Tumor-Specific Urinary Matrix Metalloproteinase Fingerprinting: Identification of High Molecular Weight Urinary Matrix Metalloproteinase Species

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Abstract

Purpose: We have previously reported that matrix metalloproteinases MMP-2, MMP-9, and the complex MMP-9/NGAL can be detected in urine of patients with a variety of cancers including prostate and bladder carcinoma. In addition, we also detected several unidentified urinary gelatinase activities with molecular weights >125 kDa. The objective of the current study was to identify these high molecular weight (HMW) species, determine their potential as predictors of disease status, and ask whether a tumor-specific pattern existed based on urinary MMP analysis.

Experimental Design: Chromatography, zymography, and mass spectrometry was used to identify HMW gelatinase species of ~140, 190, and >220 kDa in urine of cancer patients. To determine whether a tumor-specific pattern of appearance existed among the MMPs detected, we analyzed the urine of 189 patients with prostate or bladder cancer and controls.

Results: The ~140, >220 kDa, and ~190 HMW gelatinase species were identified as MMP-9/tissue inhibitor of metalloproteinase 1 complex, MMP-9 dimer, and ADAMTS-7, respectively. The frequency of detection of any MMP species was significantly higher in urine from prostate and bladder cancer groups than controls. MMP-9 dimer and MMP-9 were independent predictors for distinguishing between patients with prostate and bladder cancer (P < 0.001 for each) by multivariable analysis.

Conclusions: This study is the first to identify a tumor-specific urinary MMP fingerprint that may noninvasively facilitate identification of cancer presence and type. This information may be of diagnostic and prognostic value in the detection and/or clinical monitoring of disease progression and therapeautic efficacy in patients with bladder or prostate cancer.

Matrix metalloproteases (MMP) comprise a family of proteolytic enzymes that have been implicated in tumor growth, invasion, and metastasis in experimental cancer models and in human tumors (1–8). Two members of this family in particular, MMP-2 and MMP-9, degrade type IV collagen, fibronectin and laminin, major components of the basement membrane, and are commonly used as markers of the malignant phenotype. MMP activity is regulated by a group of four distinct tissue inhibitors of metalloproteases (TIMP; refs. 1, 5, 9).

Overexpression of MMPs in tumor tissue and stroma can result in increased levels of MMP activity in various body fluids. Increased presence of MMP-2 and MMP-9 has been detected in the serum and plasma of tumor-bearing rats and in humans with malignant tumors (10–15). An increase in MMP-9 levels has been reported in the tissue of animals bearing prostate and bladder tumors (16, 17). We have previously reported that MMPs can be detected in urine from patients with a variety of cancers and are independent predictor of disease status (18–22). The MMP species detected in urine from cancer patients include MMP-2 (~72 kDa), MMP-9 (~92 kDa; ref. 18), a complex of MMP-9 with human neutrophil gelatinase–associated lipocalin (NGAL; ~125 kDa; refs. 19, 20), and several unidentified high molecular weight (HMW) gelatinase species. Since our original report, other groups have confirmed our findings of elevated levels of MMP-2 and MMP-9 in urine from prostate and bladder cancer patients (23–30). Despite the potential importance of HMW MMPs in predicting the presence of disease, the identity of several of these HMW MMPs has remained unknown.

The objective of the current study was to identify and characterize these as yet unidentified HMW gelatinase activities in urine from cancer patients and to determine whether their presence might be relevant to disease status. Using a combination of mass spectrometry, substrate gel electrophoresis, and fractionation, we have now identified three HMW gelatinase
species in urine from cancer patients. These include ~140 kDa gelatinase identified as a complex of MMP-9 and its endogenous inhibitor TIMP-1, and a >220 kDa gelatinase species identified as MMP-9 dimer. In addition, a novel ~190 kDa gelatinase band was identified as a disintegrin and metalloproteinase with thrombospondin motifs-7 (ADAMTS-7).

