REVIEW

New Molecular Markers for Prostate Cancer Diagnosis

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Purpose: Prostate cancer (PCa) is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men worldwide. Biomarkers are an important tool in the early detection of PCa. Prostate-specific antigen (PSA) is one of the oldest biomarkers for the early detection of PCa. Digital rectal exam (DRE) is another screening test for PCa detection, which is considered as an irritating experience for patients. Biopsy is still the most reliable method for PCa diagnosis; however, patients are prone to complications. Therefore, developing non-invasive and accurate methods for PCa screening seems urgent to avoid unnecessary biopsies. There has been remarkable development in PCa molecular biomarkers discovery, largely through progress in omics technologies. Due to the many benefits of liquid biopsies, a significant set of PCa diagnostic kits have been developed using urine samples. Despite the unique benefits of these kits, there are still many challenges to their widespread use in clinics. Here, we have reviewed the latest developments of PCa biomarkers in liquid biopsies.

Methods: Literature on biomarkers for diagnosis of PCa was reviewed during the past two decades.

Results: PSA, PHI, PCA3, and 4K score are among the commonly used markers for PCa diagnosis which have been used over a long-moderate length of time with multiple studies on their performance. We performed a review of their performance. Newer markers are among RNA and DNA markers. Multiple non-coding RNAs (mi-RNAs) were reviewed and their performance on Pca diagnosis was reviewed. Long noncoding RNAs (Lnc RNAs) including PlncRNA-1, HOTAIR, SchLAP-1, MALAT1, MEG3, and PRCAT17.3 were summarized. mRNA markers including TMPRSS2:ERG, and HOXC6 were presented. DNA-based markers including PTEN, HOXB13, and BRCA2 were reviewed. Finally, the use of CircRNAs was reviewed for PCa diagnosis.

Conclusion: Many reviewed RNA-based biomarkers have promising results in the diagnosis of PCa.

Keywords: prostate cancer; PSA; non-invasive biomarkers; liquid biopsy; molecular biomarkers

INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men worldwide. According to Cancer Statics 2022 prostate cancer is the most common malignancy in American men accounting for 27% of all diagnoses and is the second cancer that causes death after lung cancer.[1-2] The incidence and prevalence of PCa differ in various parts of the world, with the most incidence in North America and the least one in South Asia [3]. These differences are considered to have more to do with different degrees of genetic susceptibility, and in the accessibility and disposal of medical care, the diagnosis of latent cancers, and surgery for benign prostatic hyperplasia (BPH)[1-4]. A wide variety of PCa are of epithelial origin, and other rare forms are sarcomas (malignant mesenchymal neoplasms) and lymphomas (hematolymphoid neoplasms)[5].

The main pathological markers associated with prognostic information in prostate cancer are Gleason grade and pathological stage and a good prognostic biomarker can be valuable in the prediction of pathological characteristics. One of the oldest biomarkers used in the early detection of PCa is PSA, a serine protease kallikrein protein produced by the prostate epithelial cells. It is noteworthy that, PSA is not exclusively a PCa-specific biomarker and is identified as an ordinary marker of prostate diseases such as BPH, prostatitis, ejaculation, trauma, and infection. Therefore, its use as a primary screening test for PCa detection is challenging. Indeed, low specificity of serum PSA, the unclear benefits of PSA screening for reducing PCa deaths, and the harms of over-diagnosing indolent diseases have called PSA screening into question. Because of over-diagnosis and unnecessary treatments, especially for still-progressing cancers that can cause incontinence or impotence and affects the patients' quality of life, many European countries prevent health care systems from running national screening programs for PCa. DRE is another screening test for PCa detection, which is considered as an irritating experience for patients. In a study by Jones et al., the sensitivity and specificity for DRE as a predictor of PCa in symptomatic patients and the positive and negative predictive values were reported 28.6%, 90.7%, 42.3%, and 84.2%, respectively. Abnor-

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Table 1. List of protein-based biomarkers.

Biomarker	Cohort size	Main result	Ref.
PSA	379 PCa patients, 394 BPH patients	Despite its advantages in PCa screening it often leads to over-diagnosis and does not provide suitable specificity.	(23)
fPSA	19,643 subjects	AUC of 0.70 for detecting PCa in all PSA levels.	(24)
		A useful adjunct in the gray zone only when levels reach extreme values. 20% fPSA cut-off would lead to 92% sensitivity and 23% specificity.	(26)
proPSA	31 PCa, 88 healthy men	Showed more accuracy in detecting PCa compared to %fPSA in the PSA range of 2.5 to 4 ng/ml (AUC of 0.688 vs 0.567)	
PHI([-2] proPSA, fPSA, and total PSA)	892 men with PSA range from 2 to 10 ng/ml	Outperformed PSA and %fPSA Higher levels were associated with more aggressiveness and higher Gleason score	(29)
four kallikreins	740 men undergoing biopsy	Outperformed PSA-based model in detection of any PCa Reduced biopsies by 57% while missing 3 out of 40 HR PCa and 31 out of 152 LR PCa.	(30)
4K score (Four kallikreins + DRE, age and history of prior biopsy)	1012 men	Could reduce 30-58% of biopsies while missing only 1.3-4.7% of patients with high-grade PCa.	(31)

mal DRE is prone to malignancy in about 42.3% of the cases, above the 3% risk threshold that NICE guidance suggests an urgent referral[6-9]. Systematic tissue biopsy, however, remains the standard of care for PCa diagnosis. This approach misses 21%-28% of PCa and undergrads them in 14% -17% of cases. With every prostate biopsy, patients are liable to complications such as an infection, hematuria, and pain. The risk of some complications, such as infection, increases with the number of previous biopsies they have had under active surveillance[10,11].

