**EFEMP1** as a Novel DNA Methylation Marker for Prostate Cancer: Array-based DNA Methylation and Expression Profiling

Yong-June Kim¹,*, Hyung-Yoon Yoon¹, Seon-Kyu Kim¹, Young-Won Kim¹, Eun-Jung Kim¹, Isaac Yi Kim², Wun-Jae Kim¹,*

¹From the Department of Urology, College of Medicine, Chungbuk National University, Cheongju, South Korea, ²Dean and Betty Gallo Prostate Cancer Center and Section of Urologic Oncology, Division of Urology, Department of Surgery, The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ

*Corresponding Author

**Yong-June Kim:** E-mail: urokyj@cbnu.ac.kr

Department of Urology, College of Medicine, Chungbuk National University, 62, Kaeshin-dong, Heungduk-ku, Cheongju, Chungbuk, 361-711, South Korea

Tel: +82-43-269-6134; Fax: +82-43-269-6144

**Wun-Jae Kim:** E-mail: wjkim@chungbuk.ac.kr

Department of Urology, College of Medicine, Chungbuk National University, 62 Kaesin-dong, Heungduk-gu, Cheongju 361-763, South Korea

Tel: +82-43-269-6137; Fax: +82-43-269-6144
Grant support: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0001735 and 2009-0068819).

Request for reprints: Yong-June Kim, Department of Urology, College of Medicine, Chungbuk National University, 62, Kaeshin-dong, Heungduk-ku, Cheongju, Chungbuk, 361-711, South Korea. Phone: +82-43-269-6134; Fax: +82-43-269-6143, E-mail: urokyj@cbnu.ac.kr

Running Header: DNA methylation profiles in prostate cancer

Keywords: Diagnosis; EFEMP1; Methylation; Neoplasm; Prostate
Translational Relevance

To identify novel methylation markers at the whole-genome level, rigorous selection criteria, as well as confirmatory studies and validation using human tissue specimens, are mandatory. In the current study, DNA methylation status and gene expression levels were considered simultaneously in a screen for candidate genes. Methylation status and expression levels of potential targets were then verified by various molecular techniques. The validity of the final candidate methylation marker was determined in a relatively large sample of human prostate tissues. Independent validation using microarray gene expression data from the Gene Expression Omnibus (GEO; series GSE6919) representing a Western population was also performed. To the best of our knowledge, this investigation is the first to identify *EFEMP1* as a novel methylation marker for prostate cancer. Further large-scale validation studies with human samples and functional investigation of *EFEMP1* will improve our understanding of its role in the tumorigenic process and its clinical relevance.
Abstract

Purpose: Abnormal DNA methylation is associated with many human cancers. The aim of the present study was to identify novel methylation markers in prostate cancer (PCa) using microarray analysis, and to test whether those markers could discriminate normal and PCa cells.

Experimental Design: Microarray-based DNA methylation and gene expression profiling was carried out using a panel of PCa cell lines and a control normal prostate cell line. The methylation status of candidate genes in prostate cell lines was confirmed by real-time reverse transcriptase polymerase chain reaction, bisulfite sequencing analysis and treatment with a demethylation agent. DNA methylation and gene expression analysis in 203 human prostate specimens including 106 PCa and 97 benign prostate hyperplasia (BPH) was performed. Further validation using microarray gene expression data from the Gene Expression Omnibus (GEO) was also carried out.

Results: Epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) was identified as a lead candidate methylation marker for PCa. The gene expression level of EFEMP1 was significantly higher in tissue samples from patients with BPH as compared to PCa ($P < 0.001$). The sensitivity and specificity of EFEMP1 methylation status in discriminating between PCa and BPH reached 95.3% (101/106)
and 86.6% (84/97), respectively. From the GEO data set, we confirmed that the expression level of *EFEMP1* was significantly different between PCa and BPH.

**Conclusion:** Genome-wide characterization of DNA methylation profiles enabled the identification of *EFEMP1* aberrant methylation patterns in PCa. *EFEMP1* might be a useful indicator for the detection of PCa.
Introduction

Although prostate cancer (PCa) continues to be a frequent cause of morbidity and death throughout the world, it can only be diagnosed by prostate biopsy. The indications for a prostate tissue biopsy include a finding of elevated serum prostate specific antigen (PSA) and/or the results of a digital rectal examination. However, a significant proportion of biopsies are negative due to the low sensitivity and specificity of these tests (1, 2). Moreover, the biopsy procedure itself can induce pain, discomfort, anxiety and complications, and increases medical costs (3). These limitations have spurred interest in other molecular parameters that could be used for more accurate, noninvasive diagnosis of PCa. To date, several potential biomarkers for the detection of PCa have been identified through molecular biological and genetic studies, but their predictive value remains to be conclusively verified (4-8).

