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# Low Expression of Mucin-4 Predicts Poor Prognosis in Patients With Clear-Cell Renal Cell Carcinoma

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**Abstract:** Mucin-4 (MUC4), a member of membrane-bound mucins, has been reported to exert a large variety of distinctive roles in tumorigenesis of different cancers. MUC4 is aberrantly expressed in clear-cell renal cell carcinoma (ccRCC) but its prognostic value is still unveiled. This study aims to assess the clinical significance of MUC4 expression in patients with ccRCC.

The expression of MUC4 was assessed by immunohistochemistry in 198 patients with ccRCC who underwent nephrectomy retrospectively in 2003 and 2004. Sixty-seven patients died before the last follow-up in the cohort. Kaplan–Meier method with log-rank test was applied to compare survival curves. Univariate and multivariate Cox regression models were applied to evaluate the prognostic value of MUC4 expression in overall survival (OS). The predictive nomogram was constructed based on the independent prognostic factors. The calibration was built to evaluate the predictive accuracy of nomogram.

In patients with ccRCC, MUC4 expression, which was determined to be an independent prognostic indicator for OS (hazard ratio [HR] 3.891; $P < 0.001$), was negatively associated with tumor size ($P = 0.036$), Fuhrman grade ($P = 0.044$), and OS ($P < 0.001$). The prognostic accuracy of TNM stage, UCLA Integrated Scoring System (UISS), and Mayo clinic stage, size, grade, and necrosis score (SSIGN) prognostic models was improved when MUC4 expression was added. The independent prognostic factors, pT stage, distant metastases, Fuhrman grade, sarcomatoid, and MUC4 expression were integrated to establish a predictive nomogram with high predictive accuracy.

MUC4 expression is an independent prognostic factor for OS in patients with ccRCC.

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**Abbreviations**: AIC = Akaike information criteria, C-index = Harrell concordance index, ccRCC = clear-cell renal carcinoma, ECOG PS = Eastern Cooperative Oncology Group performance status, EGF = epidermal growth factor, HER2/ErbB2 = human epidermal growth factor receptor 2, HR = hazard ratio, MUC4 = mucin-4, MVI = microvessel invasion, OS = overall survival, RCC = renal cell carcinoma, SSIGN = Mayo clinic stage size grade and necrosis score, TMA = tissue microarrays, UISS = UCLA Integrated Scoring System.

## INTRODUCTION

Renal cell carcinoma (RCC) accounts for 2% to 3% of all adult malignancies, constituting more than 90 % primary tumor arising from kidney.[1] It would account for an estimated 61,560 new patients and 14,080 deaths in the United States in 2015, and worse still, the incidence is still increasing.[2] Clear-cell renal carcinoma (ccRCC) is the most common histology subtype of RCC, which represents 80% to 90% of all the RCC patients.[3] Surgery is the mainstay of treatment for localized RCC. However, approximately 30% of patients undergoing nephrectomy experience local recurrence or distant metastasis, often leading to a poor prognosis. Although several existing prognostic systems and algorithms have been developed with well-performed prognostic ability, they still need to be improved. Combining the conventional models with molecular biomarkers could provide more individualized prognostic stratification based on molecular characteristics of the tumor. And unlike many tumors, RCC responds poorly to the chemotherapy and radiotherapy while it is sensitive to immunotherapy.[4] Thus, more efforts should be made to explore of RCC biomarkers, which might improve the current prognostic models and provide a new target for immunotherapy.

Mucins are heterogeneous family of large *O*-glycoproteins. They are composed of a long peptidic chain called apomucin on which are linked hundreds of oligosaccharidic chains. The family is subdivided into 3 structural and functional classes: secreted and gel-forming mucins (MUC2, MUC5AC, MUC5B, and MUC6), membrane-bound mucins (MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC15, MUC16, and MUC17), and soluble mucins (MUC7, MUC8, and MUC9).[5] They are produced by different kinds of glandular epithelial cells of respiratory, gastrointestinal, and urogenital tracts to be involved into protection of cells from distinctive kinds of injury,[6] renewal, and differentiation of epithelium and the modulation of cell adhesion and cell signaling.[7] Moreover, mucins have become the molecules of interest

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for its prognostic value and as therapeutic target for various cancer in recent years.[8–10]

Mucin-4 (MUC4), normally expressing in normal stomach, ovary, salivary gland, colon, and lung, is a membrane-bound mucin located at chromosome locus 3q29.[11] With its epidermal growth factor (EGF)-like domains, MUC4 acts as a modulator of the human epidermal growth factor receptor 2 (HER2/ErbB2) receptor tyrosine kinase and facilitate tumorigenesis and/or tumor growth in pancreatic carcinoma[12] and gallbladder carcinoma.[13] The prognostic significance of MUC4 expression is tissue-dependent and varies with the type of malignancy. For example, it has been shown that MUC4 expression was related to aggressive tumor behavior or a poor outcome in lung adenocarcinoma,[14] ovarian cancer,[15] and breast cancer.[16] On the other hand, its expression was associated with better patients' survival in mucoepidermoid carcinomas of the salivary gland,[17] prostate cancer,[18] and bladder cancer.[19] As a promising molecular involved in the numerous malignancies progression, to date, MUC4 has not been studied about its prognostic and therapy value in ccRCC. Recently, it was revealed that MUC4 gene was significantly mutated in whole-exome analysis of ccRCC compared to the normal tissue,[20] which indicated that MUC4 might be a pivotal molecule involved in the tumorigenesis of ccRCC.

