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Prostasomes as a source of diagnostic biomarkers for prostate cancer

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Introduction

Prostate cancer (PCa) is the most frequent cancer type diagnosed in men, with a yearly incidence of approximately 382,000 cases, resulting in 89,000 deaths in 2008 in Europe alone (1). The mortality of men diagnosed with PCa varies greatly between countries but, on average, has declined over the years. This decline is attributed to both increased detection of latent disease and improved PCa treatment (2). Additionally, the increase in life expectancy contributes to a rising number of elderly individuals with diagnosed PCa. Indeed, around 30% of men over the age of 65 has been estimated to have PCa; however, only a minority of these patients will experience invasive PCa during their lifetimes. Thus, it is critical to differentiate between aggressive and nonaggressive forms of PCa (3).

PCa diagnosis is typically performed via classical rectal examination. This technique is rather inefficient, with an estimated 23%–45% of PCas being missed and roughly 50% of PCa being diagnosed at an advanced stage (4). Suspected PCa patients usually undergo prostate biopsies or serial biopsies for active surveillance. However, biopsy analysis may underestimate the grade or extent of pathology or miss tumor tissue altogether as a consequence of the multifocal nature and heterogeneity within many prostate tumors. This holds true both for strict morphological analysis and detection of cancer-specific biomarkers within biopsies. In contrast, biomarkers that are released in blood or urine are more likely to represent the status of the entire prostate. Hence, there is urgency for the discovery and implementation of blood- or urine-borne markers that reliably indicate PCa (5). Such biomarkers would make active PCa surveillance less invasive, thereby reducing costs and eliminating the potential complications of biopsy sampling.

Several blood-derived molecular biomarkers for PCa have become available over the years (6). Currently, prostate-specific antigen (PSA) is the most extensively used first-line biomarker in blood for diagnosis of PCa (7). PSA is a protease produced by prostate epithelial cells. Its major known physiological function is to liquefy the semen coagulum. As an apically secreted product, PSA is mainly deposited into the prostate ducts, but it can also be detected in small quantities in the blood of healthy men. Transformed PCa cells lose cell polarity, increasing the release of PSA into blood; thus, high blood PSA titers may be indicative of PCa. However, only 25%–40% of men with elevated PSA levels are actually diagnosed with PCa after prostate biopsy (8, 9), and although high PSA levels may indicate small, localized, and low-grade malignant tumors, they may also result from benign prostatic hyperplasia (10). Furthermore, PSA levels may rise as a consequence of prostatitis or urinary tract infection and are highly variable between healthy individuals (11–13). Conversely, between 20%–40% of PCa are missed by PSA testing, and population-based screening for PSA reduced PCa mortality only by an estimated 20%. Tests that determine the ratios of distinct molecular forms of PSA — including pro-PSA, free PSA, and total PSA — may more accurately detect PCa; however, these measurements suffer from similar limitations as classical serum PSA tests (14, 15). Thus, PSA tests lack sufficient specificity to efficiently discriminate between benign prostate disease and aggressive PCa (11–14, 16), necessitating intense efforts to identify novel biomarkers that are more specific for PCa.

PCa antigen 3 (*PCA3*) is a PCa-specific antigen that is overexpressed in greater than 90% of PCa and can also be detected in urine (17, 18). *PCA3* is a noncoding RNA (ncRNA) with a very short ORF that has been widely evaluated for its diagnostic potential. A clinical test measuring *PCA3* in urine, normalized to less variable mRNA encoding for PSA, has been introduced (19). Compared with PSA, *PCA3* may have higher specificity for the prediction of