Epigenetic alterations such as abnormal DNA methylation are associated with many types of human cancer. In fact, aberrant DNA methylation has been recognized as one of the early events in tumorigenesis (9-11). Thus, differences in methylation patterns between normal and tumor cells could potentially be exploited for the detection of tumor cells in biopsy specimens or to identify tumor-derived DNA in body fluids (7, 10). The advent of high-throughput microarray technology makes possible...
comprehensive insights into the molecular basis of human diseases (12). With this technology, genome-wide DNA methylation patterns and RNA expression levels in tumor specimens can be evaluated simultaneously, and molecular targets or gene classifiers that are specific for tumor cells can be identified (5, 13).

The ability to detect the presence or absence of PCa would be an invaluable tool for guiding appropriate management strategies. The aim of the present study was to identify novel epigenetic markers that can discriminate between normal and PCa cells using microarray analysis of DNA methylation and RNA expression patterns.
Materials and Methods

Cell Lines and human tissues Samples

The human PCa cell lines LNCaP-FGC (KCLB #21740), DU-145 (KCLB #30081) and PC-3 (KCLB #21435), and the normal human prostate epithelial RWPE-1 cell line (ATCC #CRL-11609) were purchased from the Korean Cell Line Bank (Seoul, South Korea) and American Type Culture Collection (Manassas, VA), respectively. DU-145 cells were maintained in Dulbecco’s Modified Eagle’s Medium (WelGENE Inc., Daegu, South Korea), LNCaP cells were maintained in RPMI1640 Medium (WelGENE Inc., Daegu, South Korea), and PC-3 cells were cultured in Nutrient Mixture F-12 Ham Medium (GIBCO, Carlsbad, CA). RWPE-1 cells were cultured in Defined Keratinocyte-SFM (GIBCO, Carlsbad, CA). All media was supplemented with 10% fetal bovine serum (WelGENE Inc., Daegu, South Korea) and L-glutamine, and cells were incubated in a humidified atmosphere with 5% CO₂ at 37°C.

A total of 203 human prostate tissue samples were obtained from our institute and used for the validation of the screening results: 97 benign prostate hyperplasia (BPH) and 106 PCa (Table 1). Patients with PCa underwent radical prostatectomy (RP) or palliative transurethral resection (TUR); patients with BPH underwent TUR. Study design and validation strategies are outlined in Fig. 1.
All tissues were macro-dissected within 15 minutes of surgical resection. Each prostate specimen was confirmed by pathological analysis of a part of the tissue sample in fresh, frozen sections of the RP/TUR specimen, and then frozen in liquid nitrogen and stored at -80 °C until use. The collection and analysis of all samples was approved by the local institutional review board and informed consent was obtained from each subject.

**DNA and RNA extraction**

Genomic DNA was extracted by standard methods using the Wizard Genomic DNA Purification System (Promega, Madison, WI). Total RNA was extracted with TRIzol reagent (Life Technologies, NY) according to the manufacturer’s protocol. The quality and integrity of the RNA were confirmed by agarose gel electrophoresis and ethidium bromide staining, followed by visual examination under ultraviolet light. cDNAs were synthesized from 1 μg of total RNA by random priming using a First-Strand cDNA Synthesis Kit (Amersham Biosciences, Buckinghamshire, UK).

**DNA methylation profiling in cell lines**

Methylation patterns were assayed using the genome-wide Illumina Infinium HumanMethylation27 BeadChip array (Illumina Inc., San Diego, CA), which enables interrogation of 27,578 CpG dinucleotides covering 14,495 genes. Methylation assays
were carried out according to the manufacturer’s protocol. Bisulfite conversion of
genomic DNA was performed using the EZ DNA Methylation Kit (Zymo Research,
Orange, CA). Fluorescence signals corresponding to C or T nucleotides were measured
and the data were used to assign a quantitative measure of methylation level (β value).
Each methylation data point represents the fluorescent signal from the methylated (M)
and unmethylated (U) allele. Background intensity was computed from a set of negative
controls and subtracted from each analytical data point. The ratio of the fluorescent
signals from the two alleles was then computed. The β value represents a quantitative
measure of the DNA methylation level of specific CpG islands, and ranges from 0
(completely unmethylated) to 1 (completely methylated).

