The Elevated Expression of a Mismatch Repair Protein Is a Predictor for Biochemical Recurrence After Radical Prostatectomy

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Abstract

Purpose: **The inability to predict clinical outcome of prostate cancer is a major impediment to effective treatment decisions and patient counseling. New markers of recurrence are needed to improve the accuracy of risk assessment and treatment of prostate cancer. Our previous studies identified a mismatch repair protein, PMS2, to be elevated in prostate cancer; here, we investigate the prognostic potential of this marker. We hypothesized that the elevation of PMS2 would correlate with disease outcome.**

Experimental Design: **Retrospective quantitative immunohistochemistry was done to measure PMS2 in high-grade cancers of 166 men treated by radical prostatectomy with a biochemical recurrence rate of 56%. Associations between PMS2 levels, pathologic variables, and biochemical recurrence over time were determined.**

Results: **The mean level of PMS2 protein was consistently higher in both cancer-associated benign epithelium and cancer cells of patients who recurred, compared with nonrecurrent patients. PMS2 was an independent predictor of time-to-recurrence in Cox multivariate analyses and significantly stratified patients based on outcome. PMS2 was able to improve the sensitivity of total percent Gleason 4/5 as a risk factor for recurrence in this cohort.**

Conclusions: **PMS2 protein levels were shown to be a predictor of time-to-recurrence after surgery. This study is the first to document that the elevation of a mismatch repair protein negatively correlates with prognosis and has implications in patient diagnosis and molecular profiling.** (Cancer Epidemiol Biomarkers Prev 2009;18(1):57–64)

Introduction

Prostate cancer risk assessment for recurrence is done with the help of two well-established clinical prognostic markers: blood serum prostate-specific antigen (PSA) and pathologic Gleason grading of the cancer (1-4). These markers are useful for patients that present with either benign (low risk) or aggressive (high risk) cancer; however, they are uninformative for patients with less severe disease presentations (5, 6). These intermediate risk patients, characterized by localized cancer, a Gleason score of 7 and a preoperative PSA level between 2.1 and 10.0 ng/mL, may develop an aggressive form of the disease during their lifetime. A lack of reliable markers for these patients impairs the clinician's ability to plan appropriate treatment regimens and novel biomarkers are needed.

Generally, only mismatch repair (MMR) defects or loss have been described in association with cancer (7-12). Our recent discovery that a MMR protein, PMS2, is specifically elevated in prostate cancer was unexpected. The PMS2 protein is part of the MMR machinery and forms a heterodimeric complex with its homologue MLH1 (13). This complex is thought to promote the recruitment of downstream proteins after the recognition of mismatches or damage in DNA. *PMS2* overexpression has been shown to result in an increased mutation frequency (14), a resistance to methylation-induced apoptosis (14) and correlate with microsatellite instability (15).

The purpose of this study was to define the prognostic potential of PMS2 elevation in a cohort of intermediate risk prostate cancer patients. We hypothesized that patients with biochemical recurrence would have significantly higher levels of PMS2 than nonrecurrent patients. Here, we report that PMS2 elevation is a predictor of biochemical recurrence after surgery in intermediate risk patients and that it enhances the sensitivity of Gleason grading in prostatectomy samples. This finding identifies a potential new marker for recurrent prostate cancer and uncovers a potentially novel pathway for targeted cancer therapeutics.

Materials and Methods

Patients. Retrospective analysis was done on 166 of 379 prostatectomy samples collected at Stanford University Medical Center between 1983 and 1992 (16). All

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Table 1. Pathologic descriptors and patient demographics of prostate cancer patient cohort

Total number of patients	166		
% recurrence*	56		
	Median	Range	Average
Age distribution (y)	66	35-76	64
Time to recurrence (d)	256	0-3,048	1,797
PSA values			
Presurgical (ng/mL)	16.4	2-266	22.4
At time of recurrence* (ng/mL)	0.2	0.07-12	0.72
Last PSA† (ng/mL)	0.1	0-1,366	33.24
Volume of largest cancer (cc)	4.37	0.4-45	6.12
Total cancer volume (cc)	4.8	0.4-45	6.58
Gleason grade			
% Grade 3	60	0-98	55.7
% Grade 4	30	2-100	36.1
% Grade 5	0	0-90	3.9
% Intraductal	0	0-60	4.3
% high grade (4/5) tumor	35	2-100	40
Prostate weight (grams)	46	20-366	51.5

