Preoperative Serum DNA GSTP1 CpG Island Hypermethylation and the Risk of Early Prostate-Specific Antigen Recurrence Following Radical Prostatectomy

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Abstract

Purpose: Hypermethylation of the CpG island at the promoter region of the \( \pi \)-class glutathione S-transferase gene (GSTP1) is the most common somatic genome abnormality in human prostate cancer. We evaluated circulating cell-free DNA GSTP1 CpG island hypermethylation as a prognostic biomarker in the serum of men with prostate cancer.

Experimental Design: Prostate cancer DNA GSTP1 CpG island hypermethylation was detected using a restriction endonuclease quantitative PCR technique. We analyzed preoperative serum from 85 men with clinically localized prostate cancer treated with radical prostatectomy and from 35 men with a negative prostate biopsy. We then assayed preoperative serum from a data set of 55 pairs of men with clinically localized prostate cancer treated with radical prostatectomy, matched for Gleason score, comprising 55 men suffering prostate-specific antigen (PSA) recurrence (median, 2 years) and 55 men who were free of disease at last follow-up (median, 3 years). The association of serum GSTP1 CpG island hypermethylation and PSA recurrence was determined.

Results: Circulating cell-free DNA with GSTP1 CpG island hypermethylation was not detected in the serum of men with a negative prostate biopsy but was detected in 12% of men with clinically localized disease and 28% of men with metastatic cancer \((P = 0.003)\). In the matched data set, eight men (15%) who developed PSA recurrence were positive for DNA with GSTP1 CpG hypermethylation, whereas no patient who was free of disease was positive for GSTP1 CpG island hypermethylation (McNemar test, \( \chi^2 = 6.1, P = 0.01 \)). In a multivariable analysis that accounted for recognized prognostic factors, the presence of serum DNA with GSTP1 CpG island hypermethylation was the most significant predictor of PSA recurrence (hazard ratio, 4.4; 95% confidence interval, 2.2, 8.8; \( P < 0.001 \)).

Conclusion: Our study suggests that GSTP1 CpG island hypermethylation may be an important DNA-based prognostic serum biomarker for prostate cancer.

Prostate cancer is the most commonly diagnosed solid organ malignancy among men in the United States, accounting for an estimated 230,000 new cases in 2004 alone (1). Primary therapy in the form of radical prostatectomy is one of the principal treatment modalities available for men with clinically localized disease. Whereas approximately two thirds of patients treated with curative intent by surgery will remain disease free 15 years following radical prostatectomy, those who experience early prostate-specific antigen (PSA) recurrence, particularly within the first 2 years of surgery, are prone to develop metastatic lesions and are likely to succumb to prostate cancer (2, 3). Preoperative identification of men at risk for early disease progression following radical prostatectomy could improve patient selection for local therapies and identify early those at risk for relapse as potential candidates for adjuvant protocols; thus, accelerating the delivery of multimodal therapies to those who may potentially benefit the most.

Somatic genome alterations (e.g., mutations, deletions, rearrangements, amplifications, and DNA methylation) are relatively frequent events in human cancers. Of these, DNA hypermethylation has been noted the most common (4, 5). Dinucleotides consisting of a cytosine preceding a guanosine (CpG) in the DNA sequence are susceptible to the actions of a class of enzymes, DNA methyltransferases, that catalyze...
the transfer of a methyl group to the cytosine ring, yielding 5-methyl cytosine (5mC). Clusters of Cpg dinucleotides, termed Cpg islands, occur with increased frequency in the promoter area of approximately half of all genes in the human genome. Hypermethylation of promoter-associated Cpg islands is thought to repress transcriptional activity of the downstream gene, resulting in loss of production of mRNA and corresponding protein (6).

