



Extracellular vesicles in prostate cancer: a narrative review

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Abstract: Over the past decade, there has been remarkable progress in prostate cancer biomarker discovery using urine- and blood-based assays. A liquid biopsy is a minimally invasive procedure to investigate the cancer-related molecules in circulating tumor cells (CTCs), cell-free DNA, and extracellular vesicles (EVs). Liquid biopsies have the advantage of detecting heterogeneity as well as acquired resistance in cancer. EVs are cell-derived vesicles enclosed by a lipid bilayer and contain various molecules, such as nucleic acids, proteins, and lipids. In patients with cancer, EVs derived from tumors can be isolated from urine, plasma, and serum. The advances in isolation techniques provide the opportunity to use EVs as biomarkers in the clinic. Emerging evidence suggests that EVs can be useful biomarkers for the diagnosis of prostate cancer, especially high-grade cancer. EVs can also be potent biomarkers for the prediction of disease progression in patients with castration-resistant prostate cancer (CRPC). EVs shed from cancer and stromal cells are involved in the development of tumor microenvironments, enhancing cancer progression, metastasis, and drug resistance. Here, we provide an overview of the use of EVs for the diagnosis of clinically significant prostate cancer as well as for predicting disease progression. We also discuss the biological function of EVs, which regulate cancer progression.

Keywords: Extracellular vesicles (EVs); exosomes; prostate cancer; biomarker; tumor microenvironment

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Introduction

The number of newly diagnosed prostate cancer was estimated to be 1,276,000, and the number of deaths caused by prostate cancer was estimated to be 358,000 worldwide in 2018 (1). Histological grading using the Gleason score (GS) remains the gold standard for predicting the clinical outcome in prostate cancer (2). Randomized clinical trials demonstrated the potential benefits of radical prostatectomy in patients with GS ≥ 7 (3,4). Further, patients with GS 9–10 are associated with an increased risk of metastasis (5,6). However, prostate cancers with a GS of 6 have a low capacity for metastasis and death and are thus considered insignificant cancers (7,8). To date, serum

prostate-specific antigen (PSA) levels are widely used for the detection and screening of prostate cancer. However, PSA-based screening is reportedly associated with false-positive results, biopsy complications, and overdiagnosis (9,10). Thus, the development of new detection methods is required to preferentially identify patients with clinically significant prostate cancer (GS ≥ 7) and to concurrently avoid unnecessary biopsies in men without cancer or with insignificant cancer.

Over the past decade, there has been remarkable progress in prostate cancer biomarker discovery using blood- and urine-based assays (11). The 4Kscore, including total PSA, free PSA, intact PSA, and human kallikrein 2, demonstrated excellent diagnostic performance in detecting GS ≥ 7 prostate

cancer (the area under receiver operating characteristic curve, AUC =0.82) (12). The prostate health index, which combines total PSA, free PSA, and [-2]pro-PSA, could identify GS \geq 7 prostate cancer (AUC =0.707) (13). The Mi-Prostate Score, which combines total serum PSA, urine transmembrane protease serine 2:v-ets erythroblastosis virus E26 oncogene homolog (*TMPRSS2:ERG*) fusion gene, and prostate cancer antigen 3 (*PCA3*) score, improved the detection of GS \geq 7 prostate cancer (AUC =0.77) (14). A 2-gene based urinary test that combines homeobox C6 and distal-less homeobox 1 mRNA levels could detect GS \geq 7 prostate cancer (AUC =0.85 for full cohort) (15). The ExoDx Prostate IntelliScore (EPI) urine exosome assay, which includes exosomal RNA levels of *PCA3*, *ERG*, and *SPDEF*, improved the discrimination of GS \geq 7 prostate cancer (16). Thus, the development of novel assays provides a significant improvement in prostate cancer diagnosis and treatment decisions.

Prostate cancer is commonly multifocal and exhibits distinctive profiles, such as allelic gains of *MYC*, deletions of *PTEN*, *TP53*, and *NKX3-1*, and genomic rearrangement of *TMPRSS2: ERG* fusion (17-19). Furthermore, prostate cancer is a highly heterogeneous disease (20). In metastatic castration-resistant prostate cancer (CRPC), aberrations of *AR*, *ETS* genes, *TP53*, and *PTEN* are frequent (40–60% of cases), and aberrations of the DNA repair genes *BRCA2*, *BRCA1*, and *ATM* are substantially more frequent (20%) in CRPCs than in primary prostate cancers (21). A liquid biopsy is a minimally invasive procedure to investigate the cancer-related molecules in circulating tumor cells (CTCs), cell-free DNA, and extracellular vesicles (EVs). Liquid biopsies have the advantage of detecting heterogeneity as well as acquired resistance in cancer (22). In this review, we focus on the use of EVs for the diagnosis of clinically significant prostate cancer as well as for predicting disease progression. We further discuss the biological function in EVs on prostate cancer progression. We present the following article in accordance with the NARRATIVE REVIEW reporting checklist (available at <http://dx.doi.org/10.21037/tau-20-1210>).

