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Review

Recent advances in extracellular vesicle research for urological cancers: From technology to application

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A B S T R A C T

Urological malignancies, including prostate cancer, bladder cancer and kidney cancer, are major causes of morbidity and mortality worldwide. Because of the high incidence, diversity in biology, and especially direct interaction with urine, urological cancers are an important resource for both scientists and clinicians for novel diagnostic and therapeutic discovery. Extracellular vesicles (EVs) are lipid bilayer encapsulated particles released by cells into the extracellular space. Since EVs work as a safe way to transport important biological information through the whole body, they are now recognized as an important mechanism of cell–cell communication and have opened a new window for us to gain a better understanding of cancer biology, novel diagnostics, and therapeutic options. In recent years, numerous evolutions in EV technologies and novel biological and clinical findings continue to be reported in the research field of urological cancers. This comprehensive review aims to give an update of recent advances in EV technologies and summarize the state-of-the-art knowledge of EVs related to prostate cancer, bladder cancer and kidney cancer, particularly focusing on the potential of EV as biomarkers and their biological roles in promoting cancer and metastasis.

1. Introduction

Urological malignancies, including prostate cancer (PCa), bladder cancer (BCa) and kidney cancer (renal cell carcinoma, RCC) are major causes of morbidity and mortality worldwide. Approximately 326,000 new diagnoses of urological cancers and over 63,000 deaths are estimated to occur in the United States in 2018, which contribute approximately 20% and 10% to the total estimated new diagnoses and deaths respectively of all cancer types [1]. Because of the high incidence, diversity in biology, and especially direct interaction with urine, urological cancers are an important resource for both scientists and clinicians for novel diagnostic and therapeutic discovery [2,3].

Extracellular vesicles (EVs) are lipid bilayer encapsulated particles released by cells into the extracellular space. At first, they were considered to be a way cells dispose of waste but now it has been confirmed that cells pack a variety of molecular cargos, including protein, lipids, RNAs and DNA, into EVs and actively release them to the extracellular space [4–6]. The released EVs can act in a paracrine way or travel to a distant body site, where they can be accepted by target cells and play a functional role in the new host cell. For instance, in the recipient cells, messenger RNA (mRNA) cargos can be translated into proteins and protein cargos can directly function. Since EVs work as a safe way to transport important biological information through the whole body, they are now recognized as another mechanism of cell–cell communication [7,8]. EVs are also secreted by plant cells, bacteria, mycobacteria and fungi, suggesting an important evolutionarily conserved mechanism of intercellular communication [9,10].

EVs represent a wide range of subpopulations that differ not only in size but also in molecular cargo and function. Currently, there is not a standard way for straightforward classification. Some EVs are named by their origin (e.g. prostasomes are EVs released by prostate epithelial cells and large oncosomes are EVs released by cancer cells). Additionally, it is common to name EVs according to their surface markers (e.g. CD9 positive EVs) since more marker-based capture technologies and studies have been developed. Previously, many review papers classified EVs into exosomes and microvesicles (MVs) according to their biogenesis and size. However, there is a substantial overlap in their sizes, and therefore the term 'MVs' is too narrow to accurately represent all the EV subtypes with similar biogenesis. The biogenesis of the exosome starts from the inward budding of the plasma membrane that forms the early endosome and then late endosome after a series of maturation events. After the membrane of the late endosome buds inward to form several intraluminal vesicles (ILVs), it becomes a multivesicular body (MVB). Some MVBs will fuse with lysosomes and their cargo will be degraded, while the others fuse with cell surface and the released ILVs are called exosomes. Ectosomes, including but not limited

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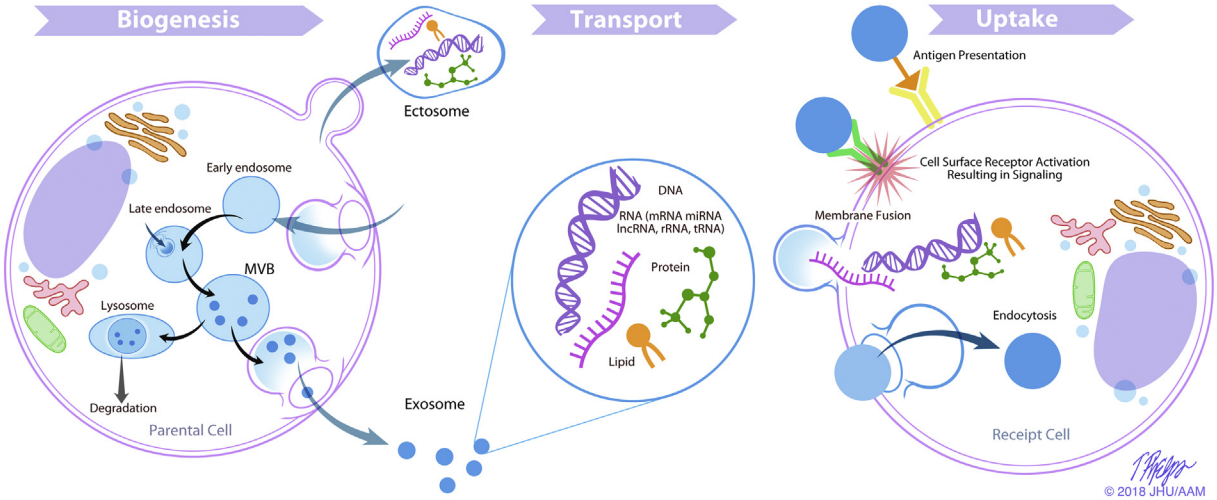

Fig. 1. The schematic diagram of EV biogenesis, transportation and uptake.
Biogenesis: The biogenesis of the exosome starts from the inward budding of the plasma membrane that forms the early endosome and then late endosome after a series of maturation events. After the membrane of the late endosome buds inward to form several intraluminal vesicles (ILVs), it becomes a multivesicular body (MVB). Some MVBs will fuse with lysosomes and their cargo will be degraded, while the others fuse with cell surface and the released ILVs are called exosomes. Ectosomes, including but not limited to MVs, are ubiquitously assembled at and released from the plasma membrane through direct outward budding Transportation: EVs provide a safe way to transport a variety of molecular cargo in human circulation, including proteins, RNAs and sometimes DNA. Uptake: EVs can either directly activate cell surface receptors via protein and bioactive lipid ligands or release their cargo into recipient cells by membrane fusion or endocytosis. EVs can also participate in immune regulation by transferring both MHC molecules and antigens.

to MVs, are ubiquitously assembled at and released from the plasma membrane through direct outward budding [11]. EVs can contain cytoplasmic and membrane proteins, RNA (mRNAs, miRNAs and other ncRNAs) and sometimes DNA. The protein and RNA cargo in EVs can be very different from their parental cells and EVs provide a safe way to transport them in human circulation [12]. Upon uptake, EVs can either directly activate cell surface receptors via protein and bioactive lipid ligands or release their cargo into recipient cells by membrane fusion or endocytosis. EVs can also participate in immune regulation by transferring both MHC molecules and antigens [13]. The biogenesis, release and uptake mechanisms of EVs have been recently reviewed [14,15] (Fig. 1).

EVs have been demonstrated to participate in many normal physiological processes, including blood coagulation, immunomodulation, embryo implantation, pregnancy, etc. [16–18]. EVs' release and uptake can be modulated in response to environmental changes (hypoxia, irradiation, injury, cellular stress, etc.) [19,20]. Besides normal physiology, EVs are also involved in almost all the pathophysiological processes including cancer. Multiple studies in different cancer types have demonstrated that EVs play an important role in cell proliferation, migration, angiogenesis, immune suppression, invasion, epithelial-to-mesenchymal transition (EMT) and metastasis [21–23]. Since theoretically all types of cells can secrete EVs and they can be found in almost all kinds of human body fluids, EVs have opened a new window to gain a better understanding of cancer biology, novel diagnostics, and therapeutic options.

Recently a survey reported a 733-fold increase in the number of publications on EVs, and a 1000-fold increase in citations containing EV-related terms in the last 9 years [24]. Although there have been many review articles in the EV-field, very few of them have provided comprehensive knowledge on EVs in the field of urological cancers [25–28]. This review aims to outline current technical advances in EV research. Furthermore, we are trying to answer questions as ‘how much do we know about EVs’ biological roles on urological cancers?’, ‘what EV-derived biomarkers could we expect to aid in diagnostics and/or prognostics?’, ‘how heterogeneous are the results from the currently available studies?’, and ‘how should we improve our future EV research?’

2. EV isolation in urological cancer research

EV isolation is one of the most important steps in EV research, since different isolation methods determine the yield and purity of EV products and impact downstream analysis. To date, there are basically four types of classic EV isolation techniques - differential and density gradient ultracentrifugation (UC), size-based techniques, immunoaffinity capture-based techniques, and precipitation reagents. Newer microfluidic techniques are also being developed. All of the techniques have strengths and weaknesses. The working principles, advantages and disadvantages of each technique are summarized in Table 1.

2.1. Biological samples available for urological cancer research

The most common biological samples for EV studies are serum, plasma, pre- or post- digital rectal examination (DRE) urine, prostatic fluid, seminal plasma, tissue, and conditioned cell culture medium. Fig. 2 illustrates the published and unpublished, but potential, resources for EVs in urological cancer research. Each sample type has its own features with potential strengths and weaknesses. The concentration of EVs in serum or plasma is usually higher than other types of samples. It was reported that serum was superior to plasma in terms of yields of exosome and exosomal micro RNA (miRNA) but had more albumin contamination [29]. Moreover, since additional EVs are released during serum preparation, plasma may be preferred [30,31]. However, there is a lack of comparative, conclusive studies on this topic. Two different types of urine samples can be collected to inform PCa research. Several studies demonstrated that the biomarker levels were significantly higher in post-DRE than pre-DRE urine [32,33]. Prostatic fluid can also be utilized for prostate cancer research after isolation of prostasomes, but the sample volume is relatively low and sample acquisition is more difficult compared to urine. Seminal plasma is a special vehicle for transportation and storage of spermatozoa, and it contains EVs that may provide information regarding male reproductive physiology, male infertility and PCa [34,35]. Tissue derived EVs have attracted considerable attention, containing information from cancer cells as well as cells in the tumor microenvironment.

Standardized collection, processing and storage of biological samples are essential to assure EV research quality. The variables of donors

Table 1
The working principle, advantages and disadvantages of each extracellular vesicle isolation technique.

Techniques	Working principle	Advantages	Disadvantages
Differential and density-gradient ultracentrifugation	Differential ultracentrifugation: Use multiple-step sequential centrifugation to remove impurities based on particle density and size; Density-gradient ultracentrifugation: combine differential ultracentrifugation with density gradient, which bases on difference in density	Differential ultracentrifugation: requires relatively lower cost; suits for various samples and volumes, especially large volume; Density-gradient ultracentrifugation: is the current "gold standard" for EV purity	Requires ultracentrifuge machine; time-consuming and labor-intensive procedure; unsuitable for very low sample volume; relatively low yield
Size-based techniques	Separate EVs from other particles based on their size difference	Simple and fast; high EV yield	High force may induce damage to vesicles; potential contamination with other particles in overlapping size; loss of EVs due to adherence to membranes or filter pores
Immunoaffinity capture-based techniques	EVs are captured based on specific interactions between membrane-bound antigens and pre-functionalized magnetic beads, coated with antibodies	Ideally suitable for the isolation of selected EVs	Can only capture a certain subtype of EVs; need to know the surface marker expression on target EVs; non-specific binding; unsuitable for large-volume samples
EV precipitation	Alter solubility or dispersibility by using water-excluding polymers, followed by centrifugation at low speed	Simple and fast; does not require specialized equipment; high EV yield	Lower purity with non-EV contaminants such as proteins and polymeric materials
Microfluidics-based techniques	Use microfluidics devices to isolate EVs based on a variety of properties like immunoaffinity, size, density, etc.	Fast, time efficient, labor-saving, high yield, good for low sample volume	Lack of validation; expensive; most of these are in exploration phase; not suitable for large sample volume

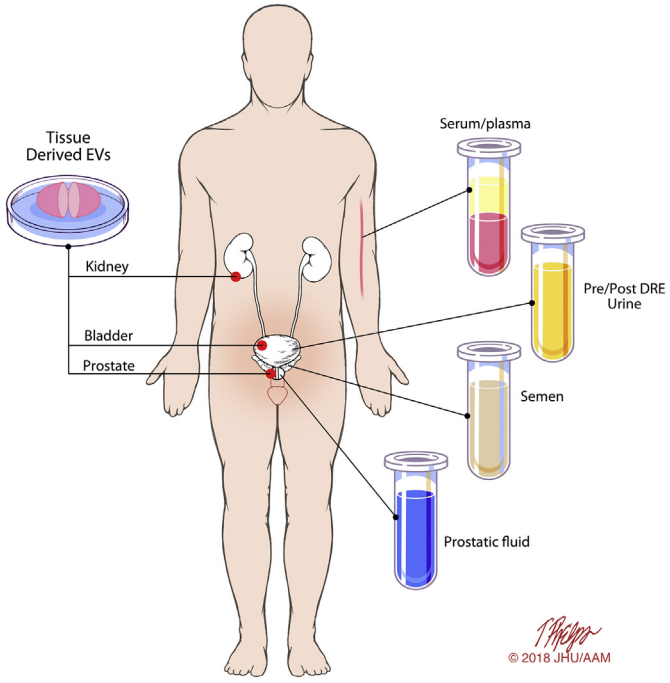

Fig. 2. The schematic diagram of the published/potential sample resourses for EVs in urological cancer research.
The most common biological samples for EV studies in the field of urological cancers are serum, plasma, urine (pre- or post- DRE), prostatic fluid, seminal plasma and tissue (cancer and/or adjacent normal tissue).

need to be recorded, such as gender, age, disease stage, and co-morbidities. In order to prevent hemolysis, it is suggested to process blood as soon as possible. For post-DRE urine, the first urine should be collected after DRE. The use of EV-depleted serum is recommended when isolating EVs from conditioned medium in cell culture. All the samples should be shipped on ice to the sample library or laboratory with minimal time between collection and processing. Samples can be stored at −80 °C after pre-processing (low speed centrifuge) to remove cell contamination or debris. Repeated freezing-thawing should be avoided [36–38].

2.2. Classic EVs isolation methods

2.2.1. Differential and density gradient UC

UC-based techniques are the current gold standard and the most widely used methods for EVs isolation from cell culture media and biofluids [39,40]. There are two types of UC: differential UC and density gradient UC. All centrifugations should be performed at 4 °C. Differential UC usually consists of multiple steps at different centrifugal forces and durations to isolate EVs from cells and debris, based on their density and size. This method is a relatively simple with drawbacks as EV loss during processing and co-isolation of other particles, such as protein aggregates and apoptotic bodies, with the EVs. In conventional differential UC protocols the pellet after centrifugation at ~10,000 *g* is discarded, but these pellets may contain large cancer cell-derived oncosomes that play an important role in regulating the tumor microenvironment [41].

The "purest" EVs are isolated by density gradient UC, which separates the mixtures according to their different flotation densities. Exosomes have a buoyant floatation density of 1.10 to 1.21 g/mL, while lipids have a density of ~1 g/mL, and proteins and RNA > 1.3 g/mL [42]. Density gradients, therefore, can be used to purify and isolate EV classes from soluble proteins, free RNA and protein-RNA complexes. The most commonly used solution in density gradient UC is sucrose. However, in some reports, 5–40% iodixanol gradients are used instead of sucrose due to the concern that sucrose may inhibit some biological effects of EVs [43,44]. It should be noted that the density in sucrose and iodixanol are not the same. The exosomes can be collected at the section containing 8.4–12% iodixanol.

2.2.2. Size-based isolation techniques

Size-based techniques such as ultrafiltration and size exclusion chromatography (SEC) are also relatively common methods, primarily isolating EVs based on their size and molecular weight. The first step of size-based techniques is usually replaced by a low-speed centrifugation due to EVs deformation caused by the force of normal filtration. Ultrafiltration is a three-step sequential method, utilizing a 100-nm membrane filter, followed by tangential flow filtration with 500 kDa molecular weight cut-off (MWCO) hollow fibers, followed by a 100 nm track-etch filter [45]. Ultrafiltration is an appropriate technique for large-volume biofluid samples such as urine or cell-conditioned medium. In SEC, the particles pass through the SEC porous column at different rates depending on their size [46]. The sample volume for SEC

should not exceed 10% of the resin volume, so it can only be used for clinical samples with a limited volume. The major impurities in these size-based isolation techniques are contaminants over the size cutoff, including viruses, protein aggregates, and very large proteins. Some new size-based techniques have been recently developed, including hydrostatic filtration dialysis (HFD) and the exosome total isolation chip (ExoTIC) [47,48].

2.2.3. Immunoaffinity capture-based techniques

Multiple proteins, that are located on the membrane of EVs, can be utilized as targets for immunoaffinity capture. The classic immunoaffinity capture-based technique is the microplate-based immunoaffinity capture approach, but antibody-coated magnetic beads are gaining popularity [49,50]. Selecting an EV surface target is the key step of this method. The most commonly used proteins to isolate EV subpopulations are members of the tetraspanin family, including CD63, CD9 and CD81, which are commonly but not always expressed on EV membranes. Organ- or disease-specific molecules are employed to obtain specific EVs (e.g., CD34 for acute myeloid leukemia, prostate specific membrane antigen (PSMA) for PCa, A33 for colorectal cancer, and CSPG4 for melanoma) [51–54]. Dual-antibody-functionalized immunoaffinity systems can be used to increase capture if one antibody is insufficient [55].

2.2.4. Co-precipitation methods

Five decades ago, polymer-based precipitation methods were used to isolate viruses and other macromolecules by altering the solubility or dispersibility through the use of a water-excluding polymer, followed by an incubation step at 4 °C and centrifugation at low speed [56]. The most commonly used polymer is polyethylene glycol (PEG). Most of the commercially available isolation kits use this method to isolate EVs, such as the Total Exosome Isolation Kit from Invitrogen (Aus), the ExoSpin Exosome Purification Kit from Cell Guidance Systems (USA) and the ExoQuick kit from System Biosciences (USA) [57,58]. As both EVs and non-EV components are simultaneously collected by precipitation, this method should be considered a way to concentrate EVs, rather than to isolate them. Although this method is used for primary isolation, it can be used after EVs isolation by other methods, for example, UC [37].

2.3. Microfluidics-based techniques

Microfluidics-based isolation techniques are emerging methods to isolate EVs by utilizing the properties of EVs, such as size, density, immunoaffinity and/or viscoelasticity.

