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Review

Current advances of liquid biopsies in prostate cancer: Molecular biomarkers

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Prostate cancer (PCa) incidence is increasing and endangers men's lives. Early detection of PCa could improve overall survival (OS) by preventing metastasis. The prostate-specific antigen (PSA) test is a popular screening method. Several advisory groups, however, warn against using the PSA test due to its high false positive rate, unsupported outcome, and limited benefit. The number of disease-related biopsies performed annually far outweighs the number of diagnoses. Thus, there is an urgent need to develop accurate diagnostic biomarkers to detect PCa and distinguish between aggressive and indolent cancers. Recently, non-coding RNA (ncRNA), circulating tumor DNA (ctDNA)/ctRNA, exosomes, and metabolomic biomarkers in the liquid biopsies (LBs) of patients with PCa showed significant differences and clinical benefits in diagnosis, prognosis, and monitoring response to therapy. The analysis of urinary exosomal ncRNA presented a substantial correlation among Exos-miR-375 downregulation, clinical T stage, and bone metastases of PCa. Furthermore, the expression of miR-532-5p in urine samples was a vital predictive biomarker of PCa progression. Thus, this review focuses on promising molecular and metabolomic biomarkers in LBs from patients with PCa. We thoroughly addressed the most recent clinical findings of LB biomarker use in diagnosing and monitoring PCa in early and advanced stages.

INTRODUCTION

The prostate-specific antigen (PSA) test is one of the most widely used screening tests for men at risk for prostate cancer (PCa).[1] Recently, several advisory groups recommended against using PSA tests because of unsubstantiated outcomes. PSA tests have high false positive rates, reducing their benefit relative to the implications of unnecessary follow-ups.[2] PCa-related mortality is not decreasing as fast as it should. Indeed, over the past few decades, PCa mortality among African American patients who are obese significantly worsened.[3–5] Other lines of investigation are needed to provide a better knowledge of prostate malignancies. Many PSA-positive men develop asymptomatic PCa. Most of these tumors do not progress or they develop slowly, averting the need for active medical intervention.[6] Nevertheless, invasive, painful biopsies are routinely recommended in the absence of other biomarkers. Gleason scoring (GS) by pathologists based on microscopic biopsy aids clinical prognosis. Screening depends on highly trained pathologists yet is ultimately subjective. Clinical evaluation could be more cost effective and objective. Conversely, among PCa diagnoses, approximately a third of the GS of PCa biopsies are inaccurate and underestimate the score, followed by metastasis within 5 years, which challenges clinical diagnosis and threatens patients' lives. Therefore, discovering more accurate and focused biomarkers for PCa grading is crucial and timely. Recently developed tests may differentiate aggressive cancers from their indolent counterparts. Developing and commercializing an objective diagnostic and prognostic method for PCa detection and evaluation in a non-invasive manner could significantly benefit men.

LIQUID BIOPSIES FOR DIAGNOSIS AND MONITORING OF PCa

Liquid biopsy (LB) is a biopsy alternative using biofluids. It is a sensitive and non-invasive tool for diagnosing and monitoring cancers.[7] LB is minimally invasive, requiring a small sample of blood, semen, or urine, and can detect cancer cells or genetic material that solid tumor cancers release into body fluids.[3] As presented in Figure 1, LB could address the following critical areas: diagnosis, prognosis, and therapeutics; monitoring spread to other organs; identifying a tumor's genetic changes or mutations; determining optimal treatments for individual patients; and assessing treatment efficacy. The detection of circulating tumor cells (CTCs) attracted attention to LB as an alternative diagnostic tool for PCa. The presence of CTCs in LB represents an efficient prognostic and predictive biomarker. LB can assess the therapeutic responses of advanced disease earlier than conventional diagnostic methods, can detect early metastatic development, and can monitor the efficacy of therapies.[8,9] Numerous studies have evaluated androgen receptor (AR) splice variants expressed by CTCs as indicators of drug resistance (abiraterone and enzalutamide).[10,11] A newly published study highlighted CTCs in the seminal biopsies of patients with PCa.[12] In a clinical setting, these biopsies yielded 613 CTCs per 1.7 mL in patients with PCa compared with 6 mL in healthy donors. Higher CTC numbers in seminal biopsies were significantly correlated with increased PSA levels.[12] The CTC level was elevated primarily in patients with metastatic castration-resistant PCa (mCRPC). Still, early diagnoses of localized PCa, of benign prostatic hyperplasia (BPH), and of advanced stages of PCa need more sensitive molecular biomarkers. Molecular tumor profiling

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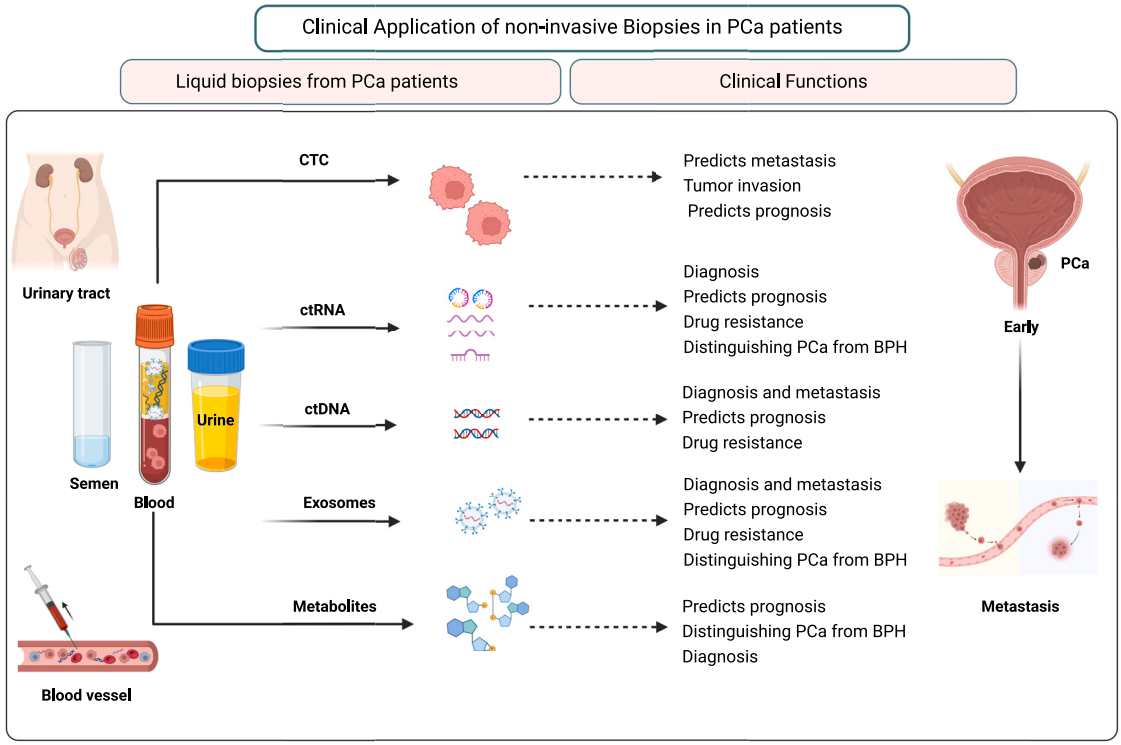

