Application of extracellular vesicles in the diagnosis and treatment of prostate cancer: implications for clinical practice

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Abstract

Extracellular vesicles (EV) are cell-derived lipid bilayer-delimited structures that are responsible for the transfer of various biomolecules, providing an important means of intercellular communication. Recent studies have shown that EV, particularly exosomes and large oncosomes contain a range of biomolecules including miRNA and proteins, which have crucial roles in prostate cancer (PCa) progression, metastasis and treatment resistance. This includes not just EV released from PCa cells, but also from other cells in the prostate tumor microenvironment. EV released from PCa patients have a unique composition compared to EV secreted from healthy controls and patients with benign prostatic diseases. As such, EV show promise to be used as liquid biopsy biomarkers to diagnose PCa, both as an adjunct and alternative to the invasive current gold standard – transrectal ultrasound guided biopsy of the prostate. Additionally, various biomolecules in EV could be utilized to guide treatment decisions in PCa by stratifying patients based on PCa risk, as well as predicting response to the plethora of agents used in PCa including hormonal therapy, chemotherapy, immunotherapy and targeted therapy. Here, we present a summary of the current evidence on the role of EV in PCa progression, metastasis and resistance, and discuss the application of EV in PCa diagnosis and treatment to optimize patient outcomes.

Introduction

Prostate cancer (PCa) is the second most common cancer in men worldwide, with 1,280,000 newly diagnosed cases and over 350,000 deaths in 2018.[1,2] With the incidence of PCa doubling over the past two decades, there is an urgent need for early and accurate diagnosis, and prompt treatment to increase survival rate.[3] Unfortunately, the gold standard for PCa diagnosis involves a transrectal ultrasound guided prostate biopsy (TRUS) which is highly invasive to patients.[4] In recent years, rapid advances have been made in the study of extracellular vesicles (EV) in PCa, prompting the prospect of a novel, less invasive and more accurate means of diagnosis. EV are nanoscale vesicles bounded by a lipid bilayer and are released by nearly all cell types that have a role in intercellular communication. Although there has been extensive debate on the nomenclature of these vesicles, more recently a consensus has been reached by the International Society of Extracellular Vesicles (ISEV) to create a defined terminology based on size, functionality, and experimental isolation techniques.[5-7] EV are generally categorized into different types – exosomes, microvesicles, and apoptotic bodies.[8] In addition, due to the prominent

roles that EV play in various cancers, a fourth category called large oncosomes (LO) has been defined. LO refer to large, micrometer-sized EV derived from cancer cells, containing oncogenic cargo and remnants of the tumor cells they originated from, and were initially described in human glioblastoma and PCa.[9,10] Studies to date on PCa have demonstrated that exosomes and LO are the most relevant categories of EV to be involved in prostate carcinogenesis, tumor progression and metastasis.[11-14] Understanding the role of exosomes and LO in PCa is critical as they could potentially serve as a source of PCa-specific markers in liquid biopsy (LB), and be utilized to diagnose and prognosticate PCa, and guide treatment protocols. This review will summarize the latest evidence on the role of exosomes and LO, 1) in prostate carcinogenesis, progression and metastasis; 2) as markers in LB; and 3) as prognostic markers to guide treatment decisions in PCa. A summary of candidate PCa biomarkers identified in EV and their potential applications in PCa diagnosis and management has been presented in **Figure 1**.

Exosomes and their relevance to prostate cancer

Exosomes are small EV (30-150 nm) produced by all eukaryotic cells, and are primarily involved in intercellular communication.[15] Exosomes are produced by the invagination of the plasma membrane, forming early endosomes that then mature to form multivesicular bodies (MVB) containing intraluminal vesicles (ILV).[15] In response to different stimuli, MVB will fuse with the plasma membrane and release the ILV into the extracellular space, which once released are known as exosomes.[15] Exosomes are surrounded by a lipid bilayer membrane enriched in cholesterol, sphingomyelin, ceramide and phosphatidylserine.[15] Prior to the release of exosomes from cells, their variable cargo content is sorted, including proteins, lipid and genetic materials, which is regulated mainly by endosomal sorting complexes required for transport (ESCRT) machinery that includes proteins such as Alix and the tumor susceptibility gene 101 (Tsg101).[15] In addition to ESCRT, there are other processes responsible for cargo sorting and exosome biogenesis.[16] These processes involve proteins such as sphingomyelinase and syntenin, together with ADP-ribosylation factor 6 (ARF6) and its effector phospholipase D2 (PLD2).[16-18] Several factors are known to induce exosome release, including but not limited to hypoxia and interference with endolysosomal trafficking.[19,20] Following their release, exosomes are taken up by the target cells through different mechanisms including endocytosis, micropinocytosis, phagocytosis or direct membrane fusion, triggering numerous downstream effects.[15,21] In the context of cancer,