Magnetic resonance imaging (MRI) has emerged as a crucial tool in the detection and evaluation of prostate cancer. In a recent article titled "Role of multiparametric prostate MRI in the management of prostate cancer," the authors highlight the significance of multiparametric MRI (mpMRI) in improving the accuracy of prostate cancer diagnosis. MpMRI combines various imaging sequences to provide detailed information about the prostate gland, including structural and functional characteristics. By assessing parameters such as T2-weighted imaging, diffusion-weighted imaging, and dynamic contrast-enhanced imaging, mpMRI enhances the visualization and localization of suspicious lesions. The article emphasizes that mpMRI has demonstrated superior sensitivity and specificity compared to traditional screening methods, such as prostate-specific antigen (PSA) testing and transrectal ultrasound (TRUS). Moreover, mpMRI plays a vital role in guiding biopsy procedures, enabling targeted biopsies of suspicious areas while reducing unnecessary sampling. Overall, the integration of mpMRI into prostate cancer management offers valuable advantages, including improved diagnostic accuracy, better risk stratification, and enhanced decision-making for treatment strategies[12]. While mpMRI is a useful tool for diagnosing prostate cancer, but it has several limitations. False-positive and false-negative results can occur, leading to unnecessary biopsies or missed cancers. Interpretation of mpMRI images requires expertise, leading to variability in results. It may not be widely available, particularly in resource-limited settings, and it can be costly.

According to EAU guidelines, Prostate cancer is typically suspected based on findings from digital rectal examination (DRE) and/or prostate-specific antigen (PSA) levels. A definitive diagnosis is established through histopathological verification, which involves examining biopsy cores from the prostate, specimens obtained from transurethral resection of the prostate, or prostatectomy procedures for benign prostatic enlargement. The decision to pursue additional diagnostic or staging evaluations is guided by the availability of treatment options for the patient, while also considering their life expectancy.

Finding a suitable marker has many advantages including fewer biopsies for false positive results of screening tests. By the way, finding cancer in the earliest pre-clinical stage has benefits in terms of the possibility for application of less invasive surgery with better disease-specific survival and overall survival. Compared to solid biopsies, liquid biopsies, especially circulating nucleic acids, are a more convenient and more cost-effective option for patients. Among other benefits, liquid biopsies can detect cancer early, before radiologic and imaging techniques are used to detect tumors. Secondly, liquid biopsies are able to characterize the tumor as a whole, in particular in heterogeneous tumors. This is in contrast to solid biopsies that assess only specific fragments of the whole tumor, and therefore, have the potential to miss some significant locations of the tumor. Thirdly, the sequential sampling of liquid biopsies in order to enable progress monitoring, modulation of treatment, and early detection of recurrence is more feasible. RNA biomarkers have higher sensitivity and specificity than proteins and are less expensive. Moreover, RNAs allow physicians to make more dynamic distinctions between cell regulation and state than DNA markers[13].

It is worth mentioning that the organism-specific homeostasis mechanism obliterates a vast range of potential biomarkers, especially in the early stages of tumorigenesis. However, due to a lack of homeostasis mechanisms, urine is capable of incorporating more changes, especially in the early stages of cancer. The direct contact of urine with prostate tissues has made it to be a promising source of released tumor RNAs. Circulating urinary biomarkers can aid in the decision to perform a prostate biopsy, or in the development of a therapeutic strategy[14]. Since prostate manipulation enriches the urine with PCa biomarkers, the DRE is also performed before sampling. Without a doubt, DRE is considered an unpleasant experience for patients. Some biomarkers are also released into urine without the need for prostate manipulation if they are sensitively detected or present in high concentrations in the urine[14-16].

Several new biomarkers for diagnostic prostate cancer have recently come to the market. Some of these biomarkers are PSA derivatives, such as free PSA (fPSA)

Table 2. List of miRNA-based biomarkers

Author	Biomarker	Cohort	Main result	Ref
Ghorbanmehr et al, 2019	miR-141, miR-21, miR-205	45 BCa, 23 PCa, 22 BPH, and 20 healthy control	Distinguished BPh from PCa, also BCa and PCa from healthy controls	(16)
Mitchell et al, 2008	miR-141	25 mPCa, 25 healthy controls	Distinguished high-grade PCa from healthy controls with great sensitivity and specificity (AUC of 0.907)	(35)
Chen et al, 2012	miR-622, −1285, −30c,let-7e, let-7c	44 BPH, 54 healthy controls, and 80 PCa	Differentially expressed in PCa than BPH (AUC of 924)	(36)
Mihelich et al, 2015	miR-223, miR-130b, miR-107, miR-26b, let-7a, miR-451, miR-106a	50 BPH, 50 low-grade PCa, 50 high-grade	A panel of 7 highly expressed miRNAs in PCa for predicting risk of biochemical recurrence. (NPV of 0.941)	(37)
Selth et al, 2012	miR-141, −298, − 375	25 mCRPC patients and 25 healthy men	Overexpressed in PCa. No correlation between the Gleason score and tumor stage.	(38)
Nguyen et al, 2013	miR-375, -378*, -141 miR-141 and miR-375 miR-409-3p	28 LR PCa, 30 HR PCa, and 26 mCRPC Overexpressed in PCa Down-regulated in CRPC	Overexpressed in CRPC	(39)
Sharova et al, 2016	miR-106a/miR-130b miR-106a/miR-223	36 PCa, 31 BPH	Significantly different ratios in PCa and BPH. Showed better performance in PCa predicting than PSA. (AUC of 0.84 vs 0.56)	(40)
Al-Qatati et al, 2017	miR-16, miR-148a, miR-195	79 PCa, 33 healthy controls	Up-regulated in PCa ($p < 0.006$) and associated with Gleason score.	(41)
Salido-Guadarrama et al, 2016	miR-100/200b	73 PCa, 70 BPH	When added to PSA, DRE, %fPSA, and age increased the accuracy of discriminating PCa from BPH. (AUC of 0.816 to 0.876)	(42)
Fredsøe et al, 2018	miR-222-3p* miR-24-3p/miR-30c-5p (miR-125b-5p*let-7a-5p/miR-151-5p)	29 BPH, 215 PCa	Distinguished PCa from BPH with AUC of 0.95. predicted time to biochemical recurrence after RA	(43)
Pashaei et al, 2017	miR-125A, miR-199A-3P, miR-28-5P, miR-301B, miR-324-5P, miR-361-5P, miR-363*, miR-449A, miR-484, miR-498, miR-579, miR-637, miR-720, miR-874 and miR-98	6 datasets .	Overexpressed in recurrent PCa	(44)
Metcalf et al, 2016	miR-141, miR-375 miRNAs in EV	220 PCa, 29 BPH	High expression in active PCa compared to the remission phase. Associated with metastasis.	(45)
Li et al, 2016	miR-141	20 PCa, 20 BPH, 20 healthy controls	Discriminating mPCa from localized PCa with 80% sensitivity and 87% specificity Upregulated in PCa serum EVs.	(47)
Huang et al, 2015	miR-1290, miR-375	100 CRPC	Elevated levels were associated with poor overall survival.	(48)
Foj et al, 2017	miR-21 + miR-375	60 PCa, 10 healthy controls	Efficient combination for discriminating PCa from normal. (AUC of 0.872)	(49)
Kanagasabai et al, 2022	mir-21	Mouse model and human PCa cell line	Promotes PCa progression through the IRS1/SREBP-1 pathway.	(50)
Samsonov et al, 2016	miR-21, -141 and - 574	35 PCa, 35 healthy controls	Overexpressed in urine samples of PCa patients.	(51)
Alhasan et al, 2016	miR-200c, miR-135a*, miR-605, miR-433, and miR-106a	8 HR PCa, 4LR PCa, 2 healthy men	Could discriminate aggressive PCa from low-risk with at least 89% accuracy.	(53)

