

REVIEW ARTICLE

# Prostate Corpuscles: Looking for Biomarkers of Early Prostate Cancer

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**Abstract:** Prostate cancer is the second most diagnosed disease in men in the world, and the mortality rate has been rising in recent years. At present, there are two early screening tests which are determination of plasma concentration of prostate specific antigen and prostate rectal contact. However, these tests do not have the best specificity and sensitivity for the detection. Although different studies have searched for new biomarkers through the implementation of new generation sequencing, mass spectrometry and other technologies, the same shortcomings still exist, so they cannot be translated in clinical practice; Therefore, the discovery of new prostate cancer biomarkers is a challenge for the scientific community.

Prostate corpuscles correspond to extracellular vesicles secreted by normal prostate or tumor tissues and can be detected in different liquids. Structurally, prostate bodies are different from other exosomes because their size, membrane composition and specific protein content make them a potential and new source of clinical biomarkers.

In this regard, this review reviews protein biomarkers isolated from prostaglandin glands present in different biological fluids for potential diagnosis of prostate cancer. To this end, a systematic search was conducted on PubMed for proteomic studies of prostate cancer, including extracellular vesicles, exosomes and prostaglandin glands, as well as blood, urine, semen and other biological samples.

**Keywords:** Biomarkers, Prostate cancer, Extracellular vesicle prostate, Protein

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## 1. Introduction

Prostate cancer is the second most common tumor in men<sup>[1]</sup>. It is estimated that 1.1 million men worldwide were diagnosed with PCa in 2012, which is also the fifth leading cause of cancer death in men<sup>[2]</sup>. More than 6,500 new cases are diagnosed every year in China, and more than 2,400 men die because of this tumor disease<sup>[3]</sup>.

Prostate specific antigen (PSA) determination and prostate rectal contact are two early screening tests for the diagnosis of prostate cancer<sup>[4]</sup>. PSA is a protein produced by prostate, and its increased expression may be caused by a variety of reasons, including benign prostatic hyperplasia (BPH), prostatitis<sup>[5]</sup>, aging<sup>[6]</sup> and PCa. Although this is a non-invasive and sensitive test<sup>[7]</sup>, its specificity is very low, resulting in false positive diagnosis. In fact, the probability of diagnosing PCa in patients with PSA greater than 4 ng/ml is 21%, that is, 75% of “over evaluation”, while for tumor diseases that may be painless, “over diagnosis” is 30%–50%<sup>[8]</sup>. Even some PCa patients are characterized by low PSA levels. In this

context, it has been reported in recent years that the prostate health index (PHI) is a new formula that combines three forms of PSA (total PSA, free PSA and proPSA) into a single score, which can be used for the diagnosis and management of PCa<sup>[9]</sup>. This study showed that the level of phi in patients with chronic prostatitis could be reduced by 10% compared with that in patients with chronic prostatitis. However, Phi has no effect on patient stratification, so we continue to look for better biomarkers for the diagnosis, prognosis and follow-up of PCa<sup>[10]</sup>.

As for rectal contact, this test causes discomfort because it involves digital scanning of glands through the rectum. In addition, it only allows touching the anterior surface of the prostate, so the efficiency is quite low. It is estimated that 23% to 45% of PCas are ignored and about 50% of PCas are diagnosed at an advanced stage<sup>[7]</sup>. Therefore, in many cases, the early diagnosis of PCa fails. On the other hand, other markers, such as the marker encoded by PCA3 gene (anti gene 3 gene in prostate cancer) are translated into prostate tumor specific proteins, which are detected in the urine and prostate fluid of patients. The level of PCA3 in the urine of about 95% of PCA patients is higher than that of healthy controls. In several published studies, they analyzed the effectiveness of this biomarker in detecting PCa in urine samples after prostate massage. The first study reported 66% sensitivity and 89% specificity<sup>[11]</sup>, and the second study reported 74% and 91% sensitivity and specificity<sup>[12]</sup>. These results have aroused interest in developing a commercially available urine test.

The PROGENA-PCA3 test is based on the transcriptional amplification of PCA3 mRNA in urine. The results produced a score that was evaluated in the clinical context of each patient. This urinary PCA3 test is used together with serum PSA test to improve the detection threshold of PCa<sup>[13]</sup>. So far, this is the only commercial urine biomarker with obvious advantages, such as prostate volume will not affect the results (such as serum PSA test), and identify high-risk patients with PCa, so as to improve the diagnostic level by reducing unnecessary biopsies.

Patients with suspected PCa should usually undergo prostate biopsy for active monitoring. However, due to the multifocality and heterogeneity of prostate tumors, histopathological analysis may underestimate the degree or degree of the disease and even do not allow the observation of tumor tissues<sup>[14]</sup>. Therefore, there is an urgent need to find and implement easily available markers carried in blood, urine or other body fluids in order to detect diseases early, actively monitor PCa in a minimally invasive manner, reduce the cost of health system, and avoid potential complications by sampling prostate tissue through transrectal biopsy.

