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Review

Molecular Biomarkers for the Diagnosis of Prostate Cancer—A Review

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Abstract

Prostate cancer (PCa) is diagnosed in a significant proportion of men worldwide and constitutes a serious public health challenge. The incidence of this illness increases with age, with worsening quality of life and mortality in aggressive PCa. Early detection and accurate prognostic assessment are essential for making appropriate therapeutic decisions and monitoring disease progression. However, this is challenging because standard diagnostic tools often lack sensitivity and yield ambiguous results. Advances in the understanding of prostate cancer have provided valuable data for detection, monitoring, and systemic anticancer therapy, significantly impacting prognosis and quality of life. During carcinogenesis, prostate tumor cells release numerous biological substances into the bloodstream that can be studied. Liquid biopsies allow for the analysis of tumor components in body fluids, and the diagnostic value of these biopsy tests as a surrogate for tumor tissue is increasing. This article provides a narrative review of the global literature on molecular biomarkers of prostate cancer, such as circulating tumor DNA (ctDNA), microRNAs, circulating tumor cells (CTCs), circulating extracellular vesicles (EVs), and metabolomic and proteomic markers.

Keywords: tumor biomarkers; prostate cancer

1. Introduction

Prostate cancer (PCa) is one of the most common malignant tumors of the male urinary system worldwide, with an increasing incidence and mortality rate. The number of new cases is estimated at approximately 1.5 million, and the number of deaths is estimated at approximately 400,000 worldwide. PCa is the second most common cancer worldwide, and it was the fifth leading cause of cancer death among men in 2022. Worse still, bone metastases and recurrence are common, which worsen the prognosis. The etiology and risk factors for prostate cancer are not fully understood. Few environmental factors have been identified to date, with only older age, family history, and certain genetic disorders considered to be risk factors for prostate cancer [1,2]. Unfortunately, prostate cancer remains asymptomatic for a long time and is often diagnosed very late. Basic diagnostics for this cancer include a digital rectal examination, serum prostate-specific antigen (PSA) testing, biopsy analysis, and histological analysis. It is extremely important to include as many patients as possible in screening tests to detect PCa at its earliest stages. However, these methods sometimes fail to accurately diagnose the development of prostate cancer or prostate hyperplasia. This applies primarily to PSA results, which are sensitive to inflammation and benign prostatic changes,

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leading to low specificity and sensitivity in prostate cancer prognosis [3]. Therefore, finding a new clinical diagnostic marker is crucial [4–6]. In recent years, liquid biopsy techniques have emerged as promising, non-invasive methods demonstrating the potential to improve early cancer detection. Liquid biopsy, as a rapidly developing technique in cancer research, can be useful at all stages of medical procedures: in early diagnosis, cancer progression, therapy monitoring, and assessment of the risk of recurrence [3]. Liquid biopsy can involve various fluids, but the most commonly used material is blood because it provides the widest range of biological analytes, such as circulating tumor DNA (ctDNA), microRNAs, circulating tumor cells (CTCs), circulating extracellular vesicles (EVs), and metabolomic and proteomic markers (Table 1 and Figure 1) [7].

Table 1. Diagnostic performance of liquid biopsy in prostate cancer.

Group/Markers	Significance	References
Circulatingtumor cells	Prognostic, monitoring, response to treatment	[8–33]
Circulating free microRNAs	Early diagnosis, metastasis, monitoring, response to treatment	[34–52]
Circulating free DNA	Diagnosis, prognostic, monitoring, response to treatment	[53–57]
Circulating extracellular Vesicles	Early diagnosis, metastasis, prognostic	[58–68]
Metabolomic markers	Early diagnosis, prognostic	[69–75]
Proteomics markers	Early diagnosis, prognostic, monitoring	[76–78]

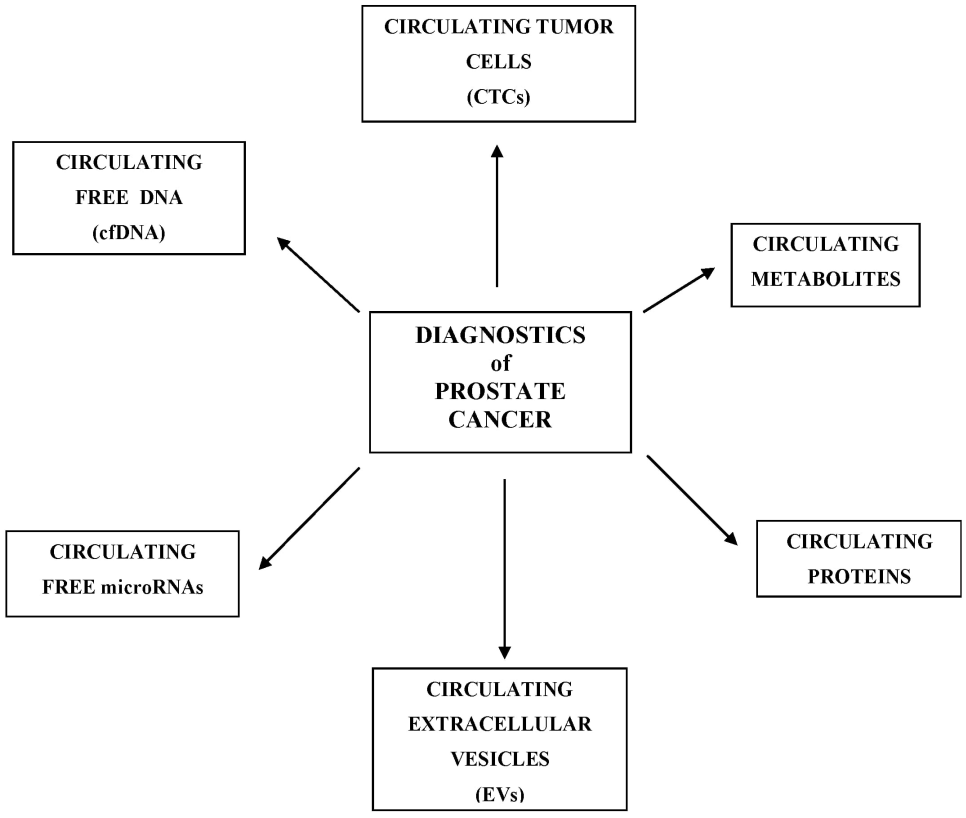


Figure 1. Division of prostate cancer biomarkers.