Gene expression profiling in cell lines

For the integrated analysis of global methylation status and gene expression
levels, we used the genome-wide HumanHT-12 Gene Expression BeadChip (Illumina
Inc., San Diego, CA), which covers 48,804 genes. Gene expression analysis was
performed according to the manufacturer’s protocol. The details of the experimental
method have been previously described (14, 15). In brief, the quality and integrity of the
RNA were confirmed by agarose gel electrophoresis and ethidium bromide staining,
followed by visual examination under ultraviolet light. Five hundred nanograms of total
RNA were used for labeling hybridization according to the manufacturer’s protocol. Arrays were scanned with an Illumina Bead Array Reader confocal scanner (BeadStation 500GXDW; Illumina Inc., San Diego, CA), according to the manufacturer’s instructions. Initial microarray gene expression data were obtained using the gene expression analysis module of Bead Studio software (version 3.1.3, Illumina Inc., San Diego, CA).

**Real-time reverse transcriptase polymerase chain reaction (RT-PCR)**

To verify the influence of methylation status on gene expression, RT-PCR analysis of selected genes was performed using a Rotor Gene 3000 PCR system (Corbett Research, Mortlake, Australia). GAPDH was analyzed in parallel as an internal control. RT-PCR reaction mixtures containing primers and SYBR Premix EX Taq (TAKARA BIO INC., Otsu, Japan) were analyzed in micro-reaction tubes (Corbett Research, Mortlake, Australia). Spectral data were captured and analyzed using Rotor-Gene Real-Time Analysis Software 6.0 Build 14 (Corbett Research, Mortlake, Australia). Gene expression was normalized to the expression of GAPDH.

**5-aza 2’-deoxycytidine (AZA) treatment**

PCa cell lines were cultured until confluent and then treated with AZA (Sigma Aldrich, St. Louis, MO). Cells were exposed continuously to AZA (8.8 μM) for 3 days.
The duration and dosage of AZA treatment were based on previous results showing that 8.8 μM AZA induces the re-expression of silenced genes without excessive cell death (16, 17).

**Bisulfite genomic sequencing (BGS)**

The methylation status of candidate genes was analyzed using bisulfite-modified genomic DNA, in which unmethylated, but not methylated, cytosine has been converted to uracil. Genomic sequencing of both strands of the candidate gene promoter was carried out after bisulfite treatment of genomic DNA. Following agarose gel electrophoresis, amplified fragments were excised from the gel and purified using the Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA). Methylated nucleotides were verified by sequencing of the fragments using an ABI Prism 3700 XL DNA analyzer (Applied Biosystems, Foster, CA). Cytosine peaks in the chromatogram with a height of 0.4 or more relative to the thymine peak height were considered as indicative of methylation in BGS (18).

**Statistical analysis**

DNA methylation and gene expression profile data were normalized using quantile normalization in the R language environment (version 2.10.0, available at http://www.r-project.org/). Measured gene expression intensity was log2 transformed.
and median centered across genes and samples. As a means of comparing profile patterns between DNA methylation and gene expression arrays, the microarray data for DNA methylation were pooled with the microarray data set for gene expression using lookup table information provided from the microarray platform manufacturer (Illumina Inc., San Diego, CA). Two criteria were applied to detect genes whose methylation level differed significantly between PCa and control cells: (1) DNA methylation level ($\beta$) in cancer and control cells differed by at least 0.5; and (2) gene expression level between cancer and control cells differed by at least 2-fold. To further validate genes identified in the initial cohort, we analyzed microarray data from prostate cancer tissues and organ donor prostate tissues, as reported by Yu et al. (available at http://www.ncbi.nlm.nih.gov/geo/ under the data series accession number GSE6919) (19).
Results

Identification of differentially methylated and expressed genes in PCa and normal cell lines

Genome-wide methylation and expression profiles from three different PCa cell lines (LNCaP-FGC, DU-145, and PC-3) were compared to control RWPE-1 prostate cells. The complete set of microarray data from the prostate cell lines is available online (http://www.ncbi.nlm.nih.gov/geo/) under the data series accession number GSE23388. Using highly stringent selection criteria ($\beta$ difference $> 0.5$), we identified 106 unique genes that were hypermethylated in all three PCa cell lines. Of these 106 unique hypermethylated genes, 13 exhibited at least a 2-fold difference in gene expression in all three cell lines as compared to control cells. To identify regulatory CpG islands in the promoters of these 13 candidate genes, we analyzed the locations of 5′ CpG islands (provided by the manufacturer) and putative transcriptional start sites using all available genome databases. The results of this analysis revealed that five of the 13 candidate genes contained CpG islands that were located upstream of the true transcriptional start site.