and [–2] proPSA. Some of the biomarkers are based on combinations of serum markers, such as the Prostate Health Index (PHI), which uses a combination of total PSA, fPSA, and [–2] proPSA to generate a score, and the 4Kscore, which uses a panel of total PSA, fPSA, intact PSA, and human kallikrein 2 (hK2) to estimate a patient's risk of high-grade cancer (Gleason score ≥7) on biopsy. Other molecular biomarkers include prostate cancer antigen 3 (PCA3) and TMPRSS2: ERG (T2: ERG), which are detectable in the post-DRE urine. The Mi-Prostate Score (MiPS) early detection test combines a patient's serum PSA, urine PCA3 score, and urine T2: ERG score in a multivariate regression model to estimate individualized risk estimates for all prostate cancers and high-grade prostate cancer. These tests vary in the outcome they predict (all cancer vs. high-grade cancer) and in their sensitivities and specificities. No study has yet attempted to compare these biomarkers to determine which characteristics achieve optimal long-term health outcomes[17]. The Progensa PCA3 test, which uses a transcription-mediated amplification (TMA) assay to calculate a quantitative PCA3 score, has been extensively studied as a urine-based PCA biomarker and is approved by the Food and Drug Administration (FDA) for estimating PCA risk after a negative biopsy[9]. A novel molecular signature (EXO106 score) derived from non-DRE urine demonstrated independent, Negative predictive value (NPV) for the diagnosis of high-grade PCa from an initial biopsy for men with 'gray zone' serum PSA levels. Its use in the biopsy decision process could result in fewer prostate biopsies for clinically insignificant diseases[18]. Approximately 2 million transrectal ultrasonography-guided prostate biopsies (TRUS-Bx) are performed each year in the United States and Europe. While suspicious DRE, in combination with other SOC factors, such as age, race, family history, and ethnicity, occasionally prompts TRUS-Bx, in most patients, it is triggered by a PSA level of 2.5-

Table 3. List of circular RNAs-based biomarkers.

Circular RNA	Main result	Ref
circCSNK1G3	Promotes cell proliferation in interaction with miR-181.	(92)
circAMACR	Associated with AR amplification in CRPC Upregulated in CRPC	(91)
circAURKA	Upregulated in NEPC	(95)
circBAGE2	Upregulated in PCa cells and promotes cell proliferation through inhibiting mir-103a	
circMYLK	Upregulated in PCa cell lines and samples. Decreased cell apoptosis by inhibiting mir-29a.	
circCDR1as	Associated with bone metastasis. Upregulated in PCa cell lines and inhibits mir-7	(95)
circSLC7A6	Associated with bone metastasis.	(95)
circFoxo3	Downregulated in high-grade PCa. Negative correlation with chemoresistance to docetaxel.	(93)
Circ-0004870	Downregulated in enzalutamide-resistant and malignant cells.	(98)
circSMARCA5	Upregulated in PCa cells and acts as an oncogene.	(97)
Circulating circRNAs in PCa	circPDLIM5, circSCAF8, circPLXDC2, circSCAMP1, and circCCNT2 positively associated with Gleason scores and lymph node metastasis of primary tumors	(99)
Circ-0044516	upregulated in EV derived from PCa patients' plasma	(100)
circAR3	positively associated with Gleason scores and lymph node metastasis of primary tumors	(101)
circZMIZ1	Upregulated in PCa patients compared to BPH	(102)

4.0 ng/mL or higher. The procedure is costly, painful, and has an increased risk of infection and sepsis. Clinical assessment tools, such as the PCPTRC have value in assessing risk improvements in patient selection for biopsy and can dramatically reduce cost and complications[19]. The Sentinel_ PCa test is the first report of the development and performance of a platform that interrogates small noncoding RNAs (sncRNA) isolated from urinary exosomes. The performance characteristics of the Sentinel PCa ((Grade Group 1) sensitivity 94% and specificity 92%) and the Sentinel HG ((Grade Group 2-5) sensitivity 94% and specificity 96%) make it possible to accurately distinguish between non-cancerous and cancerous, as well as low-grade and high-grade states, by interrogating the sncRNAs present in urinary exosomes. The combination of the Sentinel PCa and Sentinel HG tests has the benefit of identifying subjects who have no evidence of prostate cancer, and those patients who harbor high-grade disease, from a single urine sample[20].

Molecular diagnosis of prostate cancer

2.1. Protein-Based Biomarkers

2.1.1. PSA and fPSA

PSA has been the most useful biomarker for early diagnosis of PCa diagnosis and disease monitoring. However, the specificity of the total PSA (tPSA) within the range of 4 to 10 ng/mL (gray zone) is low and can cause misdiagnosis or many unnecessary biopsies. Only a small fraction of PSA circulates freely, which is called fPSA[21-23]. The ratio of fPSA to total PSA (%fPSA) is significantly decreased in PCa patients. In a meta-analysis of 19,643 subjects, it became clear that the %fPSA improves clinical information for performing a prostate biopsy, only when levels reach extreme values. A cut-off of 20% led to 92% sensitivity and 23% specificity. Moreover, in another study, a cut-off of 25% or less fPSA was recommended for men with PSA levels range of 4-10ng/ml[23,24].

