Pathol. Oncol. Res. (2015) 21:969–976
DOI 10.1007/s12253-015-9915-x

RESEARCH

Molecular and Clinicopathological Aspects of Prostate Cancer in Bulgarian Probands

Anita Tsvetkova · Albena Todorova · Tihomir Todorov · Georgi Georgiev · Ivanka Drandarska · Vanyo Mitev

Received: 4 September 2014 / Accepted: 17 February 2015 / Published online: 10 March 2015
© Arányi Lajos Foundation 2015

Abstract To correlate the molecular data to the clinicopathological parameters in Bulgarian prostate cancer patients. *PCA3* overexpression, *TMPRSS2-ERG* gene fusion, *GSTP1* promoter hypermethylation, somatic mutations in the *AR* gene and the IVS1-27G > A polymorphism in the *KLF6* gene were studied. A total of 148 patients were analyzed: 16 aggressive PCa, 83 non-aggressive PCa, 25 BPH and 24 chronic inflammatory diseases. Real-time RT-PCR, DNA sequencing, and bisulfite conversion of DNA, were applied. All cases with aggressive PCa before treatment were tested positive for *PCA3* overexpression, expression of a *T2-ERG* gene fusion product and *GSTP1* promoter hypermethylation. No somatic mutations were detected in the *AR* gene and all patients showed normal *KLF6*-IVS1-27G > A genotype. The *TMPRSS2-ERG* positive status correlates with moderate to poorly differentiated prostate tumors and it is considered as unfavorable disease predictor. Positive *GSTP1* promoter hypermethylation seems to be highly specific and the earliest epigenetic change in the prostate gland, which indicates the beginning of the pathological process. The appearance of positive molecular markers in blood was considered as a predictor of PCa dissemination. *GSTP1* promoter hypermethylation was found as the earliest and a long-lasting epigenetic marker in blood samples of PCa patients, which makes it suitable as a marker for treatment follow-up. The molecular profile of prostate cancer needs to be strictly monitored during the course of disease treatment, which is of a great help in determining the patient's individual therapy response.

Keywords CRPC · Molecular subtyping · Prostate cancer · *PCA3* · *TMPRSS2:ERG* gene fusions · Prognosis

Abbreviations

ADT	Androgen deprivation therapy
BPH	Benign prostatic hyperplasia
CNS	Central nervous system
CRPC	Castration-resistant prostate cancer
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
GS	Gleason score
GWAS	Genome wide association studies
mRNA	Messenger Ribonucleic Acid
PCa	Prostate cancer
PCR	Polymerase Chain Reaction
PIA	Proliferative inflammatory atrophy
PIN	Prostatic intraepithelial neoplasia
PSA	Prostate-Specific Antigen
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
SNP	Single nucleotide polymorphism
TNM	Tumor Node Metastasis
TURP	Transurethral Resection of the Prostate

A. Tsvetkova (✉) · A. Todorova · V. Mitev
Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria
e-mail: annita_25@abv.bg

A. Todorova
e-mail: todorova_albena@abv.bg

V. Mitev
e-mail: mitev@medfac.acad.bg

A. Tsvetkova · A. Todorova · T. Todorov
Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria

T. Todorov
e-mail: tisho.todorov@abv.bg

G. Georgiev
Department of Urology, Military Medical Academy, Sofia, Bulgaria
e-mail: georgiev61@yahoo.com

I. Drandarska
Department of Pathology, Military Medical Academy, Sofia, Bulgaria
e-mail: iva.bg@abv.bg

Introduction

PCa is a common neoplasm and increasingly prevalent cause of cancer death among men. The molecular mechanisms underlying its clinicopathological behaviour and progression remain poorly understood. PCa is a heterogeneous disease that can be indolent for decades in some patients, or can be life-threatening and lethal in a short time in others. The conventional approach nowadays, including serum PSA screening, Gleason grading, TNM staging is not sufficient to select PCa affected men whose tumours are characterized by androgen independence and require immediate alternative therapy, from those that would suffice with vigilant clinical observation [1]. There is an urgent need for molecular markers with high PCa specificity to be introduced in the clinical practice for molecular subtyping of patients with aggressive, androgen-refractory disease and for monitoring of the metastatic spread of tumor cells.