In this study, we have examined the expression pattern of MUC4 in ccRCC tissues to study its potential utility as a diagnostic marker and to develop a better understanding of its role in ccRCC.

## PATIENTS AND METHODS

### Patients

The Research Medical Ethics Committee of Fudan University approved this study. In total, 198 patients with clear-cell renal cell carcinoma (ccRCC) who underwent nephrectomy (radical or partial) at Zhongshan Hospital, Shanghai, China between 2003 and 2004 were estimated. The patients included were those who had undergone surgery alone as a therapeutic intervention across all tumor stages (stage I–IV) with confirmed postoperative histopathology diagnosis. This retrospective cohort was a consecutive patient population. Patients who had surgical margin involvement, unspecified tumor location, multiple primary malignancy within the kidney; patients who received preoperative neoadjuvant and/or postoperative adjuvant therapy, and patients died within the first month after surgery, lost the follow-up data, suffered from bilateral renal cancer, mixed type RCC and/or familial RCC were excluded. The following clinicopathological information of each patient was collected: age, gender, tumor size, pT stage, pN stage, presence of distant metastasis, TNM stage, Fuhrman grade, histological tumor necrosis, histological microvessel invasion (MVI), the Eastern Cooperative Oncology Group performance status (ECOG PS) and the presence of histological sarcomatoid. The postoperative pathological data and radiographic reports from each patient was estimated and redistributed according to 2010 AJCC TNM classification. ECOG PS score was evaluated to each patient when disease was diagnosed. Physical examination, laboratory studies, chest imaging and abdominal ultrasound or CT scan were performed postoperatively every 6 months for the first 2 years and 12 months for the next 5 years. Overall survival (OS) was calculated from the date of surgery to the most recent follow-up or the day of death. Median follow-up was 106 months which ranged from 11 to 120 months.

### Western Blot and Immunohistochemistry

Western blot was performed as described previously.[21] We constructed tissue microarrays (TMA) in this study with duplicate 1.0-mm tissue cores from 2 different areas. The MUC4 expression level between 2 tissues cores in most specimens were of excellent concordance. Anti-MUC4 antibody (diluted 1:300; Abcam, Cambridge, UK) was adopted for immunohistochemistry staining and Western blot. As presented in Document S1, http://links.lww.com/MD/A918, all the cases were stained at once and the mean score of the 2 corresponding cores was adopted. All slides were analyzed using Nikon Eclipse Ti Microscope (Nikon Corporation, Tokyo, Japan) and Leica DM6000 B (Leica Microsystems, Wetzlar, Germany). Two independent uropathologists who were blinded to the clinicopathological data evaluated and scored the staining intensity of specimens. A semiquantitative H score was used by multiplying the staining intensities (0: negative, 1: weak staining, 2: moderate staining, and 3: strong staining) and distribution areas (0–100) for each sample. The score ranged from 0 to 300.

### Statistical Analysis

MedCalc software (version 11.4.2.0; MedCalc, Mariakerke, Belgium) and Stata 12.0 (StataCorp, College Station, TX) were used to perform statistical analysis in the study. OS curves of subgroups were calculated with Kaplan–Meier method and log-rank test. Categorical data were analyzed by $\chi^2$ test, while numerical data were analyzed by Student *t* test. Hazard ratios (HRs) and 95% confidence intervals were calculate by univariate and multivariate Cox proportional hazard models, while HRs and 95% confidence intervals (CIs) of subgroups were calculated by univariate Cox proportional hazard model. The prognostic accuracy of the prognostic models was assessed by Harell concordance index (C-index). Moreover, we performed the nomogram and calibration with the R programming language version 3.2.2 with the "rms" package (R Foundation for Statistical Computing, Vienna, Austria). All *P* values in the study were 2-tailed with differences and considered significant at values of $P < 0.05$.

## RESULTS

### Immunohistochemical Finding

In order to confirm whether the expression of MUC4 is associated with the tumor development and progression in ccRCC, firstly we evaluated the MUC4 expression by IHC analysis in tumor tissue specimens from 198 ccRCC patients. A semiquantitative H score was adopted as previously described. As shown in Figure 1, specific staining was noted with variable staining intensity in different specimens (score ranges from 20 to 240). Median score (92) was adopted to dichotomize all samples into low expression group and high expression group. The score ranged of low expression group was 20 to 92 (Figure 1A, n = 101), and the score ranged of high expression group was 93 to 240 (Figure 1B, n = 97). Four preserved clinical tissue specimens were used to conduct the comparison of results between IHC and immunoblot analysis to further identify the specificity of MUC4 antibody. High concordance of the results between IHC and immunoblot was presented as Figure S1, http://links.lww.com/MD/A918. That is, the high staining MUC4 specimen correlated with high expression by immunoblot, and vice versa.