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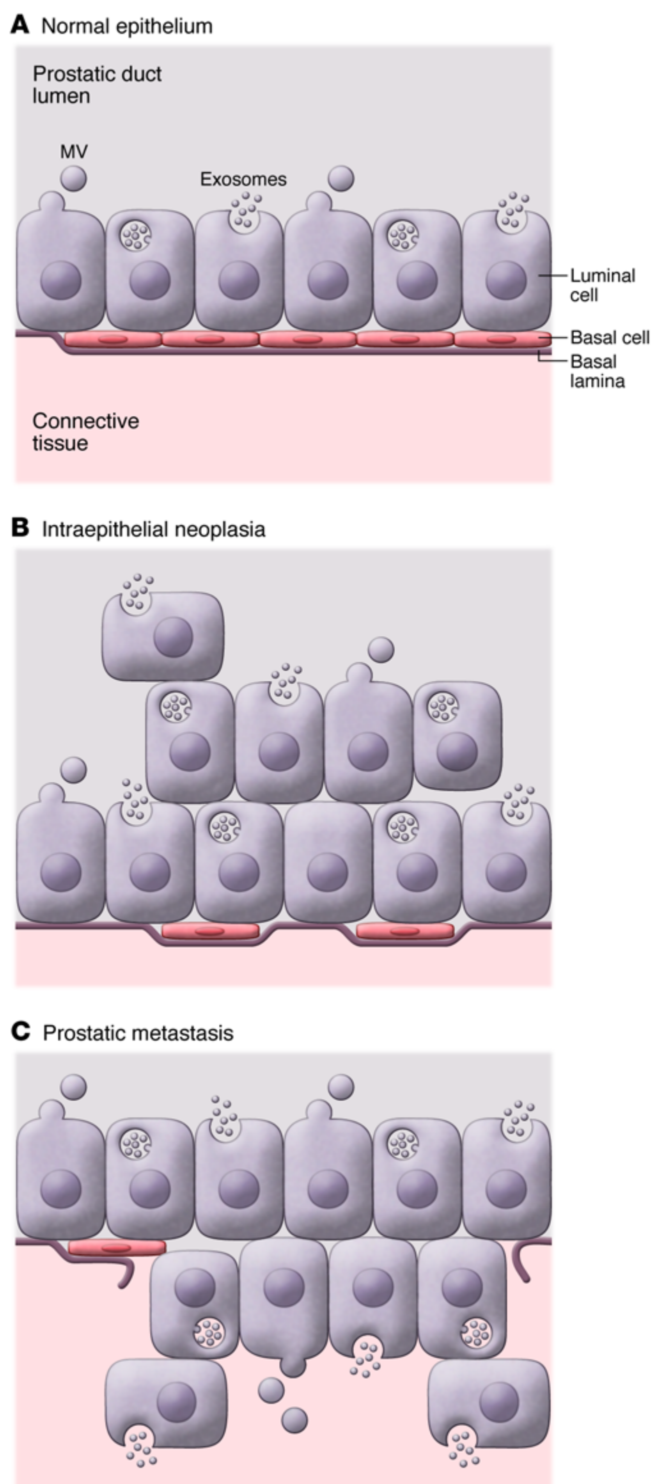

Figure 1. Morphologic changes and prostasome release during PCa progression. (**A**) Normal prostate epithelium with secretory luminal cells and basal cells, which are found between the luminal cells and the underlying basal lamina (neuroendocrine cells are not shown). Epithelial cells release prostasomes into the prostatic duct. Prostasomes can be formed by inward budding of MVE and are then secreted as exosomes by fusion of the MVE delimiting membrane with the plasma membrane. Alternatively, prostasomes may represent microvesicles (MV), which are formed by outward budding and pinching directly from the plasma membrane. (**B**) Intraepithelial neoplasia is characterized by loss of basal cells, neoplasia of luminal cells, and an intact basal lamina. (**C**) Metastasis, characterized by loss of the basal lamina, loss of polarity of luminal cells, and release of prostasomes into the underlying tissue and blood. The release of apoptotic bodies and intact cells is not shown.

PCa, but its sensitivity is relatively low and indicatory concentrations providing an optimum balance between sensitivity and specificity are still debated (20).