Figure 1. Biomarkers in the liquid biopsies of patients with PCa

The graphic depicts the significance of liquid biopsies (LBs) as a non-invasive diagnostic method in PCa. LBs can find cellular, molecular, and metabolomic biomarkers that predict the aggressiveness of PCa and metastasis and prognosis during treatment. Further, LB can identify PCa from other indolent diseases and monitor and predict the response to anticancer therapy. The picture depicts the several types of LBs appropriate for PCa, the molecular, cellular, and metabolic targets that can be used in studying PCa, and the expected outcomes of those targets. Additionally, the diagram illustrates the stages of PCa that can be monitored using LBs, from the early to the metastatic stages.

can enhance personalized cancer therapies and predict responses to drug applications and tumor relapse potential.[13] Monitoring specific and sensitive molecular biomarkers like RNA (including coding and non-coding RNA), DNA-like circulating free tumor DNA (cell-free DNA [cfDNA]), and cell-free circulating tumor DNA (ctDNA), proteins (enzymes, interleukins, chemokines), exosomes (Exos), and the metabolite profile should support therapeutic intervention and prevent disease deteriorating consequences.[14,15] LB can utilize urine, blood, synovial fluid, cerebrospinal spinal fluid (CSF), amniotic fluid, and umbilical cord cells.[14,16–18] Although LB is not yet widely used in clinical practice, it is the proper technique for care in several areas.[17,19] LB is clinically significant in the early detection and diagnosis of PCa.[20–22] The FDA has approved several single-gene and multigene assays to detect genetic alterations in plasma cfDNA. These assays were approved as companion diagnostics for a specific molecularly targeted cancer therapy.[23] Recently, kits for detecting urine-obtained exosomal biomarkers such as *PCA3*, *ERG*, and *SPDEF* genes entered commercial use for early detection of PCa.[24,25]

Urinary Exo molecular signature as LB biomarkers for diagnosis of PCa

Exos are microendosomal biovesicles that range in size from ~30 to 200 nm.[26] Exos play crucial roles in orchestrating the cell-to-cell communications that facilitate cellular and molecular physiological responses.[27] Exos' cargos, including nucleic acids, functional proteins, and metabolites, contain highly sensitive biomarkers to diagnose and monitor cancer.[28] PCa biomarkers were first reported in urinary Exos (UEs) such as PCA3 and TMPRSS2:ERG in 2009.[29] That study reported detectable PSA mRNA in the UE of newly diagnosed patients before any drug courses. UE is highly accurate at detecting diagnostic biomarkers (see Table 1). A recent study analyzed the mRNA expression of *ERG*, *PCA3*, *PSMA*, *CK19*, and *EpCAM* in the UE of patients with PCa compared with healthy controls.[30] Biomarkers were significantly higher in the UE of patients with PCa than in healthy controls. Further, the GS was closely correlated with levels of the UE genes *ERG*, *ARV7*, and *PSMA*.[30] Another study analyzed protein differential expression in UE from 16 patients with PCa compared with 15 healthy controls.[31] The study found 246 proteins with differential expression between the groups; most were upregulated in patients with PCa. Seventeen UE proteins were identified as PCa biomarkers, including TM256, LAMTOR1, VATL, ADIRF, and some RAb proteins.[31] A study of 21 urine samples used UE to monitor prostate tissue mRNA signatures, comparing results of patients pre-radical prostatectomy (RP) with corresponding RP tissue.[32] UE offered 81% accuracy (17/21) for non-invasive detection of TMPRSS2. A systematic review of 3,224 patients evaluated the sensitivity and specificity of UE as a

