New targets for molecular diagnosis of prostate cancer: beyond the era of PSA

Maciej Salagierski[1,2], Grégoire Robert[3], Marek Sosnowski[2], Jack A. Schalken[1]

[1]Department of Urology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[2]I[st] Urology Department, Medical University of Łódź, Poland
[3]Department of Urology, Pellegrin University Hospital, Bordeaux, France

KEY WORDS

prostate cancer ◗ prognosis ◗ molecular markers ◗ PCA3 ◗ TMPRSS2:ERG ◗ GSTP1

ABSTRACT

The incidence of prostate cancer has risen in most countries in the last decade. Unfortunately, standard diagnostic prostate cancer markers are not only lacking in cancer specificity but are also unable to differentiate between clinically insignificant and aggressive disease or to predict cancer progression. This frequently leads to unnecessary prostate biopsies and over-treatment of patients with indolent tumors. Therefore, there is an increased need to discover a more reliable molecular marker or a set of markers allowing for the early identification of patients with aggressive or clinically relevant prostate cancer and to determine the prognosis of the disease.

The significant improvement in technology and understanding of genetics has led to the identification of serum and urine molecular DNA and RNA biomarkers including: GSTP1 and PCA3. Furthermore, a new prostate cancer specific genetic aberration has been identified, namely TMPRSS2:ERG gene fusion. In the most recently published study, the metabolomic profile of prostate cancer has been extensively explored.

New molecular markers are showing an increased specificity in prostate cancer detection. However, a panel of multiple biomarkers appears necessary to precisely characterize this very heterogeneous disease. Genetic alterations and metabolic changes accompanying prostate cancer progression might have relevant therapeutic implications.

This review aims at presenting some of the recently developed prostate cancer molecular biomarkers and considers their clinical performance.

INTRODUCTION

Due to increasing overall life expectancy, the incidence of prostate cancer (PCa) is rising steadily. Currently, PCa constitutes the most common cancer in men and second leading cause of cancer death in Europe affecting on average one in six men during their lifetime. Many of the prostate malignancies detected in screened populations are clinically indolent i.e. not leading to a cancer specific death (there is only a 3.4% chance of death due to PCa [1]). Clinically, this situation indicates over-detection and as a consequence, over-treatment in a substantial number of patients. Nevertheless, some prostate tumors exhibiting an aggressive clinical behavior demand immediate treatment. Unfortunately, serum prostate-specific antigen (PSA), being the widely accepted and standard screening tool for prostate cancer detection, is unable to determine the progression of PCa or its clinical prognosis nor does it aid in the early identification of patients with a life-threatening disease requiring rapid radical treatment [2, 3]. Moreover, PSA is not PCa specific with only a 25-40% positive predictive value of PSA between 4.0 and 10.0 ng/ml, meaning no tumor is found on biopsy in nearly 75% of men within this range of PSA level [4]. Repetition of the first negative biopsy, although strongly recommended in case of PSA elevation, is also not very reliable. As a result, the role of PSA as a screening tool has been recently 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 on prostate cancer specific survival. Therefore, there is an urgent need for the development of new and improved diagnostic and prognostic tumor markers that could both improve the specificity in PCa detection and differentiate patients according to the aggressiveness of their disease. Nevertheless, the heterogeneity of PCa and its marked variability in progression make implementation of new assays extremely challenging.

During the last decade, due to a significant advancement in genetics and cell biology, new emerging molecular markers have been widely investigated [5]. Their appearance has been the natural consequence of recently described genetic changes in PCa including gene fusions and messenger RNA (mRNA) alterations. A great amount of work has been done so far in an effort to develop new assays based on recent discoveries. Several significant DNA and RNA biomarkers as well as a gene fusion transcript product have been identified including: glutathione *S*-transferase pi gene (GSTP1), prostate cancer gene 3 (PCA3 a.k.a. DD3), and the fusion transcript TMPRSS2:ERG (Tab. 1). It should be noted that multiple and time consuming validation steps precede the clinical introduction and application of an assay (Fig. 1). The marker which has already gained a relevant interest and has been introduced into clinical practice is PCA3. However, the heterogeneity of PCa among individuals it affects makes it extremely difficult to implement one reliable, highly specific and sensitive, test. Perhaps a few markers (a marker panel) will be necessary to stratify patients according to the aggressiveness of the disease and to predict clinical progression of PCa.