Our study also identifies, for the first time, a tumor-specific fingerprinting pattern based on the detection of MMPs in urine of patients with prostate or bladder tumors. Four MMP species were reproducibly detected in the urine of cancer patients: MMP-2, MMP-9, MMP-9/NGAL complex, and MMP-9 dimer. A tumor-specific urinary MMP (uMMP) fingerprint was found by comparing samples from prostate and bladder cancer patients. Although MMP-2 and MMP-9/NGAL complex were detected with comparable frequency in the urine of these patients, MMP-9 (~92 kDa) was detected with significantly higher frequency in the urine of patients with prostate cancer compared with those with bladder cancer. Frequency of positive expression of MMP-9 dimer (>220 kDa) was significantly higher in patients with bladder compared with prostate cancer.

Materials and Methods

**Urine collection and processing.** One hundred and eighty-nine samples were analyzed in this study, including samples from patients diagnosed with organ-confined prostate (n = 103) and bladder (n = 41) cancer, and controls (n = 45). Cancer groups and controls were comparable with respect to age both in terms of the mean and the range. Urine was collected according to the institutional bioethical guidelines pertaining to discarded clinical material (18). Specimens were obtained before surgical or other therapeutic intervention. Samples were collected in sterile containers and immediately frozen at -20°C. Urine was tested for presence of blood and leukocytes using Multistix 9 Urinalysis Strips (Bayer) and samples containing blood or leukocytes were excluded. Protein concentration of urine was determined by the Bradford method using bovine serum albumin as the standard (18, 31).

**Substrate gel electrophoresis.** Gelatinases in urine were detected using gelatin zymography as described previously (18). Briefly, urine (40 μL) from controls or cancer patients and pure (2 ng) MMP-2, MMP-9, MMP-9 dimer, or MMP-9/TIMP-1 complex were mixed with sample buffer and resolved via electrophoresis. Substrate digestion was conducted as previously described (18). Gels were stained with Coomassie and imaged using Bio-Rad Imager. Bands of enzyme activity were detected as zones of clearance on a background of

Translational Relevance

The present study identifies a tumor-specific fingerprinting pattern, based on the detection of matrix metalloproteinases in urine of cancer patients, which may noninvasively facilitate identification of cancer presence and type. This information may be of diagnostic and prognostic value in the detection and/or clinical monitoring of disease progression and therapeutic efficacy in patients with bladder or prostate tumors.

Fig. 1. HMW gelatinase species in urine from cancer patients. A, representative urine samples from cancer patient analyzed by zymography. Arrows, previously unidentified uMMps. Latent and active forms of MMP-9 are indicated. B, substrate gel electrophoresis and comigration studies of urine from cancer patients (1-5) with pure MMP-9 monomer and dimer (lane 2 and 8). C, MMP-9 dimer detected in urine from cancer patients (1-2) by immunoblot. D, immunoprecipitation of MMP-9 dimer. Urine containing MMP-9 dimer activity (lane 1) was treated with nonimmune control antibodies (lane 2) or anti-MMP-9 antibodies for 30 min (lane 3) or 1 h (lane 4). The MMP-9 dimer, MMP-9/NGAL, and MMP-9 species were specifically precipitated by the anti-MMP-9 antibody but not by the control antibody.
uniform blue staining. To correlate gelatin-degrading activities in urine, purified MMP and MMP-complexes were loaded onto each gel for comigration studies. Zymograms were evaluated independently in a binary fashion (presence or absence) by two investigators in a blinded manner.

**Immunoblot analysis.** Immunoblotting was used to verify the identity of MMP-9 and the various MMP complexes in urine. Equal amounts of proteins (20 μg) were separated by SDS-PAGE under nonreducing conditions and treated as previously described (31). Monoclonal antibodies against human MMP-9 (MAB13415; Millipore) and TIMP-1 (Ab-1; Millipore) were used. For detection of ADAMTS-7, urine (100 μg) was incubated with 50 μL gelatin sepharose beads for 16 h at 4°C. Subsequently, the sample was spun down, supernatant was discarded, and sample buffer was added. After 1 h of incubation at room temperature, samples were analyzed as described above using anti-ADAMTS-7 antibodies (CL1ADAMTS-7; Cedarlane Labs; ab28596; Abcam) at 1 μg/mL each.

