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 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)
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. Busssemakers 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. Busssemakers 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 IncRNAs have not been well studied. IncRNAs are ncRNAs that are longer than 200 nucleotides. Some IncRNAs have fairly high tissue specificity, and examination of their expression may lead to earlier diagnoses and wider targeted therapy choices. The abnormal expression of IncRNA 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 IncRNA 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 IncRNA, 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 IncRNA 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 IncRNAs, 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 cm³. 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 held 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 75% 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
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 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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