exosomes could be released from tumor cells, or surrounding stromal and immune cells in the tumor microenvironment, where they are known to mediate a series of events essential for tumor growth, drug-resistance, and progression.[22-25] As such, studying exosomes as potential PCa diagnostic markers has recently become an area of intense interest. To study the cargo content of exosomes, they need to first be isolated through various techniques including but not limited to differential centrifugation, density gradient ultracentrifugation, size-exclusive chromatography, immunoprecipitation, and most recently microfluidics.[26,27,] After isolation, exosomes are identified through a combination of analyses for their size, morphology and specific molecules using numerous methods including nanoparticle tracking analysis (NTA), nanoscale flow cytometry (nanoFACS), transmission electron microscopy (TEM), and Western blot analysis.[27-29] Exosomal cargo can further be comprehensively analyzed through proteomic, lipidomic, transcriptomic, and genomic analyses using various techniques such as antibody array, mass spectroscopy, and next-generation sequencing.[30] The specific roles of exosomes in prostate cancer, as shown through numerous preclinical and clinical studies, will be discussed in this review.

Large oncosomes and their relevance to prostate cancer

LO are large (1-10 μm) cancer-derived EV produced by outward budding from the plasma membrane of cancer cells, and are responsible for the intercellular transfer of oncogenic signals.[11] To study LO, similar methods of isolation and analysis can be adopted as those used for exosomes.[27,30] Since LO arise from the plasma membrane, as do microvesicles, it is thought that the mechanism of biogenesis of the two is comparable.[31] For instance, the small GTP-binding protein ARF6, which is highly expressed in microvesicles and is involved in microvesicle shedding, is also highly expressed in LO and thought to be responsible for oncosome release.[12,32] In the context of PCa, several mechanisms contributing to the abscission of LO have been identified, including silencing of cytoskeletal regulator diaphanous-related formin-3 (DIAPH3) by ERK and stimulation of epidermal growth factor (EGFR) and Akt1.[10,33] The concept of LO was first described by Al-Nedawi et al. (2008), who demonstrated intercellular transfer of EGFRvIII from EGFRvIII-expressing glioma cells to cells lacking EGFRvIII through LO, triggering a cascade of oncogenic signaling pathways including MAPK and Akt, and alteration in the expression of genes regulated by EGFRvIII including Bcl-xL, p27 and VEGF.[9] This transformed the target cells to attain a more aggressive phenotype, and provided the first evidence that

phenotypic transformation in cancer cells could be induced by another mechanism – oncosome exchange – in addition to the traditional paracrine signaling.[9] Similar transfer of pro-tumorigenic properties by means of LO has been observed in other cancers, such as breast cancer and PCa, suggesting that LO play a critical role in cancer progression and could serve as a therapeutic target.[10,34] Additionally, LO have been known to confer drug resistance to cancer cells.[35] The use of LO as a diagnostic tool and potential therapeutic targets in cancer, as demonstrated through various preclinical and clinical studies will be discussed in detail in this review.

Exosomes and large oncosomes in prostate cancer progression

It is now well established that cancers are not merely a clump of malignant cells, but are comprised of a stroma that includes, but is not limited to, immune cells, fibroblasts, endothelial cells, cytokines, growth factors, extracellular matrix (ECM), and EV.[36] All of these components together form the tumor microenvironment.[36] Malignant cells have constant and dynamic interaction with various cells in the tumor microenvironment through the release of extracellular signals in an autocrine manner or using EV as signal carriers.[36] EV could be released from both tumor cells and other cells in the tumor microenvironment, which exert distinct and unique effects on one another.[37] They mediate events which facilitate tumor progression, metastasis and drug-resistance.[37]

