EARLY DETECTION OF PROSTATE CANCER FROM BODY FLUIDS USING DNA PROMOTER METHYLATION

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Abstract: Prostate cancer is the second most common cancer and the second leading cause of cancer death in men. Epigenetic alterations including hypermethylation of gene promoters are believed to be the early events in neoplastic progression and thus these methylated genes can serve as biomarkers for the early detection of cancer from clinical specimens, such as: tissue biopsies, serum and urine.Early detection of PCa from body fluids, can be done by molecular detection of some epigenetic biomarkers, such as: GSTP1, RASSF1A and RARβ2 gene promoters

INTRODUCTION

Prostate cancer (PCa) is the most common malignancy diagnosed in men and is the second leading cause of cancer-related death in the United States (1) The metastatic potential of tumor cells and its possible dissemination to secondary sites are critical factors related to its mortality rates.In spite of the high incidence and mortality rates, the molecular mechanisms involved in oncogenesis and progression of prostate cancer are still poorly understood , especially related to the progression to the metastatic form. Prostate cancer etiology remains obscure and its tumors vary from indolent forms, with low evolution rates, to extremely aggressive ones with rapid growing rates (2). The methods that have been used to characterize the genetic alterations found in this neoplastic disease include familiar studies designed to map someheredi tary loci, chromosomal studies to identify aberrations that could localize oncogenes or tumor suppressor genes and intense studies of gene expression. All these studies reflect many signaling pathways that influence the carcinogenetic process.The slower progression of prostate cancer compared with other types of cancers makes it particularly amenable to early diagnosis with significantly better outcomes.

The discovery of serum marker PSA twenty years ago significantly altered the detection and follow up of prostate cancer (3). PSA is an androgen-regulated serine protease produced by both prostate epithelial cells and prostate cancer, and is the most commonly used serum marker for cancer. The detection of prostate-specific antigen (PSA) in the blood is currently the most widely used test to screen for early PCa. With the widespread use of PSA screening, the realized incidence of prostate cancer has increased, due to earlier detection, and reduced mortality.

There are limitations to the utility of PSA testing. In patients with normal digital rectal exams and PSA levels in the normal range (<4ng/mL), up to 5% of these patients have a high-grade cancer (4). The specificity of intermediate PSA levels (4 to 10 ng/mL) as an indication of prostate cancer is reported to be only 20% .(5). Identification of early disease-stage biomarkers has potential value for prevention, treatment, and early detection of several cancers, including prostate cancer.

Biomarkers are cellular, biochemical and molecular (proteomic, genomic and epigenetic) alterations by which normal, abnormal or simply a biologic process can be recognize or monitored. They are used to measure and evaluate normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. In the field of cancer research and detection, a biomarker refers to a substance or process that is indicative of the presence of cancer in the body. It might be either a molecule secreted by a malignancy itself or a specific response of the body to the presence of cancer. Biomarkers are measurable in biological media such as: tissues, cells or fluids (6).

For the past decade, the discovery of epigenetic biomarkers, specifically hypermethylation of cytosine-phosphodiester-guanine (in consecutive DNA bases) (CpG) islands, has been in development for the detection of prostate cancer.

DNA mutations have widely been described as the underlying basis of cancer. Epigenetic modifications are heritable DNA changes affecting gene expression and including micro RNA (miRNA)-associated silencing, histone modification, and DNA methylation.(7).

The most frequent and best understood epigenetic mechanismis DNA methylation. DNA methylation is the enzyme-mediated chemical addition of a methyl group (CH_3) to a nucleotide base pair. Methylation above normal levels is referred to as hypermethylation. Abberant methylation of CpG sequences is one of the most common alterations occurring in human cancer.

It was observed that human prostate cancer cells have hypermethylated CpG islands in the glutathione S-transferase pi (GSTP1) gene (8) Glutathione S-transferase Pi is a member of a large family of enzymes that conjugate reactive chemical species to glutathione for detoxification. It is reported that CpG islands in GSTP1 are hypermethylated in >80% of hepatocellular carcinomas, ~30% of breast cancers, and >90% of prostate cancers (9).