NOTE: The cohort used in this study consisted of 166 cancers from men who had localized peripheral zone cancer. Their pathologic descriptors and demographics are listed here.
* Recurrence as determined by PSA of >0.05 ng/mL after prostatectomy.
† Last PSA: either at time of death or at the last 5-y follow-up visit.

patients were treated by prostatectomy alone and all tumors were extensively analyzed for total tumor volume, the volume of the largest tumor, the total percent Gleason grade represented by all cancer in the prostate, serum PSA, age, tumor location (peripheral zone versus transition zone), nodal involvement, prostate weight, and 5-yr follow-up (16). Disease recurrence was defined by biochemical failure, which was described as two consecutive PSA values above a cutoff point of 0.07 ng/mL for PSA measured by the Tosoh method, and 0.2 ng/mL for measurements by less-sensitive methods.

From this larger cohort, patients were excluded that had cancer consisting of only Gleason pattern 3, as these patients have less than a 5% chance of recurrence (17). Our final cohort consisted of 166 cancers from men who had localized peripheral zone cancer with at least 2% Gleason grade 4/5 cancer. Within this cohort and despite surgical treatment, 56% of patients developed biochemical recurrence with an average PSA value of 0.72 ng/mL at time of recurrence (Table 1).

Histopathologic Evaluation. Surgically removed prostates had been subjected to a comprehensive histopathologic review according to the method described