### Correlation of MUC4 Expression With Clinicopathological Factors of ccRCC Patients

Totally, 198 patients [137 men (69.2%) and 61 women (30.8%)] who were aged between 26 and 80 years (median 54

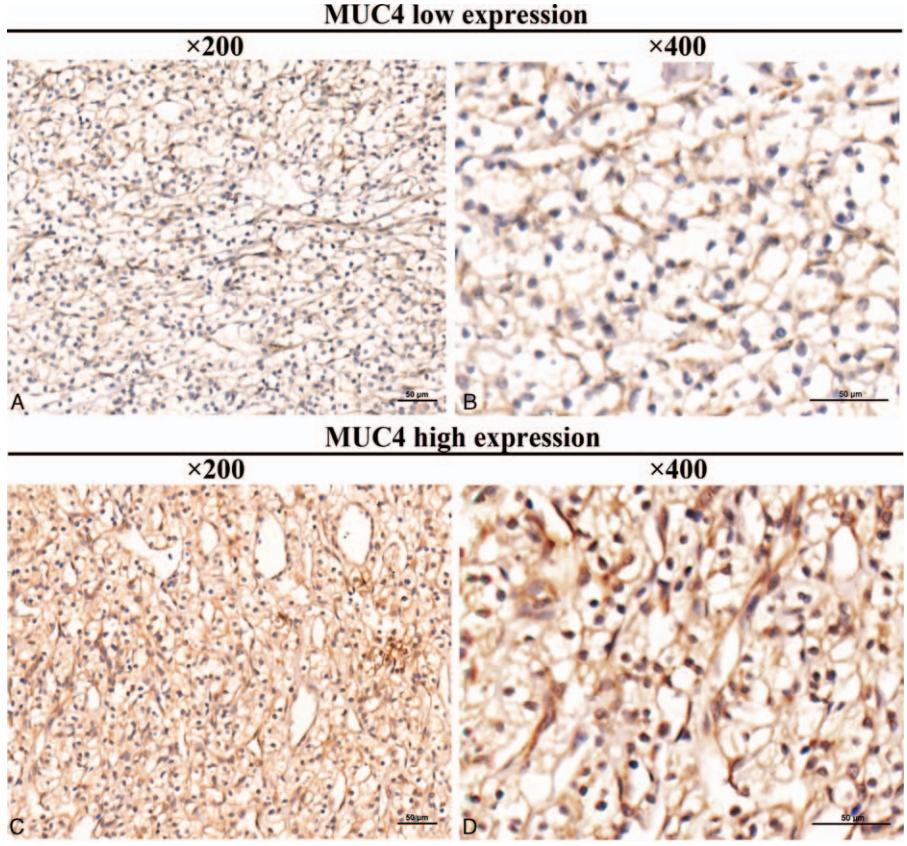


**FIGURE 1.** Mucin-4 (MUC4) expression in clear-cell renal cell carcinoma (ccRCC) tissues. (A) Representative MUC4 immunohistochemical image of low MUC4 expression in ccRCC tissue at 200 × optical magnification. (B) Representative MUC4 immunohistochemical image of low MUC4 expression in ccRCC tissue at 400 × optical magnification. (C) Representative MUC4 immunohistochemical image of high MUC4 expression in ccRCC tissue at 200 × optical magnification. (D) Representative MUC4 immunohistochemical image of high MUC4 expression in ccRCC tissue at 400 × optical magnification. Scale bar: 50 μm.

years) were included in this study as described in Table 1. Tumor size ranged from 1.0 to 18.0 cm (median 4.0 cm), and histological necrosis was observed in 48 (24.2 %) patients. Moreover, lymph node or distant metastasis was presented in 8 patients at the time of surgery. Among all cases, the patients distribution of TNM stage I, II, III, and IV was 121, 18, 52, and 7, respectively (61.1%, 9.1%, 26.3%, and 3.5%) while the patients proportion of Fuhrman grades 1, 2, 3, and 4 was 31, 85, 53, and 29, respectively (15.7%, 42.9%, 26.8%, and 14.6%). And 33 (16.7%) patients were assessed as ECOG PS $\geq 1$.

The association between MUC4 expression levels and clinicopathological variables has been determined. Patients with higher MUC4 expression trended to have smaller tumor size ($P = 0.036$) and higher Fuhrman grade ($P = 0.044$). And other clinicopathological characteristics were of no statistically significant association with MUC4 expression.

## Correlations Between MUC4 Expressions With Clinical Outcomes of ccRCC Patients

To identify the prognostic value of MUC4 in ccRCC, we compared the OS between different subgroups according to MUC4 expression level by Kaplan–Meier survival analysis. Sixty-seven patients died before the last follow-up in the cohort. Patients with low MUC4 expression had shorter OS than those in MUC4 high expression group ($P < 0.001$) as presented in Figure 2.