Table 1. Molecular biomarkers in the liquid biopsies of PCa

LB type	Screened target	Biomarkers	The expression level	Predictive function	Reference
Urine	UE-mRNA	*PSA*, *PCA3*, *TMPRSS2*, *ERG*	high/low (PCa vs. healthy)	diagnosis	29,32,35
	UE-mRNA	*ERG*, *PCA3*, *PSMA*, *CK19*, *EpCAM*, *KLK3*	high/low (PCa vs. healthy)	diagnosis	30,36
	UE-protein	TM256, LAMTOR1, VATL, ADIRF, some RAb	high/low (PCa vs. healthy)	diagnosis	31
	UE-mRNA	*PSA* and *PSMA*	low	drug response in patients with PCa (predicts a response to radiotherapy)	37
	UE-protein	AMACR	high/low (PCa vs. BPH and healthy)	diagnosis and distinguishing PCa from BPH and NC	38
	UE-mRNA	*flotillin 2*, *TMEM256*, *Rab3B*, *LAMTOR1*	high in PCa vs. normal	prediction of PCa scores and progression	39
	UE-mRNA	*GATA2*	high in PCa worse cases	biomarker of aggressiveness	40
	UE-mRNA	*TMEM256*, *Granulin*, *CHMP4A*, *CHMP4C*, *ADIRF*, *AMBP*, *FABP5*, *PCYOX1*	high/low (PCa vs. healthy)	diagnosis	24,41
	UE-lipids	lactosylceramide phosphatidylserine	high/low (PCa vs. healthy)	diagnosis	42
	UE-lncRNA	*SChLAP1*, *PCA3*, *SPRY4-IT1*, *PCATs*, *TRPM2-AS*	high/low (PCa vs. healthy)	diagnosis	21
	UE-miRNA	miR-375	up/down (PCa vs. metastasis)	bone metastasis	43
	UE-miRNA	miR-451a, miR-486-3p, miR-486-5p	down/up (PCa vs. metastasis)	bone metastasis	43
	UE-miRNA	miR-26a-5p, miR-532-5p, miR-99b-3p	high/low (PCa vs. healthy)	diagnosis and tumor progression	44
	UE-miRNA	miR-196a-5p and miR-501-3p	low/high (PCa vs. healthy)	diagnosis	45
	miRNA	miR-222-3p, miR-24-3p	down/up in PCs vs. BPH and normal	distinguish PCa from BPH	46,47
	miRNA	miR-30c-5p, miR-21	up/down in PCs vs. BPH and normal	distinguish PCa from BPH	46,47
	miRNA	miR-95	upregulated in PCa	PCa aggressiveness	47
	miRNA	miR-19a and miR-19b	up/down in PCa vs. BPH	biochemical recurrence cancer; classify cancer aggressiveness' diagnosis	47
	UE-miRNA	miR-532-3p and miR-6749-5p	up/down in PCa vs. BPH	differentiate benign and malignant PCa	48
	UE-miRNA	miR-107 and miR-574–3p	up/down in PCa vs. healthy	diagnosis	49
	UE-miRNA	miR-2909	upregulated	aggressiveness biomarker	50
	UE-miRNA	miR-574-3p, miR-141-5p, and miR-21-5p	up/down in PCa vs. healthy	diagnosis	51
	UE-lncRNA	lincRNA-p21	upregulated	malignancy biomarker	52
Serum/ plasma	Exos-miRNA	miR-141	up/down in PCa vs. BPH	metastasis biomarker	53
	Exos-miRNA	miR-744	upregulated in PCa	poor prognosis and lymph node metastasis	54
	Exos-miRNA	miR-130a-3p, miR-150-5p	down/up in PCa vs. healthy	diagnosis	55
	Exos-miRNA	miR-365a-3p, miR-148a-3p, miR-145-5p	up/down in PCa vs. BPH	diagnosis	55
	miRNA	miR-182-5p, miR-375-3p, miR-410-5p	up/down in PCa vs. BPH	distinguishing PCa from BPH	56,57
	Exos-miRNA	miR-423-3p	expressed in castration-resistant PCa	PCa castration resistance	58
	miRNA	miR-20a and miR-26a	up/down in recurrence vs. treated	monitor recurrence	59
	circRNA	circAR3	up/down in treatment vs. untreated	response to therapy	60
	circRNA	circZMIZ1	up/down in PCa vs. BPH	diagnosis and potential target for therapy	61
	circRNA	circSMARCC1	up/down in PCa vs. healthy	cancer proliferation, metastasis, and potential target for therapy	62
	circRNA	circFOXO3	up/down in PCa vs. healthy	oncogenic biomarker	63
	miRNA	miR-16, miR-148a, miR-195	up/down in PCa vs. healthy	diagnosis	64
	miRNA	miR-4289, miR-326, miR-152-3p, miR-98-5p	up/down in PCa vs. healthy	diagnosis and potential targets for in early PCa	65

(*Continued on next page*)

Table 1. *Continued*

LB type	Screened target	Biomarkers	The expression level	Predictive function	Reference
Semen	Exos-miRNA	miR-142-3p, miR-142-5p, miR-223-3p	up/down in PCa vs. healthy and BPH	diagnosis, prognosis, and distinguishing biomarkers	66
	miRNA	miR-142-3p, miR-223-3p, miR-93-5p	up/down in PCa vs. healthy	diagnosis in combined with PSA levels	67
	miRNA	miR-30d-5p, miR-93-5p,	upregulated in aggressive PCa	aggressiveness and prognosis	67
	miRNA	miR-375-3p, miR-182-5p, miR-21-5p	up/down in PCa vs. BPH	diagnosis	56

diagnostic tool for urinary tumors, including PCa.[33] UE reached 83% sensitivity and 88% specificity. The summary receiver operating characteristic curve was 0.92 (95% confidence interval [CI]: 0.89–0.94).[33] Further, a study analyzed UE and serum-Exos and found significant alterations in isolated Exos from PCa compared with healthy controls.[34] In another study, 229 men had repeat biopsies using UE; this method performed well in monitoring PCa-specific genes like PSA.[35]

UE levels are promising biomarkers to evaluate treatment response among patients with PCa. Researchers tested PCa biomarkers PSA and PSMA in patients treated with neoadjuvant androgen deprivation therapy before radical radiotherapy. The study found that UE decreased 2-fold and presented PSA and PSMA biomarkers (20/24) compared with healthy controls.[37] A recent study analyzed UE biomarkers in 272 urine samples of patients who underwent prostatic biopsy. After purifying UE, researchers tested the expression of a-methylacyl-CoA racemase (AMACR) by ELISA.[38] UE performed well at distinguishing (1) PCa, (2) BPH plus non-significant PCa (nsPCa), and (3) a healthy prostate . Thus, UE is sensitive for early PCa identification and is clinically significant in diagnosis and prognosis.[38]

UE is useful in determining the expression levels of biomarkers that are related to PCa progression and aggressiveness. For example, in 2017, researchers investigated protein biomarkers in UE collected from patients with PCa. Expression of *flotillin 2*, *TMEM256*, *Rab3B*, and *LAMTOR1* differed, showing that UE can efficiently predict PCa scores and progression.[39] UE can also determine the expression of endothelial transcription factor GATA-2 (GATA2) mRNA, the biomarker of aggressive PCa, according to a study of 128 males with elevated PSA serum levels.[40] GATA2 mRNA in UE was reduced in patients treated with prostatectomy and upregulated in patients with a positive PCa biopsy, indicating the efficacy of UE as an LB to diagnose and monitor PCa.