In vitro studies have shown that all types of human

cells release extracellular vesicles (VES), so it is common to observe them in different body fluids<sup>[15]</sup>. These VES are released from normal, apoptotic and necrotic cells and have a variety of functions, including participating in intercellular communication, especially the process of adaptive immune response<sup>[16]</sup>. In addition, the molecular mechanism and chemical composition of VES formation have been described in detail in the past decade.

Prostatic fluid contains two types of VES, namely prostatic corpuscles and exosomes: prostatic corpuscles (150–500 nm), which are produced by prostatic ductal epithelial cells, which are a normal part of semen and play a role in male fertility; And extracellular bodies, which are special nano vesicles (30–100 nm)

It has a cup-shaped shape and is actively secreted by a variety of normal and tumor cells<sup>[16]</sup>. A large number of exons were found in the serum, urine and tumor effusion of cancer patients<sup>[16]</sup>.

Due to the unique cancer specific content of VEs in cancer cells and the presence of prostate bodies in the blood and urine of patients with PCa, it is assumed that VEs can provide useful markers for PCa. This idea was supported by earlier studies that compared the proteomic characteristics of VES isolated from prostate tumor and non-tumor cell line cultures, in which a variety of proteins that may constitute biomarkers of PCa candidate proteins were identified<sup>[17]</sup>. In this regard, the purpose of this review is to introduce the latest progress of PCa diagnostic/prognostic protein biomarkers isolated from prostaglandin glands and other VEs. **Table 1** lists the revised biomarkers, distinguishing the advantages, disadvantages and potential uses of the reported studies (see **Table 1**).

## 2. Methodology

A systematic search for proteomics of prostate cancer was conducted on PubMed. The criteria are as follows: It involves the exons and prostaglandin glands, as well as biological samples such as blood, urine and semen. After manual revision, 39 original articles were included, 16 for blood, 7 for urine, 9 for semen and 7 for other biological sources. The bibliography was collected from March to July 2018.

## 3. Prostate gland and PCa

The prostate body was originally characterized by *Gunnar Ronquist* at the end of 1970<sup>[18]</sup>. The formation of prostate corpuscles begins with acinar epithelial cells of human prostate. Prostaglandins enter the glandular light through extracellular events and then fuse with the adjacent membrane. You can also see that the storage capsule can be completely transferred from the interior of the cell to the acinar light through the plasma membrane.

This process is called diacytosis. The storage sac corresponds to a polyhedron originating from the late endoderm<sup>[19]</sup>. This process is shown in the **Figure 1**.

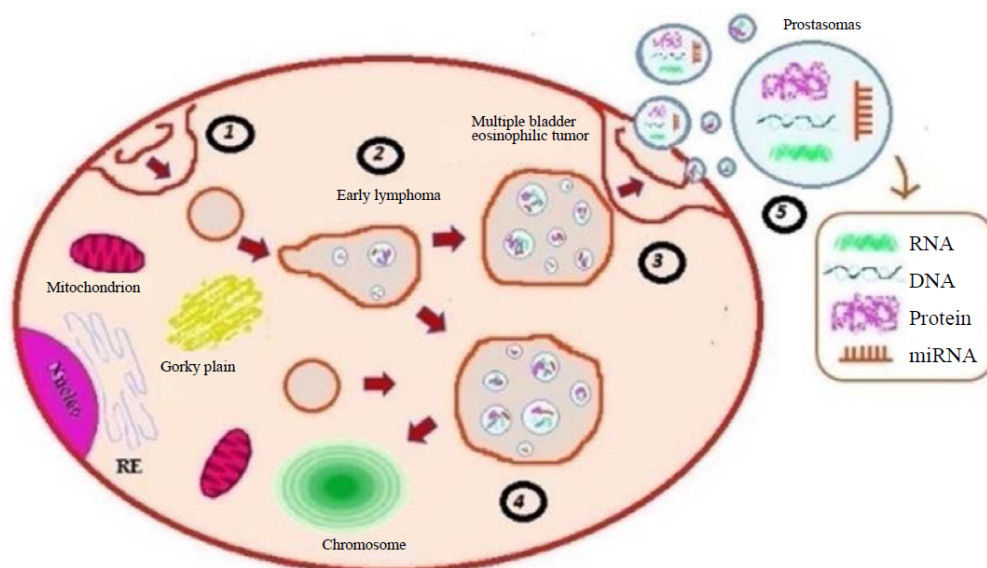
Different studies have shown that these prostate bodies have a variety of physiological functions, usually related to the function of mother cells. They can carry molecules such as lipids or proteins into sperm during sperm entering oocytes, and have the following functions: acting as intercellular messenger between prostate secretory cells and spermatocytes. As a “reservoir”,

sperm can be used according to environmental conditions<sup>[18]</sup> to regulate the formation of sperm cells, which is called the process of sperm reaching the ability to bind to the zona pellucida of oocytes, experiencing acrosome reaction and obtaining hyperactive movement; This hyperactivated movement is necessary for sperm cells to fall off from the oviduct epithelium, where the mucus swims through the follicular cell layer around the oocyte, thus penetrating the zona pellucida, leading to correct fertilization<sup>[15]</sup>.