## Low MUC4 Expression Is an Independent Indicator of Poor Prognosis in Patients With ccRCC

In order to identify the clinical significance of MUC4 expression postoperatively in the cohort, univariate analysis was applied for OS. As presented in Table 2, low MUC4 expression was shown to be a significant negative prognostic indicator for patients with ccRCC in analysis of OS (HR 3.058; 95% CI: 1.798–5.181; $P < 0.001$). In addition, tumor size ($P < 0.001$), pT stage ($P < 0.001$), pN stage ($P = 0.002$), distant metastases ($P < 0.001$), Fuhrman grade ($P < 0.001$), necrosis ($P = 0.005$), ECOG PS ($P < 0.001$), MVI ($P = 0.016$), and sarcomatoid ($P < 0.001$) were identified to be statistically significant and affected OS of patient with ccRCC. In order to investigate the prognostic value of MUC4 expression, the identical clinicopathological variables which show statistically significance in the univariate analysis were performed to derive risk evaluation by Cox multivariate regression. And we found that MUC4 expression (HR 3.891; 95% CI: 2.083–7.246), pT stage ($P = 0.005$), distant metastasis ($P < 0.001$), Fuhrman grade ($P = 0.002$), sarcomatoid ($P = 0.021$) could be recognized as independent prognostic indicator for OS of patients with ccRCC. In summary, MUC4 expression could be an independent prognostic factor of patients with ccRCC.

**TABLE 1.** Correlation Between MUC4 Expression and Patient Characteristics

| | Patients (n = 198) | | MUC4 Expression | | |
|---|---|---|---|---|---|
| **Characteristic** | **Number** | **%** | **Low (n = 101)** | **High (n = 97)** | ***P**** |
| Age, y† | | | | | 0.132 |
| Mean ± SD | 55.1 ± 11.4 | | 56.3 ± 11.1 | 53.8 ± 11.7 | |
| Median | 54 | | 56 | 54 | |
| Range | (26–80) | | (29–79) | (26–80) | |
| Gender | | | | | 0.906 |
| Female | 61 | 30.8 | 31 | 30 | |
| Male | 137 | 69.2 | 70 | 67 | |
| Tumor size, cm† | | | | | 0.036 |
| Mean ± SD | 4.6 ± 2.7 | | 4.9 ± 2.7 | 4.1 ± 2.6 | |
| Median | 4.0 | | 4.5 | 3.8 | |
| Range | (1.0–18.0) | | (1.5–18.0) | (1.0–16.0) | |
| pT stage | | | | | 0.356 |
| pT1 | 127 | 64.1 | 60 | 67 | |
| pT2 | 19 | 9.6 | 9 | 10 | |
| pT3 | 50 | 25.3 | 31 | 19 | |
| pT4 | 2 | 1.0 | 1 | 1 | |
| pN stage | | | | | 0.972 |
| pNx + pN0 | 195 | 98.5 | 99 | 96 | |
| pN1 | 3 | 1.5 | 2 | 1 | |
| Distant metastasis | | | | | 0.233 |
| M0 | 192 | 97.0 | 96 | 96 | |
| M1 | 6 | 3.0 | 5 | 1 | |
| TNM stage | | | | | 0.174 |
| I | 121 | 61.1 | 55 | 66 | |
| II | 18 | 9.1 | 9 | 9 | |
| III | 52 | 26.3 | 32 | 20 | |
| IV | 7 | 3.5 | 5 | 2 | |
| Fuhrman grade | | | | | 0.044 |
| 1 | 31 | 15.7 | 10 | 21 | |
| 2 | 85 | 42.9 | 42 | 43 | |
| 3 | 53 | 26.8 | 34 | 19 | |
| 4 | 29 | 14.6 | 15 | 14 | |
| Necrosis | | | | | 0.744 |
| Absent | 150 | 75.8 | 78 | 72 | |
| Present | 48 | 24.2 | 23 | 25 | |
| ECOG PS | | | | | 0.899 |
| 0 | 165 | 83.3 | 84 | 81 | |
| ≥1 | 33 | 16.7 | 17 | 16 | |
| MVI | | | | | 0.888 |
| Absent | 159 | 80.3 | 82 | 77 | |
| Present | 39 | 19.7 | 19 | 20 | |
| Sarcomatoid | | | | | 0.222 |
| Absent | 182 | 91.9 | 90 | 92 | |
| Present | 16 | 8.1 | 11 | 5 | |
| UISS category | | | | | 0.057 |
| Low risk | 67 | 33.8 | 28 | 39 | |
| Mediate risk | 113 | 57.1 | 60 | 53 | |
| High risk | 18 | 9.1 | 13 | 5 | |
| SSIGN category | | | | | 0.093 |
| 0–3 | 134 | 67.7 | 60 | 74 | |
| 4–7 | 53 | 26.8 | 33 | 20 | |
| 8+ | 11 | 5.6 | 6 | 5 | |

ECOG PS = Eastern Cooperative Oncology Group performance status; MUC4 = mucin-4, MVI = microvascular invasion, SD = standard deviation, SSIGN = the Mayo clinic stage, size, grade, and necrosis score, TNM stage = tumor, node and metastasis stage, UISS = the UCLA Integrated Scoring System.

*$P < 0.05$ is considered statistically significant, $t$ test for continuous variables and $\chi^2$ test for categorical variables.

†The results of continuous variables are presented as mean ± SD (standard deviation).

