



Mini-review

PCA3: From basic molecular science to the clinical lab

John R. Day*, Matthias Jost, Mark A. Reynolds, Jack Groskopf, Harry Rittenhouse

Gen-Probe Incorporated, San Diego, CA, United States

ARTICLE INFO

Article history:

Received 13 September 2010

Accepted 20 October 2010

Keywords:

PCA3
DD3
Prostate cancer
Diagnostic
Marker

ABSTRACT

Prostate cancer is the second leading cause of cancer deaths in men in the United States. Use of the serum prostate specific antigen (PSA) test to screen men for prostate cancer since the late 1980s has improved the early detection of prostate cancer, however low specificity of the test translates to numerous false positive results and many unnecessary biopsies. New biomarkers to aid in prostate cancer diagnosis are emerging and prostate cancer gene 3 (PCA3) is one such marker. PCA3 is a noncoding RNA that is highly over-expressed in prostate cancer tissue compared to benign tissue. A non-invasive test for PCA3 was developed using whole urine collected after a digital rectal exam (DRE). Numerous clinical studies have demonstrated the utility of PCA3 for the diagnosis of prostate cancer and some studies suggest that PCA3 may also have prognostic value. The use of PCA3 in combination with serum PSA and other clinical information enhances the diagnostic accuracy of prostate cancer detection and will enable physicians to make more informed decisions with patients at risk for prostate cancer.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Prostate cancer is the second leading cause of cancer deaths in men in the United States [1]. Since its initial approval in 1986, the serum prostate specific antigen (PSA) test has been used for monitoring prostate cancer patients and was quickly adopted as a screening tool in combination with the digital rectal exam (DRE). While the sensitivity of serum PSA is high for detecting prostate cancer, the specificity is quite low, resulting in many negative biopsies. An improvement to the low specificity of serum PSA is needed to prevent unnecessary and uncomfortable biopsy procedures. Furthermore, there is an increasing need to distinguish prostate cancers that will become aggressive and metastatic from those cancers that will remain indolent and cause no ill effects. The side effects of prostate cancer treatment can be difficult. New biomarkers for aiding the diagnosis of prostate cancer have emerged

and have shown promise for the detection of cancer and delineation between indolent and more aggressive cancers. One such new marker is prostate cancer gene 3 (PCA3), a noncoding RNA that is highly over-expressed in prostate cancer tissues.

2. PCA3 – the gene

PCA3, initially referred to as DD3, was first discovered in 1999 using differential display analysis, a method to compare mRNA expression levels between benign and malignant tissue [2]. In 95% of the 56 human prostatectomy specimens examined, PCA3 was highly over-expressed in prostate tumors relative to adjacent non-cancerous tissue. Using Northern blot analysis, normal prostate and benign prostatic hyperplasia (BPH) tissue from the same subjects expressed little to no PCA3 [2]. A sensitive real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of PCA3 demonstrated relatively low levels of expression in normal prostate and BPH tissues whereas the median up-regulation of PCA3 in prostate cancer cells relative to non-malignant tissue was about 60-fold [3,4]. Furthermore, no PCA3 was detected

* Corresponding author. Address: Gen-Probe Incorporated, 10210 Genetic Center Drive, San Diego, CA 92121, United States. Tel.: +1 858 410 8000.

E-mail address: John.Day@gen-probe.com (J.R. Day).

in other tissues or tumors [2,3]. These initial studies demonstrated that PCA3 is over-expressed in prostate cancer and a potentially useful biomarker for the detection of prostate cancer.

The PCA3 gene is located on chromosome 9q21–22 and was originally described as consisting of four exons with alternative polyadenylation at 3 different positions in exon 4 (Fig. 1) [2]. Due to alternative splicing, exon 2 was found to be absent from all but 5% of cDNA clones analyzed. The most frequent transcript (exons 1, 3, 4a and 4b) was found in 60% of the cDNA clones. More recently, additional complexity of the PCA3 gene has been described with 4 new transcription start sites, 2 new differentially spliced exons, and 4 new polyadenylation sites reported (Fig. 1) [5]. Transcripts either containing the newly identified exons or initiated at one of the newly identified transcription start sites were expressed at low levels, representing a minority of the PCA3 transcripts [5,6]. One important observation within the PCA3 cDNA sequence was the presence of a large number of stop codons in all three reading frames; the lack of an extensive open reading frame (ORF) suggested that PCA3 was a noncoding RNA (ncRNA) [2]. With the identification of new transcription start sites, four new putative open reading frames were predicted with amino acid lengths ranging from 70 to 82 [5]. Studies of these potential PCA3 polypeptides have not yet been conducted, but comparison of the predicted sequences with known proteins has revealed no homology or indication of their function [6]. Furthermore, using subcellular fractionation the majority of processed PCA3 mRNA was found within the nucleus at steady-state [7,8]. Nuclear localization of transcripts and the high frequency of stop codons together support the hypothesis that PCA3 is an untranslated, non-coding RNA.