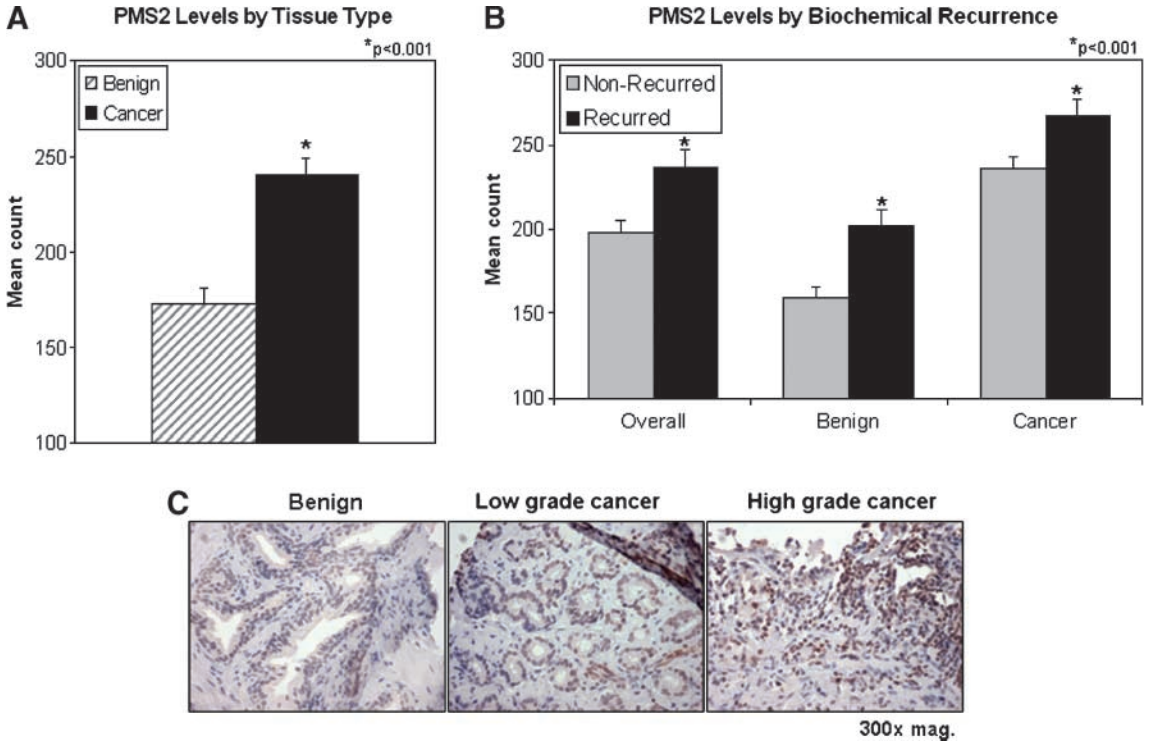

Figure 1. PMS2 protein levels are elevated in recurrent prostate cancer patients. **A,** mean and SD of PMS2 counts in cancer-surrounding benign (*hatched bar*) and cancer tissue (*black bar*); *, $P < 0.001$; **B,** mean and SD of PMS2 counts of patients with biochemical recurrence (*gray bars*) and no recurrence (*black bars*), stratified by tissue type (e.g., benign, cancer, or the average of both "overall"); *, $P < 0.001$; **C,** representative immunohistochemical staining of PMS2 in benign cancer-associated or cancer tissue of a patient with nonrecurrent disease (patient 1) or recurrent disease (patient 2). Notice the difference in PMS2 staining in cancer-associated benign cells and the similarity in the cancer cells. Pictures taken under ×300 magnification.

Table 2. Spearman correlation analyses between PMS2 levels in either benign or cancer tissue and pathologic variables

Variable	PMS2 in cancer		PMS2 in benign	
	Coeffic.	Pr>|r|	Coeffic.	Pr>|r|
Age	0.04	0.60	−0.08	0.34
PSA	0.20	0.01	0.02	0.77
Largest cancer volume	**0.22**	**0.05**	**0.15**	**0.05**
Total tumor volume	**0.19**	**0.01**	0.12	0.12
Prostate weight	−0.11	0.18	**−0.20**	**0.01**
% Gleason 4/5	0.09	0.22	**0.15**	**0.05**

NOTE: Spearman correlation analyses between PMS2 levels in either benign or cancer tissue and pathologic variables. To determine the correlation between PMS2 elevation and the pathologic variables in Table 1, a Spearman analyses was done. The resulting coefficients and *P* values are listed here. Bolded numbers indicate those variables that significantly correlated with PMS2 elevation.
Abbreviation: Coeff, coefficient.

previously (18). Briefly, each specimen was fixed in formalin, serially blocked at 3-mm intervals, and embedded in paraffin. A 5-μm-thick section was cut from each block and stained with H&E for histologic assessment. The cancer volume was calculated by tracing the exact tumor outline on each slide, determining the area of tumor at each level of section with a digitizing pad, summing the tumor areas at different levels multiplied by the section thickness, and correcting the volume for tissue shrinkage during processing. Tumor grade was determined according to the Gleason system (1). The Stanford modified Gleason scale was used to estimate the proportion of the largest cancer in each case that was poorly differentiated (grades 4 and 5) or well-differentiated (grades 1, 2, and 3; ref. 19). The percentage of each cancer occupied by Gleason grades 4 and 5 (% Gleason grade 4/5) was estimated by a single pathologist (J.E. McNeal). This measurement of tumor grade was shown to be the strongest predictor of PSA failure (20).

Immunohistochemistry. Four-micron sections were cut using a microtome and dried onto charged glass slides at least overnight. These sections were used for subsequent immunohistochemical analysis as previously described (15). The PMS2 antibodies used were from BD Biosciences at 1:100 dilution. To verify specificity of staining, various PMS2 antibodies (Sigma, BD Biosciences, Invitrogen) were used on near-identical serial sections. Patterns and staining levels were highly repeatable in all samples tested.

Microscopy and Quantitation. Images were captured using a Nikon Eclipse 50i inverted microscope attached to a Nikon Micropublisher 3.3 RTV camera. The pictures were captured at ×300 magnification and analyzed using the NIH shareware program, *ImageJ*. For the quantification of staining, a modified ''H score'' method was used as described previously (15). Briefly, the modified H-score specified that a score of ''zero'' designates no stain and a score of ''three'' designates darkest stain. The scores of ''one'' and ''two'' reflect cells that have intermediate staining. Staining and quantification were done blindly to the patient outcome or disease status.