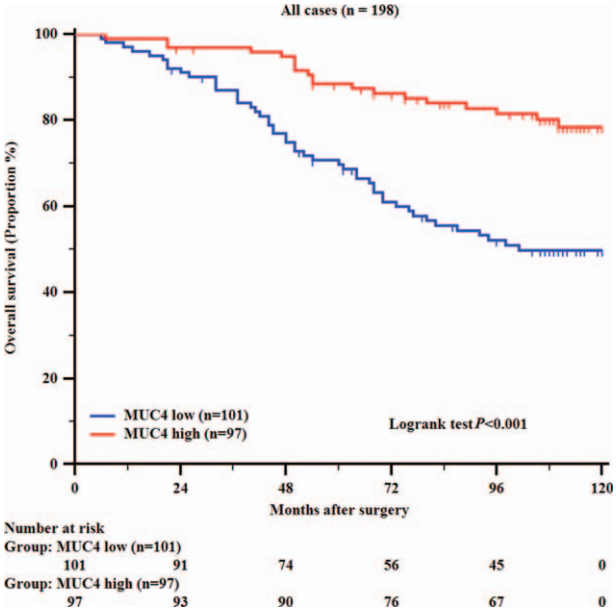


**FIGURE 2.** Kaplan–Meier analysis of overall survival (n = 198) of patients with clear-cell renal cell carcinoma (ccRCC) based on mucin-4 (MUC4) expression. *P*-value was calculated by log-rank test.

## Subgroup Analysis of MUC4 Expression in OS

To further evaluate the prognostic power of MUC4 expression in distinctive clinicopathological variable subgroups, the HRs of MUC4 expression level were investigated in dichotomized TNM stage, Fuhrman grade, the Mayo clinic stage, size, grade, and necrosis score (SSIGN) and the University of Los Angeles integrated staging system (UISS) scoring systems. As presented in Figure 3, MUC4 was significantly correlated with OS among all the patients (HR 2.681; 95% CI: 1.602–4.484; $P < 0.001$). Interestingly, In TNM stages III + IV for OS, HR ratio (95% CI) of MUC4 expression was 6.667 (2.571–17.241) and C-index was 0.658 with statistical significance ($P < 0.001$), while the HR ratio of MUC4 expression for TNM stage I + II were of no statistical significance ($P = 0.220$). Additionally, the MUC4 low expression was also strongly associated with OS in Fuhrman grade 2, 3, and 4 (HR 3.759, 4.202, and 3.268; 95% CI: 1.383–10.204, 1.235–14.286, and 1.353–7.874) with higher C-index (0.643, 0.656, and 0.647) compared to the Fuhrman grade 1 subgroup which had no statistical significance ($P = 0.909$). Low MUC4 expression was associated with poor outcome in all SSIGN category (HR 2.203, 2.801, and 9.901; 95% CI: 1.003–4.831, 1.206–6.494, and 1.160–90.909; $P = 0.047$, 0.010, and 0.002, respectively). In the other hand, MUC4 expression was related to OS in the group of UISS mediate risk and high risk (HR 2.695 and 10.309; 95% CI: 1.416–5.128 and 1.319–83.333; $P = 0.003$ and 0.027, respectively) but no statistical significance in the relatively low risk group. In the TNM stage, SSIGN category, and UISS category prognostic models, MUC4 shows more accurate prediction in higher risk group with greater C-index value.

## Extension of Prognostic Models With MUC4 Expression for ccRCC Patients

In order to further confirm the prognostic power of MUC4 expression, MUC4 expression was integrated into conventional prognostic models like TNM staging system, the Mayo clinic stage, size, grade, and necrosis score (SSIGN), and the University of Los Angeles integrated staging system (UISS) scoring systems, respectively. Harrell concordance index (C-index) and Akaike information criteria (AIC) analysis were applied to investigate the prognostic accuracy. As shown in Table 3, the C indices were 0.699, 0.742, and 0.685, respectively, when assessed with the TNM, SSIGN, and UISS outcome algorithms alone. The C indices were improved to 0.726, 0.757, and 0.723, respectively, when MUC4 expression signature was replenished for OS. Furthermore, lower AIC values were presented among all combined models than their conventional model alone.

## Prognostic Nomogram for Survival of Patients With ccRCC

Moreover, we constructed a prognostic nomogram via integrating all the independent prognostic indicators from Table 2 for OS (Figure 4A). The calibration plot for the probability of overall-survival at 3-, 5-, or 10-year after surgery presented an optimal agreement between actual observation and the prediction by nomogram (Figure 4B–D).

# DISCUSSION

Aberrant expression, glycosylation, and localization of mucins are characteristic events of different malignancies (ovarian, pancreatic, lung, and colon). MUC4 was implicated in tumor development, growth, metastasis, chemotherapeutic agent resistant, and tumor immunity. In the present study, the high expression level of MUC4 in tumor tissue of ccRCC patients was significantly related with smaller tumor size, lower Fuhrman grade, and better clinical outcomes. It was indicated that low expression level of MUC4 could be identified as an independent prognostic factor in patients with ccRCC. Meanwhile, loss of MUC4 expression had precise prediction of the poor prognosis in patient with higher Fuhrman grade and patient in higher risk group in multiple prediction models. Moreover, a nomogram was made to predict the prognosis of patients based on MUC4 and other clinicopathologic characteristics.