Cpg island hypermethylation within the regulatory region of the \( \gamma \)-class glutathione S-transferase gene (\( \text{GSTP1} \)) has been observed to be the most prevalent somatic genome abnormality in human prostate cancer (7, 8). \( \text{GSTP1} \) encodes an enzyme that acts as a carcinogen detoxifier by catalyzing conjugation reactions with reduced glutathione (7). Somatic \( \text{GSTP1} \) Cpg island hypermethylation has been noted in >90% of prostate cancer tissues, whereas it has not been observed in the normal prostate (9). It is important to note that DNA with \( \text{GSTP1} \) Cpg island hypermethylation is rarely seen elsewhere in the body, occurring with significance only in cancers of the liver and breast (10). Recently, analyses of bodily fluids from men with prostate cancer have revealed DNA containing \( \text{GSTP1} \) Cpg island hypermethylation, suggesting a possible role for this epigenetic phenomenon as a novel biomarker for prostate cancer (8, 9, 11). Using a restriction endonuclease quantitative PCR–based strategy that is capable of detecting a single hypermethylated Cpg island allele, we sought to further characterize DNA-harboring \( \text{GSTP1} \) Cpg island hypermethylation in the preoperative serum of men with prostate cancer and to determine its association with PSA progression in men with initially clinically localized disease following radical prostatectomy (12).

### Materials and Methods

**Sample collection and DNA isolation.** All serum samples were collected before prostate biopsy and stored at \(-80^\circ\text{C}\). Eighty-five consecutive men who underwent radical prostatectomy in 1999 at The Johns Hopkins Hospital for localized prostate cancer served as the initial study population (Table 1). Thirty-five men without evidence of cancer on prostate biopsy served as a control population. Sera from 18 men with metastatic prostate cancer were also examined. Based on our findings from the 85 consecutive patients (initial cohort), a subsequent group of 55 matched pairs of men with clinically localized prostate cancer treated with radical prostatectomy was created (validation cohort). This group was composed of 55 men who developed PSA recurrence and 55 men who did not experience PSA recurrence at last follow-up. Men in this data set were matched on the basis of pathologic Gleason score. Pathologic stage and duration of follow-up were used as secondary matching criteria. DNA was extracted from 600 \( \mu \text{L} \) of archived serum from each patient using the Qiagen Blood Mini Kit (Valencia, CA).

The primary end point of the study was PSA recurrence which was defined as a single post-prostatectomy PSA level of \( >0.2 \) ng/mL. Routine PSA follow-up consisted of a measurement 3 months postoperatively and annually thereafter. All of the men studied had an undetectable serum PSA level 3 months after radical prostatectomy.

To show the efficacy of our assay, DNA from prostate tissues were examined for \( \text{GSTP1} \) Cpg hypermethylation. Genomic DNA from paraffin-embedded, formalin-fixed samples (\( n = 2 \)) or fresh frozen blocks (\( n = 19 \)) was extracted using the Qiagen DNA Mini Kit or as described previously (13). Prostate cancer tissue samples were obtained from radical prostatectomy specimens. Benign prostates were obtained from 13 cadaveric tissue donors, none of which contained histologic evidence of malignancy. Sample preparation was done as previously described (13). All studies were conducted with the approval of the Institutional Review Board.

