Alteration in Urinary Matrix Metalloproteinase-9 to Tissue Inhibitor of Metalloproteinase-1 Ratio Predicts Recurrence in Nonmuscle-invasive Bladder Cancer[1]

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ABSTRACT

Purpose: **The purpose is to assess the prognostic significance of matrix metalloproteinase (MMP)-9 in patients with bladder cancer using a combination of ELISA (to measure MMP-9 in voided urine) and immunohistochemistry (to study MMP-9 in bladder tumors). The relationship between MMP-9 and its principal inhibitor, tissue inhibitor of metalloproteinase (TIMP)-1 (in voided urine samples) was also studied.**

Experimental Design: **A total of 134 patients with bladder tumors (7 cis, 76 T_a, 27 T_1, 24 T_2-T_4; 40 G1, 43 G2, and 44 G3), 33 patients with benign urological conditions, and 36 healthy volunteers was studied. Samples from 106 patients with bladder cancer and 12 controls were stained for MMP-9. Clinical follow-up data were available on 116 patients (median: 25 months; range: 4–36 months).**

Results: **MMP-9 was present in all urine samples analyzed. There were no differences between patients with cancer and patients with benign disorders. However, patients had significantly higher urinary MMP-9 than normal volunteers ($P = 0.0167$). Urinary MMP-9 was associated with bladder tumors of advanced stage ($P = 0.0065$) and large size ($P < 0.0001$) but not with grade ($P = 0.14$), multiplicity ($P = 0.31$), recurrence ($P = 0.55$), progression ($P = 0.83$), or survival ($P = 0.55$). Low MMP-9:TIMP-1 ratios in patients with nonmuscle-invasive tumors were associated with higher recurrence rates ($P = 0.0035$). Sixty percent (64 of 106) of bladder tumor specimens expressed MMP-9 compared with none of 12 normal urothelial biopsies ($P < 0.0001$). MMP-9 staining was associated with tumor size ($P = 0.014$), disease progression ($P = 0.005$), and poor disease-specific survival ($P = 0.022$) but was unrelated to tumor stage ($P = 0.46$), grade ($P = 0.26$), multiplicity ($P = 0.85$), or recurrence rate ($P = 0.62$).**

Conclusions: **High urinary MMP-9 levels are associated with large bladder tumors. A low urinary MMP-9:TIMP-1 ratio may indicate a higher risk of intraluminal nonmuscle-invasive tumor recurrence and may assist in planning follow-up surveillance protocols.**

INTRODUCTION

Metastatic disease is responsible for the majority of cancer-related deaths and remains the greatest barrier to cancer cure. MMPs[3] are a family of zinc-dependent endopeptidases intimately associated with the process of local tumor cell invasion and metastasis (1). In addition to degrading extracellular matrix, the MMPs play a key role in maintaining a supportive local environment that promotes tumor cell growth both at the primary and metastatic sites (2). Regulation of MMP activity is controlled within cells and tissues at different levels, including transcriptional control, secretion from the cell as inactive precursors, and direct inhibition by a group of specific molecules, the TIMPs of which there are four members: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (3).

The most widely studied members of the MMP family are the gelatinases (MMP-2 and MMP-9). These enzymes are particularly important because of their ability to degrade type IV collagen, an important component of basement membrane, thereby facilitating a critical, early step in the process of tumor cell invasion (4). Many experimental studies have shown a significant association between tumor progression and increased expression of the gelatinases (5, 6). In human bladder cancer, most clinical studies have examined the activities of the gelatinases in samples of voided urine, bladder wash cytology, and tumor tissue using gelatin zymography (7–10). In general, increased gelatinolytic activity has been associated with bladder tumors of advanced stage and grade in these studies. Disruption of the balance between expression of MMPs and their inhibitors, the TIMPs, may facilitate tumor progression and recurrence. TIMP-1 binds and inhibits MMPs with 1:1 stoichiometry (11), binding MMP-9 in particular, whereas TIMP-2 specifically in-

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[3] The abbreviations used are: MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; BPH, benign prostatic hyperplasia; TCC, transitional cell carcinoma; TURP, transurethral resection of the prostate.

