Review Article

Function of *PCA3* in prostate tissue and clinical research progress on developing a *PCA3* score

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Abstract: Prostate cancer gene 3 (*PCA3*, also known as *DD3*) is a new biomarker that could improve the accuracy of prostate cancer diagnosis. It is a great biomarker with fairly high specificity and sensitivity. The incidence of prostate cancer is rising steadily in most countries. The commonly used prostate-specific antigen (PSA) test once gave people hope for early diagnosis of prostate cancer. However, the low specificity of the PSA test has resulted in a large number of unnecessary biopsies and overtreatment. During the past decade, many new prostate cancer biomarkers have been found. Among these, *PCA3* is the most promising. Due to its great performance in distinguishing prostate cancer from other prostate conditions, *PCA3* could likely be applied for early diagnosis of prostate cancer, patient follow-up, prognosis prediction, and targeted therapy. After years of research, we have obtained some knowledge about the sequence of *PCA3* gene. We have also determined the relationship between *PCA3* and the proliferation of prostate cancer cells and learned some information about how *PCA3* affects tumor-related genes and proteins. A *PCA3* score has been created, and it has been used in a variety of studies. Some researchers have even applied *PCA3* to targeted therapy and obtained a good effect *in vitro*. This review describes the current state of research, and explores the future prospects for *PCA3*.

Keywords: Prostate cancer gene 3 (*PCA3*); lncRNA; prostate cancer (PCa); prostate-specific antigen (PSA)

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Introduction

Despite ever-advancing medical developments, effective treatments for advanced prostate cancer (PCa) are still very limited. Therefore, it is very important to detect and diagnose PCa early. Wide-spread use of prostate-specific antigen (PSA) to screen men for PCa has greatly improved early detection. However, although the serum PSA test is highly sensitive, it lacks adequate specificity for PCa, especially within the PSA grey zone from 4.0 to 10.0 ng/mL (1), which leads to numerous unnecessary biopsies and treatments. These unnecessary procedures not only bring a heavy medical burden but also discomfort, hematuria, and a risk of urinary obstruction to the effected patients. Furthermore, in addition to PCa, the very common conditions of prostatitis and benign prostatic hyperplasia also cause elevated PSA levels, which are often affected by many other factors, such as age, prostate massage, and biopsy. Although doctors often use four as a critical value, many confounding factors make its clinical reference value extremely limited. Therefore, PSA is a good, but imperfect biomarker, and a more specific tumor marker is needed. New biomarkers should have high sensitivity, the ability to distinguish cancer with greater specificity than PSA, and differentiate indolent cancers from aggressive cancers. Prostate cancer gene 3 (*PCA3*), also known as *DD3*, was first discovered in 1999 by Bussemakers and his colleagues (2). Due to its high specificity, it is now considered the most promising new biomarker; its PCa specificity approaches 100% in tissue. In recent years, a large number of studies

have deepened our understanding of *PCA3*. However, further studies are still needed.

PCA3 is a long non-coding RNA (lncRNA)

The *PCA3* gene was mapped to chromosome 9q21-22, in antisense orientation within intron 6 of the Prune homolog 2 gene (*PRUNE2* or *BMCC1*) (3,4), spanning a region of approximately 25 kb. No homology to any gene present in the computer databases was detected. *PCA3* is likely one of the most PCa-specific genes described thus far. Bussemakers *et al*. tested six human PCa cell lines, including ALVA-31, DU-145, JCA-1, LNCaP, PC-3, PPC-1, and TSU-pr1, and *PCA3* expression was only detected in LNCaP PCa cell line. *PCA3* is only significantly expressed in androgen receptor (AR)-positive PCa cells, although it is expressed at very low levels in the adjacent nonneoplastic tissue and BPH cells. No *PCA3*-related product was detected in any other normal human tissue. Due to its very short open reading frame and striking feature of a high density of stop codons in all three reading frames, it was designated as a non-coding RNA (ncRNA). Until now, whether the level of *PCA3* in tissue is significantly correlated with tumor volume is still controversial, even though most studies did not find a relationship between them.

PCA3 has been shown to be a better biomarker than telomerase reverse transcriptase (hTERT) (5). *PCA3* consists of four exons and three introns, the most common posttranslational modifications are alternative splicing at exon 2 and alternative polyadenylation at exon 4. Exons 1, 3, 4a, and 4b are present in 65% of *PCA3* transcripts.