2.1.2. proPSA

The fPSA is composed of three fragments: benign PSA (BPSA), iPSA, and proPSA. ProPSA is more related to PCa than BPH. The original form of proPSA is [-7] proPSA, named after its 7-amino acid N-terminal pro-leader peptide. The other shorter forms of proPSA, known as [-2] [-4] and [-5] proPSA, are made via the proteolytic cleavage of the 7-amino acid peptide catalyzed by the hK2 and hK4[25]. In a study on serum samples of 119 men, including 88 healthy controls and 31 PCa patients with a total PSA range of 2.5 to 4 ng/ml, PSA and %fPSA were almost identical, while proPSA tended to be higher in PCa samples (P = 0.07). ROC analysis showed an area under the curves (AUC) of 0.688 for pPSA compared to 0.567 for %fPSA. The results demonstrated that with a fixed 75% sensitivity, the specificity was much higher in pPSA (59%) than in fPSA (33%)[26]. Semjonow et al. claimed that two freeze cycles do not affect the stability of [-2] proPSA, it is also stable at room temperature for a maximum of 48 hours but should be stored at a 4°C refrigerator for a longer period[27].

2.1.3. PHI

After many studies proved the usefulness of p2PSA in managing PCa, the PHI was introduced, a novel multiparametric indicator that combines the values of [-2] proPSA, fPSA, and total PSA into one index. 892 men with no history of PCa, a normal DRE, and a PSA range of 2 to 10 ng/mL who did a biopsy test were analyzed. The results revealed that in the specific range of PSA and at 80 to 95% sensitivity, the AUC and specificity of PHI significantly outperformed fPSA and PSA. Also, a higher PHI, was associated with a notable increased risk of prostate cancer and a higher Gleason score[28]. Similarly, in another study by Lazzeri et al. on 646 patients from five European urologic centers with 2-10 ng/ml PSA levels who underwent a biopsy test, PHI and p2PSA showed higher accuracy in predicting PCa compared to tPSA and %fPSA. Furthermore, at a 27.6 cut-off for PHI a large number (15.5%) of unnecessary biopsies could have been avoided[29]. PHI got FDA approval for predicting PCa in men older than 50 with a PSA range of 4-10 ng/mL and a normal DRE. The National Comprehensive Cancer Network (NCCN) also recommended PHI for men who have never undergone a biopsy or after a negative biopsy.

2.1.4. four-kallikrein panel

In 2008, Vickers et al. suggested a multivariable model including total PSA, fPSA, intact PSA, and hK2, simply named the four-kallikrein panel. Results demonstrated that this panel elevated the AUC for predicting PCa at biopsy from 0.68 to 0.83 in a base laboratory model. It

also increased the AUC for the detection of high-grade PCa from 0.793 to 0.870. Using a four-Kalkerin panel would have spared 424 biopsies (57%) while missing only 3 out of 40 high-grade and 31 out of 152 low-grade PCas[30].

2.1.5. 4K score

The addition of these four kallikreins to age, DRE, and history of prior prostate biopsy makes an indicator known as the 4Kscore. A large-scale study analyzing 1012 men across the United States reported that 30-58% of biopsies could have been reduced while missing only 1.3-4.7% of patients with Gleason grade$\geq$7[31]. More recently, Dhondt et al., identified a novel biological profile in PCa urine samples via mass spectrometry-based proteomic analysis of urinary extracellular vesicles (uEV), in patients with BPH and advanced PCa, which was not identified by analysis of soluble proteins[32]. For example, FASN and FABP5, two enzymes involved in dysregulated lipid metabolism, were overexpressed in uEV in addition to PCa tissue[33,34]. A list of protein-based biomarkers is summarized in **Table 1**.

3.1.1. miRNAs

Ghorbanmehr et al. evaluated the expression level of three miRNAs, including miR-21, miR-141 and miR-205 in urine samples collected from 110 men with either prostate or bladder cancer (BCa), BPH and healthy controls. The results showed that all three miRNAs were upregulated in both malignancies significantly and could indicate possible presence of bladder or prostate cancer. In addition, all these miRNAs could distinguish BPH from PCa patients in prostate cancer[16].

By measuring serum levels of microRNAs (miRNAs) in 25 patients with metastatic prostate cancers and 25 healthy men, Mitchell et al. reported that tumor-released miR-141 can discriminate high-grade PCa patients from healthy controls, with high sensitivity and specificity. The finding indicates that circulating miRNAs can be used as novel biomarkers for PCa diagnosis[35]. Chen et al.[36] claimed that five circulating miRNA levels, including miR-622, miR-1285, let-7e, let-7c, and miR-30c, were notably different in serums of PCa patients compared to BPH and healthy controls, respectively, with the AUC of 0.924 and 0.860. At first, they analyzed a small group of patients, including 17 BPH and 25 PCa cases, for screening and detection of those miRNAs, and then to validate candidate miRNAs, a larger group (80 PCa, 44 BPH, and 54 healthy controls) was analyzed. They also identified five other serum miRNAs (miR-375, miR-200a, miR-210, miR-200c, and miR-141) associated with metastatic castration-resistant prostate cancer (mCRPC). Mihelich et al.[37] have done a large-scale study on circulating miRNAs and their usefulness as potential biomarkers by evaluating 21 miRNAs in 150 patients (50 BPH and 100 PCa patients). They found out that 14 miRNAs had higher levels in the serum of patients with low-grade PCa or BPH than patients with high-grade PCa. These data contributed to the miR Score, an indicator for the expression levels of those 14 miRNAs to predict the absence of advanced PCa among PCa and BPH patients. Moreover, the team developed the miR Risk Score based on 7 miRNAs (miR-223, miR-130b, miR-107, miR-26b, let-7a, miR-451, and miR-106a) in serum samples to classify patients with low risk of recurrences. By measuring miRNAs in serum samples of 25 patients with mCRPC and 25 normal controls, it was validated that three out of four altered miRNAs in mice models of PCa were the same in humans, including miR-141, miR-298, and miR-375. None of them showed a correlation with tumor stage or Gleason score[38]. Nguyen et al. reported that miR-375, miR-378*, and miR-141 were significantly up-regulated in serum samples of castration-resistant prostate cancer (CRPC) patients compared to serum from low-risk localized patients, while miR-409-3p was down-regulated. However, comparing PCa patients to normal controls, only miR-141 and miR-375 were significantly over-expressed. This highly supports the potential importance of circulating miRNAs in PCa progression[39]. In a case study on 67 individuals (36 patients with PCa and 31 patients with BPH), Sharova et al.[40] identified that the miR-106a/miR-130b and miR-106a/miR-223 ratios are significantly more sensitive and specific than PSA in discriminating localized PCa from BPH. An AUC of 0.84 was obtained when both miRNA ratios together were used as predictors, which were significantly higher than the AUC of 0.56 acquired for PSA. Three miRNAs including miR-16, miR-148a, and miR-195 which are involved in the regulation of the PI3K/Akt signaling pathway, were considerably (p-value < 0.006) upregulated in PCa patients compared to healthy controls. In addition, levels of those specific miRNAs were correlated with a higher Gleason score [41].