Statistical Analysis. A mixed model approach was used to examine predictors of PMS2 levels. This model accounted for multiple measurements taken from each patient (e.g., PMS2 in cancer and benign tissue) and was used to examine the impact of other patient level characteristics such as tumor grade, age, PSA, tumor volume, etc.

$$Y_{ijk} = \mu + \alpha_j + \beta_{k(i)} + e_{ijk}$$

where Y_{ijk} is the outcome (i.e., PMS2 level) measured on the *i*th individual in the *j*th tissue type (benign/cancer) in the *k*th replicate sample (1st or 2nd field for each tissue type); μ is the grand mean; α_j is the fixed cancer effect; $\beta_{k(i)}$ is the random effect of the k^{th} replicate nested within the i^{th} patient; and e_{ijk} is the error term.

To examine the correlation between PMS2 and other variables, a nonparametric Spearman correlation was used to account for the fact that several of the measurements were not normally distributed. A Stepwise Cox Proportional Hazards regression model was fit to determine if study variables were significant predictors of the time to recurrence. Only significant ($P \leq 0.05$) predictors are reported. For each of the individuals in this study, data concerning the time to relapse (if relapse occurred) was available. Survival curves and the Log-Rank test were done to examine whether PMS2 levels were predictive of recurrence. A 4-level stratification variable was also created, based on both PMS2 levels and total percent Gleason 4/5.

SAS version 9.1 for Windows was used to perform all statistical analyses. For all statistical tests done, we used two-tailed tests and considered an α level of 0.05 for statistical significance. We report actual *P* values associated with each significant test unless the observed *P* value was <0.001 where it would be reported as <0.001.

Results

Study Population. As described in the methods, a cohort consisting of 166 patients with cancers containing >2% Gleason grade 4/5 and treated by radical prostatectomy were included in this study. This group of

Table 3. Stepwise Cox Proportional Hazards regression model analyses for time to biochemical recurrence

Variable	DF	Variable estimate	SE	χ^2	Pr>χ^2	Hazard ratio
Preop PSA	1	0.015	0.002	35.215	<0.001	1.015
% Gleason 4/5	1	1.010	0.211	23.022	<0.001	2.747
PMS2 (B)	1	0.003	0.001	9.3311	0.002	1.004

NOTE: Stepwise Cox Proportional Hazards regression model analyses for time to biochemical recurrence. A multivariate stepwise Cox Proportional Hazards regression model analysis was done to determine which pathologic variables, if any, predicted time-to-recurrence. This table displays those variables that were significant. PMS2 (B): PMS2 levels in benign tissue.