As a widely expressed glycoprotein, Mucins exhibit pivotal functions in the surface of normal epithelial cells to provide protection and lubrication.[22] In addition, the roles of mucins in the cancerous lesions development were under intensively studied. Aberrant expression of mucins is likely associated with cancer biology by influencing cellular growth, differentiation, transformation, adhesion, invasion, and immune surveillance.[23] Interestingly, although deviant expression of MUC4 has been reported in various malignancies, the role that MUC4 plays in different kind of tumor may be of great disparity. The expression of MUC4 was correlated with poor clinical outcome of pancreatic cancer, lung adenocarcinoma, cholangiocarcinoma, epithelial ovarian carcinoma, and colorectal adenocarcinoma.[10,12,14,24–26] Conversely, MUC4 overexpression is positively associated with low-grade salivary tumor. MUC4-expresssing salivary gland mucoepidermoid tumors are related to improved patient survival and prolonged time to suffer recurrence.[17] Additionally, in the urogenital system, downregulation of MUC4 expression was exhibited in the prostate carcinoma tissue compared to the benign prostate regions in prostate cancer and the loss of MUC4 expression was observed in urothelial carcinoma.[18,19] Due to the heterogeneity of distinctive cancer, MUC4 might exhibit antilogous functions in tumorigenesis and progression of cancer.

**TABLE 2.** Univariate and Multivariate Cox Regression Analysis of Overall Survival (n = 198)

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| **Characteristic** | **HR (95% CI)** | ***P**** | **HR (95% CI)** | ***P**** |
| Age | 1.015 (0.993–1.037) | 0.174 | | |
| Gender | | 0.735 | | |
| Female | Reference | | | |
| Male | 0.915 (0.547–1.529) | | | |
| Tumor size, cm | 1.185 (1.105–1.272) | <0.001 | 1.071 (0.962–1.193) | 0.212 |
| pT stage | | <0.001 | | 0.005 |
| pT1 | Reference | | Reference | |
| pT2 | 2.690 (1.306–5.543) | 0.007 | 1.455 (0.599–3.535) | 0.410 |
| pT3 | 3.404 (2.017–5.744) | <0.001 | 2.803 (1.498–5.249) | 0.001 |
| pT4 | 3.727 (0.511–27.164) | 0.197 | 5.836 (0.417–81.647) | 0.192 |
| pN stage | | 0.002 | | 0.076 |
| pNx + pN0 | Reference | | Reference | |
| pN1 | 13.462 (4.046–44.796) | | 4.189 (0.867–20.230) | |
| Distant metastases | | <0.001 | | <0.001 |
| M0 | Reference | | Reference | |
| M1 | 18.927 (6.982–51.305) | | 13.376 (3.632–49.265) | |
| Fuhrman grade | | <0.001 | | 0.002 |
| 1 | Reference | | Reference | |
| 2 | 1.990 (0.686–5.766) | 0.207 | 1.079 (0.340–3.418) | 0.898 |
| 3 | 3.294 (1.132–9.588) | 0.030 | 2.118 (0.686–6.540) | 0.194 |
| 4 | 10.313 (3.562–29.855) | <0.001 | 3.870 (1.146–13.063) | 0.030 |
| Necrosis | | 0.005 | | 0.784 |
| Absent | Reference | | Reference | |
| Present | 2.064 (1.255–3.396) | | 1.093 (0.582–2.053) | |
| ECOG PS | | <0.001 | | 0.064 |
| 0 | Reference | | Reference | |
| ≥1 | 2.901 (1.733–4.856) | | 1.767 (0.970–3.219) | |
| MVI | | 0.016 | | 0.382 |
| Absent | Reference | | Reference | |
| Present | 1.967 (1.168–3.311) | | 1.317 (0.712–2.434) | |
| Sarcomatoid | | <0.001 | | 0.021 |
| Absent | Reference | | Reference | |
| Present | 5.487 (3.040–9.905) | | 2.372 (1.146–4.908) | |
| MUC4 expression | | <0.001 | | <0.001 |
| High | Reference | | Reference | |
| Low | 3.058 (1.798–5.181) | | 3.891 (2.083–7.246) | |

CI = confidence interval, ECOG PS = Eastern Cooperative Oncology Group performance status, HR = hazard ratio, MUC4 = mucin-4, MVI = microvascular invasion.

*$P < 0.05$ is considered statistically significant.

Although MUC4 is gradually recognized as a prognostic biomarker in many malignancies, the exact functional role that MUC4 plays in ccRCC is still blurred. In this study, we discovered that high expression level of MUC4 predicted a longer survival time of ccRCC patients. Shinagare et al[27] suggested that an exophytic growth pattern was associated with the MUC4 mutation, which is associated with better survival. Similar to our study, Singh et al[18] observed the loss of MUC4 during development of prostate cancer. During the study, they observed that the expression level of MUC4 was much lower in the prostatic adenocarcinoma tissue compared to the adjacent benign tissue and the increased expression of MUC4 was observed when prostate cancer cell lines treated with inhibitor of histone deacetylases and DNA methyltransferase. The detailed mechanism that how MUC4 work in the tumorigenesis and development in urinary system is remaining vague, and Singh et al suggested that the epigenetic mechanism might be regulating the MUC4 expression during pathogenesis of prostate cancer. Further studies need to be carried out in urinary system derived cancer to decipher the mechanism of aberrant MUC4 downregulation.

