

Prostate cancer patients with Maspin-negative tumors can live over a decade[§]

Research Article

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Summary

Background/Purpose: Maspin is a newly discovered tumor suppressor gene. Previous studies in prostate cancer suggest this gene's expression correlates with higher tumor grade and predicts biochemical relapse. To date, however, no study has examined the prognostic impact of maspin expression on survival in patients with prostate cancer. The current study was undertaken to provide descriptive data on the predictive impact of maspin expression on survival in prostate cancer patients. **Methods:** As part of a multi-institutional clinical trial in patients with androgen-independent prostate cancer, this preliminary investigation stained 11 diagnostic prostate biopsies for maspin and prostate specific antigen (PSA). Normal prostate tissue within these biopsies served as positive controls. All 11 patients were followed prospectively from the time of trial enrollment. **Results:** All 11 tumors stained positively for PSA and negatively for maspin. Within the cohort, there was a median survival of 123 months (range: 27 to 127 months) with 6 of 11 patients still alive. Metastatic prostate cancer was the cause of death in all 5 deceased patients. **Conclusions:** Although maspin is a tumor suppressor gene, patients with maspin-negative tumors can nonetheless live for over a decade. Hence, maspin-negativity should not be used to counsel prostate cancer patients on the prospect of a limited life expectancy.

I. Introduction

Maspin is a newly discovered member of the serpin family and has received increasing attention as a tumor suppressor gene. Mapped to chromosome 18q21.3-q23, this gene is thought to play a critical role in metastases (McGowen et al, 2000). In cell culture, maspin's 24 kilodalton gene product inhibits metastatic invasion and spread of malignant cells (Sheng et al, 1996). Although mechanisms remain uncertain, recent data from Zhang and others suggest this molecule's antiangiogenesis properties may in part explain such anti-tumor effects (Zhang et al, 2000).

Recent clinical data also suggest the importance of maspin as a tumor suppressor gene in prostate cancer patients. Machtens and others studied 84 prostate tumors (Machtens et al, 2001). They observed that positive immunohistochemistry staining for maspin, defined as the presence of a staining reaction in at least 40% of cells,

occurred in 52% of tumors. Positive-maspin staining was associated with greater tumor differentiation and earlier tumor stage. In their retrospective analysis with a median follow up of 64 months, these investigators reported a shorter disease-free survival, as defined by the absence of PSA elevation, among maspin-negative patients: 26 versus 41 months, in maspin-negative and -positive patients, respectively (P=0.04). In a second retrospective study, Zou and others examined 97 prostate tumors and observed maspin-positivity in 37% (Zou et al, 2002). Although these investigators observed that maspin expression provided no predictive value, only 27 patients within this group had manifested a biochemical recurrence after a median 59-month follow up. These investigators did, however, observe a trend to suggest that maspin expression was associated with well-differentiated tumors (P=0.05), a finding that suggests maspin does in fact predict a favorable prognosis. Taken together, the above clinical and laboratory data suggest that the presence of

maspin may carry with it a favorable prognostic effect for patients with prostate cancer and that, conversely, maspin-negativity may portend a poor prognosis.

How long do patients with maspin-negative prostate tumors actually live? Although the foregoing case control studies are robust and well planned, they were not designed to answer this question. Nor were they able to provide concrete survival data. In fact, these studies did not examine survival, the most obvious endpoint reflective of prognosis. Rather, they looked only at biochemical relapse, as manifested by prostate specific antigen (PSA) elevation -- at best only a crude surrogate for survival. Furthermore, as is the case with any retrospective investigations, the outcome data in these studies are not comparable to those gleaned prospectively. To gain an accurate clinical understanding of the prognostic effect of maspin-negativity, clinical data must be obtained in a prospective fashion. Thus, although the two large studies cited earlier suggest that maspin-negativity predicts a poor prognosis, they do not provide tangible, descriptive data to allow us to understand the clinical implications of this tumor suppressor protein.

The present exploratory investigation was undertaken to begin to answer the question posed above. The goal of this investigation was to provide prospective, illustrative data on the impact of maspin expression on survival in prostate cancer patients. As the translational component of a multi-institutional trial, this investigation relied on meticulous survival and cause-of-death data from a cohort of prostate cancer patients, thereby assembling a small but solid database that allowed for exploration of the clinical ramifications of maspin-negativity in patients with this malignancy.