2.3.1. Immunoaffinity-based microfluidic techniques

There are three types of immunoaffinity-based platforms to capture EVs: modified flat surface, modified magnetic beads, and modified microstructures and nanomaterials. Microfluidic devices with modified flat surfaces are designed to enhance the contact of EVs with a functional surface to improve the capture efficiency. For instance, the “ExoChip” is fabricated of polydimethylsiloxane and functionalized with antibodies against CD63, composed of a string of alternating circular chambers and straight narrow channels to increase retention time [59]. Ashcroft and colleagues described a detachable microfluidic device, capturing EVs on CD41 antibody-coated mica surface to improve the detection sensitivity [60]. Microfluidic devices with modified magnetic beads can isolate EVs by utilizing the interaction of EVs with beads, with subsequent separation of the beads and then application to the chip for capture and subsequent characterization analysis. Dudani and colleagues, for example, reported a microfluidic platform for inline EVs isolation and fluorescent detection using inertial manipulation of CD63 antibody-coated beads [61]. Currently, on-chip mixing, incubation, and bead trapping devices are being developed and utilized to reduce the pre-processing of samples. ExoSearch, for example, provides a continuous flow that mixes samples with magnetic beads in a serpentine channel to yield EVs [62]. Microfluidic devices with modified microstructures and nanomaterials can also improve the capture efficiency of EVs by promoting the contact frequency of EVs to the substrate. Micropillars and herringbone grooves are commonly used microstructures. For instance, Reátegui and colleagues reported a microfluidic device, the EVHB-Chip, manufactured from cyclic olefin copolymer using a micro-injecting molding process to increase the EVs yield while reducing material autofluorescence and maximizing biocompatibility [63].

2.3.2. Filtration-based microfluidic techniques

Compared to conventional ultrafiltration, filtration-based microfluidic techniques integrate various filters or filter-like materials in microfluidic chips to increase efficiency of EV isolation based on different pore size. For example, Woo and colleagues developed a lab-on-a-disc integrated with two nanofilters (Exodisc). Components of the sample < 600 nm in diameter, including EVs, flow through the first filter, and the components with a diameter < 20 nm flow through the second filter. EVs (20-600 nm) are enriched between the two filters, which provides > 100-fold higher concentration of mRNA as compared with the gold-standard UC method [64].

2.3.3. Viscoelasticity-based microfluidic techniques

Viscoelastic microfluidics separate EVs by elastic lift forces based on different sizes of EVs. Liu and colleagues developed a viscoelasticity-based microfluidic system to directly separate EVs from sample in a continuous, size-dependent and label-free manner, and achieved a high separation purity (> 90%) and recovery (> 80%) of EVs. The viscoelastic sheath fluid in this system was a diluted poly-(oxyethylene) (PEO) solution [65].

2.3.4. Acoustics-based microfluidic techniques

Acoustics-based microfluidic techniques use an acoustic wave to isolate EVs based on their size, density and compressibility. Lee and colleagues presented an acoustic nanofilter system that separates EVs in a continuous and contact-free manner by using ultrasound standing waves to exert differential acoustic force on EVs according to their size and density. This achieved a separation of EVs with an efficiency of > 90% [66]. Sehgal and colleagues developed a surface acoustic wave (SAW)-based device that integrated a Fabry-Perot type acoustic resonator into a microfluidic channel to separate EVs, which generated high-frequency acoustic waves compared to bulk acoustic wave (BAW)-based actuation, suppressed Rayleigh streaming effects on the submicrometer particles, and separated 300 and 100 nm EVs with an efficiency of 86.3% [67].

2.3.5. Field-flow fractionation-based microfluidic techniques

Field-flow fractionation is an elution-based technique with migration flow moving along the channel axis while sample retention is controlled by the rate of a secondary flow. In such a device, the driving force and the diffusion of EVs are balanced. Kang and colleagues used this method to isolate EVs from human neural stem cells and used proteomic analysis to demonstrate that this method coupled with mass spectrometry was applicable [68]. Recently, Zhang and colleagues employed a field-flow fractionation-based microfluidic technique to fractionate EVs and identified two EV subpopulations (Exo-L, 90–120 nm; Exo-S, 60–80 nm) and discovered an abundant population of non-membranous nanoparticles termed “exomeres” (~35 nm) [69].

3. Characterization of isolated EVs

The wide spectrum of EV isolation protocols results in a variation of yield, purity and remaining contamination [57,70]. The International Society for Extracellular Vesicles (ISEV) recommends a set of experiments to help researchers distinguish EVs from co-isolated non-EV

components. The purpose of the "minimal information for studies of EVs (MISEV)" is to promote the reproducibility of EV studies, setting a best-practice standard to address the characterization of EVs and cargoes [71–73]. In order to ensure the "EVs" products are actual EVs, the MISEV 2014 recommends using a combination of microscopy technique (transmission electron microscopy, TEM, etc.), particle size distribution measurement (nanoparticle tracing analysis (NTA), etc.) and western blot (WB) that include at least two positive markers of different types and a negative marker to characterize isolated EVs. In the most recent version of MISEV, MISEV 2018, the authors reported that in the past five years only about half of the EV-related articles had included EV positive markers, and only a small minority complemented positive with negative markers to track co-isolated non-EV components. In order to build bridges to reproducibility, they updated tables and outlines of suggested protocols and steps to document specific EV-associated functional activities which are essential for any type of EV studies in the up-coming future.

The characterization of multiple protein markers as quality control can be performed using semi-quantitative protocols for WB, enzyme-linked immunosorbent assay (ELISA) or fluorescence high-resolution flow cytometry (HR-FCM). The protein markers are enriched in EV-lysates but cannot be described as EV-specific. Positive markers should support the presence of membrane in the sample (transmembrane or lipid-bound extracellular proteins) as well as the sample's capacity for carrying a cargo (cytosolic proteins). Examples of transmembrane proteins associated with EVs include, but are not limited to, tetraspanins (CD9, CD63, CD81), integrins (ITG) and growth factor receptors. The cytosolic proteins commonly used are membrane-binding proteins, such as TSG101, annexins, Rab GTPases, as well as signal transduction or scaffolding proteins, such as ALIX and syntenin. Furthermore, intracellular proteins should be under-represented or absent in most EVs. These negative markers are associated with cell organelles, for example calnexin and HSP90B1 found in endoplasmatic reticulum (ER), GM130 (Golgi) or CYC1 (mitochondria) [71]. Urinary EVs can be trapped by the Tamm-Horsfall protein (THP)-complex and precipitated at low centrifuge speed, leading to a lower EV-yield and a less representative EV-population [74]. RNA analysis of urinary EVs is complicated by interference of miRNA-contamination by co-isolation of THP, just as the protein itself can corrupt mass-spectrometry data [75,76]. Additional negative markers for non-EV components are ApoB100 (LDL), ApoA1 (HDL), ApoB48 (chylomicrons), and Argonaute2 (Ago2) [37,77,78].

In order to visualize EV morphology, the isolated EVs can be imaged using microscopic methods, such as TEM, scanning electron microscopy (SEM), atomic force microscopy (AFM) or cryo-electron microscopy (Cryo-EM). TEM is a widely-accepted method which requires dehydration or fixation of the EVs [49]. The dehydration likely causes the cup-shape of the EVs on TEM images. A limitation is the sample processing time. SEM uses electrons to scan the vesicles surface, which enables 3D-imaging of the EVs. As with TEM, the EVs are cup-shaped as samples are usually fixed and dehydrated [79]. AFM requires a less laborious sample preparation, however, the AFM scanning is complex and time-consuming. EVs are pictured as a round spherical shape [80] [60]. Cryo-EM requires no staining or dehydration, but instead uses vitrification to preserve the EVs frozen and presumably in closer to the physiological morphology. Cryo-EM images EVs as round and spherical [81]. All these microscopic techniques can distinguish EVs from co-isolated non-EV-particles based on particle morphology. Additionally, EVs can be labeled with immunogold particles bound to EV-associated membrane markers, such as CD-9, CD-63, CD-81, before visualizing these subpopulations with TEM or cryo-EM [82].

Since these microscopy techniques cannot provide a representative particle concentration or size distribution, complementary analysis by a single-particle tracking method is required [71]. Most of the methods analyze the light scattering of moving particles. NTA is a wide-accepted technique that uses a monochromatic laser beam and captures the moving particles in three 60-second videos. NTA works best for particles in range of 50–1000 nm. It can analyze the total concentration of the PBS-eluted EVs, as well as concentration of different sized-particles [83,84]. Another method using scattered laser is dynamic light scattering (DLS). Particles up to 6 μm can be detected [79]. However, both NTA and DLS share a disadvantage in samples with mixed particle size. Particles > 200 nm can disturb analyses of smaller particles. Another used method is tunable resistive pulse sensing (TRPS), which detects changes in the ionic current, while a vesicle passes a membrane through a size-tunable pore [85]. Normal flow cytometry (FCM) can be used for particles > 300 nm, or after binding smaller particles to immune- or latex beads [86]. However, there is a risk of 'swarming', a term for counting multiple particles as one event, leading to underestimation of the yield [87]. The improved version, HR-FCM, can analyze smaller particles, sized around 100 nm [88].

To aid researchers in the EV-field, several tools have been developed. Exocarta is a free available database of EV proteins, RNA and lipids [89,90]. EVpedia is a similar database, but also includes data on prokaryotic and eukaryotic EVs, and includes EV network analysis [91]. Vesiclepedia, is a database that provides data on proteins, RNA, and lipids derived from multiple vesicles besides exosomes, such as apoptotic bodies, large dense-core vesicles and MVs [92]. EV-Track is a platform for researchers, to gain feedback on used methods protocols and ways of reporting them, mainly based on the MISEV 2014 [93].

4. EVs studies in prostate cancer

PCa is the most common cancer and the second leading cause of cancer death in American men. In 2018, approximately 160,000 new diagnoses of PCa and over 29,000 deaths are estimated to occur in the United States, and almost 1,276,100 new cases and 358,980 deaths of PCa are estimated to occur worldwide [1,94]. Though the reported incidence and mortality rate of PCa in Asian countries including China is much lower than in Western countries, the PCa incidence rate has been increasing rapidly in China with an annual 12.6% change since 2000 [95].

Although the overall survival (OS) of PCa patients has improved, several challenges remain in PCa biology, diagnostics and therapy. For example, evidence strongly suggests that many detected PCa, especially low-grade tumors, will remain indolent for the patient's lifetime [96,97]. To distinguish patients with indolent diseases from those with aggressive ones and avoid unnecessary treatment is of great importance. In addition, reliable non-invasive biomarkers are needed to help monitor indolent disease, instead of repetitive invasive biopsies [98]. On the other hand, it is well accepted that metastatic PCa remains incurable [99,100]. The biology underlying metastasis remains undercharacterized. Androgen deprivation therapy (ADT) is given as the most effective initial treatment to metastatic patients. However, after the initial response, almost all metastatic diseases will eventually progress despite the low levels of testosterone in the systemic circulation [101]. This disease stage is termed castration resistant PCa (CRPC). Several new agents against CRPC such as abiraterone or enzalutamide are effective, but unfortunately, approximately 15–25% of patients with CRPC do not respond to first-line treatment with these new agents [102–104]. Biomarkers are needed to predict response, duration of response, as well as the biology underlying metastasis as well of issues of sensitivity and resistance. EVs, as circulating vesicles with abundant RNA, protein and other molecular cargos, have the potential to serve as biomarker candidates and to inform biology.

4.1. EVs as biomarkers

Serum prostate-specific antigen (PSA) is one of the most well-known cancer biomarkers and has been used as a screening and monitoring tool for many years. Although it has a contribution to the drop of PCa mortality in general, the poor specificity associated with PSA screening

has also contributed to overtreatment [105,106]. Several other FDA approved, non-EV-based assays provide prognostic information regarding risk of high-grade PCa at initial biopsy, including: the prostate health index blood test (PHI), which combines total PSA, free PSA, and pro-PSA; and the 4-kallikrein (4K) blood test, which incorporates kallikrein-related peptidase 2 (hK2), intact PSA, free PSA, and total PSA [107,108]. In addition, two urine tests that require a DRE prior to collection include the PCA3 assay, which detects PCA3 transcript levels, and a test that combines total serum PSA with the PCA3 assay and the expression of the TMPRSS2:ERG fusion gene [109,110]. The accuracies of these assays for prediction of high-grade PCa with a Gleason score (GS) ≥7 are still suboptimal (AUCs range from 0.68 to 0.71). EV-incorporated cargos, including proteins, nucleic acids, lipids and metabolite compositions, have been widely applied in the discovery of novel PCa biomarkers. Although no currently available EV-derived biomarkers have sufficient clinical utility to guide decision-making in PCa, many studies have demonstrated that novel EV-derived biomarkers are able to discriminate healthy from pathologic origin, distinguish different disease states, or predict prognosis or treatment efficiency. This all implies a great potential of EVs to become the next generation liquid biopsy target for PCa [111–113].

4.1.1. RNA based biomarkers

mRNA-, miRNA- and long non-coding RNA- (lncRNA) based biomarkers have been summarized in Table 2. Almost half of these studies used UC as their EVs isolation method, and the rest generally used precipitation or ultrafiltration to isolate EVs.

Antonarakis and colleagues first reported the analysis of androgen receptor splice variants 7 (AR-V7) with a circulating tumor cell (CTC) assay to predict the response of abiraterone or enzalutamide [102]. Del Re and colleagues developed a new approach to assess AR-V7 by highly sensitive digital droplet polymerase chain reaction (ddPCR) in plasma-derived EV RNA [114]. Median progression-free survival (PFS) was significantly longer in patients with AR-V7-negative EVs compared to those with AR-V7-positive EVs (20 vs. 3 mo, $p < .001$), and OS was significantly shorter in EV AR-V7-positive participants at baseline compared with negative ones (8 mo vs. not reached, $p < .001$). Royo and colleagues found urinary EV CDH3 transcript level was significantly lower in PCa patients than in benign prostate hyperplasia (BPH) patients [115]. CDH3 mRNA expression was also significantly decreased in PCa tissue, which implied urinary EVs could represent a non-invasive tool to inform about the molecular alterations in PCa. Anterior gradient 2 (AGR2) is a gene predominantly expressed in mucus-secreting tissues or in endocrine cells and its expression is significantly higher in PCa tissues [116,117]. Neeb and colleagues assessed the expression level of AGR2 wild-type and five other AGR2 splice variants (SV) in exosomes found in urine and demonstrated that urinary EV AGR2-SV-G and SV-H had a better performance in predicting PCa than serum PSA (AUC: 0.94 and 0.96 vs. 0.72) [118].

Research on EV non-coding RNA markers are mainly focusing on miRNAs and lncRNAs, especially miRNAs, because they are the largest RNA population in most of the EVs [119]. miRNAs are short non-coding RNA molecules with an average length of 22 nucleotides. They are transcribed as RNA hairpins and processed into mature miRNAs that bind to complementary mRNA to alter gene expression [120,121]. Among all the PCa EV miRNA markers, miR-141, miR-375 and miR-21 are the most studied and demonstrate consistent trends across multiple studies. They are significantly higher expressed in PCa and involved in fundamental events of carcinogenesis, such as cell proliferation, EMT, apoptosis and/or AR regulation. Before recognition that these miRNAs are EV cargo, they had been considered circulating biomarkers for PCa that correlated with GS and lymph-node status [122,123]. In plasma or serum EVs, Bryant and colleagues demonstrated that miR-375 and miR-141 were significantly increased in both serum exosomes and MVs from patients with metastatic PCa compared with non-recurrent PCa patients [124]. Endzeliņš and colleagues could distinguish PCa from BPH patients using whole plasma miR-375. If plasma EVs was used to study the same patient groups, the miR-21-level had the best diagnostic performance [125]. Huang and colleagues found higher levels of plasma exosomal miR-375 were significantly associated with poor OS in CRPC and validated this result in a cohort of 100 CRPC patients [126]. In urinary EVs, Foj and colleagues demonstrated miR-21 and miR-375 were significantly upregulated in PCa, but no differences were found for miR-141 in urinary exosomes [127]. Samsonov and colleagues found significant upregulation of miR-141 and miR-21 were associated with PCa [128]. Koppers-Lalic and colleagues showed miRNA isoforms with 3′ end modifications of miR-21, miR-375 and miR-204 provided a better diagnostic tool than that of the mature miRNAs in urinary EVs (sensitivity: 72.9% vs. 70.8%; specificity: 88% vs. 72%; AUC: 0.866 vs. 0.766) [129]. Among other EV miRNAs, miR-1290 in serum EVs was significantly higher in patients with PCa than those with BPH, and an elevated level of miR-1290 was found associated with poor OS in CRPC patients [126,130]. Plasma EV-incorporated let-7a levels, in contrast to whole plasma let-7a levels, were found useful to distinguish PCa patients with GS ≥ 8 from those with GS ≤ 6. In urine, the let-7c level significantly differed in PCa patients and healthy controls [125,127]. Serum exosomal miR-654 and miR-379 enabled prediction of response to radiotherapy, and decreased miR-34a levels predicted docetaxel (DOC) resistance [131,132]. Other EV miRNAs/lncRNAs with potential diagnostic or prognostic values include miR-19b, miR-130b, miR-145, miR-196a, miR-200c, miR-501, miR-521, miR-572, miR-574, miR-885, miR-1246, miR-2909, lincRNA-p21, SAP30L-AS1, SChLAP1, etc. (Table 2).

PCA3 and TMPRSS2:ERG have demonstrated clinical utility as biomarkers. PCA3, a lncRNA, has been proposed as urinary biomarker for PCa since 2003 [133]. In 2009, Nilsson and colleagues reported that after DRE, PCA3 transcripts were detected in urinary EVs from all PCa patients, while the mRNA transcripts for the fusion gene TMPRSS2:ERG were only detected in half of the patients with high GS and not in low risk patients [134]. Several studies have compared the diagnostic utility from whole urine, urinary EVs or urinary pellets, before or after a DRE. These studies have confirmed that DRE increases the total RNA volume in different type of samples [33,135,136]. In 2015, Donovan and colleagues established the EXO106 score (the sum of normalized PCA3 and ERG RNA levels) that demonstrated good clinical performance in predicting biopsy results for both any cancer and high-grade disease [137]. Based on this finding, the same group later established the FDA approved ExoDx Prostate Intelliscore (ExoDx) to predict high-grade PCa at initial biopsy [138,139]. In an independent validation cohort of 519 patients, ExoDx demonstrated a sensitivity of 91.89% and a specificity of 33.96%, while 27% of biopsies would have been avoided, missing only 5% of patients with dominant pattern 4 high-risk GS 7 disease. Recently, a prospective, multicenter diagnostic validation study for combined TMPRSS2:ERG and PCA3 RNA urinary test demonstrated a sensitivity of 93% and a specificity of 33% in detecting aggressive PCa, and 42% of unnecessary biopsies could have been averted [140]. This assay is based on whole urine RNA after DRE, while ExoDx is a test based on urinary EV RNA without prior DRE. Due to the similar diagnostic performance, a head-to-head comparison on diagnostic value, cost effectiveness and patient satisfaction may be necessary in the near future.