Disease markers research is the focus of many laboratories across the world. As important groups of new markers are under investigation, we might soon be provided with a reliable molecular diagnostic tool for PCa detection and prognosis. Our objective is

Table 1. New prostate cancer biomarkers. The only clinically approved molecular marker remains PCA3. GSTP1+ methylation marker and fusion transcript marker TMPRSS2:ERG have already passed phase 5 trial.

Biomarker	Substrate	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
GSTP1+ methylation	Urine/Bx	yes	yes	ongoing	ongoing	yes	no
$PCA3^{DD3}$	Urine	yes	yes	yes	yes	End 2006 PROGENSATM Gen-Probe CE-marked	2007/2008 PROGENSATM Gen-Probe
TMPRSS2:ERG	Urine	yes	yes	yes	ongoing	yes	no

to review the PCA3 test and its application as well as some other potentially clinically relevant molecular biomarkers.

1. PCA3

1.1 Identification of PCA3

PCA3 is a non-coding RNA and the most specific prostate malignancy marker described so far (PCA3 is not expressed in any other human tissue) and has already been introduced into clinical practice [6, 7]. The presence of PCA3 RNA was first reported by Bussemakers et al., in 1999. The gene encoding PCA3 is located on chromosome 9q21-22. The PCA3 RNA is highly overexpressed in 95% of tumors when compared to benign or normal prostate tissue. Hessels et al. reported an average 66-fold up-regulation of PCA3 in PCa tissue when compared with normal prostate tissue. In addition, an average 11-fold up-regulation was revealed in prostate tissue specimens containing less than 10% of PCa cells [6]. Several years later an assay that detects PCA3 in urine has been developed.

1.2 PCA3 Assay

Optimally, for diagnostic purposes, a biomarker should be detectable with non-invasive methods, for instance by using blood or urine samples. As cancerous cells with high levels of PCA3 are shed from the prostate into the urine, the levels of PCA3 RNA can be measured not only in prostate tissue specimens but also in the urinary sediments after prostatic massage. The collection 20-30 mL of voided urine after a digital rectal examination (DRE) (three strikes per prostate lobe) is required to perform the test. The only assay available commercially, APTIMA PCA3 test (Gen-Probe Incorporated, San Diego, CA), quantitatively detects the expression of PCA3 RNA in urine and prostatic fluids using transcription-mediated amplification [7]. In order to assess 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. Deras et al., reported the 14% positive biopsy rate for men with PCA3 score <5 versus almost 70% positive biopsy rate for PCA3 score over 100 [8]. As the PCA3 score of 35 yielded the greatest diagnostic utility, demonstrating the optimal balance between specificity and sensitivity, it has been adopted as a cut-off score. The average sensitivity and specificity of the PCA3 urine test is relatively high – 66% and 76% respectively (versus 47% specificity for serum PSA level) [9].

Phase 1:
- Exploratory study using homemade first generation test

Phase 2:
- Establishment of a reproducible assay; both inter- and intra-assay variability should be assessed

Phase 3:
- Retrospective-or prospective analysis of biomarker using standardized/second generation test

Phase 4:
- Prospective multi-center evaluation of biomarker

Phase 5:
- Clinical implementation / commercialization of a biomarker

Phase 6:
- CE-marked test (EC)/FDA-approval of test (US)

Fig. 1. Six necessary steps (phases) required of a new assay before it can finally be introduced into clinical practice.