II. Materials and methods

A. Overview

This study comprised the translational component of a phase II trial conducted within the North Central Cancer Treatment Group (NCCTG). Twenty-two institutions participated. The trial had examined the antineoplastic effects of green tea in patients with androgen independent prostate cancer, as defined by the Prostate Specific Antigen Working Group (Bubley et al, 1999). The clinical results of this trial of 43 evaluable patients showed that green tea carried no antineoplastic effects and have been previously reported (Jatoi et al, 2003). At the time of patient registration, all sites were given the option of sending diagnostic, paraffin-embedded tissue blocks to the NCCTG Operations Office.

B. Clinical follow up

As part of patient monitoring while receiving the study agent, patients met with their oncologists for a history, physical examination, and laboratory testing once a month. Patients who appeared stable on treatment over 6 months were then evaluated at two-month intervals. Patients who stopped therapy were followed at 6-month intervals until death. Oncologists were asked to provide information on cause of death.

C. Immunohistochemistry

Tissue blocks were stained for maspin and PSA. Each tissue block was cut into sections that were 5 microns in thickness and mounted on charged glass slides. Sections were deparaffinized and hydrated. Antigen retrieval was performed

with 1mM EDTA plus steam. The sections were then exposed to 0.3% hydrogen peroxide to quench indigenous peroxidase activity. They were then incubated with a monoclonal anti-maspin antibody at a 1:10 dilution for 60 minutes at room temperature. Envision Plus (Dako Corporation, Carpinteria, California, USA) was used as the secondary antibody according to the manufacturer's directions.

PSA staining was accomplished similarly. Tissue sections were blocked with protein block (Dako Corporation, Carpinteria, California, USA) to prevent non-specific binding of antibody. Slides were incubated with a PSA antibody at a dilution of 1:2200. AEC chromogen was used as the substrate for visualizing the antibody staining. Slides were counterstained with Gill's Hematoxylin.

All slides were reviewed by a pathologist who provided an estimate of the percentage of maspin staining in the sample. If at least 40% of cells were staining for maspin (Machtens et al, 2001), the sample was scored as positive. PSA staining was assessed similarly and was done to provide confirmation of prostate cancer within the sample.

D. Statistics

Kaplan-Meier curves were constructed for all patients who had paraffin-embedded slides submitted. A log-rank test was used to compare survival between patients whose slides were maspin-negative and -positive. A P-value < 0.05 was deemed statistically significant. All other data are presented descriptively.

III. Results

A total of thirteen paraffin-embedded tissue blocks from thirteen separate prostate cancer patients were received. One tissue block did not include an adequate malignant tissue to allow for immunohistochemistry staining, and the other was mislabeled to the point where correlative clinical history was untraceable. Thus, a total of 11 tissue blocks were evaluated.

Eight of the samples were from the biopsy obtained at the time of the original prostate cancer diagnosis. Three represented biopsy material from patients with a prior diagnosis of prostate cancer within the preceding 2 years.

All tumor specimens from these 11 patients showed strongly positive PSA staining, or staining within > 40% of prostate tumor cells.

With normal prostate tissue on these biopsies serving as a positive control, all the prostate tumors showed negative maspin-staining, as indicated by < 40% staining on visual inspection, in keeping with the threshold defined by Machtens et al, (2001). The sample with the most positive staining demonstrated staining in 10% of cells (**Figure 1**).

Kaplan Meier survival curves show a median survival of 123 months (range: 27 to 127 months) within the cohort with 6 of 11 patients still alive. Metastatic prostate cancer was the cause of death in all 5 deceased patients, all of whom had received hormonal manipulation as primary therapy for their prostate cancer.

A comparison of patients with weak versus those with absolutely negative maspin immunohistochemistry staining showed no statistically significant differences with regard to survival: 123 versus 127 months, respectively (P= 0.72, log rank test) (**Figure 2**).

