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Significance of MMP-2 Expression in Prostate Cancer: an Immunohistochemical Study

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ABSTRACT

Prostate cancer is the most common cancer in North American men. Currently available prognostic factors inadequately predict which cancers will be aggressive and which will lead an indolent course. This study was aimed at investigating the role of matrix metalloproteinase (MMP)-2 in prostate cancer disease-free survival. We correlated MMP-2 expression by malignant prostatic epithelium and stromal cells with prostate cancer disease-free survival in 187 stage pT3NxM0 prostate carcinomas using immunohistochemistry. MMP-2 was expressed by cancer cells in 131 cases (70.0%) and by stromal cells in 142 cases (75.9%). MMP-2 expression by stromal cells was not associated with progression ($P = 0.7270$). However, in multivariate analyses, adjusting for the Gleason score, tumor-node-metastasis stage, and initial serum prostate-specific antigen, MMP-2 expression by >50% of malignant epithelial cells was associated with decreased disease-free survival (hazard ratio, 4.267; $P = 0.0012$). Increased MMP-2 expression by malignant prostatic epithelia is an independent predictor of decreased prostate cancer disease-free survival.

INTRODUCTION

Prostate cancer is the most common cancer in North American men. Although it is the second leading cause of death in men older than 60 years (1), only 2.9% of the new patients will die of prostate cancer (1, 2). In 1999, the prostate cancer-specific 15-year survival rate after prostatectomy was 91% (3). These data suggest that some patients would benefit from a more conservative therapy instead of the standard treatments currently available (radiotherapy and radical prostatectomy). However, our current prognostic markers are not sufficiently precise to allow us to accurately and reliably predict which patients will have aggressive tumors and which will not. The identification of new prognostic factors that better predict cancer behavior might benefit a substantial number of men with prostate cancer.

A group of endopeptidases known as MMPs[4] are a potential target for cancer therapy. The MMP family includes >20 zinc-dependent proteinases that degrade various components of the extracellular matrix such as fibrillar and nonfibrillar collagen, proteoglycans, glycoproteins, and denatured collagen (4, 5). Because they are the only enzymes known to degrade the extracellular matrix and the basement membrane, they are thought to play a major role in tumor cell metastasis. Moreover, MMPs have been shown to be involved in the release of growth factors that enhance tumor growth and aggressiveness (4–7). MMPs are inhibited by four endogenous TIMPs (4, 5, 8) as well as by a number of synthetic inhibitors (9). These inhibitors might prove useful as therapeutic agents in the treatment of cancer.

MMP-2 belongs to the gelatinase subfamily of the MMPs (10). Gelatinases are distinguished by their fibronectin-like gelatin-binding domain, which allows them to degrade nonfibrillar and denatured collagen (5, 6). MMP-2 overexpression has been reported in many neoplasms (4) including ovarian (11–13), urothelial (14–16), cutaneous (17, 18), gastric (19), breast (20, 21), and cervical (22) cancers. Besides its direct proteolytic actions, MMP-2 activates another major gelatinase called MMP-9 (23). In a knockout mouse model, MMP-9 has been shown to be involved in prostate cancer pathogenesis (24) and has been associated with vascular endothelial growth factor release in pancreatic cancer (25). These data suggest that MMP-2 may not only be an independent predictor of increased tumor aggressiveness but also be important in the activation of other proteases that are directly involved in tumor angiogenesis (26).

An increased expression of MMP-2 has been reported in prostate cancer (27–35). Four groups have investigated the relationship between MMP-2 expression and prostate cancer progression (31, 33–35). Relationships between MMP-2 expression and the GS and the pathological TNM stage have been described (31, 34). Similarly, Ross *et al.* (35) found a relationship among MMP-2, TIMP-2, and advanced cancer stage in 138 prostate cancers of all stages. Using an *in situ* hybridization approach on 41 patients, Wood *et al.* (33) found that TIMP-1 and MMP-2 expression are independent predictors of poor post-radical prostatectomy outcome. These data prompted us to investigate the role of MMP-2 in prostate cancer progression using a large and uniform cohort of prostate cancer patients having undergone radical prostatectomy and having a long term follow-up.