4.1.2. Protein based biomarkers

Unlike RNA biomarker studies, almost all of the protein biomarker studies utilized UC as the EV isolation method, likely reflecting that mass spectrometry requires high sample purity and as little protein contamination as possible.

PSMA is a transmembrane glycoprotein overexpressed in prostate cancers. The degree of PSMA expression in tumor tissue positively correlates with tumor stage [154]. Many studies have looked at PSA and/or PSMA expression level in EVs and tried to identify their diagnostic and/or prognostic values. Both PSA and PSMA proteins were

Table 2
Extracellular vesicle RNA biomarkers for prostate cancer.

Marker type	Marker name	Sample type	EV isolation method	Marker type	References
mRNA	TMPRSS2, ERG, TMPRSS2:ERG	Post-DRE urine, pre-DRE urine	UC, filtration, Exosome Diagnostics Urine Clinical Sample Concentration Kit	Diagnostic and prognostic	[33,134,136–138,141]
	AR-V7	Plasma, urine	exoRNeasy kit (Qiagen), Exodisc	Prognostic	[114,142]
	AGR2 SV-G and SV-H	Urine	UC	Diagnostic	[118]
	BIRC5	Pre-DRE urine	Filtration	Diagnostic	[141]
	CDH3	Urine	UC	Diagnostic	[115]
miRNA	miR-21-5p	Plasma, post-DRE urine, pre-DRE urine, cell medium	UC, SEC, ExoQuick, lectin-induced precipitation	Diagnostic and prognostic	[125,127–129,143]
	miR-141	Plasma, serum, pre-DRE urine, cell medium	UC, filtration, ExoQuick, lectin-induced precipitation	Diagnostic and prognostic	[124,128,130,144]
	miR-375	Plasma, serum, post-DRE urine	UC, filtration, ExoQuick	Diagnostic and prognostic	[124,126,127,129]
	miR-200a-5p, miR-200b, miR-200c-3p	Plasma, serum, urine	SEC, filtration, nanowire	Diagnostic and prognostic	[124,125,145]
	miR-1290	Plasma, serum, cell medium	ExoQuick	Diagnostic and prognostic	[126,130,143]
	let-7a, let-7c	Plasma, post-DRE urine	UC, SEC	Diagnostic and prognostic	[125,127]
	miR-17, miR-20a	Plasma, serum, cell medium	Filtration, ExoQuick	Prognostic	[124,143]
	miR-19a, miR-19b	Urine, cell medium	UC, filtration	Diagnostic	[146,147]
	miR-23a, miR-23c	Plasma, serum, cell medium	UC, filtration	Prognostic	[124,144]
	miR-34a, miR-34b	Cell medium	UC	Prognostic	[131,144]
	miR-130b	Plasma, serum	UC, filtration	Diagnostic and prognostic	[124,148]
	miR-135a, miR-135b, miR-519c-5p	Urine, cell medium	UC, nanowire	Diagnostic	[145,147]
	miR-488-3p	Cell medium	UC	Diagnostic and prognostic	[144,147]
	miR-572	Plasma, serum	Filtration, ExoQuick	Diagnostic and prognostic	[124,130]
	miR-7-5p, miR-93-5p, etc	Cell medium	ExoQuick	Prognostic	[143]
	miR-18a, miR-449a, etc.	Cell medium	UC	Diagnostic	[147]
	miR-28-5p, miR-105-5p, etc	Urine	Nanowire	Diagnostic	[145]
	miR-107, miR-198, etc	Plasma, serum	Filtration	Diagnostic and prognostic	[124]
	miR-145	Serum, pre-DRE urine	ExoQuick, UC + hydrostatic filtration dialysis	Diagnostic and prognostic	[130]
	miR-196a-5p, miR-501-3p	Pre-DRE urine	UC	Diagnostic	[149]
	miR-204	Post-DRE urine	UC	Diagnostic	[129]
	miR-521, miR-885	Serum	Exiqon miRCURY™ exosome isolation kit	Diagnostic	[20]
	miR-574-3p	Plasma, serum, pre-DRE urine	UC + lectin-induced precipitation, filtration	Diagnostic	[128]
	miR-888 cluster	EPS	Precipitation (Ymirite)	Diagnostic and prognostic	[150]
	miR-1246	Serum	Total Exosome Isolation Reagent (for serum)	Prognostic	[151]
	miR-2909	Urine	Exiqon miRCURY™ exosome isolation kit	Prognostic	[152]
	miR-3176, miR-5004-5p, etc	Cell medium	UC	Prognostic	[144]
lncRNA	PCA3	Post-DRE urine, pre-DRE urine	UC, filtration, Exosome Diagnostics Urine Clinical Sample Concentration Kit	Diagnostic and prognostic	[33,134–138,141]
	lncRNA-p21	Post-DRE urine	Urine Exosome RNA Isolation Kit (Norgen Biotek)	Diagnostic	[153]
	SAP30L-AS1, SChLAP1	Plasma	Total Exosome Isolation Reagent (for plasma)	Diagnostic and prognostic	[55]

present in 20/24 urinary EV samples from PCa patients and not detected in any healthy controls [155]. Similarly, plasma PSMA-positive EV concentration was statistically different among patients with BPH, and low-risk, intermediate-risk, and high-risk PCa (21.9, 43.4, 49.2, 59.9 ng/mL, respectively, $p < .001$). Additionally, plasma PSMA-positive EV concentration could be used to differentiate PCa from BPH (AUC = 0.943, at the cutoff value of 28.2 ng/mL, the optimal sensitivity and specificity were 91.7% and 83.3%, respectively) [156]. Biggs and colleagues demonstrated that PSMA positive plasma EV number could distinguish PCa patients with GS ≥ 8 from patients with GS ≤ 7 [157]. A similar result was observed in Logozzi and colleagues study using nanoscale flow cytometry to sort CD81 + /PSA + EVs from plasma and the result demonstrated that only PCa patients had significantly high level of EVs expressing both CD81 and PSA [158].

Taxanes, a class of microtubule-targeting anticancer agents such as DOC, is currently used as the first-line chemotherapy for CRPC. However, there is a finite amount of time before resistance is acquired [159,160]. In order to predict or monitor the development of drug resistance, several studies have been investigating EV protein cargos. P-glycoprotein (P-gp) encoded by multi drug resistance protein 1 (MDR1) gene is a member of the super family of adenosine triphosphate–binding cassette transporters, which acts as a drug efflux pump and contributes to the development of resistance against chemotherapy [161,162]. Kato and colleagues found the level of P-gp was higher in exosomes as well as cell lysates from DOC-resistant PC3 cells than in those from untreated PC-3 cells [163]. The P-gp level in serum exosomes was relatively higher in clinically DOC-resistant patients than in treatment naïve patients. Kharaziha and colleagues conducted a comparative proteomics analysis for exosomes secreted from DU145 cell lines that were respectively sensitive and resistant to DOC and identified a list of DOC-resistance related proteins [164]. Eventually, they found that MDR-1/3 and PABP4 could be detected in the EVs isolated from DOC resistant CRPC patients' serum. Proteomic analysis for exosomes from DOC-resistant PC3 and normal PC3 demonstrated that integrin β4 and vinculin were upregulated in exosomes derived from chemo-resistant cell lines [165].

Table 3 lists other EV proteins markers with potential diagnostic or prognostic values.

4.1.3. Lipids and metabolites biomarkers

Several studies have focused on lipid composition and/or small molecule metabolites of EVs in PCa (Table 4). Lipid metabolism is often disturbed in cancer cells, and this is expected to be reflected in a different lipid composition in normal versus cancer cells [191,192]. Llorente and colleagues compared the lipidome of urinary exosomes between 15 PCa patients and 13 healthy controls and found the levels of 9 lipids species were significantly different [193]. The highest significance was shown for phosphatidylserine (PS) 18:1/18:1 and lactosylceramide (LacCer) d18:1/16:0, and the combinations based on 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 were able to distinguish PCa and healthy controls with 93% sensitivity and 100% specificity. Yang and colleagues also compared the lipidome of urinary exosomes between PCa patients and healthy controls and found an increase in phosphatidylglycerol (PG) 22:6/22:6, a significant decrease in (16:0,16:0)- and (16:1, 18:1)- diacylglycerol (DAG) species, and an increase in triacylglycerol (TAG) in patients with PCa [194]. In urinary EVs, Puhka and colleagues demonstrated the levels of glucuronate, D-ribose 5-phosphate and isobutyryl-L-carnitine in all pre-prostatectomy patients' samples were significantly lower compared to healthy controls and post-prostatectomy samples after normalization [195]. Clos-Garcia and colleagues identified 76 metabolites from urinary EVs that exhibited significant differential abundance between PCa and BPH, including phosphathidylcholines, acyl carnitines, citrate and kynurenine [196].

4.2. EVs biological roles in prostate cancer

Since EVs have been found to be produced by all studied cell types, and their cargo offer a safe way to transfer important biological information between cells, EVs provide a window to better understand the biology of PCa [198]. EVs have been found to be involved and play important roles in key biological behaviors of PCa, including communication between cancer and stromal cells, cancer and immune cells and horizontal communication between cancer cells. Such a network mediated by EVs changes the understanding of PCa progression, metastasis, and therapeutic resistance (Fig. 3).

4.2.1. Communication between cancer and non-immune stromal cells

The PCa stroma is a dynamic complex of resident mesenchymal cells, extracellular matrix, vasculature, lymphatics, nerves, and a spectrum of immune cell types. These components together with the cancer cells, constitute the tumor microenvironment. Bidirectional interactions between cancer cells and surrounding stromal components contribute to PCa progression, metastasis and resistance to anticancer therapeutics [199,200].

Cancer-associated fibroblasts (CAFs) are a major constituent of the tumor microenvironment in many cancers. Accumulating evidence suggests that paracrine signals from cancer cells can both recruit and activate CAFs, which can also target cancer cells by secreting several growth factors [201,202]. PCa cells derived exosomes trigger TGF-β1-dependent fibroblast differentiation to a distinctive myofibroblast phenotype which was able to support angiogenesis in vitro and accelerate tumor growth *in vivo* [203]. In an orthotopic rat model, cancer derived EVs activated rat prostatic fibroblasts through the upregulation of genes such as HGF, CXCL5 and IL-10 [204]. The EVs from more aggressive tumors were more efficient at inducing this activated myofibroblast phenotype which could support tumor growth. Minciacchi and colleagues demonstrated the PCa cell derived large oncosomes could be uptaken by normal prostate fibroblasts and the internalization of these large oncosomes induced reprogramming of these fibroblasts as reflected by high levels of α-SMA, IL-6, and MMP-9 [41].

Though the majority of studies have focused on EVs produced by cancer cells and their effects on tumor microenvironment, several studies also demonstrated the role of CAF-derived EVs on cancer cells. Santi and colleagues found that CAFs had an enhanced production and delivering of EVs to cancer cells and were able to actively transfer proteins, glycolytic enzymes, and lipids to PCa cells, increasing their proliferation rate [205]. Similarly, Zhao and colleagues demonstrated that exosomes secreted by PCa patient-derived CAFs could reprogram the metabolic machinery of PCa cancer cells by inhibition of mitochondrial oxidative phosphorylation and increasing glycolysis and glutamine-dependent reductive carboxylation in target cancer cells [206]. Cancer cells under nutrient-deprived or nutrient-stressed conditions are able to utilize CAF-derived exosomes metabolite cargo (including amino acids, lipids, and tricarboxylic acid-cycle intermediates) for central carbon metabolism and promoting tumor growth. CAF-derived miR-409-3p and miR-409-5p conferred a cancer-associated stroma-like phenotype and promoted tumor induction and EMT in vitro and *in vivo* [207].

Bone is the most common site of lethal PCa metastases. Approximately 90% of patients with metastatic disease will develop bone metastases that are predominantly of the osteoblastic bone forming type [208,209]. Karlsson and colleagues demonstrated that EVs isolated from the murine PCa cell line TRAMP-C1 dramatically decreased fusion and differentiation of monocytic osteoclast precursors to mature, multinucleated osteoclasts [210]. Significantly higher expression of miR-141-3p was found in EVs from metastatic PCa patients' blood. When released by PCa cells and transferred to osteoblasts, miR-141-3p promotes osteoblast activity and induces the formation of osteoblastic bone metastases in vitro and *in vivo* [211]. Following uptake, miR-141-3p reduced the protein levels of its target gene DLC1 and

Table 3
Extracellular vesicle protein biomarkers for prostate cancer.

Marker name	Sample type	EV isolation method	Marker type	References
PSMA	Plasma, pre-DRE urine, cell medium, EPS urine	UC, PEG/DEX aqueous two-phase system, nanoscale flow cytometry	Diagnostic and prognostic	[155–157,166–169]
PSA	Plasma, post-DRE urine, pre-DRE urine, EPS urine	UC, UC + butanol	Diagnostic and prognostic	[155,158,166,167,170,171]
ANXA1, 2, 3, 5	Tissue, cell medium	UC	Diagnostic	[167,168,172]
Flotillin2	Pre-DRE urine, EPS-urine	UC	Diagnostic	[166,167,173]
PPAP	Pre-DRE urine, post-DRE urine, EPS-urine	UC	Diagnostic	[166,167,170]
ALIX	Cell medium, EPS-urine	UC	Diagnostic	[167,174]
Claudin3	Plasma	UC	Diagnostic and prognostic	[166,175]
FASN	Cell medium	UC	Diagnostic	[168,174]
Fibronectin, haptoglobin	Plasma	SEC, ExoQuick	Diagnostic and prognostic	[176,177]
GDF15	EPS-urine, cell medium	UC	Diagnostic	[167,168]
Integrin αVβ3	Plasma, serum, cell medium	UC, DGUC	Diagnostic and prognostic	[52,178]
LAMTOR1, PARK7, Rab3B, TMEM256	Pre-DRE urine	UC	Diagnostic	[166,173]
Survivin	Plasma, serum	UC, ExoQuick	Diagnostic and prognostic	[179,180]
TGM4	Post-DRE urine, EPS-urine	UC	Diagnostic	[167,170]
TRFL, ZA2G	Pre-DRE urine, EPS-urine	UC	Diagnostic	[166,167]
ADIRF, Rab2A, etc.	Pre-DRE urine	UC	Diagnostic	[166]
ADSV	Post-DRE urine	UC	Diagnostic	[170]
Afamin, CCL16, etc.	Urine	UC + SEC	Prognostic	[177]
AMBP, CHMP4A, CHMP4C, FABP5, Granulin,	Post-DRE urine	UC	Diagnostic and prognostic	[181]
ATXN7L1, ceruloplasmin precursor, etc.	Plasma	ExoQuick	Diagnostic	[176]
cathepsin-D, CCL15, etc.	Plasma	SEC	Prognostic	[177]
CD151, CDCP1	Cell medium	UC	Prognostic	[182]
cIAP-2, XIAP	Plasma, serum	ExoQuick	Prognostic	[179]
CLSTN1, FLNC	Cell medium	UC	Diagnostic	[168]
c-Src	TRAMP murine plasma	ExoQuick	Diagnostic	[183]
δ-catenin	Urine	UC	Diagnostic	[184]
EGFR	Plasma, serum	UC	Diagnostic	[185]
EphrinA2	Serum	UC	Diagnostic	[186]
GGT	Serum	SEC	Diagnostic	[165]
GLPK5, SPHM	Post-DRE urine	UC	Prognostic	[170]
Integrin subunits α3 and β1	Urine	UC	Diagnostic and prognostic	[187]
Integrin αVβ6	Cell medium	UC	Diagnostic	[188]
Integrin β4, vinculin	Cell medium	UC and CD9 magnetic beads selection	Prognostic	[189]
KLK2, LEG3, etc.	EPS-urine	UC	Diagnostic	[167]
MDR-1, MDR-3, PABP4	Serum	UC	Prognostic	[164]
PGP	Serum	UC	Prognostic	[163]
p-Met	Serum	UC	Diagnostic	[148]
PTEN	Plasma	UC	Diagnostic	[190]
XPO1	Cell medium	UC	Diagnostic	[174]

activated p38MAPK-signaling, which increased the expression of osteoblastic osteoprotegerin and further promoted bone formation. By targeting ARHGAP1 and FAM134A, miR-940 promotes the osteogenic differentiation of human mesenchymal stem cells in vitro [212].

+ + +Lyden and colleagues have hypothesized that the spread of cancer cells to secondary organs is facilitated by the release of EVs from the primary tumor microenvironment. These EVs induce the formation of a specialized environment at distal sites, the pre-metastatic niche, which is more recipient to metastasis [8,213] Besides fibroblasts and osteoblasts, PCa-derived EVs also have an ability to reprogram adipose derived stem cells (ASCs) [214]. The tumorigenic reprogramming of ASCs was associated with trafficking of oncogenic factors by PCa cell-derived EVs, including H-ras and K-ras transcripts, miR125b, miR130b and miR155, as well as the Ras superfamily of GTPases and Rab1a, Rab1b and Rab11a.

4.2.2. Communication between cancer and immune cells

T lymphocytes are primary mediators of the adaptive immune

Table 4
Other extracellular vesicle biomarkers for prostate cancer.