Exosomes and LO secreted by cells in the tumor microenvironment contribute to tumor progression in many ways, such as enhancing the proliferation of tumor cells, dampening the anti-tumor immune response, and promoting angiogenesis.[37] For instance, previous *in vitro* study using LNCaP and DUCaP cells has demonstrated that tumor-derived exosomes enriched with CD9, a tetraspanin protein involved in adhesion, motility, membrane fusion, and cell signaling, as well as an upstream regulator of the androgen receptor, stimulate the proliferation of PCa cells in androgen-restrictive conditions.[38] Another *in vitro* study by Minciacchi et al. (2015) demonstrated that large LO contain GOT1 which promotes glutamine metabolism in recipient DU145 PCa cells, thus providing an abundant substrate for PCa cells and stimulating their proliferation.[39] Additionally, Ciardello et al. (2019) discovered that LO derived from DU145 PCa cells, harboring αV-integrin on their surface, augmented the adhesion and invasion of neighboring cells via the Akt pathway.[11] Reduction in PCa invasion was observed when the LO were incubated with αV-integrin blocking antibody and Akt inhibitor.[11]

The above findings matched our previous study (2016), which demonstrated a significant increase in cell migration in LNCaP and RWPE-1 cells when AR positive, AR negative, and benign epithelial prostate cells were incubated with PCa-derived exosomes.[40] As part of the same study, we showed for the first time that tumor volume and serum PSA levels were higher in xenograft-bearing mice infused with DU145 cell-derived exosomes.[40] In a study by Webber et al. (2015), DU145 cell-derived exosomes were found to act as a carrier for TGF-β, which transformed lung fibroblasts to cancer associated fibroblasts (CAF) or myofibroblasts through the activation of TGF-β/Smad3 signaling.[24] This transformation influenced cancer progression in a number of ways. First, myofibroblasts promoted pathological fibrosis which altered ECM dynamics, resulting in enhanced local invasiveness while facilitating tumor progression.[41] Second, miR-21 and miR-409 contained within myofibroblast-derived exosomes promoted PCa proliferation and invasion.[42,43] Like exosomes, LO harboring sustained Akt1 kinase activity have been shown to reprogram normal prostate fibroblasts to a myofibroblast-like phenotype, which is capable of angiogenesis and can in turn promote tumor progression in mice.[44] Another mechanism by which EV can promote cancer progression is by dampening the anti-tumor immune response. For instance, Lundholm et al. (2014) demonstrated that through an *in vitro* study that 22Rv1 PCa cells-derived exosomes bearing ligands for NKG2D (ULBP1-2 and MICA/B) can bind to NKG2D receptors found in NK and CD8+ T-cells derived from healthy donors, causing downregulation of NKG2D receptors and disruption of the anti-tumor functions of NK and CD8+ T-cells.[23] Furthermore, DU145 cell-derived exosomes containing prostaglandin E2 (PGE2) were found to stimulate CD73 expression on dendritic cells, resulting in the inhibition of TNFα and IL-12 production which in turn impaired CD8+ T-cell function.[45]

Similarly, human-derived macrophages and peripheral blood mononuclear cells were found to be suppressed when incubated with LO derived from DIAPH3-silenced DU145 PCa cells, which was mediated by the horizontal transfer of miR-125a contained in LO.[33] More recently, Poggio et al. (2019) demonstrated that exosomal PD-L1 derived from wild type TRAMP-C2 cells substantially dampened T-cell mediated immune response, as evidenced by significant reduction in the proportion of CD8+ T cells in a preclinical C57BL/6 mouse model.[46] In line with this observation, genetic blockade of exosomal PD-L1 produced significant anti-tumoral immunity.[46] A recent in-vitro study by Guan et al. (2020) demonstrated an exosome-mediated functional miR-95 transfer from THP-1 and M2 tumor-associated macrophages (TAM) to PC3 and DU145 PCa

cells upon their co-culture.[47] This process led to enhanced cell proliferation, invasiveness, and epithelial-to-mesenchymal transition (EMT), resulting in a more metastatic PCa phenotype.[47] All of the above studies demonstrated an EV-mediated bidirectional communication of bio-active molecules between stromal and cancerous cells, which is believed to be pivotal in cancer progression, EMT, pre-metastatic niche formation, and metastasis.