Moreover, patients with late TNM stage (III + IV) could be stratified by MUC4 expression while those in the early TNM stage (I + II) could not be markedly stratified. Similarly, MUC4 expression helps to identify the prognosis of mediate and high risk patients both in UISS models while MUC4 was significantly associated with OS in all risk groups of SSIGN models. Furthermore, both the value of C-indices and HR were greater in higher Fuhrman grade and higher risk groups of TNM, SSIGN, and UISS models. Thus, we assumed that the expression of MUC4 might

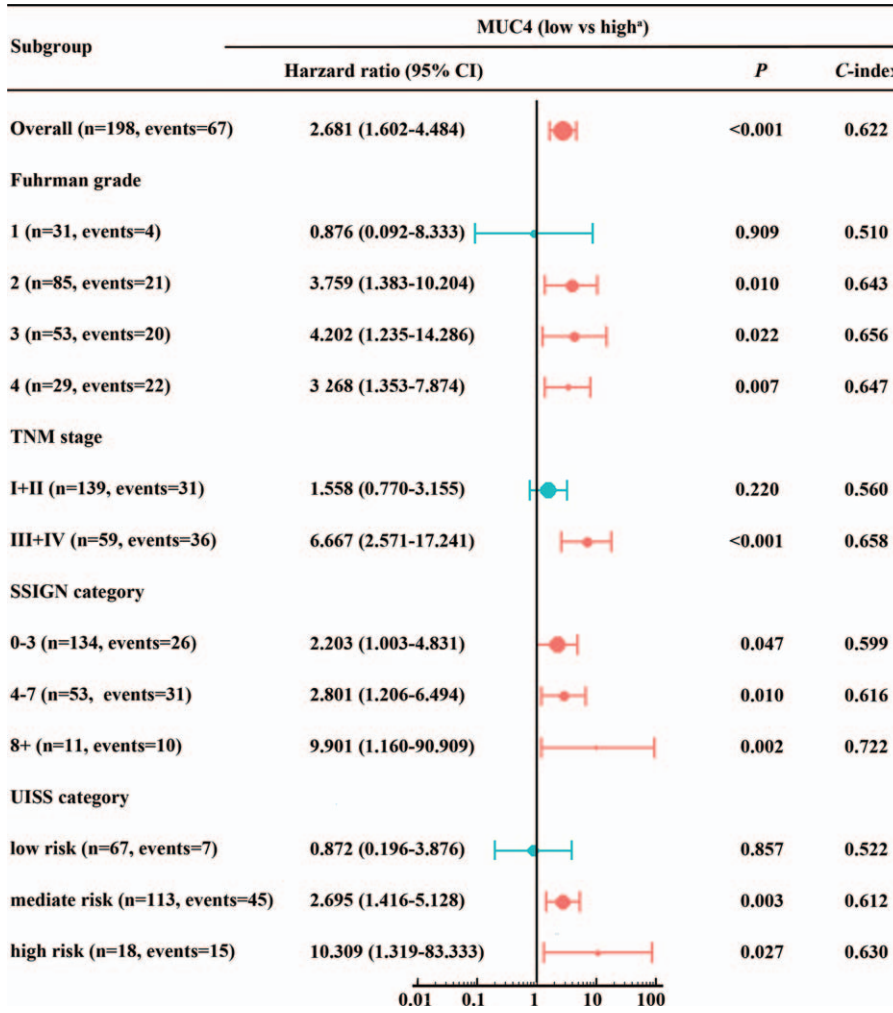


**FIGURE 3.** Subgroup analysis of mucin-4 (MUC4) expression for overall survival among patients classified by Fuhrman grade, TNM stages, the Mayo clinic stage, size, grade, and necrosis score (SSIGN) and University of Los Angeles integrated staging system (UISS) with results expressed using hazard ratios. The red lines represent the groups of patients that can be significantly stratified with MUC4 expression ($P < 0.05$). The blue lines represent the groups of patients that cannot be significantly stratified with MUC4 expression. *P*-value was 2-tailed. C-index was Harrell concordance index. [a]Reference group.

**TABLE 3.** Comparison of the Prognostic Accuracy of the Prognostic Models and MUC4 Expression

| | Overall Survival | |
|---|---|---|
| **Model** | **C-Index** | **AIC** |
| MUC4 | 0.622 | 661.160 |
| TNM stage | 0.699 | 637.653 |
| TNM stage + MUC4 | 0.726 | 622.833 |
| SSIGN | 0.742 | 620.677 |
| SSIGN + MUC4 | 0.757 | 609.864 |
| UISS | 0.685 | 626.528 |
| UISS + MUC4 | 0.723 | 616.842 |

AIC = Akaike information criteria, C-index = Harrell concordance index, MUC4 = mucin-4, SSIGN = the Mayo clinic stage, size, grade, and necrosis score, TNM stage = tumor, node and metastasis stage, UISS = University of Los Angeles integrated staging system.

play an important role in the late stage of tumor progression, perhaps via inhibition of metastasis. Additionally, the high MUC4 expression is associated with lower Fuhrman grade as shown in Table 1. Furthermore, patients with worse differentiation (Fuhrman grade 2, 3, and 4) of ccRCC could be significantly stratified by MUC4, but those with better differentiation (Fuhrman grade 1) could not. It indicated that MUC4 expression might also be associated with the tumor differentiation. However, further exploration is needed to clarify their relation.