Marker type	Marker name	Sample type	EV isolation method	Marker type	Reference
Lipid	22:6/22:6-PG, (16:0,16:0)- and (16:1, 18:1)-DAG, TAG	Urine	UC	Diagnostic	[194]
	HexCer (d18:1/16:0), LacCer (d18:1/16:0), PC 16:0–18:2, PE O-18:0/18:1, PE P-16:0/20:4 (PE O-16:1/20:4), PE P-18:1/20:4, PS 16:0–18:1, PS 18:1/18:1, PS 18:0–18:1	Urine	UC	Diagnostic	[197]
Metabolites	D-ribose 5-phosphate, glucuronate, isobutyryl-L-carnitine	Urine	UC	Diagnostic	[195]
	acyl carnitines, citrate, dehydroepiandrosterone sulphate, kynurenine, phosphathidylcholines, etc.	Urine	UC	Diagnostic	[196]

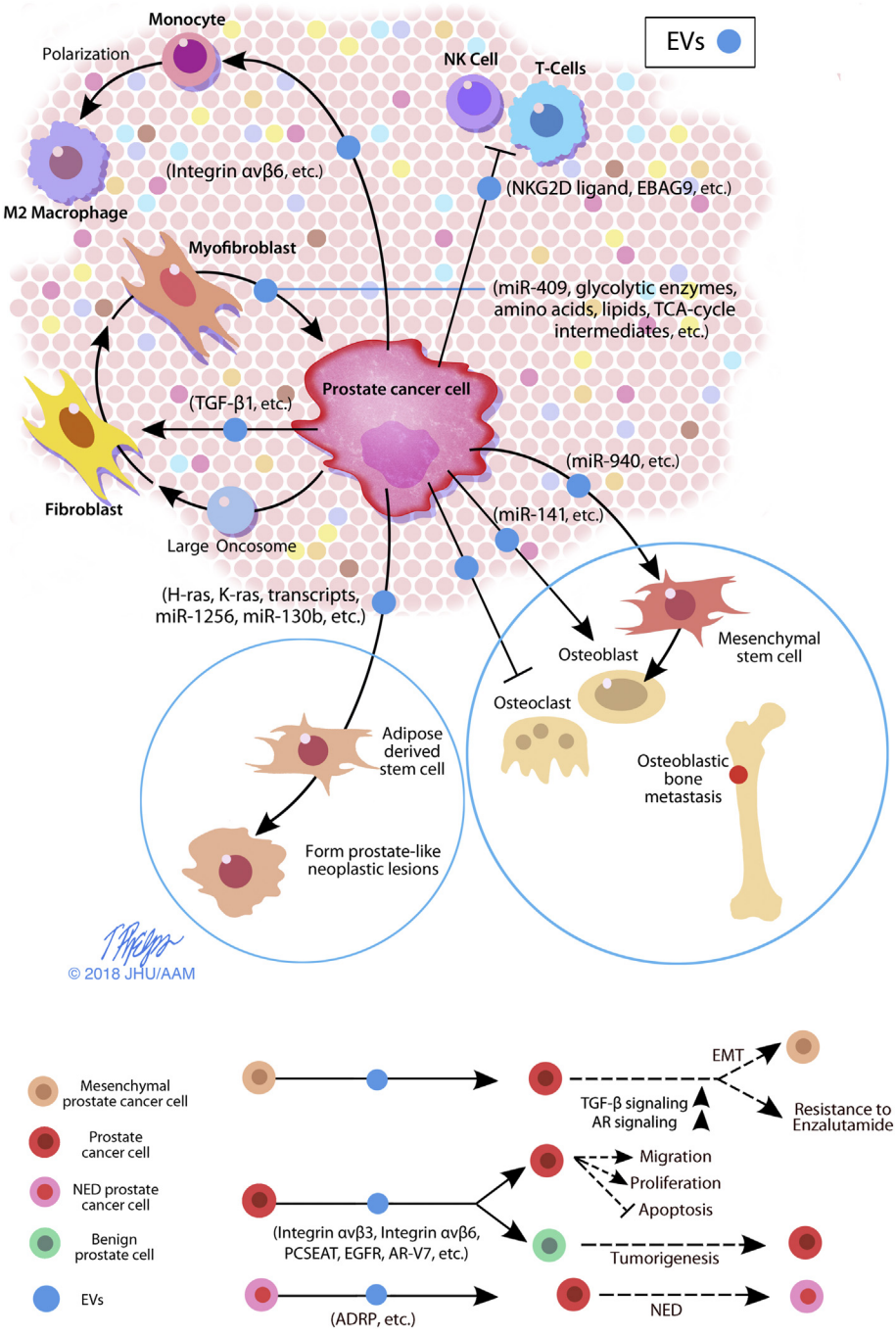

Fig. 3. The schematic diagram of cell-cell communication mediated by EVs in PCa.
The PCa stroma is a dynamic complex of resident mesenchymal cells, extracellular matrix, vasculature, lymphatics, nerves, and a spectrum of immune cell types. These components together with the cancer cells, constitute the tumor microenvironment. Within the microenvironment, PCa cells derived exosomes can trigger TGF-β1-dependent fibroblast differentiation to a distinctive myofibroblast phenotype. PCa cell derived large oncosomes can be uptaken by normal prostate fibroblasts and the internalization of these large oncosomes induced reprogramming of these fibroblasts as reflected by high levels of α-SMA, IL-6, and MMP-9. In return, CAFs have an enhanced production and delivering of EVs to PCa cells and are able to actively transfer proteins, glycolytic enzymes, and lipids to PCa cells, increasing their proliferation rate. PCa cells are able to inhibit the cytotoxic function of immune cells, like NK and CD8+ T cells, by releasing NKG2D ligand-expressing exosomes or transfer EBAG9 to T cells. TAMs characterized by a M2-polarized phenotype contribute to tumor aggressiveness. The integrin αvβ6 can be transferred from PCa cells to monocytes by exosomes to promote M2 polarization. Bone is the most common site of lethal PCa metastases. At metastatic site, EVs from PCa cells can dramatically decrease fusion and differentiation of monocytic osteoclast precursors to mature, multinucleated osteoclasts and induce the formation of osteoblastic bone metastases by transferring miR-141-3p and miR-940. PCa-derived EVs also have an ability to reprogram ASCs to form prostate-like neoplastic lesions by the trafficking of oncogenic factors including H-ras and K-ras transcripts, miR125b, miR130b and miR155, as well as the Ras superfamily of GTPases and Rab1a, Rab1b and Rab11a.
EVs also play an important role in the horizontal transfer of biological information among cancer cells. PCa cells are able to transfer a variety of molecular cargos to other PCa cells and benign prostate cells, including integrins, PCSEAT, EZH2, EGFR, EGFRvIII, AR and AR-V7. These horizontal transfers can reduce apoptosis, increase cell proliferation and induce cell migration, as well as promote tumorigenesis. Moreover, some special phenotypes of PCa cells, such as mesenchymal type PCa cells and PCa cells that undergo NED, are able to induce EMT and/or NED in their neighbor cancer cells by their EVs.

response and play an important role in the tumor surveillance. Cancer cells often obtain ability to evade the immune system, a process known as immune escape. Generally, the presence of intratumoral T cell infiltration was demonstrated to be low in PCa [215,216]. In a 3D heterotypic spheroid model composed of PC3-CD63-GFP cells and human peripheral blood mononuclear cells, PCa-derived EVs were found to interact with B cells, CD3+ T cells and CD8+ T cells [217]. Lundholm and colleagues found that the NKG2D ligand-expressing PCa-derived exosomes selectively induced downregulation of NKG2D, an activating cytotoxicity receptor, on NK and CD8+ T cells in a dose-dependent manner, leading to impaired cytotoxic function *in vitro* [218,219]. Incubation of healthy donors' lymphocytes with exosomes isolated from serum or plasma of CRPC patients triggered downregulation of NKG2D in the effector lymphocytes subgroup. Theoretically, EVs could also carry tumor antigens and thus contribute to immune activation. In order to examine the impact of PCa-derived EVs on immune response, Salimu and colleagues inhibited the EVs secretion of DU145 cells. Significantly stronger tumor-antigen-specific T cell responses were observed [220]. The enhanced T cell response could be prevented by adding purified exogenous DU145-EVs to the system, demonstrating the dominant effect of tumor-derived EVs is immunosuppression, instead of antigen delivery. Estrogen receptor-binding fragment-associated antigen 9 (EBAG9) in cancer cells can suppress T cell infiltration into the tumor in vivo, while it functions as a limiter for T cell cytotoxicity in host immune cells [221]. EVs from EBAG9-overexpressing PCa cells were able to transfer EBAG9, potentially facilitating immune escape of tumors by inhibiting T cell cytotoxicity and modulating immune-related gene expression in T cells [222].

In most solid cancers, a major component of the tumor stroma are macrophages referred to as tumor-associated macrophages (TAMs)

characterized by a M2-polarized phenotype that contributes to tumor aggressiveness [223]. EVs from orthotopic rat Dunning R-3327 prostate tumors were able to increase macrophage endocytosis and the mRNA expression associated with M2 polarization in vitro [204]. The integrin αvβ6 can be transferred from cancer cells to monocytes by exosomes to promote M2 polarization, while downregulation of αvβ6 in exosomes inhibits M2 polarization in recipient monocytes [224].

4.2.3. Communication between cancer cells

EVs are not only involved in communication between PCa cells and other non-prostate cells, but also appear to play an important role in horizontal transfer of biological information among cancer cells. For example, the gene encoding the cytoskeletal regulator DIAPH3 is frequently lost in metastatic PCa. EVs isolated from DIAPH3-silenced PCa cells can be uptaken by other PCa cells, activating AKT1 and androgen-signaling which results in increased proliferation of the recipient cells [225]. Similarly, EVs isolated from LNCaP and DU145 cells can significantly reduce apoptosis, increase cell proliferation and induce cell migration in other LNCaP and RWPE-1 cells *in vitro* and in vivo [226].

Languino and colleagues have demonstrated the ability of PCa cells to transfer integrins, such as αvβ6 and αvβ3, to other PCa cells and benign prostate cells [178,188]. It was demonstrated that de novo integrin expression in recipient cells was not a result of a change in mRNA levels, but instead is a consequence of EV-mediated transfer of this integrin between different cells. These transferred integrins were capable of promoting adhesion and migration of recipient cells. Other molecular cargos, such as PCSEAT, EZH2, EGFR, EGFRvIII, AR and AR-V7, can also be transferred from PCa cells to promote tumorigenesis [227,228]. Similarly, transfer of PCa derived tetraspanins CD9 and CD151 to non-cancerous prostate cells enhances the migratory and invasive capabilities of the non-tumorigenic prostate cellular population [229].

EVs released by modified mesenchymal-like PCa cells promote mesenchymal features in recipient epithelial-like PCa cells, which was accompanied by a modulation of AR signaling and activation of TGF-β signaling pathway. This resulted in enhanced migration and invasiveness, as well as increased resistance to the novel AR antagonist, enzalutamide [230]. Lin and colleagues demonstrated that IL-6 induced neuroendocrine differentiation (NED) was mediated by PPARγ leading to elevated adipocyte differentiation-related protein (ADRP) associated with adiposome accumulation [231]. ADRP protein can be detected in EVs released from these cells and these EVs are capable of inducing NED of other PCa cells in a paracrine fashion. EVs isolated from GS 8 PCa patients' tissues could significantly induce soft agar colony formation of non-malignant prostate cells, a hallmark of malignant cells. EVs derived from PCa patients' biopsy tissues and plasma were able to induce malignant features, including EMT, in non-tumor cells [232,233].

4.3. EVs as a therapeutic modality to treat prostate cancer

EV-based therapeutics tools can be broadly assigned to three categories: EV biogenesis or release inhibition, EV bio-engineering, and the application of EVs derived from one type of cells to effect another [234].

Inhibition of EVs release by calpeptin in PCa cells treated with DOC resulted in a 3-fold increase in intracellular concentrations of DOC, and 20-fold lower concentrations were needed to induce the same degree of apoptosis in PC3 cells [235]. Inhibition of EV biogenesis by chloramidine and bisindolylmaleimide-I combined with the chemotherapeutic agent caused a 62% and 59% decrease in numbers of viable PC3 cells compared to 5-FU alone [236]. Datta and colleagues optimized a quantitative high-throughput screening assay to identify compounds that modulate EV biogenesis and/or release by aggressive PCa [237]. They have found > 20 candidates out of > 4500 compounds and begun validation. For instance, they demonstrated that manumycin-A was able to inhibit the biogenesis and secretion of EVs by CRPC cells, but not by normal prostate cells. Also no effects were observed on cell growth.

MVA-BN-PRO (BN Immuno Therapeutics) is an EV bio-engineered candidate immunotherapeutic agent for the treatment of PCa that encodes 2 tumor-associated antigens, PSA and prostatic acid phosphatase (PAP). In 2011, Rountree reported fusing these antigens with the C1C2 domain of the lactadherin protein and displayed the on EV-membranes, which increased immune responses against the antigens [238]. Both exosomes and MVs delivered paclitaxel to recipient cells through endocytosis, leading to the release of the drug and increase of cytotoxic effect [239].

Human mesenchymal stem cells (MSCs) labeled with Venofer, an iron oxide carbohydrate nanoparticle, release EVs that contained iron oxide and were efficiently endocytosed by tumor cells, enabling ablation following induction of hyperthermia using an external alternating magnetic field [240]. Wang and colleagues co-cultured paclitaxel-resistant DU145 cell lines with purified EVs from a human non-cancerous prostate epithelial cell line, which resulted in an increased sensitivity to paclitaxel [241]. Alcayaga-Miranda and colleagues studied the angiogenic activity of menstrual stem cells secreted EVs on PC3 cells, and demonstrated the suppression of the secretion of pro-angiogenic factors by the PC3 cells in a ROS-dependent manner [242] Similarly, Peak and colleagues characterized placental-derived stem cell exosomes (PLSCExo) and found PLSCExo treatment strongly inhibited the viability of enzalutamide-sensitive and -resistant PCa cell lines, while no effect was found on the viability of a non-neoplastic human prostate cell line [243].

5. EVs studies in bladder cancer

BCa is the 4th most common cancer and the 8th leading cause of cancer death in American men. In 2018, approximately 81,100 new diagnoses of BCa and over 17,200 deaths are estimated to occur in the United States, and almost 549,400 new cases and 200,000 deaths of BCa are estimated to occur worldwide [1,94]. Approximately 75% of BCa are non-muscle invasive BCa (NMIBC) and the other 25% are muscle invasive BCa (MIBC), of which 50% are diagnosed with distant metastases [244].

EVs released into biofluid, especially urine, carry the signature of the BCa, and therefore, should be investigated as diagnostic biomarkers (Table 5). Several groups have exploited the protein content of BCa EVs. Welton and colleagues conducted the first proteomics analysis of EVs derived from BCa cells, detecting 353 exosomal proteins by mass spectrometry, and found seven proteins enriched in BCa cell EVs, including MUC1, integrin β1, integrin α6, CD36, CD44, CD10, 5T4 and CD73 [245]. Smalley and colleagues identified 9 differentially expressed proteins, of which 8 proteins were elevated in the urinary EVs from BCa patients, including 5 EGFR pathway associated proteins [246]. Another comparative and targeted proteomic analysis of urinary EVs revealed a strong association of TACSTD2 with BCa [247]. Jeppesen and colleagues analyzed proteins from the membrane and lumen of BCa derived EVs and identified several proteins potentially involved in the metastatic process, including vimentin and hepatoma-derived growth factor, casein kinase II α, and annexin A2 [248]. Other studies have identified exosomal transaldolase, periostin, α1-antitrypsin, H2B1K, LASS2, and GALNT1 as potential biomarker proteins for BCa [249–252].

Armstrong and colleagues analyzed miRNA of matched FFPE-tumor tissue, plasma, urine exosomes and WBCs, and found miR-451, miR-16, let-7a-5p, let-7b-5p were common to the matched samples [253]. Berrondo and colleagues reported lncRNA HOTAIR could facilitate the progression of BCa through altering expression of EMT genes and these may function as biomarkers for BCa [254]. Several other studies have identified multiple exosomal miRNAs that may both inform BCa biology and serve as potential predictive biomarkers, including miR-375, miR-146a, miR-21-5p, lncRNA-UCA. Furthermore, a panel including

Table 5
Extracellular vesicle biomarkers for bladder cancer.

Marker type	Marker name	Sample type	EV isolation method	Marker type	References
mRNA	GALNT1, LASS2	Urine	UC	Diagnostic	[249]
	GPRC5A, KRT17, SLC2A1	Urine	Exosome isolation tube (Hitachi Chemical Diagnostics)	Diagnostic and prognostic	[265]
miRNA	miR-21-5p	Urine	UC, Urine Exosome RNA Isolation Kit (Norgen Biotek)	Diagnostic	[253,257]
	miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-205-5p	Urine, cell medium	Exosome RNA Isolation Kit (Norgen Biotek), Total Exosome Isolation kit (for urine)	Diagnostic and prognostic	[253,266]
	miR-513b-5p	Urine, cell medium	Total Exosome Isolation kit (for urine), Nanowire	Diagnostic and prognostic	[145,266]
	miR-16-1-3p, miR-23b-3p, miR-28-5p, etc	Urine	Nanowire	Diagnostic	[145]
	miR-26a, miR-93, miR-191, miR-940	Urine	UC	Diagnostic	[256]
	miR-141-3p, miR-224-5p, miR-429-3p, etc.	Cell medium	Total Exosome Isolation kit (for urine)	Prognostic	[266]
	miR-375, miR-146a	Urine	UC	Prognostic	[255]
	miR-4454, miR-720/3007a, miR-29b-3p	Urine	Urine Exosome RNA Isolation Kit (Norgen Biotek)	Diagnostic	[253]
lncRNA	MALAT1	Urine	UC, Urine Exosome RNA Isolation Kit (Norgen Biotek)	Diagnostic and prognostic	[254,267]
	PCAT-1	Urine, serum	Urine Exosome RNA Isolation Kit (Norgen Biotek), ExoQuick	Diagnostic and prognostic	[267,268]
	HOTAIR, HOX-AS-2, HYMA1, LINC00477, LOC100506688, OCT4, OTX2-AS1, SOX2	Urine	UC	Diagnostic	[254]
	PTENP1	Plasma	ExoQuick	Diagnostic	[269]
	SNHG16, UBC1	Serum	ExoQuick	Diagnostic and prognostic	[268]
	SPRY4-IT1	Urine	Urine Exosome RNA Isolation Kit (Norgen Biotek)	Diagnostic	[267]
	UCA1	Serum	ExoQuick	Diagnostic	[258]
Protein	ES8L2	Urine	UC	Diagnostic and prognostic	[246,270]
	MUC1	Urine	UC	Diagnostic and prognostic	[245,270]
	5 T4, CD10, CD36, CD44, CD73, integrin subunits α6 and β1	Urine	UC	Diagnostic	[245]
	Alpha1-antitrypsin, histone H2B1K	Urine	UC	Diagnostic and prognostic	[250]
	Annexin A2, casein kinase II α, hepatoma-derived growth factor, vimentin	Cell medium	UC	Prognostic	[248]
	EPS8L1, GTPase Nras, MUC4, Resistin, Retinoic acid-induced protein 3, etc.	Urine	UC	Diagnostic	[246]
	EZRI, PDCD10, UROM, etc.	Urine	UC	Diagnostic and prognostic	[270]
	Periostin	Urine	UC	Diagnostic	[251]
	TACSTD2	Urine	UC	Diagnostic and prognostic	[247]
	Transaldolase	Urine	UC	Diagnostic	[252]

exosomal miR-26a, miR-93, miR-191, and miR-940 can discriminate between cancer and cancer-free patients with a sensitivity of 88% and specificity of 78% [255–258].