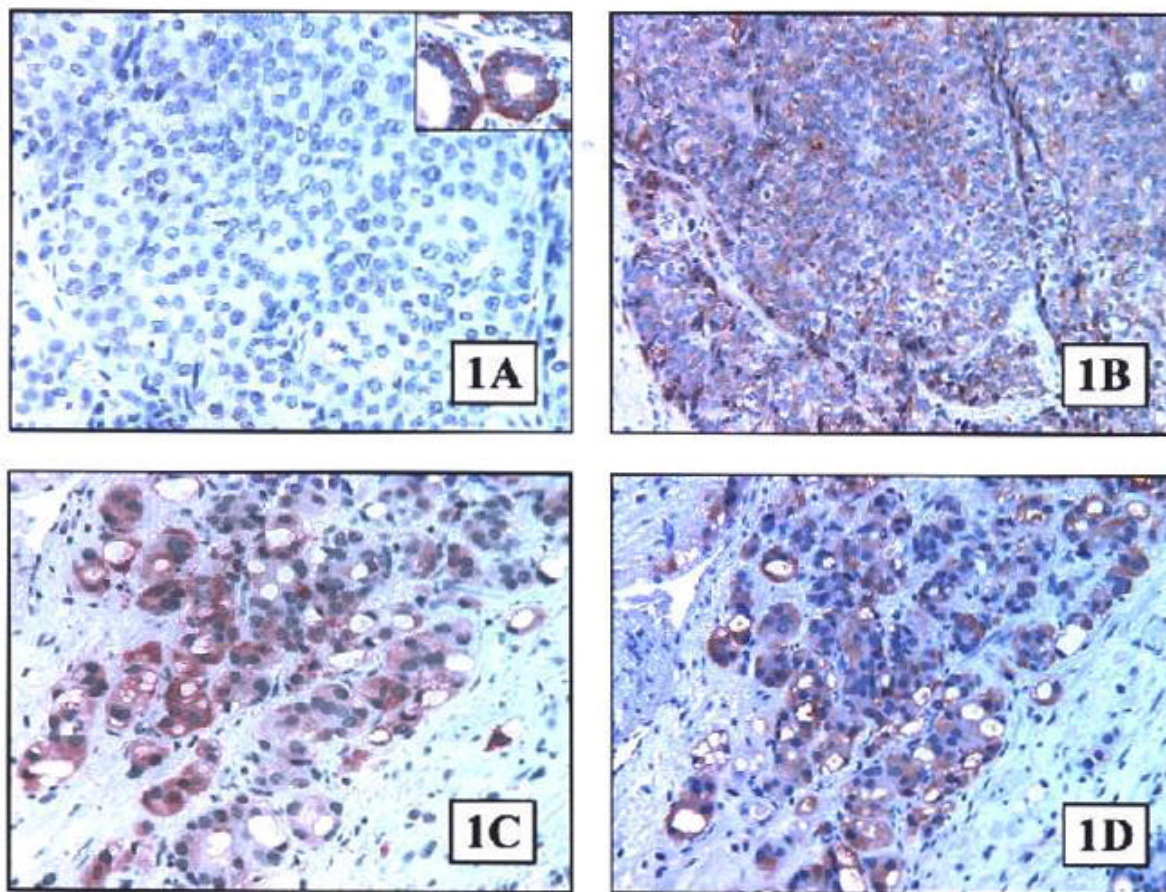


Figure 1. **A.** prostate cancer with negative maspin staining (magnification 200x); insert shows residual normal prostate glands with positive maspin staining within the specimen from the same patient (magnification of insert 200x). **B** the same specimen as in Figure 1A but with positive PSA staining (magnification 200x). **C** invasive prostate cancer with positive staining for maspin (magnification 200x). Maspin-positive tumor cells constitute 10% of the tumor in this specimen. **D** shows the same specimen as in Figure 1C with positive staining for PSA.

