The role of extracellular vesicles in prostate cancer with clinical applications

Yu-Ling Tai1,2, Chun-Jung Lin1, Tsai-Kun Li3, Tang-Long Shen2, Jer-Tsong Hsieh1 and Benjamin P C Chen4

1Department of Urology, University of Texas Southwestern Medical Center, Dallas, Texas, USA
2Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan
3Department and Graduate Institute of Microbiology, College of Medicine, National Taiwan University, Taipei, Taiwan
4Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Correspondence should be addressed to J T Hsieh: jt.hsieh@utsouthwestern.edu

Abstract

In mammalian cells, extracellular vesicles (EVs) derived from the endosomal system carry many different kinds of bioactive molecule to deliver to recipient cells in a paracrine or endocrine manner. EVs can mediate local and systemic intercellular communications, including reeducating stromal cells, remodeling the architecture of the tumor microenvironment, modulating cancer metabolism and metastases, or even conferring drug resistance. Because the molecular and functional characteristics of prostate cancer (PCa) evolve over time, the bioactive molecule profiles/signatures of tumor-derived EVs (TDEs) reflect the real-time status of cancer cells. TDEs appear to be valuable diagnostic and prognostic biomarkers as well as potential therapeutic vehicles, suggesting their essential role in precision medicine of disease management. We summarized critical aspects of TDEs in PCa and discussed their potential clinical applications.

Introduction

PCa is a typical androgen-dependent disease, the most frequently diagnosed cancer, and the leading cause of cancer death in men worldwide (Siegel et al. 2018). Currently, androgen-deprivation therapy (ADT) is the standard care for patients with metastatic PCa (Perlmutter & Lepor 2007); however, despite its high effectiveness, most patients progress to castration-resistant PCa (CRPC). Although the new generation of anti-androgen agents such as abiraterone or enzalutamide can prolong patient survival (Chen et al. 2009), CRPC eventually acquires a resistant phenotype that leads to mortality. Thus, treatment-induced resistance and lineage development have become a major challenge in patients with advanced PCa (Roubaud et al. 2017).

EVs such as endosome-derived exosomes or plasma membrane-derived ectosomes (microvesicles) function as novel mediators in either short- or long-distance communication (Tai et al. 2019). Although EVs can be produced by many normal cell types, TDEs can carry specific bioactive molecules that regulate the communication between tumor cells and their local or distant microenvironments (Zhao et al. 2016, Lin et al. 2017). Notably, a variety of bioactive molecules such as nucleic acids, proteins, metabolites, and lipids has been found in EVs (Tai et al. 2018, 2019); the EV membrane structure can prevent bioactive molecules from degradation (Koga et al. 2011). In general, EV is a key mediator to transfer bioactive molecules from donor cells to recipient cells during cancer malignancy (Tai et al. 2019). For example, the modification of tumor microenvironments by TDEs has been reported; pancreatic cancer cells-secreted TDEs contain miR-155 that is able to convert normal fibroblasts into cancer-
associated fibroblasts (CAFs) by targeting TP53INP1 mRNA expression (Pang et al. 2015). Also, TDEs can modulate the immune systems by inducing the polarization of tumor-associated macrophages (Ying et al. 2016). Regarding the TDE-mediated cancer metastasis, TDEs with specific integrin expression patterns can determine organotropic metastasis (Hoshino et al. 2015). For example, integrins α6β4 or α5β1-bearing TDEs are associated with lung or liver metastasis, respectively (Hoshino et al. 2015). TDEs can further establish pre-metastatic niche by educating distant microenvironments (Peinado et al. 2012). Additionally, TDEs can disrupt the function of the blood-brain barrier by modulating tight junction and lead to brain metastasis (Tominaga et al. 2015). On the other hand, some studies indicate a significant role of EVs derived from tumor microenvironments in the regulation of cancer cells. For example, the mitochondrial DNA packaged in EVs derived from CAFs can modulate the escape from therapy-induced metabolic dormancy in hormonal therapy-resistant cancer (Sansone et al. 2017). Also, Wnt-bearing EVs derived from fibroblasts confer chemo-resistant phenotype of cancer cells by activating Wnt/β-catenin signaling pathway (Hu et al. 2019b).