MATERIALS AND METHODS

Population. The patient cohort included all patients who underwent radical prostatectomy at l'Hôtel-Dieu de Québec Hospital between 1991 and 1997 having pT3NxM0 disease and follow-up PSA measurements. Patients were excluded from the study if they had received neoadjuvant hormonal therapy. Patients' charts were scanned by experienced research nurses to retrieve clinical information (age, tumor stage, initial and follow-up serum PSA level, status at last follow-up). Investigations were performed after approval by the Laval University Institutional Review Board.

Disease Recurrence and Survival. Prostate cancer disease-free survival was the primary end point used in our study. Prostate cancer was considered to have recurred in the following circumstances: (*a*) two consecutive PSA measurements above 0.3 ng/ml; (*b*) a last recorded PSA value >0.3 ng/ml; (*c*) radiological evidence of local recurrence or metastases; or (*d*) the initiation of adjuvant hormonal or radiation therapy.

Histology. All of the radical prostatectomies were performed by one surgeon (Y. F.). The excised prostates were handled and sectioned following a standardized method described by Vaillancourt *et al.* (36). GS, TNM stage, and surgical margin status were recorded for every patient. Slides not showing evidence of prostate cancer were eliminated from the study.

IHC. IHC was performed using the avidin-biotin complex method previously described by Hsu *et al.* (37). Briefly, one representative 5-μm tissue section was cut from a paraffin-embedded sample of the radical prostatectomy specimen. Sections were deparaffinized and rehydrated in graded alcohols and then incubated with normal goat serum for 20 min. Sections were incubated at room temperature for 1 h with a mouse monoclonal antibody to MMP-2 (MMP-2 VC2; Neomarkers, Fremont, CA; dilution 1/50). Afterward, sections were incubated with a biotinylated secondary antibody (Dako, Carpinteria, CA) and then exposed to a streptavidin complex (Dako). Complete reaction was revealed by 3,3′-diaminobenzidine, and the slide was counterstained with

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[4] The abbreviations used are: MMP, matrix metalloproteinase; PSA, prostate-specific antigen; IHC, immunohistochemistry; GS, Gleason score; MMP-2a, activated MMP-2; TIMP, tissue inhibitor of metalloproteinase; TNM, tumor-node-metastasis.

hematoxylin. Breast cancer specimens known for their high expression of MMP-2 were used as positive controls. Negative controls consisted of tissue sections incubated with PBS (0.16 M, pH ~7.5) instead of the primary antibody.

Each case was evaluated blindly by two independent readers (D. T. and B. T.). The immunolabeling of cancer cells and stromal cells was evaluated separately. The number of cells expressing the marker was assessed using a semiquantitative four-grade scale (0%, <10%, 10–50%, or >50%). The immunolabeling of normal or hyperplastic prostate glands, prostatic intraepithelial neoplasia, basal cells, vessel walls, and endothelial cells was also noted. The intensity of labeling was evaluated using a four-grade scale (0, +, ++, or +++). The localization of labeling relative to the tumor front, defined as the border between malignant and nonmalignant cells, was also recorded. Labeling patterns were defined as either homogeneous or heterogeneous according to the amount of consistency of labeling within the tumor specimen. Cases in which the two observers had obtained different results were collectively reviewed, and a consensus was obtained.