The “central dogma” of molecular biology is that genetic information flows in one direction with proteins as the end product. A growing body of evidence, however, has emerged to describe the role of the RNAs that are not translated into proteins, ncRNAs. Various types of ncRNAs have been implicated in gene regulation via modification of

chromatin structure, alterations to DNA methylation, RNA splicing, RNA editing, transcriptional gene silencing, post-transcriptional gene silencing, and enhancement of gene expression [9–11]. Noncoding infrastructural RNAs such as tRNAs, rRNAs, spliceosomal uRNAs, and snoRNAs have been known and studied for a long time. Small ncRNAs such as microRNAs (miRNAs) and small interfering RNAs (siRNAs) are also well-studied in the roles they play in post-transcriptional gene silencing, and are reported to be aberrantly expressed in many cancers [9]. Messenger-RNA-like long ncRNAs have been identified in a wide range of organisms and their function is an active area of research. PCGEM1 is one such ncRNA that is prostate specific and over-expressed in prostate cancer [12]. Upregulation of the gene was found to be significantly higher in African-American men and in men with a family history of prostate cancer [13]. Functional studies revealed that PCGEM1 promotes cell proliferation and inhibits apoptosis [13,14]. These data provide insight into a possible role that PCA3 may play as an analogous prostate specific ncRNA. Interestingly, the PCA3 gene was found to be embedded in exon 6 of the *BMCC1/PRUNE2* gene, but transcribed in the antisense orientation [5]. *BMCC1* has been implicated in cellular proliferation, apoptosis and cellular transformation. Clarke et al. [5] observed an increased expression of *BMCC1* in prostate cancer tissues and an induction by dihydrotestosterone (DHT), whereas Salagierski [6] observed no increase in cancer tissue and repression of expression by DHT. While the *BMCC1* story has yet to be sorted out, the overlap of the gene with PCA3 is intriguing. Clarke [5] speculates that PCA3 may play a role in chromatin opening to facilitate transcription. Alternatively, because PCA3 is an intronic antisense transcript, it may induce cleavage and down-regulation of *BMCC1* mRNA or inhibition/modulation of *BMCC1* splicing. Salagierski et al. [6] suggests that androgen repression of *BMCC1* could be via transcriptional interference by PCA3. While the function of the PCA3 transcript remains an open question, it is possible that PCA3 is a *cis*-acting regulator of *BMCC1*. Alternatively, PCA3 could affect gene regulation in *trans* in a manner analogous to PCGEM1.

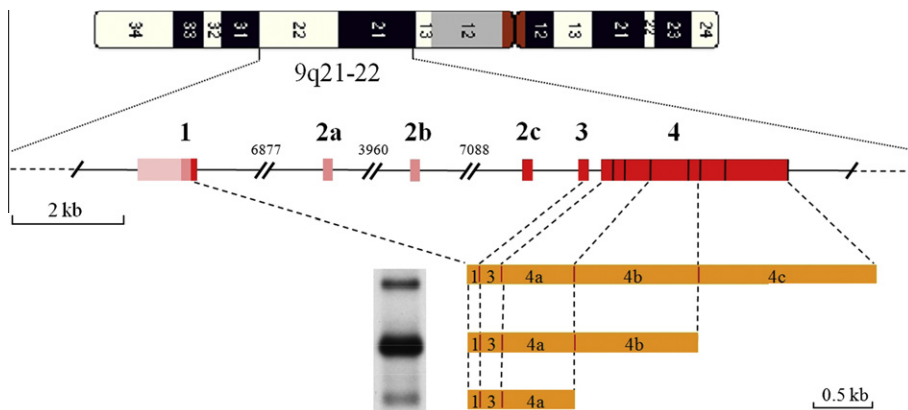


Fig. 1. The PCA3 gene is mapped to chromosome 9q21–22. As originally described in Bussemakers et al. [2], the PCA3 transcription unit consisted of 4 exons (red boxes 1, 2c, 3 and 4) and 3 polyadenylation sites in exon 4 (orange boxes 4a, 4b, and 4c). Exon 2, often skipped by alternative splicing, is absent from the 3 transcripts shown in the Northern blot. Clarke et al. [5] identified 2 new exons (2a and 2b), 4 new polyadenylation sites (vertical lines in exon 4), and 4 new transcription start sites (exon 1, pink and light pink boxes). (Courtesy of Gerald Verhaegh.)

New discoveries about ncRNAs and their function within the cell will undoubtedly lead to greater understanding of gene regulatory processes.