**Restriction endonuclease quantitative PCR.** A variety of techniques to detect DNA with \( \text{GSTP1} \) Cpg island hypermethylation in prostatic tissues and bodily fluids have been described (9). We used the previously described restriction endonuclease quantitative PCR method (12). This method allows the quantification of the amount of total DNA and methylated DNA in each sample and is able to detect as few as one methylated \( \text{GSTP1} \) allele. Briefly, DNAs extracted from serum samples were subjected to restriction enzyme digestion with \( \text{HpaII} \) (NEB, Beverly, MA). \( \text{HpaII} \) cuts the sequence CCGG but does not cut the methylated form of this sequence, C\(^{\text{mC}}\)GG. If the Cpg island does not contain C\(^{\text{mC}}\)GG, the DNA is cut and no product can be detected after PCR amplification. Typically, 4 \( \mu \text{L} \) of DNA was incubated at 37° C for 5 hours with 60 units of restriction enzyme, 2 \( \mu \text{L} \) of 10× restriction enzyme buffer (NEB) and the appropriate amount of DNase-free water to give a final reaction volume of 20 \( \mu \text{L} \). To ensure complete digestion, an additional 30 units of restriction enzyme, 1 \( \mu \text{L} \) of restriction enzyme buffer and 6 \( \mu \text{L} \) of DNase-free water were added to each reaction and incubated at 37° C overnight. As individual sample controls, PCR amplification was done on undigested DNA and DNA digested with the restriction enzyme \( \text{MspI} \) (NEB) using the above protocol. \( \text{MspI} \) cuts both the unmethylated and methylated sequence; thus, no PCR product should be detected. All PCR reactions were carried out in an iCycler real-time thermal cycler (Bio-Rad, Hercules, CA) using a specific primer set (5´ primer, ACTCACTGGTGGCGAAGACT; 3´ primer, GACCTGGGAAAGAGGGAAAG; ref. 14) that brackets six \( \text{HpaII}/\text{MspI} \) recognition sites. PCR conditions were set as follows: 95° C for

| Table 1. Patient characteristics of men with no evidence of cancer on prostate biopsy and men with clinically localized prostate cancer treated with radical prostatectomy |
|-----------------|-------------------|-------------------|
| Biopsy negative, initial cohort, \( n = 46 \% \) | Clinically localized cancer, \( n = 85 \% \) |
| Age (y) | 60.2 | 58.6 |
| Range | 40-79 | 40-71 |
| PSA (ng/mL) | 2.3 | 6.2 |
| Range | 0.1-4.0 | 0.9-15.5 |
| Pathologic Gleason score* | 5-6 | 65 (76) |
| | 7 | 17 (20) |
| | 8-10 | 2 (2) |
| Pathologic stage | 59 | 78 (92) |
| Organ confined | — | — |
| Nonorgan confined | — | 7 (8) |
| Positive extraprostatic extension | — | 6 (7) |
| Positive seminal vesicle invasion | — | 1 (1) |
| Positive surgical margins | — | 2 (2) |
| No. with \( \text{GSTP1} \) Cpg island | 0 | 10 (12) |
| hypermethylation detected | | |
| \( \text{GSTP1} \) Cpg island hypermethylation index (range) | 0-30.3 | |

*Pathologic Gleason score unavailable for one patient with clinically localized disease.
### Results

**Detection of DNA with GSTP1 CpG island hypermethylation in prostate cancer tissues and preoperative serum.** Among the serum samples assayed, DNA with GSTP1 CpG island hypermethylation was not present in any of the men with a negative prostate biopsy, 12% (10 of 85) of men with clinically localized prostate cancer, and 28% (5 of 18) of patients with hormone-refractory metastatic disease (Fig. 1). More advanced disease was associated with a higher proportion of men with serum DNA GSTP1 CpG island hypermethylation (P = 0.003). GSTP1 hypermethylation index was calculated as (methylated DNA - nonspecifically amplified DNA) × 100.

### Table 2. Univariate Cox proportional hazards model with respect to PSA progression of 85 consecutive patients (initial cohort) with clinically localized prostate cancer treated with radical prostatectomy

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GSTP1 CpG island hypermethylation</strong></td>
<td>24.0 (2.5-23.2)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Preoperative PSA (continuous)</strong></td>
<td>1.2 (0.9-1.6)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Preoperative PSA (≤10, &gt;10)</strong></td>
<td>6.3 (0.7-61.1)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Clinical stage (T1c versus T2)</strong></td>
<td>1.2 (0.1-11.7)</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Biopsy Gleason score (≥7 versus &lt;7)</strong></td>
<td>4.9 (0.7-35.1)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Pathologic Gleason score</strong></td>
<td>10.0 (1.0-95.8)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