Statistical Analyses. The relationships between the IHC data and traditional prostate cancer prognostic factors (age, initial PSA serum level, GS, pathological TNM stage, margin status) were evaluated by Pearson's χ^2 test or Fisher's exact test. Kaplan-Meier curves were developed to compare prostate cancer disease-free survival between the various groups of immunohistochemical MMP-2 labeling. These curves were compared for statistical significance using the log rank test. $P < 0.05$ was considered statistically significant. A Cox proportional hazards model was developed to assess whether the amount of MMP-2 immunolabeling was a significant prognostic marker independent of other traditional predictors of prostate cancer disease-free survival. A trend test was used to test whether the different labeling categories were distributed along a progressive order. The analyses were performed with SAS statistical software version 8.2 (SAS, Cary, NC).

RESULTS

Population and Tumor Characteristics. The initial study population consisted of 207 cases of stage pT3NxM0 prostate cancer. Of these, 20 cases were excluded because adequate follow-up was lacking or because the paraffin-embedded specimen was inadequate for analysis, leaving 187 patients for the present analysis. Characteristics of the patient population are shown in Table 1. Median patient age was 64 years (range, 44–74), and the mean serum PSA level was 14.5 ng/ml (range, 1.7–126). Thirty-four patients had initial serum PSA value >20 ng/ml and 65 patients (34.8%) experienced disease recurrence. Median follow-up time was 4.61 years.

Immunohistochemistry. A summary of MMP-2 labeling results is shown in Table 2. Stromal cells expressed MMP-2 in 142 cases (75.9%; Fig. 1), and prostate cancer cells expressed MMP-2 in 131 cases (70.0%; Fig. 2). No specific pattern of labeling or localization along the tumor front was found. The intensity of labeling was variable. Immunolabeling of MMP-2 was also present in benign prostatic epithelial glandular cells in 169 cases (90.3%) and was generally limited to either the basal cells (Fig. 3) or the secretory cells but, in some cases, both secretory and basal cells expressed the marker. MMP-2 labeling of endothelial cells and of smooth muscle cells in either vessel walls or prostatic stroma was also found.

Table 1 *Prevalence of tumor pathological substages and GSs*

Pathological stage	*N*	Substage	No. of cases	%
T	187	T3A	92	49.2
		T3B	39	20.9
		T3C	56	29.9
N		N0	138	73.8
		N1	31	16.6
		N2	18	9.6
M		M0	187	100.0
GS	187	2–6	113	60.4
		7	45	24.1
		8–10	29	15.5

Table 2 *Cohort immunolabeling description*

	N	Category	No. of cases	%
Cancer				
Expression	187	0	56	29.9
Percentage		<10	73	39.0
		10–50	41	21.9
		>50	17	9.1
Intensity	187	0	56	29.9
		+	29	15.5
		++	66	35.3
		+++	36	19.3
Localization	187	Near[a]	82	43.9
		Far[a]	12	6.4
		Near and far[a]	36	19.3
		No labeling	57	30.5
Heterogeneity	187	Homogeneous[b]	57	35.8
		Heterogeneous	120	64.2
Stroma				
Expression	187	0	45	24.1
Percentage		<10	78	41.7
		10–50	45	24.1
		>50	19	10.2
Intensity	187	0	45	24.1
		+	59	31.6
		++	64	34.2
		+++	19	10.2
Localization	187	Near[a] (1)	38	20.3
		Far[a] (2)	24	12.8
		Near and far[a] (3)	38	20.3
		Near benign glands (4)	0	0.0
		(1) + (4)	30	16.0
		(2) + (4)	11	5.9
		(3) + (4)	1	0.5
		No labeling	45	24.1
Heterogeneity	187	Homogeneous[b]	68	36.4
		Heterogeneous	119	63.6
Benign glands				
Expression	187	0	18	9.6
Percentage		<10	66	35.3
		10–50	85	45.5
		>50	18	9.6
Intensity	187	0	18	9.6
		+	19	10.2
		++	77	41.2
		+++	73	39.0
Localization	187	Near[a] (1)	12	6.4
		Far[a] (2)	4	2.1
		Near and far[a] (3)	9	4.8
		Near benign glands (4)	16	8.6
		(1) + (4)	25	13.4
		(2) + (4)	1	0.5
		(3) + (4)	0	0.0
		Nonapplicable	120	64.2
Heterogeneity	186	Homogeneous[b]	21	11.3
		Heterogeneous	165	88.7
Other				
Basal cells	184	Labeling	139	75.5
		No labeling	45	24.5
Smooth muscle	184	Labeling	95	51.6
		No labeling	89	48.4
Vessel walls	184	Labeling	39	21.2
		No labeling	145	78.8
Endothelium	117	Labeling	61	52.1
		No labeling	56	47.9