3. Development of a PCA3-based urine test

The observation that PCA3 is highly over-expressed in prostate cancer tissue led researchers to explore the potential use of PCA3 to non-invasively predict biopsy outcome. Hessels et al. [4] used time-resolved fluorescence (TRF) RT-PCR to detect PCA3 mRNA in centrifuged urine sediments harvested from urine specimens that were collected following a digital rectal exam (DRE). The levels of PSA mRNA were measured, as well. PSA mRNA is expressed in benign and cancer prostate cells at approximately the same level, with a modest (1.5-fold) decrease in PSA expression in prostate cancer cells [15]. A ratio of PCA3 to PSA mRNA was therefore used to normalize for the variable numbers of prostate cells collected by the procedure [4]. In a study of 108 men undergoing a prostate biopsy due to elevated serum PSA, 24 of them were found to have prostate cancer. PCA3 results compared to biopsy outcome revealed an area under the receiver operating curve (ROC AUC) of 0.72 (95% CI: 0.58–0.85) [4]. Using the PCA3/PSA ratio cutoff with the greatest diagnostic accuracy, the sensitivity of PCA3 was 67% and the specificity was 83%. These data were verified in a large European multi-center study and altogether indicated that a urinary PCA3 test had potential for aiding in the diagnosis prostate cancer [4,16].

An isothermally amplified version of the PCA3 test was developed by DiagnoCure Incorporated using nucleic acid sequence-based amplification (NASBA) technology and real-time fluorescence detection in nucleic acid extracts from post-DRE urine sediments [17,18]. PSA mRNA was amplified along with PCA3 mRNA as a qualitative control for the presence of an adequate number of prostate cells for analysis. One study using the NASBA PCA3 test consisted of 201 patients and resulted in a PCA3 sensitivity and specificity of 82% and 76% [18]. Another study of 517 patients from multiple centers showed an overall sensitivity and specificity of 66% and 89% [17]. Notably, the performance of PCA3 was consistent across subject groups having low (<4 ng/mL), mid (4–10 ng/mL) and high (>10 ng/mL) serum PSA values. The accuracy of the PCA3 test was 81%, whereas serum PSA was only 47% using a 4 ng/mL cutoff [17]. These studies using a NASBA assay confirmed the utility of PCA3 in post-DRE urine sediments demonstrated by the earlier RT-PCR TRF assay.

Development of a commercially available, semi-automated, quantitative PCA3 assay was performed by Gen-Probe Incorporated using specific target capture, transcription-mediated amplification (TMA) and detection with chemiluminescent DNA probes [19]. Instead of using urine sediments, the TMA assay was optimized to measure PCA3 in whole urine samples mixed with an equal volume of a detergent-based stabilization buffer. The elimination of the need for urine centrifugation and RNA extraction, as well as performing all assay steps in a single tube, improved the usability of the assay. The assay was designed to quantify all known PCA3 mRNA isoforms by targeting

the Exon 3/Exon 4a junction; the assay does not detect unspliced PCA3 RNA. Like the TRF RT-PCR assay, PSA mRNA is also measured quantitatively and a ratio of PCA3 to PSA mRNA is calculated to detect prostate cancer cells in a “background” of normal prostate cells that express low levels of PCA3. In the commercial Gen-Probe assay, the PCA3/PSA mRNA ratio is multiplied by 1000 to yield a PCA3 Score. In the initial study using the TMA assay, the PCA3 Score performed similarly to previous studies; the assay demonstrated a sensitivity of 69%, specificity of 79% and an AUC of 0.746 (95% CI: 0.574–0.918) [19]. A larger follow-up study of North American men and two European studies confirmed that PCA3 performance was independent of serum PSA level and, in contrast to serum PSA, PCA3 was unaffected by prostate size [20–22]. A key finding of several studies was that the quantitative PCA3 Score correlated with the risk of a positive biopsy [20,21,23]. The Gen-Probe PCA3 assay was cleared for use in Europe in 2006 under the name PROGENSA® PCA3 and since then several studies have validated the performance of the TMA assay with similar results (reviewed in [24]).

4. DRE and pre-analytical factors

The collection procedure for the urine sample used for PCA3 analysis has been extensively investigated. Reproducible and robust results have been reported by several laboratories [19,20,23,25,26]. The effects of DRE, time of urine collection, storage conditions, and shipment to clinical laboratories have now been well described and documented. Following a DRE the first voided urine is collected and transferred to a transport tube containing a formulated buffer to stabilize the mRNA in the specimen. The DRE significantly increases the PCA3 mRNA signal in the urine specimen. The working hypothesis is that a manipulation of the prostate by DRE releases prostate cells into the urinary tract, including cancer cells if present. Without DRE about 80% of the urine specimens will still have enough prostate cells for an informative PCA3 result [25]. With DRE the informative rate is greater than 95% with the TMA Gen-Probe technology using whole urine [19,20,23,25,26]. The previous RT-PCR and NASBA technologies using urine sediments provided less than 90% informative specimens and a cumbersome urine sediment procedure. In addition, a rigorous DRE, sometimes referred to as a prostate massage, was used in these early assays. Confusion sometimes arises regarding the type of DRE required for the current PCA3 TMA assay due to the early literature. For the clinical research studies of the Gen-Probe TMA PCA3 assay an “attentive” DRE involving three strokes for each of the two lobes was recommended to ensure a uniform procedure in the trial design. A DRE consisting of three strokes per lobe is currently recommended in the PROGENSA® PCA3 package insert to standardize the procedure. However, the performance of PCA3 has been reported to be independent of DRE procedure, including no DRE [25]. Although a DRE is required for a high informative rate, no significant difference in median PCA3 Scores for informative samples was reported when comparing pre-DRE urine with post-DRE urine following a 3-stroke