Application of EVs for the diagnosis of prostate cancer

EVs are classified based on their cellular origin, biological function, and biogenesis. The three main classes of EVs, which are determined by their biogenesis, are exosomes (40–120 nm in size), microvesicles (50–1,000 nm in size), and apoptotic bodies (500–2,000 nm in size) (23). EVs are

cell-derived vesicles enclosed by a lipid bilayer, whereas exosomes are derived from the endolysosomal pathway, and microvesicles are generated by budding from the plasma membrane (24,25) (*Figure 1*). EVs derived from cancer cells can be isolated from bodily fluids (26-29). The advances in isolation techniques provide the opportunity to use EVs as biomarkers (30). Ultracentrifugation is the most common technique for EV separation and concentration, following various other techniques, such as density gradient, filtration, size-exclusion chromatography, precipitation, and magnetic bead capture (31). Protein content-based EV characterization is based on EV markers, such as tetraspanins (CD9, CD63, and CD81), flotillin-1, tumor susceptibility gene 101 (TSG101), heat-shock proteins (HSP70 and HSP90), and the major histocompatibility complex (MHC) class I and class II proteins (32,33). The International Society for Extracellular Vesicles proposed guidelines for studies of EVs (34). Different EV enrichment methods yield subpopulations with different protein and RNA contents (35). Thus, the procedures for EV isolation from biofluids need to be optimized and standardized for clinical application (35,36).

Sequence analysis of EVs derived from human plasma showed that microRNAs (miRNA) were the most abundant RNA species (76%), followed by other RNA species, such as ribosomal RNA (9%), long non-coding RNA (lncRNA, 3%), fragments of coding sequence (1%), and others (37). miRNAs expression alters with the development and progression of prostate cancer (38-40), and the expression of cancer-related genes is regulated by miRNAs in prostate cancer cells (41-43). lncRNAs derived from prostate cancer EVs harbor miRNA seed regions, suggesting a function of EVs in prostate cancer progression (44). Prostate cancer cell-derived EVs also carry genomic DNA fragments (45,46) and contain a wide range of proteins (47). Proteomic analysis demonstrated that tumor-derived EVs have distinct protein profiles of membrane-bound and soluble proteins in prostate cancer cells (48-50). Lipids are essential components of the cellular membrane, and lipid species, such as phospholipids and glycosphingolipids, have potential to be biomarkers for malignant tumors, including prostate cancer (51-54). Lipidomic analysis revealed that specific lipid species are enriched in EVs compared to their parent cells (55,56). Thus, EVs contain various molecules, including nucleic acids, proteins, and lipids (*Figure 1*). EVs derived from urine, plasma, serum, and semen can be potential biomarkers for the diagnosis and monitoring of prostate cancer (57-59). In this narrative review, we identified the relevant studies using

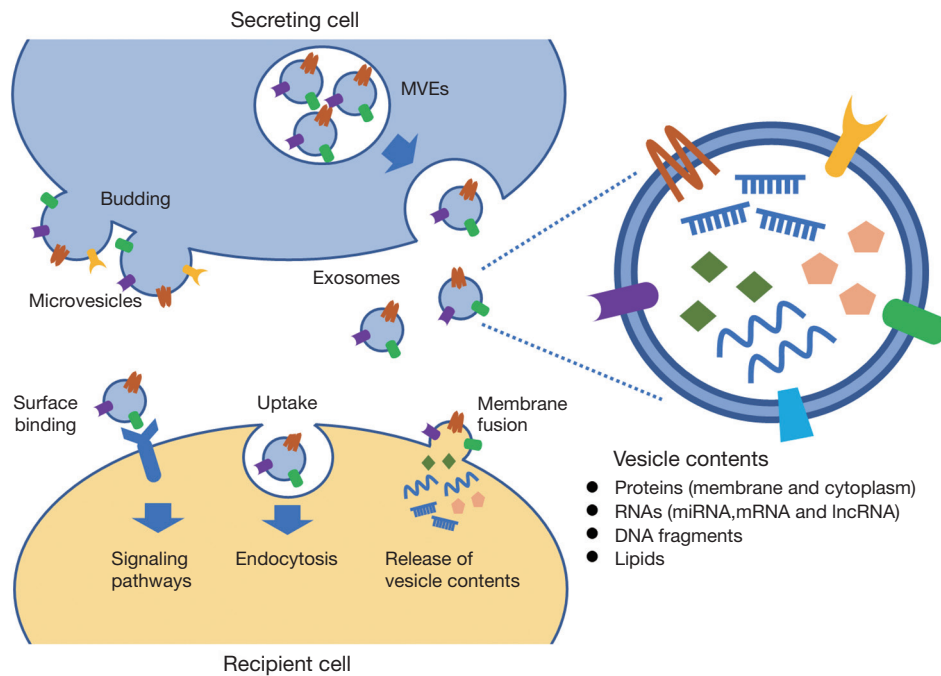


Figure 1 Biogenesis and biological function of EVs. Microvesicles are formed by budding of the plasma membrane. Exosomes are formed as intraluminal vesicles within the lumen of multivesicular endosomes (MVEs), and then, released by the fusion of MVEs with the plasma membrane. EVs are enclosed by a lipid bilayer, containing various molecules, such as proteins, nucleic acids and lipids. EVs are the mediators of intercellular communication, via the transfer of vesicle contents from the secreting cells to the recipient cells by uptake and membrane fusion. EVs also initiate intracellular signaling pathways via surface binding.

electronic databases, including PubMed, Medline and Web of Science.