Recently, EVs derived from PCa or PCa’s surrounding microenvironment have been shown to actively regulate phenotypic changes including metabolism (Zhao et al. 2016), proliferation (Soekmadji et al. 2017), invasive/metastatic features (El-Sayed et al. 2017) associated with stromal reprogramming (Webber et al. 2015), and drug resistance (Corcoran et al. 2012, Panagopoulos et al. 2013). However, the procedures for EV isolation, storage, recovery, and the characterization from biofluids, such as plasma, serum, or urine, need to be optimized and standardized for clinical applications. The International Society for Extracellular Vesicles recently provided guidelines for EV studies (Thery et al. 2018), which are expected to be modified as new techniques are developed in the EV field (Thery et al. 2018). Nevertheless, many studies have provided valuable information for elucidating the potential role and application of EVs in PCa management. We provide a comprehensive overview of EVs in PCa development, progression, and drug resistance (Table 1). Additionally, we discuss the potential clinical applications of EVs (Table 2).

**TDEs in PCa proliferation**

Given the critical role of androgenic steroid hormones in PCa proliferation, increased secretion of CD9 positive TDEs was found in response to dihydrotestosterone (DHT) treatment (Soekmadji et al. 2017). Functionally, CD9-enriched EVs induce the growth of androgen receptor (AR)-positive PCa cells under androgen-deprived conditions (Soekmadji et al. 2017). In addition, a newly detected constitutively active AR splicing variant (AR-V7) in TDEs can stimulate the proliferation of PCa cells in the absence of DHT (Read et al. 2017). These studies indicate an important effect of EV on PCa proliferation regardless of either hormone-naïve or androgen-deprived conditions. Also, EVs can deliver EGF receptor variant III (EGFRvIII) to the nucleus of recipient PCa cells (Read et al. 2017); accumulating EGFRvIII, a mutant of EGFR without the ligand-binding domain, in the nucleus of CRPC is associated with poor overall patient survival (Edwards et al. 2006).

**TDEs in PCa metastasis**

TDE-mediated intercellular communication is a key regulator of PCa metastasis (El-Sayed et al. 2017). Recent studies have indicated that epithelial-to-mesenchymal transition (EMT) is a critical step in PCa metastasis (Lo et al. 2017). Indeed, TDEs have been shown to alter the EMT of normal or non-tumorigenic recipient cells (Souza et al. 2018). For example, mesenchymal-like TDE facilitates mesenchymal characteristics in recipient epithelial-like PCa cells (El-Sayed et al. 2017). The transformed recipient PCa cells exhibit modulated androgen receptor signaling and increased activity of transforming growth factor β signaling (El-Sayed et al. 2017). Functionally, mesenchymal-like TDEs confer increased migratory and invasive abilities for recipient epithelial-like PCa cells (El-Sayed et al. 2017). Likewise, the uptake of metastatic TDEs is shown to efficiently promote migration in recipient PCa cells (Lazaro-Ibanez et al. 2017). Because hypoxia seems to promote cancer progression, recent studies found that TDE is required for hypoxia-mediated PCa progression (Ramteke et al. 2015). For example, TDEs derived from PCa cells in hypoxic conditions exhibit increased metalloproteinase activity while TDEs in normoxic condition do not (Ramteke et al. 2015). Functionally, TDEs secreted under hypoxic conditions enhance the motile and invasive abilities of recipient naïve PCa cells (Ramteke et al. 2015), suggesting that the status of the tumor microenvironment affects PCa progression by regulating TDE contents. Through a paracrine mechanism, transferring integrin αvβ6 from donor PCa to recipient PCa via TDEs increases the migration of recipient PCa (Fedele et al. 2015). Additionally, integrin αvβ3-bearing TDEs are transferred from metastatic PCa cells to benign
The role of exosome in prostate cancer

