

MUC1 Expression Is Correlated With Nuclear Grade and Tumor Progression in pT1 Renal Clear Cell Carcinoma

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Abstract

We studied, by immunohistochemical analysis, the expression of MUC1 and epithelial membrane antigen in 44 stage pT1 renal cell carcinomas (RCCs). Six patients had a metastatic evolution. The percentage of stained cells was determined for each tumor. All tumors and normal adjacent renal parenchyma were stained. In normal kidney, distal convoluted tubules and collecting ducts stained strongly with an apical distribution. In tumors, there was a significant statistical correlation of the MUC1 expression level with the nuclear grade and with tumor progression. High-grade tumors had more stained cells than did low-grade tumors. Metastatic tumors also were more stained than nonmetastatic lesions. By using the Kaplan-Meier method and the log-rank test, we observed that patients with fewer than 10% of stained cells had no metastatic evolution. In contrast, patients with 70% or more stained cells had significantly lower metastasis-free survival rates. We conclude that MUC1 is expressed in RCC and is associated with tumor progression in pT1 RCC.

The *MUC1* gene is located on chromosome 1q21-24 and encodes a transmembrane glycoprotein.¹ *MUC1* is a member of the mucin family, which is membrane-associated and membrane-secreted. MUC1 also is known as polymorphic urinary mucin, or PUM, and epithelial membrane antigen (EMA).¹⁻⁴ MUC1 is distributed widely on the apical membrane of many glandular epithelia such as of the breast, colon, pancreas, lung, and kidney.³⁻⁵ MUC1 is supposed to have a role in cell adhesion, cellular polarity, and signal transduction. In many epithelial cancers, up-regulation frequently is observed with a loss of polarized cellular expression and diffuse circumferential distribution. These variations of expression in carcinomatous cells are suspected to participate in the metastatic dissemination by destabilization of cell-cell and cell-extracellular matrix interactions.^{6,7} In several studies, the level of MUC1 expression was correlated with the metastatic spread and the prognosis.⁸⁻¹⁰ In the kidney, MUC1 is expressed in normal distal convoluted tubules, collecting ducts, and clear renal cell carcinoma (cRCC).¹¹ In the study by Fujita et al,¹² the expression of MUC1 was correlated inversely with the prognosis for RCC. The authors studied 51 RCCs classified with the TNM system of 1987 with only 2 pT1 tumors.¹² Our aim was to study a series of 44 localized cRCCs reclassified as pT1 tumors following the 1997 TNM classification¹³ to evaluate MUC1 as a marker of tumor progression and prognosis.

Materials and Methods

Case Selection

From January 1992 to December 1995, all cases coded as cRCC were retrieved from the archives of the Department

of Pathology, Lille University Hospitals, Lille, France. Of 118 cases, 44 were classified as stage pT1 using the 1997 TNM system.¹³ In all cases, histologic slides were available and reviewed (based on the international classification) (X.L.). Cytologic nuclear grading was applied as described by Fuhrman et al.¹⁴ All tumors were fixed in buffered formalin, and specimens were embedded in paraffin. All patient histories and follow-up data were recorded (L.Z.).

Immunohistochemical Studies

Immunohistochemical studies were conducted on formalin-fixed, paraffin-embedded tissues sections 4 μ m thick using an automated immunostainer (ES, Ventana Medical Systems, Strasbourg, France). Following deparaffinization, immunohistochemical analysis was performed using a 3-step indirect process based on the streptavidin-biotin complex. The primary antibodies used were directed against MUC1 and EMA. Antibody to MUC1 recognizes the tandem repeat sequences¹⁵ (monoclonal M8; pretreatment by microwave 20 minutes; dilution, 1:50). The sections were incubated for 32 minutes with goat serum to block the nonspecific antibody-binding sites. Antibody against EMA (dilution, 1:25; DAKO, Glostrup, Denmark) was used with a pretreatment by pressure cooker for 1 minute 30 seconds with citrate buffer (pH 6.0). Endogenous biotin was blocked by adding an excess amount of avidin followed by washing and the addition of free biotin. Slides were counterstained with hematoxylin. Positive and negative controls were added for each automated immunohistochemical run. Negative controls consisted of slides run without the primary antibody and negative renal structures (proximal convoluted tubules, glomeruli). Normal bronchus was used as the external positive control and distal convoluted tubules as the internal positive control.