Urine EVs for the diagnosis of prostate cancer

In 2009, Nilsson *et al.* demonstrated the presence of two prostate cancer RNA biomarkers, *PCA3* and *TMPRSS2:ERG*, in EVs isolated from the urine of patients with prostate cancer (60). Since then, a line of evidence has suggested that urine EVs are useful biomarkers for the diagnosis of prostate cancer, especially high-grade cancer (Table 1).

The sum of the normalized *PCA3* and *ERG* RNA levels in urine EVs along with the standard of care (SOC) was sufficient to discriminate $GS \geq 7$ prostate cancer (AUC =0.803) (61). The EPI test, including the RNA levels of *PCA3* and *ERG* in urine EVs, in combination with SOC improved the discrimination of $GS \geq 7$ prostate cancer in training (n=255) and validation cohorts (n=519) (AUC =0.77 and 0.73, respectively) (16). Although urine collected after prostate massage contains a variety of prostate cancer-derived molecules (84), the

commercially available EPI test can effectively detect RNA levels in EVs from non-digital rectal examination urine samples. Further research supports the clinical value of the EPI test for the detection of high-grade prostate cancer in men presenting for initial biopsy with a PSA level of 2–10 ng/mL (85,86). The ability of urine EVs to accurately reflect prostate tissue mRNA expression was validated by comparing *TMPRSS2:ERG* derived from urine EVs versus corresponding radical prostatectomy tissues (n=21), and the urine EVs had an overall accuracy of 81% for the non-invasive detection of *TMPRSS2:ERG* (64). The use of urine EVs as potential RNA biomarkers was also examined via the detection of known prostate cancer-associated genes, such as *TMPRSS2:ERG*, baculoviral IAP repeat containing 5 (*BIRC5*), *ERG*, *PCA3*, and *TMPRSS2* (64). The combination of urine EV-derived *PCA3* and prostate cancer susceptibility candidate (*PRAC*) RNA levels improved the detection of $GS \geq 7$ prostate cancer (AUC =0.736) (65). Multiple gene scores, including RNA levels of *GATA2* along with those of *PCA3* and *TMPRSS2:ERG*, in urine EVs improved the

Table 1 Urine EVs for prostate cancer diagnosis

Target molecules	Target type	Sample	Detection methods	Performance	Refs
ERG, PCA3 and SPDEF	RNA	Urine	qRT-PCR	AUC of 0.803 for the detection of \geq GS7 PCa by PCA3 and ERG RNA levels in combination with SOC	(61)
ERG, PCA3, and SPDEF	RNA	Urine	qRT-PCR	EPI test in combination with SOC improved discrimination of \geq GS7 PCa in training and validation cohorts (AUC =0.77 and 0.73, respectively)	(16)
LincRNA-p21	RNA	Urine	qRT-PCR	The lincRNA-p21 levels were significantly higher in PCa than in BPH (AUC =0.663)	(62)
CDH3	RNA	Urine	qRT-PCR	The decreased abundance of CDH3 transcript in urine EVs from PCa patients	(63)
BIRC5, ERG, PCA3, TMPRSS2:ERG, and TMPRSS2	RNA	Urine	qRT-PCR	BIRC5 (AUC =0.674), ERG (AUC =0.785), PCA3 (AUC =0.681), TMPRSS2:ERG (AUC =0.744), and TMPRSS2 (AUC =0.637) are upregulated in PCa	(64)
PCA3 and PRAC	RNA	Urine	qRT-PCR	AUC of 0.736 for the detection of GS \geq 7 PCa by PCA3 and PRAC	(65)
GATA2, PCA3 and TMPRSS2-ERG	RNA	Urine	qRT-PCR	GAPT-E (GATA2, PCA3, and TMPRSS2-ERG) score improved discrimination of high-grade PCa in training and validation cohorts (AUC =0.85 and 0.71, respectively)	(66)
miR-574-3p, miR-141-5p, and miR-21-5p	miRNA	Urine	qRT-PCR	AUC of 0.85, 0.86, and 0.65 for the detection of PCa by miRNA-574-3p, miR-141-5p, and miR-21-5p, respectively	(67)
miR204-5p, miR21-5p, and miR-375	miRNA	Urine	Stemloop RTPCR	AUC of 0.866 for the diagnosis of PCa by the three miRNA-isoformes	(68)
miR-19b	miRNA	Urine	qRT-PCR	Sensitivity/specificity for the detection of PCa were 93%/100% in total vesicles and 79%/95% in exosome-enriched fractions, respectively	(69)
miR-21 and miR-375	miRNA	Urine	qRT-PCR	A panel combining miR-21 and miR-375 improved discrimination of PCa (AUC =0.872)	(70)
miR-145	miRNA	Urine	qRT-PCR	miR-145 levels in combination with serum PSA differentiate PCa from BPH (AUC =0.863)	(71)
miR-196a-5p and miR-501-3p	miRNA	Urine	qRT-PCR	AUC of 0.73 and 0.69 for the detection of PCa by miR-196a-5p and miR-501-3p, respectively	(72)
miR-2909	miRNA	Urine	qRT-PCR	miR-2909 levels correlated with the GS in PCa	(73)
miR-21-5p and miR-200c-3p	miRNA	Urine	qRT-PCR	miR-21 is upregulated and miR-200c is downregulated in PCa	(74)
miR-30b-3p and miR-126-3p	miRNA	Urine	qRT-PCR	AUC of 0.663 and 0.664 in discriminating PCa from negative biopsy by miR-30b-3p and miR-126-3p, respectively	(75)
A platform that interrogates small noncoding RNAs	sncRNA	Urine	Affymetrix miR 4.0 arrays	The miR Sentinel™ PCa Test demonstrated a sensitivity of 93% and specificity of 90% for the prediction of GS \geq 7 PCa	(76)
ITGA3 and ITGB1	Protein	Urine	WB	ITGA3 and ITGB1 were abundant in urine exosomes of metastatic PCa	(77)