**Table 1.** Prostate associated protein that is considered as a potential marker of prostate cancer

Biological fluid	Protein	Advantage	Shortcoming	Practicability	Reference
Blood	Surviving	It is related to tumor severity and mortality	Small sample cost	Diagnostic/therapeutic response	[23], [28], [29]
	PTEN	Expression was enhanced only in tumor cells	Further research is needed	Help control cell growth	[31]
	NKG2D	Associated with immune response and cancer progression	Further research is needed	Immunotherapy treatments	[35]
	PSMA	Present in ves components isolated from the urine of patients with PCa	Further research is needed to confirm this finding.	High potential as PCa biomarkers	[17]
	Ramto	PCa secretion increased significantly	Further research is needed	High potential as PCa biomarkers.	[39]
Urine	TMPSR2: Electroretinogram	Increase in PCa, require a small number of samples	A larger number of people are needed in the study	Diagnosis/monitoring PCa tumor	[16]
	PCA-3	Tumor phenotype severity classification	Research needs to be conducted in a larger population	Diagnosis/monitoring PCa tumor/disease classification	[16]
	Appendix A3	High specificity as a PCa biomarker	Supplement PSA	Epidemiological surveillance	[16]
	TM256	It has high sensitivity to distinguish PCa	Research needs to be conducted in a larger population	High potential as PCa biomarkers.	[39]
	CD13 and DPPIV	Abundant LS concentration in serum of patients with PCa	Further research is needed	Prostaglandin gland study	[43]
Semen	Galectina-3	Functions related to sperm regulation, immunosuppression and PCa progression.	Further research is needed	PCa progress	[45]
	Chromatograph	Expression of in prostate cancer metastatic cells	More tests are needed	Degree of malignancy	[47]
	Kinase and ATPase	Significant increase in activity: phosphorylation	More tests are needed	Types of immunotherapy/drug intervention in patients with PCa	[48]
Other sources	Connexin	Its expression is related to the increased proliferation of hepatoma cells	Other sources and tumors need further study	Possible biomarkers of PCa	[49]
	Caveolin-1 and MRP1	Its increase leads to cell growth arrest and inhibits the cell invasion of PCa	Further investigation is needed	Diagnosis/prognosis/progress of disease	[52], [53]

Source: author.



**Figure 1.** Prostate formation. The cellular pathway of prostate secretion in extracellular space. (1) Vesicles grow inward from the cell surface. (2) These vesicles are invaginated into the endoderm to form the early metastasis of the inner sac to the endoderm, resulting in multivesicular endodermoma (MVE). (3) MVE can be located on the cell surface (4) or mature lysosomes, where the gallbladder nest will be digested (destroyed). (5) Finally, if continue fuses with the plasma membrane through exocytosis, it releases its contents, including the internal capsule that is converted to prostaglandins when secreted from the cell. Source: author

In addition to the prostate, other epithelial cells of the male reproductive tract also release VEs, which are mixed with the real prostate body during semen excretion<sup>[15]</sup>, such as the epididymal epithelium that produces VEs, which is believed to be removed from the plasma membrane in the form of gonadotropins. These vesicles then fuse with the sperm plasma membrane, allowing specific cell membrane subsets and cytosolic proteins to be transferred to sperm cells.

The prostate body is rich in protein; In a study conducted by Utleg *et al.*<sup>[20]</sup>, samples were collected from ejaculation samples of 5 healthy volunteers and further identified by shotgun proteomics. A total of 139 proteins were identified, of which 128 were not previously described as prostaglandin components. It is worth mentioning that many proteins reported in this study are regulated by androgens, such as PSA, serine transmembrane protease 2 (TMPRSS2), prostate specific transglutaminase, and some of them are highly specifically expressed in this tissue, such as PAP and prostate stem cell antigen (PSCA)<sup>[15]</sup>.

Poorly differentiated PCa tumors and metastatic cells have the ability to synthesize and output high concentrations of prostaglandins, because in the process of carcinogenesis, the tissue structure changes, which is conducive to the release of prostaglandins into the interstitial space. In addition, it is well known that tumor cells tend to use the host's physiological system to obtain support for nutrition, growth and metastasis. It seems that there are several prostaglandin gland that can help fertilize sperm cells, promote the transition from normal

cells to tumor cells, and help poorly differentiated cancer cells survive and metastasize<sup>[21]</sup>.

#### 4. Prostatic fluid

The general concept that exosomes isolated from blood can be used as potential biomarkers for cancer detection has recently been approved for pancreatic cancer, because in this model, exosomes of tumor cells are identified as cancer specific and unique content<sup>[22]</sup>.