Large oncosomes and exosomes in prostate cancer metastasis

EV have been shown to mediate the development of a pre-metastatic niche, which is an important step to metastasis. As our team has previously demonstrated, exosomes play a unique role in enabling communication among multiple cell types and inducing phenotypic changes by modulating the tumor microenvironment.[48] This occurs through the transfer of bio-active molecules, such as genetic materials, lipids, receptor shuttling, and functional proteins, just to name a few. Their unique and targeted ability allows exosomes to take on the role of the 'seed' as proposed by Stephen Paget over 100 years ago in his 'seed and soil' doctrine.[49]

Previous studies have shown that elevated levels of miR-141 contained in exosomes derived from PCa patients, which are involved in osteoblastogenesis, can assist in the colonization of bone by PCa cells as higher level of serum miR-141 is associated with more bone lesions.[22,50] The PCa-derived exosomes act by increasing the levels of receptor activator for nuclear factor-κB ligand (RANKL) and the release of relevant growth factors such as fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), and transforming growth factor-β (TGF-β).[22,51,52] These biomolecules then initiate the process of reprograming the stromal compartment. As described above, the transformation of normal stromal fibroblasts into myofibroblasts leads to a further upregulation and secretion of growth factors, creating a vicious cycle and a 'pro-metastatic' niche.[24,53,54] The initiation of metastasis is known to be facilitated by EMT, for which exosomes are one of the significant mediators.[55] For instance, patient cancer-associated prostate stromal fibroblasts-derived exosomes containing miR-409 can promote EMT via the inhibition of stromal antigen 2 (STAG2) and Ras suppressor 1 (RSU1) in-vitro and in-vivo.[55] Furthermore, a number of integrins, particularly integrin α3, integrin β1, and integrin αvβ6 contained in exosomes derived from various PCa cell lines such as LNCaP, PC3 and RWPE cells can mediate EMT by inducing alterations in cell-ECM interactions, through binding to membrane Type-1 matrix metalloproteinase (MT1-MMP).[56-58] The tumor microenvironment can also be primed for

metastasis through the action of MMP9 and MMP2 harbored in LNCaP/MyrAkt1 PCa cell-derived LO, which degrade the ECM and facilitate tumor invasion.[12] These studies shed light on the role of PCa-derived exosomes in creating a pre-metastatic niche by modulating the tumor microenvironment, as well as by triggering the PCa metastatic process through EMT. Exosomes may therefore have great potential to be utilized as diagnostic and prognostic biomarkers, and more importantly as therapeutic targets in the fight against metastatic PCa.

Large oncosomes and exosomes as liquid biopsy biomarkers for prostate cancer

LB is a non-invasive diagnostic approach whereby biomarkers from biological fluids such as blood, urine, saliva or cerebrospinal fluid are analyzed to detect disease and guide treatment decisions.[59] The use of LB has been studied in various types of cancer, with mostly promising results.[59] In PCa, LB has several advantages over TRUS which is the current gold standard for PCa diagnosis. First, LB eliminates the potential complications of TRUS such as rectal bleeding, hematuria, dysuria, infection and sexual dysfunction for several weeks.[60] Second, LB analysis can be performed more rapidly than TRUS which usually takes several days. This is now aided by the increasing availability of commercial assays such as ExosomeDx, developed by Bioteche which allows high-throughput analysis of EV through LB.[61] Third, LB can offer more sensitive and specific detection of tumor cells, which may provide earlier cancer detection and thus offer a more favorable alternative in monitoring cancer progression and treatment response compared to conventional imaging and PSA. Last but not least, in addition to delivering a diagnosis, LB can provide additional valuable information compared to TRUS, which can be used to guide treatment decisions and improve outcomes. A variety of biological markers can be used in LB, including circulating tumor cells (CTCs), cell-free DNA (cfDNA) and EV.[59] Of these, EV may be considered the most superior since they are more abundant than CTCs, and more stable than cfDNA since they are surrounded by a lipid bilayer.[62,63]