Analysis of changes in gene expression levels by AZA treatment

To confirm that DNA methylation resulted in gene silencing of the five
identified candidate genes, we performed gene expression analysis by RT-PCR. The expression levels of all 5 genes were significantly decreased in PCa cells as compared to control cells (each $P < 0.05$) (Data not shown). To determine whether the silencing of these genes could be reversed, PCa cells were treated with the DNA methyl transferase inhibitor AZA, and then expression was analyzed by RT-PCR. Expression of three of the five candidate genes was significantly enhanced in response to AZA treatment in all three PCa cell lines, as compared to the corresponding untreated control cells; the remaining two genes were either unchanged or expression was altered in only one of the cell lines (Data not shown). These three final candidate genes, identified as protein phosphatase 1 regulatory (inhibitor) subunit 14C (PPP1R14C), epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1), and Islet1 (ISL1), were subjected to further analysis as putative methylation-silenced genes in PCa cells.

**Confirmation of CpG island hypermethylation using BGS in prostate cell lines**

To determine whether the final three candidate genes were inactivated in PCa cells by promoter hypermethylation, we performed BGS using bisulfite-modified genomic DNA from prostate cell lines. BGS of CpG sites that were included in the
array confirmed that the methylation patterns of *PPP1R14C*, *EFEMP1*, and *ISL1* were significantly different in PCa and normal prostate cells. The majority of the CpG sites in the promoter regions of the three candidate genes were either completely or partially methylated in all three PCa cell lines, but not in control cells.

*Validation of methylated genes in human prostate tissues*

In a preliminary validation experiment, we analyzed the methylation status of the three candidate methylated gene markers in a small set of validated human prostate tissue samples (10 PCa and 10 BPH) by BGS. Of the three candidate markers, *EFEMP1* was the best performing methylated marker in terms of sensitivity and specificity for PCa versus control BPH (Data not shown). For further validation of *EFEMP1*, we analyzed 106 PCa and 97 control BPH tissue specimens (including those analyzed in the preliminary validation) by RT-PCR and BGS. The primer sequences used for RT-PCR and BGS and the results of BGS of *EFEMP1* promoter CpG island methylation are illustrated in Figure 2. The level of expression of *EFEMP1* was significantly higher in BPH as compared to PCa samples (*P* < 0.001) (Fig. 3A). As presented in Table 2, *EFEMP1* was methylated in 95.3% (101/106) and 13.4% (13/97) of PCa and BPH samples, respectively. Similar results were obtained even after stratification by stage, Gleason score, and PSA level. The validity of *EFEMP1* as a methylation marker in PCa
cells was also evaluated using an independent set of microarray data from a Western population, which included prostate cancer tissues (n = 90) and organ donor prostate tissues (n = 18) (19). Similar to the current set of results, the expression level of EFEMP1 was significantly lower in PCa as compared to normal controls in the independent dataset ($P < 0.001$) (Fig. 3B).
Discussion

Using microarray analysis to detect novel epigenetic markers that are relevant to PCa, we identified significant differences in the methylation patterns of three genes in PCa and normal cell lines. Further analysis revealed an association between gene silencing due to promoter hypermethylation and reduced gene expression levels for all three genes in PCa cell lines. In validation experiments using human specimens, \textit{EFEMP1} emerged as the strongest candidate methylation marker, being methylated in most human PCa tissues but rarely methylated in non-cancerous prostate tissue. Altered expression of \textit{EFEMP1} in PCa versus BPH was confirmed in an independent microarray dataset.

Analysis of aberrant DNA methylation is gaining traction in the areas of cancer risk assessment, diagnosis, therapy monitoring, and novel drug targets for several different types of cancer (5, 11, 13, 20-23). Recently, the feasibility of detecting aberrant DNA methylation in tissue and body fluids and the potential role of DNA methylation as a tumor marker for PCa were reported (7, 20-22). Some methylation markers, such as \textit{GSTP1} and \textit{RASSF1A}, exhibited high sensitivity and specificity for the detection and monitoring of PCa (7). Although histologically confirmed prostate tissues were used, the possibility of unrevealed PCa in BPH patients and undetected small fraction of
methylated DNA molecule might affect our results of sensitivity and specificity. Nonetheless, in terms of methylation frequency, our results were comparable to previous reports (7, 20-22). The sensitivity and specificity of EFEMP1 methylation in distinguishing PCa and benign tissue reached 95.3% and 86.6%, respectively. Moreover, the frequency of EFEMP1 methylation in PCa was independent of serum PSA level, Gleason score and stage. While these findings are promising, large-scale clinical validation studies using primary human cancer tissue and body fluids are currently underway at our institute to confirm EFEMP1 as a diagnostic PCa methylation marker.