Immunoreactivity was scored as previously described by Fujita et al¹²: A cell was estimated as positive when the cytoplasm, the whole cell membrane, or both were stained. When only the apical side was stained, the cell was judged to be negative. The percentage of positively stained cells (positive rate) was determined for each tumor.

Statistical Analysis

The Mann-Whitney *U* test was used to compare the percentage of cells staining in groups with and without metastatic evolution. The percentages of cells staining were log transformed to explain the relationship with nuclear grade by a linear regression model. The time to metastasis-free survival was calculated from the date of surgery. Deaths resulting from disease were treated as an endpoint for disease survival. All other deaths were regarded as censored events. The method of Kaplan-Meier was used for graphic representation of the survival data. Univariate influence of the cytologic nuclear grade and percentage of cells staining were analyzed using the log-rank test. No multivariate

analysis was carried out owing to the moderate number of events. Differences were defined as statistically significant at *P* less than .05. Statistical analysis were performed using SPSS software, version 9.0 (SPSS, Chicago, IL).

Data are given as mean \pm SD unless stated otherwise.

Results

Patients and Tumor Classification

Patients were 30 men (68%) and 14 women (32%) aged 58.3 ± 10.1 years (range, 31-78 years) at the time of diagnosis. The size of tumors was 4.3 ± 1.5 cm (range, 1.7-7 cm). There were 36 tumors of low nuclear grade (Fuhrman grade 1, 15 tumors; Fuhrman grade 2, 21 tumors) and 8 of high nuclear grade (Fuhrman grade 3). Initially at the time of surgery, all patients were classified as N0 M0.

The follow-up period was 72.2 ± 26.5 months (range, 4-110 months), and the median was 75.5 months. During the follow-up period, 6 patients (14%) developed visceral metastases (lungs, bone, eye). Of these patients, 4 died of the disease, and 2 are alive with metastases. Five patients died of another cause.

Two patients with metastatic disease had tumors of nuclear grade 2, and 4 patients had tumors of nuclear grade 3.

Immunohistochemical Results

Normal Kidney Adjacent to Carcinoma

In each case, a part of normal kidney was present with distal convoluted tubules and collecting ducts always strongly and diffusely stained at the apical border **Image 1**.

Renal Clear Cell Carcinomas

The levels of expression of MUC1 in the 44 cRCCs tested are summarized in **Table 1**. MUC1 expression was found in all cRCCs with staining of $29.6\% \pm 29.4\%$ (range, 1%-85%). Staining of $14.8\% \pm 21.2\%$ (range, 1%-60%) was observed in grade 1, $29.1\% \pm 27.7\%$ (range, 1%-70%) in grade 2 **Image 2**, and $58.7\% \pm 28.1\%$ (range, 10%-85%) in grade 3. By using a linear regression model, we observed a strong linear relationship between the log of the percentage of cells stained and the nuclear grade (*P* < .005) **Figure 1**.

In the group of patients without metastasis, staining of $25.1\% \pm 27.3\%$ (range, 1%-80%) was observed. In the group of patients with metastases, staining was $58.3\% \pm 28.0\%$ (range, 10%-85%) **Image 3**. A significant difference was found between these groups (*P* < .01).

The log-rank test demonstrated that nuclear grade significantly affected survival time (*P* = .0036), and the Kaplan-Meier curves showed that the difference was largely due to the poorer survival for patients with grade 3 tumors **Figure 2**.



Image 1 MUC1 apical expression in normal distal convoluted tubules (anti-MUC1, ×400).