[a] According to tumor front.
[b] Homogeneous cases include cases with no labeling.

Statistical Analyses. Kaplan-Meier curves showed no significant association between the amount of MMP-2 labeling by stromal cells and prostate cancer disease-free survival (Fig. 4*A*). However, a trend to lower disease-free survival was noted when MMP-2 expression levels by cancer cells increased ($P = 0.0679$; Fig. 4*B*).

Univariate analyses showed no correlation between the amount of MMP-2 labeling by stromal cells and disease-free survival, but the hazard ratio of developing recurrent prostate cancer was significantly higher in cases in which prostate cancer cells expressed increasingly higher amounts of MMP-2 (Table 3). This relationship remained significant in multivariate analyses (Table 3) adjusting for other

prognostic factors that were found prognostically significant (TNM stage, GS, preoperative PSA level; Table 4). No individual association was found between MMP-2 expression by prostate cancer cells and other prognostic factors (Table 5). MMP-2 expression by >50% of the cancer cells was a significant predictor of prostate cancer recurrence (hazard ratio, 4.267; $P = 0.0012$; Table 3). A multivariate trend test confirmed that the risk of developing recurrent prostate cancer increases with MMP-2 expression (hazard ratio, 1.539; $P = 0.0030$; Table 3). MMP-2 labeling by benign prostate glands, basal cells, vessel walls, and endothelial cells provided no additional information (data not shown).

DISCUSSION

Evidence that matrix metalloproteinase-2 expression is important in the pathogenesis of prostate cancer is rapidly accumulating (27–35). To date, four groups have evaluated the role of the MMP-2 protease as a prognostic factor in prostate cancer (31, 33–35). These reports have shown that MMP-2 expression is correlated with disease stage and the GS (31, 34, 35). One group has shown that MMP-2 may be a prognostic factor independent of disease stage and grade (33). In the current study, we show that in a large and uniform cohort of patients who underwent radical prostatectomy, MMP-2 expression in >50% of prostate cancer cells is a major and independent predictor of decreased prostate cancer disease-free survival.

This study uses PSA failure as a surrogate end point for disease recurrence because it is currently recognized as the most sensitive indicator of residual or recurrent disease after radical prostatectomy (3, 38). In fact, clinically detectable recurrent prostate cancer occurring in the absence of a detectable serum PSA is a rare event (3, 39). With a median follow-up of 4.62 years, we found that 65 of 187 patients (34.8%) with pT3NxM0 disease treated by radical prostatec-

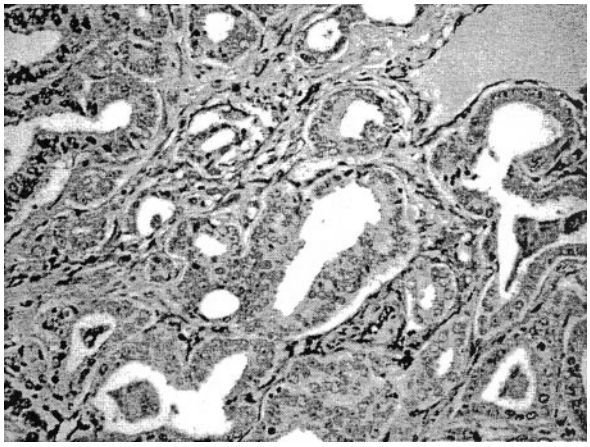

Fig. 1. MMP-2 expression by stromal cells (IHC).