As mentioned earlier, membrane secreted VEs initially develops in the vesicular endoplasm in the cell. In this process, proteins and nucleic acids are enPCasulated in the exosome. Once released into the extracellular space, these VEs enter the circulation<sup>[17]</sup>. Especially in PCa, due to the loss of cell polarity, it helps to release the prostate body into the interstitial space, so as to promote circulation<sup>[23]</sup>, which may be separated from the blood for further research.

For several years, some blood derived molecular biomarkers for the detection of PCa are available<sup>[24]</sup>. However, due to the low sensitivity of these antibodies to prostaglandins and prostaglandins in the blood, it has been proved for the first time that these antibodies can be induced by prostaglandins/prostaglandins. These antibodies can be detected in the blood of patients with PCa. However, these anti prostacyclinoma antibodies are rarely specific and do not allow PCa stratification<sup>[25]</sup>.

However, it must be considered that not only the prostaglandin glands exist in the blood, but also other prostate bodies are actively secreted by most nucleated



cells, resulting in pollution, thus transforming the separation process of prostaglandin glands into a complex process<sup>[17]</sup>. In a study conducted by Tavoosidana *et al.*<sup>[23]</sup>, they proposed a method for isolating prostaglandin glands. In this method, the detection of these multi protein microvessels depends on the simultaneous recognition of five different epitopes in at least four proteins, namely aminopeptidase N (CD13) and tissue factor (CD142), a cell membrane associated glycoprotein, as the receptor and key cofactor of coagulation cascade VII and VIIa. By using different monoclonal or polyclonal antibodies to detect the adequacy of prostaglandin glands in the plasma of patients with PCa, they modified the proximity ligation test (PLA), which is a sensitive and specific protein detection mechanism, in which relevant molecules must be recognized by antibodies<sup>[26]</sup>, and then quantified by real-time PCR.

Plasma was collected from two groups of PCA patients and the control group and matched by age: the first group included 20 patients with PSA of 94–2,706 ng/ml and 20 controls with PSA level lower than 2.5 ng/ml. The second group was composed of 59 patients with PCa and PSA of 1.1–39.1 ng/ml. Compared with the control group of 20 patients with PSA of 1.7–14.8 ng/ml, the results showed that both cases were benign. Relevant prostaglandin body levels were detected in blood samples from patients with PCa, which was 7 times higher than 20 samples in the control group<sup>[23]</sup>.

Using this study, we successfully demonstrated that the prostate body could detect elevated levels in the plasma of patients with PCa. In addition, the results also showed that this analysis can distinguish between patients with medium and high Gleason score and patients with low Gleason score, which is related to tumor invasiveness. In conclusion, due to its high sensitivity and specificity to prostaglandins in blood samples, the test conducted in this study is expected to be used as a method to separate these vesicles, but the test should continue<sup>[23]</sup>.

On the other hand, the anti-apoptotic protein survivin belongs to a known oncoprotein family, which is isolated from blood derived extracellular vesicles expressed in most malignant tumors<sup>[27]</sup>, which is why it has been studied as a potential biomarker of different types of cancer, including PCa. In a study of blood samples from African American PCa patients, this protein was significantly increased compared with men and controls from other ethnic groups<sup>[28]</sup>. It should be emphasized that PCa is often more aggressive among African Americans than men from other ethnic groups; This finding describes the tumor biological differences of PCa in different races. In particular, the authors believe that the increased expression of survivin in VEs in African

American PCA patients may affect tumor invasiveness and contribute to the mortality observed in this population<sup>[28]</sup>. This protein can be used as a new biomarker and potential therapeutic target, and may have clinical application value in improving the health of patients with PCa, because survivin has been proved to exist outside cells and contained in exons<sup>[29]</sup>. In addition, it is also expressed in PCa, and its regulation makes cancer cells sensitive to chemotherapeutic drugs. Khan *et al.*<sup>[30]</sup> conducted a study with enzyme-linked immunosorbent assay and Western blot to study the exosomal survivin in the plasma of patients with different manifestations of PCa and compare its expression levels in the control group and patients with benign prostatic hyperplasia (BPH). To this end, they collected samples from the plasma of 10 healthy volunteers and 28 patients with PCa, and selected 10 low-level (Gleason 6) and 10 high-level (Gleason 9); In addition, they collected samples from 8 patients with advanced chemotherapy and 20 patients with BPH. In their study, survivin was detected in all control groups, PCa and BPH patients, but their results were different in all cases, with high expression in exons of PCA patients with Gleason score of 6 and 9 and patients with recurrence after chemotherapy. However, there was no significant difference in survivin levels among subjects with low or high Gleason scores. In addition, although the exons of BPH patients also contain survivin, the expression level of survivin is significantly lower than that of PCA patients. In conclusion, this study shows that plasma survivin level can be used as a diagnostic or monitoring tool for PCa<sup>[30]</sup>.