UE could also prevent unnecessary biopsies. A clinical study evaluated the predictive role of UE for high-grade PCa at initial biopsy.[68] UE gene expression and PSA measurement plus standard of care (SOC) improved the distinction between benign disease and GS7+ vs. GS6: area under the curve (AUC) 0.77 (95% CI, 0.71–0.83) vs. SOC AUC 0.66 (95% CI, 0.58–0.72) (P = 0.001).[68] Hence, a predetermined cut point could have prevented 138 of 519 (27%) unnecessary biopsies. In a previous study, researchers compared UE biomarkers in patients with PCa to urinary sediment (cell pellet) and whole urine before and after digital rectal examination (DRE).[36] PCa was diagnosed in 52% of patients. *PCA3*, *ERG*, and *KLK3* mRNA expression levels were significantly elevated after DRE compared with non-PCa controls. Further, UE expressed more sensitivity than cell pellets in detecting PCa biomarkers.[36] UE was highly sensitive to several significant PCa biomarkers like *TMEM256*, *Granulin*, *CHMP4A*, *CHMP4C*, *ADIRF*, *AMBP*, *FABP5*, and *PCYOX1*, with high levels in patients with PCa.[24,41] Some studies reported UE lipids as biomarkers for PCa diagnosis.[42] Mass spectrometry demonstrated that the levels of nine lipid species were significantly different between patients with PCa and healthy patients. Patients with PCa had elevated levels of lactosylceramide and phosphatidylserine (PS).[42] Overall, UE offers sensitive and specific detection of PCa biomarkers; this makes UE an efficient form of LB for PCa diagnosis and treatment response monitoring.

UE-ncRNAs as LB biomarkers for diagnosis of PCa

When non-coding RNAs (ncRNAs) are longer than 200 nucleotides, they are classified as long ncRNAs (lncRNAs).[69] In patients with PCa, several studies reported lncRNA-specific upregulated biomarkers like *SChLAP1*, *PCA3*, *SPRY4-IT1*, *PCATs*, and *TRPM2-AS*.[21] LB biomarkers offer precise, swift, non-invasive diagnostics. Thus, as listed in Table 1, specific microRNAs (miRNAs) in LB predict PCa diagnosis and prognosis. A recent study of PCa biomarkers investigated the UE miRNA profile of patients with PCa by miRNA sequencing (miRNA-seq) and validated the results by RT-PCR.[43] Exos-miR-375 was significantly downregulated in patients with PCa, while Exos-miR-451a, Exos-miR-486-3p, and Exos-miR-486-5p were significantly upregulated.[43] The study found a substantial correlation among Exos-miR-375 downregulation, clinical T stage, and bone metastases. Another study analyzed molecular biomarkers in UE from 21 non-PCa patients and 6 patients with PCa.[44] It found 21 miRNAs differentially expressed in patients with PCa vs. healthy controls. The researchers validated these results by qPCR, using UE from 28 non-biochemical recurrence (BCR) patients and 26 BCR patients. Notably, miR-26a-5p, miR-532-5p, and miR-99b-3p showed significant upregulation among patients with PCa, and miR-532-5p was a vital predictive biomarker of PCa progression.[44] In another study, miRNA-seq of UE from 20 patients with PCa identified five molecular biomarkers: miR-92a-1-5p, miR-196a-5p, miR-143-3p, miR-501-3p, and miR-34a-5p. These biomarkers were significantly decreased in patients with PCa compared with controls.[45] The study singled out miR-196a-5p and miR-501-3p as promising biomarkers for PCa diagnosis.

The first extensive studies of dysregulated miRNA expression in PCa tissue have improved knowledge of miRNA participation in PCa pathophysiology. A recent study profiled miRNAs in 29 urine biopsies from patients with BPH compared with 215 biopsies from patients with clinically localized PCa.[46] The study revealed that miR-222-3p, miR-24-3p, and miR-30c-5p could distinguish patients with BPH from patients with PCa, suggesting the value of miRNA biomarkers as a PCa diagnostic tool.[46] Another study analyzed urine from 52 patients with PCa compared with 12 non-cancerous prostate tissues (NPTs). Results showed that the overexpression of miR-95 is linked to aggressive PCa.[47] The levels of miR-21 in urine samples distinguished patients with PCa from patients with BPH. Additionally, the expression of miR-19a and miR-19b in urine biopsy can be used as a prognostic biomarker for BRC in treated patients.[47] Such signals can classify PCa according to the disease's aggressiveness and distinguish between tumor and non-tumor tissue.[70]

A recent study investigated biomarkers in UE-ncRNA that can differentiate BPH from PCa instead of using a transrectal prostate biopsy.[48] Researchers analyzed 28 urine LBs from patients with PCa and 25 from patients with BPH. Exos-miR-532-3p and Exos-miR-6749-5p were differentially expressed in patients with PCa. The study concluded that UE-miRNA can differentiate benign and malignant PCa.[48] A previous study reported that miR-107 and miR-574-3p were significantly upregulated in the UE of patients with PCa compared with controls.[49] That study found that UE-miRNA was superior to traditional biomarkers such as PCA3.[49] Another study investigated the diagnostic role of miR-2909 and miR-615-3p in UE from 90 patients with PCa, 60 patients with bladder cancer, 10 patients with BPH, and 50 healthy controls.[50] Exos-miR-2909 was clearly expressed in patients with PCa and functioned as an aggressiveness biomarker in patients with PCa.

A study compared UE-miRNA from 35 patients with PCa with healthy controls and PC cell lines. It found that miR-574-3p, miR-141-5p, and miR-21-5p were significantly upregulated in patients with PCa and that they showed promise for PCa diagnosis.[51] However, miRNA-205 and miRNA-214 were significantly downregulated in the UE of patients with PCa.[71] Another study tested the expression of lincRNA-p21 in 30 UE from patients with PCa and 49 UE samples from patients with BPH.[52] lincRNA-p21 was expressed differently in the two groups, serving as a PCa malignancy biomarker.[52] Overall, UE-ncRNAs are clinically significant biomarkers in PCa diagnosis and monitoring disease development and aggressiveness.