Gene fusion transcripts have also been used as PCa markers. Chromosomal rearrangements are a common feature of carcinomas, and frequent gene fusions in PCa include androgen-regulated gene transmembrane protease serine 2 (*TMPRSS2*) and two ETS transcription factors, *ETV1* and the v-ets erythroblastosis virus E26 oncogene homolog (*ERG*) (21). Urine-based tests of the fusion gene transcript *TMPRSS2:ERG* have a high specificity but low sensitivity (20). Combinational testing of *PCA3* and *TMPRSS2:ERG* (22) or multiplex testing of *TMPRSS2:ERG* together with transcripts of *PCA3*, *GOLPH2*, and *SPINK1* (23) improved the detection of PCa; however, such tests remain unreliable, and the search for better PCa markers in either blood or urine is ongoing. Herein, we discuss blood- and urine-borne prostate-specific extracellular vesicles (EVs, known as prostasomes) as a potential source of biomarkers for PCa.

Prostasomes

Prostasomes are EVs that range in diameter between 50 nm and 0.5 μm and are released into the extracellular environment by prostate epithelial cells (Figure 1). They were first described in 1982 as vesicles with a capacity to promote progressive motility of sperm cells (24). Other proposed physiological activities of prostasomes include regulation of sperm cell capacitation, acrosome reaction, and immune suppression within the female reproductive tract (9, 25). The majority of prostasomes constitutes 50–100 nm vesicles that are formed in prostate epithelial cells by inward budding of endosomal delimiting membranes. These multivesicular endosomes (MVEs) have also been referred to as storage vesicles (26, 27). Prostasomes are released into the lumen of the prostatic ductal system of the prostate gland as a consequence of MVE fusion with the apical plasma membrane of the epithelial cell (Figure 1). Because of their derivation from MVEs, prostasomes can be regarded as the equivalent of exosomes produced by other cell types (28). Importantly, storage vesicles in prostate epithelial cells are not the exclusive source of EVs in seminal plasma. Vesicles may also derive by direct shedding from the prostate epithelial cell plasma membrane, an EV type referred to as microvesicles, and these too can be considered true prostasomes (Figure 1). In addition, seminal plasma contains EVs from other sources within the male genital tract, including the epididymal ducts, vesicular glands, and bulbourethral glands (9). Thus, although the term prostasomes has been generally applied to all EVs that can be isolated from semen, only part of the EV population in semen truly derives from the prostate. Prostate secretions, including prostasomes, are mixed with other components of seminal plasma and sperm cells during ejaculation (26, 29) but also leak into urine (see below).

Like all EVs, prostasomes are composed of cytosolic contents surrounded by a lipid bilayer containing membrane proteins, and this orientation is dictated by the cytosolic outward direction of

budding during their formation, irrespective of whether this occurs at MVEs or at the plasma membrane. The protein composition of prostasomes isolated from seminal plasma has been analyzed by mass spectrometry, amongst other techniques (9, 30, 31). Over 400 distinct proteins have been identified, although some of these might originate from EVs that derive from sources other than the prostate or they may constitute contaminants. Many of the identified proteins, including aminopeptidase N (29), tissue factor (32), and dipeptidyl peptidase IV (33), are not exclusively expressed by prostate epithelial cells and may also associate with EVs other than prostasomes (9). Identification of prostate-specific membrane proteins, including TMPRSS2, prostate-specific transglutaminase, and prostate stem cell antigen (PSCA), confirmed the prostatic origin of at least part of the isolated EVs at the molecular level.

Prostasomes and PCa

In general, both healthy and diseased cells shed EVs, although their release may be enhanced upon malignant transformation (34–37). Tumor cell–derived EVs have been described for different types of cancer (38) and are reported to contain cancer-specific content, including oncoproteins, mutant transcripts, and cancer-specific microRNAs (miRNAs) (39, 40). Both neoplastic and metastatic PCa cells have been demonstrated to release prostasomes (27, 36, 41–46). Observations that prostasomes isolated from PCa patients stimulated in vitro tumor cell proliferation and invasion are consistent with the general idea that EVs from tumor cells can stimulate tumor growth and metastasis and can modulate surrounding cells to promote tumor growth (9, 47–50).