NOTE: The variables surgical margin status, seminal vesicle invasion, pathologic lymph node status, extraprostatic extension, and organ-confined status were not evaluable as they produced a nonconvergent model due to sparse data.
Assessment of preoperative serum DNA with GSTP1 CpG island hypermethylation in a matched cohort of men with clinically localized prostate cancer. Based on the above results and the fact that the initial consecutive cohort of men had only a small number of PSA recurrences (n = 4), a second data set was studied (validation cohort), consisting of 55 pairs of men with clinically localized prostate cancer, dichotomized by the presence or absence of PSA recurrence following radical prostatectomy (see Materials and Methods, Table 3). Eight men (15%) among the 55 who developed PSA recurrence were also positive for GSTP1 CpG island hypermethylation in the serum, whereas no patient who was free of disease at last follow-up was positive for GSTP1 CpG island hypermethylation. Thus, the odds ratio of PSA recurrence for men positive for serum GSTP1 CpG island hypermethylation in this model approached infinity (e.g., 8/0). That is, in all eight pairs (100%) with a positive assay, the recurrent patient was serum positive for GSTP1 CpG island hypermethylation whereas the nonrecurrent matched patient was negative (McNemar test, $\chi^2 = 6.1$, $P = 0.01$). Median time to PSA recurrence for the 55 men who experienced biochemical relapse was 2 years (range, 1-5) and the median follow-up time for the 55 men who were free of disease was 3 years (range, 1-7).

Univariate analysis and prostate-specific antigen–free survival of 55 matched pairs of men. Univariate Cox proportional hazards analysis of the paired matched data set (validation cohort) identified positive serum GSTP1 CpG island hypermethylation, preoperative PSA, surgical margin status, and positive lymph nodes as factors significantly associated with PSA recurrence post-prostatectomy (Table 4). Median time to PSA recurrence following radical prostatectomy was 2 and 4 years for men who were either positive or negative.

### Table 3. Patient characteristics of a matched cohort (validation data set) of men with clinically localized prostate cancer treated with radical prostatectomy

<table>
<thead>
<tr>
<th></th>
<th>NED*, n = 55</th>
<th>PSA recurrence, n = 55 (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>59.7</td>
<td>58.6</td>
<td>0.39</td>
</tr>
<tr>
<td>Range</td>
<td>48-70</td>
<td>43-71</td>
<td></td>
</tr>
<tr>
<td>Preoperative PSA (ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.9</td>
<td>9.9</td>
<td>0.11</td>
</tr>
<tr>
<td>Range</td>
<td>1.4-23.9</td>
<td>1.8-38</td>
<td></td>
</tr>
<tr>
<td>Median follow-up, y (range)</td>
<td>3 (1-7)</td>
<td>4 (1-14)</td>
<td></td>
</tr>
<tr>
<td>Median time to PSA failure, y (range)</td>
<td>—</td>
<td>2 (1-5)</td>
<td></td>
</tr>
<tr>
<td>Biopsy Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-6</td>
<td>34 (63)</td>
<td>24 (44)</td>
<td>0.09</td>
</tr>
<tr>
<td>7</td>
<td>14 (26)</td>
<td>25 (46)</td>
<td></td>
</tr>
<tr>
<td>8-10</td>
<td>6 (11)</td>
<td>6 (9)</td>
<td></td>
</tr>
<tr>
<td>Pathologic Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-6</td>
<td>12 (22)</td>
<td>12 (23)</td>
<td>0.99</td>
</tr>
<tr>
<td>7</td>
<td>31 (56)</td>
<td>29 (55)</td>
<td></td>
</tr>
<tr>
<td>8-10</td>
<td>12 (22)</td>
<td>12 (23)</td>
<td></td>
</tr>
<tr>
<td>Pathologic stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ confined</td>
<td>16 (29)</td>
<td>11 (21)</td>
<td>0.32</td>
</tr>
<tr>
<td>Nonorgan confined</td>
<td>39 (71)</td>
<td>42 (79)</td>
<td></td>
</tr>
<tr>
<td>Positive extraprostatic extension</td>
<td>38 (69)</td>
<td>42 (79)</td>
<td>0.23</td>
</tr>
<tr>
<td>Positive seminal vesicle invasion</td>
<td>8 (15)</td>
<td>9 (17)</td>
<td>0.73</td>
</tr>
<tr>
<td>Positive surgical margins</td>
<td>12 (22)</td>
<td>23 (43)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pathologic lymph node positive</td>
<td>0</td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td>No. with GSTP1 CpG island hypermethylation detected</td>
<td>0</td>
<td>8 (15)</td>
<td>0.006</td>
</tr>
<tr>
<td>Hypermethylation index (range)</td>
<td>0-34.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*No evidence of disease.
†Two men with unknown data.
respectively, for preoperative serum GSTP1 CpG island hypermethylation (log-rank \( P < 0.001 \); Fig. 2B). No patient who was positive for serum DNA with GSTP1 CpG island hypermethylation was free of biochemical recurrence beyond 3 years from surgery. As expected in this matched data set, pathologic Gleason score was not associated with PSA recurrence.