The most promising recently described genetic markers with benefits in PCa molecular subtyping are epigenetic fluctuations (e.g., *GSTP1* promoter hypermethylation), gene fusions (e.g., *TMPRSS2-ERG* gene fusion), and mRNA alterations (e.g., *PCA3* overexpression).

Here we report 148 Bulgarian patients affected by different prostatic pathologies, including PCa, aiming to correlate their molecular profile to the clinicopathological data. In the subgroup of differentiated aggressive PCa, somatic mutations in the *AR* gene and IVS1-27G > A polymorphism in the *KLF6* gene were additionally studied.

Materials and Methods

Patients and Samples The study group consisted of 148 sporadic patients affected by different prostate pathologies that can be divided in four groups: 16 aggressive PCa, 83 nonaggressive PCa, 25 BPH and 24 chronic inflammatory disease of the prostate gland. All patients were selected on the basis of their elevated serum PSA levels >3 ng/mL and abnormal DRE. The patients' age varied between 37 and 87 years (mean age of 66). Eighteen fresh-frozen prostate tissues (13 from prostatectomy and 5 from adenomectomy), 98 "tru-cut" biopsies, 31 TURP, 13 urine and 88 blood samples were collected in the urological outpatient clinic. For histological examination, 8–12 cores were taken from the suspected zones of the prostate gland, following the World guidelines instructions [2]. For molecular testing, 1 or 2 additional prostatic cores were taken predominantly from the peripheral prostatic zone. The latest cores were used freshly, without fixation for the purpose of RNA extraction.

The patients signed informed consent for genetic testing.

RNA and DNA Extraction Total RNA was extracted using the TRIzol reagent (Ambion, US). Total DNA was extracted using the AmpliSens DNA isolation kit (Ecoli s.r.o, Slovak Republic).

PCA3 expression levels were measured by reverse transcription (Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit, Lithuania) and real time PCR analysis with Universal Master Mix (Applied Biosystems, Foster City, CA), using previously published primers [3].

TMPRSS2-ERG gene fusions *T2-F1*/*ERG-R4*; *T2-F1*/*ERG-R6*; *T2-F*/*ERG-R* and *T2-F2*/*ERG-R2* were studied by RT-PCR analysis [4] and direct sequencing of the obtained PCR products (BigDye Terminator Cycle Sequencing kit v.3.1, Applied Biosystems, Foster city, CA).

The *GSTP1* promoter hypermethylation was assessed by bisulfite conversion of DNA (Zymo research, EZ DNA Methylation TM Kit, USA), followed by methylation sensitive PCR [5].

AR gene mutations and IVS1-27G > A polymorphisms were analyzed by direct sequencing of PCR products of the *AR* coding region or the *KLF6* region of interest, respectively.

Pathological examination was performed on hematoxylin-eosin-stained sections from formalin-fixed paraffin embedded prostate tissues. Sections were graded according to the GS system.

Results

The clinical, histological and molecular findings for the aggressive and indolent PCa subgroups are provided in Tables 1 and 2, respectively.

I. Aggressive PCa (16 patients): Histological and clinical results in this group of patients showed an aggressive subtype of PCa, as perivascular and perineural infiltration (Fig. 1a, b) and metastases in cranial and skeletal bones were found. This group was additionally split in two subgroups: 11 patients with CRPC tumors (Table 1, #1–11) and five patients with androgen-dependent PCa, poor prognosis and risk for biochemical recurrences (Table 1, #12–16). All but two patients from this group (for #9 and #10 no tissue was available for analysis) showed *PCA3* overexpression, by means of real-time qPCR. For patients #9 and #10, urine samples (post-ADT) were *PCA3* negative. Elevated *PCA3* was also found in four blood samples (Table 1 #1, 2, 3 and 16).

   Expression analysis of *TMPRSS2*:*ERG* gene fusion products revealed only one type of fusion transcript: *TMPRSS2* exon 1 fused to *ERG* exon 4 (Fig. 2a). A SNP c.51G > A, p.Ser17Ser was detected by sequencing in one patient (Fig. 2a). T*2ex1-ERGex4* positive fusion status was found in all tissue samples in the aggressive subgroup. Again, positive results were obtained in patients tested

Table 1 Overview of molecular profile and clinicopathological parameters in 16 Bulgarian prostatic adenocarcinoma patients, forming an aggressive PCa subgroup

