NONINVASIVE BIOMARKERS IN THE DIAGNOSIS OF PROSTATE CANCER

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Abstract: Prostate cancer is responsible for 3% of all deaths in the Western world in men over 55 years old. Molecular biomarkers represent an important diagnostic tool in prostate cancer, because at a specific stage, they reflect the physiologic state of a cell and might be vital for the identification of early prostate cancer and subjects at risk of developing this disease. Many biomarkers have been described in human serum, urine, seminal fluid and histological specimens that exhibit varying capacities to detect prostate cancer, to monitor the disease progression, help in predicting the disease recurrence and therapeutic treatment efficacy.

INTRODUCTION

Biomarkers are cellular, biochemical, and molecular (proteomic, genetic and epigenetic) alterations by which a normal, abnormal, or simply a biologic process can be recognized or monitored. They are used to measure and evaluate normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers are measurable in biological media, such as tissues, cells, or fluids. Identification of early disease-stage biomarkers has potential value for the prevention, treatment, and early detection of several cancers, including prostate cancer.

Prostate cancer (PCa) represents the second leading cause of cancer death in the USA and Western Europe. In this ''post-genomic'' era, the recent application of increasingly sophisticated molecular approaches to the study of PCa have resulted in a rapid increase in the identification of somatic genome alterations and the role of heritable factors in this disease. All these findings are leading to a new understanding of the pathogenesis of PCa and to the generation of new targets for diagnosis, prognosis, and prediction of therapeutic response (22). PCa cells, like other cancer cells, usually contain a large number of somatic genome alterations (15) that contribute to the cancer phenotype. Some of the somatic alterations are genetic (changes in DNA sequence), such as: point mutations, deletions, amplifications, and translocations. Other changes are epigenetic, including modifications in deoxycytidine methylation patterns and chromatin structure.

Since its discovery, more than 20 years ago, prostate-specific antigen (PSA) has been the most valuable tool in the detection, staging, and monitoring of PCa.

 The European Randomized Study of Screening for Prostate Cancer (ERSPC) evaluated the screening procedures and based on the knowledge of tumor characteristics and prevalence predictions of biopsy -detectable cancer per PSA range, ERSPC study group implemented changes in the screening protocol. Since 1997, the ERSPC study group accepted PSA values of more than or equal to 3ng/ml as the standard biopsy indication. Digital rectal examination (DRE) and transrectal ultrasound (TRUS) have been discarded as initial screening tests (4).

Many PCa screening trials have showed that approximately 50% of patients with serum PSA values greater than 10ng/ml had advanced disease. Most patients with serum PSA values less than 10ng/ml were diagnosed with early stage disease (14). These findings have led to the conclusion that men with serum PSA values between 3ng/ml and 10ng /ml most likely have clinically localized disease and would benefit from curative treatment.

Widely accepted as a prostate tumor biomarker, PSA has turned out to be organ specific but not PCa specific. PSA levels have been reported to be increased in men with benign prostatic hyperplasia (BPH) and prostatitis. This overlap in serum PSA values between men with nonmalignant prostatic disease and PCa is the limitation of PSA as a tumor biomarker. Upon the detection of serum PSA values greater than 3ng/ml, the conventional diagnostic approach is traditional sextant TRUS-guided prostate biopsies. The low specificity of serum PSA results in a negative biopsy rate of 70-80%. In case of persistent rising serum PSA levels, repeated biopsies are indicated, which have at least 10% of probability of demonstrating cancer (23).

Moreover, if the combined use of serum PSA, DRE, and TRUS biopsy do indicate clinically confined PCa, 40% of these men are found to have already extracapsular disease upon radical prostatectomy(8).

1. Prostate cancer antigen 3 (PCA3):

PCA3, also known as DD3, is a non-coding RNA produced almost exclusively in the prostate. The gene encoding PCA3 is located on chromosome 9q21-22.The PCA3 RNA is over-expressed in more than 95% of malignant tumors, when compared to benign or normal prostate tissue. Also, an assay which detects PCA3 in urine has been developed a few years ago (2).

As cancerous cells with high levels of PCA3 RNA are shed into the urine from the prostate, the levels of PCA3 RNA can be measured not only in prostate tissue specimens, but also in the urinary sediments after prostatic massage. To perform this test it is necessary to collect 20-30 ml of voided urine after a DRE. APTIMA® (Gen-Probe) PCA3 test the only assay available, detects quantitatively the expression of PCA3 RNA in urine and prostatic fluids using transcription -mediated amplification (12).

In order to asses the probability of PCa detection on prostate biopsy, the quantitative PCA3 score was developed. The PCA3 score is defined as PCA3-RNA/PSA-mRNA ratio, meaning that PCA3 expression is standardized with the PSA expression used as a housekeeping gene. The PCA3 score correlates with the likelihood of positive biopsy: the higher the PCA3 score, the greater the probability of a positive biopsy (5).