Table 1 Breakdown of tumor-node-metastasis stage and WHO grade of bladder tumours in 134 patients who submitted voided urine specimens for analysis (Urine ELISA). Clinical follow-up data were available on 116 patients as indicated (Followed up). The stages and grades of the 106 bladder tumours stained immunohistochemically for MMP-9 are also shown (Immunohistochemistry)

Stage	*cis*	T_a	T_1	T_2+
Urine ELISA	7	76	27	24
Followed up	5	72	21	18
Immunohistochemistry	4	68	18	16
Grade		G1	G2	G3
Urine ELISA		40	43	41
Followed up		40	41	30
Immunohistochemistry		36	38	28

activates MMP-2 (12). We have previously noted that a low urinary MMP-1:TIMP-1 ratio is associated with bladder tumors of advanced stage and grade (13). Others have shown that an imbalance between serum levels of MMP-2 to TIMP-2 may predict tumor recurrence in patients with advanced urothelial malignancy who have previously undergone potentially curative resection (14).

Most bladder cancers (70–80%) present as nonmuscle-invasive papillary tumors that have a high incidence (50–80%) of intraluminal recurrence but less frequently (5–20%) progress to invade bladder wall muscle. Tumor recurrence can be broadly predicted by tumor size at presentation (15) or the combination of number of tumors at presentation plus presence or absence of recurrence at 3 months (16). The molecular mechanisms underlying recurrence of nonmuscle-invasive bladder cancer have not been well characterized as yet, but evidence is emerging that MMPs and TIMPs may play an important role. In a study of 51 nonmuscle-invasive bladder cancers, increased expression of MMP-9 and TIMP-2 mRNA in tumor tissue strongly correlated with tumor recurrence (17). Only one previous study has measured actual urinary MMP-9 levels in patients with bladder cancer using ELISA (18). Urinary MMP-9 was detectable in voided urine specimens from patients with bladder tumors but not from normal volunteers. Tumor recurrence rates were not evaluated, but it was found that the balance between MMP-9 and TIMP-1 was preserved in noninvasive tumors where the basement membrane barrier was not breached (18).

To additionally evaluate the significance of MMP-9 in voided urine samples from patients with bladder cancer, we measured urinary MMP-9 concentrations by ELISA. The ratio of urinary MMP-9 to TIMP-1 was also calculated because the significance of this relationship has not previously been determined. In addition, samples of bladder tumor tissue were stained immunohistochemically for MMP-9 to determine whether urinary MMP-9 levels were related to MMP-9 expression in corresponding bladder tumors.

PATIENTS AND METHODS

Patients. Freshly voided urine was collected from patients with suspected or known carcinoma of the bladder. Urine samples were obtained from 134 patients with bladder cancer [107 males, 27 females; mean age: 70 ± 11 years (range: 35–90 years)], 33 patients with benign urological conditions [29 males, 4 females; BPH: $n = 19$, cystitis: $n = 14$, mean age: 68 ± 11 years (range: 34–80 years)], and 36 healthy volunteers [16 males, 20 females; mean age: 39 ± 14 years (range: 21–78 years)]. Bladder tumors were staged using the Tumor-Node-Metastasis classification (19) and graded according to the WHO system (Ref. 20; Table 1). Complete clinical follow-up data were available on 116 patients with a median follow-up time of 25 months (range: 4–39 months; Table 1). Of the 98 patients in this subgroup with nonmuscle-invasive bladder cancer, 30 (31%) had newly diagnosed tumors. There were 68 (69%) patients with recurrent disease. Those with high-grade lesions (cis/G3) had completed a 6-week induction course of intravesical Bacille Calmette-Guerin therapy, whereas all other patients had received a single postoperative intravesical instillation of mitomycin-C. Tumor tissue was obtained from 106 of 134 patients with bladder cancer (Table 1). Normal urothelium was collected from 12 men [mean age: 71 ± 12 years (range: 57–86 years)] with no previous history of TCC, undergoing TURP for bladder outlet obstruction secondary to BPH. Ethical committee approval was granted for this procedure, and all patients gave consent to undergo bladder biopsy at the time of TURP.