Because DNA methylation is reversible, it is considered a good therapeutic target. Drugs that target epigenetic alterations, such as DNA methylation inhibitors, restore the activity of genes by targeting aberrant heterochromatic regions, ultimately leading to the reactivation of tumor-suppressor genes and/or other genes that are crucial for normal cellular function (24). EFEMP1 methylation was reversed by treatment with the DNA methyl transferase inhibitor AZA, which supports EFEMP1 as a putative therapeutic target for the treatment of PCa.

EFEMP1, also known as fibulin-3, belongs to the fibulin family, a widely-expressed family of extracellular matrix proteins that regulate cell proliferation in a context-specific manner (25). EFEMP1 mediates cell-to-cell and cell-to-matrix
communication, provides organization and stability to extracellular matrix structures, and appears to function as an antagonist of angiogenesis (26). Interestingly, EFEMP1 is a binding partner of tissue inhibitor of metalloproteinase-3 (TIMP-3) (27). Fibulin-3 and another fibulin family member, fibulin-5, can antagonize tumor angiogenesis in vivo (26), which suggests that concerted deregulation of a set of antiangiogenic factors, such as fibulin-3 and TIMP-3, might contribute to tumorigenesis. Inactivation of EFEMP1 due to DNA hypermethylation has been reported recently in lung cancer and hepatocellular carcinoma (28, 29). To the best of our knowledge, the current study is the first to identify EFEMP1 as a methylation marker for PCa. Although the precise function of EFEMP1 and whether it has a causal relationship to PCa have yet to be determined, further large-scale validation studies with human samples and functional investigation of EFEMP1 will improve our understanding of its role in tumorigenesis and its clinical relevance.

Comprehensive insight into the molecular basis of human diseases is becoming a reality with microarray-based clinical research, and new molecular targets at the whole-genome level are rapidly emerging (5, 30, 31). The number of putative cancer-specific methylation markers is vast, and genome-wide techniques will likely bring to light new markers with improved sensitivity and specificity. To date, genome-wide
screening for regions that are differentially methylated in normal and tumor cells has typically resulted in the isolation of many differentially methylated DNA fragments (10, 32). However, to identify bona fide candidate methylation markers, rigorous selection criteria, confirmation of methylation status and expression level, verification of re-expression upon exposure to demethylating agents, and validation in human tissues are mandatory (10). In the current study, DNA methylation status and gene expression levels were considered simultaneously in a screen for candidate genes. Methylation status and expression levels of potential targets were verified by BGS and RT-PCR, and reversal of methylation-induced gene silencing by treatment with AZA was confirmed. The validity of the lead candidate methylation marker was determined using human prostate tissue samples from a relatively large patient population. The gene expression level of \textit{EFEMP1} was also independently verified using microarray data deposited in the on-line Gene Expression Omnibus (GEO) database. The current results, derived from a whole genome analysis and confirmed by several independent techniques, strongly support \textit{EFEMP1} as a novel candidate epigenetic marker for the detection of PCa, and possibly a candidate therapeutic target as well. Continued effort along these lines will enhance the accuracy of PCa detection and diagnosis, and lead to new therapies that target specific molecular defects, thereby significantly lowering the
morbidity associated with PCa.

In conclusion, characterization of the DNA methylation profiles of PCa cells at the genome-wide level resulted in the identification of *EFEMP1*, a gene that is aberrantly methylated in PCa. This methylation marker could assist in more accurate detection and monitoring of PCa.
Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0001735 and 2009-0068819).
References


Cancer Res 2010;17: 2131-7.


27. Klenotic PA, Munier FL, Marmorstein LY, Anand-Apte B. Tissue inhibitor of


Figure legends

**Fig. 1.** Study Design and Validation Strategies. AZA, 5-Aza 2’-deoxycytidine; *EFEMP1*, *EGF*-containing fibulin-like extracellular matrix protein 1.