This comprehensive review assessed the diagnostic utility of various biomarkers for prostate cancer. This manuscript is a narrative review with a structured literature search. Three independent authors (W.J., B.M., and S.O.) conducted a comprehensive online search of articles from three databases: Embase, PubMed, and Web of Science. Publications were published between 2012 and 2026, with a particular focus on the last 5 years. Compilation of these databases yielded satisfactory results, achieving an overall recovery rate of approximately 90%. Ultimately, 83 published reviews were included, resulting in a total of 1270 relevant references in our databases (Figure 2). The search terms used were "prostate cancer," "microRNAs," "circulating tumor DNA," "circulating tumor cells," "circulating extracellular vesicles," "metabolomic and proteomic markers," and "diagnostic relevance." We adopted the inclusion criteria for eligible articles reviewed as part of our full-text review: articles addressing the association between specific biomarkers and prostate cancer diagnosis. Studies were considered eligible if they met the following criteria: the diagnostic ability of a specific biomarker to detect prostate cancer was reported, and all cancers were diagnosed based on histological examination. We included articles written in English that reported empirical data on biomarkers related to the diagnosis or prognosis of prostate cancer. The exclusion criteria were as follows: editorials; commentaries; letters to the editor; meta-analyses; protocol papers; studies not involving liquid biopsy or a biospecimen; and non-human or in vitro studies.

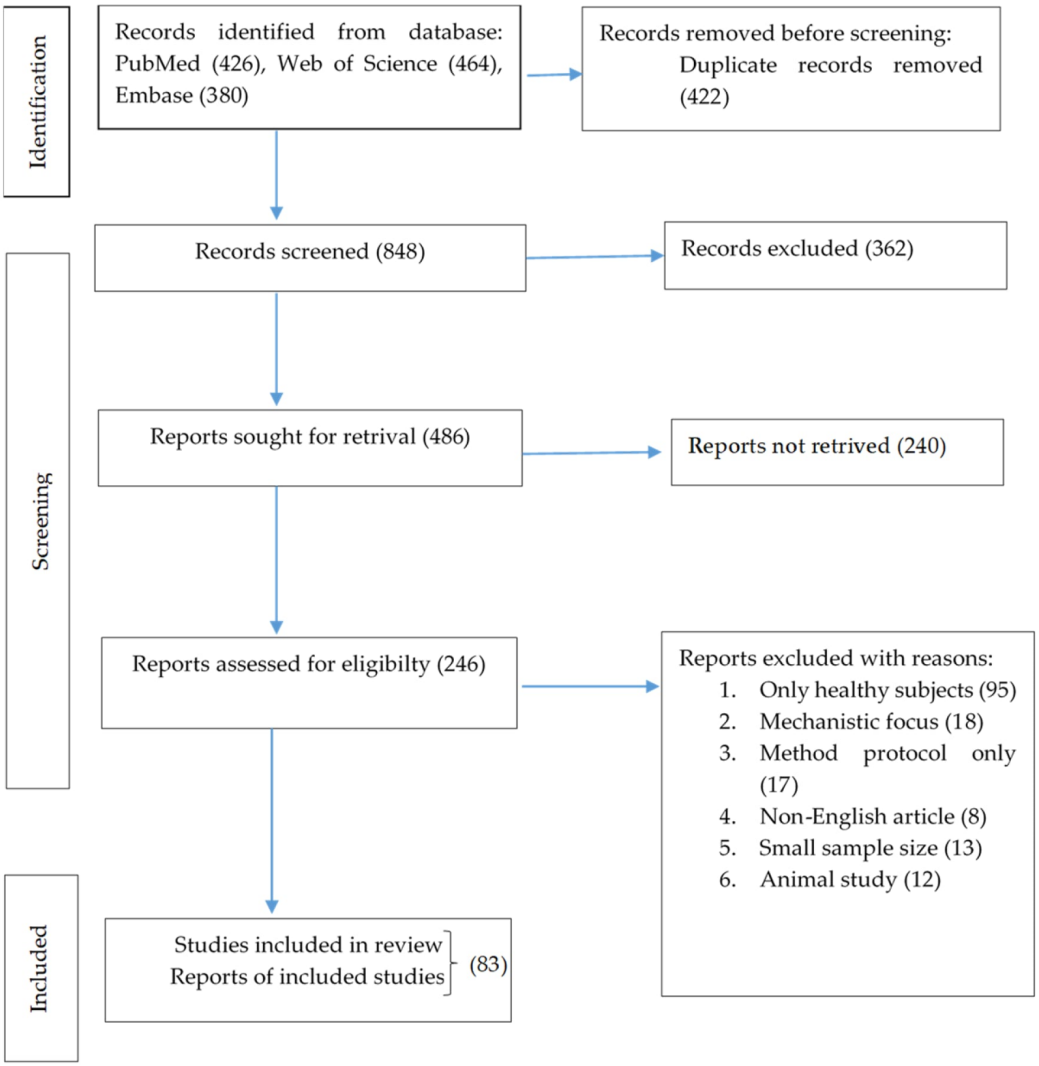


Figure 2. Flow diagram of the selection of studies.

2. Circulating Tumor Cells

Circulating tumor cells (CTCs) are cancer cells of epithelial or mesenchymal origin that, as a result of exfoliation from the primary tumor or metastases, enter body fluids (blood, cerebrospinal fluid, urine, or saliva) [8]. CTCs participate in the development of metastasis and the migration of tumor cells to secondary sites via the circulatory and lymphatic systems. CTCs have the ability to survive in the bloodstream, migrate through extravasation into the interstitial space, and then form a distant tumor in a new microenvironment. Circulating tumor cells are released from the primary tumor actively or passively, also during biopsy or surgery, entering blood vessels. Active epithelial–mesenchymal transfer of CTCs is stimulated by factors such as interleukin-6 (IL-6) and transforming growth factor-β (TGF-β). Circulating tumor cells are characterized by positive expression of epithelial markers, including cytokeratins (CK) and epithelial cell adhesion molecules (EpCAM). They are also negatively expressed for the white blood cell-specific marker CD45 [9]. In the blood, circulating tumor cells can travel alone, as homotypic clusters (CTC-CTC), or as heterotypic clusters (e.g., covered with platelets, neutrophils, or other blood cells). Therefore, the neutrophil-to-lymphocyte ratio in CTC-positive patients can be used to stratify patients with metastatic prostate cancer [10,11]. CTCs contain cellular and subcellular components that can be used in further research, such as intact DNA for analyzing various mutations and discovering new markers, RNA for assessing gene expression, and various biomarkers for proteomic studies.