The general concept that EVs isolated from blood can serve as biomarkers for cancer was recently validated for pancreatic cancer (51). In this study, glypican-1–carrying exosomes were detected in the serum of pancreatic cancer patients with absolute specificity and sensitivity, although it should be noted that other tumors, including breast cancer and gliomas, also express glypican-1. *KRAS* is a frequently mutated gene in pancreatic cancer, and mutant *KRAS* mRNA was found in glypican-1–carrying exosomes from all tested patients with *KRAS* mutations (51). Findings that EVs from cancer cells have unique, cancer-specific contents (52) — together with the observation that prostasomes are present in both the blood and urine of PCa patients (53–56) — suggested the hypothesis that prostasomes may provide useful markers of PCa. This idea was supported further by early studies in which proteome and RNA profiles of EVs isolated from cultured prostate tumor cell lines and immortalized prostate epithelial cells were compared, identifying several potential candidate biomarker proteins for PCa (44, 57–59).

Prostasome-associated PCa protein markers in blood

The first attempts to demonstrate the presence of prostasomes in a PCa patient's blood focused on the detection of anti-prostasome antibodies (53, 54, 60). In healthy individuals, excretory ducts form a closed compartment with the basement membrane surrounding the prostate epithelial cells, thereby hiding prostasomes from the immune system (61). The loss of cell polarity in prostate malignancies (61) allows release of prostasomes into the interstitial space and into circulation (refs. 46, 55, and Figure 1). By crossing these barriers, prostasomes may stimulate the adaptive immune system to produce prostasome-directed autoantibodies, which can be detected in blood from PCa patients (53, 60). However, there is no consensus on whether titers of prostasome-directed antibodies correlate with PCa grade or metastasis (54, 62, 63), leaving little evidence that prostasome-specific antibodies can be used as reliable prognostic markers for PCa.

Proteins that are exclusively expressed by PCa cells — as compared with healthy prostate epithelial cells or any other cell type — and are incorporated into prostasomes have yet to be identified. The detection of prostasomes in blood is further complicated by the concomitant presence of EVs from many other sources. Nevertheless, detection of high levels of a single protein, even when ubiquitously expressed, in a total EV fraction from blood may be sufficient to detect PCa, as exemplified by a study in which the antiapoptotic protein survivin was found to be significantly increased in EVs isolated from the plasma of PCa patients compared with plasma from patients with preinflammatory benign prostate hyperplasia or healthy controls (64). In another study, the tumor suppressor PTEN was approximately 10-fold higher in EVs isolated from PCa patients compared with normal subjects (65). However, by colocalizing multiple markers on the same EV in a so called proximity ligation assay, it may be possible to detect PCa-derived prostasomes in blood with greater sensitivity compared with single-marker analyses (55). Tavoosidana and colleagues (55) first recruited prostasomes with anti–aminopeptidase-N antibodies, after which the coincident presence of four prostasomal proteins was probed with DNA-conjugated antibodies to generate an amplifiable reporter. With this assay, PCa patients, especially those with high prostatectomy Gleason scores, were found to have elevated concentrations of prostasomes in their blood compared with healthy controls, confirming the potential of combinational analysis of blood-borne prostasomal proteins for PCa diagnosis. Prostasome proteins in serum may also be used to follow therapy efficacy in patients. For example, P-glycoprotein encoded by multidrug resistance protein 1 (*MDR1*) in blood EVs was relatively higher in docetaxel-resistant patients than in therapy-naive patients (66).