**Multivariable analysis and prostate-specific antigen–free survival of aggregate patient population.** Both subject groups were then combined for subsequent analysis (\( n = 192 \); three men from the consecutive data set were also in the matched paired data set). To minimize a bias towards finding a positive association between GSTP1 CpG island hypermethylation and PSA recurrence that may have resulted from study design, we incorporated an additional variable in the multivariable Cox proportional hazards model (i.e., Dataset) that would serve to remove the artificial effect of increased risk of PSA recurrence in the matched pair data set, where 50% by definition developed PSA recurrence. In the multivariable Cox proportional hazards model, positive serum GSTP1 CpG island hypermethylation status was the most significant predictor of PSA recurrence (HR, 4.4; 95% CI, 2.2-8.8; \( P < 0.001 \); Table 5). The effect of DNA with GSTP1 CpG island hypermethylation did not differ significantly between the two data sets (\( P = 0.093 \) for interaction). Pathologically positive lymph nodes and positive surgical margins were also significantly associated with biochemical recurrence. The 3-year PSA-free survival rate for men with either positive or negative serum GSTP1 CpG island hypermethylation was 41% and 74%, respectively (log-rank \( P = 0.01 \); Fig. 2C).

**Association of preoperative serum DNA GSTP1 CpG island hypermethylation status with established prognostic factors.** None of the previously defined prognostic features associated with PSA recurrence displayed a statistically significant relationship with GSTP1 CpG island hypermethylation status (Table 6; refs. 16, 17). These findings suggest that preoperative serum GSTP1 CpG island hypermethylation positivity may be an independent risk factor for biochemical relapse following radical prostatectomy.

**Discussion**

In the present study, we have shown that men with clinically localized prostate cancer with a positive preoperative serum analysis for GSTP1 CpG island hypermethylation, a marker of prostate cancer DNA, were at significant risk to experience PSA recurrence within the first several years following radical prostatectomy. This is the first use of a cancer-specific molecular biomarker as a blood test for prostate cancer prognosis. For all men with clinically localized disease, those with a positive preoperative serum assay for DNA GSTP1 CpG island hypermethylation were at 4.4 times greater risk to develop biochemical relapse than those with a negative test. Among the established preoperative and postoperative predictors of

**Table 4. Univariate Cox proportional hazards model with respect to PSA progression of 55 matched pairs of men (validation cohort) with clinically localized prostate cancer treated with radical prostatectomy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP1 CpG island hypermethylation</td>
<td>3.0 (1.4-6.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>Preoperative PSA (continuous)</td>
<td>1.0 (1.0-1.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Preoperative PSA (( \leq 10, &gt;10 ))</td>
<td>1.2 (0.7-2.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 versus T1c</td>
<td>1.1 (0.6-2.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Biopsy Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 versus ( \leq 6 )</td>
<td>1.7 (1.0-3.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>8-10 versus ( \leq 6 )</td>
<td>1.2 (0.5-3.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>( \geq 7 ) versus ( &gt;7 )</td>
<td>1.6 (1.0-2.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Pathologic stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical margin status</td>
<td>1.8 (1.1-3.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Extraprostatic extension</td>
<td>1.5 (0.8-2.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td>1.1 (0.5-2.2)</td>
<td>0.83</td>
</tr>
<tr>
<td>Positive lymph node(s)</td>
<td>3.3 (1.5-7.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>Organ confined disease</td>
<td>0.7 (0.4-1.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>Pathologic Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 versus ( \leq 6 )</td>
<td>1.0 (0.5-1.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>8-10 versus ( \leq 6 )</td>
<td>1.2 (0.6-2.6)</td>
<td>0.65</td>
</tr>
<tr>
<td>( \geq 7 ) versus ( &gt;7 )</td>
<td>1.2 (0.6-2.2)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