Although the significant contribution of circulating tumor cells to the development of metastatic lesions has been confirmed, the mechanisms by which CTCs enter the blood and then travel to various other organs, creating secondary lesions, are highly complex and have not been fully elucidated. However, CTCs are gaining increasing importance as potential prognostic and predictive biomarkers for various cancers, including prostate cancer, as they constitute a valuable source of tumor-specific information and can be frequently repeated during disease progression and therapy. Circulating tumor cells occur in various body fluids in small numbers (e.g., up to 10 cells in 10 mL of blood), but they are nevertheless easily accessible for testing through non-invasive and easy sampling of body fluids. Liquid biopsy is an alternative examination method to conventional tissue biopsy and may be advantageous over tumor tissue biopsy in prostate cancer due to the high heterogeneity of this tumor. Furthermore, whole-genome sequencing of CTCs provides more information about metastatic tumors [12]. However, unlike tissue biopsy, CTC analysis does not allow for simultaneous, direct assessment of the tumor microenvironment.

Most studies on the use of CTCs as prognostic biomarkers for prostate cancer have focused on metastatic tumors, and there are few studies on early-stage prostate cancer [13,14]. Ma et al., in their meta-analysis, confirmed the diagnostic utility of circulating tumor cells, demonstrating a strong correlation between CTC count and overall survival and progression-free survival [15]. Keller & Pantel focused their attention on examining the expression of different receptors on CTC surfaces or gene expression in CTCs [16]. Casanova-Salas et al., in turn, examined various biomarkers and confirmed the prognostic significance of CTCs in metastatic prostate cancer [17]. Some studies have assessed the diagnostic value of CTCs as a screening test for prostate cancer. The overall negative predictive value (NPV) was in the range of 57.4–98%, and the positive predictive value (PPV) was 18–65%. This low PPV severely limits the use of CTCs in screening [18,19]. Qiao et al., in their latest study, found that the number of CTCs can be an independent predictive factor for prostate cancer and, in combination with PSA and the Prostate Imaging Reporting and Data System (PI-RADS), can increase the diagnostic effectiveness [20]. Although CTCs are detected in both localized (including low- and intermediate-risk) and metastatic prostate cancer, according to Liu et al., their usefulness in localized prostate cancer is very limited [21].

The primary difference between detecting CTCs in advanced disease and using them for screening lies in the number of cells present in the blood, the objective of the analysis, and the level of technical sensitivity required. In advanced cancer, CTCs are used to monitor treatment and are relatively easier to detect; in contrast, screening (in healthy or asymptomatic individuals) involves attempting to capture individual cells at a very early stage, which places extremely high demands on the technology.

Most researchers found no correlation between CTC counts and clinical symptoms, such as PSA level, stage, or lymph node lesions [22,23]. However, when combined with other CTC-associated markers, CTCs had greater prognostic value. The tumor suppressor gene CD82 deserves special attention, as it plays a role in cell adhesion to the protein matrix. Murray et al. found that the 36-, 60-, and 120-month biochemical-free survival for CD82-positive CTCs was 93%, 93%, and 69%, respectively, whereas in the absence of such cells, these values were 62%, 44%, and 16%, respectively. Therefore, CD82 expression indicates a prognosis similar to that in the absence of CTCs [24]. There was also no correlation between baseline CTC counts and stage in patients undergoing radical prostatectomy [23,25]. Although Liu et al. found that a number of bi-phenotypic CTCs (cells with markers of both epithelial and mesenchymal cells) predicted extraprostatic extension, the odds ratio (OR) was 1.383, and the area under the curve (AUC) was 0.72 [21]. Interestingly, minimally invasive interventions (e.g., brachytherapy) cause a short-term increase in CTC counts. Tsumura et al. did not detect circulating tumor cells before the procedure; however, immediately after brachytherapy, CTCs were detected in almost 12% of patients. This is probably the result of damage to the stroma and, consequently, the release of epithelial cells into the bloodstream. The same is observed after transrectal biopsy under ultrasound guidance [26,27]. Circulating tumor cells may be diagnostically useful for monitoring treatment response. Murray et al. examined the blood and bone marrow of 321 patients with localized PCa one month after radical prostatectomy, analyzing the number of CTCs and the presence of micrometastases. CTC-positive patients with bone marrow micrometastases had a higher incidence of early treatment failure compared to patients with only micrometastases. Therefore, these results may provide a basis for implementing appropriate clinical procedures in patients at high risk of prostate cancer recurrence [24]. The number of CTCs greater than one in patients with prostate cancer after radical prostatectomy is a significant predictor of poorer 5-year overall survival but not the absence of metastases [28].

The process of CTC analysis consists of two independent yet complementary steps: enrichment and identification. Enrichment is the initial stage of selection and sample concentration, which is necessary because CTCs are extremely rare (averaging 1 cell per billion blood cells). Identification is the stage used to confirm that the isolated cells are indeed CTCs rather than contaminants. In the context of liquid biopsy for cancer, it is crucial to distinguish between the enrichment (isolation) phase and the identification (detection) phase of circulating tumor cells. Relying solely on epithelial markers is associated with significant biological limitations. There is no universal technique for detecting circulating tumor cells. There are several methods based on different principles of operation and showing differences in sensitivity. The CTC detection process involves two fundamental steps: enrichment (isolation) and actual detection (identification). Most techniques involve capturing cells showing signs of epithelial differentiation. These may include the epithelial membrane marker EpCAM (CellCollector) or cytokeratin expression (CellSearch) [29,30]. Physical isolation based on cell centrifugation and isolation of CTCs of a specific size and mass can also be used [31]. A commonly used FDA-approved isolation method, CellSearch, is based on the use of antibodies to isolate circulating tumor cells (positive isolation) or remove other cells (negative isolation). Then, multiplex polymerase chain reaction (PCR) is used to properly detect CTCs [32].