**Immunoprecipitation.** Urine (50 μL) containing MMP-9 dimer activity was mixed with equal volume of radioimmunoprecipitation assay buffer [0.1 mol/L Hepes (pH 8.0), 0.15 mol/L NaCl, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS] and MMP-9 antibody (44236; EMD Chemicals) or a nonimmune serum control and treated as described previously (19). Antibody-antigen complexes were pelleted by centrifugation at 10,000 × g, and the supernatant was subjected to zymography to detect any remaining gelatinase activity.

**Identification of urinary ADAMTS-7.** Urine (25 mL) was concentrated (Amicon YM-5; Millipore), diluted with 1 volume of 50 mmol/L Tris (pH 7.5; buffer A) and applied to a HiTrap Q Sepharose column (GE Healthcare). A gradient of 0 to 500 mmol/L NaCl in buffer A was applied. Fractions containing ∼190 kDa gelatinase activity were pooled, the NaCl concentration adjusted to 200 mmol/L, and loaded onto Gelatin Sepharose column (GE Healthcare) pre-equilibrated with buffer A containing 200 mmol/L NaCl (buffer B). Elution was done using a stepwise gradient of 5%, 10%, and 20% DMSO, respectively, in buffer B. Eluted fractions containing the ∼190 kDa gelatinase were concentrated (Vivaspin 500; Sartorius) and resolved using SDS-PAGE. Gels were stained with Sypro Ruby (Bio-Rad), and protein bands were excised from the gel, subjected to tryptic digestion, and analyzed by Tandem MS/MS mass spectrometry (Perceptive STR; Applied Biosystems). Using the SEQUEST (32) search program, the peptide maps generated were searched against a FASTA database of public domain proteins. Peptide matches were filtered according to their xCorrelation values, percentage of masses matched, molecular weight, and number of observations of peptides and proteins.

**Statistical analyses.** Percentages of individuals with positive expression for different uMMP species (MMP-9, MMP-2, MMP-9/NGAL complex, and MMP-9 dimer) were compared between cancer patients and controls using Fisher’s exact test. Power analysis indicated that a sample size of 40 patients in each cancer group (prostate and bladder) and 40 controls would provide 80% power (two-tailed α, 0.01; β, 0.20) to detect significant differences in expression for each
uMMP species (version 6.0, nQuery Advisor; Statistical Solutions). Sensitivity, specificity, and accuracy were determined for each uMMP species using standard formulae. Sensitivity (true-positive rate) is defined as the percentage of patients with the target disease who have a positive MMP test result; specificity (true-negative rate) is defined as the percentage of individuals without the target disease who have a negative MMP test result; accuracy is determined as the percentage of correct test results (true positives and true negatives divided by the number of tests). Likelihood ratio for a positive test result was defined as the ratio of its probability of occurrence if cancer is present to its probability of occurrence if cancer is absent (sensitivity/1-specificity; ref. 33). Multiple logistic regression was applied to estimate the probability of prostate and bladder cancer based on the MMPs that were identified as multivariate predictors (34). Statistical analysis was done using SPSS (version 16.0, SPSS, Inc.). Conservative two-tailed P values of <0.01 were considered statistically significant to protect against type I errors arising from multiple comparisons (34).