As *PCA3* does not encode a protein, the only molecule that can be tested is the mRNA, and its expression is mainly restricted to the nuclear and microsomal compartments (6).

PCA3 in tissue

Even though *PCA3* is a promising biomarker for early detection of PCa and targeted therapeutic approaches, its functional role in PCa cells and PCa biology are unknown. Associations between *PCA3* and the AR signaling pathway have been investigated. Ferreira *et al*. transfected LNCaP cells with an siRNA directed against *PCA3*, and found that *PCA3* silencing decreases cell growth and survival and induces apoptotic cell death (6). *PCA3* may modulate PCa cell survival. LNCaP cells transfected with *siPCA3* showed a lower proportion of cells in G0 phase and a higher percentage of pyknotic nuclei. This is not only an indication of cells undergoing apoptosis but also of cell growth suppression. Transfection of *siPCA3* also counteracted the AR signaling cascade, and significantly down-regulated the expression of the other seven AR target genes. *PCA3* expression is up-regulated by AR signaling. Dihydrotestosterone (DHT) treatment increased the expression of AR and *PCA3*, and this DHT-induced up-regulation can be reversed by AR antagonists such as flutamide. Therefore, they thought that *PCA3* expression is androgen-regulated via activation of AR-mediated signaling. However, *Akt* and *ERK* phosphorylation levels were not modified in *siPCA3*-transfected LNCaP cells, suggesting that *PCA3* modulates the survival of LNCaP cells mainly through signals downstream of AR signaling. *PCA3* is mostly expressed in the nuclear and microsomal cell compartments, and no PCA3 is expressed in primary prostate stromal cell cultures.

Previous studies have shown that the *PCA3* promoter has no known initiator motif, no *TATA*-box, no *CAAT*-box, and no *GC*-rich regions at consensus positions. Zhou *et al*. found a short tandem repeat polymorphism (TAAA) in the promoter region of *PCA3* gene (7). This short repeat polymorphism includes five polymorphisms and eight genotypes. They also suggested that the presence of these short tandem repeat polymorphisms may be a risk factor for PCa. According to a retrospective study of 321 patients, eight genotypes were divided into three groups according to the number of *TAAA* repeats: ≤10, 11, and ≥12. The group with ≤10 *TAAA* repeats was associated with a lower relative risk for PCa than the other groups. This result implied that this short tandem repeat polymorphism might be one unit of the transcriptional initiation site of *PCA3* gene and an increased number of repeats may up-regulate *PCA3* transcription. However, no association was found between this short tandem repeat polymorphism and Gleason score in prostate carcinoma patients.

Whether PCa-specific expression of *PCA3* is restricted to exon 4 or if both exon 4 and exon 3 are PCa-specific is still a point of contention. Bussemakers showed that exon 2 was only present in 5% of cDNA clones, and exons 1, 3, and 4a were the most frequently found in cDNA clones (2). Exon 3 and exon 4 are in the prostate-specific region of *PCA3* gene. Gandini *et al*. thought that the prostate-specific expression of *PCA3* was restricted to exon 4, and that the region between exon 1 and exon 3 was not prostate-specific (8). Because they found that the *PCA3* transcript in several non-prostate cell lines could also be amplified when using a primer set located in exon 1 and exon 3. When

primers located in exons 1 and 4 were used, the *PCA3* band was only found in LNCaP cell line. Tao *et al*. repeated this experiment, and obtained the same result as that of Bussemakers; but they did not identify any spliced *PCA3* variants in non-PCa cells (9).

Clarke *et al*. undertook a more detailed investigation of *PCA3* and its chromosomal locus. They identified 4 new transcription start sites, four polyadenylation sites, and two new differentially spliced exons in an extended form of *PCA3* (4). In their studies, the novel transcripts with start sites located at 1,150 bp, 699 bp, 640 bp, and 136 bp were termed *PCA3* isoforms 1-4, respectively, and the original transcript was named *PCA3-5*. Clarke observed that a forward primer based on *PCA3* isoform 4 (*PCA3-4*) together with a reverse primer for exon 2 efficiently amplified *PCA3* in PCa and metastasis samples but failed to detect *PCA3* in BPH samples. Furthermore, they also found that amplification of *PCA3* using the *PCA3-4F* primer together with a primer corresponding to exon 2a or a reverse primer for exon 2b could better discriminate PCa and metastatic samples from BPH. However, Salagierski did not find any relevant diagnostic advantage of the new *PCA3* isoform (*PCA3-TS4*) over the "classical" *PCA3* isoform in their studies (3). Additionally, *PCA3-TS4* appears to be a minor *PCA3* transcript. They confirmed that the previously described classical *PCA3* isoform was still the best target for diagnostic purposes. Clarke once thought *PCA3* and *BMCC1* were overlapping genes in reverse orientation that appeared to be co-regulated; however, Salagierski did not observe this relationship.