ELISA. After collection, freshly voided urine samples were centrifuged at 1200 × *g* for 5 min, and the supernatant was stored at −20°C until analyzed. Before analysis, samples were slowly thawed and gently mixed. All measurements were performed in duplicate. Urinary MMP-9 was measured using a commercially available ELISA system [Biotrak MMP-9 (human) ELISA system, code RPN 2614; Amersham Life Science, Little Chalfont, Buckinghamshire, United Kingdom]. The assay measures pro-MMP-9 and the pro-MMP-9/TIMP-1 complex, but not active MMP-9, the MMP-9/TIMP-2 complex or MMP-9 complexed with alpha-2 macroglobulin. The assay does not detect other members of the MMP family and is based on a two-step sandwich ELISA format using two different antibodies directed against different epitopes of MMP-9. Urinary TIMP-1 was measured using a commercially available ELISA kit [Biotrak TIMP-1 (human) ELISA system, code RPN 2611; Amersham Life Science) as described previously (13). The TIMP-1 assay measures total TIMP-1, both free and complexed with MMPs.

Immunohistochemistry. Five-μm thick sections were cut from formalin-fixed bladder tumor tissue embedded in paraffin. After dewaxing and rehydration through graded alcohol serials, antigen retrieval was performed by autoclaving sections in 850 ml of 0.01 M citrate buffer (pH 6.0) at 120°C in a pressure

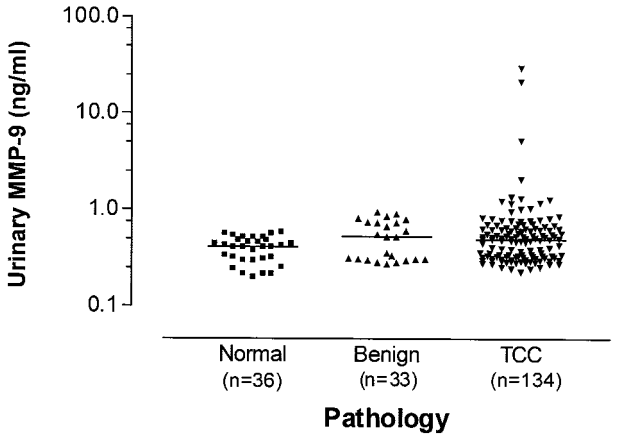

Fig. 1 Urinary MMP-9 concentrations in samples from healthy volunteers (normal), benign lower urinary tract conditions (benign), and patients with TCC of the bladder. *Bars* represent median urinary MMP-9 concentrations.

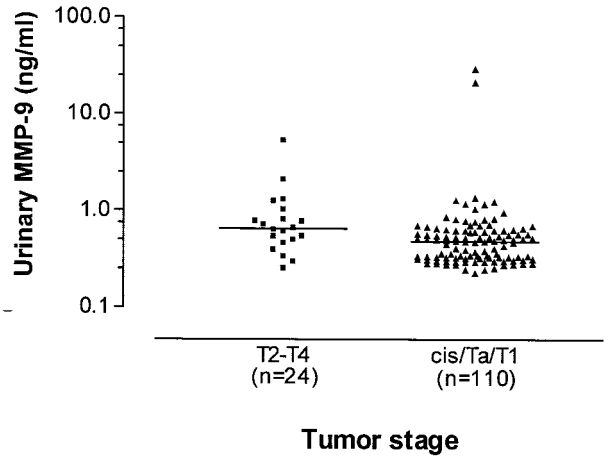

Fig. 2 Urinary MMP-9 concentrations in samples from patients with muscle-invasive bladder tumors (T_2–T_4) compared with samples from patients with noninvasive (*cis*/T_a/T_1) tumors. *Bars* represent median urinary MMP-9 concentrations.

cooker for 20 min. Slides were incubated with an anti-MMP-9 mouse monoclonal antibody, clone 56-2A4 (Oncogene Research Products) diluted 1:50 in PBS, at room temperature for 2 h. Using Western Blotting, the antibody specifically recognized MMP-9 and did not cross-react with other members of the MMP family. Sections of colon and breast carcinoma served as positive controls. Negative controls were obtained by omitting the primary antibody from bladder tumor sections. Normal urothelial biopsies obtained during TURP from the 12 patients with BPH (but no history of bladder cancer) were also stained for MMP-9.