Since the content of exosomes reflects its cellular origin, these exosomes may contain carcinogenic protein or tumor suppressor protein RAS, which may lead to positive or negative effects related to cancer progression. In the study conducted by Gabriel *et al.* in 2013, in addition to 8 healthy volunteers aged 50 and 65, 30 samples of PCA patients before advanced (T3/T4) prostatectomy were collected<sup>[31]</sup>. Invasive or metastatic PCa is associated with the reduction or loss of PTEN, an effective tumor suppressor protein. Therefore, the results of this immunometastasis study show that PTEN is incorporated into the exosomes of blood circulation in patients with PCa, but interestingly, healthy subjects do not have this expression in their exosomes. The results of this study reveal a new mechanism of tumor cells regulating PTEN expression through exosomes. Taken together, the expression of PTEN in the exosomes of prostate cancer is a unique feature of tumor cells. The expression of PTEN in patients with prostate cancer may be a risk factor for metastasis or recurrence after prostatectomy. However, further studies are needed to confirm these findings<sup>[31]</sup>.

Extracellular microRNA (miRNA) embedded in circulating exosomes can be used as a biomarker of cancer prognosis. In a study conducted by Huang *et al.* In 2015, they sequenced RNA from 50 healthy subjects and 36 patients with PCa to determine the exosomal miRNA, of which 23 patients were resistant to castration. miRNA was detected by real-time fluorescence PCR. This study identified two miRNA candidates (miR-1290 and miR-375). However, cancer-related miR-375 is involved in a variety of cancers, including PCa. The regulation of PCa is related to both the metastasis of PCa and the survival of patients with esophageal cancer; On the other hand, compared with miR-375, miR-1290 has a better prediction of prognosis, but its increased expression in serum is helpful to distinguish low-stage pancreatic cancer from breast cancer, so its direct effect on PCa is uncertain. However, in this study, they observed some significant differences in results, suggesting that the surrounding exosomal miRNA may be a sensitive biomarker affecting the prognosis of CRP patients<sup>[32]</sup>.

Another study by Bryant *et al.*<sup>[33]</sup> proposed the analysis of changes in circulating miRNA as a potential biomarker for PCa diagnosis, stratification and prediction. For this purpose, they collected plasma samples from 78 patients with PCa (12 without metastasis, 51 M0 and 15 M1) and 28 controls (PSA > 10 ng/ml), and analyzed 742 miRs using circulating microparticles (CMV) by real-time PCR. In their results, they obtained 12 different numbers of Mir, of which 11 significantly increased the CMV of PCA patients compared with the control group, while the concentration of miR-181a-2 decreased. Subsequently, they analyzed the CMV of 55 patients with non-metastatic PCa. They identified 10 different degrees of miR, 9 miR showed a significant increase and miR-181a-2 showed a decrease. Then, they compared the miR spectra of 16 patients with metastatic PCa and 55 patients with non-metastatic PCa, and found that there were 16 quantitative differences in Mir between patients with metastatic PCa and patients with non-metastatic PCa; 15 miR showed a higher concentration, while the expression of miR-572 decreased significantly in men with metastatic and non-metastatic PCa. This leads to the conclusion that changes in Mir concentrations in PCA patients can be used for diagnostic discovery and stratification<sup>[33]</sup>.

Cancer cells produce a large number of VEs, so it is believed that tumor exosomes affect immune response and may contribute to cancer progression<sup>[34]</sup>. In a study conducted by Lundholm *et al.*<sup>[35]</sup> analyzed the negative regulatory ability of exosomes from tumor cells on the expression of NKG2D, a cytotoxic receptor expressed by a variety of immune cells, including NK cells, NKT cells, CD8 and T cells; its loss in cancer is very important in immunosuppression. In a study, the blood samples of 18

patients with chronic myeloid leukemia before chemotherapy and 8 healthy people were collected; by flow cytometry, they found that compared with the control group, the expression of NKG2D in circulating lymphocytes of CRPC patients decreased, and tumor derived exons induced negative regulation of NKG2D in T cells, NK cells and CD8 cells. According to these findings, the secretion of extracellular bodies may be the mechanism of PCa tumor immune escape<sup>[35]</sup>.

## 5. Urinary prostate

Urine is an easily available body waste and an ideal place for the determination and analysis of biomarkers. Urine is a complex mixture of proteins, salts, urea and filtration and secretion metabolites, which may be different not only in physiological conditions, but also in diseases affecting the urogenital system<sup>[36]</sup>.

At present, it is known that prostate specific protein (PSMA), prostatic acid phosphatase and prostatic transaminase<sup>[17]</sup> are prostate glands in the VES part separated from urine by detecting prostate specific protein.

Urine contains intact prostate tumor cells and apoptotic bodies produced by these cells. Cells and most apoptotic bodies are much larger than prostate bodies, so they can be easily separated by differential centrifugation<sup>[37]</sup>, so urine is an important source for the study of prostate body related biomarkers.