Results

**Identification of HMW gelatinase species in urine of cancer patients.** In addition to MMP-2 (~72 kDa, 18), MMP-9 (~92 kDa, 18), and MMP-9/NGAL complex (~125 kDa, 19-22), urine samples from cancer patients also contain several previously unidentified HMW gelatinases (Fig. 1A). These include species migrating at >220, ~190, and ~140 kDa, respectively. The first HMW gelatinase identified in this study was the >220 kDa species. When urine containing this activity was subjected to zymography, we found that it comigrated with pure MMP-9 dimer (Fig. 1B). Urine contained three immunoreactive forms of MMP-9. As previously reported, the ~92 kDa and ~125 kDa gelatinase species were the monomeric and MMP-9/NGAL complex form of MMP-9, respectively (18, 19). The >220 kDa species was identified as MMP-9 dimer (Fig. 1C). These results were further verified by immunoprecipitation using anti–MMP-9 antibodies. The nonimmune control antibody failed to immunoprecipitate any of the MMP activities; however, MMP-9 dimer, MMP-9/NGAL, and MMP-9 were all specifically precipitated by the anti–MMP-9 antibody (Fig. 1D). Taken together, these data confirm the identity of the >220 kDa gelatinase in urine of cancer patients as being MMP-9 dimer.

Interestingly, the ~140 kDa gelatinase appeared to comigrate with purified preparation of MMP-9/TIMP-1 complex (Fig. 2A). Under physiologic conditions, active MMP-9 is known to form a complex with TIMP-1, an inhibitor of MMPs (35). Anti–TIMP-1 antibodies detected the ~140 kDa band in urine from cancer patients (Fig. 2B). This antibody also detected pure MMP-9/TIMP-1 complex and TIMP-1 (~28 kDa; Fig. 2C). To validate this finding, 15 urine samples containing the ~140 kDa gelatinase were subjected to immunoblotting. Six of these samples (40%) indicated the presence of MMP-9/TIMP-1 complex (Fig. 2B and C).

**Identification of the ~190 kDa gelatinase in urine.** An ~190 kDa gelatinase species was detected in urine from [Fig. 3. Identification of ~190 kDa gelatinase species in urine. A, the ~190 kDa gelatinase species (arrow) was detected in representative urine samples by zymography. B, enrichment of the ~190 kDa species via fractionation. Fractions eluted from gelatin sepharose column were analyzed by zymography, and the ~190 kDa gelatinolytic activity was detected in the fraction eluted after the application of 20% DMSO (lane 2) but not in the flow through or wash fractions (lane 3 and 4). C, Sypro Ruby staining (left) and zymography (right) of ~190 kDa species. D, immunoblot analysis of ADAMTS-7. ADAMTS-7 was detected in urines from cancer patients (right) but not controls (left).]
patients with a variety of cancers including prostate, brain, bladder, breast, and liver carcinomas (Fig. 3A). To identify this species, we pooled and fractionated urine. The enriched fraction collected from a Gelatin Sepharose column contained several distinct ~68, 92, 125, 190, and >250 kDa gelatinases (Fig. 3B, lane 2), whereas the flow through and wash fractions did not contain these activities (Fig. 3B, lane 3 and 4). Enrichment of the ~190 kDa band in the fractionated sample was evident from the appearance of strong gelatinase activity (Fig. 3C, right). Distinct protein bands of ~92, 125, and >220 kDa (Fig. 3C, left) were excised and subjected to Tandem MS/MS. Consistent with previous reports, the ~92 kDa band was identified as human MMP-9 (NP_004985, 11 peptides), the ~125 kDa species contained both MMP-9 (9 peptides) and NGAL (NP_005555, 4 peptides; refs. 18–22). The ~190 kDa gelatinase (Fig. 3C, lane 2) was identified as disintegrin and metalloprotease with thrombospondin motifs-7 (ADAMTS-7; NP_055087).

Three distinct peptides spanning the amino acid sequence of human ADAMTS-7 were identified (data not shown). To confirm these results, urine from patients with breast, bladder, or prostate carcinoma and age-matched controls was analyzed. An ADAMTS-7–specific antibody (ab28596) recognized the ~190 kDa species (Fig. 3D, right) in these urines, whereas no such species was detected in control urines (Fig. 3D, left). A second ADAMTS-7 antibody (CL1ADAMTS-7) gave similar results (data not shown). Based on this cross reactivity and molecular weight, the ~190 kDa gelatinase in urine was identified as the mature form of ADAMTS-7.