Sections were scored based on the intensity of cytoplasmic staining for MMP-9, where normal tissue/cellular architecture had been preserved. Staining intensities were scored as 0, 1+, 2+, 3+, or 4+ representing absent, weak, moderate, strong, and very strong staining, respectively. By definition, negative control sections were scored as 0, whereas positive control sections were scored as 4+. Only sections that were scored as strong (3+) or very strong (4+) were considered positive. Consensus scoring of sections was performed by two independent observers (G. C. D., J. E. N.) using a light microscope (×400 magnification). At the time of scoring, both observers were blinded to all clinical and pathological details related to the bladder tumors assessed. There was 81% concordance in scoring of sections between observers after the initial independent assessment of tumor MMP-9 status. Where disagreement arose, scores differed by one grade only. In these discordant cases, a consensus view was reached by reviewing the sections jointly with an experienced urological histopathologist (M. C. R.).

Statistical Analysis. Statistical analyses were performed with a computer software package. All tests of significance were two-sided. Data were presented as medians with 95% CIs and distributions were compared using nonparametric (Mann-Whitney *U* test, Kruskal-Wallis test) analyses. Proportions were compared using contingency tables, and correlations were determined using Spearman's rank test. Follow-up time and time to recurrence were calculated from the date of surgery to the date of the relevant event. Progression-free survival was defined as the interval between the date of surgery and the date of diagnosis of disease progression. Disease-specific survival was calculated as the interval between the date of surgery and the date of death from bladder cancer. Assessments of recurrence-free, progression-free and disease-specific survival rates were performed by log-rank analysis. P of <0.05 was considered significant.

RESULTS

During the follow-up period, 47 patients (48%) with cis/T_a/T_1 tumors had a recurrence, and of these, 27 (28%) had a positive 3-month check cystoscopy. Fourteen patients (12%) had tumors that progressed. Six patients with muscle-invasive tumors developed metastatic disease, whereas 8 patients with T_a/T_1 tumors progressed ($T_a \rightarrow T_1$, $T_a \rightarrow T_1$ + *cis*, $T_a \rightarrow$upper tract T3 tumor, and 5 $T_1 \rightarrow T_2$). Of the 10 patients who died, three succumbed to causes other than bladder cancer. The 7 patients that died of bladder cancer had high-grade muscle-invasive tumors at presentation.

Urinary MMP-9 Concentrations. MMP-9 was present at detectable levels in all urine samples analyzed from patients with bladder cancer, patients with benign urological conditions (cystitis, BPH) and normal volunteers. On entry to the study, patients with newly diagnosed bladder cancer had equivalent levels of urinary MMP-9 to patients with recurrent disease [0.55 ng/ml (CI: 0.438–0.661) *versus* 0.493 ng/ml (CI: 0.446–0.548), $P = 0.65$]. There was no significant difference in median urinary MMP-9 concentrations between patients with bladder cancer [0.495 ng/ml (CI: 0.41–0.53 ng/ml)] and patients with benign urological disorders [0.542 ng/ml (CI: 0.316–0.743 ng/ml)]. However, all patient groups had significantly higher urinary MMP-9 concentrations than normal volunteers [0.414 ng/ml (CI: 0.327–0.458 ng/ml); Kruskal-Wallis test, $P = 0.0167$; Fig. 1].

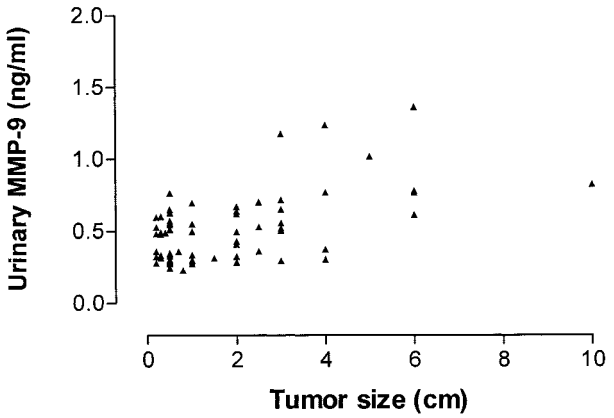

Fig. 3 *Scatter plot* showing the correlation between urinary MMP-9 concentrations and maximum tumor diameter recorded for each patient. Spearman's rank correlation coefficient, $r = 0.452$, $P < 0.0001$.