**Table 5. Multivariable Cox proportional hazards model with respect to PSA progression for all men with clinically localized prostate cancer treated with radical prostatectomy (\( n = 192 \))**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical margins</td>
<td>1.8 (1.0-3.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Pathologic lymph node status</td>
<td>2.4 (1.0-5.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>GSTP1 CpG island hypermethylation</td>
<td>4.4 (2.2-8.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*NOTE: Three of the men in the matched pair data set were also represented in the group of 85 consecutive patients. Model also adjusted for data set indicator.*

**Table 6. Association of positive serum GSTP1 CpG island hypermethylation status with established prognostic factors from aggregate patient sample (\( n = 192 \))**

<table>
<thead>
<tr>
<th>Established prognostic factor</th>
<th>( P ) (chi-square)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative PSA (( \leq 10, &gt;10 ))</td>
<td>0.74*</td>
</tr>
<tr>
<td>Biopsy Gleason score</td>
<td>0.21</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>0.25</td>
</tr>
<tr>
<td>Pathologic Gleason score (( \geq 7 )</td>
<td>0.95</td>
</tr>
<tr>
<td>Pathologic stage</td>
<td></td>
</tr>
<tr>
<td>Extraprostatic extension</td>
<td>0.11</td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td>0.72</td>
</tr>
<tr>
<td>Organ confined</td>
<td>0.11</td>
</tr>
<tr>
<td>Positive lymph node(s)</td>
<td>0.60</td>
</tr>
<tr>
<td>Positive surgical margins</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*Fisher’s exact test.
PSA recurrence (i.e., preoperative PSA, clinical stage, biopsy Gleason score, pathologic Gleason score, extraprostatic extension, seminal vesicle invasion, positive pathologic lymph nodes, pathologic organ confined status, and positive surgical margins), preoperative positive DNA GSTP1 CpG island hypermethylation status was the single most powerful predictor of PSA recurrence. In addition, we noted that positive GSTP1 CpG island hypermethylation was not significantly associated with any of the aforementioned variables. These results suggest that DNA with GSTP1 CpG island hypermethylation is a promising independent DNA-based prognostic serum biomarker for prostate cancer.

The presence of cell-free prostate cancer DNA in the serum of men with localized disease portended a poor prognosis. What is the source of cell-free DNA in the blood? Whereas multiple hypotheses exist, the true origins of this phenomenon remain unknown. Potential explanations include the release of DNA by normal cells undergoing apoptosis and the liberation of nucleic acids by tumor cells undergoing necrosis (18, 19). DNA released from fragile cells during the clotting process after blood sampling may be an additional source of serum cell-free DNA (20), although this contribution is thought to be negligible (18). Our finding that prostate cancer DNA, assessed by GSTP1 hypermethylation, in the preoperative serum of men with localized prostate cancer is associated with a poor prognosis may relate to previously described characteristics of more aggressive tumors (i.e., increased access to circulation, increased cell turnover, and the presence of micrometastases; refs. 21–25). Any of these traits, alone or in combination, might increase the likelihood that cell-free prostate cancer DNA would be present in the serum. What is the fate of circulating cell-free DNA? The clearance of blood-borne DNA is poorly understood. Plasma nucleases as well as hepatic and renal clearance mechanisms are thought to play an important role in clearing the blood of free nucleic acids (26, 27). The half-life of tumor associated circulating DNA is estimated to be in the order of hours (28, 29).