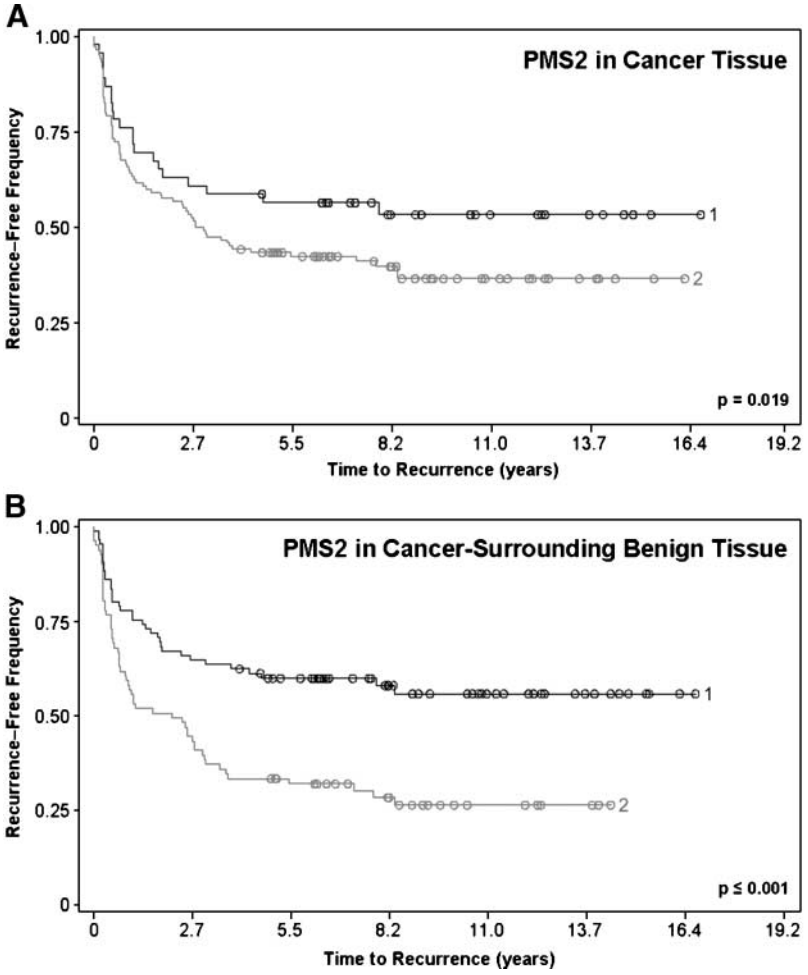

Figure 2. PMS2 protein levels in cancer-associated benign tissue stratifies patients based on recurrence and enhances the predictive power of Gleason grading. Kaplan-Meier analysis of time to biochemical recurrence using total percent Gleason 4/5 and PMS2 measurements. Analyses were done using (**A**) only PMS2 in cancer tissue ($P = 0.019$), (**B**) only PMS2 in cancer-surrounding benign tissue ($P < 0.001$), or (**C-D**) a combination of PMS2 in cancer-surrounding benign tissue and total percent Gleason 4/5. **A,** low PMS2 (1,999 ± 143 d; *black*; *line 1*) versus high PMS2 (1,264 ± 141 d; *red*; *line 2*); **B,** low PMS2 (1,797 ± 188 d; *black*; *line 1*) versus high PMS2 (1,542 ± 121 d; *red*; *line 3*); **C,** low PMS2 or low percent Gleason 4/5 (1,874 ± 114 d; *black*; *line 1*) versus high PMS2 or high percent Gleason 4/5 (657 ± 158 d; *red*; *line 2*); **D,** low PMS2 and low percent Gleason 4/5 (2,355 ± 154 d; *black*; *line 1*), high PMS2 and low percent Gleason 4/5 (1,651 ± 188 d; *green*; *line 2*), low PMS2 and high percent Gleason 4/5 (418 ± 51 d; *blue*; *line 3*), or high PMS2 and high percent Gleason 4/5 (657 ± 158 d; *red*; *line 4*). Median time-to-recurrence values are reflected in the parentheses. Each drop is a recurrence event and each circle is end-of-data for a patient. *P* values were computed from Log-Rank test.

patients was selected because although patients with only Gleason grade 3 (score of 6) tumor are not likely to recur, patients with only Gleason grade 5 (score of 10) are extremely likely to recur. Therefore, we chose to study patients with an uncertain risk of recurrence; those patients typically have a combination of Gleason grade 4 and 5 tumor (score of 7-9). Accordingly, this group had a 56% rate of recurrence and average PSA value of 0.72 ng/mL at time of recurrence. Thus, for each individual, recurrence was uncertain and additional prognostic factors could be useful. PSA levels were measured by the sensitive Tosoh assay, which has been previously shown to successfully detect PSA recurrence at 0.07 ng/mL (21, 22). We defined biochemical failure as having consecutive PSA values above 0.07 ng/mL, a definition that has been used extensively in previous studies (23-27). Table 1 includes the descriptive statistics on the clinical and pathologic variables collected for this cohort.

PMS2 Protein Levels in Human Prostate Cancer. We determined the PMS2 protein levels of cancerous and cancer-associated benign tissue immunohistochemically. The intensity of PMS2 stain was quantitated using an H-score semiquantitative method as done previously (15). The variability for this method (calculated for the same sample analyzed at different time points) was ~10 H-score counts. Within this cohort, PMS2 protein levels were elevated in prostate cancer (Fig. 1A). PMS2 elevation was elevated in 67% (111 of 166) of patients with the mean cancer PMS2 count of 240 compared with the mean normal PMS2 count of 173 ($P < 0.001$). This finding validated our previous results (15) and confirmed that PMS2 elevation associates with prostate cancer.

We next stratified patients based on biochemical recurrence (Fig. 1B). As seen in the left-most pair of bars, recurrent patients had statistically higher levels of PMS2 than nonrecurrent patients ($P < 0.001$). When further stratified by tissue type (e.g., benign or cancer), we show that cancer PMS2 levels are significantly higher in cancerous tissue than in patient-matched cancer-associated benign tissue. However, in both cases, PMS2 levels were elevated in patients with biochemical recurrence. Characteristic staining of PMS2 in a recurrent versus nonrecurrent patient is shown in Fig. 1C.