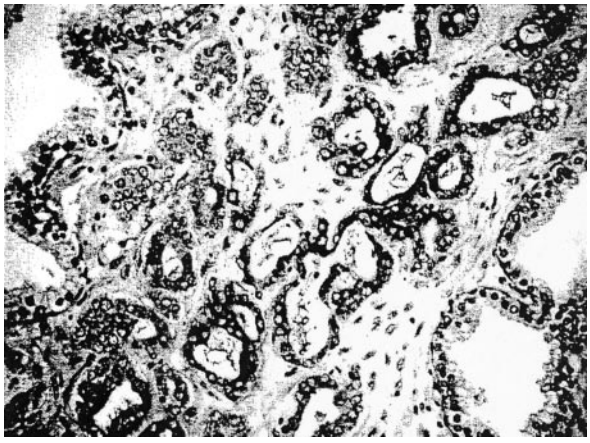

Fig. 2. MMP-2 expression by cancer cells (IHC).

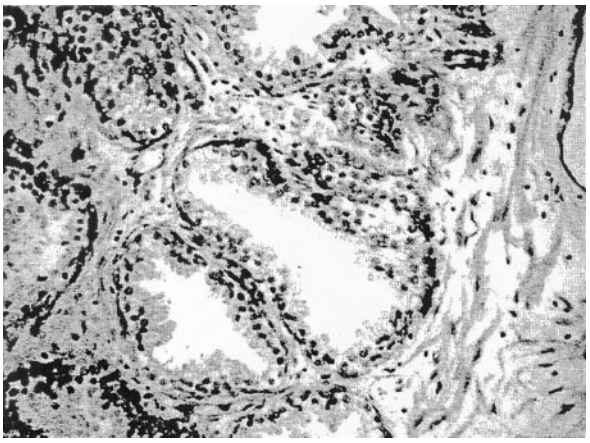

Fig. 3. MMP-2 expression by basal cells only (IHC).