A serious limitation to the use of CTC assays in routine diagnostics is the isolation method and the similarity of circulating tumor cells to monocytes and leukocyte aggregates. Furthermore, CTCs can disappear very quickly after release into the bloodstream, so the timing of blood collection may be important [33]. Another limitation is the large difference in the number of cells assayed using the different available systems. Comparing CTCs in peripheral blood using three commercially available systems: CellSearch, CellCollector, and Epispot, Kuske et al. found results ranging from 37% for CellSearch to 54.9% for CellCollector and 58.7% for Epispot [31].

3. Circulating Free miRNA

Another interesting group of molecular biomarkers for prostate cancer are extracellular microRNAs (miRNAs), which are characterized by high stability regardless of conditions (Table 2). The presence of microRNAs can be assessed in various biological materials, such as tumor tissue, blood, plasma, serum, urine, and seminal fluid. MicroRNAs are small, non-coding RNAs consisting of approximately twenty nucleotides. They are involved in regulating gene expression at both the post-transcriptional and/or translational stages. They are also responsible for cell balance and intercellular communication in healthy and cancer cells. MicroRNAs influence the growth, apoptosis, and differentiation of cancer cells. MicroRNA analysis allowed for the precise identification of the origin of tumor tissue in various cancers [34]. Various biological processes in cancer tissue may result in changes in microRNa concentrations in body fluids. MicroRNAs in these fluids may come not only from the primary tumor or micrometastases but also from stimulated neighboring tissues. Blood-derived dissemination of cancer cells is often observed in patients without clinical evidence of distant metastases. This is likely a systemic reaction to cancer, involving the development of cells from other tissues, including red blood cells, leukocytes, and platelets, within the tumor [35,36]. The expression of microRNAs has been found to be tissue-specific, indicating that microRNAs may be useful as diagnostic, prognostic, and predictive biomarkers for cancer [37]. Numerous studies have shown that some microRNA signatures may be specific to different cancers. For example, microRNA-21 is a widely studied miRNA whose expression has been found in many malignancies, including non-small cell lung, breast, and colorectal cancer [38,39]. On the other hand, results from various studies suggest that microRNA panels outperform single-marker assays and demonstrate diagnostic accuracy comparable to or higher than PSA, particularly within the diagnostic gray zone of PSA results. The diagnostic performance of microRNAs in studies varied widely, from modest single-marker AUCs of approximately 0.56 for microRNA-133a-3p to near-perfect panel performance achieved by multi-marker panels [40]. In independent cohorts, the most consistently dysregulated miRNAs included micoRNA-21, microRNA-125b, microRNA-141, microRNA-221, and microRNA-375 [41–44]. Matin et al. studied a panel consisting of four microRNAs—microRNA-98-5p, microRNA-152-3p, microRNA-326, and microRNA-4289—and found higher levels in plasma from prostate cancer patients compared to healthy controls. The diagnostic power of this panel, determined by AUC, was 0.88, which was higher than that previously reported for PSA [45]. Liu et al. found even higher diagnostic power for prostate cancer by examining a panel of five serum microRNAs: microRNA-17-3p, microRNA-1185-2-3p, microRNA-1207-3p, microRNA-4417, and microRNA-4732-5p. The area under the curve was 99.96%, sensitivity was 99.3%, and specificity was 100% in a group of 425 patients. Although these results are very satisfactory, they should be interpreted very carefully. Such ideal diagnostic accuracy, especially in early-phase studies, raises concerns about potential overfitting and highlights the need for validation in multicenter prospective cohorts with more heterogeneous groups [46]. Other studies have demonstrated relatively high levels of microRNA-98 and microRNA-326 in the plasma

of patients with prostate cancer compared to several other malignancies. However, these levels are not characterized by relatively low disease specificity. An advantage, however, is that microRNA-98 may also have prognostic significance, as it has been reported to be elevated in recurrent cases after radical prostatectomy, indicating its potential dual role in diagnosis and disease monitoring [47]. Various microRNAs found in blood are being studied not only for their diagnostic power but also for their biological significance and role in the mechanisms of carcinogenesis in prostate cancer. In recent years, several circulating microRNAs, microRNA-9-3p, microRNA-330-3p, and microRNA-345-5p, have been found to be significantly overexpressed in the serum of prostate cancer patients compared to controls, confirming their potential oncogenic role and diagnostic utility. This is particularly true for microRNA-345-5p, which has shown promise as both a diagnostic and therapeutic biomarker [48]. Another interesting type of microRNA is microRNA-183, which directly targets the PSA 3′ UTR. This microRNA increases PSA expression at the mRNA and protein levels. Clinical studies also confirm a positive correlation between microRNA-183 levels in the prostate of patients and circulating PSA levels, suggesting a regulatory role for this microRNA in PSA secretion. Furthermore, detected microRNA-183 levels correlate with increased cell proliferation and disease progression, confirming its potential oncogenic role and importance in early PSA-based detection [49].

Table 2. Selected microRNAs in prostate cancer.