2. Glutathione-S transferase P1 (GSTP1):

The most described epigenetic alteration in PCa is the hypermethylation of the GSTP1 gene. Promoter hypermethylation of GSTP1 is located on chromosome 11q13 and represents the most frequent DNA alteration in prostatic carcinoma, being specifically detectable in more than 90% of prostatic carcinomas, including early stages (13).It has been reported in approximately 6% of proliferative inflammatory atrophy (PIA) lesions and in 70% of prostatic intraepithelial neoplasia (PIN) lesions (6).

Goessl et al demonstrated that GSTP1 gene is hypermethylated in 75% of serum, 50% of ejaculates, and 37% of urine samples from patients harboring PCa, but it does not appear hypermethylated in BPH or normal samples Hypermethylation of GSTP1 gene has been detected in more than 90% of prostate tumors, whereas no hypermethylation has been observed in in BPH and normal prostate tissue.In another study, hypermethylation of the GSTP1 gene has been detected in 50% of ejaculates from PCa patients, but not in men with BPH.Due to the fact that ejaculates are not always easily obtained from PCa patients, hypermethylation of GSTP1 was determined in urinary sediments obtained from PCa patients after prostate massage. Cancer could be detected in 77% of these sediments .A technique known as methylation-specific PCR (MSP) is used to detect the promoter hypermethylation. This technique requires a tumor-to-normal ratio of only 0.1-0.001%. This means that by using this technique, hypermethylated alleles from tumor DNA can be detected in the presence of 10^4-10^5, excess amounts of normal alleles (10).

3.α-methylacyl Co-enzyme A racemase (AMACR):

AMACR is an enzyme involved in the oxidative metabolism and synthesis of branched-fatty acids found in dairy products and red meat. Besides being strongly produced in PCa tissue(18), the enzyme is encode by a gene located on chromosome 5p13, in a region that contains polymorphisms associated with PCa (11). Many studies have demonstrated that immunohistochemical staining of AMACR can aid to differentiate benign prostatic tissue from cancerous prostatic tissue, with a 97% diagnostic sensitivity and a 92% specificity (19).

Circulating levels of AMACR mRNA in serum and urine can be measured by reverse-transcription PCR analysis (16-17). The concentration of AMACR protein is low in serum and but, it can be detected in urine by Western blotting. (7).

4. Circulating tumor-associated DNA:

Dissemination of tumor cells represents a prerequisite for the metastasis process; thus early detection of these cells in the circulation can be useful for assessing the prognosis of PCa patients. Tumor cells have been detected from serum , urine, urine sediments and seminal liquid with reverse-transcription PCR , which has proved to be analytically sensitive and to be very useful for increasing the diagnostic accuracy of staging and prediction of disease recurrence with specific biomarkers for the prostate (20).

5. Transforming growth factor β 1: (TGF-β1):

Transforming growth factor (TGF-β1) is a widely acting growth factor involved in a variety of molecular processes, such as cellular differentiation, immune response, angiogenesis, and proliferation. Many studies have demonstrated the role of TGF-β1 in PCa progression. Increased levels of TGF-β1 in tissue from PCa patients have been correlated with tumor grade and stage, and with lymph node metastasis (21).

The plasma levels of TGF-β1 can be measured by enzyme-linked immunosorbent assay (ELISA). Studies have shown that preoperative plasma levels of TGF-β 1 are increased in PCa patients, and can be correlated with extracapsular extension, seminal vesicle invasion,metastasis and biochemical recurrence (1).

6. Prostate-specific membrane antigen (PSMA):

Prostate-specific membrane antigen (PSMA) is a trans-membrane glycoprotein that is expressed on the surface of prostate epithelial cells. The expression of PSMA appears to be restricted to the prostate. No overlap in PSMA expression has been found between benign prostatic hyperplasia (BPH) and PCa, indicating that PSMA is a diagnostic promising biomarker (24). It has been shown that high PSMA expression in PCa cases has been correlated with tumor grade, pathological stage, aneuploidy and biochemical recurrence. Its clinical utility as a diagnostic or prognostic biomarker for PCa has been hindered by the lack of a sensitive immunoassay for this protein. Recently, a combination of Protein Chip (Ciphergen Biosystems) arrays and SELDI-TOF MS has led to the introduction of a protein biochip immunoassay for the quantification of serum PSMA. It was demonstrated that the average serum PSMA levels for PCa patients was higher compared with those of men with BPH and healthy controls.

RT-PCR studies have shown that PSMA in combination with its splice variant PSMA' could be used as a prognostic biomarker for PCa. In the normal prostate, PSMA' expression is higher than PSMA expression. In PCa tissues, the PSMA expression is more dominant. Therefore, the ratio of PSMA to PSMA' is highly indicative of disease progression.

CONCLUSION

Since prostate cancer is a heterogenous disease, it is clear that new biomarkers are needed for the early detection of this disease.

New tests, based on GSTP1 hypermethylation, PCA3, AMACR, PSMA genes, which are overexpressed in PCa, will enable the noninvasive detection of prostate cancer in body fluids, such as: serum, plasma, urine or ejaculates.