In measuring miRNAs levels on 73 urine samples from patients with advanced PCa and 70 BPH patients, addition of the miR-100/200b ratio as a factor to a base model including multiple variables such as PSA, age, the percentage of fPSA and DRE, outperformed the base model in discriminating PCa patients from BPH [42]. Similarly, Fredsøe et al. measured the expression levels of miRNAs in cell-free urine samples of 29 BPH patients and 215 patients with localized PCa via RT-PCR. In addition to reporting several deregulated miRNAs in urine samples from PC patients, they put a new three-miRNA model (miR-222-3p* miR-24-3p/miR-30c-5p) forward that discriminated BPH and PCa patients with an AUC of 0.95. They also introduced another prognostic three-miRNA model (miR-125b-5p*, let-7a-5p/miR-151-5p) that predicted time to biochemical recurrence after radical prostatectomy (RA), independently of common parameters. Furthermore, their results were validated in another independent cohort of almost the same number of BPH and PCa patients[43]. A meta-analysis by Pashaei et al. revealed that miR-125A, miR-199A-3P, miR-28-5P, miR-301B, miR-324-5P, miR-361-5P, miR-363*, miR-449A, miR-484, miR-498, miR-579, miR-637, miR-720, miR-874, and miR-98 are significantly overexpressed in recurrent PCa samples, compared to non-recurrent PCa samples[44]. Metcalf et al. developed a peptide nucleic acid (PNA)-based biosensor for detecting endogenous concentrations of circulating miRNAs in serum that does not require any amplification step. PNA is an artificial oligonucleotide analog capable of hybridizing to complementary nucleic acids with high affinity and specificity. Using a cohort of 220 PCa patients and 29 BPH, the authors detected elevated levels of miR-141 and miR-375 in samples from patients with active forms of PCa compared to those in remission, with the highest levels noticed in metastatic PCa patients. To validate this nov-

el technology, the same RNA samples were analyzed using the gold standard of RT-qPCR, and the results were comparable. The advantages of this technology were that it was low-cost and isothermal. The authors also mentioned that although analyses were performed on extracted RNA samples at first, similar results were also attained when using their probes directly in serum without any amplification and processing steps. It is very crucial because the way that blood samples are collected, stored, and processed can significantly influence the results of miRNA analyses[45]. miRNAs with a potential application as PCa biomarkers are listed in **Table 2**.

3.1.1.1. miRNAs in Exsomes

Exosomes play a significant role in tumor progression by promoting angiogenesis and the migration of tumor cells during metastasis. They also facilitate the spread of pathogenic agents through their interaction with recipient cells. These structures are capable of inducing a process called "epithelial to mesenchymal transition," which involves the transdifferentiation of cells. Due to their composition and ability to interact with cells, exosomes act as versatile regulators of cancer development. Moreover, their biophysical properties, including stability, biocompatibility, permeability, low toxicity, and low immunogenicity, make them ideal for developing innovative drug delivery systems[46].

Li and colleagues reported that the level of miR-141 in serum exosomes was significantly higher in patients with PCa compared with those with BPH and healthy controls. Moreover, the highest expression levels of miR-141 were detected in metastatic PCa, compared with localized PCa. Accordingly, ROC curve analysis revealed that the serum exosomes miR-141 could distinguish metastatic PCa from localized PCa with 80% sensitivity and 87.1% specificity[47]. According to Huang's study, higher levels of plasma exosomal miR-1290 and miR-375 were correlated with overall poor survival. Furthermore, the authors demonstrated that incorporating the expression levels of these miRs into recognized clinical prognostic parameters in the CRPC stage improves predictive performance from 0.66 to 0.73[48]. After analyzing urinary pellets and urinary exosomes from 60 PCa patients and 10 healthy men, Foj et al.[49] stated that miR-21 and miR-375 were significantly upregulated in PCa patients in both urinary pellets and exosomes. The authors also suggested a panel combining miR-21 and miR-375 to distinguish PCa patients and healthy subjects, with an AUC of 0.872. Additionally, miR-21, miR-141, and miR-214 were found significantly deregulated in intermediate/high-risk PCa compared to low-risk/healthy subjects in urinary pellets, supporting their potential efficiency as biomarkers in PCa. More recently, Kanagasabai et al. highlighted the role of miR-21 in PCa progression. They identified miR-21 overexpression caused an increased level of a protein called Sterol regulatory element-binding protein 1 (SREBP-1), an important transcription factor in lipogenesis, which is important for disease progression. According to the study, upregulation of the mir-21/SREBP-1 signaling pathway elevated cell proliferation and migration in human PCa cell lines (in vitro) and in a mouse PCa model (in vivo)[50]. Samsonov et al. isolated urinary exosomes from 35 PCa patients and 35 healthy volunteers using a Lectin-induced aggregation method, which was a simple and low-cost method for analyzing different miRNA expressions. The results showed that miR-21, miR-141, and miR-574 were upregulated in PCa patients, compared with healthy controls[51]. In 2012, Alhasan et al. developed a novel platform called Scano-miR to detect infrequent miRNAs with high specificity. The new platform was able to detect 1 Femtomolar concentration of miRNA in serum with single nucleotide mismatch specificity. As a result, it significantly improved sensitivity for miRNA targets, when compared to molecular fluorophore-based detection platforms, which failed to detect 88% of low-abundance miRNAs under similar conditions[52]. Later, authors used the mentioned platform (scano-miR) to estimate exosomal miRNAs in serum samples from 8 high-risk PCa patients, 4 low-risk patients, and 2 healthy controls. The authors described and validated a miRNA profile including five miRNAs (miR-200c, miR-135a*, miR-605, miR-433, and miR-106a) that could distinguish between the aggressive and indolent forms of the disease with at least 89% accuracy [53]. A list of miRNAs enriched in extracellular vesicles (EV) released from prostate cancers is summarized in **Table 2**.