1.3 Clinical performance

Haese et al., in a group of 463 patients showed that men with a PCA3 score over 35 have a 39% chance of a positive repeat biopsy compared to 22% likelihood in men with lower than 35 PCA3 score [10]. This study also demonstrated that PCA3 score is significantly higher in men with a positive biopsy (median value of 33.7) than in men with a negative biopsy (median value of 19.5). Moreover, the negative predictive value of the test is very high, reaching – depending on a PCA3 cut-off value – 90%. The authors reported that in their group by using a threshold PCA3 score of 20, a 44% reduction of repeat biopsy could have been achieved while missing only 9% of clinically significant cancers (Gleason score ≥7). In addition, PCA3 allows detection of the precancerous lesion in PCa described as high grade prostatic intraepithelial neoplasia (HGPIN). Popa et al., demonstrated PCA3 expression by over 90% of HGPIN tissue [11]. Another study by Haese et al. demonstrated that PCA3 score in HGPIN patients was 16% higher than in tissue of men without this lesion [10].

It is valuable to emphasize that unlike PSA screening, PCA3 score is independent of prostate volume, the number of prior biopsies, and is unaffected by patients' age. What's more, no correlation was revealed between urine PCA3 score and serum PSA level. Furthermore, the test is not influenced by principal causes of noncancerous PSA elevations i.e. benign prostatic hyperplasia (BPH) and prostatitis. It also seems unrelated to the pharmacotherapy of the prostate including the application of type I and II 5-alpha reductase inhibitors.

Thus, clinical application of PCA3 assay appears much broader than only testing men with an elevated PSA and a negative biopsy. PCA3, being a highly specific PCa test, appears extremely useful for detecting the presence of PCa in men with frequently observed alternative causes of PSA elevation, including inflammation of the gland and increase of its size. The test might also be applicable for establishing treatment decisions in men undergoing active surveillance management by helping clinicians to select patients requiring rapid treatment inclusion e.g. demanding prompt surgery or radiotherapy. In addition, PCA3 could constitute a valuable tool in PCa screening enabling detection of clinically relevant tumors in men with normal or low (<4 ng/mL) PSA values. Furthermore, the PCA3 test might also be helpful in monitoring patients with the presence of HGPIN e.g. increase in PCA3 value would trigger biopsy decision.

1.4 Prognostic value

As the results of different studies are conflicting, the questionable aspect of the PCA3 score remains its ability to assess PCa aggressiveness or its clinical behavior. In one of the recently published papers, Gils et al. did not reveal significant association between PCA3 score and any of PCa's prognostic parameters including Gleason grade, tumor volume, and tumor stage [12]. On the contrary, Nakanishi et al. have described a relevant relationship between the preoperative PCA3 score and the radical prostatectomy specimens tumor volume. The average PCA3 score was statistically lower in low volume (less than 0.5 cc) tumors. This study also showed that increasing PCA3 score was associated with a higher Gleason score [13]. Marks et al. supported the findings of Nakanishi et al., demonstrating the presence of a higher PCA3 score in men with a Gleason score ≥7 [14]. The existing discrepancies between the studies might be related to different populations selected for the investigations.

To sum up, with the current status of knowledge, the PCA3 assay might be a useful clinical tool for biopsy making decisions enabling the avoidance of unnecessary biopsies and an extremely helpful test for screening patients with unspecific causes of PSA elevations. Despite very promising data, further investigations and prospective clinical trials are required especially to assess the prognostic performance of the test. The presence of positive prostate biopsy findings despite the negative PCA3 result (low PCA3 score does not exclude PCa) [10] does not support PCA3 test as the only and sufficient screening tool for PCa detection.