Symbol	Expression	Materials	Biomarker
microRNA-21	Up	Plasma, tissue	Prediction of survival
microRNA-133a-3p	Down	Plasma	Diagnosis, prognosis
microRNA-125b	Down	Plasma, tissue	Diagnosis, prognosis
microRNA-141	Down	Whole blood	Early detection, prognosis
microRNA-221	Down Up	Whole blood, tissue	Early detection, monitoring recurrences, diagnosis, prediction of survival
microRNA-98-5p	Up	Plasma	Diagnosis, prognosis
microRNA-152-3p	Up	Plasma	Diagnosis, prognosis
microRNA-326	Up	Plasma	Diagnosis, prognosis
microRNA-4289	Up	Plasma	Diagnosis, prognosis
microRNA-17-3p	Up	Serum	Prediction of survival
microRNA-1185-2-3p	Up	Serum	Diagnosis, prognosis
microRNA-4417	Up	Serum	Early detection, prognosis
microRNA-1207-3p	Up	Serum	Diagnosis, prognosis
microRNA-4732-52	Up	Serum	Early detection
microRNA-9-3p	\Up	Serum	Early detection
microRNA-330-3p	Up	Serum	Prediction of survival
microRNA-345-5p	Up	Serum	Early detection, response to treatment
microRNA-183	Up	Plasma	Early detection, prognosis
microRNA-222	Up	Tissue	Early detection, prognosis

Many types of microRNAs exhibiting high levels in the blood of prostate cancer patients exhibit expression changes in tumor tissue, which has been confirmed in numerous

studies. Sun et al. found that aggressive prostate cancers tend to overexpress oncogenic microRNA-21, microRNA-125b, microRNA-221, and microRNA-222 [50]. Zhang et al., in turn, found that aggressive prostate cancers tended to overexpress oncogenic microRNA-21, microRNA-125b, microRNA-221, and microRNA-222. They also described microRNA-35-5p as an apoptosis-inducing factor in prostate cancer cells and proposed it as a potential biomarker [51].

The primary method for quantitatively detecting low concentrations of microRNAs with high sensitivity and specificity is quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Bioluminescence assays are another method for sensitive analysis of microRNA expression based on isothermal rolling circle amplification. In recent years, hybridization-based methods, such as in situ hybridization with blocked nucleic acid probes, have become widely used for simultaneous screening of large numbers of microRNAs. Deep sequencing based on next-generation sequencers is a very useful technique due to the ability to process millions of reads in a short time. Circulating microRNAs in various body fluids can also be studied using high-throughput profiling, sequencing, and microRNA microarray techniques combined with qRT-PCR [52].

Although the levels of many microRNAs are elevated in patients with prostate cancer, multicenter studies in large populations and uniform method validation are lacking. Furthermore, analytical platform variability currently prevents definitive determination of these markers with high confidence. Further research is needed to determine the role of each microRNA and explain how their circulating levels are related to tumor biology, disease status, and therapeutic response.

4. Circulating Tumor DNA

A developing tumor can cause genetic alterations, resulting in the appearance of freely circulating fragmented DNA chains, also known as circulating tumor DNA (ctDNA). These originate from exfoliated tumor tissue as a result of processes occurring in cancer cells, such as apoptosis or necrosis. Physiologically, the amount of circulating free DNA (cfDNA) in the blood is low due to its removal by phagocytosis. Significantly higher cfDNA concentrations in the blood have been observed in patients with advanced solid tumors, which have been analyzed in detail [53]. Circulating free tumor DNA testing is a new, non-invasive diagnostic approach that allows for real-time assessment of the heterogeneous molecular landscape of late-stage prostate cancer. This test provides the same data as invasive tumor biopsy and overcomes the limitations of traditional tissue biopsies and PSA testing. Analysis of circulating DNA enables the detection of tumor-specific mutations, methylation disorders, and extracellular DNA fragmentation, as well as providing information on transcriptomic changes and broader epigenetic data. Even in locally advanced prostate cancer, where cFDNA levels are often low, the mere detection of circulating DNA can be very useful in prognostication [54]. Although the amount of cfDNA in the circulation of early-stage prostate cancer patients is low, the use of new, sensitive, and specific techniques allows it to be used to detect genetic abnormalities associated with the presence of cancer. Chen et al. using the cfMeDIP-seq method, the cfDNA methylome distinguished localized from metastatic prostate cancer with 98.9% accuracy. Even with low free tumor DNA fractions (<2%), cfMeDIP observed characteristic epigenetic signatures associated with immunosuppression and poor prognosis [55]. Brikun et al., in turn, confirmed the effectiveness of methylation panels in diagnosing prostate cancer, achieving a non-invasive urine test with a sensitivity of 94% and a specificity of 71–76% [56].

Detailed cfDNA analysis enables the detection of tumor progression and drug resistance at an early stage of the disease. These studies provide more accurate information at a very early stage and increase the effectiveness of therapy. Circulating tumor DNA is

significantly easier to detect in the blood plasma of patients with advanced or metastatic prostate cancer than in individuals with a localized form of the disease. For this reason, in routine oncological practice, liquid biopsy tests that analyze ctDNA are currently primarily used in the context of advanced, castration-resistant prostate cancer. This enables the identification of genetic mutations, the monitoring of treatment response, and the early detection of mechanisms of resistance to the therapies being administered.

High plasma ctDNA levels correlate with poorer survival; therefore, ctDNA may be a useful prognostic factor. Additionally, unchanged ctDNA levels after surgical treatment for early-stage prostate cancer may indicate disease recurrence and enable referral of patients for further therapy. Pope et al. detected ctDNA in 19 (9.3%) of 118 patients with localized prostate cancer after prostatectomy, which correlated with significantly shorter biochemical recurrence-free survival and metastasis-free survival compared to patients with negative ctDNA in their blood [57].

Although determining circulating tumor DNA levels allows for genomic characterization, helpful in making therapeutic decisions, especially in clinical situations where tissue biopsy is difficult, such as bone-only disease, the widespread clinical implementation of ctDNA analysis is hampered by several analytical challenges, such as low sensitivity in localized disease and low disease burden, and the risk of false positives due to clonal hematopoiesis. Moreover, it is necessary to improve the standardization of pre-analytical stages and the interpretation and reporting of post-analytical data in individual institutions.