Prostasome-associated PCa protein markers in urine

Prostate fluid constitutively leaks into urine and prostate massage (usually as a consequence of digital rectal examination) before urine collection increases the amount of prostasomes in urine (37, 67–69). The presence of prostasomes in EV fractions isolated from urine was confirmed by detection of prostate-specific proteins, including prostate-specific membrane antigen (PSMA), prostatic acid phosphatase, and prostate transglutaminase (67, 70–73). One advantage of collecting EVs from urine, as compared with blood, is that such isolates are more enriched in prostasomes relative to other constituents, although tissues within the urogenital system other than the prostate, including the kidney (74) and bladder (75), also contribute EVs to urine. Moreover, urine also contains intact PCa cells and PCa cell–derived apoptotic bodies (74). Cells and most apoptotic bodies are considerably larger than prostasomes and can thus be easily separated from prostasomes by differential centrifugation. Proteomic profiling of EVs isolated from urine identified hundreds of proteins, many of which may indeed associate with EVs from sources other than the prostate or non–EV-related particles

(68, 76–79). In a recent study using mass spectrometry, the protein compositions of EVs isolated from 16 preoperative urine samples of PCa patients were compared with those from 15 healthy individuals, and as many as 246 proteins were found to be differentially expressed (56). Of these, 17 proteins displayed sensitivities above 60% at 100% specificity at a detection threshold with positive values for patient samples and negative values for control samples. The transmembrane protein TM256 had the highest sensitivity (16 of 17 patients), and it was suggested that by combining this protein with a panel of some of the other identified markers, it might be possible to fully differentiate PCa patients from healthy individuals. Although these findings require confirmation in larger cohorts of patients, this study very strongly supports the feasibility of developing highly sensitive and specific PCa markers based on urinary EVs. It should be noted that TM256 and the other proteins that were enriched in the EV fraction from PCa patient urine are ubiquitously expressed, and their increased expression may be explained through elevation of EV release upon transformation of prostate epithelial cells.

Prostasome-associated mRNA and ncRNA PCa markers

The general concept that EVs carry RNA molecules and may serve as a vehicle for RNA transfer between cells was first demonstrated for mast cell–derived EVs (80). The idea that EVs elicit epigenetic effects by transferring selected mRNA and miRNA species between cells has revolutionized concepts concerning the mechanisms of intercellular signaling, including the interactions of tumor cells with surrounding tissues. These ideas have also led to investigations into the utility of EV-associated RNAs as biomarkers for disease. Because cellular RNA profiles are unique for each type of cancer, their EVs can be expected to contain quantitatively and qualitatively distinct RNA signatures. Indeed, EVs from many sources were found to contain selected sets of RNA molecules, including mRNA, miRNA, and other ncRNAs (28). Packaging of RNAs in EVs is an active, nonrandom process, and the underlying molecular mechanisms are just beginning to emerge (81). For example, it has been proposed that sumoylated heterogeneous nuclear ribonucleoproteins (hnRNPs) facilitate incorporation of selected miRNAs by binding of consensus motifs at their 3′ end (82). Posttranscriptional 3′ uridylation may also help direct ncRNAs into EVs (83). Consensus motifs for RNA sorting into EVs have also been proposed for mRNAs (84–86). Incorporation of mRNA into EVs may also involve their 3′-untranslated region (87) or interaction with mRNA-specific miRNAs (84). Further investigations will be required to fully define the mechanisms by which specific RNAs are incorporated into EVs.

The potential of mRNAs from blood-derived EVs to serve as cancer biomarkers is illustrated by studies of patients with colorectal cancer (88) and glioblastoma (89). To our knowledge, changes in mRNA content of EVs isolated from blood of PCa patients have not yet been reported; however, several studies have examined mRNA in EVs isolated from urine. As discussed above, transcripts for *TMPRSS2:ERG*, *PCA3*, *GOLPH2*, and *SPINK1* have all been detected in the urine of PCa patients with some predictive value for PCa (20, 22, 23, 67). In most of these studies, it was not clear whether these transcripts were associated with cells, apoptotic bodies, or EVs. EVs that were isolated from urine of PCa patients and separated from cells and large apoptotic bodies by differential centrifugation were found to contain splice variant transcripts of the anterior gradient 2 gene (*AGR2*), which had greater specificity than serum PSA as a marker for PCa (90). Other studies demonstrated the presence of both *TMPRSS2:ERG* and *PCA3* in EV fractions from the urine of PCa patients (67, 69). In a recent study, a molecular signature (EXO106 score), which is based on the combined normalized expression of *ERG* and *PCA3* isolated with exosomes from urine samples from a cohort of 195 patients, was used to predict PCa (91). It should be noted that the methodology for exosome isolation in this study does not exclude the presence of sources other than exosomes. The reported negative and positive predictive values of the EXO106 score for high-grade PCa were 97.5% and 34.5%, respectively (91). *TMPRSS2:ERG* was found in EVs isolated from the blood of human PCa xenograft–bearing mice (92), supporting the idea that such transcripts may also be traceable in EVs from the blood of PCa patients. Importantly, mRNA testing could potentially be used to track cancer-specific (rare) genomic mutations or reorganizations in a background of WT mRNA sequences by using digital PCR approaches, as has been exemplified for the detection of mutant isocitrate dehydrogenase 1 (*IDH1*) transcripts in EVs from the cerebrospinal fluid of glioma patients (93).

