DNA Methylation Markers for Prostate Cancer with a Stem Cell Twist
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Abstract

This perspective on Ibragimova et al. (beginning on page 1084 in this issue of the journal) highlights the potential role of DNA methylation–based markers in the early detection of prostate cancer (PCa), with a focus on the global reactivation of expression of genes epigenetically silenced in PCa cell lines. Novel findings of these investigators identified four genes methylated specifically in PCa, including the stem cell marker TACSTD2, which seems to discriminate PCa (methylated TACSTD2) from prostatic intraepithelial neoplasia (unmethylated). These genes add significantly to the list of epigenetic markers showing promise for clinical early detection of PCa in the near future. Cancer Prev Res; 3(9):1053–5. ©2010 AACR.

Prostate cancer (PCa) is one of the most prevalent malignant neoplasms in men living in Western countries. In the United States, it accounted for 25% of all noncutaneous cancer cases in men and 9% of cancer-related mortality, with an estimated 192,280 new cases and 27,360 deaths in 2009 (1). Locally advanced or metastatic disease is associated with considerable morbidity and mortality. Therefore, efficient early detection could greatly alleviate the burden of PCa.

Current efforts to identify individuals with early-stage PCa, who are most amenable to curative treatments, rely on screening methods based mainly on putative PCa-specific biomarkers. Measuring prostate-specific antigen (PSA) in the serum of at-risk men has become the mainstay of PCa screening programs over the past 20 years. Although the introduction of PSA in routine clinical practice has increased the rate of detecting organ-confined PCa and is associated with a decrease in mortality (2, 3), the role of serum PSA has been challenged mainly because of its relatively low specificity, especially at the lower end of its spectrum of levels, where most curable PCa occurs. It is important to note that there is growing concern in the medical community that PSA-detected PCa is overtreated, subjecting a large number of men to unnecessary therapy-related morbidity (3). Therefore, research devoted to finding new PCa biomarkers that could replace or be an adjunct to serum PSA as a screening tool has flourished in recent years.

The last decade has witnessed the progressive acknowledgment of the role of epigenetics in the development and progression of human cancers in general and PCa in particular. Indeed, there is growing evidence that epigenetic alterations are early events in tumorigenesis and may even precede better-known genetic alterations (4).Instances of aberrant DNA methylation at CpG-rich sites in the regulatory regions of several genes are currently the strongest candidates among cancer-related epigenetic alterations for becoming epigenetic-based cancer biomarkers because this methylation is relatively easy to screen for, even in samples with low tumor DNA content such as body fluids (blood, urine; refs. 4, 5). Moreover, the emergence of high-throughput techniques allows for the simultaneous analysis of multiple gene promoters in a large number of clinical samples.

Initial efforts to find specific DNA methylation markers for PCa followed a “candidate gene” approach. Genes with a lost or reduced expression in PCa tissue or cell lines became candidates for assessing epigenetic silencing through aberrant promoter methylation. This approach discovered some of the most robust methylation-based biomarkers for PCa detection, of which GSTP1 is one of the best-known examples (6, 7). GSTP1 is hypermethylated in 80% to 90% of primary prostate tumors and at much lower frequencies in liver, kidney, and breast neoplasms (20–30%; ref. 8). Extended studies of PCa methylation profiling identified other genes that, when added to GSTP1 in panels, augmented the sensitivity of testing for GSTP1 methylation alone, even in urine samples (9). The candidate gene strategy is very laborious, however, requiring the full screening of a large number of genes, of which only a minority will show promise as specific cancer biomarkers.
Genome-wide analysis of the cancer methylome recently has become possible and provides interesting results that are tested in tissues and other clinical samples to identify potential cancer biomarkers. One method that provides valuable information about epigenetically regulated genes is based on the dynamics of epigenetic alteration, which can be pharmacologically modulated and reversed. As reported in this issue of the journal, Ibragimova and colleagues (10) exposed PCa cell lines to both 5-aza-2-deoxycytidine (a DNA-demethylating agent) and trichostatin A (an inhibitor of histone deacetylases), thus allowing for the reexpression of epigenetically silenced genes detected in an expression-array platform. They initially identified a set of almost 3,000 upregulated genes, from which they selected a panel of 45 genes following comparative expression analysis of normal and cancerous prostate cells. After removing 15 genes (including GSTP1) already reported in the literature to be methylated in PCa, they conducted novel work that identified and validated the previously unknown methylation of seven other genes (KRT7, TACSTD2, SLC15A3, GADD45d, IFI30, ANX2, and AQP3) in PCa by direct bisulfite sequencing. These genes were extensively methylated in PCa cell lines, representing less than 0.3% of all the genes that surfaced from the array analysis. This result clearly shows the power of global methylation analysis to uncover and pinpoint potential biomarkers among a very large number of putative candidates. Of importance, the validity of the methods and data analysis of Ibragimova et al. is supported by the presence of GSTP1 in their data set as one of the most frequently methylated genes, as would be predicted from previously published data.

In an obvious next step, these investigators validated the seven newly identified genes in tissue samples, which came from 35 PCa and 19 lesions of prostatic intraepithelial neoplasia (PIN), a putative PCa precursor. Including PIN in this setting provided some interesting insight into the timing of the gain of methylation of the studied genes during prostate carcinogenesis. The validation analyses found that three (KRT7, TACSTD2, and SLC15A3) of the seven genes identified in vitro were frequently methylated (17-66%) in PCa tissues. These results highlight the differences between cancer cell lines and primary cancer tissues. Indeed, findings in cancer cell lines may be misleading and require an extensive analysis, as performed by Ibragimova et al., of tumor tissues to ascertain the validity of the biomarkers in a “real-life” setting.

Another interesting finding of this study is the differential methylation of TACSTD2 between PCa and PIN lesions. Indeed, most of the genes previously known to be methylated in PCa were also methylated in PIN, although less frequently (11, 12). If confirmed in a larger series, the differential TACSTD2 finding will be an important step toward discriminating cells originating in PCa from cells originating in PIN lesions (e.g., in urine), thus increasing the specificity of methylation-based assays for early detection of PCa. Also, with expression in a subset of prostate basal cells with stem cell features (13) and silencing by promoter methylation in PCa but not in PIN, TACSTD2 might represent an “epigenetic switch” that renders preinvasive neoplastic cells prone to becoming invasive, thus advancing our biological understanding of prostate carcinogenesis.

A limitation of the Ibragimova et al. study is its lack of testing in body fluids (plasma and/or urine), where detection of CpG island hypermethylation could have a clear clinical value (14) and would add to the novelty of the findings. It also would be important to extend the number of specimens in the tissue validation set to ascertain whether all PIN lesions indeed are unmethylated at the TACSTD2 promoter. Last, it would be interesting to determine whether these novel epigenetic markers are complementary, and whether their combined use might increase the rate (sensitivity) of PCa detection.

In conclusion, the data provided by this novel study add significantly to our knowledge of the PCa methylome, revealing some promising PCa biomarkers that deserve further exploratory studies in body fluids to assess their power to detect early PCa in the clinic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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