EVs derived from BCa cell lines have been shown to participate in several biological functions. Yang and colleagues demonstrated that exosomes from BCa cells could inhibit the tumor cell apoptosis through activation of AKT- and ERK pathways [259]. Urinary EVs from BCa patients promoted angiogenesis and migration of BCa cells and endothelial cells by activating EGFR signaling, which containing EDIL-3 [260]. Selective secretion of the tumor-suppressor miRNA, miR23b, into exosomes in a RAB27-dependent manner resulted in the acquisition of metastatic properties [261].

The feasibility for EV-mediated therapeutics in BCa therapy is less developed [262]. Jin and colleagues demonstrated that BCa cell-derived microparticles could markedly enhance the effects of intravesical chemotherapy by increasing the pH of lysosomes, leading to the migration of drug-loaded lysosomes along microtubule tracks towards the nucleus [263]. Greco and colleagues found that human embryonic kidney 293 (HEK293) cell- and MSC-derived exosomes could be used as a delivery vector to transport PLK-1 siRNA into BCa cells, which resulted in the silencing of PLK-1 [264].

6. EVs studies in renal cell carcinoma

RCC is the 6th most common cancer and the 10th leading cause of cancer death in American men and the 10th most common cancer in American women. In 2018, approximately 65,300 new diagnoses of RCC and over 14,900 deaths are estimated to occur in the United States, and almost 403,200 new cases and 175,000 deaths of RCC are estimated to occur worldwide [1,94] Clear cell RCC (ccRCC) is the most common histological subtype [271]. EVs have been shown to participate in RCC progression, and several publications have reported the predictive value of EVs for the diagnosis and prognosis of RCC (Table 6).

Exosomal lipidomics analysis based on a hyphenated microLC-Q-TOF-MS platform demonstrated a different lipid composition in RCC patients' and healthy donors' urinary exosomes [272]. A comparative analysis performed, using differential protein profiling, identified MMP-9, podocalyxin (PODXL), dickkopf-related protein 4 (DKK4), carbonic anhydrase IX (CAIX) and ceruloplasmin as the urinary EVs-derived, diagnostic biomarkers for RCC [273]. De Palma and colleagues found that urinary exosomal GSTA1, CEBPA and PCBD1 were non-invasive markers to diagnose ccRCC [274]. Zhang and colleagues demonstrated serum exosomal miR-210 and miR-1233 had potential for the diagnosis and post-treatment monitoring for ccRCC, with 70% sensitivity and 62.2% specificity for miR-210 and 81% sensitivity and 76% specificity for miR-1233 [275]. Urinary miR-126-3p, miR-449a, miR-34b-5p and miR-486-5p may serve as biomarkers to confirm the diagnosis of ccRCC [276]. Du and colleagues evaluated the prognostic value of plasma exosomal miRNAs in metastatic RCC by RNA sequencing and confirmed the independent predictive value of miR-let-7i-5p for OS (P = .006, HR = 0.566) [277]. Similarly, high serum exosomal miR-224 expression was a significant independent risk factor related to PFS, CSS and OS in multivariate analysis for ccRCC patients [278] However, to date, there are no consistent biomarkers across studies for RCC.

Increasing evidence demonstrates that EVs affect RCC tumorigenesis, including proliferation, invasion, migration, immune evasion, metastasis, and drug resistance. Exosomes from a metastatic RCC cell line can promote the proliferation and invasion of primary RCC cells. In vitro analyses revealed that 786-0 RCC cell line-derived exosomes could increase migration and invasion of 786-0 cells, while decreasing the adhesive ability by upregulating CXCR4 and MMP-9 expression [279]. Jingushi and colleagues extracted EVs directly from surgically resected ccRCC tissues, and found that azurocidin (AZU1) was highly enriched and could disrupt vascular endothelial cell morphology [280]. To help evade immune surveillance, exosomal HSP70 was able to trigger p-STAT3 of myeloid-derived suppressor cells in a TLR2-MyD88-dependent manner [281]. RCC cell-derived exosomal FAS ligand inhibited Jurkat T cell proliferation and trigger apoptosis [282]. A recent study performed by Xia and co-workers provided evidence that ccRCC-derived exosomal TGF-β1 induced natural killer cell dysfunction by regulating the TGF-β/SMAD pathway [283]. Furthermore, RCC stem cells-derived EVs may induce a pro-tumorigenic phenotype in MSCs through an increased expression of genes associated with cell migration (CXCR4, CXCR7), matrix remodeling (COL4A3), angiogenesis and tumor growth (IL-8, Osteopontin and Myeloperoxidase). In return, EV-stimulated MSCs enhanced migration of RCC cells and induced vessel-like formation [284]. CD105-positive renal cancer stem cell-derived MVs triggered angiogenesis and promoted the formation of a pre-metastatic niche and lung metastasis through a set of pro-angiogenic mRNAs (VEGF, FGF2, angiopoietin1, ephrin A3, MMP2, MMP9, etc.) and miRNAs (miR-142-5p, miR-200c, etc.) [285]. In addition, exosome-transmitted lncARSR had the capacity of promoting sunitinib resistance via competitively binding miR-34/miR-449 to facilitate AXL and c-MET expression in RCC cells [286].

To date, there was only one study investigating EVs in RCC therapy. Exosomes derived from IL-12-anchored RCC cells induced immunoregulatory effects by promoting T cell proliferation and increasing the release of INF-γ and cytotoxic effects of T cells [287].

7. Summary and future directions

The emerging work on the biology, diagnostic and therapeutic potential of EVs has generated much excitement, changing our understanding of urological cancers. Since specific molecular cargo contained in circulating EVs have great promise as robust mirrors of tumor biology, EVs have been increasingly utilized as diagnostic, prognostic, and predictive biomarkers. To do this, several challenges must be

Table 6
EV biomarkers for kidney cancer.

Marker type	Marker name	Sample type	EV isolation method	Marker type	References
mRNA	GSTA1, CEBPA and PCBD1	Urine	UC	Diagnostic	[274]
miRNA	miR-150-5p	Urine, cell medium	Urine Exosome RNA Isolation Kit, UC	Diagnostic	[276,288]
	miR-let-7i-5p, miR-26a-1-3p, miR-615-3p	Plasma	ExoQuick	Prognostic	[277]
	miR-15, miR-210, miR-1233	Serum	Total Exosome Isolation Reagent (for serum) + EpCAM magnetitc isolation beads (Invitrogen)	Diagnostic	[275]
	miR-34b-5p, miR-126-3p, miR-449a, miR-486-5p	Urine	Urine Exosome RNA Isolation Kit	Diagnostic	[276]
	miR-205	Cell medium	UC	Diagnostic	[288]
	miR-224	Serum	Total Exosome Isolation kit (for serum)	Prognostic	[278]
lncRNA	lncARSR	Cell medium	UC	Prognostic	[286]
Protein	Azurocidin	Cell medium, tissue, serum	UC, Total Exosome Isolation kit (for serum)	Diagnostic	[280]

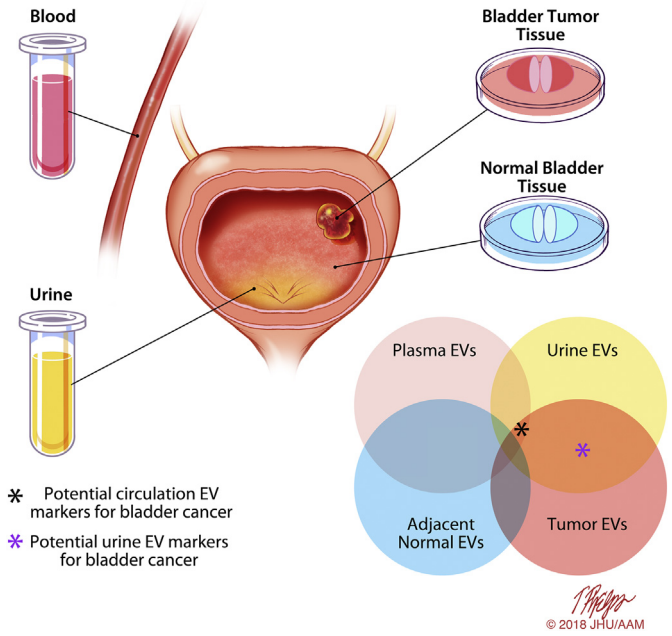

Fig. 4. The schematic diagram of the design of a precise EV biomarkers exploration plan.
In order to identify tumor-derived EVs from the whole EV population in biofluids, this figure demonstrates a design of a precise EV biomarker exploration plan, which utilizes the current available methodologies and aim to find the "cancer specific" EV markers. For example, in BCa, EV markers shared by EVs from a patient's urine and tumor tissue only, but not from plasma or adjacent normal tissues, can be considered as potential urinary EV biomarkers for BCa. If certain EV markers are shared by EVs from a patient's urine, plasma and tumor tissue, but not from adjacent normal tissues, they can be considered as potential circulating EV biomarkers for BCa.

overcome, including the standardization of methods for EV isolation from complex tissues and biofluids, as well as quantification and analysis of EVs on a single particle level. More importantly, it remains to be understood why despite the long list of findings in EVs studies, only a few consistencies have been observed. Research on EVs in the field of RCC and BCa is still at a modest stage, compared to PCa and other cancer types. Urologic malignancies have the advantage of urine as an additional, readily accessible biofluid to query. In order to identify tumor-derived EVs from the whole EV population in biofluids, Fig. 4 demonstrates a design of a precise EV biomarker exploration plan, which utilizes the current available methodologies and aim to find the "cancer specific" EV markers.

Conflicts of interest

No conflict of interests.

Author contributions

L.D., R.C.Z. and Y.W. researched data for the article and wrote the manuscript. All authors made a substantial contribution to discussion of content and reviewed and edited the manuscript before submission.

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin. 68 (2018) 7–30, https://doi.org/10.3322/caac.21442.
[2] A. Di Meo, J. Bartlett, Y. Cheng, M.D. Pasic, G.M. Yousef, Liquid biopsy: a step forward towards precision medicine in urologic malignancies, Mol. Cancer 16 (2017) 80, , https://doi.org/10.1186/s12943-017-0644-5.
[3] B. Dhondt, et al., Urinary extracellular vesicle biomarkers in urological cancers: from discovery towards clinical implementation, Int. J. Biochem. Cell Biol. 99 (2018) 236–256, https://doi.org/10.1016/j.biocel.2018.04.009.
[4] C. Harding, Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes, J. Cell Biol. 97 (1983) 329–339, https://doi.org/10.1083/jcb.97.2.329.
[5] G. Raposo, B lymphocytes secrete antigen-presenting vesicles, J. Exp. Med. 183 (1996) 1161–1172, https://doi.org/10.1084/jem.183.3.1161.
[6] H. Valadi, et al., Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007) 654–659, https://doi.org/10.1038/ncb1596.
[7] M. Colombo, G. Raposo, C. Thery, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289, https://doi.org/10.1146/annurev-cellbio-101512-122326.
[8] A. Becker, et al., Extracellular vesicles in cancer: cell-to-cell mediators of metastasis, Cancer Cell 30 (2016) 836–848, https://doi.org/10.1016/j.ccell.2016.10.009.
[9] Q. An, R. Huckelhoven, K.H. Kogel, A.J. van Bel, Multivesicular bodies participate in a cell wall-associated defence response in barley leaves attacked by the pathogenic powdery mildew fungus, Cell. Microbiol. 8 (2006) 1009–1019, https://doi.org/10.1111/j.1462-5822.2006.00683.x.
[10] B.L. Deatherage, B.T. Cookson, Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life, Infect. Immun. 80 (2012) 1948–1957, https://doi.org/10.1128/IAI.06014-11.
[11] H. Shao, et al., New technologies for analysis of extracellular vesicles, Chem. Rev. 118 (2018) 1917–1950, https://doi.org/10.1021/acs.chemrev.7b00534.
[12] J.S. Schorey, S. Bhatnagar, Exosome function: from tumor immunology to pathogen biology, Traffic 9 (2008) 871–881, https://doi.org/10.1111/j.1600-0854.2008.00734.x.
[13] E.L.A. S, I. Mager, X.O. Breakefield, M.J. Wood, Extracellular vesicles: biology and emerging therapeutic opportunities, Nat. Rev. Drug Discov. 12 (2013) 347–357, https://doi.org/10.1038/nrd3978.
[14] G. van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat. Rev. Mol. Cell Biol. 19 (2018) 213–228, https://doi.org/10.1038/nrm.2017.125.
[15] C. D'Souza-Schorey, J.S. Schorey, Regulation and mechanisms of extracellular vesicle biogenesis and secretion, Essays Biochem. 62 (2018) 125–133, https://doi.org/10.1042/EBC20170078.
[16] D.W. Greening, S.K. Gopal, R. Xu, R.J. Simpson, W. Chen, Exosomes and their roles in immune regulation and cancer, Semin. Cell Dev. Biol. 40 (2015) 72–81, https://doi.org/10.1016/j.semcdb.2015.02.009.
[17] M. Malloci, et al., Extracellular vesicles: mechanisms in human health and disease, Antioxid. Redox Signal. (2018), https://doi.org/10.1089/ars.2017.7265.
[18] L. Cruz, J.A.A. Romero, R.P. Iglesia, M.H. Lopes, Extracellular vesicles: decoding a new language for cellular communication in early embryonic development, Front. Cell Dev. Biol. 6 (2018) 94, , https://doi.org/10.3389/fcell.2018.00094.
[19] P. Kucharzewska, M. Belting, Emerging roles of extracellular vesicles in the adaptive response of tumour cells to microenvironmental stress, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.20304.
[20] G.K. Panigrahi, et al., Exosomal microRNA profiling to identify hypoxia-related biomarkers in prostate cancer, Oncotarget 9 (2018) 13894–13910, https://doi.org/10.18632/oncotarget.24532.
[21] B. Dos Anjos Pultz, et al., The multifaceted role of extracellular vesicles in metastasis: priming the soil for seeding, Int. J. Cancer 140 (2017) 2397–2407, https://doi.org/10.1002/ijc.30595.
[22] R.H. Blackwell, K.E. Foreman, G.N. Gupta, The role of cancer-derived exosomes in tumorigenicity & epithelial-to-mesenchymal transition, Cancers (Basel) 9 (2017), https://doi.org/10.3390/cancers9080105.
[23] M. Shen, X. Ren, New insights into the biological impacts of immune cell-derived exosomes within the tumor environment, Cancer Lett. 431 (2018) 115–122, https://doi.org/10.1016/j.canlet.2018.05.040.
[24] S. Roy, F.H. Hochberg, P.S. Jones, Extracellular vesicles: the growth as diagnostics and therapeutics; a survey, J. Extracell. Vesicles 7 (2018) 1438720, , https://doi.org/10.1080/20013078.2018.1438720.
[25] M. Nawaz, et al., The emerging role of extracellular vesicles as biomarkers for urogenital cancers, Nat. Rev. Urol. 11 (2014) 688–701, https://doi.org/10.1038/nrurol.2014.301.
[26] A.J. Tompkins, et al., The emergence of extracellular vesicles in urology: fertility, cancer, biomarkers and targeted pharmacotherapy, J. Extracell. Vesicles 4 (2015) 23815, , https://doi.org/10.3402/jev.v4.23815.
[27] K. Junker, J. Heinzelmann, C. Beckham, T. Ochiya, G. Jenster, Extracellular vesicles and their role in urologic malignancies, Eur. Urol. 70 (2016) 323–331, https://doi.org/10.1016/j.eururo.2016.02.046.