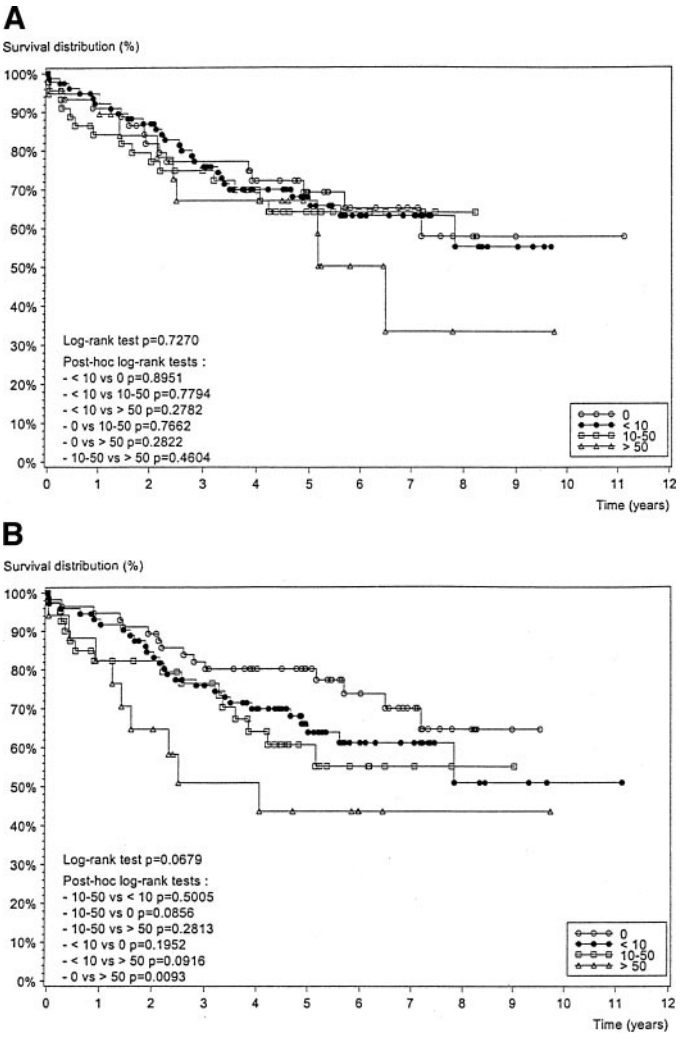

Fig. 4. Kaplan-Meier disease-free survival curves. *A*, MMP-2 expression by stromal cells; *B*, MMP-2 expression by cancer cells.

Table 3 *Disease-free survival rates according to MMP-2 percentage of expression*

	Univariate			Multivariate[a]		
	HR[b]	95% CI	*P*	HR	95% CI	*P*
Cancer cells						
0%	1.000			1.000		
<10%	1.145	0.60, 2.19	0.6804	1.107	0.57, 2.15	0.7650
10–50%	1.802	0.83, 3.92	0.1367	1.990	0.92, 4.32	0.0821
>50%	3.796	1.61, 8.97	0.0024	4.267	1.77, 10.27	0.0012
Trend test	1.394	1.08, 1.79	0.0098	1.539	1.16, 2.05	0.0030
Stroma						
0%	1.000					
<10%	1.040	0.55, 1.96	0.9039			
10–50%	1.137	0.56, 2.32	0.7263			
>50%	1.553	0.68, 3.55	0.2973			

[a] Adjusted for pathological stage (T and N), initial serum PSA level, and GS at surgery.
[b] HR, hazard ratio; CI, confidence interval.

Table 4 *Hazard ratios of potential prognostic factors on disease-free survival*

	HR[a]	95% CI	*P*
Age at time of study[b]	0.992	0.95; 1.03	0.6930
Initial serum PSA level[b]	1.018	1.01; 1.02	0.0002
T pathological stage			
T3A	1.000		
T3B	2.807	1.42; 5.56	0.0031
T3C	4.456	2.47; 8.04	<0.0001
N pathological stage			
N_0	1.000		
N_1	2.504	1.38; 4.54	0.0025
N_2	6.750	3.62; 12.57	<0.0001
Gleason score			
2–6	1.000		
7	2.632	1.46; 4.75	0.0013
8–10	4.547	2.52; 8.21	<0.0001

[a] HR, hazard ratio; CI, confidence interval.
[b] Continuous variable.

tomy had experienced either a clinical or biochemical treatment failure.

The pattern of MMP-2 expression in prostate cancer cells and stromal cells observed in the current study is consistent with that previously reported by other groups (27, 40). As shown in Table 2, we observed the highest levels of MMP-2 expression in malignant prostate glands and in stromal fibroblasts. However, MMP-2 expression was also noted in normal prostatic epithelial cells as well as in prostatic and vascular smooth muscle, albeit at a much lower level. This tends to support the concept of constitutive but differentially regulated MMP-2 expression (41).