2. TMPRSS2: ERG

2.1 Molecular mechanism

Gene rearrangements are frequent in hematologic malignancies especially in leukemia and lymphomas. BCR-ABL fusion protein (BCR gene from chromosome 9 and ABL gene on chromosome 22) leads to the development of chronic myeloid leukemia (CML). The discovery of imatinib (Gleevec), the drug targeting the kinase domain of the BCR-ABL fusion, has revolutionized the management of CML by significantly improving patients' survival. However, genetic alterations (considered to be the initial event in oncogenesis) have rarely been reported in solid tumors, which account for more than 80% of cancer-related deaths [1]. Importantly, genetic aberrations were also suggested to occur in almost all human malignancies [15]. Therefore, the identification of new cancer specific fusion products might constitute a cornerstone for the implementation of targeted drug therapy not only limited to hematologic malignancies.

In recent studies, gene rearrangements involving androgen regulated gene- TMPRSS2 (trans-membrane protease, serine 2) and ETS transcription factor genes (ERG and ETV) have been identified in patients diagnosed with PCa. TMPRSS2:ERG- fusion constitutes the most common variant occurring in approximately 40-70% (~50%) of PCa patients. Considering the high prevalence of PCa, TMPRSS2:ERG- fusion is the most common genetic aberration described so far in human malignancies [16]. Both genes are located on chromosome 21; TMPRSS2 at 21q22.3 and ERG at 21q22.2. The predominant mechanism for gene fusion is the loss of 2.8 Mb of genomic DNA between TMPRSS2 and ERG [17]. The gene rearrangement occurs almost exclusively in patients with marked overexpression of ERG (95% of cases), the gene which is currently considered a key oncogene in PCa [18].

2.2 Clinical performance

It should be noted that the TMPRSS2:ERG fusion product can be found in 20% of prostatic intraepithelial neoplasia (PIN) cases but not in benign prostate tissue specimens or proliferative inflammatory atrophy (PIA) [19]. TMPRSS2:ERG rearrangement can be, similarly to PCA3 gene, detected in urine after DRE [20]. The detection of TMPRSS2:ERG fusion in urine as described by Hessels et al. has over 90% specificity and 94% positive predictive value for PCa detection [20]. Given the high specificity of the test, fusion status in ERG positive men may shortly serve in clinic as a viable biomarker for establishing the presence or absence of PCa [16].

2.3 Prognostic value

The data on the association between TMPRSS2:ERG fusions and patient's outcome remains conflicting. Most of the studies suggest that TMPRSS2:ERG fusion prostate cancer contributes to a more aggressive cancer phenotype which is associated with higher tumor stage and prostate cancer-specific death [21, 22]. Demichelis et al. in the cohort of 252 patients under active surveillance for T1a-b, Nx, M0 tumors with a median follow-up of 9.1 years demonstrated that fusion transcript was associated with metastases and lethal prostate cancer [18]. The fusion-positive tumors had also significantly higher Gleason score. However, in the mentioned study the TMPRSS2:ERG fusion product was identified only in 15% of PCa patients, which is much less than found by other authors. On the contrary, the most recently published study by Gopalan et al. [23] showed no correlation between TMPRSS2:ERG gene rearrangement and outcome in patients treated with radical prostatectomy. In this study the clinical association between the presence of fusion transcript and outcome was analyzed on a large group of 521 PCa patients. The presence of the fusion product was associated with lower PCa stage. Interestingly enough, the aggressive tumor behavior i.e. metastatic disease was associated with a copy number increase of TMPRSS2:ERG loci on chromosome 21.

The contradictory findings on the association of TMPRSS2:ERG with patient's outcome i.e. described so far both improved and worsened patients prognosis might have been related to different study groups as well as varied populations included in the investigations.

Targeting gene rearrangement appears an important innovative approach in solid tumors management. As a consequence, in the near future, we may possibly expect the appearance of fusion specific therapeutic solutions for men affected with PCa.

3. DNA methylation biomarkers

3.1 Molecular mechanism

Formation and progression of cancer is considered to be associated with the aberrant hypermethylation of cytosine guanine (CpG) dinucleotide of promoter regions in specific genes. It has been frequently suggested in the number of tumors [24-27] that hypermethylation can be helpful in early detection and prognosis of cancer [28, 29]. Hypermethylation appears to be closely associated with PCa development being regarded as a key player in the initiation of the disease. Several candidates' genes have been evaluated so far for their performance in PCa diagnosis including e.g. GSTP1, RASSF1A, APC, TIG1, DAPK, and MGMT.