Patient	Serum PSA ng/mL	Gleason grade in biopsies	Additional clinicopathological parameters	*TMPRSS2-EGR* fusion transcripts[a]	Tumor staging (TNM)	*PCA3* expression	*GSTP1* promoter hyper-methylation	*AR*	IVS1-27G > A	Flow of PCa specific markers in blood circulation
1	126	4+3=7	Perineural infiltration	1→4	pT2a NxMxG2	Overexpression	Positive	No somatic mutation	Normal genotype	*PCA3* overexpression, *T2ex.1-ERGex.4*, *GSTP1* promoter hyper-methylation
2	111	/2+3/=5 left lobe /4+3/=7 right lobe	Perineural infiltration, extraprostatic extension	1→4	Multiple bone metastases	Overexpression	Positive	No somatic mutation	Normal genotype	*PCA3* overexpression *GSTP1* promoter hyper-methylation
3	99	/4+3/=7 left lobe /2+3/=5 right lobe	Perineural infiltration, extraprostatic extension	1→4	Multiple bone metastases	Overexpression	Positive	No somatic mutation		*PCA3* overexpression *GSTP1* promoter hyper-methylation
4	115	/2+3/=5 left lobe /4+3/=7 right lobe	Perineural and perivascular infiltration capsular invasion	1→4	–	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation
5	130	/4+5/=9	Perineural and perivascular infiltration extraprostatic extension	1→4	pT3bNxMx	Overexpression	Positive	No somatic mutation		*GSTP1* promoter hyper-methylation
6	80	/4+4/=8	Perineural and perivascular infiltration extraprostatic extension	1→4	pT4 NxMx	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation
7	106	/4+5/=9	Angiolymphatic invasion extraprostatic extension	1→4	–	Overexpression	Positive	No somatic mutation		*T2ex.1-ERGex.4*, *GSTP1* promoter hyper-methylation
8	110	/4+4/=8	Perineural infiltration, extraprostatic extension; lymphonodal invasion PCa-related death	1→4	pT4	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation
9	110	/4+4/=8	Ductal invasive prostate adenocarcinoma, extraprostatic extension; lymphonodal invasion PCa-related death	N.D.[b]	Multiple bone metastases	Overexpression	Positive	No somatic mutation		*GSTP1* promoter hyper-methylation
10	57	/4+5/=9	Perineural infiltration	N.D.[b]	–	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation
11	67	/2+4/=6	Perineural infiltration	1→4	G1pT2bN0 (0/6) MxVn.	Overexpression	Positive	No somatic mutation		*GSTP1* promoter hyper-methylation
12	73	/2+2/=4	Perineural infiltration	1→4	–	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation
13	99	/4+5/=9	Perineural infiltration, extraprostatic extension	1→4	pT4	Overexpression	Positive	No somatic mutation		*GSTP1* promoter hyper-methylation

Table 1 (continued)

Patient	Serum PSA ng/mL	Gleason grade in biopsies	Additional clinicopathological parameters	*TMPRSS2-EGR* fusion transcripts[a]	Tumor staging (TNM)	*PCA3* expression	*GSTP1* promoter hyper-methylation	*AR*	IVS1-27G > A	Flow of PCa specific markers in blood circulation
14	117	/4+4/=8	Seminal vesical invasion; perineural infiltration, extraprostatic extension;	1→4	Multiple bone metastases	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation
15	96	/4+4/=8	Perineural infiltration, extraprostatic extension	1→4	–	Overexpression	Positive	No somatic mutation		*GSTP1* promoter hyper-methylation
16	80	/5+4/=9	Perineural and perivascular infiltration extraprostatic extension PCa-related death	1→4	pT4 multiple bone metastases	Overexpression	Positive	No somatic mutation	Normal genotype	*GSTP1* promoter hyper-methylation *PCA3*overexpression, *T2ex.1-ERGex.4*

N.D. Not detectable

[a] Only the presence of one type of TMPRSS2-ERG gene fusion was determined in our group: TMPRSS2-ex1 /ERG-ex4 (1→4)

[b] Urine sediments were investigated, after ADT and tissue samples were not available for molecular studies

before ADT; the patients #9 and #10, tested after treatment, showed a negative fusion profile in urine. The positive fusion profile in blood was obtained only in three patients (Table 1 #1, 7 and 16).

Hypermethylation of the *GSTP1* gene promoter was found in all patients and in all samples tested, including blood (Fig. 2b and Tables 1 and 2).

