mass lesion instead of becoming hypoxic and necrotic, which result in acquiring the access to vascular system, leading to tumor dissemination [25].

Increasing evidence has emerged that MUC18 could play a crucial role in angiogenesis.

MUC18 was over expressed in tumor blood vessels, and up-regulation of MUC18 showed a close association with tumor angiogenesis [30]. By knocking down MUC18, there appeared a hindered vascular development [31]. In invasive micropapillary carcinoma of the breast, MUC18 expression in tumor cells was highly correlated with MVD [32]. The direct evidence for MUC18 in tumor angiogenesis is MUC18's interaction with VEGFR-2 in endothelial cells and function as a co-receptor for VEGFR-2 [33]. It was also illustrated that the different VEGFR-2 co-receptors could trigger different downstream signals, resulting in different cell functions. Therefore, it is of particular interest to elucidate the function of MUC18 as the co-receptor of VEGFR-2.

A recent research found that vascular promotion treatment led to a reduced cancer growth and metastasis, suggesting anti-angiogenesis was not the one-way therapy to inhibit the tumor [34]. A brand new strategy that balances pro- and anti-angiogenesis can be used to deal with malignance diseases some day. At present, the angiogenesis role of MUC18 in ccRCC is far from fully elucidation and need further exploration in the future. Limitations of our study are the retrospective design and relatively small study cohort. A multicenter and prospective study is needed to validate the results.

In conclusion, we have identified MUC18 as an independent prognosticator in ccRCC and could be incorporated with T stage, N stage, M stage and ECOG PS to give a better stratification for patients with ccRCC after surgery.

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## Disclosure of conflict of interest

None.

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