Fontenete and his colleague studied and analyzed the frequency of the polymorphism *PCA3*-845 *G>A*, and found that carriers of the *GA* and *AA* genotype had a higher risk for metastatic PCa (10). Moreover, an allele carrier had an increased risk for developing metastatic PCa. There was an increased risk for PCa or metastasis in carriers of the *A* allele, which is located in the promoter region of the *PCA3* gene, although they did not find a statistically significant association between this allele and Gleason grade. Further study found no link between allele carriers and disease progression with hormonal castration resistance in patients undergoing androgen blockade therapy; however, it still suggests a link between *PCA3* and metastatic PCa.

Protein-coding genes account for only approximately 2% of the human genome, and although the remaining 98% of the transcriptional output of the human genome was once regarded as "transcriptional noise", these ncRNAs have been implicated in gene expression regulation via modification of chromatin structure, DNA methylation, RNA splicing, RNA editing, and by many other means (11). Previous studies on ncRNAs mainly focused on microRNAs, and lncRNAs have not been well studied. lncRNAs are ncRNAs that are longer than 200 nucleotides. Some lncRNAs have fairly high tissue specificity, and examination of their expression may lead to earlier diagnoses and wider targeted therapy choices. The abnormal expression of lncRNA was considered an early event in some tumors, including PCa, breast cancer, liver cancer, and colorectal cancer, among others. Although the number of ncRNA genes that may play important regulatory roles in cancer biology has increased during the past decade, functional data are only available for a small subset of these genes. However, progress has been made toward understanding their functions. For example, the function of an lncRNA named urothelial carcinoma associated 1 (*UCA1*) (12) in bladder cancer has been relatively well studied. *UCA1* influences *AKT* expression and the phosphorylation of *CREB*, which affects the cell cycle and many downstream genes. *HOTAIR* is a biomarker that plays a vital role during breast cancer progression (13). *HOTAIR* overexpression is indicative of a higher possibility of cancer invasiveness and metastasis. *HOTAIR* can bind to and targets the PRC2 complex and leads to altered histone H3 lysine 27 methylation. As a newly identified lncRNA, *ncRAN* was found to enhance human bladder cancer growth, invasion, and survival (14). *H19* levels were markedly increased in gastric cancer cells and tissues (15). *H19* upregulation increased gastric cancer cell proliferation, inhibited cell apoptosis, and positively regulated the growth of gastric cancer cells. Moreover, *H19* was associated with *p53* and activation of *E2F1*, which facilitate the growth of other tumors like breast cancer (16). *ROR*, which functions as a negative regulator of *p53*, modulates *p53*-regulated cellular processes (17). These two molecules form an autoregulatory feedback loop, and *p53* can also regulate *RoR* expression. *RNA-ROR* does not induce *p53* phosphorylation or acetylation, instead, it regulates *p53* levels though a posttranscriptional regulation mechanism. *ROR* keeps *p53* levels low even after DNA damage. Another lncRNA called *PCGEM1* is also a highly prostate-specific, non-protein-coding and androgen-regulated gene (18-20). It promotes cell proliferation, inhibits doxorubicin-induced apoptosis, and delays the induction of *p53* and $p21^{Waf1/Cip1}$. *PCGEM1* overexpression also affects cell proliferation through Rb phosphorylation. We hypothesize that the function of *PCA3* may be similar to that of *PCGEM1*.

We now know that lncRNAs, including *PCA3*, are

associated with the recurrence, metastasis, and prognosis of many different cancers. It has also been shown that when overexpressed, some lncRNAs behave like oncogenes that can promote the matrix invasion of cancer cells and tumor growth. We know that lncRNAs play important roles in the regulation of tumor-related gene and protein expression, and most studies have suggested that this regulation is the result of co-regulation by many different modulators.