The log-rank test demonstrated that the degree of staining for MUC1 significantly affected survival time ($P = .0012$), and the Kaplan-Meier curves demonstrated that the difference was largely due to the poorer survival for patients with tumors with 70% or more cells staining for MUC1 **Figure 3**.

The results obtained with antibody to EMA were similar to those for MUC1.

Discussion

MUC1 belongs to the family of human mucins that are large O-glycoproteins expressed by epithelial cells. Mucins are thought to be implicated in cell protection, adhesion, and signaling.²⁻⁴ MUC1 is a transmembrane mucin and is a major component of many glandular epithelia. MUC1 frequently is up-regulated and abnormally glycosylated in carcinoma.^{6,16} The overexpression of MUC1 is associated with a loss of polarity and circumferential distribution in tumor cells.¹⁷ This abnormal overexpression is suspected to destabilize cell-cell adhesion and cell-extracellular matrix adhesion and to protect tumor cells from immune recognition and then to favor metastases. Satoh et al¹⁸ showed that in MUC1-transfected human pancreatic cancer cells in nude mice, the number of spontaneous lung metastases was higher than in control cells.

In many studies, carcinomas associated with metastatic evolution and poor prognosis frequently overexpressed MUC1.^{8,10,19,20} In the prostate, MUC1 expression seems to be correlated with an advanced Gleason pattern and histopathologic stage.²¹

In the normal kidney, MUC1, also known as EMA, is largely expressed in the distal convoluted tubules and in the

Table 1
Levels of Expression of MUC1 in the 44 Clear Renal Cell Carcinomas

Variable	No. of Cases	Mean ± SD for the Percentage of Positively Stained Cells
Grade		
1	15	14.8 ± 21.2
2	21	29.1 ± 27.7
3	8	58.7 ± 28.1 ($P < .005^*$)
Metastatic evolution		
Positive	6	58.3 ± 28.0
Negative	38	25.1 ± 27.3 ($P < .01^\dagger$)

* Linear regression.

† Mann-Whitney *U* test.

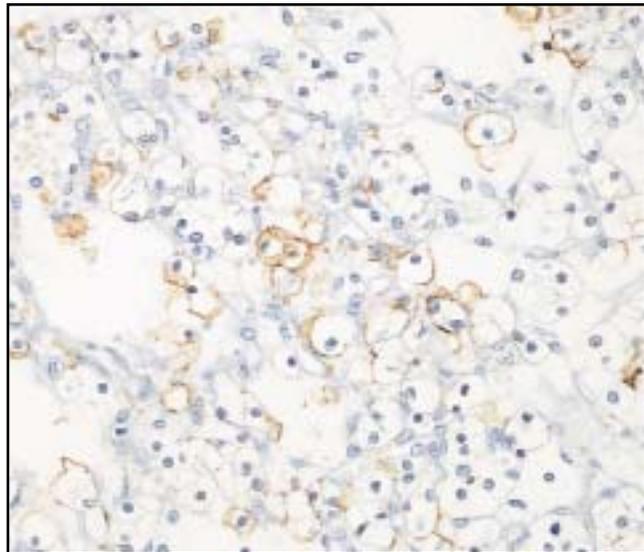


Image 2 Focal staining is observed in low-grade tumor (anti-MUC1, ×400).

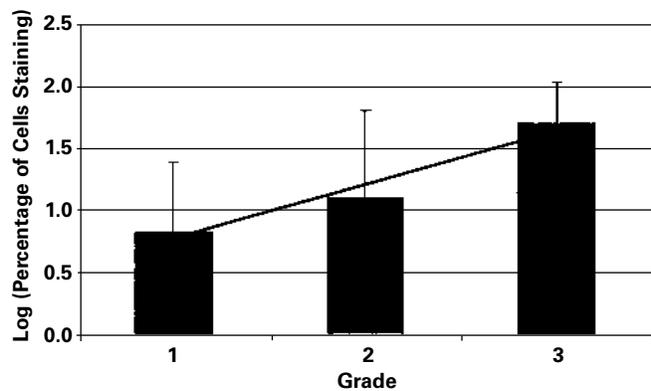


Figure 1 Relationship between the logarithm of the percentage of cells staining and the cytologic nuclear grade. Linear regression curve: log of percentage of cells staining = 0.412 grade + 0.346 ; t test = 3.185; $P < .005$.