Protein-induced osteoblast activity
Induce migration of prostate stromal cells
Singh Karlsson
Induce NED of PCa
Induce differentiation of PCa-promoting stromal cells
et al.
Induce procoagulant activity in PCa
Reduce PCa proliferation
Induce mesenchymal stem cell differentiation
Induce PCa proliferation
Induce osteoblast activity
Induce prostate tumorigenesis
Reduce T-cell activation and induce PCa growth
Immunosuppression of dendritic cell function
Reduce PCa proliferation
Induce differentiation of PCa-promoting stromal myofibroblasts
Induce mesenchymal stem cell differentiation into pro-angiogenic and pro-invasive myofibroblasts
Induce procoagulant activity in PCa microenvironment
Induce migration of prostate stromal cells

Table 1 Functional effects of EV bioactive molecules in PCa development and progression.

<table>
<thead>
<tr>
<th>EV bioactive molecules</th>
<th>Type of bioactive molecules</th>
<th>Functional effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRP</td>
<td>Protein</td>
<td>Induce NED of PCa</td>
<td>(Lin et al. 2017)</td>
</tr>
<tr>
<td>Amino acids and tricarboxylic acid-cycle intermediates</td>
<td>Metabolite</td>
<td>Modulate PCa metabolism</td>
<td>(Zhao et al. 2016)</td>
</tr>
<tr>
<td>AR and AR-V7</td>
<td>Protein</td>
<td>Induce PCa proliferation</td>
<td>(Read et al. 2017)</td>
</tr>
<tr>
<td>BRN4 and BRN2</td>
<td>mRNA</td>
<td>Induce oncogenic reprogramming of PCa cells to NEPC</td>
<td>(Bhagirath et al. 2019)</td>
</tr>
<tr>
<td>Caveolin-1</td>
<td>Protein</td>
<td>Induce PCa stemness</td>
<td>(Lin et al. 2019)</td>
</tr>
<tr>
<td>hsa-miR-940</td>
<td>miRNA</td>
<td>Increase osteogenic differentiation of mesenchymal stem cells</td>
<td>(Hashimoto et al. 2018)</td>
</tr>
<tr>
<td>Integrin αvβ3</td>
<td>Protein</td>
<td>Induce cell adhesion and migration</td>
<td>(Singh et al. 2016)</td>
</tr>
<tr>
<td>Integrin αvβ6</td>
<td>Protein</td>
<td>Induce M2 polarization in monocytes</td>
<td>(Li et al. 2015)</td>
</tr>
<tr>
<td>Integrin αvβ6</td>
<td>Protein</td>
<td>Induce PCa adhesion and migration</td>
<td>(Fedele et al. 2015)</td>
</tr>
<tr>
<td>miR-34a</td>
<td>miRNA</td>
<td>Induce docetaxel sensitivity of PCa</td>
<td>(Corcoran et al. 2014)</td>
</tr>
<tr>
<td>miR-143</td>
<td>miRNA</td>
<td>Reduce PCa proliferation</td>
<td>(Kosaka et al. 2012)</td>
</tr>
<tr>
<td>miR-375</td>
<td>miRNA</td>
<td>Induce osteoblast activity</td>
<td>(Li et al. 2019)</td>
</tr>
<tr>
<td>miR-409</td>
<td>miRNA</td>
<td>Induce prostate tumorigenesis</td>
<td>(Jossen et al. 2015)</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Protein</td>
<td>Reduce T-cell activation and induce PCa growth</td>
<td>(Poggio et al. 2019)</td>
</tr>
<tr>
<td>Prostaglandin E2</td>
<td>Prostanoid fatty acid derivative</td>
<td>Imunosuppression of dendritic cell function</td>
<td>(Salimu et al. 2017)</td>
</tr>
<tr>
<td>PTEN</td>
<td>Protein</td>
<td>Reduce PCa proliferation</td>
<td>(Gabriel et al. 2013)</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Protein</td>
<td>Induce differentiation of PCa-promoting stromal myofibroblasts</td>
<td>(Webber et al. 2015)</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Protein</td>
<td>Induce mesenchymal stem cell differentiation into pro-angiogenic and pro-invasive myofibroblasts</td>
<td>(Chowdhury et al. 2015)</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Protein</td>
<td>Induce procoagulant activity in PCa microenvironment</td>
<td>(Ali Saleh et al. 2018)</td>
</tr>
<tr>
<td>Transferring hyaluronidase 1</td>
<td>Protein</td>
<td>Induce migration of prostate stromal cells</td>
<td>(McAtee et al. 2019)</td>
</tr>
</tbody>
</table>