PCa is a disease that is related to many different genes. Mutation of genes often influences the expression of its mRNA and protein. E-cadherin is a protein that is important for the maintenance of epithelial integrity and cell-to-cell interactions (21). Loss of function E-cadherin mutation is associated with metastasis and invasion. *PCA3* may act on E-cadherin though some signaling pathway. Polycomb group (PcG) proteins work in multiprotein complexes called Polycomb Repressive Complexes (PRCs). These are important tumor-related proteins that can repress transcription through chromatin modification. In cancer, PcG target genes are frequently epigenetically silenced by DNA methylation, and lncRNAs may regulate PcG proteins. More than 8,100 lncRNAs have been found during the past decade. Experimental evidence suggests that some lncRNAs can influence PRCs and retarget them to an occupancy pattern resembling that of the embryonic state. Approximately 20% of all human lncRNAs have been shown to bind to the PRC2 complex (22), and they may further guide PcG proteins to their target genes. EZH2 is a critical component of the Polycomb repressive complex 2 (PRC2) (23,24). It functions as a H3K27 methyltransferase when associated with PRC2. Ectopic expression of *HOTAIR* in epithelial cancer cells induces genome-wide retargeting of PRC2 to an occupancy pattern more resembling that in embryonic fibroblasts, leading to altered histone H3 lysine 27 methylation, gene expression, and increased cancer invasiveness and metastasis (13,24). Ming Luo and colleagues showed that *H19* can increase bladder cancer metastasis by associating with *EZH2*. Furthermore, *H19* could inhibit the expression of E-cadherin (22). As we mentioned before, both E-cadherin and PPC2 have certain correlations with *PCA3*. We put forward the idea that PCA3 may play a role similar to that of *H19*. However, the relationship between *PCA3* and PRC2 is not clear, and further studies are needed.

Expression of *PCA3* in peripheral blood

Although there are very few studies on *PCA3* in circulating cells (25), here has been some progress. Extraction of *PCA3* mRNA from peripheral blood has many limitations. One is the lack of reliable methods to correct for differences in RNA extraction yield. The expression of housekeeping genes is not as constant as shown in early reports, instead, it varies greatly in different experimental conditions (26,27). In Vaananen's study, only 2 of 67 prostatic carcinoma patients were limit of quantification (LOQ) + for *PCA3* mRNA (28). Healthy individuals and patients with other prostatic disorders were negative in all PCR replicate samples. In Marangon's study, they found *PCA3*-positive blood samples in patients with BPH and prostatic intra-epithelial neoplasia, and cancer (25). This result is in stark contrast to Vaananen's finding. Whether *PCA3* is only highly up-regulated in PCa is still controversial. Furthermore, even in Vaananen's study, *PCA3* did not show sufficient sensitivity. *PCA3* expression levels were lower than PSA levels, and *PCA3* was only detected in a sub-fraction of blood samples from patients with high PCa burdens.

Development and controversy of *PCA3* score

Assays using the first voided urine following a digital rectal examination (DRE) have progressed significantly over the past decade. In 2003, Hessels *et al*. demonstrated for the first time the possibility of translating the PCa specificity of *PCA3* at the tissue level into a specific test for diagnosis (29). They tested 108 urine samples and reported a sensitivity of 67%, specificity of 83%, positive predictive value of 53%, and negative predictive value of 90%. Since PCa cells with high *PCA3* levels can be shed from the prostate into the urine, *PCA3* RNA can be measured in urine sediments after DRE. Using time-resolved fluorescence (TRF) RT-PCR, *PCA3* mRNA and PSA mRNA can be detected in centrifuged urine sediment. A *PCA3* score is currently being used in some research studies. The *PCA3* score is the ratio of *PCA3* mRNA to PSA mRNA multiplied by 1,000. PSA mRNA is used to normalize the test for the number of prostate cells in the urine sediment. During the past few years, commercial methods for *PCA3* measurement that are well suited to large-scale testing have progressed greatly. The transcription-mediated amplification (TMA) assay, which uses specific target capture, can measure *PCA3* in whole urine samples mixed with an equal volume of a detergent-based stabilization buffer instead of urine sediments. TMA does not require the urine centrifugation step, which makes it a much more convenient test to determine the *PCA3* score.