One blood sample (Table 1 #1) was found to be triple positive for *PCA3*, *GSTP1* and *T2ex1-ERGex4*.

In the whole group of 16 patients with aggressive PCa, no somatic mutations were detected in the *AR* gene.

The IVS1-27G > A polymorphic variant in the *KLF6* gene showed normal genotype in all patients analyzed.

II. Nonaggressive (indolent) PCa (83 patients): The *PCA3* overexpression was detected in 75 patients from the group (75/83=90.4 %). The rest of *PCA3* negative patients showed PSA levels in the range between 3.0 and 10.0 and GS 4–6 tumors.

All but one patient lacked expression of the *TMPRSS2ex1-ERGex4* fusion transcript. The fusion positive patient had a GS 2+2=4 tumor.

The *GSTP1* promoter hypermethylation was found to be positive in 100 % of nonaggressive PCa probands.

III. BPH (25 individuals): No positive *PCA3* expression was detected in BPH patients. All patients also were negative for the *TMPRSS2-ERG* gene fusions. In nine out of 25 BPH patients (36 %) *GSTP1* promoter hypermethylation was detected, most probably due to additional pathological alterations, such as PIN and cystic changes in the prostate gland.

IV. Chronic inflammatory disease of the prostate gland (24 cases): No *PCA3* expression and no *TMPRSS2-ERG* gene fusions were found in this group of patients. Eleven patients (11/24=45.8 %) showed *GSTP1* promoter hypermethylation. In these patients also the presence of PIA was demonstrated. The rest 13 *GSTP1* negative patients were PIA free.

Discussion

Linkage-studies (based on multiple case families) and GWAS suggest that predisposition to PCa may be mediated through multiple polymorphic alleles or mutations in genes with low penetrance. The experimental data confirms that the mechanism contributing to prostate cancer development will be rather regulatory than coding [6, 7].

Numerous molecular abnormalities have been described in association with PCa carcinogenesis. Most promising genetic alterations involved in disease progression, metastatic and androgen-refractory PCa are the occurrence of gene fusions, mRNA expression level alterations and epigenetic modifications.

Table 2 Molecular and clinicopathological data of patients with primary/indolent PCa

Clinicopathological parameters	Number of cases	*TMPRSS2ex1-ERGex4* fusion	*PCA3* overexpression	*GSTP1* promoter hypermethylation
PSA ng/mL	*N*=83			
0.0–3.0	0 (83) 0 %	–	–	–
3.0–10.0	43 (83)	N.D.	35 (83)[a]	43 (83)
10.1–20	13 (83)	N.D.	13 (83)	13 (83)
20.1–85.0	27 (83)	N.D.	27 (83)	27 (83)
Gleason score				
/4–6/	51	1 (51)[b]	43 (51)[a]	51 (51)
/7/	21	N.D.	21 (21)	21 (21)
/8–9/	11	N.D.	11 (11)	11 (11)
Pathologic stage	66		19(19)	19(19)
Local/ Organ confined	(pT2a)19 (pT2b)8	N.D.	8(8)	8(8)
Regional/ Extracapsular extension	14 (pT3a)5 (pT3b)3	N.D.	5(5) 3(3)	5(5) 3(3)
Distant/Seminal vesical extension	3	N.D.	3 (3)	3 (3)
Remarks	–	TMPRSS2ex1-ERGex4 fusion transcript was found in 1 (83) samples. 0.83 %	*PCA3* overexpressed levels were detected in 75 (83) PCa samples. 90,361 %	*GSTP1* promoter hypermethylation was detectable in 83 (83) PCa samples. 100 %

N.D Not detectable

[a] 8 samples showed PSA value within the range of 3.0–10.0 and Gl. score range /4–6/ and did not show *PCA3* expression

[b] TMPRSS2ex1-ERGex4 fusion transcript was the only one, detected in "tru-cut" biopsy from the patient with Gl.grade /2+2=4/

PSA is well- established as a biochemical marker of choice in PCa diagnosis and prognosis, but its accuracy and specificity are often limited [8–16].

The application of more predicative markers in the clinical practice, able to assist and improve PCa early detection and prognosis, is extremely needed.

Here we report molecular profile in 148 patients affected by different prostate pathologies in association to clinicopathological findings.