In our previous study (2012), we found more than 50 exosomal proteins with potential biomarker applications (e.g. CLSTN1, FASN, FLNC, and GDF15) in different PCa cell line-derived exosomes.[48] Since then, EV have been studied in a range of human body fluids, primarily in urine and blood serum for use as LB markers in PCa. For instance, EV derived from the plasma of PCa patients have been shown to contain several proteins at greatly elevated levels compared to healthy controls, such as PTEN, CK18 and PSA.[39,64,65] Other EV biomarkers can be used to

distinguish between PCa and other prostate pathologies such as benign prostatic hyperplasia (BPH). For example, levels of PSMA, survivin, GGT and PSA were shown to be significantly increased in EV obtained from PCa patients compared to BPH patients.[66-68] While blood-based EV biomarkers may appear promising, a major challenge in their translation to clinical use arises from the potential presence of contaminants following EV isolation. An example is plasma lipoproteins with similar buoyant density and physical size as EV, which can potentially produce false negatives and positives if the isolate is used for clinical diagnosis.[69] In addition to blood-derived EV, urine-derived EV have been studied extensively as LB biomarkers for PCa. Øverbye et al. (2015) showed that 221 proteins were elevated in urinary exosomes of PCa patients relative to healthy controls in a comprehensive proteomic analysis.[70] Of these, the combination of two proteins, transmembrane protein 256 (TM256) and LAMTOR1, could produce high specificity and sensitivity of up to 100% in PCa diagnosis.[70] Urine-derived EV from PCa patients have been found to contain elevated levels of several other proteins compared to healthy controls, including VAT, ADIRF, Flotillin1, Flotillin2, Rab3B, FABP5 and PARK7, which have potential to be explored as new PCa biomarkers.[70-72] A multi-panel test that combines a selection of these biomarkers could be useful in further maximizing diagnostic accuracy.

EV to guide treatment in prostate cancer

EV have great potential to be utilized as an alternative or complementary method in PCa diagnosis, management and optimization of survival outcomes. Currently, the management of newly diagnosed non-metastatic PCa is dependent on the assessed risk. Low risk PCa, defined by PSA<10, Gleason grade group (GGG) 1, and clinical stage T1-T2a is typically managed by active surveillance, while high risk, defined by PSA>20, GGG 4-5, and clinical stage ≥T3 is treated by radical prostatectomy or radiotherapy with or without adjuvant therapy.[73] The stratification of PCa based on the aforementioned criteria has several limitations. First, PSA is known to be a non-specific PCa marker as it is also elevated in a number of benign prostatic diseases including BPH and prostatitis.[74] Second, GGG is determined by analyzing the prostate obtained by the TRUS procedure, which is an invasive procedure. Recently, the application of EV biomarkers to stratify PCa patients into low-risk and high-risk groups has been explored. Using proteomics and immunoblotting analysis, Sequeiros et al. (2017) identified an array of proteins, including CD63, putative glycerol kinase 5 (GLPK5), SPHM, PSA and prostatic acid phosphatase (PAPP), in

urinary EV which was more elevated in patients with high-risk PCa compared to low-risk PCa.[75] Additionally, higher quantities of Cav-1 and αV-integrin-positive LO were found in the plasma of patients with high-grade PCa relative to low-grade PCa.[11,76] These biomarkers could be combined to produce a multi-panel test kit to improve stratification accuracy. Thus, EV could provide a solution for addressing some of the limitations of current standard practices in PCa risk stratification, thereby preventing the overtreatment or undertreatment of patients, and ultimately optimizing patient outcomes.

The initial selection of pharmacotherapy for patients with locally advanced or metastatic PCa remains a challenge due to the lack of biomarkers that can accurately predict therapeutic response. EV may be exploited to address this problem, since they can be utilized as biomarkers of therapeutic response to the various agents used in PCa, including but not limited to docetaxel and novel anti-androgens. Peak et al. (2017) showed that enzalutamide-resistant C4-2B-R cells secreted 2.67-fold higher levels of exosomes relative to enzalutamide-sensitive C4-2B-S cells.[77] Proteomics analysis revealed that exosomes released from C4-2B-R cells contained higher levels of AR-V7 than those from C4-2B-S cells.[77] In the same study, inhibition of exosome biogenesis using GW4869 and dimethyl amiloride enhanced the sensitivity of C4-2B-R cells to enzalutamide, implicating the role of AR-V7 in providing resistance to hormonal treatment.[77] Additionally, Re et al. (2017) demonstrated that AR-V7 in plasma-derived exosomal RNA could be used to predict response in metastatic PCa patients treated with abiraterone or enzalutamide in the second-line setting.[78] The median progression-free survival and overall survival were significantly shorter in AR-V7-positive patients relative to AR-V7-negative patients (3 vs 20 months, $p<0.001$; 8 months vs not reached; $p<0.001$ respectively).[78] Thus, PCa patients who are AR-V7-positive as assessed through LB could be offered other treatment options such as docetaxel to optimize survival outcomes, whereas patients who are AR-V7-negative could be offered androgen deprivation therapy (ADT).