Although various tissues in the male genitourinary system, such as kidney, testis, etc.<sup>[38]</sup> contribute to VEs in urine, a special advantage of using this liquid as a source of biomarkers is that it is often found to be rich in prostaglandin glands. It may be due to the special function of prostate body in PCa tumorigenesis, angiogenesis and immune response evasion<sup>[36]</sup>.

In one study<sup>[40]</sup>, the proteome of urinary exosomes was analyzed using mass spectrometry to determine the differentially expressed proteins in PCA patients compared with healthy controls; Exosomes were isolated from the urine of 15 normal subjects and 17 patients with PCa. Analysis showed that 246 proteins were differentially expressed in the two groups, of which 221 proteins were overexpressed in PCa. In particular, 37 of these proteins were selected according to specific criteria, such as sensitivity, specificity and time of expression change. These 37 proteins together have 100% specificity. 17 of them showed more than 60% individual sensitivity. Although some proteins show high sensitivity and specificity to PCa as individual biomarkers, it is possible to fully differentiate PCa from healthy controls by combining them in a single test. Transmembrane protein 256 (TM256) had the highest sensitivity, 94% (16 of 17 cases). Similarly, the LAMTOR protein complex showed very high specificity in PCa samples. These

results demonstrate the potential application of urinary exosomes in the diagnosis and clinical treatment of PCa.

Another study<sup>[16]</sup> analyzed the urinary exosomes of 9 patients with PCa and divided them into the following four groups: Patients with untreated new diagnosis, diagnosis, androgen deprivation therapy (ADT) and confirmed bone metastasis; from very limited exogenous RNA, they successfully confirmed the existence of two known PCa biomarkers by nested PCR; PCA-3 and TMPRSS2:ERG. mRNA transcription of fusion gene TMPRSS2 ERG was detected in patients with high Gleason score and high PSA level, but not in patients with low-risk tumors, while *pca-3* transcription was detected in all patients after mild prostate massage. This study shows that the search for biomarkers by including mRNAs and exosomal miRNAs is helpful not only for the detection of cancer, but also for the classification of tumor phenotypic severity and treatment response.

At present, about 1,000 miRNAs have been found in humans, and each miRNA may be related to or target genes that affect important signaling pathways of several cancer progression. Some miRNAs have been found to have functional changes or differences in PCa and seem to affect anti apoptotic activity<sup>[33]</sup>.

In a population study<sup>[33]</sup>, urine samples of 135 men after transrectal prostate massage were collected. Five selected miRNAs were analyzed by RT-qPCR. It was found that miR-107 and miR-574-3p in the urine of PCA patients were significantly higher than those in the control group; Both miRNAs can identify the presence of PCa from urine samples (consistency index 0.66–0.74), which seems to be more accurate than the standardized PCA3 of urinary PSA (consistency index 0.61), which allows us to observe that, for example, a separate test for miR-107 concentration level may be a clinically useful diagnostic test for non- metastatic PCa.

## 6. Seminal fluid

Seminal fluid (SF) is composed of high concentration protein, soluble ions and small molecules. About 40% of SF is prostate material released after ejaculation<sup>[40]</sup>.

As a source of PCa specific biomarkers, SF has many advantages over blood and urine. First, compared with other body fluids, the prostate component in SF is highly enriched. In fact, PSA was originally described in SF, where the concentration is about 5–6 orders of magnitude higher than that in serum. Second, unlike malignant prostatic epithelial cells and their products, they only enter the circulation after breaking through the tissue barrier, and the cells and their secretions are naturally released in the SF of normal and malignant glands. Both factors suggest that biomarkers will be detected in SF rather than in blood, highlighting the potential of this liquid in early detection, including pre-malignant lesions. Third, SF contains not only free prostate cells, but also

detectable tumor cells before biopsy diagnosis of PCa. The proliferation rate of these cells has potential value in monitoring patients with low-grade diseases<sup>[41]</sup>.

As SF is a diverse and abundant molecular medium, which is composed of nucleic acids, proteins, lipids, sugars, small metabolites and ions<sup>[42]</sup>, it makes it a candidate for prostaglandin glands as biomarkers of PCa. In recent years, people have tried to use different technologies to identify PCa specific biomarkers. In a recent study by Carlsson *et al.*<sup>[43]</sup>, they isolated the prostate to compare the functional and biochemical characteristics of prostates from three different sources, including semen. The semen samples were centrifuged for 20 minutes, and other possible cells in sperm and seminal plasma were separated and divided into 15 samples. The prostate bodies isolated from the three samples were analyzed by flow cytometry and enzyme-linked immunosorbent assay (ELISA); the high proportion of some enzymes, cholesterol and phospholipids is a common feature. In addition, the membrane proteins CD13, CD26, CD10 and CD46 previously associated with seminal plasma (SP) are also present in the three prostaglandin glands, and the concentrations of CD13 and DPPIV in this liquid are also very high. These results show that prostaglandin glands can be found in prostate from different sources, and further research on prostaglandin glands has great prospects.

