

Applicability of noninvasive biomarkers in prostate cancer diagnosis

Raluca DUMACHE, MD, PhD^a; Maria PUIU, MD, PhD^d; Dragos MICLEA, MD, PhD student^b; Bogdan BUMBACILA, PhD student^c; Florin MICLEA, MD, PhD^b

^aDepartment of Biochemistry, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania

^bDepartment of Urology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania

^cDepartment of Clinical Pharmacy, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania

^dDepartment of Medical Genetics, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania

ABSTRACT

Prostate cancer represents the second leading cause of male cancer-related deaths worldwide. Better indicators of the presence of prostate cancer are needed to avoid unnecessary treatment, predict disease course and develop more effective therapy. Many molecular biomarkers have been described in human serum, urine, seminal fluid and histological specimens that exhibit varying capacities to detect prostate cancer and predict disease course.

Key words: prostate cancer (PCa), benign prostatic hyperplasia (BPH), prostate-specific antigen (PSA), biomarkers; prostatic intraepithelial neoplasia (PIN); proliferative inflammatory atrophy (PIA); digital rectal examination (DRE)

INTRODUCTION

Biomarkers are cellular, biochemical and molecular (proteomic, genomic and epigenetic) alterations by which 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. 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

Address for correspondence:

Raluca Dumache, MD, PhD student "Victor Babes" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, Zip Code 300041, Timisoara, Romania
email address: rdumache@gmail.com

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 (1).

Prostate cancer represents the most common cancer in men and the second leading cause of cancer death in Europe affecting on average one in six men during their life time (2). The biomarker currently used for PCa diagnosis is PSA.

Many of the prostate malignancies detected in screened populations are clinically indolent, they do not lead to a cancer specific death (there is only a 3.4% chance of death due to PCa)(3). Clinically, this situation indicates over-detection and as a consequence, over-treatment in a substantial number of patients. Some prostate tumors exhibiting an aggressive clinical behavior demand immediate treatment. PSA, being widely accepted and standard screening tool for PCa detection, is unable to determine the progression of PCa or its clinical prognosis, nor does it aid in the early identification of patients with life-threatening disease requiring rapid radical treatment (4). Moreover, PSA is not PCa specific with only a 25-40% positive predictive value of PSA between 4.0 and 10.0ng/ml, meaning no tumor is found on biopsy in nearly 75% of men within this range of PSA level (5). Increased levels of serum PSA have been reported in men with BPH and prostatitis.

Repetition of the first negative biopsy, which is strongly recommended in case of PSA elevation, is also not reliable.

Because of these, the role of PSA as a screening tool has been questioned, not only due to its diagnostic limitations and an elevated number of unnecessary biopsies performed, but also because of the potential risk of unnecessary therapies without a relevant impact of PCa survival (6).

Therefore, noninvasive biomarkers that can accurately identify men who have early stage, organ-confined PCa and who would gain prolonged survival and improved quality of life from early radical intervention are needed. □

PCA3 (PROSTATE CANCER ANTIGEN 3)

PCA3 is a non-coding RNA and the most specific prostate malignancy biomarker described so far. The gene encoding PCA3 is lo-

cated on chromosome 9q21-22. The PCA3 RNA is highly over-expressed in approximately 95% of tumours when compared to benign or normal prostate tissue (7). As cancerous cells with high levels of PCA3 RNA are shed from the prostate into the urine, the levels of PCA3 RNA can be measured in the urinary sediments after prostatic massage. To perform the test it is necessary to collect about 20-30 ml of voided urine after a digital rectal examination. The assay available, APTIMA® (Gen-Probe) PCA3 test, detects quantitatively the expression of PCA3 RNA in urine and prostatic fluids using transcription-mediated amplification (8).

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 biopsies: the higher the PCA3 score, the greater the probability of a positive biopsy. PCA3 score is not influenced by prostate volume, the number of priori biopsies, and is unaffected by patients' age, and the test is not influenced by the principal causes of PSA elevations: BPH and prostatitis (9).

PCA3 being a highly specific PCa test, is very useful for detecting the presence of PCa in men with alternative causes of PSA elevation, including inflammation of the gland and increase of its size. □

TMPRSS2: ERG

Genetic aberrations occur in almost all human malignancies. In the last years, many studies have demonstrated that in patients with PCa, gene rearrangements involving androgen regulated gene- TMPRSS2 and ETS transcription factor genes (ERG and ETV) are present. The genes are located on chromosome 21. TMPRSS2 is located at 21q22.3 and ERG at 21q22.2 (10). TMPRSS2: ERG -fusion represents the most common variant which occurs in 40-70% of patients with PCa. The gene rearrangement occurs exclusively in patients with over-expression of ERG (95%), the gene which is currently considered a key oncogene in PCa (11).

The product of TMPRSS2: ERG fusion is found in about 20% of PIN cases, but it does not appear in benign prostate tissue specimens or proliferative inflammatory atrophy (PIA). TMPRSS2: ERG rearrangement can be detected in urine after DRE (12-13).

α-METHYLACYL-COA RACEMASE (AMACR)

α-methylacyl CoA racemase (AMACR) is an enzyme involved in the oxidative metabolism and synthesis of branched fatty acids found in dairy products and red meat. The enzyme is encoded by a gene located on chromosome 5p13.3 which contains polymorphisms associated with PCa. Many studies made with microarray demonstrated that AMACR appears over expressed in PCa.

Recently it has been shown that decreased AMACR production could have a prognostic value in predicting biochemical recurrence and death due to PCa. Circulating concentrations of AMACR mRNA in serum and urine have been detected by reverse-transcription PCR analysis (RT-PCR) (14).