Aggressive PCa All probands in this group, tested before ADT were found to be positive for *PCA3* overexpression, *T2ex1-ERGex4* fusion and *GSTP1* promoter hypermethylation. The *T2-F1/ERG-R4* fusion was predominantly detected in patients with higher GS 7, 8 and 9, as previously reported [17–20] and only in one patient with a GS 6 tumor, typically associated with better prognosis. An association between the detected *T2-F1/ERG-R4* fusion profile and the higher tumor stage (assessed by TNM as T3 and T4) was noticed, whereas in T2 tumor stage such an association was not found. The detected SNP, 31 nucleotides apart from the T2-ERG fusion point, which falls in exon 2 of the *ERG* gene, has an uncertain contribution to the PCa predisposition or clinical behaviour, but considering the extremely polygenic and heterogeneous PCa nature it is worth mentioning.

With respect to the *GSTP1* promoter hypermethylation, most probably this is the earliest epigenetic alteration indicating the presence of (pre)cancerous cells in the prostate. The detection of *GSTP1* hypermethylation in blood samples of PCa patients makes it a suitable marker for treatment follow-up. Actually, patient #1 (having the triple positive blood sample before treatment) was subjected to ADT with Docetaxel and after 1 year follow-up the blood sample was found to be positive only for *GSTP1* promoter hypermethylation. This finding was interpreted as a good therapy response, which coincides with the result from the clinical exams. On the contrary, in patient #16 we obtained the same molecular result, but it was associated with poor clinic-histological prognosis and PCa-related death.

No somatic *AR* gene mutations were detected neither in the Bulgarian CRPC patients, nor in the rest of the aggressive subgroup, which supported the hypothesis that *AR* gene mutations were comparatively rare in PCa and CRPC [21], regardless of the expected inducible effect from the supplied ADT (Taxotere (Docetaxel), Xgeva (Denosumab), Zytiga (Abiraterone Acetate) + Deltasone (Prednisone), Zometa (Zoledronic acid), etc.).

The detected normal genotypes for the IVS1-27G > A polymorphism in the *KLF6* gene contradict to the previously published data for its higher frequency in aggressive prostate cancer [22, 23].