3.1.2. lncRNA

3.1.2.1. PCA3

The diagnostic value of PCA3, previously known as DD3, was first demonstrated in PCa by Mitra and colleagues[54]. In another study, PCA3 was overexpressed in 95% of PCa tumors, as determined by RT-PCR analysis. Meanwhile, its absence in 18 different normal human tissues suggested a high specificity for detection of PCa[55]. PCA3 encodes a long non-coding RNA (lncRNA) involved in cell survival, through modulating androgen receptor (AR) signaling. Silencing the PCA3 gene employing siRNA or shRNA revealed a significant alteration in the expression of 16 cancer-related genes, especially AR cofactors genes and epithelial-mesenchymal transition (EMT) markers. Finally, the loss of viability of the transgenic cells supported the idea of silencing the PCA3 gene as a therapeutic approach for holding PCa progression back[56]. In a large-scale study of 3072 men undergoing initial biopsies, PCA3 outperformed PSA in the prediction of PCa, but not for advanced ones[57]. Moreover, PCA3 demonstrated better diagnostic performance compared to the telomerase reverse transcriptase (hTERT) gene. Comparing prostate tumor tissues with non-malignant prostate tissues, PCA3 showed a 34-fold change that was significantly higher than a 6-fold change for hTERT[21].

Assessing the diagnostic impact of PCA3 in PCa, Li and colleagues recruited 24 patients with pathologically confirmed PCa, 40 patients with benign prostatic hyperplasia (BPH), and 13 patients with urolithiasis. The urine levels of PCA3 were measured using RT-qPCR and compared among the three groups. The diagnostic sensitivity, specificity, and AUC were calculated using urine PCA3 as a reference parameter. The results showed that the urine PCA3 level in PCa patients was significantly higher compared to the other two groups ($P < 0.05$). The AUC was 0.90, while the sensitivity and specificity were 87.5% and 79.2%. In conclusion, the authors claimed urine PCA3 can serve as a biomarker for diagnosing patients with prostate cancer, providing a potential diagnostic tool for this disease[58].

In another study Merola and colleagues aimed to validate the clinical utility of the prostate cancer gene 3 (PCA3) test and evaluate its prognostic potential in

prostate cancer (PCa) patients. The researchers enrolled 407 Italian men with multiple PCa risk factors and at least one previous negative biopsy. These men underwent PCA3 testing, as well as total prostate-specific antigen (tPSA) and free PSA (fPSA and f/tPSA) tests. The results showed that the PCA3 score was significantly higher in the PCa-positive population compared to the tPSA score. Additionally, the PCA3 test outperformed the f/tPSA test. The PCA3 test demonstrated a sensitivity of 94.9% and specificity of 60.1% using a threshold of 35, while a cutoff of 51 provided the best results with sensitivity and specificity of 82.1% and 79.3%, respectively. Moreover, there was a significant association between the PCA3 score and increasing Gleason scores [59].

3.1.2.2. PlncRNA-1

PlncRNA-1 (Prostate cancer long non-coding RNA) expression was elevated in PCa patients compared to normal tissues and BPH. Its depletion in PCa cells induced apoptosis and repressed AR signaling and cell proliferation[60]. Moreover, in vitro and in vivo experiments both revealed that oncogene PlncRNA-1 can regulate the growth of prostate cancer cells through the TGF-β1 signaling pathway[61].

3.1.2.3. HOTAIR

HOTAIR (HOX transcript antisense RNA) encodes a lncRNA that binds to the androgen receptor (AR) to stabilize AR and therefore induces AR activation, independently of androgen presence. According to Zhang et al., HOTAIR was upregulated after Androgen deprivation therapy (ADP) and in CRPC. Up-regulation of HOTAIR induced PCa cell growth and proliferation but its knockdown inhibited the progress[62].

3.1.2.4. SChLAP-1

SChLAP-1 (second chromosome locus associated with prostate-1) has an elevated expression level in about 25% of PCa patients, commonly in metastatic form. Mechanistically, SChLAP-1 interferes with the switch/SNF (SWI/SNF) complex, which is a tumor suppressor, and thereby impedes its efficacy. Therefore, as was expected SChLAP-1 suppression in PCa cell lines significantly inhibited cell proliferation, while the overexpression of SChLAP-1 in normal prostate epithelial notably promoted cell proliferation[63,64]. SChLAP-1 upregulation is associated with a high risk of tumor recurrence, a poor prognosis, a higher Gleason grade, and a higher mortality[65].

3.1.2.5. MALAT1

Since Peng et al. identified MALAT1 (Metastasis-associated lung adenocarcinoma transcript 1), many studies have demonstrated its role in several cancers [66]. MALAT1 was up-regulated in PCa and its high transcript level was associated with higher PSA levels, a high Gleason score, and advanced PCa including CRPC[67,68]. A study on 434 urine samples revealed that MALAT1 was favorable for PCa diagnosis in men whose PSA level was in the range of 4-10ng/ml. MALAT1 score with an AUC of 0.742 outperformed %fPSA with an AUC of 0.627 in predicting PCa, also a 25% threshold for the MALAT1 model prevented 30.2%-46.5% of unnecessary biopsies[69].

3.1.2.6. MEG3

MEG3 (Maternally expressed gene 3) transcript is an lncRNA that acts as a tumor suppressor gene through its interaction with the p53 protein. Zhou et al. have reported that MEG3 was down-regulated in PCa, compared to BPH[64]. Moreover, they identified that MEG3 down-regulation leads to a higher level of miR-9-5p, resulting in more invasion and cell proliferation[70].

PRCAT17.3

The results of a study showed a significant upregulation of PRCAT17.3 ($P < 0.0001$) and PRCAT38 ($P < 0.0002$) expression levels in human prostate cancer tissues when compared to benign prostatic hyperplasia (BPH) tissues. This suggests that these markers could be potentially useful in distinguishing between prostate cancer and BPH.

Furthermore, real-time RT-PCR analyses on urine samples from prostate cancer patients showed a significant elevation in prcat17.3 levels ($P < 0.0197$). AUC for prcat17.3 was calculated to be 0.72. These findings indicate that prcat17.3 may serve as a promising biomarker for prostate cancer detection when analyzing urine samples, showing potential diagnostic value in differentiating prostate cancer patients from those with BPH[71].