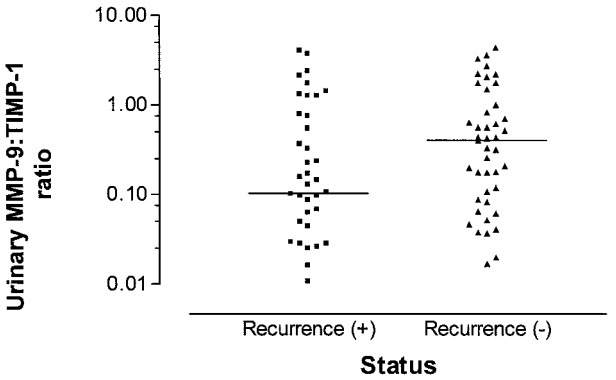

Fig. 4 Ratio of urinary MMP-9:TIMP-1 for patients with tumor recurrence ["Recurrence (+)"] at any stage during the follow-up period compared with patients who never experienced a recurrence during follow-up ["Recurrence (−)"]. *Bars* represent median urinary MMP-9:TIMP-1 ratios.

Tumor Stage and Grade: Relationship to Urinary MMP-9. Concentrations of urinary MMP-9 were significantly higher in urine samples from patients with T_2–T_4 tumors [0.648 ng/ml (CI: 0.504–0.786 ng/ml)] compared with samples from patients with *cis*/T_a/T_1 tumors [0.481 ng/ml (CI: 0.372–0.52 ng/ml); Mann-Whitney *U* test, $P = 0.0065$; Fig. 2]. In contrast, no significant variation in urinary MMP-9 concentrations with tumor grade was found [G1 (0.47 ng/ml, CI: 0.35–0.49 ng/ml) *versus* G2 (0.536 ng/ml, CI: 0.37–0.63 ng/ml) *versus* G3 (0.54 ng/ml, CI: 0.37–0.66 ng/ml); Kruskal-Wallis test, $P = 0.14$].

Urinary MMP-9: Association with Number of Tumors at Presentation, Maximum Tumor Diameter, Tumor Recurrence, Progression, and Survival. The number of bladder tumors noted at cystoscopy was unrelated to concentrations of urinary MMP-9 (Spearman's rank correlation coefficient, $r = 0.12$, $P = 0.31$). However, urinary MMP-9 levels were directly correlated with tumor size, with higher concentrations of urinary MMP-9 strongly associated with tumors of increasing diameter (Fig. 3). The median urinary MMP-9 concentration (0.42 ng/ml) was unrelated to overall recurrence-free survival (Logrank test, $\chi^2 = 0.36$, $P = 0.55$). Similarly, no correlation was found between rate of tumor recurrence and urinary MMP-9 levels (Spearman's rank correlation coefficient, $r = -0.11$, $P = 0.35$). Urinary MMP-9 concentrations were not associated with tumor progression (Logrank test, $\chi^2 = 0.046$, $P = 0.83$) or disease-specific survival (Logrank test, $\chi^2 = 0.36$, $P = 0.55$).

Ratio of Urinary MMP-9:TIMP-1. The median urinary MMP-9:TIMP-1 ratio for all patients with TCC was 0.177 (CI: 0.11–0.32). For nonmuscle-invasive (*cis*/T_a/T_1) tumors, recurrence was inversely related to the urinary MMP-9:TIMP-1 ratio. Patients who suffered a recurrence of their bladder cancer at any stage during the follow-up period had a significantly lower median urinary MMP-9:TIMP-1 ratio when compared with patients who never experienced recurrence during follow-up [0.1 (CI: 0.052–0.23) *versus* 0.4 (CI: 0.18–0.59), $P = 0.02$, Mann-Whitney *U* test; Fig. 4]. An inverse correlation was found between overall tumor recurrence rate and the urinary MMP-9:TIMP-1 ratio, but this did not reach significance (Spearman's rank correlation coefficient, $r = -0.1862$, $P = 0.086$). Patients