Studies of immunohistochemical staining of AMACR distinguished benign from cancerous prostate tissue with a 97% diagnostic sensitivity and a 92% specificity (15). □

INSULIN-LIKE GROWTH FACTORS AND BINDING PROTEINS

The IGF family consists of 2 ligands (IGF-1 and IGF-2), 2 receptors (IGFR-1, IGFR-2) and 6 binding proteins (IGFBPs 1-6). In serum, the concentrations of insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are associated with PCa. A prospective study demonstrated that increased IGF-1 and decreased IGFBP-3 concentrations have been correlated with an increased risk for developing PCa (16). The main IGFBP produced by the

prostate, which is IGFBP-2, has been reported to be increased in PCa, and serum concentrations of IGFBP-3 has been shown to be inversely correlated with the presence of bone metastases (17). □

GLUTATHIONE-S TRANSFERASE P1 (GSTP1)

The glutathione -S transferase P1 (GSTP1) gene is emerging as one of the most important tumor suppressor genes in PCa. GSTP1 can detoxify environmental electophilic carcinogens and oxidants and may play a genome caretaker role by preventing oxidant and electophilic DNA damage (18). Hypermethylation of GSTP1 gene is the most common (>90%) reported epigenetic alteration in PCa. It occurs early in cancer progression and is a promising biomarker for detecting organ-confined disease (19). In a study, Goessl et al demonstrated that gene GSTP1 appears hypermethylated in about 72% of serum, 50% of ejaculates, and 37% of urine samples from patients with PCa, but it was not found in any samples from patients with BPH (20). □

CONCLUSION

Recent developments in the field of molecular biology have provided new tools that have led to the discovery of many promising biomarkers for PCa. These biomarkers may be instrumental in the development of new screening tests that have a high specificity in the diagnostic gray zone and as such are able to reduce the number of unnecessary biopsies. □

REFERENCES

1. Parekh DJ, Ankerst DP, Troyer D et al – Biomarkers for prostate cancer detection. *J Urol* 2007; 178:2252-2259
2. McDavid K, Lee J, Fulton JP et al – Prostate cancer incidence and mortality rates and trends in the United States and Canada. *Public Health Rep* 2004; 119:174-186
3. Jamal A, Siegel R, Ward E et al – Cancer statistics, 2008. *CA Cancer J Clin* 2008; 58:71-96
4. Chodak G – Prostate cancer: epidemiology, screening, and biomarkers. *Rev Urol* 2006; 8 Suppl 2: S2-S3
5. Roobol MJ, Zappa M, Maattanen L et al – The value of different screening tests in predicting prostate biopsy outcome in screening for prostate cancer data from a multicenter study (ERSPC). *Prostate* 2007; 67:439-446
6. Jemal A, Tiwari RC, Murray et al – Cancer statistics, 2004. *CA Cancer J Clin* 2004; 54:8-29
7. Hessels D, Klein Gunnewiek JM, van OI Karthaus HF et al – DD3 (PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 2003; 44:8-15
8. Deras IL, Aubin SM, Blasé A et al – PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J Urol* 2008; 179:1587-1592
9. Van Glis MP, Hessels D, Jannink SA et al – The time-resolved fluorescence based PCA3 test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. *Clin Cancer Res* 2007; 13:939-943

10. **Mitelman F, Johansson B, Mertens F** – The impact of translocations and gene fusions on cancer causation. *Nat Rev Cancer* 2007; 7:233-245
11. **Perner S, Mosquera JM, Demichelis F et al** – TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. *Am J Surg Pathol* 2007; 31:882-888
12. **Demichelis F, Fall K, Perner S et al** – TMPRSS2: ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene* 2007; 26:4596-4599
13. **Hessels D, Smit FP, Verhaegh GW et al** – Detection of TMPRSS2: ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res* 2007; 13:5103-5108
14. **Zehenter BK, Secrist H, Zhang X et al** – Detection of α -methylacyl-coenzyme-A racemase transcripts in blood and urine samples of prostate cancer patients. *Mol Diagn Ther* 2006; 10:397-403
15. **Rubin MA, Bismar TA, Andren O et al** – Decreased α -methylacyl CoA racemase expression in localized prostate cancer is associated with an increased rate of biochemical recurrence and cancer-specific death. *Cancer Epidemiol Biomarkers Prev* 2005; 14:1424-1432
16. **Shariat SF, Lamb DJ, Kattan MW et al** – Association of preoperative plasma levels of insulin-like growth factor 1 and insulin-like growth factor binding proteins-2 and 3 with prostate cancer invasion, progression, and metastasis. *J Clin Oncol* 2002; 20:833-841
17. **Harman SM, Metter EJ, Blackman MR et al** – Serum levels of insulin-like growth factor1 (IGF-I) IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. *J Clin Endocrinol Metab* 2000; 85:4258-4265
18. **Duffy MJ, Napieralski R, Martens JW et al** – Methylated genes as new cancer biomarkers. *Eur J Cancer* 2009; 45:335-346
19. **Bryzgunova OE, Morozkin ES, Yarmoschuk SV et al** – Methylation-specific sequencing of GSTP1 gene promoter in circulating/extracellular DNA from blood and urine of healthy donors and prostate cancer patients. *Ann N Y Acad Sci* 2008; 1137:222-225
20. **Goessl C, Muller M, Straub B et al** – DNA alterations in body fluids as molecular tumor markers for urological malignancies. *Eur Urol* 2002; 41:668-676