4.1. mRNA-Based Biomarkers

4.1.1. TMPRSS2: ERG Fusion Gene

TMPRSS2 is an androgen-response gene located on 21q22.2 which encodes a protein called transmembrane protease serine 2 which is expressed in normal and malignant prostatic tissue. Serine protease enzymes are involved in many physiological and pathological processes. Its role in prostate carcinogenesis relies on gene fusion with ETS transcription factors, particularly ERG and ETV1[72-74]. ERG is an oncogene encoding a member of the erythroblast transformation-specific family of transcription factors 2, which is a key regulator of cell proliferation, differentiation, embryonic development, angiogenesis, inflammation, and apoptosis[75]. In 2005, Tomlins et al. identified chromosomal rearrangements in PCa that fuse the androgen-regulated promoter of the TMPRSS2 with the coding sequence of erythroblastosis virus E26 (ETS) gene family members, more often ERG and ETV1[76]. Following this rearrangement, the new androgen-sensitive promoter may cause overexpression of ETS gene family members due to androgen elevated levels. Consequently, overexpressed ETS members can induce neoplastic phenotype by up-regulating their target genes expression such as KLK2, MMP3, and IL1R2[76-78]. ETS gene fusions were found in approximately 50% of prostate cancers in the following studies. Up to 9 out of 10 of these ETS fusions were between TMPRSS2 and ERG[79]. ERG and TMPRSS2 fusions were measured in urine samples using qRT-PCR and presented as TMPRSS2: ERG score. A higher TMPRSS2: ERG score was linked to a higher risk of dying from PCa[71] Furthermore, the authors reported that detecting PCa with a combination of TMPRSS2: ERG score, PCA3 score, and PSA has a 90% specificity and an 80% recall[80]. Similarly, in a study on a cohort of 1244 PCa patients, Tomlins et al. indicated that incorporating serum PSA with urine TMPRSS2: ERG and PCA3 scores for diagnosing PCa outperforms serum PSA-based strategies. The addition of those two scores to the PCa prevention trial risk calculator (PCPTrc) improved the AUC from 0.639 to 0.762. The authors also highlighted the significance of these biomarkers in predicting advanced PCa (Gleason grade > 6) in needle biopsy, with an AUC of 0.779[81].

HOXC6

HOXC6 is a member of the HOX gene family that is found on human chromosome 12q13.3, and its altered expression has been linked to a variety of cancers. Zhou et al. demonstrated that relative expression levels of HOXC6 at both mRNA and protein forms were significantly higher in PCa tissues and cell lines, compared to adjacent non-cancerous and normal prostate epithelial cells. Moreover, the downregulation of HOXC6 by siRNA led to the inhibition of cell proliferation and invasion of PCa cells[82]. A large multi-center study also validated the clinical utility of urinary HOXC6 in early PCa detection[83]. Hamid et al. developed an assay for the detection of HOXC6 urinary mRNA and found out that HOXC6 was upregulated in urine samples from PCa patients in comparison to normal men[84].

5.1. DNA-Based Biomarkers

5.1.1. PTEN

One of the most deregulated pathways in PCa is PI3K-AKT. PI3K induces tumorigenesis by activating AKT/mTOR signaling pathway which inhibits apoptosis and promotes cell survival[85,86]. The phosphatase and tensin homolog (PTEN) gene is a key regulator of the PI3K/AKT signaling pathway located on chromosome 10q23. This gene is the most frequently deleted tumor suppressor gene in invasive PCa. Deletion, point mutation, and promoter hypermethylation are the main reasons for PTEN inactivation[55,87,88]. PTEN deletion is associated with poor prognosis, early biochemical recurrence, and resistance to androgen deprivation therapy, which is the principal first-line treatment of advanced PCa. In a study on 35 patients with radical prostatectomy, none of the benign glandular epithelium samples showed PTEN deletion but it was seen in 68% of PCa samples[89].

5.1.2. HOXB13

In a linkage analysis on 94 unrelated men with PCa, a rare mutation (G84E) in the HOXB13 gene was found. The G84E variant was significantly associated with early-onset disease and familial PCa. However, it is not clear If it is associated with aggressiveness or not.

5.1.3. BRCA2

In a recent study on 2932 individuals about the impact of BRCA2 mutations in PCa, it showed that the PCa incidence rate per 1,000 person-years was 19 in patients with BRCA2 mutations and 12 in normal controls. Also, the BRCA2 mutation was significantly associated with early-onset and more aggressive disease[90].

6.1. Circular RNAs (circRNAs) Biomarkers

Besides establishing a comprehensive catalog of circRNAs species in tumor tissues called MiOncoCirc, Vo et al. reported two circRNAs able to discriminate PCa subtypes from each other. circAMACR was upregulated and correlated with the amplification of androgen receptors in CRPC. Moreover, circAURKA was upregulated in neuroendocrine PCa (NEPC). According to this study, circAURKA and circAMACR are promising markers for therapy-resistant PCa. The authors also evaluated circRNAs in urine and detected 1092 circRNAs in urine samples of PCa patients, which completely overlapped with results from tissue samples. Therefore, urine samples can be a promising source for profiling circRNAs in PCa[91]. Chen et al. performed ultra-deep RNA sequencing without poly-A selection on 144 biopsies from PCa patients to fully characterize the transcriptome of localized PCa. They identified a panel of circRNAs expressed by tumor cells. Subsequently, it was shown by circular transcriptome loss-of-function screening that 11.3% of those frequent circRNAs are essential for cell proliferation. According to this study, circCSNK1G3 promotes cell proliferation via interaction with miR-181[92]. Investigating the role of circular RNA Foxo3 in PCa, Shen et al. analyzed circ-Foxo3 expression in 22 low-grade PCa, 24 high-grade PCa, and 18 normal prostate tissue samples. The authors discovered that the level of circFoxo3 expression was significantly lower in high-grade PCa, compared to low-grade PCa and normal prostate tissues. Furthermore, silencing circFoxo3 expression resulted in increased migration, PCa cell survival, invasion, and chemoresistance to docetaxel. Accordingly, circFoxo3 delivery promoted chemosensitivity to docetaxel and extended the life span of mice model[93]. Exploring expression profiles of circRNAs in three cell lines via high-throughput circRNAs sequencing, Zhang et al. reported that CDR1as (hsa_circ_0001946) was highly expressed in PCa cell lines compared to normal prostate epithelial cells. CDR1as is a circRNA containing multiple binding sites for miR-7, a miR interacting with important tumor suppressor genes or oncogenes including KLF4, RAF1, PIK3CD, IGF1R, mTOR, NOTCH1, and AKT. CDR1as functions as a miRNA sponge and causes downregulation of miR-7. According to the results from this study, circSLC7A6 and CDR1as may be associated with bone metastasis. The authors also reported that circBAGE2 (hsa_circ_0061259) is upregulated in epithelial prostate cancer cells compared to normal prostate cells. This circRNA binds to miR-103a which can impair the tumor suppressive function of mentioned miR and promote cancer cell proliferation[94,95]. In a study on both PCa tissue samples (seventeen paired PCa samples and matched non-tumor normal samples) and PCa cell lines, Dai et al. reported that circRNA Myosin Light Chain Kinase (MYLK) or hsa_circ_0141940 is significantly upregulated in PCa tissue samples and cell lines compared to normal prostatic cells. Upregulation of circRNA-MYLK increased PCa cells proliferation, migration, and disease progression; while, its silencing via si-circRNA-MYLK significantly increased PCa cell apoptosis. Due to the negative correlation between miR-29a and circRNA-MYLK, authors suggested circRNA-MYLK promotes PCa progression by downregulating miR-29a, an important tumor suppressor miRNA[96]. In another study, Kong et al. found that circ-SMARCA5 which is encoded by a gene with the same name, was upregulated in PCa cell lines. Authors also identified that DHT treatment greatly induced circ-SMARCA5 expression. According to their results, circ-SMARCA5 functioned as an oncogene in PCa by elevating cell proliferation and preventing apoptosis[97]. An FDA-approved drug, enzalutamide, provides a substantial survival benefit for men with CRPC, however, many patients develop resistance to therapy. Greene et al. demonstrated that hsa_circ_0004870 was downregulated in enzalutamide-resistant cells and decreased in malignant cells. The authors mentioned that hsa_circ_0004870 may play a critical role in the development of enzalutamide resistance in PCa through RBM39, a member of the U2AF65 proteins family[98].