PMS2 Correlations with Pathologic Variables. Next, PMS2 elevation was correlated with pathologic variables. The results from the Spearman correlation analyses are shown in Table 2. PMS2 levels in cancer (*left column*) marginally correlated with volume of the largest cancer ($P = 0.05$) and total tumor volume ($P = 0.01$) but not with age, preoperative serum PSA, prostate weight, or total

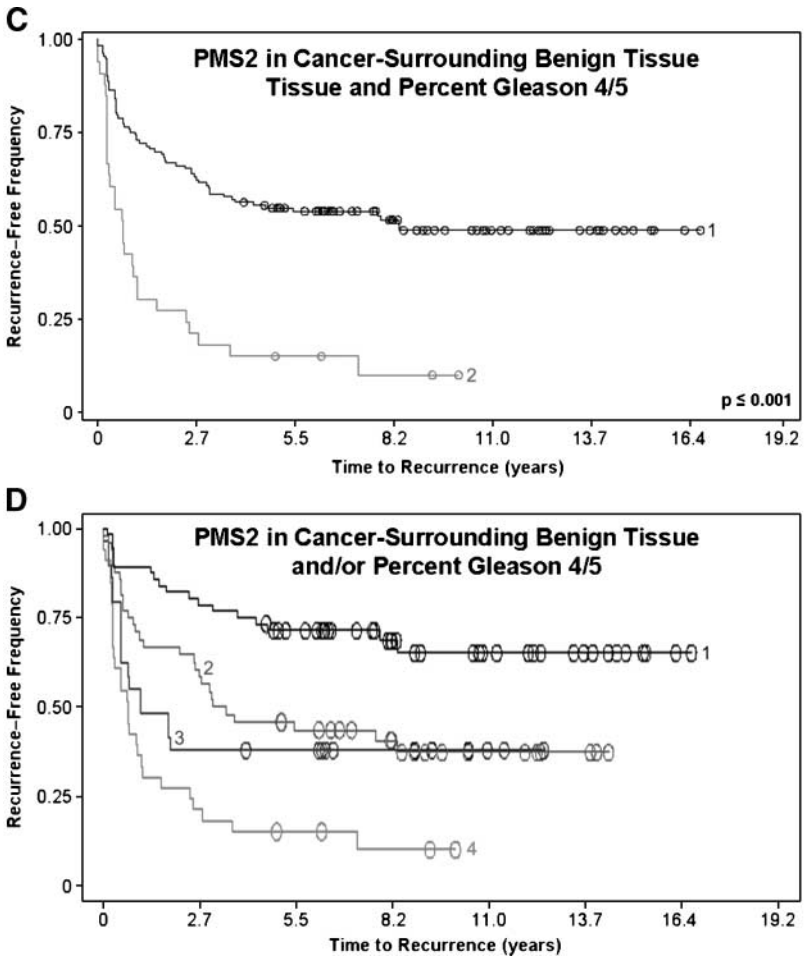

Figure 2 *Continued.*

percent Gleason 4/5. PMS2 in cancer-surrounding benign tissue (*right column*) marginally correlated with volume of the largest cancer ($P = 0.05$), prostate weight ($P = 0.01$), and total percent Gleason 4/5 but not age, PSA or total tumor volume (Table 2).

PMS2 Protein Levels and Biochemical Recurrence. We next determined if elevation of PMS2 is predictive of biochemical recurrence in this cohort. Receiver operating characteristic analyses were used to determine a cutpoint H-score of 200 for PMS2. This number best corresponded to the highest sensitivity/specificity ratio and was also approximately the median value in this cohort. It should be noted that the cutpoint used here was the most effective for this cohort and analysis but may not be as effective in a larger or different population. Further studies are needed to specifically address this aspect.