Table 1 (continued)

Table 1 (continued)

Target molecules	Target type	Sample	Detection methods	Performance	Refs
TMEM256 and LAMTOR1	Protein	Urine	MS	AUC of 0.87 for TMEM256 as biomarker for PCa. AUC 0.94 in combining TMEM256 and LAMTOR1 as biomarker for PCa	(78)
CD63	Protein	Urine	TR-FIA	AUC of 0.68 in discriminating PCa from control by CD63	(79)
CD63, GK5, SGSH, PSA and PPAP	Protein	Urine	SRM	AUC of 0.70 for the diagnosis of GS ≥ 7 (4+3) PCa by combining CD63, GK5, SGSH, PSA, and PPAP	(80)
FABP5	Protein	Urine	SRM/MRM	AUC of 0.757 for the detection of PCa, and AUC of 0.856 for the detection of \geq GS7 PCa	(81)
Flotillin 2	Protein	Urine	WB or ELISA	AUC of 0.91 for the diagnosis of PCa by flotillin 2 using western blot	(82)
Phosphatidylserine and lactosylceramide	Lipid	Urine	MS	The ratio of LacCer (d18:1/16:0) over PS 18:1/18:1 and of PS 18:0-18:2 over PS 18:1/18:1 had a sensitivity of 93% at 100% specificity in distinguishing PCa	(83)

qRT-PCR, quantitative reverse transcription-polymerase chain reaction; TR-FIA, time-resolved fluorescence immunoassay; SRM, selected reaction monitoring; MRM, multiple reaction monitoring; WB, western blotting; MS, mass spectrometry; ELISA, enzyme-linked immunosorbent assay; SOC, standard of care; EPI, ExoDx Prostate IntelliScore; PCa, prostate cancer; REF, reference.

discrimination of high-grade prostate cancer in training and validation cohorts (AUC =0.85 and 0.71, respectively) (66).

A genome-wide transcriptomic analysis of urine EVs revealed transcripts with differential abundance in prostate cancer and benign prostate hyperplasia (BPH) and identified the decreased abundance of cadherin 3, type 1 (*CDH3*) transcript in prostate cancer-derived urine EVs (63). Long intergenic noncoding RNA-p21 (*lincRNA-p21*), a p53-dependent transcriptional target gene, is associated with cancer (87). The level of *lincRNA-p21* derived from urine EVs could discriminate prostate cancer from BPH (AUC =0.663) (62).

Next-generation sequencing revealed that miRNAs have a distinct expression pattern in urine EVs isolated from patients with prostate cancer compared with that in urine EVs from healthy control males. The three most differentially expressed isomiRs, miR21, miR204, and miR375, were identified and had an AUC of 0.866 for the diagnostic performance in prostate cancer patients (68). Another next-generation sequencing study demonstrated that miR-196a-5p and miR-501-3p were downregulated in urine EVs derived from prostate cancer and had AUC values of 0.73 and 0.69, respectively, for detecting prostate cancer (72). Lectin-induced agglutination of urinary EV isolation revealed a significant upregulation of miR-574-3p, miR-141-5p, and miR-21-5p in patients with prostate cancer, with AUC values of 0.85, 0.86,

and 0.65, respectively, for detecting prostate cancer (67). Using hydrostatic filtration dialysis to isolate urine EVs effectively from patients with prostate cancer, Xu *et al.* revealed that miR-145 levels in urine EVs combined with serum PSA could differentiate prostate cancer from BPH (AUC =0.863) (71). A panel combining miR-21 and miR-375 derived from urine EVs improved discrimination of prostate cancer (AUC =0.872) (70). Detection of miR-19b versus miR-16 in total urine EVs and exosome-enriched fractions achieved 100%/93% and 95%/79% specificity/sensitivity, respectively, in distinguishing prostate cancer patients from healthy male (69). The levels of miR-30b-3p and miR-126-3p, which are overexpressed in urinary EVs derived from patients with prostate cancer, could discriminate patients with prostate cancer from biopsy-negative men (75). A platform that interrogates small non-coding RNAs (sncRNA) isolated from urinary EVs demonstrated a sensitivity and specificity of 93% and 90%, respectively, for the prediction of GS ≥ 7 prostate cancer (76). The miR-21 levels in urine EVs were upregulated in both non-metastatic and metastatic prostate cancer compared with those in BPH (74). The miR-2909 levels in urine EVs correlated with the GS in prostate cancer (73).