Exos-ncRNA as biomarkers of PCa in serum, plasma, and semen LBs

The use of LB in PCa diagnosis and monitoring has great clinical importance. It avoids repeated unnecessary biopsies that cost time, money, and hospitalization of patients with PCa. Urine LB is an applicable technique with high sensitivity and specificity. As presented in Figure 1 and in Table 1, other types of LBs, such as serum, plasma, and semen, also offer significant biomarkers for PCa diagnosis and monitoring. Li et al. analyzed miR-141 levels in Exos from the serum of patients with PCa compared with patients with BPH and healthy controls. Exos-miR-141 expression was significantly upregulated in Exos of patients with PCa compared with patients with BPH and healthy controls (3.85-fold, $p = 0.0007$ and 4.06-fold, $p = 0.0005$, respectively).[53] Exos-miR-141 in PCa is an important metastasis biomarker circulating in the serum of patients with PCa. A study analyzed 94 plasma samples of patients with PCa compared with 68 plasma samples from healthy controls by miRNA array.[54] It found that miR-744 was upregulated in patients with PCa and was associated with poor prognosis. The increase of miR-744 was significantly linked with lymph node metastasis ($p = 0.0407$) and recurrences ($p = 0.0376$).[54] In a similar context, plasma miRNA profiling found that miR-130a-3p, miR-365a-3p, miR-148a-3p, miR-145-5p, and miR-150-5p were differentially expressed in patients with PCa.[55] Notably, miR-130a-3p and miR-150-5p were downregulated in both plasma and tissue, while miR-148a-3p and miR-145-5p were upregulated. This highlights the expression of miR-150-5p in plasma as a validated biomarker in diagnosing PCa.[55] A recent study of Exos-miRNA semen biomarkers compared PCa with BPH and other cancers. Exos-miR-142-3p, Exos-miR-142-5p, and Exos-miR-223-3p were diagnostic and prognostic biomarkers and distinguished PCa from BPH.[66]

PSA and miRNA expression levels can be combined for greater predictive value. In a recent study, researchers compared the expression of diagnostic and prognostic miRNA biomarkers in seminal plasma of patients with PCa with those of patients with BPH and healthy donors.[67] miRNA biomarkers in semen plasma were significantly similar to those in PCa tissue. The differences in the expression of miR-142-3p, miR-223-3p, and miR-93-5p, combined with PSA levels, offer high specificity in diagnosing PCa. Further, combining PSA and the expression of miR-30d-5p, miR-93-5p, and miR-30d-5p can predict the aggressiveness and prognosis of PCa.[67] A recent study by Wang et al. reported high specificity when combining the expression of PSA with the expression of serum miRNA-149 in diagnosing PCa.[72] A recent study compared epigenetic miRNA biomarkers in LB with PSA value. Using PCR, researchers found miR-375-3p, miR-182-5p, miR-21-5p, and miR-148a-3p to be expressed in the blood and seminal plasma of 65 patients with PCa and 58 patients with BPH.[56] The expression of miR-182-5p and miR-375-3p in plasma was significantly different between patients with PCa and patients with BPH. Additionally, the seminal plasma of PCa showed greater expression of miR-375-3p, miR-182-5p, and miR-21-5p and performed better than PSA at diagnosing PCa.[56] In another clinical study, plasma levels of Exos-miR-423-3p were associated with castration-resistant PCa as a predictive biomarker of PCa castration resistance.[58] The analysis of plasma miRNAs in 149 patients with PCa compared with 57 healthy controls showed that miR-410-5p was significantly overexpressed in patients with PCa.[57] A study by Mohammadi et al. reported the importance of serum miR-20a and miR-26a in monitoring PCa before and after prostatectomy.[59] However, in another study, circAR3, which regulates the expression of an AR gene, had high serum levels in BPH-naive PCa.[60] Yet, circAR3 was suppressed when patients received neoadjuvant hormone therapy. It

was overexpressed among patients with PCa with high GS and lymph node metastasis but became undetectable in plasma after RP.[60]

Similarly, circZMIZ1 was higher in the plasma of patients with PCa than in patients with BPH.[61] The inhibition of circZMIZ1 also inhibited tumor cell proliferation and growth. Interestingly, circZMIZ1 is linked with the overexpression of AR and AR splice variant 7.[61] Also, circSMARCC1, a significant ncRNA in PCa, could be detected in plasma, cancer cells, and tissue. Overexpression of circSMARCC1 was associated with increased cancer proliferation and metastasis by regulating the expression of CCL20. This blocked the expression of miR-1322, leading to activated PI3K-Akt signaling. The inhibition of circSMARCC1 reduced tumor growth and metastasis. Thus, circSMARCC1 is a plasma biomarker of PCa.[62] Moreover, circ_0006404 (circFOXO3) is a crucial ncRNA in PCa pathogenesis. A recent study reported upregulated levels of circFOXO3 in the serum and tissue of patients with PCa.[63] circFOXO3 serves as an oncogenic ncRNA in PCa by targeting the expression of miR-29a-3p, which stimulates the overexpression of SLC25A15. Thus, circFOXO3 enhances tumor progression and cancer cell proliferation by upregulating the expression of SLC25A15 in PCa.[63] Overall, the importance of ncRNA in LB as a non-invasive approach is increasing clinically. This approach offers high specificity and sensitivity in PCa diagnosis and predicts prognosis and disease aggressiveness without unnecessary tissue biopsies. Further, a study suggested specific plasma miRNAs as potential therapeutic targets for PCa. The study examined the plasma miRNA profiles of 33 healthy males, 51 patients with PCa who had undergone RP, and 79 patients with PCa who had not yet received treatment.[64] The expression of miR-16, miR-148a, and miR-195 was significantly correlated with high GS and was involved in the regulation of the PI3K/Akt signaling pathway.[64] A study by Matin et al. profiled 372 cancer-associated miRNAs in plasma from patients with PCa: 60% of the samples were obtained from patients had not yet been treated, and 40% were from patients after treatment, compared with samples from healthy controls.[65] Four miRNAs, miR-4289, miR-326, miR-152-3p, and miR-98-5p, were significantly upregulated in patients with PCa compared with controls. miR-152-3p was a significant biomarker in the pathogenesis of PCa. The four miRNAs could be used as targets for treating early-stage PCa.[65]