[28] C.A. Franzen, et al., Urinary exosomes: the potential for biomarker utility, intercellular signaling and therapeutics in urological malignancy, J. Urol. 195 (2016) 1331–1339, https://doi.org/10.1016/j.juro.2015.08.115.
[29] M. Ding, et al., Comparison of commercial exosome isolation kits for circulating exosomal microRNA profiling, Anal. Bioanal. Chem. 410 (2018) 3805–3814, https://doi.org/10.1007/s00216-018-1052-4.
[30] P. Wolf, The nature and significance of platelet products in human plasma, Br. J. Haematol. 13 (1967) 269–288.
[31] Z. Andreu, et al., Comparative analysis of EV isolation procedures for miRNAs detection in serum samples, J. Extracell. Vesicles 5 (2016) 31655, , https://doi.org/10.3402/jev.v5.31655.
[32] D. Duijvesz, et al., Immuno-based detection of extracellular vesicles in urine as diagnostic marker for prostate cancer, Int. J. Cancer 137 (2015) 2869–2878, https://doi.org/10.1002/ijc.29664.
[33] R.J. Hendriks, et al., Comparative analysis of prostate cancer specific biomarkers PCA3 and ERG in whole urine, urinary sediments and exosomes, Clin. Chem. Lab. Med. 54 (2016) 483–492, https://doi.org/10.1515/cclm-2015-0599.
[34] L. Vojtech, et al., Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions, Nucleic Acids Res. 42 (2014) 7290–7304, https://doi.org/10.1093/nar/gku347.
[35] C. Yang, et al., Comprehensive proteomics analysis of exosomes derived from human seminal plasma, Andrology 5 (2017) 1007–1015, https://doi.org/10.1111/andr.12412.
[36] K.W. Witwer, et al., Standardization of sample collection, isolation and analysis methods in extracellular vesicle research, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.20360.
[37] F.A.W. Coumans, et al., Methodological guidelines to study extracellular vesicles, Circ. Res. 120 (2017) 1632–1648, https://doi.org/10.1161/CIRCRESAHA.117.309417.
[38] M.L. Merchant, I.M. Rood, J.K.J. Deegens, J.B. Klein, Isolation and characterization of urinary extracellular vesicles: implications for biomarker discovery, Nat. Rev. Nephrol. 13 (2017) 731–749, https://doi.org/10.1038/nrneph.2017.148.
[39] C. Gardiner, et al., Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey, J. Extracell. Vesicles 5 (2016) 32945, , https://doi.org/10.3402/jev.v5.32945.
[40] F. Momen-Heravi, Winston Patrick Kuo, Shidong Jia (Eds.), Extracellular Vesicles: Methods and Protocols, Springer, New York, 2017, pp. 25–32.
[41] V.R. Minciacchi, et al., MYC mediates large oncosome-induced fibroblast reprogramming in prostate cancer, Cancer Res. 77 (2017) 2306–2317, https://doi.org/10.1158/0008-5472.CAN-16-2942.
[42] K.C. Miranda, et al., Massively parallel sequencing of human urinary exosome/microvesicle RNA reveals a predominance of non-coding RNA, PLoS One 9 (2014) e96094, , https://doi.org/10.1371/journal.pone.0096094.
[43] R. Cantin, J. Diou, D. Belanger, A.M. Tremblay, C. Gilbert, Discrimination between exosomes and HIV-1: purification of both vesicles from cell-free supernatants, J. Immunol. Methods 338 (2008) 21–30, https://doi.org/10.1016/j.jim.2008.07.007.
[44] J. Small, S. Roy, R. Alexander, L. Balaj, Overview of protocols for studying extracellular RNA and extracellular vesicles, Methods Mol. Biol. 1740 (2018) 17–21, https://doi.org/10.1007/978-1-4939-7652-2_2.
[45] M.L. Heinemann, J. Vykoukal, Winston Patrick Kuo, Shidong Jia (Eds.), Extracellular Vesicles: Methods and Protocols, Springer, New York, 2017, pp. 33–41.
[46] R. Lobb, A. Möller, Winston Patrick Kuo, Shidong Jia (Eds.), Extracellular Vesicles: Methods and Protocols, Springer, New York, 2017, pp. 105–110.
[47] L. Musante, et al., A simplified method to recover urinary vesicles for clinical applications, and sample banking, Sci. Rep. 4 (2014) 7532, , https://doi.org/10.1038/srep07532.
[48] F. Liu, et al., The exosome Total isolation Chip, ACS Nano 11 (2017) 10712–10723, https://doi.org/10.1021/acsnano.7b04878.
[49] C. Thery, S. Amigorena, G. Raposo, A. Clayton, Isolation and characterization of exosomes from cell culture supernatants and biological fluids, Curr. Protoc. Cell Biol. 3 (2006), https://doi.org/10.1002/0471143030.cb0322s30 (Unit 3 22).
[50] K.W. Pedersen, B. Kierulf, A. Neurauter, Winston Patrick Kuo, Shidong Jia (Eds.), Extracellular Vesicles: Methods and Protocols, Springer, New York, 2017, pp. 65–87.
[51] C.S. Hong, L. Muller, M. Boyiadzis, T.L. Whiteside, Isolation and characterization of CD34+ blast-derived exosomes in acute myeloid leukemia, PLoS One 9 (2014) e103310, , https://doi.org/10.1371/journal.pone.0103310.
[52] S.R. Krishn, et al., Prostate cancer sheds the alphavbeta3 integrin in vivo through exosomes, Matrix Biol. (2018), https://doi.org/10.1016/j.matbio.2018.08.004.
[53] S. Mathivanan, et al., Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature, Mol. Cell. Proteomics 9 (2010) 197–208, https://doi.org/10.1074/mcp.M900152-MCP200.
[54] P. Sharma, et al., Immunoaffinity-based isolation of melanoma cell-derived exosomes from plasma of patients with melanoma, J. Extracell. Vesicles 7 (2018) 1435138, , https://doi.org/10.1080/20013078.2018.1435138.
[55] Y.H. Wang, et al., Tumor-derived exosomal long noncoding RNAs as promising diagnostic biomarkers for prostate cancer, Cell. Physiol. Biochem. 46 (2018) 532–545, https://doi.org/10.1159/000488620.
[56] G.V. Gooding Jr., T.T. Hebert, A simple technique for purification of tobacco mosaic virus in large quantities, Phytopathology 57 (1967) 1285.
[57] J. Van Deun, et al., The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3.24858.
[58] E. Zeringer, T. Barta, M. Li, A.V. Vlassov, Strategies for isolation of exosomes, Cold Spring Harb Protoc 2015 (2015) 319–323, https://doi.org/10.1101/pdb.top074476.
[59] S.S. Kanwar, C.J. Dunlay, D.M. Simeone, S. Nagrath, Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes, Lab Chip 14 (2014) 1891–1900, https://doi.org/10.1039/c4lc00136b.
[60] B.A. Ashcroft, et al., Determination of the size distribution of blood microparticles directly in plasma using atomic force microscopy and microfluidics, Biomed. Microdevices 14 (2012) 641–649, https://doi.org/10.1007/s10544-012-9642-y.
[61] J.S. Dudani, et al., Rapid inertial solution exchange for enrichment and flow cytometric detection of microvesicles, Biomicrofluidics 9 (2015) 014112, , https://doi.org/10.1063/1.4907807.
[62] Z. Zhao, Y. Yang, Y. Zeng, M. He, A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis, Lab Chip 16 (2016) 489–496, https://doi.org/10.1039/c5lc01117e.
[63] E. Reategui, et al., Engineered nanointerfaces for microfluidic isolation and molecular profiling of tumor-specific extracellular vesicles, Nat. Commun. 9 (2018) 175, , https://doi.org/10.1038/s41467-017-02261-1.
[64] H.K. Woo, et al., Exodisc for rapid, size-selective, and efficient isolation and analysis of nanoscale extracellular vesicles from biological samples, ACS Nano 11 (2017) 1360–1370, https://doi.org/10.1021/acsnano.6b06131.
[65] C. Liu, et al., Field-free isolation of exosomes from extracellular vesicles by microfluidic viscoelastic flows, ACS Nano 11 (2017) 6968–6976, https://doi.org/10.1021/acsnano.7b02277.
[66] K. Lee, H. Shao, R. Weissleder, H. Lee, Acoustic purification of extracellular microvesicles, ACS Nano 9 (2015) 2321–2327, https://doi.org/10.1021/nn506538f.
[67] P. Sehgal, B.J. Kirby, Separation of 300 and 100 nm particles in Fabry-Perot acoustofluidic resonators, Anal. Chem. 89 (2017) 12192–12200, https://doi.org/10.1021/acs.analchem.7b02858.
[68] D. Kang, S. Oh, S.M. Ahn, B.H. Lee, M.H. Moon, Proteomic analysis of exosomes from human neural stem cells by flow field-flow fractionation and nanoflow liquid chromatography-tandem mass spectrometry, J. Proteome Res. 7 (2008) 3475–3480, https://doi.org/10.1021/pr800225z.
[69] H. Zhang, et al., Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation, Nat. Cell Biol. 20 (2018) 332–343, https://doi.org/10.1038/s41556-018-0040-4.
[70] H. Kalra, et al., Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma, Proteomics 13 (2013) 3354–3364, https://doi.org/10.1002/pmic.201300282.
[71] J. Lotvall, et al., Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles, J. Extracell. Vesicles 3 (2014) 26913, , https://doi.org/10.3402/jev.v3.26913.
[72] K.W. Witwer, et al., Updating the MISEV minimal requirements for extracellular vesicle studies: building bridges to reproducibility, J. Extracell. Vesicles 6 (2017) 1396823, , https://doi.org/10.1080/20013078.2017.1396823.
[73] C. Théry, et al., Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J. Extracell. Vesicles 7 (2018), https://doi.org/10.1080/20013078.2018.1535750.
[74] P. Vyletal, A.J. Bleyer, S. Kmoch, Uromodulin biology and pathophysiology—an update, Kidney Blood Press Res. 33 (2010) 456–475, https://doi.org/10.1159/000321013.
[75] T. Pisitkun, R. Johnstone, M.A. Knepper, Discovery of urinary biomarkers, Mol. Cell. Proteomics 5 (2006) 1760–1771, https://doi.org/10.1074/mcp.R600004-MCP200.
[76] M. Wachalska, et al., Protein complexes in urine interfere with extracellular vesicle biomarker studies, J. Circ. Biomark. 5 (2016) 4, , https://doi.org/10.5772/62579.
[77] J.D. Arroyo, et al., Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5003–5008, https://doi.org/10.1073/pnas.1019055108.
[78] R.A. Boon, K.C. Vickers, Intercellular transport of microRNAs, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 186–192, https://doi.org/10.1161/ATVBAHA.112.300139.
[79] V. Sokolova, et al., Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy, Colloids Surf. B Biointerfaces 87 (2011) 146–150, https://doi.org/10.1016/j.colsurfb.2011.05.013.
[80] S. Sharma, et al., Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy, ACS Nano 4 (2010) 1921–1926, https://doi.org/10.1021/nn901824n.
[81] Y. Yuana, et al., Cryo-electron microscopy of extracellular vesicles in fresh plasma, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.21494.
[82] J.M. Street, et al., Identification and proteomic profiling of exosomes in human cerebrospinal fluid, J. Transl. Med. 10 (2012) 5, https://doi.org/10.1186/1479-5876-10-5.
[83] R.A. Dragovic, et al., Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis, Nanomedicine 7 (2011) 780–788, https://doi.org/10.1016/j.nano.2011.04.003.
[84] C. Gardiner, Y.J. Ferreira, R.A. Dragovic, C.W. Redman, I.L. Sargent, Extracellular vesicle sizing and enumeration by nanoparticle tracking analysis, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.19671.
[85] J. de Vrij, et al., Quantification of nanosized extracellular membrane vesicles with scanning ion occlusion sensing, Nanomedicine (Lond.) 8 (2013) 1443–1458,

https://doi.org/10.2217/nnm.12.173.
[86] A. Clayton, et al., Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry, J. Immunol. Methods 247 (2001) 163–174.
[87] S. Libregts, G.J.A. Arkesteijn, A. Nemeth, E.N.M. Nolte-'t Hoen, M.H.M. Wauben, Flow cytometric analysis of extracellular vesicle subsets in plasma: impact of swarm by particles of non-interest, J. Thromb. Haemost. 16 (2018) 1423–1436, https://doi.org/10.1111/jth.14154.
[88] E.J. van der Vlist, E.N. Nolte-'t Hoen, W. Stoorvogel, G.J. Arkesteijn, M.H. Wauben, Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry, Nat. Protoc. 7 (2012) 1311–1326, https://doi.org/10.1038/nprot.2012.065.
[89] S. Mathivanan, C.J. Fahner, G.E. Reid, R.J. Simpson, ExoCarta 2012: database of exosomal proteins, RNA and lipids, Nucleic Acids Res. 40 (2012) D1241–D1244, https://doi.org/10.1093/nar/gkr828.
[90] S. Keerthikumar, et al., ExoCarta: a web-based compendium of exosomal cargo, J. Mol. Biol. 428 (2016) 688–692, https://doi.org/10.1016/j.jmb.2015.09.019.
[91] D.K. Kim, et al., EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.20384.
[92] H. Kalra, et al., Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation, PLoS Biol. 10 (2012) e1001450, , https://doi.org/10.1371/journal.pbio.1001450.
[93] E.-T. Consortium, et al., EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research, Nat. Methods 14 (2017) 228–232, https://doi.org/10.1038/nmeth.4185.
[94] F. Bray, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. (2018), https://doi.org/10.3322/caac.21492.
[95] W. Chen, et al., Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2016) 115–132, https://doi.org/10.3322/caac.21338.
[96] A.J. Wein, L.R. Kavoussi, A.C. Novick, A.W. Partin, C.A. Peters, Campbell-Walsh Urology: Expert Consult Premium Edition: Enhanced Online Features and Print, 4-Volume Set, Elsevier Health Sciences, 2011.
[97] D.M. Berman, J.I. Epstein, When is prostate cancer really cancer? Urol Clin North Am 41 (2014) 339–346, https://doi.org/10.1016/j.ucl.2014.01.006.
[98] J.E. Thompson, P.D. Stricker, MRI improves active surveillance of prostate cancer however biopsy is still crucial, BJU Int. 122 (2018) E1–E2, https://doi.org/10.1111/bju.14424.
[99] J.N. Wu, K.M. Fish, C.P. Evans, R.W. Devere White, M.A. Dall'Era, No improvement noted in overall or cause-specific survival for men presenting with metastatic prostate cancer over a 20-year period, Cancer 120 (2014) 818–823, https://doi.org/10.1002/cncr.28485.
[100] E.D. Crawford, D. Petrylak, O. Sartor, Navigating the evolving therapeutic landscape in advanced prostate cancer, Urol. Oncol. 35S (2017) S1–S13, https://doi.org/10.1016/j.urolonc.2017.01.020.
[101] B.S. Taylor, et al., Integrative genomic profiling of human prostate cancer, Cancer Cell 18 (2010) 11–22, https://doi.org/10.1016/j.ccr.2010.05.026.
[102] E.S. Antonarakis, et al., AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer, N. Engl. J. Med. 371 (2014) 1028–1038, https://doi.org/10.1056/NEJMoa1315815.
[103] P. Nuhn, et al., Update on systemic prostate cancer therapies: management of metastatic castration-resistant prostate cancer in the era of precision oncology, Eur. Urol. (2018), https://doi.org/10.1016/j.eururo.2018.03.028.
[104] O. Sartor, J.S. de Bono, Metastatic prostate cancer, N. Engl. J. Med. 378 (2018) 645–657, https://doi.org/10.1056/NEJMra1701695.
[105] J.H. Hayes, M.J. Barry, Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence, JAMA 311 (2014) 1143–1149, https://doi.org/10.1001/jama.2014.2085.
[106] D. Ilic, et al., Prostate cancer screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis, BMJ 362 (2018) k3519, https://doi.org/10.1136/bmj.k3519.
[107] S. Loeb, et al., The prostate health index selectively identifies clinically significant prostate cancer, J. Urol. 193 (2015) 1163–1169, https://doi.org/10.1016/j.juro.2014.10.121.
[108] T. Nordstrom, et al., Comparison between the four-kallikrein panel and prostate health index for predicting prostate cancer, Eur. Urol. 68 (2015) 139–146, https://doi.org/10.1016/j.eururo.2014.08.010.
[109] K.K. Chevli, et al., Urinary PCA3 as a predictor of prostate cancer in a cohort of 3,073 men undergoing initial prostate biopsy, J. Urol. 191 (2014) 1743–1748, https://doi.org/10.1016/j.juro.2013.12.005.
[110] S.A. Tomlins, et al., Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment, Eur. Urol. 70 (2016) 45–53, https://doi.org/10.1016/j.eururo.2015.04.039.
[111] V. Vlaeminck-Guillem, Extracellular vesicles in prostate cancer carcinogenesis, diagnosis, and management, Front. Oncol. 8 (2018) 222, https://doi.org/10.3389/fonc.2018.00222.
[112] M. Giulietti, et al., Exploring Small extracellular vesicles for precision medicine in prostate cancer, Front. Oncol. 8 (2018) 221, https://doi.org/10.3389/fonc.2018.00221.
[113] V.R. Minciacchi, A. Zijlstra, M.A. Rubin, D. Di Vizio, Extracellular vesicles for liquid biopsy in prostate cancer: where are we and where are we headed? Prostate Cancer Prostatic Dis. 20 (2017) 251–258, https://doi.org/10.1038/pcan.2017.7.
[114] M. Del Re, et al., The detection of androgen receptor splice variant 7 in plasma-derived exosomal RNA strongly predicts resistance to hormonal therapy in metastatic prostate cancer patients, Eur. Urol. 71 (2017) 680–687, https://doi.org/10.1016/j.eururo.2016.08.012.
[115] F. Royo, et al., Comparative miRNA analysis of urine extracellular vesicles isolated through five different methods, Cancers (Basel) 8 (2016), https://doi.org/10.3390/cancers8120112.
[116] V. Brychtova, B. Vojtesek, R. Hrstka, Anterior gradient 2: a novel player in tumor cell biology, Cancer Lett. 304 (2011) 1–7, https://doi.org/10.1016/j.canlet.2010.12.023.
[117] H. Bu, et al., The anterior gradient 2 (AGR2) gene is overexpressed in prostate cancer and may be useful as a urine sediment marker for prostate cancer detection, Prostate 71 (2011) 575–587, https://doi.org/10.1002/pros.21273.
[118] A. Neeb, et al., Splice variant transcripts of the anterior gradient 2 gene as a marker of prostate cancer, Oncotarget 5 (2014) 8681–8689, https://doi.org/10.18632/oncotarget.2365.
[119] S.W. Ferguson, J. Nguyen, Exosomes as therapeutics: the implications of molecular composition and exosomal heterogeneity, J. Control. Release 228 (2016) 179–190, https://doi.org/10.1016/j.jconrel.2016.02.037.
[120] V.N. Kim, MicroRNA biogenesis: coordinated cropping and dicing, Nat. Rev. Mol. Cell Biol. 6 (2005) 376–385, https://doi.org/10.1038/nrm1644.
[121] E. van Rooij, The art of microRNA research, Circ. Res. 108 (2011) 219–234, https://doi.org/10.1161/CIRCRESAHA.110.227496.
[122] B. Kumar, et al., Cell-type specific expression of oncogenic and tumor suppressive microRNAs in the human prostate and prostate cancer, Sci. Rep. 8 (2018) 7189, https://doi.org/10.1038/s41598-018-25320-z.
[123] N. Sharma, M.M. Baruah, The microRNA signatures: aberrantly expressed miRNAs in prostate cancer, Clin. Transl. Oncol. (2018), https://doi.org/10.1007/s12094-018-1910-8.
[124] R.J. Bryant, et al., Changes in circulating microRNA levels associated with prostate cancer, Br. J. Cancer 106 (2012) 768–774, https://doi.org/10.1038/bjc.2011.595.
[125] E. Endzelins, et al., Detection of circulating miRNAs: comparative analysis of extracellular vesicle-incorporated miRNAs and cell-free miRNAs in whole plasma of prostate cancer patients, BMC Cancer 17 (2017) 730, , https://doi.org/10.1186/s12885-017-3737-z.
[126] X. Huang, et al., Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer, Eur. Urol. 67 (2015) 33–41, https://doi.org/10.1016/j.eururo.2014.07.035.
[127] L. Foj, et al., Exosomal and non-exosomal urinary miRNAs in prostate cancer detection and prognosis, Prostate 77 (2017) 573–583, https://doi.org/10.1002/pros.23295.
[128] R. Samsonov, et al., Lectin-induced agglutination method of urinary exosomes isolation followed by mi-RNA analysis: application for prostate cancer diagnostic, Prostate 76 (2016) 68–79, https://doi.org/10.1002/pros.23101.
[129] D. Koppers-Lalic, et al., Noninvasive prostate cancer detection by measuring miRNA variants (isomiRs) in urine extracellular vesicles, Oncotarget 7 (2016) 22566–22578, https://doi.org/10.18632/oncotarget.8124.
[130] Y. Xu, et al., MiR-145 detection in urinary extracellular vesicles increase diagnostic efficiency of prostate cancer based on hydrostatic filtration dialysis method, Prostate 77 (2017) 1167–1175, https://doi.org/10.1002/pros.23376.
[131] C. Corcoran, S. Rani, L. O'Driscoll, miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression, Prostate 74 (2014) 1320–1334, https://doi.org/10.1002/pros.22848.
[132] Q. Yu, et al., Nano-vesicles are a potential tool to monitor therapeutic efficacy of carbon ion radiotherapy in prostate cancer, J. Biomed. Nanotechnol. 14 (2018) 168–178, https://doi.org/10.1166/jbn.2018.2503.
[133] D. Hessels, et al., DD3PCA3-based molecular urine analysis for the diagnosis of prostate cancer, Eur. Urol. 44 (2003) 8–16, https://doi.org/10.1016/s0302-2838(03)00201-x.
[134] J. Nilsson, et al., Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer, Br. J. Cancer 100 (2009) 1603–1607, https://doi.org/10.1038/sj.bjc.6605058.
[135] S. Dijkstra, et al., Prostate cancer biomarker profiles in urinary sediments and exosomes, J. Urol. 191 (2014) 1132–1138, https://doi.org/10.1016/j.juro.2013.11.001.
[136] K.L. Pellegrini, et al., Detection of prostate cancer-specific transcripts in extracellular vesicles isolated from post-DRE urine, Prostate 77 (2017) 990–999, https://doi.org/10.1002/pros.23355.
[137] M.J. Donovan, et al., A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result, Prostate Cancer Prostatic Dis. 18 (2015) 370–375, https://doi.org/10.1038/pcan.2015.40.
[138] J. McKiernan, et al., A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy, JAMA Oncol. 2 (2016) 882–889, https://doi.org/10.1001/jamaoncol.2016.0097.
[139] J. McKiernan, et al., A prospective adaptive utility trial to validate performance of a novel urine exosome gene expression assay to predict high-grade prostate cancer in patients with prostate-specific antigen 2–10 ng/ml at initial biopsy, Eur. Urol. (2018), https://doi.org/10.1016/j.eururo.2018.08.019.
[140] M.G. Sanda, et al., Association between combined TMPRSS2:ERG and PCA3 RNA urinary testing and detection of aggressive prostate cancer, JAMA Oncol 3 (2017) 1085–1093, https://doi.org/10.1001/jamaoncol.2017.0177.
[141] P. Motamedinia, et al., Urine exosomes for non-invasive assessment of gene expression and mutations of prostate cancer, PLoS One 11 (2016) e0154507, , https://doi.org/10.1371/journal.pone.0154507.
[142] H.K. Woo, et al., Urine-based liquid biopsy: non-invasive and sensitive AR-V7 detection in urinary EVs from patients with prostate cancer, Lab Chip 19 (2018) 87–97, https://doi.org/10.1039/c8lc01185k.