Prostasome-associated miRNA PCa markers

miRNAs regulate the expression of protein-coding genes at the translational level (38, 94), and miRNA expression is altered by epigenetic repression, genomic deletion, amplification, or mutation in many types of cancer, including PCa (95, 96). The ability of some miRNAs to inhibit translation of oncogenes and tumor suppressors may explain their involvement in carcinogenesis. It appears that each tumor type is characterized by a unique miRNA expression profile (38, 49, 97–99), and aberrant expression of miRNAs in cancers offers the opportunity to use miRNA from tissue biopsies as biomarkers in cancer diagnosis, prognosis, and treatment response (100). miRNAs are also found extracellularly in blood, and analysis of blood-borne extracellular miRNA provides a less invasive means to screen patients for tumor-specific miRNA profiles compared with traditional biopsies. Indeed, changes in composition of secretory miRNAs have been reported for many different types of cancer (99–101). Selected sets of extracellular miRNAs in blood are contained by EVs (102–104); however, the majority of extracellular miRNAs in plasma is associated with membrane-free protein complexes containing Argonaute (104) and nucleophosmin (105), and to some extent with HDL particles (106).

Analysis of whole serum or plasma samples from PCa patients revealed differential expression patterns of extracellular miRNAs, identifying more than 50 miRNAs as potential blood-based PCa biomarkers (99, 107–112). In these studies, upregulation of miR-141 and miR-375 was most consistently observed in plasma or serum of PCa patients. It has been suggested that panels of two or more circulating miRNAs may reliably distinguish PCa patients from patients with benign prostatic hyperplasia and healthy controls (103, 113, 114). Most studies on extracellular miRNA in plasma did not discriminate between miRNA in EVs and other forms of circulating miRNAs. Furthermore, it should be kept in mind that when EVs were isolated from blood either by ultracen-

Table 1. PCa-associated proteins and RNA molecules in EV isolates from blood plasma or serum or urine

Molecule	Marker	Reference
PCa markers in EV fractions from blood plasma or serum		
Protein	Surviving	64
	PTEN	65
	P-glycoprotein	66
miRNA	21; 375; 574	110
	107; 130b; 141; 181a-2; 301a; 326; 331-3p; 373; 432; 484; 574-3p; 625;2110	100
PCa markers in EV fractions from urine		
Protein	ADIRF; ARL8B; ATP6V0C; LAMTOR1; LCP1; PARK7; RAB2A; RAB3B; RAB3D; RAB6A; RAB7A; S100P; SLC2A13; STEAP4; SYTL4; TM256; TSPAN6	56
mRNA/ncRNA	*AGR2* splice variants	85
	PCA3; TMPRSS2:ERG	67, 69
	PCA3; ERG	91