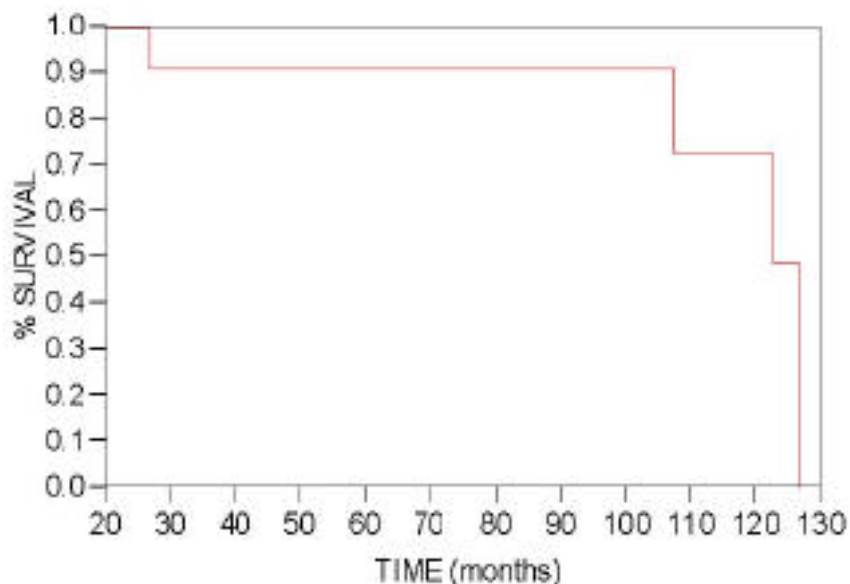


Figure 2. A comparison of patients with weak versus those with absolutely negative maspin immunohistochemistry staining showed no statistically significant differences with regard to survival: 123 versus 127 months, respectively ($P= 0.72$, log rank test). The median survival within this cohort was 123 months (range: 27 to 127 months) with 6 of 11 patients still alive.

IV. Discussion

Within this cohort of 11 patients, maspin-negativity was not associated with a markedly diminished life expectancy. Median survival within this cohort was 123 months, and six patients remain alive. Although prior retrospective studies show that patients with maspin-negative tumors carry a higher tumor grade and might suffer a shorter time until biochemical relapse, no prior study had directly evaluated the prognostic impact of maspin-negativity in terms of actual survival. The goal of this study was to provide descriptive data on patient survival as they pertain to maspin-negativity, and the data presented here show that patients with maspin-negative tumors may live for longer than 10 years. Thus, immunohistochemistry staining with maspin does not appear to be a powerful prognosticator of great clinical utility.

Three aspects of this study deserve further comment. First, all eleven tumor samples stained negatively for maspin. In effect, there was no comparative group that allowed us to state definitively that patients with maspin-positive tumors lived longer compared to patients with maspin-negative tumors. However, the absolute survival of greater than 10 years among patients whose tumors were maspin-negative allows us to conclude that although survival may be worse in the absence of maspin, in actuality it is not really that bad. Secondly, and as noted earlier, the size of this cohort was relatively small, as only a small subset of patients had had their blocks submitted. However, meticulous follow up to the time of death, coupled with the fact that the five patients who died did in fact have confirmation of death from prostate cancer, make this investigation worth reporting. The data presented here suggest that during a one-to-one encounter, maspin-negativity should not be used to counsel a patient on life expectancy, as patients may live for many years despite having a maspin-negative prostate tumor.

Third, this study did not follow patients from the time of diagnosis. Rather patients entered this investigation once they developed androgen independent prostate tumors. Although one might argue that this study "selected" long-term survivors, it is important to point out that if any "selection" had occurred, it likely occurred in a manner favoring a bleaker life expectancy for maspin-negative patients. It is possible that many patients with maspin-negative tumors were cured and thus were never eligible for this trial. It is also possible that patients who were surviving for even longer than 10 years did not have their slides sent in because of a greater likelihood of

inaccessibility that occurred with time. Hence, the findings from this investigation may not allow for accurate prediction of median survival in maspin-negative patients, but they do allow for drawing a general conclusion that maspin-negativity does not necessarily predict early demise.

In short, patients with maspin-negative prostate tumors may live for many years after their diagnosis. A more in depth understanding of maspin and how it functions as a tumor suppressor gene is of great scientific consequence. However, from a clinical standpoint, maspin-negativity should not be used to counsel prostate cancer patients on the prospect of a limited life expectancy.

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