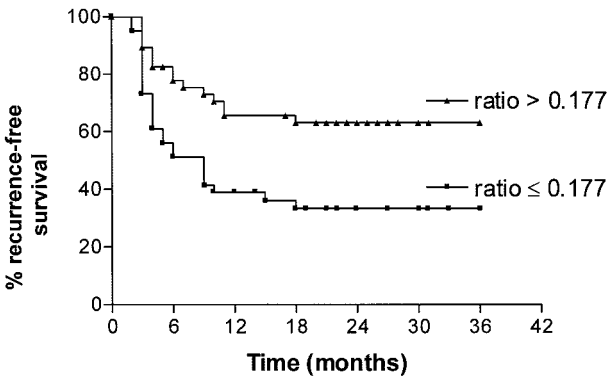

Fig. 5 Kaplan-Meier curve showing recurrence-free survival rates for patients with urinary MMP-9:TIMP-1 ratios less than, or equal to, the median urinary MMP-9:TIMP-1 level (ratio ≤ 0.177) compared with patients with ratios greater than the median urinary MMP-9:TIMP-1 level (ratio > 0.177). Logrank test, $\chi^2 = 8.5$, hazard ratio: 2.34 (CI: 1.36–4.87), $P = 0.0035$.

with urinary MMP-9:TIMP-1 ratios ≤ 0.177 had significantly poorer rates of recurrence-free survival compared with patients with urinary MMP-9:TIMP-1 ratios >0.177 ($P = 0.0035$, Fig. 5). There was no relationship between the urinary MMP-9:TIMP-1 ratio and bladder tumor stage, grade, size, or multifocality. Also, no association was found between the ratio of urinary MMP-9:TIMP-1 and progression-free or disease-specific survival.

Immunohistochemical Detection of MMP-9. Of the 106 bladder tumor tissue sections stained for MMP-9, 64 (60%) sections were scored as MMP-9 positive (3+ and 4+), whereas 42 (40%) were classed as MMP-9 negative (0, +1, and +2). A mixed pattern of MMP-9 staining was observed in bladder tumor sections with staining localized predominantly to tumor cell cytoplasm but where staining was noted in stromal fibroblasts it was usually adjacent to the tumor/stroma interface. In addition, occasional staining of endothelial cells was observed, and very strong staining was noted in polymorphonuclear leu-

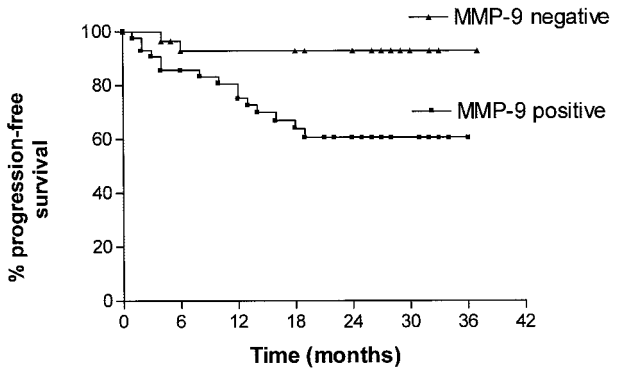

Fig. 6 Kaplan-Meier curve showing the difference in rates of disease progression (for all stages) for patients with tumors displaying positive immunohistochemical staining for MMP-9 compared with patients with MMP-9-negative bladder tumors [Logrank test, $\chi^2 = 7.85$, hazard ratio: 6.22 (CI: 1.51–10.37), $P = 0.005$].