Tumor-specific fingerprinting of uMMPs. Analysis of 189 urine samples was conducted via zymography (n = 144 patients with organ-confined prostate or bladder cancers; n = 45 healthy volunteers). Representative zymographic results indicated low or no gelatinase activity in control urines (Fig. 4A). Predominant MMPs detected reproducibly in a binary fashion, in both types of cancer specimens were MMP-9 dimer (>220 kDa), MMP-9/NGAL complex (~125 kDa), and MMP-2 (~68 kDa).

Positive expression of MMPs was compared between prostate and bladder cancer by Fisher’s exact test (Fig. 4B). Higher percentages of urines from prostate and bladder cancer patients were positive for MMP-9 dimer and MMP-9/NGAL compared with controls (Table 1). MMP-9 dimer was present with significantly higher frequency in urine of bladder compared with prostate cancer (66% versus 36%; P < 0.001) patients, whereas MMP-9/NGAL expression was similar between cancer subgroups (50% versus 49%). As compared with controls, positive rates for MMP-9 were significantly higher in patients with prostate cancer (18% versus 58%; P < 0.001) but not with bladder cancer (18% versus 29%; P = 0.31). No controls were

Fig. 4. Tumor-specific fingerprinting of uMMPs. A, substrate gel electrophoresis of MMPs in representative urine samples from healthy controls, prostate, and bladder cancer patients. Gelatinase activity was detected in the urine of both types of cancer patients at approximately ~68, 125, and 220 kDa, which corresponds to MMP-2, MMP-9/NGAL complex, and MMP-9 dimer, respectively. MMP-9 (~92 kDa) was detected with significantly higher frequency in the urine of prostate cancer patients. B, positive expression for different MMP species for cancer patients and controls. * indicates significantly higher rate of expression for cancer group compared with controls (P < 0.001 for each; Fisher’s exact test). No significant difference was observed in the MMP-9 – positive expression rate between patients with bladder cancer and controls (29% versus 18%, P = 0.31); 1, a significantly different rate of expression between and prostate and bladder cancer groups. Model indicating probability of prostate (C) and bladder (D) cancer based on combinations of two multivariate biomarkers. For both cancer groups, the estimated probability is 100% for positive MMP-2 expression; however, when MMP-2 is negative, a positive MMP-9/NGAL expression indicates a predicted probability of 86% for prostate cancer (C), whereas positive MMP-9 dimer expression indicates a predicted probability of 71% for bladder cancer (D).
positive for MMP-2 compared with 55% of prostate and 66% of bladder cancer patients, respectively ($P < 0.001$).

MMP diagnostic characteristics, based on the binary evaluation, are provided in Table 1. Likelihood ratios summarize probability of a test result for individuals with and without cancer. For example, 52 of 103 prostate cancer patients were positive for MMP-9/NGAL (sensitivity, 50%), whereas 2 of 45 controls were positive (false-positive rate, 4%). This represents a likelihood ratio for a positive test of 12.5 (50 of 4), indicating that a positive MMP-9/NGAL test result is over 12 times as likely to come from patients with prostate cancer than controls. The likelihood ratio for a negative test calculated as the false-negative rate (50%; 52 of 103) divided by specificity (96%; 43 of 45) was determined to be 0.52. Thus, a negative MMP-9/NGAL result is about half as likely to be from prostate cancer patients than controls. For bladder cancer, the sensitivity and false-positive rate were determined to be 66% and 4%, respectively, with a likelihood ratio for a positive test of 16.5 (66 of 4). Therefore, urine from a bladder cancer patient would be 16.5 times as likely as to be positive for MMP-9 dimer than controls. Similarly, a likelihood ratio for a negative test of 0.35 indicates that a negative MMP-9 dimer result is about a third as likely to come from bladder cancer patients as controls.