or 8-stroke DRE [25]. In addition, a study has demonstrated that prostate massage yielding extra prostatic secretions does not increase the clinical sensitivity or specificity compared to DRE [27]. Therefore, the current PCA3 test is robust with respect to DRE procedure.

5. Prostate cells in urine

Although the presence of prostate cells in voided urine was reported decades ago by Papanicolaou [28], the frequency of positive cytology for prostate cancer in voided urine is low [29,30]. Presumably, manipulation of the prostate increases the number and frequency of prostate cells in urine, which is supported by measured PCA3 values. Koss [31] hypothesized that prostate cancer cells in voided urine without prostate manipulation signaled advanced cancer compared to patients with cancer cells in urine only after prostate massage. Studies of prostate cells in urine have generally reported that the cells are low in number and labile requiring careful collection and storage of the samples. The origin of prostate cells has been a matter of debate. One view is that HGPIN and intraductal carcinoma of the prostate may be released into the urinary tract but invasive prostate cancer cells are blocked [32]. Sensitive immunofluorescence techniques have been recently used to increase the sensitivity of detection of prostate cancer cells in urine [33]. A study in the laboratory of Dr. Alan Meeker using antibodies to AMACR, Nkx-3 and nucleolin demonstrated a sensitivity of 36% with 100% specificity in 25 informative specimens [33]. Specific molecular probes to prostate cancer, such as PCA3, would also be expected to show high specificity and easier application since cytology could be avoided. As discussed in the above sections, the initial PCA3 tests using PCR and NASBA technology measured PCA3 mRNA in urinary sediments enriched for prostate cells. The more sensitive TMA technology has allowed for the more easily obtained whole urine sample. The clinical sensitivity of the PCA3 test has ranged from 50% to 75% in most studies. The sensitivity and specificity of the PCA3 test argues for the detection of invasive prostate cancer cells, since the reported incidence of isolated HGPIN is much lower. A number of studies have correlated urine PCA3 Score with the presence of HGPIN at biopsy, with conflicting results. Deras et al. [20] found no difference in PCA3 Score for HGPIN vs. no evidence of abnormal pathology, while Haese et al. [21] found increased PCA3 Scores in men with HGPIN; these two studies yielded equivalent diagnostic accuracy for biopsy-detectable cancer. A recent study indicated that the efficacy of PCA3 in patients with isolated HGPIN is lower than in men with benign pathology [34]. Follow-up studies of patients with apparent isolated HGPIN and elevated PCA3 Scores need to be done. A more thorough understanding of the target cells in urine will lead to a better application of the PCA3 test.

6. Performance of PCA3 in clinical studies

The clinical performance of PCA3 has been determined in several studies and reviewed elsewhere [24]. Not in-

cluded in the Vlaeminck-Guillem review, however, is the largest, international, multicenter, double-blinded study to date to evaluate PCA3 performance in men with elevated serum PSA and a previous negative biopsy. The REDuction by DUtasteride of prostate Cancer Events (REDUCE) trial was designed to evaluate the use of a drug for the chemoprevention of prostate cancer [35]. The placebo arm of the study enrolled more than 4000 subjects and a subset of 1140 subjects provided urine samples for PCA3 analysis prior to year 2 and year 4 biopsies [36]. Consistent with previous studies using the TMA PCA3 assay, a large proportion of the specimens (94%, $N = 1072$) contained sufficient RNA for PCA3 analysis. The PCA3 Score was found to correlate with the percent of biopsy-positive men; the greater the PCA3 Score the greater the probability of a positive biopsy (Fig. 2) [36]. At PCA3 Scores less than 5, 6% (7/116) of men were biopsy-positive, whereas at Scores >100 more than 57% (28/49) were biopsy-positive. Using a cutoff of 35, the clinical performance of the PCA3 Score in this study was similar to previous studies: sensitivity was 48% and specificity was 79%. Compared to serum PSA, the PCA3 Score performed significantly better ($p = 0.008$); the AUC of the PCA3 Score was 0.693 (95% CI: 0.649–0.736) and the AUC of serum PSA was 0.612 (95% CI: 0.570–0.655) (Fig. 3). A predictive model incorporating PCA3, serum PSA, % free-PSA and other clinical information significantly improved diagnostic accuracy (AUC = 0.753) (Fig. 3) compared to the model excluding PCA3 (AUC = 0.717, $p = 0.0009$) (not shown) [36]. This is consistent with other studies that have incorporated PCA3 into statistical models and nomograms [20,37–39]. One very interesting finding from the REDUCE study placebo arm was that year 2 PCA3 Scores were predictive of year 4 biopsy outcome with an AUC of 0.634 ($p = 0.0002$). These data suggest that prostate cancers that were missed by biopsy at year 2 were in fact detected by PCA3. A needle biopsy samples only a small portion of the prostate gland and is not a perfect method to diagnose cancer. In some cases a false-positive PCA3 may be a true-positive if the cancer remained undetected on biopsy. Altogether, data from the placebo arm of the REDUCE trial validated the clinical performance of