**Fig. 2.** BGS of *EFEMP1* in cell lines and human tissues. (A) Schematic representation of *EFEMP1* (accession number NM_018894.1) on chromosome locus 2p16, the location of the CpG island and the region amplified for BGS. (B) Representative results of BGS of *EFEMP1* promoter CpG island methylation status in prostate cell lines (B) and human prostate samples (C). Unmethylated and methylated bisulfate-converted sequences are indicated above the chromatograms. BGS, Bisulfite genomic sequencing; *EFEMP1*, *EGF*-containing fibulin-like extracellular matrix protein 1.

**Fig. 3.** Expression of *EFEMP1* in BPH and PCa. Two group box plot comparing expression levels of *EFEMP1* in a Korean cohort (A) and Western population cohort (B). Differences (*P*-value) between BPH and PCa patients were obtained by two sample t-test. The y-axis indicates relative expression level of *EFEMP1* (log2-transformed scale). Expression values were mean-centered across all samples to generate relative expression levels. BPH, benign prostate hyperplasia; *EFEMP1*, *EGF*-containing fibulin-like extracellular matrix protein 1; PCa, prostate cancer.
Figure 1

Cell lines

14,495 genes

Select gene differentially methylated and expressed using array-based analysis

13 genes

Identify CpG island of the candidate genes

5 genes

Analyze expression changes in AZA treated cells

3 genes

Identify methylation status (Preliminary study)

EFEMP1

Human prostate tissues

GEO dataset (GSE6919)

Internal validation
- Expression, methylation

External validation

Expression
<table>
<thead>
<tr>
<th></th>
<th>BPH (n = 97)</th>
<th>PCa (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, (year)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>67 (46–83)</td>
<td>69 (52–82)</td>
</tr>
<tr>
<td><strong>PSA (ng/mL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.6 (0.1–7.7)</td>
<td>30 (1.9–350.2)</td>
</tr>
<tr>
<td><strong>Gleason score, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>-</td>
<td>5 (4.7)</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>41 (38.7)</td>
</tr>
<tr>
<td>8-10</td>
<td>-</td>
<td>60 (56.6)</td>
</tr>
<tr>
<td><strong>T staging, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>-</td>
<td>54 (50.9)</td>
</tr>
<tr>
<td>T3</td>
<td>-</td>
<td>30 (28.3)</td>
</tr>
<tr>
<td>T4</td>
<td>-</td>
<td>22 (20.8)</td>
</tr>
</tbody>
</table>

Abbreviations: BPH, benign prostate hyperplasia; PCa, prostate cancer; PSA, prostate specific antigen.
<table>
<thead>
<tr>
<th></th>
<th>BPH (n = 97)</th>
<th>PCa (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylation rate*</td>
<td>13.4 (13/97)</td>
<td>95.3 (101/106)</td>
</tr>
<tr>
<td>PSA level (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 ng/mL</td>
<td>12.3 (8/65)</td>
<td>100 (3/3)</td>
</tr>
<tr>
<td>3–10 ng/mL</td>
<td>15.6 (5/32)</td>
<td>93.8 (15/16)</td>
</tr>
<tr>
<td>10–20 ng/mL</td>
<td>-</td>
<td>93.8 (15/16)</td>
</tr>
<tr>
<td>≥ 20 ng/mL</td>
<td>-</td>
<td>95.8 (68/71)</td>
</tr>
<tr>
<td>T staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>-</td>
<td>96.3 (52/54)</td>
</tr>
<tr>
<td>T3</td>
<td>-</td>
<td>93.3 (28/30)</td>
</tr>
<tr>
<td>T4</td>
<td>-</td>
<td>95.5 (21/22)</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>-</td>
<td>100 (5/5)</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>95.1 (39/41)</td>
</tr>
<tr>
<td>8–10</td>
<td>-</td>
<td>95.0 (57/60)</td>
</tr>
</tbody>
</table>

*Methylation rate is expressed as percentage (no. of methylated samples/total number of samples).
Abbreviations: BPH, benign prostate hyperplasia; EFEMP1, EGF-containing fibulin-like extracellular matrix protein 1; PCa, prostate cancer; PSA, prostate specific antigen.
Clinical Cancer Research

EFEMP1 as a Novel DNA Methylation Marker for Prostate Cancer: Array-based DNA Methylation and Expression Profiling

Yong-June Kim, Hyung Yoon Yoon, Seon-Kyu Kim, et al.

Clin Cancer Res Published OnlineFirst May 13, 2011.

Updated version
Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-10-2817

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.