Proteomic analysis of urinary EVs by mass spectrometry identified proteins differentially expressed in patients with prostate cancer compared with the expression of these proteins in healthy male controls. The combination of transmembrane

Table 2 Plasma and serum EVs for prostate cancer diagnosis

Target molecules	Target type	Sample	Detection methods	Performance	Refs
SAP30L-AS1 and SChLAP1	lncRNA	Plasma	qRT-PCR	AUC of 0.922 by combining SAP30L-AS1 and SChLAP1 in discriminating PCa from controls	(89)
Circ_0044516	circRNA	Blood	qRT-PCR	Circ_0044516 was upregulated in EVs from PCa patients and cell lines	(90)
miR-141 and miR-375	miRNA	Plasma and Serum	qRT-PCR	miR-141 and miR-375 were upregulated in metastatic PCa	(91)
miR-141	miRNA	Serum	qRT-PCR	AUC of 0.869 in discriminating metastatic PCa from localized PCa by miR-141	(92)
miR-200c-3p, miR-21-5p and Let-7a-5p	miRNA	Plasma	qRT-PCR	miR-200c-3p and miR-21-5p differentiate between PCa and BPH (AUC =0.68 and 0.67, respectively). Let-7a-5p distinguish \geq GS8 PCa (AUC =0.68)	(93)
miR-1246	miRNA	Serum	qRT-PCR	AUC of 0.926 in discriminating PCa from control by miR-1246	(94)
miR-125a-5p and miR-141-5p	miRNA	Plasma	qRT-PCR	AUC of 0.793 in distinguishing PCa from control by miR-125a-5p/miR-141-5p ratio	(95)
Survivin	Protein	Plasma	WB	Survivin was significantly increased in PCa compared with BPH and control	(96)
PTEN	Protein	Plasma	WB	PTEN was detected in EVs derived from the plasma of PCa patients but not in the plasma of normal subjects	(97)
PSMA	Protein	Plasma	ELISA	Plasma PSMA-positive EV levels differentiated PCa from BPH (AUC =0.943)	(98)
GGT1	Protein	Serum	Fluorescent probe	Serum exosomal GGT activity was significantly higher in patients with PCa than in those with BPH	(99)
CLDN3	Protein	Plasma	ELISA	CLDN3 levels predicted \geq GS8 disease in patients with localized PCa (AUC =0.705)	(100)
PSA	Protein	Plasma	ELISA and flow-cytometry	PCa patients have high levels of EVs expressing both CD81 and PSA	(101)
PSA	Protein	Plasma	ELISA and flow-cytometry	Plasmatic levels of PSA-expressing exosomes distinguish PCa from BPH by ELISA and flow-cytometry (AUC =0.982 and 0.960, respectively)	(102)
EphrinA2	Protein	Serum	ELISA and WB	AUC of 0.906 in differentiating patients with PCa from those with BPH	(103)

qRT-PCR, quantitative reverse transcription-polymerase chain reaction; WB, western blotting; ELISA, enzyme-linked immunosorbent assay; PCa, prostate cancer; BPH, benign prostatic hyperplasia; REF, reference.

protein 256 (TMEM256) and late endosomal/lysosomal adaptor, MAPK and MTOR activator 1 (LAMTOR1) provided an AUC of 0.94 for detecting prostate cancer (78). Then, antibody-based methods, such as western blotting or enzyme-linked immunosorbent assay, were used to validate the proteins which were identified by the mass spectrometry; a receiver operating characteristic curve of flotillin 2 showed an AUC of 0.91 (82). Shotgun proteomics of EVs in urine by iTRAQ (isobaric tag for relative and absolute quantitation) analysis

identified protein candidates for prostate cancer diagnosis (81). Subsequent analysis of these candidates by selected reaction monitoring/multiple reaction monitoring (SRM/MRM) revealed that the levels of fatty acid-binding protein 5 (FABP5) were higher in prostate cancer than in healthy individuals and could discriminate GS \geq 7 prostate cancer (AUC =0.856) (81). Targeted proteomics and immunoblotting techniques defined a set of protein-combination panels in urinary EVs in which a combination of CD63, glycerol kinase 5 (GK5),

N-sulfoglucosamine sulfohydrolase (SGSH), PSA, and prostatic acid phosphatase (PPAP) had an AUC of 0.70 for the diagnosis of GS ≥ 7 (4+3) prostate cancer (80). Immuno-based detection of urine EVs by CD63 had an AUC of 0.68 (79).

Targeted metabolomics analysis of urine EVs demonstrated a variety of metabolic alterations in prostate cancer, including phosphatidylcholines, acylcarnitines, citrate, and kynurenine (88). Lipidomic analysis indicated that the lipid composition of urinary EVs, including phosphatidylserine and lactosylceramide, was significantly different between patients with prostate cancer and healthy control individuals (83).

Plasma and serum EVs for the diagnosis of prostate cancer

Plasma and serum EVs have been reported as useful biomarkers for the diagnosis of prostate cancer (*Table 2*).

A comprehensive qRT-PCR screening identified differentially quantified miRNAs in plasma from patients with prostate cancer (91). The expression of miR-141 and miR-375 was upregulated in plasma- and serum-derived EVs from patients with metastatic prostate cancer (91). miR-141 derived from serum EVs had an AUC of 0.869 in discriminating patients with metastatic prostate cancer from those with localized prostate cancer (92). The miR-125a-5p/miR-141-5p ratio in plasma EVs had an AUC value of 0.793 in distinguishing patients with prostate cancer (95). The EV-incorporated and whole plasma cell-free miRNA profiles were clearly different. Plasma EVs had Let-7a-5p levels that could distinguish patients with GS ≥ 8 prostate cancer (AUC =0.68) (93). A NanoString nCounter technology revealed the profile of serum EV-derived miRNAs from patients with aggressive prostate cancer and identified miR-1246 as a potential diagnostic marker (94). miR-1246 had an AUC value of 0.926 in discriminating prostate cancer from controls. miR-1246 is a tumor suppressor miRNA that is downregulated in prostate cancer clinical tissues and selectively released in EVs (94).