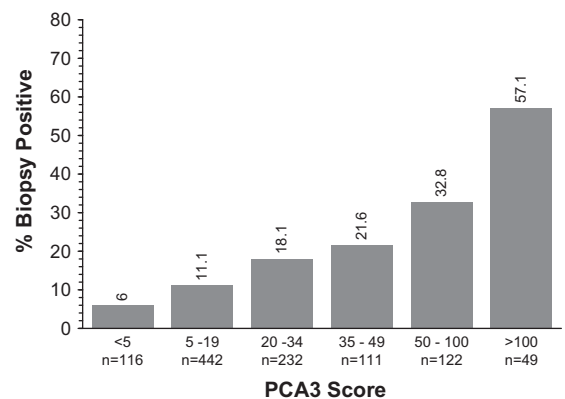


Fig. 2. Percent of men with positive biopsy vs. PCA3 Score range. The number of men in each group is shown [36].

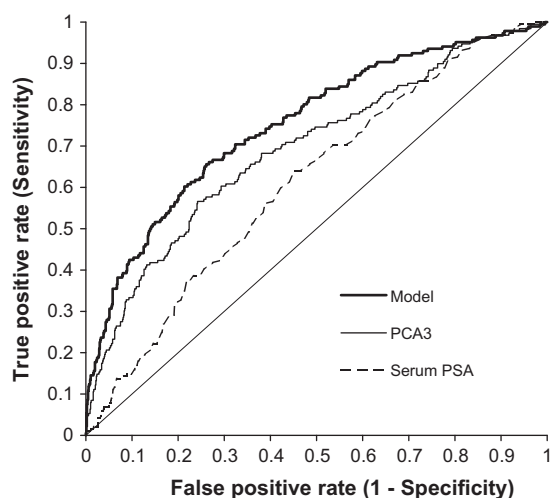


Fig. 3. ROC curve analysis for a multivariate logistic model (includes PCA3 as continuous variable, serum PSA, % fPSA, prostate volume, age, and family history of PCa) in comparison to univariate PCA3 (continuous), and serum PSA [36].

PCA3 in the largest study to date and indicated that PCA3 could predict future biopsy outcome.

In addition to predicting the presence or absence of cancer, there is some evidence that PCA3 may correlate with determinants of cancer aggressiveness such as tumor size, Gleason score and extracapsular extension (ECE). This could be explained by the premise that larger, more invasive tumors are more likely to shed cells into the prostatic urethra following physical manipulation by DRE. Two independent studies of men undergoing a prostatectomy showed that the PCA3 Score significantly correlated with the total tumor volume ($p = 0.008$ and $p < 0.01$) [40,41]. Nakanishi [40] reported that the PCA3 Score was also significantly associated with prostatectomy Gleason score (6 vs. 7 or greater, $p = 0.005$) and “significant” cancer ($p = 0.007$), a classification based on dominant tumor volume and Gleason score (dominant tumor volume ≥ 0.5 cc and Gleason score ≥ 7). While Whitman [41] did not find a significant association with prostatectomy Gleason score, their study showed that PCA3 was an independent predictor of ECE. They also showed that PCA3 combined synergistically with serum PSA and biopsy Gleason score to greatly improve ECE predictive ability [41]. A more recent study did not find the same correlations with PCA3; however, the study used post-DRE urine sediments instead of whole urine and cannot be directly compared [42]. These data, while in need of further study and verification, suggest that the PCA3 Score may be useful in the identification of prostate cancers that require more aggressive treatment.

7. Conclusion

PCA3 is a prostate-specific gene that is highly over-expressed in prostate cancer. PCA3 is likely a noncoding RNA, yet its function is still under investigation. One hypothesis is that PCA3 plays a role in gene regulation in a manner analogous to PCGEM1. PCA3 ncRNA has been

shown to be useful as a biomarker for prostate cancer in post-DRE urine using at least 3 different molecular techniques. A sensitive, urine-based, quantitative test was developed by Gen-Probe Incorporated and released commercially in Europe in 2006 under the brand name PROGENSA® PCA3. A clinical trial is being conducted in the United States to obtain US Food and Drug Administration approval for the assay.