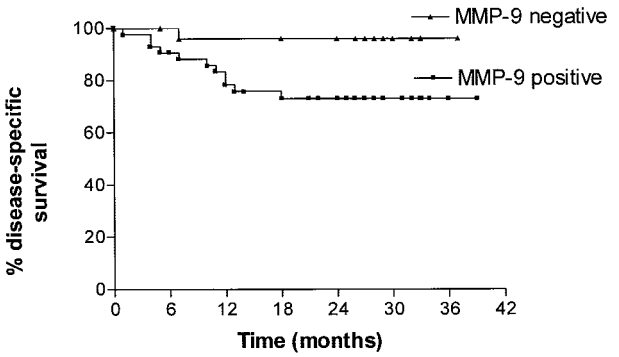

Fig. 7 Kaplan-Meier curve comparing disease-specific survival rates for patients with tumors displaying positive immunohistochemical staining for MMP-9 compared with patients with MMP-9-negative bladder tumors [Logrank test, $\chi^2 = 5.277$, hazard ratio: 7.56 (CI: 1.22–12.36) $P = 0.022$].

kocytes. Negative control sections from which the primary antibody had been omitted showed a complete absence of staining for MMP-9, whereas all 12 sections of normal urothelium obtained from the control patients with no previous history of urological malignancy showed weak (+1) or absent (0) staining for MMP-9. There was no relationship between the concentrations of MMP-9 or TIMP-1 detected in urine samples from patients with bladder cancer and the immunohistochemical status of corresponding bladder tumor tissue sections stained for MMP-9 (Mann-Whitney *U* test, $P = 0.23$ and $P = 0.85$, respectively).

A positive correlation was noted between MMP-9 staining and increasing tumor size (Spearman's rank correlation coefficient, $r = 0.294$, $P = 0.014$). Furthermore, patients who underwent disease progression had a higher proportion of MMP-9-positive tumors than patients with tumors that did not progress ($P = 0.005$, Fig. 6). Disease-specific survival was also associated with MMP-9 status: patients with MMP-9 positive tumors had higher death rates from bladder cancer than patients with MMP-9-negative tumors ($P = 0.022$, Fig. 7).

There was no significant difference in the proportion of muscle-invasive tumors staining positively for MMP-9 compared with *cis*/T_a/T_1 tumors (68% T_2–T_4 *versus* 56% *cis*/T_a/T_1, Fisher's exact test, $P = 0.46$). MMP-9 staining was also unrelated to tumor grade (45% G_1 *versus* 61% G_2 *versus* 67% G3, χ^2 test, $P = 0.26$). There was no correlation between MMP-9 status and tumor multifocality (Spearman's rank correlation coefficient, $r = -0.0235$, $P = 0.85$) or recurrence rate (Spearman's rank correlation coefficient, $r = 0.069$, $P = 0.62$), nor was there an association between bladder tumors that stained positively for MMP-9 and overall recurrence-free survival (Logrank test, $\chi^2 = 0.226$, $P = 0.635$).

DISCUSSION

We have noted that urinary MMP-9 concentrations increased with advancing tumor stage and tumor size but were unrelated to tumor grade, recurrence, progression, or survival. It is therefore likely that higher urinary MMP-9 concentrations simply reflect tumor burden rather than loss of differentiation. In the only previous study to measure concentrations of urinary MMP-9 in bladder cancer, 33 patients submitted voided urine samples before surgery. Urinary MMP-9 was measured in the range 0.9–15.2 ng/ml using a one-step enzyme immunoassay, and concentrations of urinary MMP-9 were not associated with tumor stage or grade (18).

In our study, although concentrations of MMP-9 were higher in samples from patients with bladder cancer than normal volunteers, no difference was noted in levels of urinary MMP-9 between patients with bladder cancer and those with benign urological conditions (cystitis or BPH). This lack of specificity calls into question the source of urinary MMP-9. It is known that increased levels of MMP-9 can be detected in the urine by zymography in patients with tumors outside the urinary tract compared with healthy individuals without cancer (9). Presumably, in such cases, MMP-9 is either filtered from the blood or actively secreted by the kidney. In bladder cancer, urinary MMP-9 is likely to arise from the serum as a result of hematuria and also directly from the bladder wall at the site of the tumor. In benign urological conditions, raised levels of urinary MMP-9 are probably mediated by cytokines elaborated by neutrophils and mast cells in response to acute inflammation.