It has been shown that long-term treatment of PCa adenocarcinoma with ADT and novel anti-androgens can promote their differentiation into the neuroendocrine subtype which is substantially more aggressive, with a median cancer-specific survival of less than 2 years.[79-81] A different treatment protocol is necessary for the neuroendocrine phenotype, which is typically managed with cisplatin-based chemotherapy regimens in combination with etoposide.[82] This

coupled with the aggressive nature of the tumor mandates detection as early as possible. While there is no established biomarker in clinical practice which could predict neuroendocrine differentiation, Bhagirath et al. (2019) recently identified two transcription factors, BRN4 and BRN2, in serum-derived EV of PCa patients that were shown to be responsible for the oncogenic reprogramming of PCa adenocarcinoma to the neuroendocrine subtype.[83] These EV biomarkers could be used to predict neuroendocrine differentiation in PCa patients who have been treated long-term with ADT and novel anti-androgens to allow early detection and prescription of the correct treatment.

Various markers in PCa-derived EV could be used to predict patient response to docetaxel. Kharaziha et al. (2015) demonstrated that exosomes derived from DU145 taxane-resistant PCa cells (DU145 Tax-Res) and PCa patients not responding to taxanes contained a unique set of proteins, particularly MDR-1, MDR-3, Endophilin-A2 and PABP4, which were not found in exosomes derived from taxane-sensitive (DU145 Tax-Sen) DU145 PCa cells and PCa patients responding to taxanes.[84] Furthermore, increased levels of exosomal MDR-1/P-gp, integrin β4 and vinculin, and decreased levels of exosomal miR-34a were associated with docetaxel resistance.[13,85-87] Interestingly, increased serum exosomal P-gp levels were not associated with cabazitaxel resistance, implying that patients could be offered cabazitaxel if there was evidence of increased serum exosomal P-gp, particularly if they have previously failed to respond to docetaxel.[87] . Finally, the aforementioned exosomal biomarkers could be used to select the use of docetaxel or abiraterone in the metastatic setting, where the choice between the two agents would be guided by disease volume – currently an equivocal biomarker as two large phase 3 clinical trials evaluating docetaxel in low- and high-volume PCa showed different results.[88,89]

EV could find additional applications in predicting the response of PCa patients to immunotherapy. This could help overcome some of the challenges faced in using imperfect biomarkers to predict responses to immunotherapy, such as PD-L1 and CTLA-4 expression. While a high PD-L1 expression generally correlates with a higher response rate to anti-PD-1 therapy, this may not be true in PCa due to low surface PD-L1 expression in PCa cells, since the majority of PD-L1 expression is in exosomes.[46] In light of this, high exosomal PD-L1 could be a better predictor of response to anti-PD-L1 therapy, especially considering the recent findings from the KEYNOTE-199 study where PD-L1 expression on prostate biopsy was found to be not predictive

of response rate (objective response rate: 5% in PD-L1 positive and 3% in PD-L1 negative tumors).[90] Leading on from this, an antibody designed to specifically target exosomal PD-L1 may provide an improved predictor for the response to anti-PD-L1 therapy. Additionally, promising results from the use of EV in cancer immunotherapy for other cancers provide inspiration for similar uses in PCa. For instance, Morishita et al (2016) demonstrated that the delivery of exosomes containing melanoma-associated antigen and streptavidin-lactadherin to immunized B16-BL6 tumor-bearing mice produced an effective anti-tumor response.[91] A similar therapy could be tested for the treatment of PCa, whereby EV could be loaded with PCa-specific antigens such as prostate-specific membrane antigen, prostate stem-cell antigen, and MAGE-C2/CT10 as potential targets of immunotherapy.