Multivariable regression confirmed two MMPs (MMP-2, $P < 0.001$; MMP-9/NGAL, $P = 0.003$) as independently predictive in differentiating patients with prostate cancer from controls. When MMP-2 is positive, the probability of prostate cancer is 100% regardless of MMP-9/NGAL status (Fig. 4C). When MMP-2 is negative, the probability of prostate cancer is 86% when MMP-9/NGAL is positive and 44% when MMP-9/NGAL is negative. For bladder cancer, MMP-2 and MMP-9 dimer proved to be independently predictive (Fig. 4D). Specifically, when MMP-2 is positive, the probability of bladder cancer is 100% regardless of whether MMP-9 dimer is positive or negative; however, when MMP-2 is negative, the probability is high (71%) if MMP-9 dimer is positive and low (17%) when it is negative. MMP-2 is the most powerful diagnostic predictor for both cancer groups.

We also identified MMP-9 dimer and MMP-9 as multivariable predictors for differentiating prostate from bladder cancer ($P < 0.001$ each). Positive expression of MMP-9 dimer was 1.5-fold higher in frequency in urine from bladder compared with prostate cancer patients, whereas MMP-9 expression was 2-fold higher in prostate compared with bladder cancer patients, respectively. MMP-2 ($P = 0.73$) and MMP-9/NGAL ($P = 0.69$) were not discriminatory. In short, although prostate and bladder cancer patients often test positive for MMP-2 and MMP-9/NGAL, differences relate to MMP-9 monomer and dimer.

### Discussion

In this study, we report for the first time the identity of several HMW species in urine from cancer patients. These are MMP-9 dimer, MMP-9/TIMP-1 complex, and ADAMTS-7. Among the members of the MMP family, MMP-9 is unique in that when present in excess relative to its endogenous inhibitor TIMP-1, it can form dimers. MMP-9 dimer has been identified in a variety of MMP-9-producing cells including neutrophils and normal breast epithelial cells (36, 37) and is a component of normal plasma (38). Enzymatic activity of the monomeric and dimeric forms of MMP-9 does not differ; however, the dimer can be activated by stromelysin with much lower efficiency (10-fold) than the monomer (39). The existence of the more stable, slow-activating MMP-9 dimer might serve as a regulatory mechanism during extracellular matrix degradation (39). Therefore, it is conceivable that in our study, expression of an excess level of MMP-9 relative to TIMP-1 by the primary tumor and surrounding stroma resulted in elevated levels of both monomeric and dimeric forms of this protease in urine.

MMP-9 is inhibited by TIMP-1 (37, 39). Several studies that measured free MMP-9 and TIMP-1 levels in bladder, breast, and gastric tumor tissue (26, 40, 41) and biological fluids including urine, plasma, and serum from cancer patients (15, 26, 42) reported that the ratio of MMP-9 versus TIMP-1 expression may be an important indicator of tumor progression and a predictor of tumor recurrence. MMP-9/TIMP-1 complex was recently reported to be a serum marker for fibrosis in children with chronic hepatitis B (43). However, very little is known about MMP-9/TIMP-1 complex and its correlation with cancer. We found that urine from cancer patients indicated the presence of ~140 kDa species consistent with MMP-9/TIMP-1 complex when probed with TIMP-1–specific antibody. In the current

### Table 1. MMP diagnostic performance characteristics in differentiating prostate and bladder cancer patients from each other and from controls