Staining for MMP-9 localized predominantly to bladder tumor cells with cytoplasmic immunoreactivity, but strong staining was also noted in polymorphonuclear leukocytes. Where stromal staining occurred, it was strongest at the tumor/stromal interface. These observations are generally consistent with other immunohistochemical studies of MMP-9 in bladder cancer (18, 21). Levels of MMP-9 in urine were unrelated to the presence of MMP-9 detected immunohistochemically in bladder tumor tissues. A disadvantage of the scoring method used in the immunohistochemical aspects of this study is that cells have to be allocated to a category of staining intensity, whereas in reality, staining intensity is a continuum from absent to weak to moderate to strong. ELISA measures actual concentrations of MMP-9 protein present in urine, whereas immunohistochemistry is relatively subjective, semiquantitative, and does not determine tumor tissue concentrations of MMP-9. A more precise relationship between levels of MMP-9 in urine samples and

bladder tumor tissue specimens might be determined by measuring MMP-9 levels in the supernatant of tissue lysates of snap-frozen fresh bladder tumors.

Although MMP-9 staining was strongly associated with the presence of bladder cancer and was completely absent from biopsies of normal urothelium, the prognostic significance of positive MMP-9 immunoreactivity in bladder cancer is questionable. The immunohistochemical expression of MMP-9 was not associated with tumor stage, grade, multiplicity, or recurrence rate, although both tumor progression and disease-specific survival were related to MMP-9 status in univariate analysis. The patients studied comprised a heterogeneous group with many cancers of different stages and grades. All patients who died of bladder cancer had muscle-invasive disease, and the majority of patients that suffered disease progression had high-grade tumors, most of which (77%) were MMP-9 positive. However, muscle-invasive tumors were underrepresented in this series. To determine whether the immunohistochemical detection of MMP-9 has true prognostic significance in bladder cancer, additional studies and longer clinical follow-up are required, where a greater proportion of patients have muscle-invasive disease, permitting a formal multivariate analysis to be performed.

Most bladder tumors are nonmuscle invasive at presentation, and a significant proportion of these will recur, placing a heavy burden of surveillance on both the patient and clinician. Few studies have examined the significance of the MMP-TIMP system in this major subgroup of patients with bladder cancer. In one such study, using Northern blotting to assess mRNA levels in nonmuscle-invasive bladder cancer, Hara *et al.* (17) noted that increased expression of both MMP-9 and TIMP-2 were associated with significantly higher rates of tumor recurrence. However, the balance or ratio between these markers was not assessed in this study. It is interesting to note that the concept of TIMPs as simple inhibitors of MMPs in tumor systems has evolved and studies have demonstrated a clear association between increased levels of TIMP expression and poor prognosis in a number of solid malignancies, including colorectal (22), lung (23), gastric (24), and breast cancer (25). We have previously noted that increased urinary concentrations of TIMP-1 in patients with bladder cancer were associated with stage progression and that a low urinary MMP-1:TIMP-1 ratio was found in patients with bladder tumors of advanced stage and grade (13). This study shows that the ratio of urinary MMP-9 to TIMP-1 was unrelated to tumor stage, grade, size, multiplicity, and progression. However, where the ratio of urinary MMP-9 to TIMP-1 was low, indicating a significant excess of TIMP-1 over MMP-9, the recurrence rate of T_a/T_1 tumors was high and overall recurrence-free survival was poor. It is now well recognized that TIMP-1 is a multifunctional protein with a variety of activities that are independent of MMP-inhibition (3), including the ability to stimulate growth of many different cell lines (26). This unique property may, in part, explain why patients are more likely to experience tumor recurrence when TIMP-1 is present in excess of MMP-9. Measuring the ratio of urinary MMP-9 to TIMP-1 may prove useful in planning follow-up cystoscopic surveillance protocols or intravesical chemotherapy regimens for patients with bladder tumors. For patients with low ratios, undergoing cystoscopy at more frequent intervals along with more aggressive intravesical chemotherapy may be appropriate. Additional prospective studies of the ability of an altered urinary MMP-9:TIMP-1 ratio to predict tumor recurrence in patients with nonmuscle-invasive bladder cancer are warranted.

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