REFERENCES

Burger MJ, Tebay MA, Keith PA, et al (2002). Expression analysis of δ-catenin and prostate-specific membrane antigen: their potential as diagnostic markers for prostate cancer.
 Int. J. Cancer, 100: 228-237

Deras IL, Aubin SM, Blasé A, et al. (2008) PCA3: a molecular urine assay for predicting prostate biopsy outcome. J Urol; 179: 1587-1592

Djavan B, Zlotta A, Remzi M et al.(2007) Optimal predictors of prostate cancer on repeat biopsy: a prospective study of 1051 men. J Urol: 163: 1144-1148

Etzioni R, Urban N, Ramsey S, et al. (2003).The case for early detection. Nature Rev Cancer; 3:243-252

Goessl C, Muller M, Straub B ,et al. (2002).DNA alterations in body fluids as molecular tumor markers for urological malignancies. Eur Urol, 41; 668-676

Goessl C, Krause H, Muller M (2000).Fluorescent methylation-specific polymerase chain reaction for DNA-based detection of prostate cancer in bodily fluids. Cancer Res, 60:5941-5945

Hara N, Kasahara T, Kawasaki T, Bilim V, Obara K, Takahashi T, et al. (2002). Reverse-transcription polymerase chain reaction detection of prostate specific antigen, prostate-specific membrane antigen, and prostate stem cell antigen in one milliliter of peripheral blood: value for staging of prostate cancer. Clin Cancer Res, 8: 1794-9

Hessels D, Klein Gunnewiek JM, Van OI Karthaus HF et al. (2003). DD3 (PCA3)-based molecular urine analysis for the diagnosis of prostate cancer.Eur Urol, 44: 8-15

Hoque MO, Topaloglu O, Begum S, et al.(2005).Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. J Clin Oncol, 23: 6569-6575

Jiang Z, Wu CL, Woda BA, Iczkowski KA, Chu PG, Tretiakova MS, et al. (2004).α-methyl-acyl CoA racemase: a multi-institutional study of a new prostate cancer marker. Histopathology 45:218-25

Luo J, Zha S, Gage WR, et al. (2002). α-methylacyl -CoA racemase: a new molecular marker for prostate cancer. Cancer Res., 62:2220-2226

Nakanishi H, Groskopf J, Fritsche HA, et al (2008). PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance.
J Urol, 179: 1804-1809

Nakayama M, Bennett CJ, Hicks JL, et al (2003). Hypermethylation of the human glutathione S-transferase P1 gene (GSTP1) CpG island is present in a subset of proliferative inflammatory atrophy lesions but not in normal or hyperplastic epithelium of the prostate: a detailed study using laser-capture microdissection . Am J Pathol, 163: 923- 933

Schroder FH (2001). Diagnosis, characterization and potential clinical relevance of prostate cancer detected at low PSA ranges. Eur Urol, 39(Suppl 4): 49-53

Parekh D J, Ankerst DP, Troyer D et al (2007).Biomarkers for prostate cancer detection. J Urol, 178: 2252-2259

Rogers CG, Yan G, Zha S, Gonzalgo ML, Isaacs WB, et al (2004).Prostate cancer detection on urinalysis for α-methylacyl coenzyme A racemase protein. J Urol; 172: 1501-3

Ross JS, Sheehan CE, Fisher HA et al (2003).Correlation of primary tumor prostate-specific membrane antigen expression with disease recurrence in prostate cancer.Clin.Cancer. Res 9: 6357-6362

Roupret M, Hupertan V, Catto JW , et al (2008).Promoter hypermethylation in circulating blood cells identifies prostate cancer progression.Int J Cancer;122:952-956

Rubin MA, Zhou M, Dhanasekaran SM ,et al.(2002).α-methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA, 287:1662-1670

Shariat SF, Menesses -Diaz A, Kim IY, Muramoto M, Wheeler TM, Slawin KM.(2004). Tissue expression of transforming growth factor -β1 and its receptors: correlation with pathologic features and biochemical progression in patients undergoing radical prostatectomy. Urology, 63: 1191-7

Shariat SF, Walz J, Roehrborn CG, Montorsi F, Jeldres C, Saad F, et al. (2008).Early postoperative plasma transforming growth factor -β1 is a strong predictor of biochemical progression after radical prostatectomy. J Urol, 179; 1593-7

Shand RL, Gelmann EP (2006).Molecular biology of prostate -cancer pathogenesis. Curr Opin Urol, 16: 123-131

Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL et al. (2004) Prevalence of prostate cancer among men with a prostate-specific antigen level < 4.0 ng per milliliter. N Engl J Med , 93: 1054-61

Xiao Z, Adam BL, Cazares LH, et al. (2001).Quantitation of serum prostate-specific membrane antigen by a novel protein biochip immunoassay discriminates benign from malignant prostate disease. Cancer Res, 61:6029-6033

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