A lncRNA termed second chromosome locus associated with prostate-1 (*SCbLAP1*) is overexpressed in a subset of prostate cancers, and elevated *SCbLAP1* levels are associated with poor outcomes (104). The combination of two lncRNAs derived from plasma EVs, *SAP30L* antisense RNA 1 (*SAP30L-AS1*), and *SCbLAP1* had adequate diagnostic value to distinguish prostate cancer from controls (AUC =0.922) (89). Among circular RNAs, circ_0044516 was upregulated in EVs from patients with prostate cancer

and controlled the proliferation and invasion in prostate cancer cells (90).

Plasma and serum EV-derived proteins are potential biomarkers for prostate cancer. The level of survivin isolated from plasma EVs was significantly increased in patients with prostate cancer compared with that in the controls (96). PTEN was incorporated in the cargo of EVs that circulate in the blood of patients with prostate cancer but not in the blood of healthy individuals (97). Plasma prostate-specific membrane antigen (PSMA)-positive EV concentration could differentiate prostate cancer from BPH (AUC =0.943) (98). Gamma-glutamyltransferase (GGT) activity in serum EVs was significantly higher in patients with prostate cancer than in patients with BPH (99). Claudin 3 (CLDN3) levels in plasma EVs identified patients with GS ≥ 8 prostate cancer (AUC =0.705) (100). The amount of plasmatic EVs expressing both CD81 and PSA was significantly higher in patients with prostate cancer than in patients with BPH and healthy control individuals (101,102). The ephrinA2 levels in serum EVs had an AUC value of 0.906 in differentiating patients with prostate cancer from those with BPH (103).

Semen EVs

Seminal fluid contains various small membranous vesicles (105). Prostatosomes, the EVs released into prostatic fluid, have been proposed to regulate spermatozoa to attain fertility (106,107). Comprehensive proteomics analysis of EVs derived from seminal plasma can identify potential biomarkers for male infertility and prostate cancer (108). The miRNA profile in semen EVs was altered in patients with prostate cancer compared with that in patients with BPH. A model including serum PSA, miR-342-3p, and miR-374b-5p had an AUC of 0.891 in detecting GS ≥ 7 prostate cancer (109).

EVs as prognostic biomarkers

EVs are considered potential biomarkers for predicting disease progression, especially in patients with CRPC (*Table 3*).

CTC counts can predict the prognosis of CRPC patients (122). Tumor-derived plasma EV levels were significantly increased in patients with CRPC and associated with worse overall survival (110,111). The detection of androgen-receptor splice variant 7 (*AR-V7*) in CTCs from patients with CRPC was associated with resistance to enzalutamide and abiraterone (123). Likewise, the detection of *AR-V7* in plasma-derived EVs predicted resistance to

Table 3 EVs as prognostic biomarkers of prostate cancer

Target molecules	Target type	Sample	Detection methods	Performance	Refs
Tumour-derived EVs	EVs	Blood	ACCEPT software	CTCs and plasma levels of tumour-derived EVs were significantly increased in CRPC patients compared with those in healthy donors	(110)
Tumour-derived EVs	EVs	Blood	ACCEPT software	Increasing tumour-derived EV counts were associated with worse OS in CRPC (HR 2.2, 95% CI, 1.3–3.5)	(111)
AR-V7	mRNA	Plasma	ddPCR	Median PFS and OS were significantly longer in AR-V7-negative than in AR-V7-positive patients with metastatic CRPC before second-line hormonal treatment (20 vs. 3 mo, P<0.001; not reached vs. 8 mo, P<0.001, respectively)	(112)
AR-V7 and AR-FL	mRNA	Urine	ddPCR	Higher AR-V7 and lower AR-FL levels in CRPC than in hormone sensitive PCa	(113)
AR-V7 and AR-FL	mRNA	Plasma	ddPCR	Median PFS and OS were significantly longer in AR-V7-negative than in AR-V7-positive patients with CRPC (20 vs. 4 mo, P<0.0001; not reached vs. 9 mo, P<0.0001, respectively)	(114)
AR-V7	mRNA	Plasma	ddPCR	AR-V7-positive was associated with a shorter PFS in patients with CRPC (median, 16 vs. 28 mo, P=0.0499)	(115)
miR-1290 and miR-375	miRNA	Plasma	qRT-PCR	Higher levels of miR-1290 and -375 were significantly associated with poor OS in patients with CRPC (P<0.004)	(116)
ACTN4	Protein	Serum	Proteomic analysis	Actinin-4 level was increased in patients with metastatic CRPC compared with that in patients with well-controlled metastatic PCa receiving primary androgen deprivation therapy	(117)
CD44v8-10	mRNA	Serum	ddPCR	CD44v8-10 mRNA copy numbers were higher in docetaxel-resistant patients than in healthy individuals and docetaxel-naïve patients	(118)
miR-654-3p and miR-379-5p	miRNA	Serum	NGS	miR-654-3p and miR-379-5p serve as biomarkers predicting the efficacy of carbon ion radiotherapy for PCa	(119)
let-7a-5p and miR-21-5p	miRNA	Serum	qRT-PCR	The expression of let-7a-5p and miR-21-5p was abundant in EVs after radiation therapy	(120)
miR-151a-5p, miR-204-5p, miR-222-3p, miR-23b-3p and miR-331-3p	miRNA	Urine	qRT-PCR	Prediction of biochemical recurrence after radical prostatectomy by five urine miRNAs and serum PSA (HR =3.12, 2.24, and 2.15 for three cohorts)	(121)