A multivariate analysis was done to determine which pathologic variables, if any, predicted time-to-recurrence. As shown in Table 3, only preoperative PSA ($P < 0.001$), percent Gleason grade 4/5 ($P < 0.001$), and PMS2 in tumor-associated benign tissue ($P = 0.002$) were independent predictors of biochemical recurrence. For all variables, the variable estimates were positive, indicating that the higher PSA, higher percent Gleason 4/5, and higher PMS2 counts all predicted time-to-recurrence. The fact that all three variables were highly significant in the same regression model indicates that each contributes independent information in the prediction of time-to-recurrence.

Figure 2 shows the Kaplan-Meier estimate of recurrence-free survival stratified by PMS2. For this analysis, ''PMS2 high'' patients corresponded to those having a PMS2 score of ≥200, whereas ''PMS2 low'' corresponded to patients with a score of <200. Figure 2A depicts the curves for PMS2 levels in cancer tissue alone ($P = 0.019$), whereas Fig. 2B depicts the curves resulting from PMS2 levels in cancer-surrounding benign tissue alone ($P < 0.001$). In both cases, patients with low PMS2 (*black lines*; *line 1*) have higher recurrence-free survival when compared with patients with high PMS2 (*red lines*; *line 2*), and according to the P value, the level of PMS2 in cancer-surrounding benign tissue was better able to stratify patients based on biochemical recurrence. For PMS2 in cancer tissue (Fig. 2A), the median time-to-recurrence for PMS2-low patients (1,999 ± 143 days) was longer than the PMS2-high patients (1,264 ± 141 days). Similar results were found for the cancer-associated benign tissue (Fig. 2B), where the median time-to-recurrence for PMS2 low patients (1,797 ± 188 days) was equally longer than the PMS2 high patients (1,542 ± 121 days).

We next determined if a combination of prognostic markers would improve the predictive value of either alone. PMS2 levels in cancer-surrounding benign tissue were combined with total percent Gleason 4/5.

Kaplan-Meier analyses of the combination of PMS2 and percent Gleason 4/5 is shown in Fig. 2C to D. For these analyses, we assigned patients that had ≥50% Gleason grade 4/5 tumor as ''high percent Gleason'' and patients with <50% Gleason grade 4/5 tumor as ''low percent Gleason.'' Total percent of Gleason 4/5 measurements were used in place of Gleason score because they were previously shown to be the best predictor of outcome with this population (16). As seen in Fig. 2C, patients who had low PMS2 or low percent Gleason 4/5 (*black*; *line 1*) had a higher recurrence-free survival (1,874 ± 114 days versus 657 ± 158 days, respectively) when compared with patients with high PMS2 or high percent Gleason 4/5 (*red*; *line 2*; $P < 0.001$). Figure 2D depicts patients that were further stratified into low PMS2 and low percent Gleason 4/5 (*black*; *line 1*), high PMS2 and high percent Gleason 4/5 (*red*; *line 4*), high PMS2 with low percent Gleason 4/5 (*green*; *line 2*), or low PMS2 with high percent Gleason 4/5 (*blue*; *line 3*).

Of these 4 subgroups, patients that had high PMS2 and high percent Gleason 4/5 had the fastest time-to-recurrence (418 ± 51 days) when compared with all other groups (low PMS2 and low percent Gleason 4/5 was 2,355 ± 154 days; high PMS2 and low percent Gleason 4/5 was 1,651 ± 188 days; low PMS2 and high percent Gleason 4/5 was 657 ± 158 days). The Kaplan-Meier curves for the low PMS2 and high percent Gleason 4/5 (*blue*; *line 3*) and the high PMS2 and low percent Gleason 4/5 (*green*; *line 2*) become virtually identical over time indicating that both groups have the same frequency of biochemical recurrence ~4 years postsurgery (Fig. 2D).

We further used two-by-two table analyses to determine the sensitivity (true positive) and specificity (true negative) of PMS2 alone and in combination with percent Gleason 4/5. As shown in Table 4, these variables for PMS2 levels in cancer-surrounding benign tissue (72%, 58%) are better than PMS2 levels in cancer (61%, 54%) and are approximately equivalent to percent Gleason 4/5 (76%, 55%). When percent Gleason 4/5 was combined with PMS2 in cancer-surrounding benign tissue, the sensitivity was increased to 88% at some expense to the specificity (48% versus 55%). These analyses indicate that PMS2 provides prognostic benefits alone and in combination with traditional clinical indicators.