<table>
<thead>
<tr>
<th>MMP species</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>LR (+)</th>
<th>LR (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostate cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-9 dimer</td>
<td>36% (37/103)</td>
<td>96% (43/45)</td>
<td>54% (80/148)</td>
<td>9</td>
<td>0.67</td>
</tr>
<tr>
<td>MMP-9/NGAL</td>
<td>50% (52/103)</td>
<td>96% (43/45)</td>
<td>64% (95/148)</td>
<td>12.5</td>
<td>0.52</td>
</tr>
<tr>
<td>MMP-9</td>
<td>56% (60/103)</td>
<td>82% (37/45)</td>
<td>65% (97/148)</td>
<td>3.2</td>
<td>0.51</td>
</tr>
<tr>
<td>MMP-2</td>
<td>58% (57/103)</td>
<td>100% (45/45)</td>
<td>69% (102/148)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Any MMP</td>
<td>74% (77/103)</td>
<td>82% (37/45)</td>
<td>77% (114/118)</td>
<td>4.1</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Bladder cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-9 dimer</td>
<td>66% (27/41)</td>
<td>96% (43/45)</td>
<td>81% (70/86)</td>
<td>16.5</td>
<td>0.35</td>
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<tr>
<td>MMP-9/NGAL</td>
<td>49% (20/41)</td>
<td>96% (43/45)</td>
<td>73% (63/86)</td>
<td>12.3</td>
<td>0.53</td>
</tr>
<tr>
<td>MMP-9</td>
<td>29% (12/41)</td>
<td>82% (37/45)</td>
<td>57% (49/86)</td>
<td>1.6</td>
<td>0.87</td>
</tr>
<tr>
<td>MMP-2</td>
<td>66% (27/41)</td>
<td>100% (45/45)</td>
<td>84% (72/86)</td>
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<td>ND</td>
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<tr>
<td>Any MMP</td>
<td>81% (33/41)</td>
<td>82% (37/45)</td>
<td>81% (70/86)</td>
<td>4.5</td>
<td>0.23</td>
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Abbreviations: LR (+), likelihood ratio for a positive test; LR (-), likelihood ratio for a negative test; ND, not defined.
study, MMP-9/TIMP-1 complex was not detected with significant frequency in urine from prostate and bladder cancer patients. We also report here the identification of ADAMTS-7 as the ~190 kDa gelatinase present in urine. To date, ADAMTS-7 has yet to be associated with human cancers. Therefore, the identification of ADAMTS-7 in urine from cancer patients in this study represents the first report correlating this protease with this disease. ADAMTS are a family of disintegrin zinc-dependent proteases that have at least one Thrombospondin type 1 motif (1, 44). ADAMTS-7 is known to bind and degrade cartilage oligomeric matrix protein, a prominent matrix component of articular cartilage. Fragments of cartilage oligomeric matrix protein as well as elevated levels of ADAMTS-7 can be detected in the cartilage, synovial fluid, and serum of arthritis patients (45). Given that ADAMTS-7 has a functional catalytic site and can degrade cartilage oligomeric matrix protein, it is not surprising that the enzyme has gelatinolytic activity as observed in the present study. Interestingly, analysis of urine from patients with prostate, bladder, and breast tumors indicated the presence of ADAMTS-7, suggesting a functional role for this protease in tumor growth and invasion.

In this study, we analyzed the expression of four distinct gelatinase species including MMP-2, MMP-9, MMP-9/NGAL complex, and MMP-9 dimer in urine of patients with primary tumors in the prostate or bladder. Taken individually, each MMP species was detected at significantly higher rates in urine from cancer patients compared with controls. Multivariate logistic regression analyses of all four uMMP species indicated that MMP-9 dimer and MMP-9 were independent predictors for distinguishing between prostate and bladder cancer. The difference in detection of MMP-9 dimer, MMP-9, and MMP-2 in the urine of these two cancer subgroups may be an important pattern that can indicate both the presence of a tumor as well as location. This first report of a tumor-specific fingerprinting pattern based on the detection of uMMPs suggests that the detection of these uMMPs may provide useful clinical information regarding the cancer type and status.

Disclosure of Potential Conflicts of Interest

M.A. Moses is a co-inventor on Children's Hospital, Boston, patents for urinary MMPs.

Acknowledgments

We dedicate this study to the memory of Dr. Judah Folkman to express our appreciation for his continued support and encouragement of our urinary biomarker research.

References

33. Jäschke R, Guyatt GH, Sackett DL. Users’ guides to the medical literature. III. How to use an article
about a diagnostic test. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. JAMA 1994;271: 703–7.


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