AR-V7, androgen-receptor splice variant 7; AR-FL, androgen-receptor full-length; ddPCR, droplet digital polymerase chain reaction; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; NGS, next-generation sequencing; CTC, circulating tumor cells; CRPC, castrate-resistant prostate cancer; PCa, prostate cancer; OS, overall survival; PFS, progression-free survival; REF, reference.

hormonal therapy in patients with CRPC (112,114,115). Higher *AR-V7* and lower *AR* full-length expressions were detected in urine-derived EVs from patients with CRPC compared with those in urine EVs from patients with hormone-sensitive prostate cancer (113).

RNA sequencing identified miRNAs in EVs, which were associated with overall survival in patients with CRPC. Among them, miR-1290 and miR-375, higher levels of which were significantly associated with poor overall

survival in patients with CRPC (116). Proteomic analysis of serum EVs revealed that the actinin-4 (ACTN4) level was higher in patients with metastatic CRPC than in patients with well-controlled metastatic prostate cancer who received primary androgen deprivation therapy (117). The *CD44v8-10* mRNA copy numbers in serum EVs were higher in docetaxel-resistant patients than in control individuals and docetaxel-naïve patients (118). A number of miRNAs are implicated in DNA repair pathways that

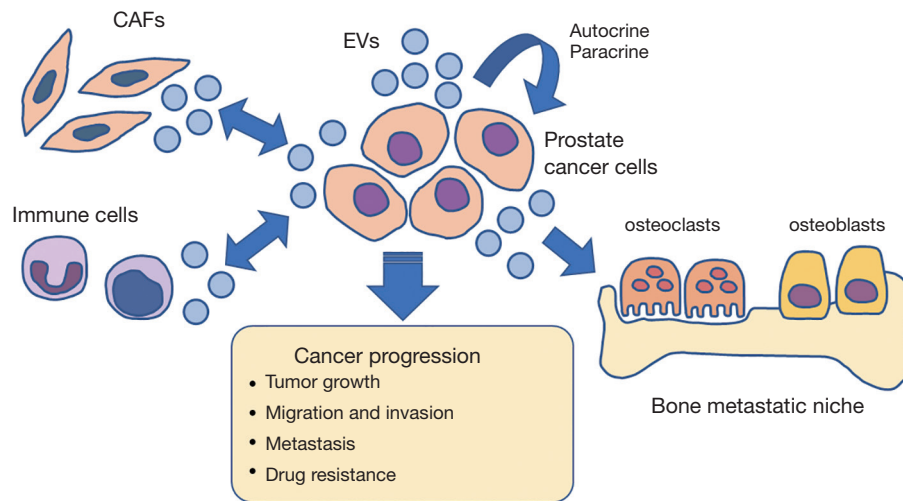


Figure 2 EVs regulate prostate tumor microenvironment. EVs shed from cancer cells and stromal cells are involved in the development of tumor microenvironment. Prostate cancer cell-derived EVs activate CAFs, in turn, CAFs shed EVs and induce proliferation, migration and invasion of cancer cells. EVs promote bone metastatic niche formation, attenuating the activity of osteoclasts and osteoblasts. EVs can enhance proliferation and invasion of tumor cells by autocrine and paracrine manner. Thus, EVs enhance cancer progression, metastasis and drug resistance. EV, extracellular vesicle; CAF, Cancer associated fibroblast.

affect radiation sensitivity (41). miRNAs in serum EVs were significantly altered after radiation therapy, indicating that they serve as potential biomarkers for predicting radiation therapy efficacy (119,120). A model, comprising five urine EV miRNAs (miR-151a-5p, miR-204-5p, miR-222-3p, miR-23b-3p, and miR-331-3p) and serum PSA, significantly predicted the time to biochemical recurrence after radical prostatectomy in three independent cohorts (hazard ratio =3.12, 2.24, and 2.15, respectively) (121). Thus, EVs may be useful to improve prostate cancer risk stratification and to guide treatment decisions.

EVs are involved in tumor microenvironment formation

Although EVs were initially considered membrane debris, in 1996, Raposo *et al.* reported that EVs derived from B lymphocytes induced T cell responses (124). In 2007, Valadi *et al.* showed that both mRNA and miRNA could be transferred between cells through EVs (125). These transferred miRNAs were shown to regulate target gene expression and recipient cell function (126-128). Since then, emerging evidence has demonstrated that EVs are important mediators of intercellular communication by transferring vesicle contents and activating signaling pathways (129-138) (Figure 1). Thus, EVs play important

roles in the maintenance of normal physiology by participating in a diverse range of biological processes (139,140). Furthermore, EVs are also involved in various pathological conditions, such as infectious diseases (141-143) and cancer (144-147). Emerging evidence suggests that EVs control tumor microenvironment and thus also play crucial roles in cancer development and progression (148-159).