Apart from immunotherapy, EV could be utilized to predict response to PARP inhibitors, which have been recently studied in PCa patients in numerous trials.[92-94] It remains a challenge to select patients who are anticipated to derive the most benefits from PARP inhibitors, with the current standard predictors being BRCA mutant phenotype and superior prior response to platinum-based therapy.[95] One of the major problems is that up to 20% of reports of BRCA mutation screening yields variants of uncertain clinical significance (VUS), which creates confusion regarding actual BRCA status and thus eligibility for PARP inhibitor therapy.[96] Patel et al. (2016) explored exosomal mutations in DNA repair genes of tumors acquired from PCa patients, and observed a trend towards a relationship between BRCA2 and MLL2 mutation ($p=0.055$), with the mutation non-randomly distributed in clusters across BRCA2 exons.[97] While these findings require further validation, MLL2 in exosomes may be useful as a biomarker in predicting response to PARP inhibitors. Furthermore, proteomic analysis of exosomes derived from BRCA1-deficient and BRCA1-proficient murine breast tumor models revealed that the levels of TOP1 and CDH3 were significantly higher in the former than the latter ($p=0.002$ and $p<0.001$, respectively), suggesting that exosomal TOP1 and CDH3 may be used to predict BRCA mutation status and thus response to PARP inhibitors.[98]

To date, numerous prospective trials have been conducted assessing the use of exosomes as LB biomarker in PCa. McKiernan et al. (2016) evaluated the results of exosome gene expression assay (EGEA), also known as ExoDx Prostate IntelliScore (EPI) test, and biopsy outcomes in 499 patients with PSA levels of 2 to 20 ng/mL, and discovered that applying an EGEA cut-off score

of >15.6 to signal a biopsy could have led to 20% of biopsies being averted and missing only 2% of all PCa with Gleason Score>7.[61] In a similar study by the same group assessing EGEA and biopsy outcomes in 503 patients with a median PSA of 5.4 ng/mL, a cut-off score of 15.6 averted 26% of unnecessary prostate biopsies and missed 7% of PCa with GGG>2.[99] Furthermore, Tutrone et al. (2020) conducted the first blinded prospective, double-arm trial of 1094 patients who were considered for prostate biopsy in which they were randomized to EPI and control arm, with all patients receiving the EPI test but only the EPI arm receiving results for their biopsy decision. This study revealed that the detection of high grade PCa (HGPC) was 30% higher in the EPI arm relative to the control arm, and predicted that 49% fewer HGPC was missed due to the EPI test.[100] These promising results led to the Food and Drug Administration (FDA) granting a Breakthrough Device Designation (BDD) to the EPI test.[101]

Problems with using EV as liquid biopsy biomarkers

Despite demonstrating great potential, there are two major limitations surrounding the use of EV as LB biomarkers, particularly when considering their use in a clinical setting. Both of these limitations are associated with the evolving discovery of new knowledge in the young field of EV research, and the relative lack of standardization of methods compared to more established fields. First, there are limitations in the current protocols used for the isolation and purification of EV, such that the homogeneity and purity of EV and of its subpopulations such as exosomes and LO following isolation are often suboptimal.[26,102] This is a particularly important problem when isolating EV from biological fluids such as serum plasma, which contain various lipoproteins and chylomicrons that have similar sizes compared to the EV of interest including exosomes and LO.[102] Second, there is a lack of standardization in the methods used for EV characterization and analysis, which makes the comparison of results among different studies arduous.[103] Going forward, it is an essential task for the EV field to improve the protocols used for EV isolation, purification and analysis, and to standardize these across PCa studies involving EV. This will help to maximize the accuracy of EV as LB biomarkers in PCa and hasten their translation to the clinic as a new generation of improved biomarkers and therapeutic targets.

Conclusion and future directions

In conclusion, EV serve as a carrier for a plethora of biomolecules including but not limited to proteins, nucleic acids and lipids, which contain oncogenic information crucial for PCa progression and metastasis. These biomolecules have shown immense potential to be utilized as LB biomarkers that could improve PCa diagnosis and aid clinicians in determining the most appropriate therapy for patients to optimize outcomes. Future directions in the field should include conducting large prospective studies to investigate the application of various recently discovered serum and urinary EV biomarkers in PCa diagnosis, and in predicting the clinical response to hormonal therapy, targeted therapy and immunotherapy. Furthermore, the use of agents targeting EV in PCa patients should be evaluated in clinical trials, while exploring any potential correlations between EV quantity and clinical outcomes. Finally, with promising preclinical results, it could be worthwhile to investigate the use of EV as a biomolecule or drug carrier in clinical trials involving PCa patients.[104,105] New findings from a rapidly and constantly evolving field point to the significant promise of EV in refining the diagnosis and management of PCa and delivering improved therapies.

Author Contributions

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