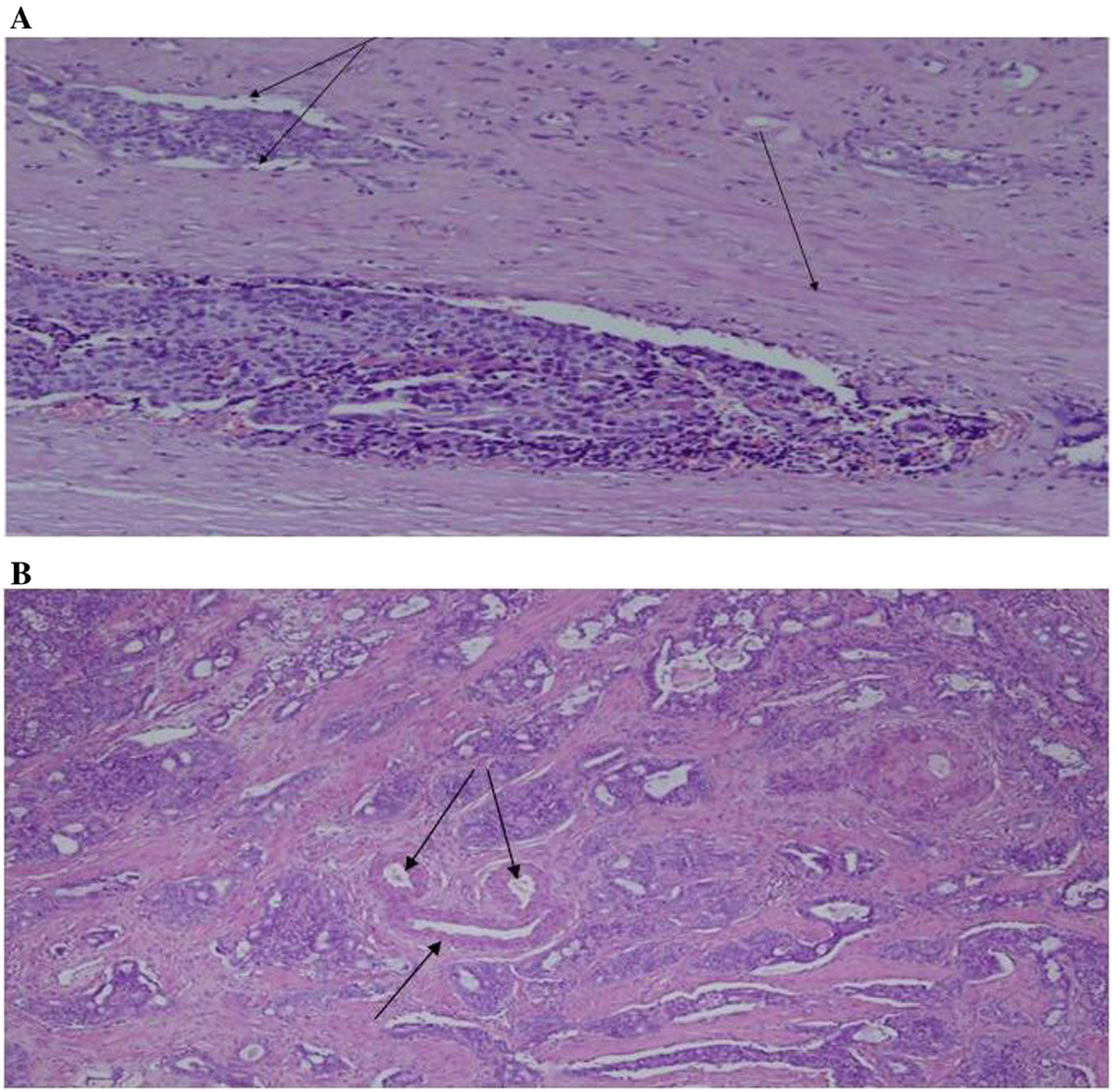

Fig. 1 **a** Presence of prostate tumor cells in lymph clefts, moderately-differentiated tumor. **b** Histological H&E stained section of PCa; GS 4+3=7, moderately-differentiated tumor. *Arrows* indicate the presence of perivascular tumor infiltrates; tumor nests and arterial vessels

Indolent PCa Group Most of the patients in this group were with primary low risk PCa, predominantly organ-confined and in quite initial disease phase (precarcinogenic). Five patients were diagnosed as primary PCa only by means of the obtained molecular profile, regardless of the negative biopsy results. Few months later, the diagnosis primary PCa in these five patients was histologically confirmed.

The detected *TMPRSS2ex1-ERGex4* fusion in a patient with a GS 4 tumor shows that Gleason grading could not be considered as the most important prognostic parameter. It is reasonable to be interpreted simultaneously with additional histological and molecular findings.

The positive *GSTP1* promoter hypermethylation seems to be highly specific marker, indicating early changes in the prostate gland.

In the groups of BPH and chronic inflammatory diseases of the prostate gland most of the molecular markers were tested negative, as published before [13, 24]. Only the *GSTP1* promoter hypermethylation was detected in 36 % of the BPH and in 45.8 % of the inflammatory disease group. The *GSTP1* positive patients showed PIN type III and cystic changes in the prostate gland or PIA. Similar findings have been reported for >70 % of cases with PIN or PIA, and these are now considered as precancerous lesions [25, 26].

In conclusion, the combined molecular examination of *PCA3* overexpression, *GSTP1* promoter hypermethylation and *TMPRSS2-ERG* gene fusions, was recommended for better diagnostics and management of the Bulgarian PCa patients. The positive *GSTP1* promoter hypermethylation seems to be highly specific and the earliest epigenetic change in the prostate gland, which indicates the beginning of the