EVs are critically involved in the development of the prostate tumor microenvironment, promoting tumor growth, invasion, bone metastasis, and drug resistance (Figure 2). Prostate cancer EVs promote the establishment of a tumor-supportive environment by inducing reprogramming of the stroma (160,161). In addition, they induce the activation of fibroblasts, which in turn increase EV shedding and induce migration and invasion of prostate cancer cells via the CX3CL1-CX3CR1 pathway (162). Prostate cancer-derived EVs also induce migration and invasion of prostate epithelial cells via integrin $\alpha 3$ (ITGA3) and integrin $\beta 1$ (ITGB1), explaining the increased levels of ITGA3 and ITGB1 in urine EVs from patients with metastatic prostate cancer (77). Prostate cancer EVs drive fibroblast differentiation to a pro-tumorigenic phenotype via the transforming growth factor β (TGF β), leading to angiogenesis and accelerated tumor growth (163,164). Atypically large EVs released by prostate cancer cells enhance the migration of cancer-associated fibroblasts

(CAFs) by intercellular transfer of functional miRNA such as miR-1227 (165). Prostate cancer EVs containing catalytically active hyaluronidase Hyal1 stimulate prostate stromal cell motility (166).

CAFs regulate the tumor microenvironment via EVs and transfer lipids and proteins to prostate cancer cells through EVs to support tumor growth (167). CAFs release miR-409 through EVs, promote epithelial-to-mesenchymal transition, and enhance prostate tumorigenesis (168). CAF-derived EVs contain intact metabolites, including amino acids, lipids, and tricarboxylic acid cycle intermediates, that are utilized by cancer cells to promote tumor growth under nutrient deprivation conditions (169).

Prostate cancer cell-derived EVs are capable of inducing osteoclast differentiation and osteoblast proliferation, which attenuate bone metastasis (170). EV-derived miR-141-3p from prostate cancer cells promotes osteoblast activity, increases osteoprotegerin expression, and regulates the microenvironment of bone metastases (171). Comprehensive expression analysis identified miRNAs that were highly expressed in EVs from osteoblastic phenotype-inducing prostate cancer cell lines (172). Among them, miR-940 significantly promoted the osteogenic differentiation of human mesenchymal stem cells by targeting *ARHGAP1* (Rho GTPase activating protein 1) and *FAM134A* (family with sequence similarity 134 member A) (172). Prostate cancer-derived EVs promoted premetastatic niche formation through EV-mediated transfer of pyruvate kinase M2 from prostate cancer cells into bone marrow stromal cells (173).

EVs secreted by tumor cells can enhance the proliferation and invasion of tumor cells in an autocrine and paracrine manner. The urokinase plasminogen activator in EVs derived from the prostate cancer cell line PC3 promotes the invasion ability of the prostate cancer cell line LnCaP (174). EVs transfer $\alpha\beta6$ integrin and its related functions between different subsets of prostate cancer cells, potentially promoting cell migration and metastasis (175). Hypoxia promotes prostate cancer aggressiveness by enhancing lipid accumulation in cells and EVs (176). Androgen enhances the secretion of CD9-positive EVs by prostate cancer cells to induce cancer cell proliferation (177). Nuclear translocation of AR and epidermal growth factor receptor through EVs stimulates the proliferation of prostate cancer cells (178). EVs derived from mesenchymal-like prostate cancer promote mesenchymal features in recipient epithelial-like prostate cancer cells, accompanied by a

modulation of AR signaling and activation of the TGF β signaling pathway (179).

Prostate cancer-derived EVs downregulate natural killer group 2, member D expression on natural killer and CD8+ T cells, thereby promoting immune evasion (180). Tumor-associated macrophages promote prostate cancer progression by EV-mediated transfer of miR-95 (181).

EVs expelled from docetaxel-resistant prostate cancer cells imparted docetaxel-resistance to parental prostate cancer cells by mediating the transfer of P-glycoprotein (MDR-1) (182). The levels of miR-34a decreased in EVs of docetaxel-resistant prostate cancer cell lines, indicating that miR-34a potentially regulates the response to docetaxel by downregulating BCL-2 levels (183). As an adaptive mechanism to enzalutamide treatment, prostate cancer cells express and secrete Brain4 (BRN4) and BRN2 in EVs that drive oncogenic reprogramming of prostate cancer cells to neuroendocrine prostate cancer, an aggressive variant of CRPC (184).

Thus, EVs shed from cancer and stromal cells contribute to the generation of the tumor microenvironment and enhance cancer progression, metastasis, and drug resistance. The cargo of EV includes various molecules, and EV is also useful as a drug delivery system (185-187). The advances in EV isolation techniques may further promote the potential use of EVs as biomarkers in the clinic.

Conclusions

EVs derived from cancer cells and stromal cells can be isolated from bodily fluids, such as urine, plasma, serum, and semen. EVs are considered useful biomarkers for detecting clinically significant prostate cancer. EVs can also be used as potential biomarkers for the prediction of disease progression and the monitoring of prostate cancer. Although the EPI test has been clinically validated for the detection of high-grade prostate cancer, the other methods still need further validation. EVs contribute to the formation of the prostate tumor microenvironment and enhance tumor proliferation, invasion, bone metastasis, and drug resistance. Future studies are needed to uncover the mechanism of cancer progression mediated by EVs.

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