3.2 Clinical performance

One of the most frequently reported hypermethylated genes in PCa patients appears to be glutathione *S*-transferase pi gene (GSTP1) [30]. Its hypermethylation occurs at a very high frequency, in up to 90% of PCa patients and in over 60% of men diagnosed with HGPIN [31]. Goessl et al. demonstrated that GSTP1 is methylated in 72% of sera, 50% of ejaculates, and 36% of urine samples from patients harboring prostate cancer [32]. In a more recent study Hoque et al.

analyzed an extended panel of nine genes for the aberrant methylation status of their promoters. The authors by comparing urinary sediments from 52 PCa patients with 91 healthy individuals demonstrated that all 52 PCa patients (100%) had at least one hypermethylated gene and 42 (80%) had at least three hypermethylated genes at the same time. They concluded that by examining a combination of only four genes (GSTP1, ARF, MGMT, and p16) they would be able to detect PCa with a very high sensitivity (87%) and specificity (100%) [33]. According to Rouprêt et al. the four methylated gene combination of GSTP1, Ras association domain family 1 isoform A (RASSF1a), retinoic acid receptor ß2 (RARbeta2), and adenomatosis polyposis coli (APC) yields 86% sensitivity and 89% specificity in discriminating malignant from healthy prostate tissue. The presence of hypermethylation was detected in prostatic fluid obtained after massage of the prostate gland [34].

3.3 Prognostic value

In the recently published study Rouprêt et al. examined promoter hypermethylation of the earlier mentioned four genes (GSTP1, RASSF1a, RARbeta2, and APC) in circulating blood cells. The study revealed that the hypermethylation increases during PCa progression. Therefore, by assessing methylation status it could be possible to timely identify patients at risk of cancer progression [35]. In the same study the authors confirmed the essential role of GSTP1 for predicting biochemical recurrence after radical prostatectomy. Rouprêt al. even postulated that GSTP1 should not only be included as a marker for PCa diagnosis but also should be used in the follow-up of PCa patients. In addition, GSTP1 testing while combined with prostate biopsy was reported to increase the sensitivity of PCa detection by 11-15% [36].

4. Metabolomic profile and risk of prostate cancer progression

In the most recently published study, Sreekumar et al. [37] explored the set of 1,126 small-molecule metabolites within 262 clinical samples of PCa progression, and was able to identify six metabolites being significantly increased during progression from benign prostate tissue to metastatic PCa. According to the authors, one of them- sarcosine, detectable in urine of men with localized disease, constitutes the key metabolite which is most significantly expressed in metastatic PCa. Moreover, the levels of sarcosine were shown to be elevated in some of the invasive PCa cell lines i.e. Du145, 22Rv1, and LNCaP. Nevertheless, the study findings demand validation. Moreover, strongly suggested by the researchers therapeutic implications of their discovery require additional meticulous investigations.

5. A gene panel for prostate cancer detection

Currently, we are still unable to discriminate between clinically important and indolent prostate malignancies e.g. a low PCA3 score does not exclude a clinically significant tumor. Therefore, some authors were trying to evaluate whether by combining novel molecular biomarkers the specificity and sensitivity of PCa detection would increase. Hessels et al., upon analyzing the urinary sediments of 108 men for the presence of both PCA3 and TMPRSS2:ERG products showed that by combining two assays the sensitivity of PCa detection markedly increases (from 63% for PCA3 alone to 73% for both tests) without compromising the specificity [20]. By that means the very satisfying diagnostic performance of PCa detection was achieved. Similar findings were reported by Laxman et al. who, by incorporating four urine biomarkers including PCA3 and TMPRSS2:ERG, achieved a 65.9% specificity and 76.0% sensitivity in PCa detection. This quantitative multiplex biomarker study also outperformed PCA3 alone in the detection of PCa [38]. Petrovics et al., by investigating a marker panel composed of ERG, PCA3, and the alpha-methylacyl-CoA racemase (AMACR) demonstrated a very promising gene panel for PCa diagnosis. The study showed that at least one of three genes included in a panel was over expressed in almost all examined PCa specimens (54 of 55) [39].