Quantitative DNA Methylation Analysis by PCR

The power of DNA methylation analysis compared to other technologies that use RNA or protein is that it is a DNA-based technology (10). DNA is much more stable than RNA and proteins. Thus, it is more feasible to isolate and analyze DNA from archieved or stored biological samples. Over the past five years, techniques for assesing DNA methylation have become more sensitive and specific and less labor intensive. The latest technology, termed MethyLight, uses a fluorescence-based real-time polymerase chain reaction (PCR) methodology that incorporetes methylation-specific PCR to enable quantitative analysis of hypermethylated alleles (11). The reported level of detection of the MethyLight technique is 1 hypermethylated alle in 10.000 unmethylated alleles or 1/10.000 (12).

The strength of DNA methylation as a potential biomarkler for the early detection of prostatic intraepithelial neoplasia is based on the following factors:

1. Methylation can be detected in circulating tumor DNA in serum or urine, and is strongly correlated with the methylation status of DNA isolated from the corresponding primary tumors (13);
2. Methylation of target genes is seldom observed in normal DNA tissue samples (14-15);
3. Laboratory evidence suggests that methylation is an early event in the carcinogenic process (16);
4. Several genes implicated in carcinogenesis undergo methylation leading to gene inactivation. These genes include: APC , GSTP1, RARβ2, RASSF1A (17-18);
5. Preliminary evidence shows that DNA methylation status as a biomarker would provide both specificity and sensitivity (19);
6. New methodologies and techniques offer promise of high-throughput and quantitative analysis of DNA methylation (20);

Hypermethylation of the GSTP1 CpG Islands are Found in Prostate Cancer

The first analysis of GSTP1 CpG island hypermethylation was made by Lee and colleagues in 1994. (21) In this study, it was determined that the promoter sequences in the GSTP1 gene were hypermethylated in every prostate carcinoma tissue sample examined. In contrast, hypermethylation was completely absent in prostate tissue from healthy individuals and those with benign hyperplasia (22).Hypermethylation of GSTP1 appears to increase with the progression of prostate cancer. Whereas hypermethylation is completely absent in healthy prostate epithelium, >90% of localized prostate cancers have GSTP1 CpG island hypermethylatio(23) These fundamental findings have laid the foundation for a number of recent reports that have investigated the detection of hypermethylation in CpG islands in clinically relevant samples.

Detection of CpG Island Hypermethylation in Urine and Blood Specimens

The close relationship between hypermethylated CpG islands of GSTP1 and prostate cancer makes detection of these changes in clinically relevant specimens a fascinating prospect. To detect epigenetic changes in a noninvasive manner, ideal samples would be either urine or peripheral venous blood. In urine, prostate cells are released through the connection of the prostatic ducts into the urethra. In peripheral blood samples, it has been established that PCR methods can detect prostate-specific DNA in specimens from men with prostate cancer (24) Prostate DNA passes into the blood through several different pathways including direct release into the circulation, as a result of prostate cell turnover, and through circulating phagocytes that have ingested prostate cancer cells. The rich vascular supply to the prostate provides a ready avenue for prostate-derived DNA to reach the bloodstream. (25)

Detection of CpG Island Hypermethylation in Urine

Due to the anatomic proximity of the prostate to the urethra, normal and disease prostate cells are regularly shed into the urine. Since >90% of prostate cancers have epigenetic changes, it was initially hypothesized that noninvasive detection of hypermethylation in urine might help improve current methods to diagnose prostate cancer.

In 2001, Cairns and colleagues reported that GSTP1 hypermethylation was detectable in 27% of patients with prostate cancer.Subsequent studies have also investigated the efficacy of GSTP1 hypermethylation to identify patients with prostate cancer versus BPH, identifying a high specificity of the test (26).