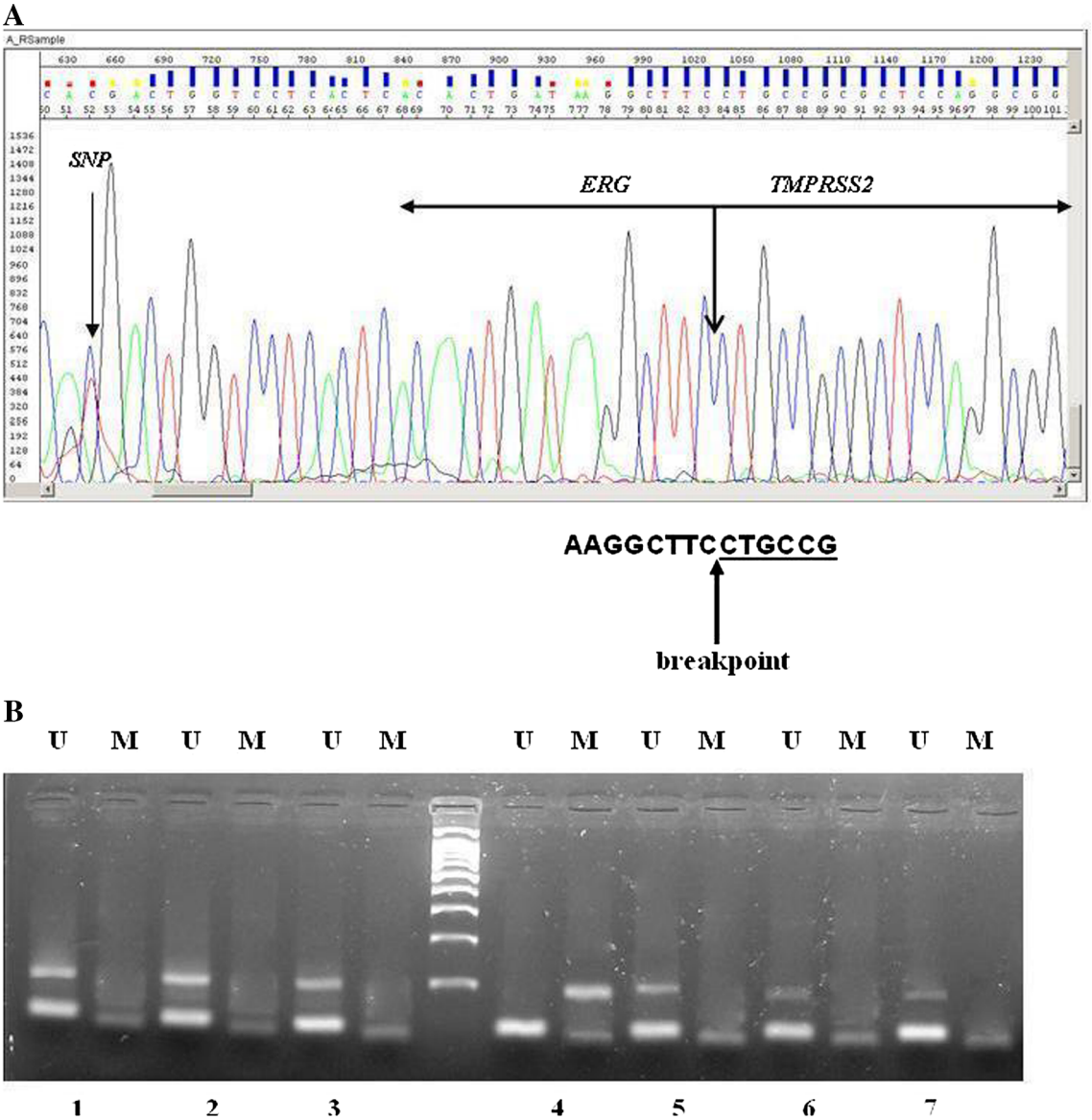

Fig. 2 **a** Sequencing profile of *TMPRSS2ex1-ERGex4* fusion detected in "tru-cut" biopsy of a GS seven prostate tumor. SNP, single nucleotide polymorphism c.51G > A, p.Ser17Ser. **b** Methylation profile of the *GSTP1* gene promoter in seven samples: #1–3, BPH (fresh tissue); #4, PCa patient ("tru-cut" biopsy); #5, venous blood from PCa patient; #6–7, BPH (urine). U, unmethylated, and M, methylated *GSTP1* alleles

pathological process. The appearance of positive molecular markers in blood (*PCA3* expression, *T2-F1/ERG-R4* gene fusion and *GSTP1* hypermethylation) was considered as a predictor of PCa dissemination, which coincides with the clinicohistological findings. Again, *GSTP1* promoter hypermethylation was determined as the earliest and as a long-lasting epigenetic marker detectable in blood, which makes it suitable for treatment follow-up.

The results from the molecular testing should be interpreted in the light of the clinic-histological data. Positive molecular status could help differentiating invasive and life-threatening subtypes of PCa. The obtained molecular profile in different samples of a single patient before and during the course of treatment is important for the strict monitoring, for choosing adequate treatment and for follow-up of the patient's response to therapy.