ctDNA/ctRNA biomarkers in PCa LBs

The formation, progression, and metastasis of PCa are linked to the DNA damage response (DDR).[73] The alteration and instability of DDR increase PCa progression, metastasis, and drug resistance[74] (see Figure 1). Thus, monitoring the circulating fraction of PCa tumor DNA (ctDNA) or RNA (ctRNA) provides an efficient non-invasive tool to measure drug response and tumor aggressiveness.[75] An early study compared plasma LB of patients with PCa to tissue to characterize mutation deletions at 21q22, 8p21, and 10q23 in metastatic PCa cases.[76] AR mutation was associated with PCa clinical progression. Thus, analyzing ctDNA in LB offered an efficient, early non-invasive detection tool for monitoring and predicting the aggressiveness and the development of PCa.[76] A clinical study sequenced 45 plasma-free circulating DNA collected at a stage of PCa metastasis.[77] The results aligned with exome sequencing data, showing mutations like the solid biopsy. Altered ctDNA in plasma and tissue showed 22 amplifications of the *AR* gene (64.7%); speckle-type pox virus and zinc finger protein (*SPOP*) mutations were 8.8%. However, the alterations in tumor suppressors *TP53*, *PTEN*, *RB1*, *APC*, *CDKN1B*, *BRCA2*, and *PIK3R1* were inactive. ctDNA alterations such as WNT and PI3K pathways appeared in plasma biopsies but not in solid biopsies.[77] A recent study sequenced plasma DNA from 151 chemotherapy-unexperienced patients with mCRPC. Sequencing covered exons of these genes: *TP53*, *AR*, *RB1*, *PTEN*, *PIK3CA*, *BRCA1*, *BRCA2*, and *ATM*. The study was a phase 2 trial of abiraterone acetate (ClinicalTrials.gov: NCT01867710) for patients with PCa.[78] A shorter overall survival (OS) was linked to plasma-based tumor-DNA detection. After one cycle of therapy, plasma gene testing improved prognosis and gave early indications of medicinal effects.[78] In another recent study, 43 patients with mCRPC received abiraterone medication. The researchers analyzed 114 sequential plasma specimens for ctDNA changes. Tumor progression was identified as the elevation in ctDNA fractions in plasma.[79] Increased plasma ctDNA after the first treatment cycle was significantly associated with increased PSA. While PSA levels were elevated in pseudo-progression, plasma ctDNA showed no significant increase. Further, the initial alteration in ctDNA was linked to drug duration. Thus, the study concluded that plasma ctDNA could monitor the response to androgen deprivation therapy.[79]

A recent study tracked the expression of methylated ctDNA in the plasma of patients with PCa, with BPH, and with *de novo* metastatic PCa (mPCa) compared with healthy controls. Methylated ctDNA for *DOCK2*, *HAPLN3*, and *FBXO30* genes was undetected in the plasma of healthy controls, patients with BPH, and patients with localized PCa but was highly expressed in patients with mPCa.[80] Those three methylated ctDNA types were useful diagnostic biomarkers for hormone-naive mPCa patients. A study by Haldrup et al. detected hypermethylated ctDNA for *ST6GALNAC3*, *ZNF660*, *HAPLN3*, and *CCDC181* in the serum of 27 patients with PCa compared with 10 patients with BPH.[81] The researchers compared the results of ctDNA in serum with solid PCa biopsies. Methylated ctDNA of ST6GALNAC3 and ZNF660 could be diagnostic biomarkers of PCa, and ZNF660 could be a prognostic biomarker.[81]

A study by Chen et al. measured the levels of cfDNA in the blood of patients with mCRPC and PCa. The concentration of cfDNA identified individuals with mCRPC but showed no significant differences between healthy controls and patients with localized PCa.[82] Further, an early comparative study reported that cfDNA levels decreased significantly in the plasma biopsies of patients with PCa treated with 1–3 cycles of chemotherapy.[83] Large (200 bp–10.4 kb) cfDNA fragments and loss of methylation at *GSTP1* or *RARB2* appeared in post-treatment samples.[83] A related study tested the effects of cytotoxic chemotherapy before and after infusion on the cfDNA in blood biopsies of patients with mCRPC.[84] The infused drug directly affected the quantity of ctDNA and the frequency of mutations, suggesting the sensitivity of ctDNA biomarkers in LB of patients with PCa.[84] A

Table 2. Metabolomic biomarkers in the liquid biopsies of PCa

LB type	Biomarkers	Predictive function	Reference
Plasma and urine	metabolites related to the urea cycle, tricarboxylic acid cycle (TCA), fatty acid metabolism, and the glycine	PCa diagnosis	93
Plasma	sarcosine, acetylglycine, and coreximine metabolites	PCa diagnosis	94
Serum	5-hydroxy-N-formylkynurenine, 2-isopropyl citrate, cytidine, D-asparagine, D-4-O-methyl-myo-inositol, and N-acetylgalactosamine-4-sulphate	diagnosis of PCa early stage	95
Plasma	trihexosylceramide and tetrahexosylceramide in glycosphingolipids	aggressiveness biomarkers	96
Serum	PC, PS, SM, and carnitine	diagnostic biomarkers	97
Serum	glycerol 1-hexadecanoate, 4-oxoretinol, 2-hydroxy-nonadecanoic acid, anandamide, palmitic acid, dl-dihydrosphingosine, 2-methoxy-6Z-hexadecenoic acid, hexadecyl acetyl glycerol, 3-oxo-nonadecanoic acid, 2-palmitoylglycerol, N-palmitoyl glycine, glycidyl stearate, N-methyl arachidonoyl amine, d-erythro-sphingosine C-15, 1-(9Z-pentadecenoyl)-2-eicosanoyl-glycero-3-phosphate, 9-octadecenal, 3Z,6Z,9Z-octadecatriene, and hexadecenal	diagnostic biomarkers	98
Serum	l-tryptophan, kynurenine, anthranilate, isophenoxazine, glutaryl-CoA, (S)-3-hydroxybutanoyl-CoA, acetoacetyl-CoA, and acetyl-CoA	diagnostic biomarkers	99
Urine	glutamate metabolism and glutamate oxaloacetate transaminase 1	distinguish patients with PCa from patients with BPH	14
Urine	hexanal, 2,5-dimethylbenzaldehyde, 4-methylhexan-3-one, dihydroedulan IA, methylglyoxal, and 3-phenylpropionaldehyde	diagnostic biomarkers	100