5. Circulating Extracellular Vesicles

Small cellular particles formed as a result of endocytosis and released outside the cell by exocytosis are called extracellular vesicles (EVs). They can arise in both normal and cancer cells. These are spheres with a diameter of 30–200 nm protected by a double lipid layer. EVs consist mainly of nucleic acids, proteins, and lipids [58]. Extracellular vesicles are responsible for cell-to-cell communication and lead to changes in the biomolecular bioactivity of recipient cells through the release of numerous biological substances. Consequently, cancer cells can release exosomes with cancer-specific biomarkers that enable the detection of primary tumor sites [59]. Higher numbers of EVs were found in cancer patients than in healthy people [60]. Numerous studies indicate that exosomes play an important role in carcinogenesis, tumor development, and metastasis of many cancers, including prostate cancer [61,62]. Therefore, extracellular vesicles may be useful as biomarkers of prostate cancer in cancer detection, monitoring of cancer metastasis, and screening. Recently, and especially within the last 3–4 years, numerous prospective clinical trials and validation studies have demonstrated that extracellular vesicles constitute a clinically important biomarker platform in prostate cancer. The significant clinical role of EV-based biomarkers is confirmed by ExoDx Prostate (Intelliscore), the first non-invasive EV-based diagnostic test certified by the Food and Drug Administration (FDA) and approved by the Clinical Laboratory Improvement Amendments (CLIA). However, the FDA has not yet granted full approval or authorization for this test; instead, it has awarded it the "Breakthrough Device Designation," making it the first exosome-based "liquid biopsy" test to receive such a distinction. This status is intended to accelerate the development and evaluation process, but it does not constitute final market authorization. CLIA certification means that the test is officially permitted for use in the U.S. market as a Laboratory Developed Test (LDT) and is performed in a specialized, certified laboratory within the United States. It is a non-invasive diagnostic test integrated with prostate cancer risk stratification algorithms [63]. The test involves quantitatively determining the expression of three genes—ERG, PCA3, and SPDEF—in urinary extracellular vesicles. This risk score (1–100) predicts the likelihood of clinically significant prostate cancer in men aged ≥50 years with a PSA of 2–10 ng/mL without requir-

ing a digital rectal exam. This test has been shown to not only provide an individualized assessment of prostate cancer risk during the initial biopsy but also prove significant in subsequent repeat biopsies [64].

Advanced research confirms that exosome-derived components can provide superior diagnostic performance compared to prostate-specific antigen testing in experimental and clinical settings. Several urinary exosome-derived microRNA signatures demonstrate high diagnostic accuracy, as evidenced by an area under the ROC curve (AUC) of approximately 0.92 and sensitivity and specificity of over 80%, significantly exceeding PSA alone (AUC ~ 0.60) in the studied cohorts [65]. Immunocapture assays based on exosomal proteins, such as carbonic anhydrase IX, have also been reported to demonstrate high sensitivity in differentiating prostate cancer from benign prostatic hyperplasia. Urinary exosomal PSMA and selected miRNA signatures (e.g., miR-1246) have achieved AUCs of 0.85–0.93 in retrospective or single-center studies. Future studies involving larger cohorts are warranted to validate these findings. Such validation could potentially lead to the development of a simple test that can be integrated with other clinical risk calculators to non-invasively predict the aggressiveness of prostate cancer [66,67].

Progress in the diagnostic use of extracellular vesicles has been made possible by the use of numerous isolation methods, such as ultracentrifugation combined with a sucrose gradient and isolation using immunobeads, such as magnetically activated cell sorting (MACS). Transmission electron microscopy (TEM) or nanoparticle tracking analysis (NTA) can also be used to analyze extracellular vesicles. Another method involves examining numerous membrane-associated proteins, e.g., ICAM-1 or integrins, which are identified using flow cytometry, Western blotting, quantitative RT-PCR, and nucleic acid sequencing [68].

6. Metabolomic and Proteomic Markers

The process of carcinogenesis in various organs causes changes in cell metabolism, which can initiate or exacerbate cancer cell proliferation. These specific metabolic pathway abnormalities, depending on the tumor type, can be used in cancer diagnosis with liquid biopsy. The development of modern research methods, such as liquid chromatography with mass spectrometry, allows for the analysis of various body fluids for the presence of different metabolites [69,70]. The recent identification of several different metabolites offers an opportunity to improve the detection and monitoring of prostate cancer treatment. Healthy prostate cells exhibit a specific metabolic profile (including high citrate and zinc concentrations) that is significantly disrupted during malignant transformation. These abnormalities, combined with other factors such as metabolic syndrome, which increases prostate cancer risk, highlight the potential of metabolite profiling to elucidate the biological processes associated with prostate cancer carcinogenesis [71]. For example, changes in lipids, polyamines, and amino acids, which are intermediate metabolites of the tricarboxylic acid cycle, are characteristic of prostate cancer. However, products such as choline, phosphatidylcholines, and sarcosine help differentiate prostate cancer cases from healthy ones [72]. Even better results are obtained with a panel of 18 serum biomarkers of inflammation and cytokines, such as vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6), which will increase the detection of aggressive prostate cancer. Combining metabolomic data with genetic markers has additionally improved diagnostic accuracy. An example is the Michigan Prostate Score 2 (MiPS2). Biomarkers utilizing exosome content (e.g., prostate cancer antigen 3 (PCA3), SAM pointed domain-containing ETS transcription factor (SPDEF), and transmembrane protease, serine 2–ETS-related gene fusion (TMPRSS2-ERG)) also enable non-invasive analysis of RNA expression in tumor cells [73].

Metabolic biomarkers can also be used as prognostic markers. Based on metabolic profiles obtained from histologically benign tissues, tumor malignancy and prostate cancer recurrence can be predicted. The obtained data regarding the prognosis of further cancer progression exceed those obtained using current gold-standard methods [74]. High glutamate levels have been shown to correlate with high diagnostic performance (area under the curve is about 90%) in distinguishing benign prostatic hyperplasia from prostate cancer. Furthermore, significant changes in the concentrations of choline, lysophosphatidylcholine, and amino acids such as aspartate, proline, and serine were observed between prostate cancer patients and controls [75].

Proteomic biomarkers include proteins, peptides, and post-translational modifications that reflect cancer cell function. The human proteome, consisting of 500,000 proteins from approximately 35,000 genes, is estimated to be highly complex due to alternative splicing and post-translational modifications. Proteomics based on modern mass spectrometry, such as stable isotope labeling of amino acids in cell culture or tandem mass tags (TMT), has enabled high-throughput protein analysis [76]. Larkin et al., examining over a thousand proteins in serum samples, found that seven target proteins, such as tumor necrosis factor (TNF), signal transducer and activator of transcription 3 (STAT3), NF-κB, and IL-6, could be useful in diagnosing prostate cancer [77]. In turn, Turiak et al. showed that prostate tumor proteins differ significantly depending on the stage of cancer advancement [78].