The clinical performance and diagnostic accuracy of PCA3 have been evaluated in numerous independent studies with reproducible results. PCA3 consistently outperforms serum PSA in diagnostic accuracy. PSA is a surrogate marker because it is not specific to prostate cancer; other conditions such as inflammation and benign prostatic hyperplasia can also cause serum PSA levels to increase. PCA3 improves upon serum PSA due to its higher specificity. Notably, the performance of PCA3 appears unaffected by the serum PSA level or prostate size. Due to its widespread use for many years and high sensitivity, serum PSA will likely be used for years to come. However, in combination with serum PSA and other clinical information, PCA3 improves diagnostic accuracy and will give physicians a more specific tool to aid in the decision to biopsy a patient.

Discrimination between aggressive or significant cancers and slow-growing or indolent cancers is perhaps the greatest unmet need in prostate cancer patient care. While further research is needed, some reports indicate that PCA3 is correlated with features of significant cancer such as tumor volume, Gleason score and extracapsular extension. PCA3 may prove to be useful to physicians to help decide if patients require aggressive treatment and follow-up or if they could be placed in a watchful waiting or active surveillance program. PCA3 may have prognostic value as well as diagnostic value.

Studies that will further validate and expand the clinical utility of the PCA3 assay are in progress. Some potential applications of the PCA3 assay include testing prior to first biopsy, deciding whether to rebiopsy men with elevated serum PSA and a previous negative biopsy, detecting recurrence following radical prostatectomy or radiation therapy, or monitoring patients receiving drug therapies that affect serum PSA levels (e.g. 5 α -reductase inhibitors). The PCA3 test is emerging as the first fully translated molecular diagnostic assay for prostate cancer cells in biological fluids, and holds promise as a valuable tool for aiding in the diagnosis of prostate cancer.

Conflict of interest

The authors are employed by Gen-Probe Incorporated.

References

- [1] American Cancer Society, Cancer Facts & Figures, American Cancer Society, Atlanta, 2010.
- [2] M.J. Bussemakers, A. van Bokhoven, G.W. Verhaegh, F.P. Smit, H.F. Karthaus, J.A. Schalken, F.M. Debruyne, N. Ru, W.B. Isaacs, DD3: a new prostate-specific gene, highly overexpressed in prostate cancer, *Cancer Res.* 59 (1999) 5975–5979.
- [3] J.B. de Kok, G.W. Verhaegh, R.W. Roelofs, D. Hessels, L.A. Kiemeny, T.W. Aalders, D.W. Swinkels, J.A. Schalken, DD3(PCA3), A very

