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 stromal 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 prostate epithelium 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 are a potential target for cancer therapy. The MMP family includes >20 zinc-dependent proteases 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’Hôpital-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 standardized technique 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 V2C2; Neomarkers, Fremont, CA; dilution 1/50). Afterwards, 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 hematoxylin.
RESULTS

Population and Tumor Characteristics. The initial study population consisted of 207 cases of stage pT3N0M0 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 subtypes and GSs

<table>
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<th>Pathological stage</th>
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<th>Substage</th>
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<tr>
<td>T</td>
<td>187</td>
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<tr>
<td></td>
<td></td>
<td>T3B</td>
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<td></td>
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Table 2 Cohort immunolabeling description

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<tr>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Localization</td>
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<td>Far$^a$</td>
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<tr>
<td>187</td>
<td></td>
<td>Near and far$^a$</td>
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<tr>
<td>Heterogeneity</td>
<td>Homogeneous$^b$</td>
<td>Heterogeneous</td>
</tr>
</tbody>
</table>

$^a$ 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. 4A). However, a trend to lower disease-free survival was noted when MMP-2 expression levels by cancer cells increased ($P = 0.0679$; Fig. 4B).

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 factors.
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 pT3N0M0 disease treated by radical prostatecto-
stromal cells. Free adjacent MMP-14 can then cleave the Asn37-Leu38 bond of pro-MMP-2, leading to intermolecular autocatalytic cleavage of the Asn80-Tyr81 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 αβ3 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 remains to be proved. Secondly, our IHC was conducted on radical prostatectomy specimens, not prostate biopsy cores. If MMP-2 is to be 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 potential novel antineoplastic agents. Studies are currently evaluating...
prionmetastat (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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