Although proteomic biomarkers have an increasing diagnostic importance, it is important to remember that broadband proteomic studies, due to their complexity and the difficulty of interpreting large amounts of data, are primarily for research purposes. However, several of the newly studied proteomic biomarkers demonstrate promising diagnostic potential and, despite discrepancies between studies, may become even more significant with improved validation. Another problem is the fact that the number of tissue samples obtained for proteomic analyses is small, and some MS methods are unable to detect large numbers of samples.

7. Limitations and Challenges

Although the biomarkers presented in this publication have potential applications in prostate cancer diagnostics, it is important to acknowledge existing limitations. Significant heterogeneity across studies regarding research designs, analytical methods, and patient populations poses a challenge. This diversity precludes meta-analysis and direct comparisons. Many studies are retrospective or employ a case–control design with small sample sizes, which may lead to errors in selection and result interpretation. Methodological differences also contributed to a lack of reproducibility. Research primarily utilizes targeted detection methods (e.g., qPCR or ELISA), which allow for the possibility of interference from various biomolecules. A necessary condition for optimizing and identifying biomolecules is their comprehensive profiling through sequencing. Standardization of the processes of quantitative determination of biomarkers and further analysis is a condition for ensuring the repeatability and comparability of research results [79]. Inconsistencies in analytical sample processing, normalization and assay calibration, and varying cutoff values resulted in a lack of comparability between studies. Another issue concerns the timing of biomarker testing. There is also significant heterogeneity regarding participant selection, which complicates the analysis of results and limits the ability to draw definitive conclusions. For example, the use of patients with benign prostatic hyperplasia (BPH) as a control group can lead to differences in baseline characteristics across the various control populations. Most studies examined and assessed them at a single time point, and only a few compared changes over time or across disease stages. However, the greatest obstacle to clinical adoption is the lack of large, prospective, blinded validation studies.

The challenge lies in implementing molecular biomarker analysis into clinical practice and therapeutic procedures. Liquid biopsy can complement standard tests, increasing the accuracy of early prostate cancer detection and reducing the number of false positives. Further work is also needed to develop user-friendly point-of-care diagnostic devices using biomarkers. The key to successful implementation of these solutions will be the involvement of clinicians knowledgeable about the biology and interpretation of biomarkers and understanding the principles of their integration into existing diagnostic procedures. The cost-effectiveness of using biomarkers in diagnostic procedures is also important, but it remains ambiguous in light of various studies [80,81]. Cost estimates depend on many factors, such as the type/stage of cancer, the specific application (e.g., screening, monitoring, treatment stratification, and response assessment), and the observation period. A comparison of the advantages and disadvantages of prostate cancer biomarkers is presented in Table 3.

Table 3. Advantages and disadvantages of prostate cancer biomarkers.

Biomarker	Advantages	Disadvantages
CTCs	They contain intact, whole DNA, RNA, and proteins. They are an excellent prognostic marker.	They show extremely low concentrations in the early stages. They require advanced and expensive isolation technologies. There is a risk of missing cells after EMT transformation.
microRNA	They are highly stable in body fluids (blood, urine). They play a significant role in key pathways of prostate cancer pathogenesis. Easy and cheap detection (qPCR technology).	They have low specificity (the same microRNAs can change during inflammation). There are no unified reference standards for standardizing results. High individual variability.
ctDNA	They allow for the precise tracking of genetic mutations and changes in gene copy number. They exhibit high specificity for cancer-related mutations. They have a short half-life (enabling real-time monitoring).	They have low sensitivity in the early stages of the disease. Sequencing costs are high. There are difficulties in distinguishing ctDNA from cfDNA derived from healthy cells.
EVs	They protect the molecules they contain (RNA, proteins) from degradation. They reflect the status of the tumor stem cell. They are present in large quantities in the urine of patients.	Isolation procedures using ultracentrifugation are difficult, time-consuming, and not very repeatable. There is a high degree of contamination with material from other tissues.
Metabolomic markers	They dynamically respond to the slightest changes in tumor cells. It is possible to test the metabolic profile directly in a patient's urine sample.	They are characterized by high susceptibility to the influence of external factors (patient's diet, lifestyle, and medications). They require the use of complex equipment (mass spectrometry, NMR).
Proteomic markers	They directly reflect the active tumor phenotype. They enable the creation of multi-protein panels, increasing diagnostic sensitivity.	Proteins are rapidly degraded by proteases in the sample. The dominance of "major" proteins (e.g., albumin) makes it difficult to detect rare markers.

8. Conclusions and Perspectives

The development of effective biomarkers for cancer detection is essential for increasing the survival rate of cancer patients and improving their quality of life. Liquid biopsy is a minimally invasive method that, unlike traditional tissue biopsy, promises to improve the diagnosis and treatment of prostate cancer. Molecular biomarkers such as circulating microRNAs, CTCs, and EVs, among others, show great potential as non-invasive tools for early detection of prostate cancer. A number of studies indicate that these biomarkers measured at the time of detection and during monitoring of the course of the disease and serially during active surveillance can provide important prognostic information. It is generally believed that tumors are highly heterogeneous; therefore, each stage should be treated as a separate condition. An additional benefit is that these circulating markers, such as extracellular vesicles found in various body fluids (e.g., blood or serum), contain tumor-derived genetic material. A review of currently known molecular biomarkers indicates that they complement PSA and raises the hope that they will be more effective, especially when used together. One piece of evidence is the demonstration that free DNA from plasma is suitable for monitoring response to treatment and early detection of mechanisms of acquired resistance [82]. From a clinical perspective, new biomarkers are more likely to serve as a supplement to, rather than a replacement for, existing diagnostic methods and parameters, including PSA. In the context of prostate cancer, for instance, they may facilitate more precise risk stratification, particularly in patients with borderline PSA levels, or assist in decision-making regarding whether to perform a biopsy. It is worth emphasizing that future studies should clearly define their intended clinical applications, as the requirements for efficacy vary significantly depending on whether the context is screening, diagnosis, or prognostic assessment. Looking ahead, multimodal diagnostic strategies—integrating the analysis of ctDNA, microRNA, proteins, and metabolites present in the blood (either alone or in combination with PSA testing)—may enable a more comprehensive assessment of tumor biology.