Many large-scale multicenter clinical studies have confirmed that the *PCA3* score can overcome the disadvantages of the low specificity of the traditional PSA test. Demonstrating the balance between specificity and sensitivity, a *PCA3* score of 35 was adopted as a cutoff. However, no significant correlation was found between *DD3* expression and tumor stage or Gleason score in Bussemakers' study. Similar to what was found for the expression of *PCA3* in tissues, many studies have not found a significant association between *PCA3* score and pathological findings. For example, Goode *et al*. tested 289 men who underwent an initial prostate biopsy and 167 who underwent a repeat prostate biopsy, and they did not find any correlation between *PCA3* score and prostate volume (30). Augustin *et al*. performed ProgensaTM *PCA3* assays in samples from 127 patients treated with radical prostatectomy for clinically localized PCa, and found that *PCA3* showed no significant correlation with tumor volume. There was also no correlation between *PCA3* score and PSA score. Other researchers, including Van Poppel and Haese (31), also could not find any correlation between *PCA3* and tumor volume or Gleason score. However, Ploussard *et al*. found that *PCA3* score was strongly correlated with tumor volume in a linear regression analysis (32). A high *PCA3* score was an important predictive factor for tumor volume >0.5 cm^3. In addition, Nakanishi *et al*. found that the *PCA3* score was significantly correlated with total tumor volume in prostatectomy specimens and was also associated with prostatectomy Gleason score in their studies of 30 men with negative biopsies and 29 men with positive biopsies (33). Auprich *et al*. confirmed that the urinary *PCA3* score represents a valuable predictor of low-volume disease and pathologically confirmed insignificant PCa (34). Gasthuisberg *et al*. analyzed data from two studies enrolling 1,009 men and hold the opinion that the *PCA3* score is associated with many pathological features of PCa, including tumor volume and Gleason score. Durand *et al*. collected that first-catch urine after DRE of 160 patients with localized PCa and found that *PCA3* scores correlated with numerous histoprognostic factors, specifically tumor volume and positive surgical margins (35). Although the *PCA3* score may has many limitations, it can indeed reduce unnecessary prostate biopsies by 67%. Utilizing combinations of different new PCa-specific markers as predictors could further enhance the diagnostic accuracy as we stated above.

Whether a new biomarker can be conveniently detected strongly influences its clinical value. PSA levels can be influenced by many factors. Unlike the PSA score, the *PCA3* score is independent of prostate volume and whether a patient has had a prior biopsy or not, and it is unaffected by age. Because it is related to AR signaling pathways, its level can be used to endocrine drugs that are used to treat PCa. *PCA3* can also detect precancerous lesions, as more than 90% of HGPIN tissues expressed *PCA3*. Regardless of these limitations, *PCA3* is a great new biomarker with excellent specificity, and its combined use with other new tumor markers may further improve its sensitivity and specificity. Many researchers hold the opinion that *PCA3* combined with *TMPRSS2:ERG* could be a good strategy. Similar to *PCA3*, *TMPRSS2:ERG* rearrangement can be detected in urine after DRE. *PCA3* and *TMPRSS2:ERG* has been identified as the most promising biomarkers of PCa (36,37). Hessels *et al*. noted that by combining the tests for *PCA3* and *TMPRSS2:ERG*, the sensitivity of PCa detection increased markedly to 73% without compromising specificity (38). Robert *et al*. tested 48 BPH, 32 NP, and 48 PCa samples and showed that most of the false-negative results obtained with the *PCA3* test could be corrected by *TMPRSS2:ERG*; therefore, the combination can improve the sensitivity of PCa diagnosis (39). Stephan *et al*. compared tests for *PCA3*, *TMPRSS2:ERG*, and the two combined, and found that the combination of multiple biomarkers yielded only moderate enhancements in diagnostic accuracy for PCa at first or repeat prostate biopsy (40). Recent studies found that sarcosine was one of the key metabolites that were significantly overexpressed in metastatic PCa. Sarcosine may contribute to changes in proteome expression during BPH progression to PCa (41). Perhaps combining tests for sarcosine and *PCA3* can achieve an optimal result. Landers holds the opinion that the use of *PCA3*, Hepsin, and PSMA is the best based on a multivariate predictive model (42). This model correctly predicted the classification of 100% of the samples in their studies. Neves *et al*. analyzed AR, SRD5A2, KLK2, PSMA, and *PCA3* transcripts and thought that the most promising marker for PCa diagnosis was positive *PCA3* detection and serum PSA, which has 92% specificity and a 94% positive predictive value (43). Whether a patient needs a prostate biopsy mainly depends on PSA level, DRE, prostate volume, and life expectancy. These are usually called best clinical judgment (BCJ) without considering the *PCA3* scores. Tombal *et al*. tested more than 1,000 patients and found that if the *PCA3* score with a cutoff score 20 was considered, BCJ with *PCA3* could avoid 64% of unnecessary repeat prostate biopsies compared with 26% for BCJ alone and 55% for *PCA3* alone (44). Furthermore, combination

of the classical BCJ with the *PCA3* score could maintain the sensitivity to detect Gleason sum ≥7 PCa. To confirm the best strategy for PCa detection, further studies are needed.