[143] C.A. Sanchez, et al., Exosomes from bulk and stem cells from human prostate cancer have a differential microRNA content that contributes cooperatively over local and pre-metastatic niche, Oncotarget 7 (2016) 3993–4008, https://doi.org/10.18632/oncotarget.6540.
[144] J. Li, et al., Exosome-derived microRNAs contribute to prostate cancer chemoresistance, Int. J. Oncol. 49 (2016) 838–846, https://doi.org/10.3892/ijo.2016.3560.
[145] T. Yasui, et al., Unveiling massive numbers of cancer-related urinary-microRNA candidates via nanowires, Sci. Adv. 3 (2017) e1701133, , https://doi.org/10.1126/sciadv.1701133.
[146] O.E. Bryzgunova, et al., Comparative study of extracellular vesicles from the urine of healthy individuals and prostate cancer patients, PLoS One 11 (2016) e0157566, , https://doi.org/10.1371/journal.pone.0157566.
[147] M. Morello, et al., Large oncosomes mediate intercellular transfer of functional microRNA, Cell Cycle 12 (2013) 3526–3536, https://doi.org/10.4161/cc.26539.
[148] A. Cannistraci, et al., C-met/miR-130b axis as novel mechanism and biomarker for castration resistance state acquisition, Oncogene 36 (2017) 3718–3728, https://doi.org/10.1038/onc.2016.505.
[149] M. Rodriguez, et al., Identification of non-invasive miRNAs biomarkers for prostate cancer by deep sequencing analysis of urinary exosomes, Mol. Cancer 16 (2017) 156, , https://doi.org/10.1186/s12943-017-0726-4.
[150] T. Hasegawa, et al., Characterization and evidence of the miR-888 cluster as a novel cancer network in prostate, Mol. Cancer Res. 16 (2018) 669–681, https://doi.org/10.1158/1541-7786.MCR-17-0321.
[151] D. Bhagirath, et al., microRNA-1246 is an exosomal biomarker for aggressive prostate cancer, Cancer Res. 78 (2018) 1833–1844, https://doi.org/10.1158/0008-5472.CAN-17-2069.
[152] S. Wani, D. Kaul, R.S. Mavuduru, N. Kakkar, A. Bhatia, Urinary-exosomal miR-2909: a novel pathognomonic trait of prostate cancer severity, J. Biotechnol. 259 (2017) 135–139, https://doi.org/10.1016/j.jbiotec.2017.07.029.
[153] M. Isin, et al., Exosomal lncRNA-p21 levels may help to distinguish prostate cancer from benign disease, Front. Genet. 6 (2015) 168, , https://doi.org/10.3389/fgene.2015.00168.
[154] S.D. Sweat, A. Pacelli, G.P. Murphy, D.G. Bostwick, Prostate-specific membrane antigen expression is greatest in prostate adenocarcinoma and lymph node metastases, Urology 52 (1998) 637–640.
[155] P.J. Mitchell, et al., Can urinary exosomes act as treatment response markers in prostate cancer? J. Transl. Med. 7 (2009) 4, https://doi.org/10.1186/1479-5876-7-4.
[156] Y.H. Park, et al., Prostate-specific extracellular vesicles as a novel biomarker in human prostate cancer, Sci. Rep. 6 (2016) 30386, , https://doi.org/10.1038/srep30386.
[157] C.N. Biggs, et al., Prostate extracellular vesicles in patient plasma as a liquid biopsy platform for prostate cancer using nanoscale flow cytometry, Oncotarget 7 (2016) 8839–8849, https://doi.org/10.18632/oncotarget.6983.
[158] M. Logozzi, et al., Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients, Cancer Lett. 403 (2017) 318–329, https://doi.org/10.1016/j.canlet.2017.06.036.
[159] I.F. Tannock, et al., Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer, N. Engl. J. Med. 351 (2004) 1502–1512, https://doi.org/10.1056/NEJMoa040720.
[160] D.R. Berthold, et al., Survival and PSA response of patients in the TAX 327 study who crossed over to receive docetaxel after mitoxantrone or vice versa, Ann. Oncol. 19 (2008) 1749–1753, https://doi.org/10.1093/annonc/mdn288.
[161] S. Marchetti, et al., In vitro transport of gimatecan (7-t-butoxyiminomethylcamptothecin) by breast cancer resistance protein, P-glycoprotein, and multidrug resistance protein 2, Mol. Cancer Ther. 6 (2007) 3307–3313, https://doi.org/10.1158/1535-7163.MCT-07-0461.
[162] M. Takeda, et al., The establishment of two paclitaxel-resistant prostate cancer cell lines and the mechanisms of paclitaxel resistance with two cell lines, Prostate 67 (2007) 955–967, https://doi.org/10.1002/pros.20581.
[163] T. Kato, et al., Serum exosomal P-glycoprotein is a potential marker to diagnose docetaxel resistance and select a taxoid for patients with prostate cancer, Urol. Oncol. 33 (2015), https://doi.org/10.1016/j.urolonc.2015.04.019 (385 e315-320).
[164] P. Kharaziha, et al., Molecular profiling of prostate cancer derived exosomes may reveal a predictive signature for response to docetaxel, Oncotarget 6 (2015) 21740–21754, https://doi.org/10.18632/oncotarget.3226.
[165] K. Kawakami, et al., Gamma-glutamyltransferase activity in exosomes as a potential marker for prostate cancer, BMC Cancer 17 (2017) 316, https://doi.org/10.1186/s12885-017-3301-x.
[166] A. Overbye, et al., Identification of prostate cancer biomarkers in urinary exosomes, Oncotarget 6 (2015) 30357–30376, https://doi.org/10.18632/oncotarget.4851.
[167] S. Principe, et al., In-depth proteomic analyses of exosomes isolated from expressed prostatic secretions in urine, Proteomics 13 (2013) 1667–1671, https://doi.org/10.1002/pmic.201200561.
[168] E. Hosseini-Beheshti, S. Pham, H. Adomat, N. Li, E.S. Tomlinson Guns, Exosomes as biomarker enriched microvesicles: characterization of exosomal proteins derived from a panel of prostate cell lines with distinct AR phenotypes, Mol. Cell. Proteomics 11 (2012) 863–885, https://doi.org/10.1074/mcp.M111.014845.
[169] T. Liu, D.E. Mendes, C.E. Berkman, Functional prostate-specific membrane antigen is enriched in exosomes from prostate cancer cells, Int. J. Oncol. 44 (2014) 918–922, https://doi.org/10.3892/ijo.2014.2256.
[170] T. Sequeiros, et al., Targeted proteomics in urinary extracellular vesicles identifies biomarkers for diagnosis and prognosis of prostate cancer, Oncotarget 8 (2017) 4960–4976, https://doi.org/10.18632/oncotarget.13634.
[171] T. Vermassen, et al., Release of urinary extracellular vesicles in prostate cancer is associated with altered urinary N-glycosylation profile, J. Clin. Pathol. 70 (2017) 838–846, https://doi.org/10.1136/jclinpath-2016-204312.
[172] K.G. Ronquist, G. Ronquist, A. Larsson, L. Carlsson, Proteomic analysis of prostate cancer metastasis-derived prostasomes, Anticancer Res. 30 (2010) 285–290.
[173] L. Wang, T. Skotland, V. Berge, K. Sandvig, A. Llorente, Exosomal proteins as prostate cancer biomarkers in urine: from mass spectrometry discovery to immunoassay-based validation, Eur. J. Pharm. Sci. 98 (2017) 80–85, https://doi.org/10.1016/j.ejps.2016.09.023.
[174] D. Duijvesz, et al., Proteomic profiling of exosomes leads to the identification of novel biomarkers for prostate cancer, PLoS One 8 (2013) e82589, , https://doi.org/10.1371/journal.pone.0082589.
[175] T.S. Worst, et al., Database-augmented mass spectrometry analysis of exosomes identifies Claudin 3 as a putative prostate cancer biomarker, Mol. Cell. Proteomics 16 (2017) 998–1008, https://doi.org/10.1074/mcp.M117.068577.
[176] D. Turay, et al., Proteomic profiling of serum-derived exosomes from ethnically diverse prostate cancer patients, Cancer Investig. 34 (2016) 1–11, https://doi.org/10.3109/07357907.2015.1081921.
[177] J.L. Welton, et al., Proteomics analysis of vesicles isolated from plasma and urine of prostate cancer patients using a multiplex, aptamer-based protein array, J. Extracell. Vesicles 5 (2016) 31209, , https://doi.org/10.3402/jev.v5.31209.
[178] A. Singh, et al., Exosome-mediated transfer of alphavbeta3 integrin from tumorigenic to nontumorigenic cells promotes a migratory phenotype, Mol. Cancer Res. 14 (2016) 1136–1146, https://doi.org/10.1158/1541-7786.MCR-16-0058.
[179] S. Khan, et al., Racial differences in the expression of inhibitors of apoptosis (IAP) proteins in extracellular vesicles (EV) from prostate cancer patients, PLoS One 12 (2017) e0183122, , https://doi.org/10.1371/journal.pone.0183122.
[180] S. Khan, et al., Plasma-derived exosomal survivin, a plausible biomarker for early detection of prostate cancer, PLoS One 7 (2012) e46737, , https://doi.org/10.1371/journal.pone.0046737.
[181] K. Fujita, et al., Proteomic analysis of urinary extracellular vesicles from high Gleason score prostate cancer, Sci. Rep. 7 (2017) 42961, , https://doi.org/10.1038/srep42961.
[182] K. Sandvig, A. Llorente, Proteomic analysis of microvesicles released by the human prostate cancer cell line PC-3, Mol. Cell. Proteomics 11 (2012), https://doi.org/10.1074/mcp.M111.012914 (M111 012914).
[183] R.M. DeRita, et al., C-Src, insulin-like growth factor I receptor, G-protein-coupled receptor kinases and focal adhesion kinase are enriched into prostate cancer cell exosomes, J. Cell. Biochem. 118 (66–73) (2017), https://doi.org/10.1002/jcb.25611.
[184] Q. Lu, et al., Identification of extracellular delta-catenin accumulation for prostate cancer detection, Prostate 69 (2009) 411–418, https://doi.org/10.1002/pros.20902.
[185] G. Kharmate, E. Hosseini-Beheshti, J. Caradec, M.Y. Chin, E.S. Tomlinson Guns, Epidermal growth factor receptor in prostate cancer derived exosomes, PLoS One 11 (2016) e0154967, , https://doi.org/10.1371/journal.pone.0154967.
[186] S. Li, et al., Exosomal ephrinA2 derived from serum as a potential biomarker for prostate cancer, J. Cancer 9 (2018) 2659–2665, https://doi.org/10.7150/jca.25201.
[187] I.V. Bijnsdorp, et al., Exosomal ITGA3 interferes with non-cancerous prostate cell functions and is increased in urine exosomes of metastatic prostate cancer patients, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.22097.
[188] C. Fedele, A. Singh, B.J. Zerlanko, R.V. Iozzo, L.R. Languino, The alphavbeta6 integrin is transferred intercellularly via exosomes, J. Biol. Chem. 290 (2015) 4545–4551, https://doi.org/10.1074/jbc.C114.617662.
[189] K. Kawakami, et al., Integrin beta4 and vinculin contained in exosomes are potential markers for progression of prostate cancer associated with taxane-resistance, Int. J. Oncol. 47 (2015) 384–390, https://doi.org/10.3892/ijo.2015.3011.
[190] K. Gabriel, et al., Regulation of the tumor suppressor PTEN through exosomes: a diagnostic potential for prostate cancer, PLoS One 8 (2013) e70047, , https://doi.org/10.1371/journal.pone.0070047.
[191] A.Z. Fernandis, M.R. Wenk, Lipid-based biomarkers for cancer, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2830–2835, https://doi.org/10.1016/j.jchromb.2009.06.015.
[192] X. Zhou, et al., Identification of plasma lipid biomarkers for prostate cancer by lipidomics and bioinformatics, PLoS One 7 (2012) e48889, , https://doi.org/10.1371/journal.pone.0048889.
[193] A. Llorente, et al., Molecular lipidomics of exosomes released by PC-3 prostate cancer cells, Biochim. Biophys. Acta (BBA) 1831 (2013) 1302–1309, https://doi.org/10.1016/j.bbalip.2013.04.011.
[194] J.S. Yang, J.C. Lee, S.K. Byeon, K.H. Rha, M.H. Moon, Size dependent lipidomic analysis of urinary exosomes from patients with prostate cancer by flow field-flow fractionation and nanoflow liquid chromatography-tandem mass spectrometry, Anal. Chem. 89 (2017) 2488–2496, https://doi.org/10.1021/acs.analchem.6b04634.
[195] M. Puhka, et al., Metabolomic profiling of extracellular vesicles and alternative normalization methods reveal enriched metabolites and strategies to study prostate cancer-related changes, Theranostics 7 (2017) 3824–3841, https://doi.org/10.7150/thno.19890.
[196] M. Clos-Garcia, et al., Metabolic alterations in urine extracellular vesicles are associated to prostate cancer pathogenesis and progression, J. Extracell. Vesicles 7 (2018) 1470442, , https://doi.org/10.1080/20013078.2018.1470442.
[197] T. Skotland, et al., Molecular lipid species in urinary exosomes as potential prostate cancer biomarkers, Eur. J. Cancer 70 (2017) 122–132, https://doi.org/