Acknowledgments This study was supported by the grants №4-D/2011 and 26-D/2012, and 17-D/2013 Medical University Sofia, Bulgaria.

Conflict of Interest The authors report no conflict of interest.

References

1. Salagierski M, Schalken JA (2010) PCA3 and TMPRSS2-ERG: promising biomarkers in prostate cancer diagnosis. Cancers 2(3): 1432–1440. doi:10.3390/cancers2031432

2. Heidenreich A, Aus G, Bolla M, Joniau S, Matveev VB, Schmid HP et al (2008) EAU guidelines on prostate cancer. Eur Urol 53(1):68–80
3. de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW et al (2002) *DD3PCA3*, a very sensitive and specific marker to detect prostate tumors. Cancer Res 62(9):2695–8
4. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW et al (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310(5748):644–8
5. Gonzalgo ML, Pavlovich CP, Lee SM, Nelson WG (2003) Prostate cancer detection by *GSTP1* methylation analysis of postbiopsy urine specimens. Clin Cancer Res 9(7):2673–7
6. Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N et al (2008) Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 40:310–315. doi:10.1038/ng.91
7. Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK et al (2008) Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40(3):316–21. doi:10.1038/ng.90
8. Mitelman F, Johansson B, Mertens F (2007) The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer 7(4):233–45
9. Morris DS, Tomlins SA, Montie JE, Chinnaiyan AM (2008) The discovery and application of gene fusions in prostate cancer. BJU Int 102(3):276–82
10. Barry M, Perner S, Demichelis F, Rubin A (2007) TMPRSS2-ERG fusion heterogeneity in multifocal prostate cancer: clinical and biologic implications. Urology 70(4):630–3
11. Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR et al (2007) TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 26(31):4596–9
12. Clark JP, Munson KW, Gu JW, Lamparska-Kupsik K, Chan KG, Yoshida JS et al (2008) Performance of a single assay for both type III and type VI TMPRSS2:ERG fusions in noninvasive prediction of prostate biopsy outcome. Clin Chem 54(12):2007–17
13. Wang J, Cai Y, Ren C, Ittmann M (2006) Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res 66(17):8347–51
14. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, Schalken JA (2007) Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res 13(17):5103–8
15. Kirby RS, Christmas TJ, Brawer MK (2001) Prostate Cancer. London
16. Schröder FH (2005) Detection of prostate cancer: the impact of the European Randomized Study of Screening for Prostate Cancer (ERSPC). Can J Urol 1:2–6, 92–3
17. Yoshimoto M, Joshua AM, Cunha IW, Coudry RA, Fonseca FP, Ludkovski O et al (2008) Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol 21(12):1451–60
18. Shen MM, Abate-Shen C (2010) Molecular genetics of prostate cancer: new prospects for old challenges genes. Genes Dev 24(18):1967–2000
19. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS et al (2007) Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 448(7153):595–9
20. Rajput AB, Miller MA, De Luca A, Boyd N, Leung S, Hurtado-Coll A et al (2007) Frequency of the TMPRSS2:ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol 60(11):1238–1243
21. Eisermann K, Wang D, Jing Y, Pascal LE, Wang Z (2013) Androgen receptor gene mutation, rearrangement, polymorphism. Transl Androl Urol 2(3):137–147
22. Narla G, Difeo A, Reeves HL, Schaid DJ, Hirshfeld J, Hod E et al (2005) A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. Cancer Res 65(4):1213–22
23. Narla G, DiFeo A, Fernandez Y, Dhanasekaran S, Huang F, Sangodkar J et al (2008) KLF6-SV1 overexpression accelerates human and mouse prostate cancer progression and metastasis. Clin Invest 118(8):2711–21
24. Bussemakers MJ, van Bokhoven A, Verhaegh GW et al (1999) DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res 59(23):5975–9
25. Brooks JD, Weinstein M, Lin X, Sun Y, Pin SS, Bova GS et al (1998) CG island methylation changes near the GSTP1 gene in prostatic intraepithelial neoplasia. Cancer Epidemiol Biomarkers Prev 7(6):531–6
26. Nakayama M, Bennett CJ, Hicks JL, Epstein JI, Platz EA, Nelson WG et al (2003) Hypermethylation of the human glutathione S-transferase-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 studyusing laser-capture microdissection. Am J Pathol 163(3):923–933