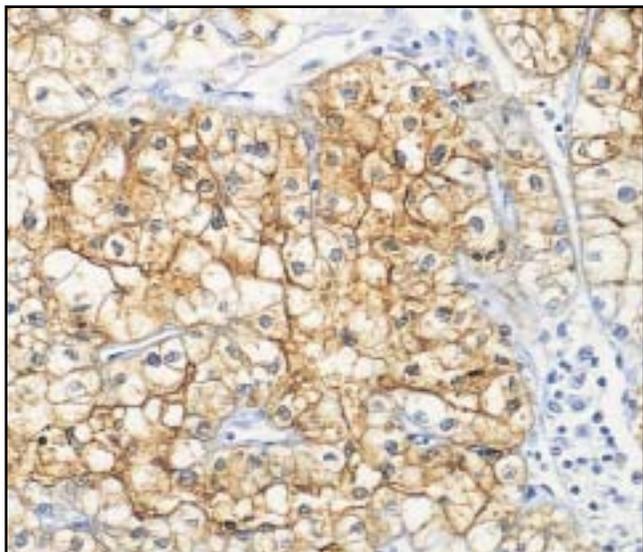


Image 3 Strong and diffuse circumferential staining in a clear renal cell carcinoma with metastatic evolution (anti-MUC1, x400).

collecting ducts with a cellular apical expression.¹¹ Hudson et al²² recently demonstrated that the transfection of MUC1 in Madin-Darby canine kidney was able to induce tubular morphogenesis, indicating that MUC1 probably is implicated in renal development.

In cRCC, which is the most frequently encountered renal tumor, it has been suggested that MUC1 is expressed in almost all tumors.¹¹ In the present study, we were able to confirm that MUC1 is expressed in all cRCCs with variable staining. To date, only 1 previous series studied the value of MUC1 in the

prognosis of RCC.¹² Fujita et al¹² evaluated the immunohistochemical expression of MUC1 in 51 cases of cRCC. These tumors were classified according to the 1987 TNM system (International Union Against Cancer) and the pathologic nuclear grading system (Japanese Urological Association). In this work, the authors observed significant differences in the percentage of stained cells between high- and low-grade tumors and between metastatic and nonmetastatic tumors. RCCs of low grade (grades 1 and 2) were less stained than were high-grade tumors (grade 3). Metastatic tumors also were more stained than localized tumors. In contrast, no difference between pT1-2 and pT3-4 tumors was noted. The survival also was correlated inversely with levels of MUC1 expression.¹² In this study,¹² only 2 pT1 tumors (TNM 1987) were studied. In 1997, the TNM classification was revised, and the maximum diameter of pT1 was increased from 2.5 to 7 cm.¹³ Using this new classification, the majority of renal tumors are classified as stage pT1. In general, pT1 RCCs were associated with a good prognosis (10-year survival, 91%).²³ But visceral dissemination remains possible and is difficult to predict with the classic prognostic factors (TNM and nuclear grading). In the present work, studying a group of 44 pT1 cRCCs (TNM 1997) we observed, like Fujita et al,¹² that MUC1 expression was correlated with Fuhrman nuclear grade. High MUC1 expression was associated significantly with tumors of high nuclear grade (Fuhrman grade 3). In addition, univariate study revealed that staining of 70% or more was associated significantly with an increased risk of visceral metastatic evolution. In contrast, no patients with fewer than 10% of stained cells had metastatic evolution. With these results, we suggest that MUC1 may be a potential prognostic