trifugation, ultrafiltration, or precipitation techniques, particulate RNA/protein complexes, such as Argonaute/miRNA complexes, might have been coisolated with EVs (28). Definitive proof of association with EVs requires separation of membranes from non-membranous complexes by equilibrium density gradient fractionation; generally, such analysis has not been performed in previous studies. In some studies, RNAse protection is taken as evidence for encapsulation of miRNA by EV membranes, but Argonaute/miRNA complexes are also resistant to RNase if protease is not applied first (104). In a study by Bryant and colleagues (103), EV-associated miRNAs were isolated using filtration and RNAse protection assays. They identified 11 miRNAs that were significantly increased and one miRNA that was significantly decreased in the plasma-derived EV fraction of PCa patients compared with healthy controls. In the same study, 16 miRNAs were increased in PCa patients with metastatic compared with nonmetastatic disease. Of these, miR-141 had already been identified in other studies as a potentially interesting blood-borne biomarker for metastatic PCa (115). Li and colleagues (116) isolated EVs using a commercial EV precipitation kit and identified three discriminating miRNAs. These clinical studies were all conducted with small numbers of subjects, and studies of large cohorts are required to further investigate the value of extracellular, circulating miRNAs as biomarkers for PCa.

miRNA has also been analyzed in urine samples. PCa cells can be collected by low-speed centrifugation from urine that is collected after transrectal examination/massage, and such non-invasive "liquid" biopsies have obvious advantages over invasive tissue collection. For total urine samples, including cells, potential miRNA biomarkers that differentiate PCa and benign prostatic hyperplasia have been reported (117, 118). In cells isolated from urine, miR-107 and miR-574-3p were found to be elevated in PCa patients compared with controls, and these miRNAs appeared to have a higher specificity for PCa than PSA-normalized urinary *PCA3* (103). In cell-free urine, miRNAs were significantly enriched in EVs, and urinary EVs have been proposed to be a good source of

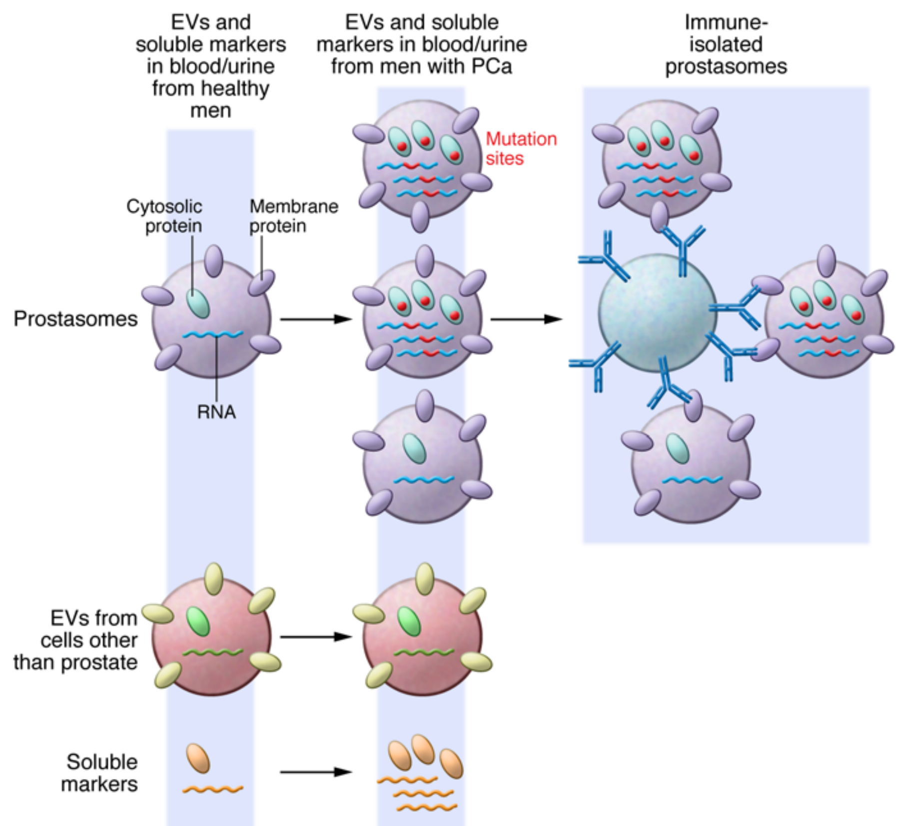

Figure 2. Isolation of prostasomes in blood and urine from other EVs and non–EV-related markers for PCa analysis. During PCa development, prostasomes may increase in number and change their content. Protein and RNA markers in prostasomes can be analyzed separately from noise signals after prostasome immunoisolation employing prostasome-specific membrane proteins.

miRNA biomarkers (119), not only for PCa but also for kidney disease (74) and bladder cancer (120, 121). In another recent study, miR-483-5p was also found to be elevated in cell-free urine from PCa patients (122). However, it has remained unanalyzed whether these cell-free miRNAs in urine are associated with EVs.