clinical trial analyzed cfDNA in blood biopsies from patients with mCRPC. The phase 3 trial studied docetaxel (ClinicalTrials.gov: NCT01308567) and cabazitaxel (ClinicalTrials.gov: NCT01308580) as first- and second-line chemotherapies. Radiological progression-free survival (rPFS) was significantly correlated with OS and with the concentration of ctDNA.[85] Another clinical study collected serum biopsies from two groups of patients with PCa (n = 196 and 133) to investigate peripheral ctRNA in patients with PCa.[86] In that study, miRNA-223, miRNA-24, and miRNA-375 significantly predicted PCa reclassification independently of clinical features. The scores of those miRNAs combined with PSA levels could predict indolent PCa.[86] A study investigated ctRNA in the serum of patients at high risk of aggressive PCa. miR-200c, miR-605, miR-135a, miR-433, and miR-106a were the circulating biomarker miRNAs significantly distinguishing indolent from aggressive PCa.[87] Another study validated the expression of ctRNA in plasma biopsies from 102 patients with PCa compared with 50 healthy individuals. PCa was substantially connected with two genes, *OR51E2* and *SIM2*, and two miRNAs, miR-200c and miR-200b.[88]

Further, ctDNA is detectable in the urine samples of patients with early PCa. A study compared urine ctDNA in 29 samples from patients with PCa with 25 samples from healthy controls for assessing the integrity of urine ctDNA in the diagnosis of PCa. The ctDNA fragments longer than 250 bp (*c-Myc*, *BCAS1*, and *HER2*) were verified by RT-PCR.[89] The study suggested that urine ctDNA could aid early diagnosis of PCa. By contrast, another study investigated urine ctDNA in 131 individuals, including patients with PCa and those with benign diseases of the urogenital tract (control group). Urine ctDNA was less accurate at diagnosing PCa than PSA levels.[90] An early study investigated the expression of PCA3 mRNA in the urine samples of 233 patients with expected PCa who presented a PSA level $\geq$2.5 ng/mL.[91] The study concluded that the expression of mRNA-PCA3 should be combined with other clinical features to diagnose PCa.[91] Further study is needed to compare urine biomarkers with peripheral circulating biomarkers.

Metabolite biomarkers in the LB of patients with PCa

An alteration in the metabolomic content of body fluids is linked to serious pathological and physiological disorders[92] (see Figure 1 and Table 2). A recent study employing liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography (GC)-MS discovered several changed metabolites related to the urea cycle, the tricarboxylic acid cycle (TCA), fatty acid metabolism, and the glycine cleavage system in plasma and urine from 89 patients with PCa, 84 patients with BPH, and 70 healthy males.[93] It provided compelling evidence that the energy and amide nitrogen metabolic pathways could be useful sources of PCa markers.[93] Another study used ultrahigh performance LC-MS (UPLC-MS) to analyze PCa metabolite diagnostic biomarkers in plasma biopsies from patients with PCa compared with age-matched, healthy individuals.[94] The results presented 19 differential metabolites in PCa plasma biopsies. Specifically, sarcosine, acetylglycine, and coreximine metabolites could be significant metabolic diagnostic biomarkers for PCa.[94] Fast UPLC-MS/MS (FPLC-MS/MS) found significant differences in serum metabolites between patients with PCa and controls for 5-hydroxy-N-formylkynurenine, 2-isopropyl citrate, cytidine, D-asparagine, D-4-O-methyl-myo-inositol, and N-acetylgalactosamine-4-sulphate.[95] Those metabolites are novel diagnostic biomarkers for early-stage PCa. Snider et al. reported metabolomic results for 159 plasma biopsies of patients with PCa receiving treatment in the North Carolina-Louisiana PCa Project.[96] About 35 small metabolites were associated with PCa aggressiveness. The researchers concluded that highly aggressive PCa was linked to trihexosylceramide and tetrahexosylceramide in glycosphingolipids that circulate in the plasma of patients with PCa.[96]

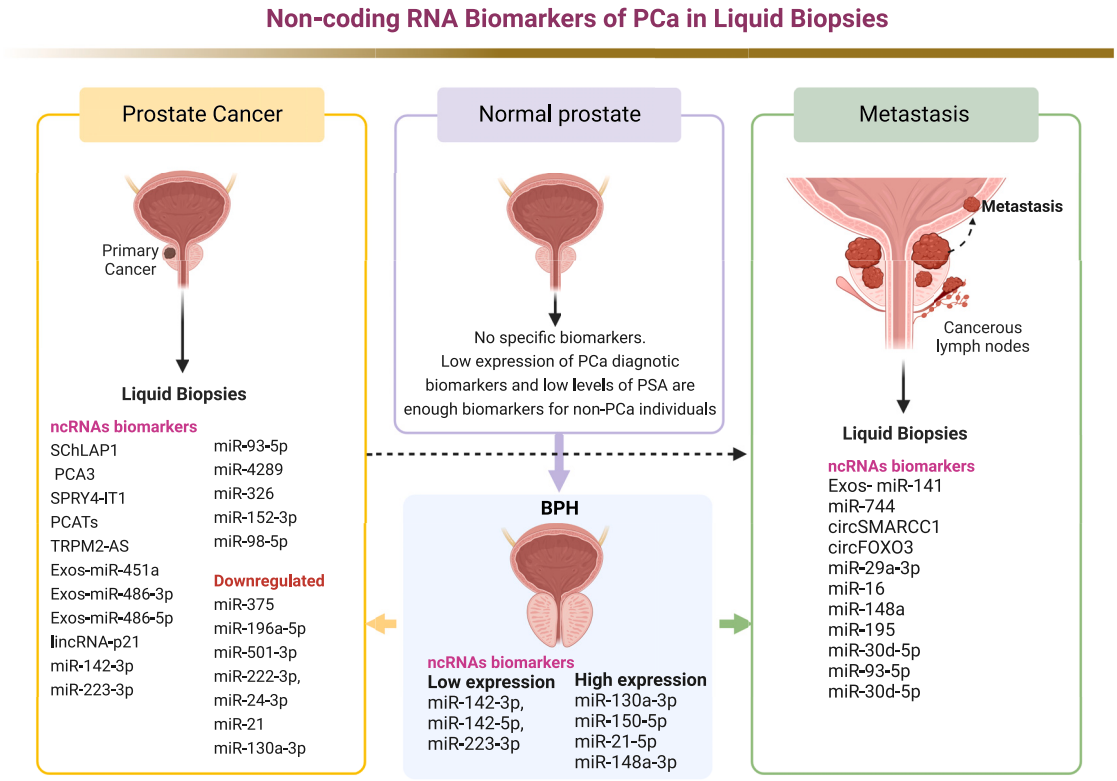

Figure 2. Non-coding RNA biomarkers in the LBs that distinguish PCa from BPH and healthy control