The association of increased MMP-2 expression by malignant epithelial cells but not by stromal cells with disease recurrence is intriguing. One group has found that MMP-2 mRNA levels were increased in stromal prostatic cells only (33), thus suggesting that MMP-2 is produced by stromal cells and not by malignant epithelium. However, our group and others have found increased MMP-2 expression in both prostatic cell types (27, 31, 32, 35). One potential explanation for the apparent discordance between the ISH and IHC findings may be the particular mechanism of MMP-2 activation. Stetler-Stevenson *et al.* (42) were the first to propose that MMP-2 activation was a stromal event. Others have since demonstrated that MMP-14, a protein anchored to the cell membrane of stromal cells, binds to TIMP-2 through its catalytic domain and then uses it as a receptor for pro-MMP-2, a protein also initially produced by the stromal cells. Free adjacent MMP-14 can then cleave the Asn^{37}-Leu^{38} bond of pro-MMP-2 leading to MMP-2 intermolecular autocatalytic cleavage of the Asn^{80}-Tyr^{81} bond. This process leads to an active and soluble form of MMP-2 called MMP-2a. MMP-2a can then bind to cancer cells via an $\alpha v\beta 3$ integrin (7, 26, 43). By IHC, using a monoclonal antibody directed against MMP-2a, Stearns and Stearns (31) found that MMP-2a was expressed only in malignant epithelium. They also showed a correlation between increased MMP-2a expression and high Gleason scores. Although the antibody used in our study cannot differentiate pro-MMP-2 from MMP-2a, our results and those of Stearns and Stearns (31) are consistent with current knowledge of the molecular biology of MMP-2 (7). Our results showing that increased MMP-2 expression in cancerous prostate cells is associated with decreased disease-free survival suggest that most MMP-2a is bound to cancer cells.

Although the results of our study are quite interesting, a few points should be clarified. Our study population consisted only of pT3NxM0 cases treated by radical prostatectomy. Whether our results are generalizable to patients with different disease stages or those preferring other treatment options remain to be proved. Secondly, our IHC was conducted on radical prostatectomy specimens, not prostate biopsy cores. If MMP-2 is to be a used as part of a pretreatment decision-making tool, its predictive power on biopsy specimens must first be validated. Despite these caveats, we think that our results are important and that they support the concept of synthetic MMP inhibitors as potential novel antineoplastic agents. Studies are currently evaluating

Table 5 *Correlation of MMP-2 expression by cancer cells with recognized prognostic factors*

	N	Expression (%)	No. of cases	%	*P*
T pathological stage					
T3A	92	0	34	37.0	0.4438
		<10	32	34.8	
		10–50	18	19.6	
		>50	8	8.7	
T3B	39	0	10	25.6	
		<10	18	46.2	
		10–50	7	17.9	
		>50	4	10.3	
T3C	56	0	12	21.4	
		<10	23	41.1	
		10–50	16	28.6	
		>50	5	8.9	
N pathological stage					
N0	138	0	41	29.7	0.9730
		<10	53	38.4	
		10–50	31	22.5	
		>50	13	9.4	
N1	31	0	11	35.5	
		<10	12	38.7	
		10–50	6	19.4	
		>50	2	6.5	
N2	18	0	4	22.2	
		<10	8	44.4	
		10–50	4	22.2	
		>50	2	11.1	
GS					
2–6	113	0	38	33.6	0.5854
		<10	41	6.3	
		10–50	24	1.2	
		>50	10	8.8	
7	45	0	11	24.4	
		<10	17	37.8	
		10–50	13	28.9	
		>50	4	8.9	
8–10	29	0	7	24.1	
		<10	15	51.7	
		10–50	4	13.8	
		>50	3	10.3	
Initial serum PSA level (ng/ml)					
<10	91	0	26	29.0	0.909
		<10	35	38.5	
		10–50	21	23.1	
		>50	9	9.9	
10–20	34	0	18	30.0	
		<10	22	36.7	
		10–50	14	23.3	
		>50	6	10.0	
>20	34	0	12	35.3	
		<10	15	44.1	
		10–50	5	14.7	
		>50	2	5.9	

prinomastat (AG-3340; Agouron/Pfizer) and neovastat (AE-941; Aeterna) as treatment options in prostate cancer and other neoplasms (10, 44). Murine studies report a reduction in tumor burden when these drugs are administered at an earlier stage (44, 45). Whether or not these agents ultimately have a role in the treatment of prostate cancer remains to be determined.

In conclusion, our study suggests that increased MMP-2 expression by malignant prostate glands may be a predictor of prostate cancer disease-free survival independent of disease stage, PSA, and GS.

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