Discussion

Currently, in the diagnosis of prostate cancer, in patients with Gleason score 7 cancer, no prognostic markers are able to distinguish between those whose cancer will and will not recur. Unfortunately, this group represents the largest number of patients at presentation. The consequences of ineffective risk assessment are underdiagnosis (increasing the number of deaths from cancer) or overdiagnosis (increasing the treatment-associated morbidity and life-altering side effects). Thus, new markers that can improve predictions of disease progression and treatment planning of patients with prostate cancer are needed.

Here, we show that PMS2, a MMR protein that is elevated in prostate cancer, predicts biochemical recurrence in a population of patients containing Gleason score 7 prostate cancer treated by prostatectomy. The levels of PMS2 were consistently higher in recurrent individuals (Fig. 1). The levels of PMS2 in both cancer and the cancer-surrounding benign epithelium were able to stratify patients based on recurrence (Fig. 2), whereas PMS2 levels in the benign epithelium surrounding the cancer were actually predictive of disease recurrence (Table 3). When used in combination, PMS2 was able to improve the prognostic sensitivity of total percent Gleason 4/5 by 12% (Table 4). The presence of PMS2 in benign tissue is reminiscent of previous reports on risk markers that were found to be present in cancer-associated, benign tissue (28, 29) and suggests that PMS2 elevation is an early event in prostate tumorigenesis.

The search for molecular prognostic indicators has produced a number of markers that can be detected immunohistochemically and that correlate with prostate cancer progression. Well-characterized markers include Bcl-2 family proteins (30-34), CDK1/p34 (35, 36), E-cadherin (37-40), insulin-like growth factor binding proteins (41, 42), Ki67 (30, 32, 43, 44), p27 (45-47), and PTEN (48, 49). Many more potential biomarkers are the subject of further investigation and verification (50, 51). Like PMS2, many of these markers are detectable in tissue samples and correlate with patient outcome. PMS2 is unique, however, in that its quantitation in histologically benign epithelium adjacent to the tumor is informative for biochemical relapse. Also, although the well-characterized markers mentioned above are primarily indicators and effectors of cellular proliferation or apoptotic activation, PMS2 elevation is associated with genomic instability and mutagenesis.

Elevation of PMS2 has previously been shown to have biological consequences including increased mutation frequency (14), increased resistance to methylation-induced apoptosis (14), reduced MMR (52), and microsatellite instability in tumors (15). PMS2 elevation even in early, preneoplastic PIN lesions (15) indicates that it may be an early event in tumorigenesis. The discovery that PMS2 levels in cancer-surrounding benign epithelium are predictive of biochemical recurrence suggests that PMS2 may be a consequence of early changes in the tumor microenvironment that contribute to aggressive tumor formation. At this point, the exact role of PMS2 elevation in tumorigenesis is still speculative but may involve aberrant and improper binding to inactivate the MMR system. This mechanism would be consistent with

Table 4. Two-by-two table analyses of PMS2 levels (in benign and cancer tissue), of total percent Gleason 4/5 and of both combined

Group	Sensitivity	Specificity
PMS2 in Cancer	61%	54%
PMS2 in Benign	72%	58%
% Gleason 4/5	76%	55%
% Gleason 4/5 + PMS2 (B)	88%	48%

NOTE: Two by two table analyses of PMS2 levels (in benign and cancer tissue), of total percent Gleason 4/5 and of both combined. To determine the sensitivity (true positive) and specificity (true negative) of PMS2 alone and in combination with percent Gleason 4/5, a two-by-two table analyses was done. The resulting percentages are shown above. PMS2 (B): PMS2 levels in benign tissue.

the biological consequences already associated with PMS2 elevation.

As this study was retrospective and would be defined as a Phase 2 biomarker study (53), further studies are required to (*a*) validate these findings in an independent patient population, (*b*) to investigate the effect of treatment (e.g., radiation, adjunct chemotherapy), and (*c*) to determine a standardized scale of PMS2 quantitation. Overall, this study confirms previous findings of a MMR protein, PMS2, being elevated in a significant proportion of prostate cancer (15) and shows that PMS2 is a novel prognostic indicator for biochemical recurrence after surgery.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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