Nomogram based on PCA3

A nomogram based on *PCA3* score could be convenient. Chun *et al*. used regression coefficients to analyze the *PCA3* assay cut-off threshold and constructed four sets of nomograms (45). They used these nomograms to help assess PCa risk at biopsy and reported good results. Auprich *et al*. used previously published prebiopsy *PCA3* gene-based nomograms and logistic regression coefficients to forecast patients' biopsy results (46). They put the results of the previously reported nomogram on the x-axis and the actual proportion of biopsy-proven PCa on the y-axis. Then, the 45° line indicates perfect agreement between the predicted probability and observed proportion of PCa cases. In their studies, the accuracy, depending on *PCA3* coding, ranged from 0.73 to 0.75, which demonstrated its clinical applicability and generalizability. Perdonà *et al*. tested 218 patients presenting with an abnormal PSA and showed that both Chun's nomogram and the PCPT calculator incorporating *PCA3* can assist in the decision to biopsy by assigning an individual risk of PCa, specifically for PSA levels <10 ng/mL (1). In addition, Hansen and his colleagues developed and validated internally the first initial biopsy specific *PCA3*-based nomogram (47). They collected 692 referred initial biopsies and biopsy data, including urinary *PCA3* score, and then used regression coefficients of logistic risk factor analyses to build the nomogram. The nomogram allows individual assessment of a man's risk of any PCa and risk of high-grade PCa. A PCA3-based nomogram could assist in the decision to biopsy. It is a wonderful tool.

PCA3 in therapeutics

The specific activity of the *PCA3* promoter in PCa cells may also be used as an additional strategy for targeted therapeutic approaches. It was previously shown that the PCa-specific expression of *PCA3* is mainly controlled by its promoter. van der Poel *et al*. used an attenuated diphtheria toxin mutant (tox176) to test the promoter's "leakiness" and cloned prostate-specific promoters, including that for *PCA3*, in an expression plasmid called *pBK* to create a new way to predict the basal promoter activity of prostate-specific gene promoters (48). As the toxicity of suicide gene therapeutics is related to basal promoter activity, predicting this can avoid unwanted side effects. They found that the basal expression of the *PCA3* promoter construct has a highly prostate-specific transactivation pattern, which suggested its potential in targeted therapy. Fan and colleagues constructed an oncolytic adenovirus carrying the therapeutic gene IL-24, in which replication is driven by the minimal *DD3* promoter (49). In their study, treatment with Ad.*DD3*-E1A-IL-24 had a significant antitumor effect on DU145 xenograft tumors in nude mice. IL-24 has been extensively shown not only to possess antiangiogenic activity but also to induce growth suppression and apoptosis in many types of carcinomas (50,51). Targeting gene-virotherapy is an attractive strategy for cancer treatment. Although DU145 is androgen-independent cell line, the *PCA3* promoter in their study showed relatively high transcriptional activity. If additional studies could identify the transcription factors that interact with the *PCA3* promoter and their binding sites, it would provide a powerful basis for the utilization of the *DD3* promoter in PCa-targeted treatment.

Conclusions

During the past few years, many new candidate biomarkers of PCa have been discovered and studied. The most specific and most promising of these is *PCA3*. In hundreds of studies, *PCA3* has been used in many different applications, including the diagnosis, treatment, and prediction of PCa, and so on. Its excellent performance has already been demonstrated in the existing studies. Although we currently have a good understanding of the role of *PCA3* in tumor genes and tissues, the picture is incomplete. Tests for *PCA3* have already been approved by the FDA to help decide whether a patient needs a prostate biopsy (44). However, we believe that if we obtain a full understanding of the roles of *PCA3* in the development and advancement of PCa, we could usher in a new era of PCa diagnosis and treatment. However, before this day arrives, many additional studies are needed.