As mentioned earlier, prostaglandins play a role in regulating sperm, immunosuppression and PCa progression. Galectin-3 is a multifunctional carbohydrate binding protein that was originally described as a proteolytic substrate of PSA and has been shown to be associated with the prostate body in human semen<sup>[44]</sup>. In addition, galectin-3 is also involved in immune regulation, cell interaction and cancer progression, including PCa<sup>[45]</sup>. In this study conducted by Block *et al.*, galectin-3 candidate ligands in prostaglandin glands were identified by mass spectrometry, and the relationship between Mac-2 binding protein (M2BP) and prostaglandin glands was studied by other immunochemical and biochemical methods. It is worth noting that galectin-3 molecule performs its function through the interaction between protein carbohydrate and sugar binding ligands. These interactions cross each other and produce ligands bound to galectin-3 to induce subsequent effects<sup>[45]</sup>. Semen samples were obtained for prostaglandin isolation. The galectin 3 binding ligands identified in the results include M2BP, CD26, PIP, peptidase IV, olfactory omedin-4 (OLF4) and spermatogenin I and II; M2BP is present on the surface of the prostate and in sperm. The research shows that M2BP has important functional significance for the prostate body, which lays a foundation for further research on the progress of PCa<sup>[45]</sup>.

Another study published by MJ *et al.* studied the prognosis or diagnostic accuracy of PCa through SF, and analyzed a sample cohort of 152 men who suspected PCa due to elevated PSA and/or abnormal rectal contact; Patients were divided into two groups: high-risk group and low-risk group<sup>[46]</sup>. The diagnostic accuracy of PCA3, a non-coding RNA associated with PCa, and Hepsin, a transmembrane tea precursor previously associated with PCa were evaluated. The combination of Hepsin, PCA3 and serum PSA can more accurately predict PCa status and clinical risk than serum PSA alone. In addition, the integration of miRNA markers with PCA3 and Hepsin further improved diagnostic specificity and risk prediction<sup>[46]</sup>.

In a new study by Dubois *et al.*, the seminal plasma of 48 male patients with PCa were first collected to evaluate the expression of chromogranin (CG) protein in prostaglandin glands and prostate cancer cells VEs. When comparing prostaglandin glands and VEs, different patterns of chromogranin peptide were observed by ELISA, indicating phenotypic changes<sup>[47]</sup>. This study shows that there is a peptide mosaic belonging to the classic member of Glutenin family not only on the surface of prostaglandin gland, but also on the surface of VEs, and the expression of prostaglandin gland is significantly different from that found in VEs. Therefore, we conclude that CGA peptide is best detected in the prostate gland of non-malignant cells, while CGB peptide is easier to detect in VEs of malignant cells; These Chromogranins are not only from prostaglandin glands, but also from PCa derived VEs, which may be of great significance in designing a new PCa malignancy test<sup>[47]</sup>.

When cells do not rely on androgen and metastasize, the characteristics of PCa will change significantly. These metastatic PCa cells can produce prostate corpuscles and export them to extracellular space; Therefore, the prostate corpuscles produced by PCa cells themselves are considered to be the main factor in the malignant process, and the resistance to the immune system is a central feature in the selection process<sup>[48]</sup>. In the study of Babiker *et al.*, they studied the expression and function of prostate protease and ATPase by culturing PCa cell line, and isolated prostaglandin glands from 30 male semen samples collected from the fertility clinic of Uppsala University Hospital; the function of protein kinase in isolated prostate cells was analyzed by phosphorylation substrate, the activity of ATPase was evaluated, and these expressions were verified by flow cytometry. The results showed that all cell-derived prostaglandins had significantly higher protein kinase activity than seminal plasma, resulting in increased phosphorylation of exogenous and endogenous substrates. In addition, C3 component and fibrinogen (two proteins whose activities are regulated by phosphorylation) are

also expressed during phosphorylation. If the overexpression of protein kinase in metastatic PCa cells can be controlled or offset, it can enhance other types of immunotherapies. All these results help to find new targets for drug intervention in PCA patients<sup>[48]</sup>.

## 7. Other prostate related sources

Other sources exist as possible prostate related biomarkers. It is well known that extracellular vesicles have different molecules, including RNA, in addition to circulating in different body fluids<sup>[38]</sup>. These VE play a biological role far away from their source and interact with other cells through different mechanisms, such as binding to receptors on the cell membrane, triggering intracellular signaling pathways to release their content<sup>[49]</sup>.

Because VE is a valuable tool for cancer detection, prognosis and treatment, the metastatic PCa PC-3 cell line was used to analyze the microbubbles released into the extracellular environment by nano column liquid chromatography and mass spectrometry; they successfully identified 266 proteins with peptide sequences because some of them have been previously identified *in vitro*, indicating that PC-3 vesicles have common characteristics<sup>[49]</sup>.