- sensitive and specific marker to detect prostate tumors, *Cancer Res.* 62 (2002) 2695–2698.
- [4] D. Hessels, J.M. Klein Gunnewiek, I. van Oort, H.F. Karthaus, G.J. van Leenders, B. van Balken, L.A. Kiemeny, J.A. Witjes, J.A. Schalken, DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer, *Eur. Urol.* 44 (2003) 8–15.
 - [5] R.A. Clarke, Z. Zhao, A.Y. Guo, K. Roper, L. Teng, Z.M. Fang, H. Samarutunga, M.F. Lavin, R.A. Gardiner, New genomic structure for prostate cancer specific gene PCA3 within BMCC1: implications for prostate cancer detection and progression, *PLoS One* 4 (2009) e4995.
 - [6] M. Salagierski, G.W. Verhaegh, S.A. Jannink, F.P. Smit, D. Hessels, J.A. Schalken, Differential expression of PCA3 and its overlapping PRUNE2 transcript in prostate cancer, *Prostate* 70 (2010) 70–78.
 - [7] J.A. Schalken, D. Hessels, G. Verhaegh, New targets for therapy in prostate cancer: differential display code 3 (DD3(PCA3)), a highly prostate cancer-specific gene, *Urology* 62 (2003) 34–43.
 - [8] J.A. Schalken, personal communication.
 - [9] J.S. Mattick, I.V. Makunin, Non-coding RNA, *Human Mol. Genet.* 15 Spec No. 1, 2006, pp. R17–29.
 - [10] M. Szell, Z. Bata-Csorgo, L. Kemeny, The enigmatic world of mRNA-like ncRNAs: their role in human evolution and in human diseases, *Semin. Cancer Biol.* 18 (2008) 141–148.
 - [11] A.M. Turner, K.V. Morris, Controlling transcription with noncoding RNAs in mammalian cells, *BioTechniques* 48 (2010) ix–xvi.
 - [12] V. Srikantan, Z. Zou, G. Petrovics, L. Xu, M. Augustus, L. Davis, J.R. Livezey, T. Connell, I.A. Sesterhenn, K. Yoshino, G.S. Buzard, F.K. Mostofi, D.G. McLeod, J.W. Moul, S. Srivastava, PCGEM1, a prostate-specific gene, is overexpressed in prostate cancer, *Proc. Natl. Acad. Sci. USA* 97 (2000) 12216–12221.
 - [13] G. Petrovics, W. Zhang, M. Makarem, J.P. Street, R. Connelly, L. Sun, I.A. Sesterhenn, V. Srikantan, J.W. Moul, S. Srivastava, Elevated expression of PCGEM1, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients, *Oncogene* 23 (2004) 605–611.
 - [14] X. Fu, L. Ravindranath, N. Tran, G. Petrovics, S. Srivastava, Regulation of apoptosis by a prostate-specific and prostate cancer-associated noncoding gene, PCGEM1, *DNA Cell Biol.* 25 (2006) 135–141.
 - [15] F.J. Meng, A. Shan, L. Jin, C.Y. Young, The expression of a variant prostate-specific antigen in human prostate, *Cancer Epidemiol. Biomarkers Prev.* 11 (2002) 305–309.
 - [16] M.P. van Gils, D. Hessels, O. van Hooij, S.A. Jannink, W.P. Peelen, S.L. Hanssen, J.A. Witjes, E.B. Cornel, H.F. Karthaus, G.A. Smits, G.A. Dijkman, P.F. Mulders, J.A. Schalken, 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.* 13 (2007) 939–943.
 - [17] Y. Fradet, F. Saad, A. Aprikian, J. Dessureault, M. Elhilali, C. Trudel, B. Masse, L. Piche, C. Chypre, UPM3, a new molecular urine test for the detection of prostate cancer, *Urology* 64 (2004) 311–315.
 - [18] M. Tinzl, M. Marberger, S. Horvath, C. Chypre, DD3PCA3 RNA analysis in urine – a new perspective for detecting prostate cancer, *Eur. Urol.* 46 (2004) 182–186.
 - [19] J. Groskopf, S.M. Aubin, I.L. Deras, A. Blase, S. Bodrug, C. Clark, S. Brentano, J. Mathis, J. Pham, T. Meyer, M. Cass, P. Hodge, M.L. Macairan, L.S. Marks, H. Rittenhouse, APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer, *Clin. Chem.* 52 (2006) 1089–1095.
 - [20] I.L. Deras, S.M. Aubin, A. Blase, J.R. Day, S. Koo, A.W. Partin, W.J. Ellis, L.S. Marks, Y. Fradet, H. Rittenhouse, J. Groskopf, PCA3: a molecular urine assay for predicting prostate biopsy outcome, *J. Urol.* 179 (2008) 1587–1592.
 - [21] A. Haese, A. de la Taille, H. van Poppel, M. Marberger, A. Stenzl, P.F. Mulders, H. Huland, C.C. Abbou, M. Remzi, M. Tinzl, S. Feyerabend, A.B. Stillebroer, M.P. van Gils, J.A. Schalken, Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy, *Eur. Urol.* 54 (2008) 1081–1088.
 - [22] D. Schilling, J. Hennenlotter, M. Munz, U. Bokeler, K.D. Sievert, A. Stenzl, Interpretation of the prostate cancer gene 3 in reference to the individual clinical background: implications for daily practice, *Urol. Int.* 85 (2010) 159–165.
 - [23] L.S. Marks, Y. Fradet, I.L. Deras, A. Blase, J. Mathis, S.M. Aubin, A.T. Cancio, M. Desaulniers, W.J. Ellis, H. Rittenhouse, J. Groskopf, PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy, *Urology* 69 (2007) 532–535.
 - [24] V. Vlaeminck-Guillem, A. Ruffion, J. Andre, M. Devonec, P. Paparel, Urinary prostate cancer 3 test: toward the age of reason?, *Urology* 75 (2010) 447–453.