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References

1. Perdonà S, Cavadas V, Di Lorenzo G, et al. Prostate cancer detection in the "grey area" of prostate-specific

antigen below 10 ng/ml: head-to-head comparison of the updated PCPT calculator and Chun's nomogram, two risk estimators incorporating prostate cancer antigen 3. Eur Urol 2011;59:81-7.
2. Bussemakers MJ, van Bokhoven A, Verhaegh GW, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res 1999;59:5975-9.
3. Salagierski M, Verhaegh GW, Jannink SA, et al. Differential expression of PCA3 and its overlapping PRUNE2 transcript in prostate cancer. Prostate 2010;70:70-8.
4. Clarke RA, Zhao Z, Guo AY, et al. New genomic structure for prostate cancer specific gene PCA3 within BMCC1: implications for prostate cancer detection and progression. PLoS One 2009;4:e4995.
5. de Kok JB, Verhaegh GW, Roelofs RW, et al. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res 2002;62:2695-8.
6. Ferreira LB, Palumbo A, de Mello KD, et al. PCA3 noncoding RNA is involved in the control of prostate-cancer cell survival and modulates androgen receptor signaling. BMC Cancer 2012;12:507.
7. Zhou W, Chen Z, Hu W, et al. Association of short tandem repeat polymorphism in the promoter of prostate cancer antigen 3 gene with the risk of prostate cancer. PLoS One 2011;6:e20378.
8. Gandini O, Luci L, Stigliano A, et al. Is DD3 a new prostate-specific gene? Anticancer Res 2003;23:305-8.
9. Tao Z, Shen M, Zheng Y, et al. PCA3 gene expression in prostate cancer tissue in a Chinese population: quantification by real-time FQ-RT-PCR based on exon 3 of PCA3. Exp Mol Pathol 2010;89:58-62.
10. Fontenete S, Nogueira A, Pina F, et al. Molecular study of the PCA3 gene: genotypic analysis of PCA3 polymorphism -845G>A and metastatic prostate cancer. Genet Test Mol Biomarkers 2012;16:418-22.
11. Qiu MT, Hu JW, Yin R, et al. Long noncoding RNA: an emerging paradigm of cancer research. Tumour Biol 2013;34:613-20.
12. Yang C, Li X, Wang Y, et al. Long non-coding RNA UCA1 regulated cell cycle distribution via CREB through PI3-K dependent pathway in bladder carcinoma cells. Gene 2012;496:8-16.
13. Gupta RA, Shah N, Wang KC, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010;464:1071-6.
14. Zhu Y, Yu M, Li Z, et al. ncRAN, a newly identified long noncoding RNA, enhances human bladder tumor growth, invasion, and survival. Urology 2011;77:510.e1-5.
15. Yang F, Bi J, Xue X, et al. Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. FEBS J 2012;279:3159-65.
16. Matouk IJ, DeGroot N, Mezan S, et al. The H19 non-coding RNA is essential for human tumor growth. PLoS One 2007;2:e845.
17. Zhang A, Zhou N, Huang J, et al. The human long non-coding RNA-RoR is a p53 repressor in response to DNA damage. Cell Res 2013;23:340-50.
18. Petrovics G, Zhang W, Makarem M, et al. Elevated expression of PCGEM1, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients. Oncogene 2004;23:605-11.
19. Bialkowska-Hobrzanska H, Driman DK, Fletcher R, et al. Expression of human telomerase reverse transcriptase, Survivin, DD3 and PCGEM1 messenger RNA in archival prostate carcinoma tissue. Can J Urol 2006;13:2967-74.
20. Srikantan V, Zou Z, Petrovics G, et al. PCGEM1, a prostate-specific gene, is overexpressed in prostate cancer. Proc Natl Acad Sci U S A 2000 Oct;97:12216-21.
21. Schalken J. Molecular diagnostics and therapy of prostate cancer: new avenues. Eur Urol 1998;34 Suppl 3:3-6.
22. Luo M, Li Z, Wang W, et al. Long non-coding RNA H19 increases bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression. Cancer Lett 2013;333:213-21.
23. Cao Q, Yu J, Dhanasekaran SM, et al. Repression of E-cadherin by the polycomb group protein EZH2 in cancer. Oncogene 2008;27:7274-84.
24. Tsai MC, Manor O, Wan Y, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010;329:689-93.
25. Marangoni K, Neves AF, Cardoso AM, et al. The endothelial nitric oxide synthase Glu-298-Asp polymorphism and its mRNA expression in the peripheral blood of patients with prostate cancer and benign prostatic hyperplasia. Cancer Detect Prev 2006;30:7-13.
26. Huggett J, Dheda K, Bustin S, et al. Real-time RT-PCR normalisation; strategies and considerations. Genes Immun 2005;6:279-84.
27. Dheda K, Huggett JF, Bustin SA, et al. Validation of housekeeping genes for normalizing RNA expression in real-time PCR. Biotechniques 2004;37:112-4,116,118-9.
28. Väänänen RM, Rissanen M, Kauko O, et al. Quantitative real-time RT-PCR assay for PCA3. Clin Biochem 2008;41:103-8.