10.1016/j.ejca.2016.10.011.
[198] R. Xu, et al., Extracellular vesicles in cancer - implications for future improvements in cancer care, Nat. Rev. Clin. Oncol. (2018), https://doi.org/10.1038/s41571-018-0036-9.
[199] C. Levesque, P.S. Nelson, Cellular constituents of the prostate stroma: key contributors to prostate cancer progression and therapy resistance, Cold Spring Harb. Perspect. Med. 8 (2018), https://doi.org/10.1101/cshperspect.a030510.
[200] K.C. Valkenburg, A.E. de Groot, K.J. Pienta, Targeting the tumour stroma to improve cancer therapy, Nat. Rev. Clin. Oncol. 15 (2018) 366–381, https://doi.org/10.1038/s41571-018-0007-1.
[201] Y. Kojima, et al., Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20009–20014, https://doi.org/10.1073/pnas.1013805107.
[202] C. Feig, et al., The pancreas cancer microenvironment, Clin. Cancer Res. 18 (2012) 4266–4276, https://doi.org/10.1158/1078-0432.CCR-11-3114.
[203] J.P. Webber, et al., Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes, Oncogene 34 (2015) 290–302, https://doi.org/10.1038/onc.2013.560.
[204] S. Halin Bergstrom, et al., Extracellular vesicles from metastatic rat prostate tumors prime the Normal prostate tissue to facilitate tumor growth, Sci. Rep. 6 (2016) 31805, , https://doi.org/10.1038/srep31805.
[205] A. Santi, et al., Cancer associated fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth, Biochim. Biophys. Acta 1853 (2015) 3211–3223, https://doi.org/10.1016/j.bbamcr.2015.09.013.
[206] H. Zhao, et al., Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism, Elife 5 (2016) e10250, , https://doi.org/10.7554/eLife.10250.
[207] S. Josson, et al., Stromal fibroblast-derived miR-409 promotes epithelial-to-mesenchymal transition and prostate tumorigenesis, Oncogene 34 (2015) 2690–2699, https://doi.org/10.1038/onc.2014.212.
[208] L.A. Kingsley, P.G. Fournier, J.M. Chirgwin, T.A. Guise, Molecular biology of bone metastasis, Mol. Cancer Ther. 6 (2007) 2609–2617, https://doi.org/10.1158/1535-7163.MCT-07-0234.
[209] D.J. Papachristou, E.K. Basdra, A.G. Papavassiliou, Bone metastases: molecular mechanisms and novel therapeutic interventions, Med. Res. Rev. 32 (2012) 611–636, https://doi.org/10.1002/med.20224.
[210] T. Karlsson, M. Lundholm, A. Widmark, E. Tumor Persson, Cell-derived exosomes from the prostate cancer cell line TRAMP-C1 impair osteoclast formation and differentiation, PLoS One 11 (2016) e0166284, , https://doi.org/10.1371/journal.pone.0166284.
[211] Y. Ye, et al., Exosomal miR-141-3p regulates osteoblast activity to promote the osteoblastic metastasis of prostate cancer, Oncotarget 8 (2017) 94834–94849, https://doi.org/10.18632/oncotarget.22014.
[212] K. Hashimoto, et al., Cancer-secreted hsa-miR-940 induces an osteoblastic phenotype in the bone metastatic microenvironment via targeting ARHGAP1 and FAM134A, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 2204–2209, https://doi.org/10.1073/pnas.1717363115.
[213] H. Peinado, et al., Pre-metastatic niches: organ-specific homes for metastases, Nat. Rev. Cancer 17 (2017) 302–317, https://doi.org/10.1038/nrc.2017.6.
[214] Z.Y. Abd Elmageed, et al., Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes, Stem Cells 32 (2014) 983–997, https://doi.org/10.1002/stem.1619.
[215] V. Bronte, et al., Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers, J. Exp. Med. 201 (2005) 1257–1268, https://doi.org/10.1084/jem.20042028.
[216] K. Adachi, K. Tamada, Immune checkpoint blockade opens an avenue of cancer immunotherapy with a potent clinical efficacy, Cancer Sci. 106 (2015) 945–950, https://doi.org/10.1111/cas.12695.
[217] L. Sadovska, et al., A novel 3D heterotypic spheroid model for studying extracellular vesicle-mediated tumour and immune cell communication, Biochem. Biophys. Res. Commun. 495 (2018) 1930–1935, https://doi.org/10.1016/j.bbrc.2017.12.072.
[218] H.G. Ljunggren, Cancer immunosurveillance: NKG2D breaks cover, Immunity 28 (2008) 492–494, https://doi.org/10.1016/j.immuni.2008.03.007.
[219] M. Lundholm, et al., Prostate tumor-derived exosomes down-regulate NKG2D expression on natural killer cells and CD8+ T cells: mechanism of immune evasion, PLoS One 9 (2014) e108925, , https://doi.org/10.1371/journal.pone.0108925.
[220] J. Salimu, et al., Dominant immunosuppression of dendritic cell function by prostate-cancer-derived exosomes, J. Extracell. Vesicles 6 (2017) 1368823, , https://doi.org/10.1080/20013078.2017.1368823.
[221] T. Suzuki, et al., EBAG9/RCAS1 in human breast carcinoma: a possible factor in endocrine-immune interactions, Br. J. Cancer 85 (2001) 1731–1737, https://doi.org/10.1054/bjoc.2001.2176.
[222] T. Miyazaki, K. Ikeda, W. Sato, K. Horie-Inoue, S. Inoue, Extracellular vesicle-mediated EBAG9 transfer from cancer cells to tumor microenvironment promotes immune escape and tumor progression, Oncogenesis 7 (2018) 7, , https://doi.org/10.1038/s41389-017-0022-6.
[223] J. Kim, J.S. Bae, Tumor-associated macrophages and neutrophils in tumor microenvironment, Mediat. Inflamm. 2016 (2016) 6058147, https://doi.org/10.1155/2016/6058147.
[224] H. Lu, et al., Exosomal alphavbeta6 integrin is required for monocyte M2 polarization in prostate cancer, Matrix Biol. 70 (2018) 20–35, https://doi.org/10.1016/j.matbio.2018.03.009.
[225] J. Kim, et al., Enhanced shedding of extracellular vesicles from amoeboid prostate cancer cells: potential effects on the tumor microenvironment, Cancer Biol. Ther. 15 (2014) 409–418, https://doi.org/10.4161/cbt.27627.
[226] E. Hosseini-Beheshti, et al., Exosomes confer pro-survival signals to alter the phenotype of prostate cells in their surrounding environment, Oncotarget 7 (2016) 14639–14658, https://doi.org/10.18632/oncotarget.7052.
[227] J. Read, et al., Nuclear transportation of exogenous epidermal growth factor receptor and androgen receptor via extracellular vesicles, Eur. J. Cancer 70 (2017) 62–74, https://doi.org/10.1016/j.ejca.2016.10.017.
[228] X. Yang, et al., The long non-coding RNA PCSEAT exhibits an oncogenic property in prostate cancer and functions as a competing endogenous RNA that associates with EZH2, Biochem. Biophys. Res. Commun. 502 (2018) 262–268, https://doi.org/10.1016/j.bbrc.2018.05.157.
[229] J.S. Brzozowski, et al., Extracellular vesicles with altered tetraspanin CD9 and CD151 levels confer increased prostate cell motility and invasion, Sci. Rep. 8 (2018) 8822, , https://doi.org/10.1038/s41598-018-27180-z.
[230] I.Y. El-Sayed, et al., Extracellular vesicles released by mesenchymal-like prostate carcinoma cells modulate EMT state of recipient epithelial-like carcinoma cells through regulation of AR signaling, Cancer Lett. 410 (2017) 100–111, https://doi.org/10.1016/j.canlet.2017.09.010.
[231] L.C. Lin, A.C. Gao, C.H. Lai, J.T. Hsieh, H. Lin, Induction of neuroendocrine differentiation in castration resistant prostate cancer cells by adipocyte differentiation-related protein (ADRP) delivered by exosomes, Cancer Lett. 391 (2017) 74–82, https://doi.org/10.1016/j.canlet.2017.01.018.
[232] K. Panagopoulos, et al., Reversal of chemosensitivity and induction of cell malignancy of a non-malignant prostate cancer cell line upon extracellular vesicle exposure, Mol. Cancer 12 (2013) 118, , https://doi.org/10.1186/1476-4598-12-118.
[233] A.G. Souza, et al., Extracellular vesicles as drivers of epithelial-mesenchymal transition and carcinogenic characteristics in normal prostate cells, Mol. Carcinog. 57 (2018) 503–511, https://doi.org/10.1002/mc.22775.
[234] F. Urabe, N. Kosaka, T. Kimura, S. Egawa, T. Ochiya, Extracellular vesicles: toward a clinical application in urological cancer treatment, Int. J. Urol. 25 (2018) 533–543, https://doi.org/10.1111/iju.13594.
[235] S. Jorfi, et al., Inhibition of microvesiculation sensitizes prostate cancer cells to chemotherapy and reduces docetaxel dose required to limit tumor growth in vivo, Sci. Rep. 5 (2015) 13006, , https://doi.org/10.1038/srep13006.
[236] U.S. Kosgodage, R.P. Trindade, P.R. Thompson, J.M. Inal, S. Lange, Chloramidine/bisindolylmaleimide-I-mediated inhibition of exosome and microvesicle release and enhanced efficacy of cancer chemotherapy, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18051007.
[237] A. Datta, et al., High-throughput screening identified selective inhibitors of exosome biogenesis and secretion: a drug repurposing strategy for advanced cancer, Sci. Rep. 8 (2018) 8161, https://doi.org/10.1038/s41598-018-26411-7.
[238] R.B. Rountree, et al., Exosome targeting of tumor antigens expressed by cancer vaccines can improve antigen immunogenicity and therapeutic efficacy, Cancer Res. 71 (2011) 5235–5244, https://doi.org/10.1158/0008-5472.CAN-10-4076.
[239] H. Saari, et al., Microvesicle- and exosome-mediated drug delivery enhances the cytotoxicity of paclitaxel in autologous prostate cancer cells, J. Control. Release 220 (2015) 727–737, https://doi.org/10.1016/j.jconrel.2015.09.031.
[240] U. Altanerova, et al., Human mesenchymal stem cell-derived iron oxide exosomes allow targeted ablation of tumor cells via magnetic hyperthermia, Int. J. Nanomedicine 12 (2017) 7923–7936, https://doi.org/10.2147/IJN.S145096.
[241] J.Q. Wang, et al., Extracellular vesicle-mediated reversal of paclitaxel resistance in prostate cancer, Crit. Rev. Oncog. 20 (2015) 407–417, https://doi.org/10.1615/CritRevOncog.v20.i5-6.120.
[242] F. Alcayaga-Miranda, et al., Prostate tumor-induced angiogenesis is blocked by exosomes derived from menstrual stem cells through the inhibition of reactive oxygen species, Oncotarget 7 (2016) 44462–44477, https://doi.org/10.18632/oncotarget.9852.
[243] T.C. Peak, et al., Exosomes secreted by placental stem cells selectively inhibit growth of aggressive prostate cancer cells, Biochem. Biophys. Res. Commun. 499 (2018) 1004–1010, https://doi.org/10.1016/j.bbrc.2018.04.038.
[244] A.R. Zlotta, What is the future of virtual cystoscopy in urology? Can. Urol. Assoc. J. 5 (2011) 38–39, https://doi.org/10.5489/cuaj.11011.
[245] J.L. Welton, et al., Proteomics analysis of bladder cancer exosomes, Mol. Cell. Proteomics 9 (2010) 1324–1338, https://doi.org/10.1074/mcp.M000063-MCP201.
[246] D.M. Smalley, N.E. Sheman, K. Nelson, D. Theodorescu, Isolation and identification of potential urinary microparticle biomarkers of bladder cancer, J. Proteome Res. 7 (2008) 2088–2096, https://doi.org/10.1021/pr700775x.
[247] C.L. Chen, et al., Comparative and targeted proteomic analyses of urinary microparticles from bladder cancer and hernia patients, J. Proteome Res. 11 (2012) 5611–5629, https://doi.org/10.1021/pr3008732.
[248] D.K. Jeppesen, et al., Quantitative proteomics of fractionated membrane and lumen exosome proteins from isogenic metastatic and nonmetastatic bladder cancer cells reveal differential expression of EMT factors, Proteomics 14 (2014) 699–712, https://doi.org/10.1002/pmic.201300452.
[249] A. Perez, et al., A pilot study on the potential of RNA-associated to urinary vesicles as a suitable non-invasive source for diagnostic purposes in bladder cancer, Cancers (Basel) 6 (2014) 179–192, https://doi.org/10.3390/cancers6010179.
[250] S.Y. Lin, et al., Proteome profiling of urinary exosomes identifies alpha 1-antitrypsin and H2B1K as diagnostic and prognostic biomarkers for urothelial carcinoma, Sci. Rep. 6 (2016) 34446, , https://doi.org/10.1038/srep34446.
[251] C.R. Silvers, et al., Identification of extracellular vesicle-borne periostin as a feature of muscle-invasive bladder cancer, Oncotarget 7 (2016) 23335–23345, https://doi.org/10.18632/oncotarget.8024.

[252] C.R. Silvers, H. Miyamoto, E.M. Messing, G.J. Netto, Y.F. Lee, Characterization of urinary extracellular vesicle proteins in muscle-invasive bladder cancer, Oncotarget 8 (2017) 91199–91208, https://doi.org/10.18632/oncotarget.20043.
[253] D.A. Armstrong, B.B. Green, J.D. Seigne, A.R. Schned, C.J. Marsit, MicroRNA molecular profiling from matched tumor and bio-fluids in bladder cancer, Mol. Cancer 14 (2015) 194, , https://doi.org/10.1186/s12943-015-0466-2.
[254] C. Berrondo, et al., Expression of the long non-coding RNA HOTAIR correlates with disease progression in bladder cancer and is contained in bladder cancer patient urinary exosomes, PLoS One 11 (2016) e0147236, , https://doi.org/10.1371/journal.pone.0147236.
[255] Z. Andreu, et al., Extracellular vesicles as a source for non-invasive biomarkers in bladder cancer progression, Eur. J. Pharm. Sci. 98 (2017) 70–79, https://doi.org/10.1016/j.ejps.2016.10.008.
[256] J.D. Long, et al., A non-invasive miRNA based assay to detect bladder cancer in cell-free urine, Am. J. Transl. Res. 7 (2015) 2500–2509.
[257] K. Matsuzaki, et al., MiR-21-5p in urinary extracellular vesicles is a novel biomarker of urothelial carcinoma, Oncotarget 8 (2017) 24668–24678, https://doi.org/10.18632/oncotarget.14969.
[258] M. Xue, et al., Hypoxic exosomes facilitate bladder tumor growth and development through transferring long non-coding RNA-UCA1, Mol. Cancer 16 (2017) 143, , https://doi.org/10.1186/s12943-017-0714-8.
[259] L. Yang, X.H. Wu, D. Wang, C.L. Luo, L.X. Chen, Bladder cancer cell-derived exosomes inhibit tumor cell apoptosis and induce cell proliferation in vitro, Mol. Med. Rep. 8 (2013) 1272–1278, https://doi.org/10.3892/mmr.2013.1634.
[260] C.J. Beckham, et al., Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression, J. Urol. 192 (2014) 583–592, https://doi.org/10.1016/j.juro.2014.02.035.
[261] M.S. Ostenfeld, et al., Cellular disposal of miR23b by RAB27-dependent exosome release is linked to acquisition of metastatic properties, Cancer Res. 74 (2014) 5758–5771, https://doi.org/10.1158/0008-5472.CAN-13-3512.
[262] N. Kumari, S. Saxena, U. Agrawal, Exosomal protein interactors as emerging therapeutic targets in urothelial bladder cancer, J. Egypt. Natl. Cancer Inst. 27 (2015) 51–58, https://doi.org/10.1016/j.jnci.2015.02.002.
[263] X. Jin, et al., Pre-instillation of tumor microparticles enhances intravesical chemotherapy of nonmuscle-invasive bladder cancer through a lysosomal pathway, Biomaterials 113 (2017) 93–104, https://doi.org/10.1016/j.biomaterials.2016.10.036.
[264] K.A. Greco, et al., PLK-1 silencing in bladder cancer by siRNA delivered with exosomes, Urology 91 (2016), https://doi.org/10.1016/j.urology.2016.01.028 (241 e241-247).
[265] T. Murakami, et al., Bladder cancer detection by urinary extracellular vesicle mRNA analysis, Oncotarget 9 (2018) 32810–32821, https://doi.org/10.18632/oncotarget.25998.
[266] S. Baumgart, et al., Exosomes of invasive urothelial carcinoma cells are characterized by a specific miRNA expression signature, Oncotarget 8 (2017) 58278–58291, https://doi.org/10.18632/oncotarget.17619.
[267] Y. Zhan, et al., Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer, Mol. Cancer 17 (2018) 142, , https://doi.org/10.1186/s12943-018-0893-y.
[268] S. Zhang, et al., Evaluation of serum exosomal LncRNA-based biomarker panel for diagnosis and recurrence prediction of bladder cancer, J. Cell. Mol. Med. (2018), https://doi.org/10.1111/jcmm.14042.
[269] R. Zheng, et al., Exosome-transmitted long non-coding RNA PTENP1 suppresses bladder cancer progression, Mol. Cancer 17 (2018) 143, , https://doi.org/10.1186/s12943-018-0880-3.
[270] J. Lee, et al., Altered proteome of extracellular vesicles derived from bladder cancer patients urine, Mol. Cells 41 (2018) 179–187, https://doi.org/10.14348/molcells.2018.2110.
[271] E. Jonasch, et al., State of the science: an update on renal cell carcinoma, Mol. Cancer Res. 10 (2012) 859–880, https://doi.org/10.1158/1541-7786.MCR-12-0117.
[272] P. Del Boccio, et al., A hyphenated microLC-Q-TOF-MS platform for exosomal lipidomics investigations: application to RCC urinary exosomes, Electrophoresis 33 (2012) 689–696, https://doi.org/10.1002/elps.201100375.
[273] F. Raimondo, et al., Differential protein profiling of renal cell carcinoma urinary exosomes, Mol. BioSyst. 9 (2013) 1220–1233, https://doi.org/10.1039/c3mb25582d.
[274] G. De Palma, et al., The three-gene signature in urinary extracellular vesicles from patients with clear cell renal cell carcinoma, J. Cancer 7 (2016) 1960–1967, https://doi.org/10.7150/jca.16123.
[275] W. Zhang, et al., MicroRNAs in serum exosomes as potential biomarkers in clear-cell renal cell carcinoma, Eur. Urol. Focus (2016), https://doi.org/10.1016/j.euf.2016.09.007.
[276] H. Butz, et al., Exosomal MicroRNAs are diagnostic biomarkers and can mediate cell-cell communication in renal cell carcinoma, Eur. Urol. Focus 2 (2016) 210–218, https://doi.org/10.1016/j.euf.2015.11.006.
[277] M. Du, et al., Plasma exosomal miRNAs-based prognosis in metastatic kidney cancer, Oncotarget 8 (2017) 63703–63714, https://doi.org/10.18632/oncotarget.19476.
[278] N. Fujii, et al., Extracellular miR-224 as a prognostic marker for clear cell renal cell carcinoma, Oncotarget 8 (2017) 109877–109888, https://doi.org/10.18632/oncotarget.22436.
[279] G. Chen, Y. Zhang, X. Wu, 786-0 renal cancer cell line-derived exosomes promote 786-0 cell migration and invasion in vitro, Oncol. Lett. 7 (2014) 1576–1580, https://doi.org/10.3892/ol.2014.1962.
[280] K. Jingushi, et al., Extracellular vesicles isolated from human renal cell carcinoma tissues disrupt vascular endothelial cell morphology via azurocidin, Int. J. Cancer 142 (2018) 607–617, https://doi.org/10.1002/ijc.31080.
[281] J. Diao, et al., Exosomal Hsp70 mediates immunosuppressive activity of the myeloid-derived suppressor cells via phosphorylation of Stat3, Med. Oncol. 32 (2015) 453, , https://doi.org/10.1007/s12032-014-0453-2.
[282] L. Yang, X. Wu, D. Wang, C. Luo, L. Chen, Renal carcinoma cell-derived exosomes induce human immortalized line of Jurkat T lymphocyte apoptosis in vitro, Urol. Int. 91 (2013) 363–369, https://doi.org/10.1159/000348747.
[283] Y. Xia, et al., Negative regulation of tumor-infiltrating NK cell in clear cell renal cell carcinoma patients through the exosomal pathway, Oncotarget 8 (2017) 37783–37795, https://doi.org/10.18632/oncotarget.16354.
[284] R.S. Lindoso, F. Collino, G. Camussi, Extracellular vesicles derived from renal cancer stem cells induce a pro-tumorigenic phenotype in mesenchymal stromal cells, Oncotarget 6 (2015) 7959–7969, https://doi.org/10.18632/oncotarget.3503.
[285] C. Grange, et al., Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche, Cancer Res. 71 (2011) 5346–5356, https://doi.org/10.1158/0008-5472.CAN-11-0241.
[286] L. Qu, et al., Exosome-transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA, Cancer Cell 29 (2016) 653–668, https://doi.org/10.1016/j.ccell.2016.03.004.
[287] Y. Zhang, et al., Exosomes derived from IL-12-anchored renal cancer cells increase induction of specific antitumor response in vitro: a novel vaccine for renal cell carcinoma, Int. J. Oncol. 36 (2009), https://doi.org/10.3892/ijo_00000484.
[288] V.C. Crentsil, H. Liu, D.F. Sellitti, Comparison of exosomal microRNAs secreted by 786-O clear cell renal carcinoma cells and HK-2 proximal tubule-derived cells in culture identifies microRNA-205 as a potential biomarker of clear cell renal carcinoma, Oncol. Lett. 16 (2018) 1285–1290, https://doi.org/10.3892/ol.2018.8751.