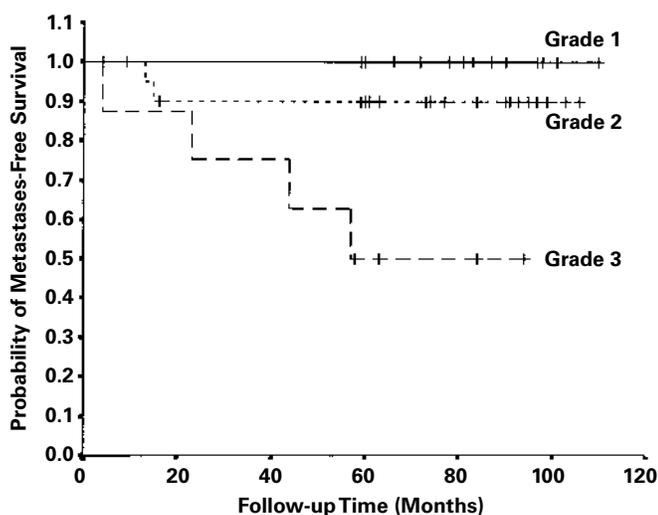


Figure 2 Kaplan-Meier survival curves for patients divided according to cytologic nuclear grade. Event = metastases; +, censored patients. Chi square = 11.24; *P* = .0036, log-rank test.

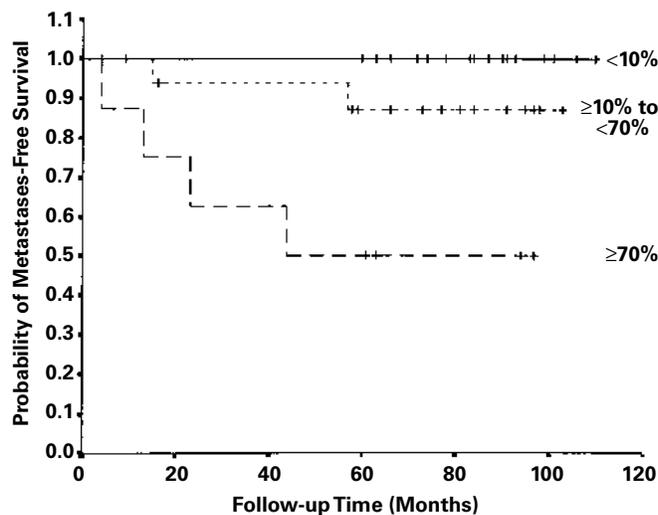


Figure 3 Kaplan-Meier survival curves for patients divided according to cell staining percentages. Event = metastases; +, censored patients. Chi square, 13.44; *P* = .0012, log-rank test.

marker. But only larger series with multivariate analysis could demonstrate whether MUC1 is a more powerful and reproducible prognostic factor than the nuclear grade.

In the present study, we also confirmed that the cellular localization of MUC1 is an important factor to evaluate MUC1 as a prognostic marker of carcinomas. Thus we observed, as in the studies about MUC1 expression in breast cancer,¹⁷ that apical staining in tumor cells is associated with a good prognosis and that circumferential membrane staining is associated with a worse prognosis. Similar results were described for a series of small cell carcinomas of the lung.²⁴

The exact mechanism of MUC1 in carcinogenesis remains unclear. Apical staining may indicate normal expression of MUC1, as in normal distal convoluted tubules. In contrast, circumferential expression may indicate deregulation of MUC1 cell trafficking and could result in destabilization of cell-cell and cell-extracellular matrix interactions.^{4,6,7,24} Schroeder et al²⁵ recently demonstrated that MUC1 also interacts with epidermal growth factor receptors and could activate the mitogen-activated protein kinase pathway.

Our results show that high-level expression of MUC1 with circumferential membrane staining is associated with high-grade tumors and with an increased risk of visceral dissemination in pT1 cRCC. We believe that further large studies should investigate MUC1 as a prognostic marker and potential therapeutic target of RCC.

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