29. Hessels D, Klein Gunnewiek JM, van Oort I, et al. DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 2003;44:8-15; discussion 15-6.
30. Goode RR, Marshall SJ, Duff M, et al. Use of PCA3 in detecting prostate cancer in initial and repeat prostate biopsy patients. Prostate 2013;73:48-53.
31. van Poppel H, Haese A, Graefen M, et al. The relationship between Prostate CAncer gene 3 (PCA3) and prostate cancer significance. BJU Int 2012;109:360-6.
32. Ploussard G, Durand X, Xylinas E, et al. Prostate cancer antigen 3 score accurately predicts tumour volume and might help in selecting prostate cancer patients for active surveillance. Eur Urol 2011;59:422-9.
33. 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;179:1804-9; discussion 1809-10.
34. Auprich M, Chun FK, Ward JF, et al. Critical assessment of preoperative urinary prostate cancer antigen 3 on the accuracy of prostate cancer staging. Eur Urol 2011;59:96-105.
35. Durand X, Xylinas E, Radulescu C, et al. The value of urinary prostate cancer gene 3 (PCA3) scores in predicting pathological features at radical prostatectomy. BJU Int 2012;110:43-9.
36. Hessels D, Schalken JA. The use of PCA3 in the diagnosis of prostate cancer. Nat Rev Urol 2009;6:255-61.
37. Tomlins SA, Bjartell A, Chinnaiyan AM, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol 2009;56:275-86.
38. 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;13:5103-8.
39. Robert G, Jannink S, Smit F, et al. Rational basis for the combination of PCA3 and TMPRSS2:ERG gene fusion for prostate cancer diagnosis. Prostate 2013;73:113-20.
40. Stephan C, Jung K, Semjonow A, et al. Comparative assessment of urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion with the serum [-2] proprostate-specific antigen-based prostate health index for detection of prostate cancer. Clin Chem 2013;59:280-8.
41. Schalken JA. Is urinary sarcosine useful to identify patients with significant prostate cancer? The trials and tribulations of biomarker development. Eur Urol 2010;58:19-20.
42. Landers KA, Burger MJ, Tebay MA, et al. Use of multiple biomarkers for a molecular diagnosis of prostate cancer. Int J Cancer 2005;114:950-6.
43. Neves AF, Araújo TG, Biase WK, et al. Combined analysis of multiple mRNA markers by RT-PCR assay for prostate cancer diagnosis. Clin Biochem 2008;41:1191-8.
44. Tombal B, Andriole GL, de la Taille A, et al. Clinical judgment versus biomarker prostate cancer gene 3: which is best when determining the need for repeat prostate biopsy? Urology 2013;81:998-1004.
45. Chun FK, Steuber T, Erbersdobler A, et al. Development and internal validation of a nomogram predicting the probability of prostate cancer Gleason sum upgrading between biopsy and radical prostatectomy pathology. Eur Urol 2006;49:820-6.
46. Auprich M, Haese A, Walz J, et al. External validation of urinary PCA3-based nomograms to individually predict prostate biopsy outcome. Eur Urol 2010;58:727-32.
47. Hansen J, Auprich M, Ahyai SA, et al. Initial prostate biopsy: development and internal validation of a biopsy-specific nomogram based on the prostate cancer antigen 3 assay. Eur Urol 2013;63:201-9.
48. van der Poel HG, McCadden J, Verhaegh GW, et al. A novel method for the determination of basal gene expression of tissue-specific promoters: an analysis of prostate-specific promoters. Cancer Gene Ther 2001;8:927-35.
49. Fan JK, Wei N, Ding M, et al. Targeting Gene-ViroTherapy for prostate cancer by DD3-driven oncolytic virus-harboring interleukin-24 gene. Int J Cancer 2010;127:707-17.
50. Poindexter NJ, Walch ET, Chada S, et al. Cytokine induction of interleukin-24 in human peripheral blood mononuclear cells. J Leukoc Biol 2005;78:745-52.
51. Caudell EG, Mumm JB, Poindexter N, et al. The protein product of the tumor suppressor gene, melanoma differentiation-associated gene 7, exhibits immunostimulatory activity and is designated IL-24. J Immunol 2002;168:6041-6.

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