Several limitations of this study should be acknowledged. Although we have concluded and presented some novel findings about MUC4 expression in ccRCC, more efforts need to be exerted in the future studies. Single cohort seems to be inadequate to reach greater reliability. And patients with ccRCC of TNM stage IV seem to be insufficient in the study. Additionally, further studies are warranted to explore the pathophysiology mechanism of MUC4 in ccRCC.

In summary, the low expression level of MUC4 was observed to be strongly associated with poor clinical outcomes

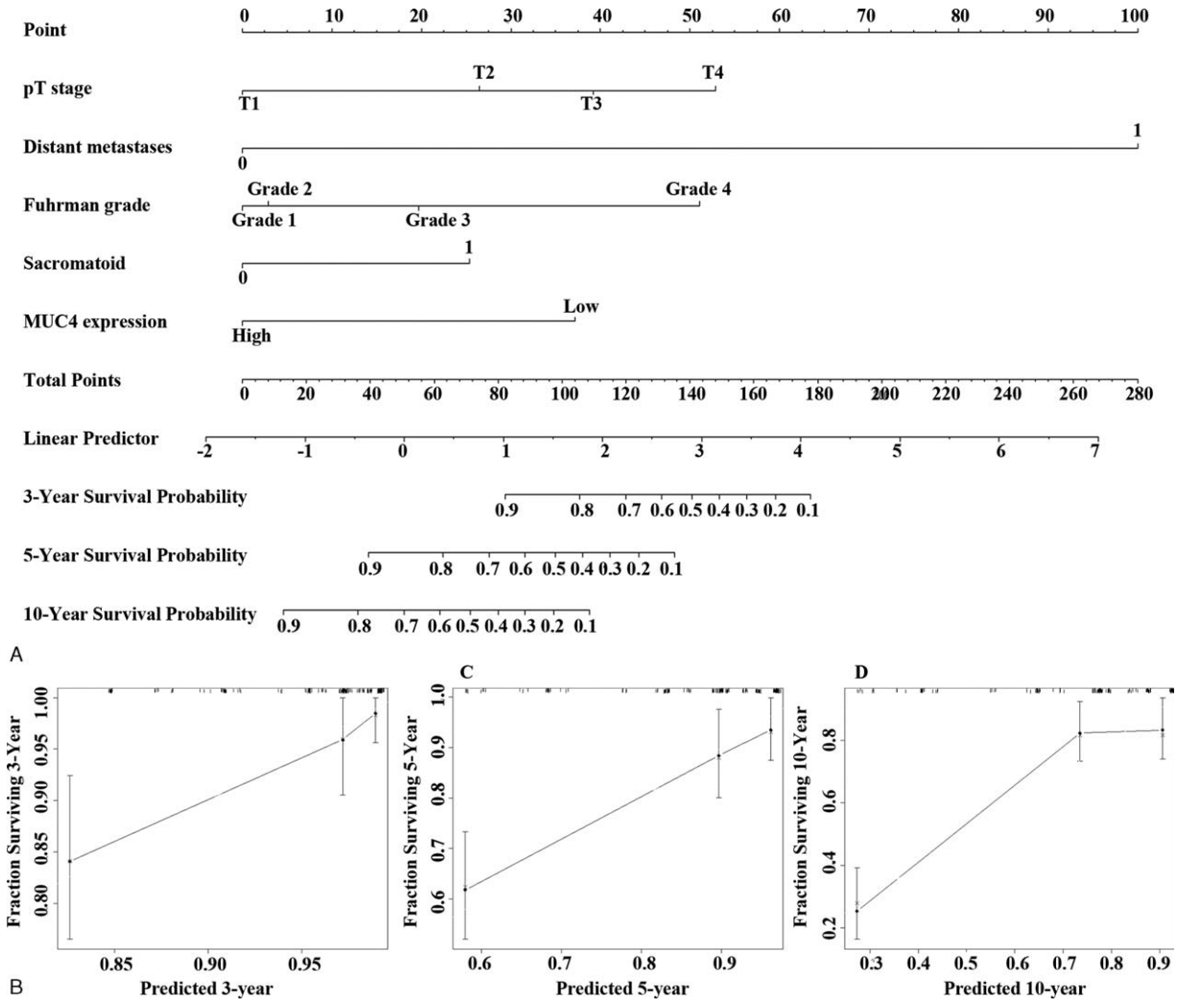


**FIGURE 4.** Nomogram and calibration plot for prediction of overall survival in patients with clear-cell renal cell carcinoma (ccRCC). Postoperative prognostic nomogram of patients with ccRCC (A). The calibration plots for predicting survival at 3 (B), 5 (C), and 10 years (D).

of patients with ccRCC. Furthermore, patients with ccRCC could be significantly stratified in TNM stage III + IV and Fuhrman grade 2, 3, and 4. Therefore, MUC4 might be of a pivotal role in the progression of ccRCC and could be integrated to the current model to predict survival of ccRCC as an independent prognosis factor, which might guide the clinical decisions. Further studies need to be performed to exploit the potential of MUC4 as a new therapeutic target for RCC therapy. However, further validation in larger cohort of tumor tissue samples is required in future studies.

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