Regardless of its origins or function, circulating cell-free DNA-based biomarkers have certain advantages over those employing RNA or protein. DNA is vastly more stable than RNA and is easier than protein to manipulate in the laboratory to identify subtle changes. As genetic mutations are very specific for neoplastic processes, their detection may seem ideal for the identification of certain cancers, such as colon cancer where over 50% of tumors contain a mutation in either K-RAS or p53 (30). Prostate cancer, however, is genetically heterogeneous both among affected men and within a diseased organ, making such a strategy problematic. The near-universal epigenetic alteration of GSTP1 CpG island hypermethylation in prostate cancer, together with the complete absence of GSTP1 CpG island hypermethylation in normal tissues, makes it an attractive DNA-based biomarker for this disease (13, 31).

Previous studies have examined DNA methylation of various genes in plasma and serum as biomarkers for a number of human cancers (5). Although these investigations are in their infancy, their specificity seems high (5). Such reports have found blood-borne hypermethylated DNA in patients with advanced-stage cancer and have noted a correlation with a diminished prognosis (32–37). In men with prostate cancer, Goessl et al. observed GSTP1 CpG island hypermethylation in the plasma of 56% of patients with pT2-3 disease and in 93% of men with pT4N+ disease (11). In nonpalpable, clinically localized prostate cancer (clinical stage T1c), Jeronimo et al. detected GSTP1 serum hypermethylation in 32% of patients examined (15).

The amount of cell-free DNA in the serum differs among normal and cancer patients. In healthy individuals the total amount of circulating DNA has been reported to be between 10 and 30 ng/mL (38, 39), compared with ~1,200 ng/mL in serum samples of patients with certain types of cancer (18). Recently, the amount of cell-free DNA in men with metastatic prostate cancer was reported to be ~40 ng/mL (40). Furthermore, in men with metastatic prostate cancer, serum PSA level and the amount of plasma DNA have been similarly associated with survival (40).

A few specific aspects of our study warrant further discussion. First, of the two patient groups that made up our study population, only the initial group of 85 men was prospectively collected. Based on our preliminary findings with this consecutive data set, we constructed a matched data set with enough statistical power to detect the predicted difference in PSA-free survival between paired groupings. Second, our chosen end point was time to PSA recurrence not time to metastasis or death. Sufficient follow-up time has not yet elapsed for the majority of men in the matched cohort to make either of the latter two end points meaningful. Moreover, PSA recurrence within the first 2 years of surgery has been associated with a higher probability of developing distant metastasis, a short PSA doubling time, and prostate cancer-specific mortality (3, 41).

How might serum GSTP1 CpG island hypermethylation status affect the clinical management of men with prostate cancer? To begin with, the fact that the assay described involves readily available laboratory reagents, requires only common molecular biological techniques and necessitates no extra procedure to be done other than a routine blood draw should make the clinical application of this serum test relatively straightforward. Its use may improve our ability to identify men who are less likely cured by surgery. Furthermore, renewed interest in adjuvant therapies for high-risk patients following radical prostatectomy, as well as recent advances in prostate cancer chemotherapeutic regimens, call for the identification of patients considered to be at greatest risk (42–45). Men diagnosed with clinically localized prostate cancer, who are serum positive for DNA GSTP1 CpG island hypermethylation and are contemplating radical prostatectomy as primary treatment, may be appropriate candidates for adjuvant therapy. Further studies prospectively evaluating the role of GSTP1 CpG island hypermethylation as an independent prognostic factor in men with clinically localized prostate cancer are indicated. This is the first demonstration of the clinical utility of a DNA-based serum biomarker in prostate cancer.

References

Preoperative Serum DNA GSTP1 CpG Island Hypermethylation and the Risk of Early Prostate-Specific Antigen Recurrence Following Radical Prostatectomy

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