Recent studies have expanded on the use of detecting GSTP1 hypermethylation in urine samples to detecting hypermethylation of an array of other gene promoters. Hoque and colleagues studied the relationship of aberrantly methylated promoters in 9 different methylation-regulated genes (p16INK4a, p14 [ARF], MGMT, GSTP1, RARβ2, CDH1 [E-cadherin], TIMP3, RASSF1A, and APC) using quantitative PCR to analyze the urine sediment from 52 patients with prostate cancer and 91 controls. These 9 genes were chosen because their expression is frequently silenced by hypermethylation and in many cases have the absence or near absence of methylation in normal prostate tissue (27). Promoter hypermethylation was identified in at least 1 of the 9 genes in all 52 prostate cancer patients. Promoter

hypermethylation of the combined subset of p16, ARF, MGMT, and GSTP1 enabled detection of 87% of prostate cancers with 100% specificity Roupret and colleagues similarly assayed urine samples obtained post-prostate massage for aberrant methylation of 10 genes (GSTP1, RASSF1A, ECDH1, APC, DAPK, MGMT, p14, p16, RARβ2, and TIMP3) by methylation-specific PCR (28).
In another study, post-prostatic massage urine samples were assayed for the presence of aberrant GSTP1 hypermethylation in men referred for diagnostic biopsies to determine its utility in potentially screening prostate cancers. In this population, the detection of hypermethylation in urine specimens had 75% sensitivity and 98% specificity for prostate cancer in contrast to biopsies which had 91% sensitivity and 88% specificity. GSTP1 methylation was detected more frequently in men with stage 3 versus stage 2 disease (100% versus 20%), suggesting a potential use for staging disease (29). This study indicates that detection of increased GSTP1 methylation in urine samples may improve the specificity of PSA and/or biopsy, helping distinguish prostate cancer from benign prostatic hyperplasia.

Serum/Plasma Detection of CpG Island Hypermethylation
Several studies have provided evidence that prostate cancer can be detected in serum and plasma. The first study to detect GSTP1 promoter hypermethylation in plasma samples found that 72% (23/32) of patients with prostate cancer had GSTP1 promoter hypermethylation compared with none of patients with benign prostatic hyperplasia (BPH). Other studies have similarly identified the utility of assaying for hypermethylated GSTP1 (and other genes such as the androgen receptor) to differentiate patients with prostate cancer from those without (30). These studies provided proof-of-concept that increased hypermethylated GSTP1 is associated with prostate cancers and is detectable in plasma and serum using quantitative PCR methods. In tissue based analysis, the detection of hypermethylation in multiple genes (including GSTP1) appears to increase diagnostic sensitivity and specificity to 96% and 100%. (31).
To determine the usefulness of multiple markers in serum samples, Ellinger and colleagues (32), recently investigated the utility of detecting CpG island hypermethylation in patients with localized prostate cancer. To assay cell-free serum from patients with prostate cancer, they used methylation-specific quantitative PCR to measure hypermethylation of CpG islands in GSTP1, TIG1, PTGS2, and Reprimo. These specific genes, which are not well characterized, were investigated because they have been implicated in the pathogenesis of prostate cancer, and hypermethylation of these genes have been identified in prostate cancer tissue.
All 4 genes displayed higher frequencies of CpG hypermethylation in patients with prostate cancer (42.3%, 9.5%, 2.4%, and 1.2%, respectively) compared with BPH patients (7.7%, 0%, 0%, 0%, respectively) and healthy controls (all 0%). Hypermethylation of GSTP1 in cell-free serum identified 4 patients with incidental prostate cancer recurrence (33).

CONCLUSIONS

Approximately 10 years ago, it was identified that CpG islands in prostate-specific genes are hypermethylated in >90% of prostate cancer cases. Since then, a series of studies have exploited this finding in the hopes of creating a way to detect prostate cancer in clinically relevant samples, such as blood and urine, in order to improve the diagnostic accuracy of prostate cancer detection and progression. In the past year, research studies have demonstrated the ability to detect hypermethylated CpG islands in blood and urine on diagnostic samples has offered promise that these tests may eventually be an improvement over our current diagnostic tools. These advances would mean fewer unnecessary prostate biopsies, and tests that more accurately differentiate prostate cancer from benign prostate disease.

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