Circulating circRNAs

A few studies have investigated the biomarker potential of circulating circRNAs in biological fluids, including

plasma and urine samples obtained from PCa patients (Table 3). Recently, in a study on 1265 non-DRE urine samples from eligible participants (the training cohort, n = 263; validation cohort 1, n = 497; validation cohort 2, n = 505) He et al. identified and validated a panel of five circular RNAs in uEV called Ccirc. These circulating circRNAs (circPDLIM5, circSCAF8, circPLXDC2, circSCAMP1, and circCCNT2) could discriminate PCa from BPH patients with significant specificity. Unlike many commercialized kits, this assay is completely non-invasive and does not require pre-collection DRE[99]. In another study, Li et al. claimed that Circ-0044516 is upregulated in EVs derived from PCa patients' blood and PCa cell lines. The authors also verified that the downregulation of Circ-0044516 inhibited cell proliferation and metastasis of PCa through overexpression of miR-29a-3p[100]. CircAR3 is a circRNA encoded by an androgen receptor gene that is reported to be highly expressed in PCa cell models and tumor samples94. Moreover, circAR3 expression level in plasma was extremely low in patients with benign prostate cancer, whereas it is upregulated in PCa patients with high-grade and lymph node metastasis. Authors also claimed that circAR3 does not affect AR signaling nor cell proliferation, and it is undetectable after radical prostatectomy which supports the idea that the origin of circAR3 in plasma is prostate[101]. Moreover, it was proved that the plasma expression level of circZMIZ1 was higher in PCa patients compared to BPH ones. Interestingly, the knockdown of circZMIZ1 in PCa cell lines inhibited cell proliferation and arrested the cell cycle at G1. The authors also claimed that circZMIZ1 could cause PCa by overexpressing the androgen receptor gene[102].

CONCLUSION AND FUTURE DIRECTION

Prostate cancer (PCa) is one of the most commonly diagnosed malignancies globally, leading to significant cancer-related deaths. The disease exhibits a broad spectrum of behavior, ranging from indolent to aggressive and fatal forms. Accurate risk stratification is crucial for therapeutic decision-making and clinical trial design, necessitating the differentiation between benign and aggressive states. Incorporating clinically valuable prognostic and predictive biomarkers can aid in the timely prevention of metastatic disease and guide therapy selection. While several biomarkers have been recommended or questioned by international guidelines, further validation through larger prospective randomized studies is necessary to determine their efficacy in PCa detection, discrimination, prognosis, and treatment effectiveness. Two biomarkers, Prostate Health Index (PHI) and 4Kscore, have shown clinical relevance in distinguishing more aggressive forms of PCa. However, the development of a new grading classification system based on molecular features that are pertinent to PCa risk stratification and tailored treatment remains a priority. This classification system would enhance our understanding of the disease and facilitate personalized treatment approaches based on individual patient characteristics. In summary, further research is needed to validate the efficacy of biomarkers in PCa detection and discrimination. Recently non-invasive metabolic approaches for PCa diagnosis has been introduced including interesting field of E-nose. Advances in diagnosis and treatment options have improved patient outcomes, with chemotherapy and targeted agents playing a crucial role in managing metastatic prostate cancer. Continued research efforts and the identification of clinically significant biomarkers will contribute to significant advances in the management of prostate cancer[103-105].

Our article introduces groundbreaking advancements in the field of prostate cancer diagnosis. Unlike previous studies, we have focused on identifying and utilizing newer and more specific biomarkers, specifically RNA biomarkers, which greatly enhance the accuracy and efficiency of prostate cancer diagnosis. The molecular biomarkers that are summarized in this review play key roles in improving diagnosis and treatment of PCa. However, how to assess and prioritize the new markers is still a question that remains to be answered, especially in patients with PSA levels in the grey zone of 4.0 to 10.0 ng/mL, to avoid unnecessary biopsies. While our study has investigated numerous biomarkers in the field of prostate cancer diagnosis, it is important to acknowledge certain limitations. Firstly, our study is a Mini Review, and as such, it may not provide the same level of comprehensive analysis as meta-analyses or systematic reviews. Additionally, the sample size and scope of the studies included in our review may have been limited, which could potentially affect the generalizability of our findings. Despite these limitations, our study provides a valuable overview of the potential of RNA biomarkers in prostate cancer diagnosis and highlights the need for future investigations to address these challenges and further refine their implementation. Despite the numerous perks of liquid biopsy, further studies and investigations in large populations are needed to validate the circulating biomarkers. Having replaced biopsies with available kits or future ones, they need to be very sensitive and accurate to elude dispensable biopsies.

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