 - [25] L.J. Sokoll, W. Ellis, P. Lange, J. Noteboom, D.J. Elliott, I.L. Deras, A. Blase, S. Koo, M. Sarno, H. Rittenhouse, J. Groskopf, R.L. Vessella, A multicenter evaluation of the PCA3 molecular urine test: pre-analytical effects, analytical performance, and diagnostic accuracy, *Clin. Chim. Acta* 389 (2008) 1–6.
 - [26] S.B. Shappell, J. Fulmer, D. Arguello, B.S. Wright, J.R. Oppenheimer, M.J. Putzi, PCA3 urine mRNA testing for prostate carcinoma: patterns of use by community urologists and assay performance in reference laboratory setting, *Urology* 73 (2009) 363–368.
 - [27] M.P. van Gils, E.B. Cornel, D. Hessels, W.P. Peelen, J.A. Witjes, P.F. Mulders, H.G. Rittenhouse, J.A. Schalken, Molecular PCA3 diagnostics on prostatic fluid, *Prostate* 67 (2007) 881–887.
 - [28] N.C. Foot, G.N. Papanicolaou, N.D. Holmquist, J.F. Seybolt, Exfoliative cytology of urinary sediments; a review of 2, 829 cases, *Cancer* 11 (1958) 127–137.
 - [29] V.A. Varma, P.S. Fekete, M.J. Franks, M.M. Walther, Cytologic features of prostatic adenocarcinoma in urine: a clinicopathologic and immunocytochemical study, *Diagnos. Cytopathol.* 4 (1988) 300–305.
 - [30] B. Krishnan, L.D. Truong, Prostatic adenocarcinoma diagnosed by urinary cytology, *Am. J. Clin. Pathol.* 113 (2000) 29–34.
 - [31] L.G. Koss, Cytology of specimens obtained by prostatic massage, *Diagnostic Cytology and its Histopathologic Bases*, J.B. Lippincott Company, Philadelphia, 1992, pp. 1001–1005.
 - [32] H. Bonkhoff, Molecular diagnostics in prostate urine: interpret results correctly, *Uro-News* 2 (2009) 45–48.
 - [33] K. Fujita, C.P. Pavlovich, G.J. Netto, Y. Konishi, W.B. Isaacs, S. Ali, A. De Marzo, A.K. Meeker, Specific detection of prostate cancer cells in urine by multiplex immunofluorescence cytology, *Human Pathol.* 40 (2009) 924–933.
 - [34] J. Morote, M. Rigau, M. Garcia, C. Mir, C. Ballesteros, J. Planas, C.X. Raventos, J. Placer, I.M. de Torres, J. Reventos, A. Doll, Behavior of the PCA3 gene in the urine of men with high grade prostatic intraepithelial neoplasia, *World J. Urol.* 28 (2010) 677–680.
 - [35] G. Andriole, D. Bostwick, O. Brawley, L. Gomella, M. Marberger, D. Tindall, S. Breed, M. Somerville, R. Rittmaster, And REDUCE Study Group, Chemoprevention of prostate cancer in men at high risk: rationale and design of the reduction by dutasteride of prostate cancer events (REDUCE) trial, *J. Urol.* 172 (2004) 1314–1317.
 - [36] S.M. Aubin, J. Reid, M.J. Sarno, A. Blase, J. Aussie, H. Rittenhouse, R. Rittmaster, G.L. Andriole, J. Groskopf, PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: validation in the placebo arm of the dutasteride REDUCE trial, *J. Urol.* 184 (2010) 1947–1952.
 - [37] D.P. Ankerst, J. Groskopf, J.R. Day, A. Blase, H. Rittenhouse, B.H. Pollock, C. Tangen, D. Parekh, R.J. Leach, I. Thompson, Predicting prostate cancer risk through incorporation of prostate cancer gene 3, *J. Urol.* 180 (2008) 1303–1308.
 - [38] F.K. Chun, A. de la Taille, H. van Poppel, M. Marberger, A. Stenzl, P.F. Mulders, H. Huland, C.C. Abbou, A.B. Stillebroer, M.P. van Gils, J.A. Schalken, Y. Fradet, L.S. Marks, W. Ellis, A.W. Partin, A. Haese, Prostate cancer gene 3 (PCA3): development and internal validation of a novel biopsy nomogram, *Eur. Urol.* 56 (2009) 659–667.
 - [39] M. Auprich, A. Haese, J. Walz, K. Pummer, A. de la Taille, M. Graefen, T. de Reijke, M. Fisch, P. Kil, P. Gontero, J. Irani, F.K. Chun, External validation of urinary PCA3-based nomograms to individually predict prostate biopsy outcome, *Eur. Urol.* 58 (2010) 727–732.
 - [40] H. Nakanishi, J. Groskopf, H.A. Fritsche, V. Bhadkamkar, A. Blase, S.V. Kumar, J.W. Davis, P. Troncoso, H. Rittenhouse, R.J. Babaian, PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance, *J. Urol.* 179 (2008) 1804–1809.
 - [41] E.J. Whitman, J. Groskopf, A. Ali, Y. Chen, A. Blase, B. Furusato, G. Petrovics, M. Ibrahim, S. Elsamoudi, J. Cullen, I.A. Sesterhenn, S. Brassell, H. Rittenhouse, S. Srivastava, D.G. McLeod, PCA3 score before radical prostatectomy predicts extracapsular extension and tumor volume, *J. Urol.* 180 (2008) 1975–1978.
 - [42] D. Hessels, M.P. van Gils, O. van Hooij, S.A. Jannink, J.A. Witjes, G.W. Verhaegh, J.A. Schalken, Predictive value of PCA3 in urinary sediments in determining clinico-pathological characteristics of prostate cancer, *Prostate* 70 (2010) 10–16.