Conclusions

The limited predictive value of PSA as a diagnostic marker for PCa has led to an intense search for novel biomarkers in blood or urine with better sensitivity and specificity. Screening the molecular composition of EVs isolated from either blood or urine has revealed candidate proteins and RNA molecules that, upon further selection, may serve as biomarkers for PCa diagnosis, differentiation, prognosis, and epidemiology (Table 1). Because PCa is highly heterogeneous, both within individual tumors and between patients, biomarker multiplex testing will increase the sensitivity and specificity of EV-based PCa diagnosis. The development of such markers will require the analysis of large patient cohorts. In addition to increasing cohort size, it will be necessary to increase the purity of EV isolates. So far, only a few studies have separated EVs from other constituents in blood or urine, and no study has separated prostate epithelial cell–derived prostasomes from other EVs within blood or urine. Plasma contains EVs from nearly all tissues, and it is likely that prostasomes constitute only a minority population, even in PCa patients. Similarly, urine contains EVs from tissues within the entire urogenital tract. Moreover, EVs that are isolated by ultracentrifugation, ultrafiltration, or precipitation techniques may still be contaminated with protein complexes that also carry RNA (28). Prostasomes contain prostate-specific membrane proteins, and these can be used as targets for immune-isolation techniques to separate prostasomes from other constituents. The immune isolation of EVs from PCa cell line culture media using anti–PSMA-coated beads provided a proof-of-principle that immune isolation could be used to enrich for prostasomes (123). Subsequent analysis of such isolated prostasomes for the presence of PCa-specific proteins or RNA molecules may not only enhance reproducibility but also the sensitivity and specificity of PCa analysis (Figure 2).

PCa foci are often heterogeneously distributed within the prostate and may be missed by prostate biopsy sampling. In contrast, prostasomes sampled from either blood or urine are representative of the overall condition of the prostate and PCa metastasis, and their use as a source of PCa biomarkers has the advantage of being semi- or noninvasive. Furthermore, mutant transcripts can be detected and quantified in a background of WT transcripts from healthy tissue and, thus, mirror the genetic diversity within a tumor (124).

Analysis of isolated prostasomes has additional advantages over the analysis of their originating PCa cells, which may also be isolated from blood or urine. First, EVs are extremely stable, and RNA within their lumen can resist exogenous RNAse. Second, EVs are known to incorporate selected sets of proteins and RNA molecules, and the molecular composition of prostasomes probably reflects their capacity to influence PCa growth and metastasis. Thus, EVs, including prostasomes, can be considered convenient packages that contain constitutively expressed prostate-specific proteins that can be employed for immune isolation, as well as PCa-specific molecular fingerprints that can serve as readouts for the status of their originating cells (Figure 2). Absolute values of single PCa biomarkers have often proven to be of little diagnostic value. This is particularly true for urine-derived markers, as their concentrations are strongly influenced by external factors such as prostate massage and the timing, frequency, and volume of urination. Finally, an additional advantage of the multicomponent composition of prostasomes is that associated PCa-specific markers can easily be normalized using constitutively expressed constituents within the same isolate.

The already-identified prostasome-associated molecules or combinations of these molecules need to be tested in large patient cohort studies to determine their specificity and sensitivity for diagnosis of PCa. We propose that immune isolation of prostasomes from either blood or urine may further increase the specificity and sensitivity of such markers and aid in the identification of novel prostasome-associated PCa-specific (mutant) RNA markers.

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