The schematic diagram illustrates significant levels of ncRNAs in LBs that distinguish PCa from other indolent forms when compared with prostates in healthy individuals. These potential biomarkers have been reported in several clinical studies that compared molecular biomarkers of LBs obtained from patients with PCa with those obtained from patients with BPH and healthy controls.

Xu et al. collected serum biopsies from 134 individuals: 49 with PCa and the rest negative for PCa tissue biopsy.[97] They found that dimethylphosphatidylethanolamine (dMePE) (18:0/18:2), phosphatidylcholine (PC) (16:0/20:2), Phosphatidylserine (PS) (15:0/18:2), Sphingomyelin (SM) (d16:0/24:1), and carnitine (C14:0) were differentially expressed in patients with PCa.[97] A related study analyzed the serum biopsies of 220 patients with PCa and BPH for metabolite differences.[98] Metabolic reprogramming, particularly in lipid metabolism, was a crucial characteristic of PCa. With a PSA level in the gray range of 4–10 ng/mL, 18 lipid metabolites (glycerol 1-hexadecanoate, 4-oxoretinol, 2-hydroxy-nonadecanoic acid, anandamide, palmitic acid, dl-dihydrosphingosine, 2-methoxy-6*Z*-hexadecenoic acid, hexadecyl acetyl glycerol, 3-oxo-nonadecanoic acid, 2-palmitoylglycerol, *N*-palmitoyl glycine, glycidyl stearate, *N*-methyl arachidonoyl amine, d-erythro-sphingosine C-15, 1-(9*Z*-pentadecenoyl)-2-eicosanoyl-glycero-3-phosphate, 9-octadecenal, 3*Z*,6*Z*,9*Z*-octadecatriene, and hexadecenal) and lipid-associated metabolites were promising diagnostic markers for the differential diagnosis of patients with PCa and patients with BPH.[98]

Another study identified metabolites in PCa serum biopsies that might play a central role in PCa progression. l-Tryptophan, kynurenine, anthranilate, isophenoxazine, glutaryl-CoA, (S)-3-hydroxybutanoyl-CoA, acetoacetyl-CoA, and acetyl-CoA were upregulated in correlation with PSA level in serum biopsies of patients with PCa.[99] In contrast, indoxyl, indolelactate, and indole-3-ethanol were depressed in the serum biopsies.[99] A recent study analyzed urine metabolites and RNA profiles in urine biopsies of patients with PCa compared with patients with BPH or healthy controls. There were significant differences in the circulating glutamate metabolism and the TCA in patients with PCa compared with patients with BPH.[14] Glutamate metabolism and glutamate oxaloacetate transaminase 1 (GOT1)-dependent redox balance played a significant role in PCa as novel biomarkers to distinguish patients with PCa from patients with BPH.[14] Similarly, a clinical study analyzed metabolomic differences in urine biopsies of patients with PCa compared with healthy controls using GS-MS and headspace solid-phase microextraction (HS-SPME/GC-MS). Six metabolites indicated PCa: hexanal, 2,5-dimethylbenzaldehyde, 4-methylhexan-3-one, dihydroedulan IA, methylglyoxal, and 3-phenylpropionaldehyde.[100] Common modifications in the expression of valine, taurine, leucine, and citrate have also been discovered across urine and tissue. In recent clinical studies, metabolomic diagnostic biomarkers in the LB of PCa showed clear patterns. Metabolomic biomarkers could assist with the early detection

of PCa, could discriminate between PCa and BPH, and could predict PCa aggressiveness. These findings indicate the urgent application of such biomarkers in the non-invasive diagnosis of PCa.

CONCLUDING REMARKS

Recently, there has been a resurgence of interest in the clinical use of LBs as a minimally invasive way to diagnose and monitor PCa. Relying on solid biopsies in PCa has serious drawbacks. Tracking molecular and metabolomic biomarkers in PCa is a viable way to reduce unnecessary tissue biopsies and improve early detection of PCa. LB can also monitor the response to anticancer medicines, which may lead to better treatment options. LB shows significant efficacy in discriminating PCa from BPH and other non-PCa disorders, as presented in Figure 2. In particular, several studies have highlighted the value of UE analysis for PCa diagnosis and predicting progression and aggressiveness. UE-ncRNAs have performed essential non-invasive diagnostic tasks in patients with PCa and predicted responses to anticancer therapy. Numerous studies have emphasized the intriguing role of ctDNA/ctRNA biomarkers in the peripheral blood and urine of patients with PCa. ctDNA and ctRNA biomarkers in LBs (serum, plasma, and urine) from patients with PCa played an important role in evaluating the response to chemotherapy and numerous anticancer medications. Further, studies of metabolomic patterns in LBs from patients with PCa suggest clinical uses for distinguishing PCa from BPH disorders. Metabolic variations in PCa urine samples, while a source of diagnostic indicators, play only a modest role in distinguishing patients with PCa from patients with BPH. On the other hand, the metabolic profiling of plasma and serum biopsies can identify PCa from BPH and other non-PCa disorders. Plasma and serum studies have also identified crucial biomarkers for PCa diagnosis and monitoring medication responses. Overall, LB is effective in diagnosing PCa, primarily through early identification of cancer-related alterations in urine, plasma, and serum samples.

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AUTHOR CONTRIBUTIONS

Article conception and design, M.A. and R.J.P.; manuscript drafting, M.A. and R.A.P.; revising manuscript and corrections, R.J.P., M.A., R.A.P., M.C.M., and V.P.; drawing figures and tables, M.A.; supervising the whole work, R.J.P. The authors reviewed the whole work and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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