This kind of innovative approach for the development of a urine multiplex test may be an important and necessary step (considering the heterogeneity of cancer) in creating a maker panel with a very high accuracy in PCa detection and in predicting prognosis of the disease. Before this could be achieved, the combination of available commercially PCA3 test and TMPRSS2:ERG assay (in clinical trials) by significantly improving testing performance could be of a great value for men with a persistent elevation of PSA and a history of negative biopsies.

CONCLUSIONS

Taken together, PSA testing holistically revolutionized the management of PCa in the last century. However considering the well-known limitation of PSA in cancer detection, especially its low specificity, the development of new markers appears necessary. These new tools are required to properly select patients who can truly benefit from treatment e.g. radical prostatectomy.

By being able to predict the progression of PCa we might soon be able not only to decrease patients morbidity related to current PCa therapies, but also to significantly reduce the cost of PCa management. New emerging biomolecular markers are showing a lot of promise in this respect. It seems very possible that one of the reviewed markers will take over the present role of PSA by itself or by being incorporated in a larger marker panel which deems necessary to fully characterize this very heterogeneous cancer.

Acknowledgement:
Maciej Salagierski was awarded a European Urological Scholarship Program grant: S-02-2008 from the European Association of Urology for performing the project: New targets for molecular diagnostics of Prostate Cancer in the Department of Urology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands.