Molecular and metabolic biomarkers can be utilized at various stages of the prostate cancer diagnostic process. Proteomic and metabolic markers, extracellular vesicles, and microRNAs demonstrate the highest diagnostic efficacy—that is, the ability to detect the condition—whereas circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) show limited diagnostic efficacy in the early stages of cancer. Nevertheless, circulating tumor cells and circulating tumor DNA exhibit significant prognostic and predictive value.

Developing technologies, such as multimodal liquid biopsy or mutation analysis supported by machine learning, enable the study of modern biomarkers (e.g., CTCs, EVs, microRNAs, and circulating extracellular DNA) and set new directions for future research. The main goals of future research are to determine optimal cutoff values for biomarkers, elucidate the interrelationships between biomarkers and MRI findings, and explore the possibility of their integration to better characterize the disease and monitor its course. The future use of multi-marker molecular panels with current diagnostic schemes will allow better assessment of risk stratification, reduce the number of unnecessary biopsies, and support more precise and faster detection of prostate cancer worldwide. This requires broad international collaboration, encompassing large groups of patients with various cancer types, and the exchange of information on treatment methods. This will allow for the development of more effective biomarkers that improve the prediction of treatment response in prostate cancer patients. Having reliable data on treatment outcomes obtained in high-throughput studies is crucial for the discovery of clinical biomarkers. Early detection of the disease and accurate prediction of treatment effectiveness may ultimately help clinicians improve survival rates in this group of patients. An additional benefit of the development of molecular biomarkers results from the expansion of biological and patho-

physiological knowledge about the process of prostate cancer carcinogenesis. Strategies based on biomolecular markers represent a promising complement to current diagnostic methods. It should be noted, however, that many of these are still in the research phase and require assay standardization, prospective validation, and the demonstration of clinical utility before they can be implemented on a wide scale.

At the current stage of research into prostate cancer biomarkers, only total and free PSA remain fully validated. Biomarkers such as CTCs, ctDNA, and EVs, despite showing high diagnostic potential, have not yet undergone the full validation process required for widespread implementation into medical standards. For instance, secondary validation often takes place primarily within small research cohorts or retrospectively as part of advanced clinical trials. There is a lack of definitive cutoff values. For clinical markers (such as PSA), laboratory procedural errors are minimized. In the case of experimental biomarkers (e.g., microRNA, ctDNA), the lack of international calibration standards remains a significant challenge.

This review presents a wide range of molecular biomarkers for prostate cancer. In our view, however, extracellular vesicles and microRNAs are of particular interest. From a clinical perspective, extracellular vesicles represent a promising class of non-invasive biomarkers that facilitate early detection, risk stratification, and treatment monitoring. Multi-omic studies utilizing extracellular vesicles are likely to prove increasingly valuable in cancer diagnosis and prognosis. From a therapeutic standpoint, modified extracellular vesicles offer an innovative platform for the targeted delivery of cytotoxic agents, small molecules, and immunomodulatory drugs—an approach particularly relevant to prostate cancer. Although therapeutic research involving extracellular vesicles remains largely in the preclinical stage, several proof-of-concept studies demonstrate the promising potential of EV-based therapies for the treatment of advanced-stage prostate cancer.

The assessment of microRNAs represents a groundbreaking frontier in the detection of prostate cancer. Their high stability and ability to reflect tumor biology make them suitable for use in non-invasive screening, more accurate prognostication, and real-time monitoring. The application of microRNA-based tools will steer the field of prostate oncology toward a more precise and personalized approach. Ultimately, integrating microRNA-based tests with existing biomarkers could enable more accurate patient stratification, reduce overtreatment, and facilitate timely therapeutic decision-making in prostate cancer.

The use of new techniques, primarily artificial intelligence, can help solve current challenges—for example, identifying diagnostic and prognostic biomarkers—which will enable physicians to more precisely select medications. Integrating this diverse data with advanced computational methods allows for a comprehensive picture of the cancer process. In the era of artificial intelligence, diagnostics based on both quantitative biomarker determination in liquid biopsy and imaging studies, combined with clinical data, can also improve the effectiveness of prostate cancer detection, treatment monitoring, and prognosis. Advances in molecular biomarker development, AI-powered analysis, and scalable technologies pave the way for effective therapeutic management.

The future of prostate cancer diagnostics focuses on replacing the traditional PSA level measurement with more precise tools, implementing optimized screening algorithms, and advancing personalized medicine. A primary challenge is to avoid overdiagnosis and unnecessary, invasive treatment of low-aggressiveness tumors, while ensuring the early detection of aggressive forms. Further development of liquid biopsies, the investigation of highly specific biomarkers, and the use of multi-marker panels (e.g., the ExoDx Prostate test) are essential. The test is included in the National Comprehensive Cancer Network guidelines (since 2019) and has FDA Breakthrough Designation status. It is also advisable to implement "smart screening" programs—which utilize algorithms based on risk stratification (factoring

in age, family history, and baseline PSA levels)—and to combine laboratory tests with rapid magnetic resonance imaging (MRI). Significant emphasis must be placed on genetic testing conducted at an early stage of the diagnostic process.

Molecular biomarkers have the potential to become a vital tool in the diagnostic process, enabling non-invasive, real-time insight into tumor progression and supporting the development of individualized treatment plans. By providing dynamic information on tumor characteristics and therapeutic responses, biomarkers—such as circulating tumor cells, microRNAs, and extracellular vesicles—are revolutionizing the diagnosis of prostate cancer and the approach to personalized treatment. While numerous challenges remain, including issues regarding standardization and cost-effectiveness, these biomarkers offer tremendous potential for enhancing cancer detection, monitoring, and patient treatment outcomes. Furthermore, new biomarkers can improve our biological understanding and offer insights into the mechanisms underlying the progression of prostate cancer. Efforts should be intensified to integrate multi-marker molecular panels with existing diagnostic methods, which could improve risk stratification, decrease the number of unnecessary biopsies, and support the precise detection of prostate cancer.

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