In particular, three proteins of interest, namely CDCP1, tetraspanin CD151 and CD147, as well as other proteins, such as TCTP and neuropilin, are associated with PCa and/or not regulated; on the other hand, a large number of connexins (actin family proteins involved in the interaction between cytoskeleton and extracellular matrix) are found in the microbubbles of PC-3. The latter seems to be a promising biomarker because its expression is associated with increased tumor cell proliferation in PCa<sup>[49]</sup>.

In the past decade, active monitoring (VA) of early PCa has been implemented because early diagnosis and treatment contribute to the survival of patients with adverse risk cancer. However, there are major concerns about over diagnosis and over treatment of patients with low-risk PCa. Although the published VA cohort varies according to the protocol used, the disease metastasis rate and PCa specific mortality are very low in the medium term (5–10 years). These results seem to be closely related to the specific criteria for selection, monitoring and intervention in the protocol, suggesting that VA and other management strategies may be more effective in the correct stratification of patients with PCa. In order to improve risk stratification, individual monitoring through specific biomarkers may be an option. If these biomarkers are circulating, the disadvantages inherent in prostate biopsy related to Treo subgroup can be avoided<sup>[50,51]</sup>.

Since some studies have shown that prostate corpuscles may be an indicator of cancer progression,



caveolin (Cav-1) has been found to be a membrane protein highly bound to cholesterol, and its increased expression is accompanied by prostate cancer cells acquiring a multidrug resistance (MDR) phenotype<sup>[52]</sup>. Because PCa is also resistant to a variety of antitumor drugs, Pellinin *et al.* observed in the prostate tissue samples of 435 patients with primary and secondary Gleason grade, Cav-1 and CD59 in the prostate corpuscles of PCa tumor cells are specific markers of these vesicles, reflecting promising results, Cav-1 leads to growth arrest and inhibition of cell invasion of PCa cell line, which may be the diagnosis or prognosis of PCa, and evaluates the progress of patients already suffering from PCa<sup>[52]</sup>.

When further studying the lymph node metastasis and bone metastasis of three prostate cancer cell lines (normal, androgen sensitive and androgen independent), in order to understand their possible role in anti-PCa chemotherapy, we found that multidrug resistance protein 1 (MRP1) existed in the lipid raft of tumor cells, and the number of pits increased with the increase of malignancy. MRP1 exists not only in the plasma membrane associated with lipid rafts, but also in the cytoplasmic accumulation associated with prostate corpuscle markers caveolin-1 and CD59<sup>[53]</sup>. Since all three cell lines show that prostaglandin glands and MRP1 are present in these cells, the prostate body can be used as a predictor of the malignancy of PCa. In addition, we conclude that there may be two different cell populations, one less invasive cell population that does not express MRP1 in the prostate gland, and the other more invasive cell population that localizes MRP1 in the prostate gland. It can be concluded that both CD59 and Cav-1 can be used as markers of prostate gland when cells are malignant, and the more prostate glands, the less plasma membrane MRP1 of cancer cells<sup>[53]</sup>.

Due to the transfer of microvascular encapsulated materials from tumor cells to normal cells, a study was conducted to determine the expression of prostate-specific genes in normal bone marrow cells cultured with PCa cells<sup>[54]</sup>. Bone marrow samples were collected from 11 patients with PCa, 1 normal person and 1 healthy person; results showed that the gene expression increased significantly. It was found that the microvessels of PCa could enter circulating monocytes, stem cells or other cells, so as to change the phenotype of PCa cells. All of these provide an opportunity for new treatment strategies, such as antibodies, to prevent the release of microtubules from cancer cells<sup>[54]</sup>.

## 8. Conclusion

PSA has low specificity and sensitivity as a diagnostic marker of PCa, which leads the scientific community to look for new biomarkers in biological sources such as urine, blood and prostate fluid. The molecular

composition of prostate-specific extracellular vesicles reveals proteins as potential biomarkers for diagnosis, stratification and prognosis of PCa. The development of these markers requires the analysis of a large number of patients. In addition, it is also necessary to improve the purity of prostate gland isolates in blood, urine or prostate fluid.

One advantage of prostaglandin glands extracted from blood or urine is that they represent the general state of the body and the development of PCa, and are minimally invasive.

The molecular composition of prostaglandin glands, such as protein and RNA molecules, as well as their stability and the ability of RNA in the lumen to resist exogenous RNases, provide the ability to affect the development and transfer of PCa. Therefore, prostate corpuscles can be considered as potential markers for the diagnosis/prognosis of prostate cancer and as a reading of their source cell status.

However, the identified prostate related molecules must be tested in cohort studies of elderly patients to determine their specificity and sensitivity in the diagnosis of PCa; similarly, the research field should be expanded to more effective methods to separate prostaglandin glands in different biological fluids for further research, because this is one of the main weaknesses of prostaglandin glands.

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## Conflict of interest

Authors declared no conflict of interest.

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