REFERENCES

1. Jemal A, Siegel R, Ward E et al: *Cancer statistics, 2008*. CA Cancer J Clin 2008; 58: 71-96.
2. Chodak G: *Prostate cancer: epidemiology, screening, and biomarkers*. Rev Urol 2006; 8 Suppl 2: S3-S8.
3. Beuzeboc P: *Prostate cancer epidemiology*. Soins 2007; 713: 32-33.
4. Roobol MJ, Zappa M, Maattanen L, Ciatto S: *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.
5. Parekh DJ, Ankerst DP, Troyer D et al: *Biomarkers for prostate cancer detection*. J Urol 2007; 178: 2252-2259.
6. Hessels D, Klein Gunnewiek JM, van Ol Karthaus HF et al: *DD3 (PCA3)-based molecular urine analysis for the diagnosis of prostate cancer*. Eur Urol 2003; 44: 8-15.
7. Schalken JA, Hessels D, Verhaegh G: *New targets for therapy in prostate cancer: differential display code 3 (DD3(PCA3)), a highly prostate cancer-specific gene*. Urology 2003; 62: 34-43.
8. Deras IL, Aubin SM, Blase A et al: *PCA3: a molecular urine assay for predicting prostate biopsy outcome*. J Urol 2008; 179: 1587-1592.
9. van Gils MP, Hessels D, van HO, 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. Haese A, de la Taille A, van PH Marberger M et al: *Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy*. Eur Urol 2008; 54: 1081-1088.
11. Popa I, Fradet Y, Beaudry G et al: *Identification of PCA3 (DD3) in prostatic carcinoma by in situ hybridization*. Mod Pathol 2007; 20: 1121-1127.
12. van Gils MP, Hessels D, Hulsbergen-van de Kaa CA et al: *Detailed analysis of histopathological parameters in radical prostatectomy specimens and PCA3 urine test results*. Prostate 2008; 68: 1215-1222.
13. Nakanishi H, Groskopf J, Fritsche HA et al: *PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance*. J Urol 2008; 179: 1804-1809.
14. Marks LS, Fradet Y, Deras IL et al: *PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy*. Urology 2007; 69: 532-535.
15. Mitelman F, Johansson B, Mertens F. *The impact of translocations and gene fusions on cancer causation*. Nat Rev Cancer 2007; 7: 233-245.
16. 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.
17. Perner S, Demichelis F, Beroukhim R et al: *TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer*. Cancer Res 2006; 66: 8337-8341.
18. 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.
19. Morris DS, Tomlins SA, Montie JE, Chinnaiyan AM: *The discovery and application of gene fusions in prostate cancer*. BJU Int 2008; 102: 276-282.
20. 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.
21. Barry M, Perner S, Demichelis F, Rubin MA: *TMPRSS2-ERG fusion heterogeneity in multifocal prostate cancer: clinical and biologic implications*. Urology 2007; 70: 630-633.
22. 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.
23. Gopalan A, Leversha MA, Satagopan JM et al: *TMPRSS2-ERG Gene Fusion Is Not Associated with Outcome in Patients Treated by Prostatectomy*. Cancer Res 2009; 69: 1400-1406.
24. Lee HS, Kim BH, Cho NY et al: *Prognostic implications of and relationship between CpG island hypermethylation and repetitive DNA hypomethylation in hepatocellular carcinoma*. Clin Cancer Res 2009; 15: 812-820.
25. Jee CD, Kim MA, Jung EJ et al: *Identification of genes epigenetically silenced by CpG methylation in human gastric carcinoma*. Eur J Cancer 2009; 45: 1282-1293.
26. Tanemura A, Terando AM, Sim MS et al: *CpG Island Methylator Phenotype Predicts Progression of Malignant Melanoma*. Clin Cancer Res 2009; 15: 1801-1807.
27. Ostrow KL, Park HL, Hoque MO et al: *Pharmacologic unmasking of epigenetically silenced genes in breast cancer*. Clin Cancer Res 2009; 15: 1184-1191.
28. Duffy MJ, Napieralski R, Martens JW et al: *Methylated genes as new cancer biomarkers*. Eur J Cancer 2009; 45: 335-346.
29. Bastian PJ, Ellinger J, von RA et al: *CpG island hypermethylation of the DNA. Perspectives of a molecular biomarker for prostate cancer*. Urologe A 2008; 47: 1205-1207.
30. 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.
31. Goessl C, Muller M, Straub B, Miller K: *DNA alterations in body fluids as molecular tumor markers for urological malignancies*. Eur Urol 2002; 41: 668-676.
32. Goessl C, Krause H, Muller M: *Fluorescent methylation-specific polymerase chain reaction for DNA-based detection of prostate cancer in bodily fluids*. Cancer Res 2000; 60: 5941-5945.
33. Hoque MO, Topaloglu O, Begum S et al: *Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects*. J Clin Oncol 2005; 23: 6569-6575.
34. Rouprêt M, Hupertan V, Yates DR et al: *Molecular detection of localized prostate cancer using quantitative methylation-specific PCR on urinary cells obtained following prostate massage*. Clin Cancer Res 2007; 13: 1720-1725.
35. Rouprêt M, Hupertan V, Catto JW et al: *Promoter hypermethylation in circulating blood cells identifies prostate cancer progression*. Int J Cancer 2008; 122: 952-956.
36. Harden SV, Sanderson H, Goodman SN et al: *Quantitative GSTP1 methylation and the detection of prostate adenocarcinoma in sextant biopsies*. J Natl Cancer Inst 2003; 95: 1634-1637.
37. Sreekumar A, Poisson LM, Rajendiran TM et al: *Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression*. Nature 2009; 457: 910-914.
38. Laxman B, Morris DS, Yu J et al: *A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer*. Cancer Res 2008; 68: 645-649.
39. Petrovics G, Liu A, Shaheduzzaman S et al: *Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome*. Oncogene 2005; 24: 3847-3852.

Correspondence
Jack A. Schalken
267 Experimental Urology
Radboud University Nijmegen Medical Center
PO Box 9101 NL-6500HB Nijmegen, The Netherlands
phone: +31 24 3614146
J.Schalken@uro.umcn.nl