Prostatic hyperplasia (BPH) epithelial cells (Singh et al. 2016). Upon integrin αvβ3-bearing TDE uptake, non-tumorigenic recipient cells exhibit increased adhesive and migratory abilities in response to vitronectin, a αvβ3 ligand (Singh et al. 2016).

Bone metastases can commonly develop in PCa patients and are the major cause of death in advanced PCa (Jin et al. 2011). Several studies indicate that TDEs modulate osteoblasts or osteoclasts in the microenvironment of bone metastasis (Karlsson et al. 2016, Hashimoto et al. 2018). For example, TDE RNA molecules are transferred from PCa cells to recipient osteoblasts, which subsequently enhance osteoblast viability (Probert et al. 2019). Reciprocally, TDE-educated osteoblasts provide a supportive environment for PCa growth (Probert et al. 2019). Likewise, miR-375-bearing TDEs derived from PCa cells facilitate osteoblast functions, such as increased alkaline phosphatase activity and overexpressed osteoblast activity-associated markers (Li et al. 2019). Additionally, hsa-miR-940-bearing TDEs derived from PCa promote osteogenic differentiation of mesenchymal stem cells by downregulating Ras homology GTPase-activating protein 1, subsequently enhancing osteoblastic lesions in the bone microenvironment (Hashimoto et al. 2018). On the other hand, TDEs significantly impair osteoclast differentiation by downregulating miR-214 and ablating NF-κB signaling (Duan et al. 2019). In a murine PCa model, TDEs derived from TRAMP-C1 PCa ablate the expression of osteoclast differentiation markers (Karlsson et al. 2016). Functionally, TRAMP-C1 TDEs reduce the proliferation and differentiation of osteoclast progenitor cells (Karlsson et al. 2016). These studies suggest that TDE is a critical mediator in remodeling the microenvironment in bone metastasis.

TDEs in PCa lineage plasticity

Clinical evidence indicates that CRPC exhibits neuroendocrine differentiation (NED) phenotypes that correlate with therapy resistance and poor survival. Treatment-induced resistance and lineage development become a clinical challenge in advanced PCa, including neuroendocrine (NE) prostate cancer (NEPC) (Roubaud et al. 2017). Due to the resistance to anti-androgen agents as well as the positive correlation between...
NEPC and the alterations of androgen receptor (AR) gene signatures, treatment-induced selection of PCa population with relative low AR expression has been suggested to regulate the PCa lineage plasticity (Roubaud et al. 2017). Based on the whole-exome sequencing of biopsy samples from patients with castration-resistant adenocarcinoma or NE histologies, a consistent result suggested that low AR expression could involve in the clonal evolution of NEPC (Beltran et al. 2016). Mechanistically, recent studies identified candidate NE drivers, such as BRN2 (Bishop et al. 2016). In Table 2, we summarize the EV bioactive molecules used as diagnostic or prognostic biomarkers in PCa.

Table 2  EV bioactive molecules used as diagnostic or prognostic biomarkers in PCa.

<table>
<thead>
<tr>
<th>EV bioactive molecules</th>
<th>Type of bioactive molecules</th>
<th>Type of biofluids</th>
<th>Analytical approach</th>
<th>Expression level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR-V7</td>
<td>RNA</td>
<td>Plasma</td>
<td>PCR</td>
<td>Predict resistance to hormonal therapy in metastatic PCa patients</td>
<td>(Del Re et al. 2017)</td>
</tr>
<tr>
<td>BRN4 and BRN2</td>
<td>mRNA</td>
<td>Serum</td>
<td>PCR</td>
<td>Upregulation in CRPC patients with NE features compared to CRPC patients with adenocarcinoma features</td>
<td>(Bhagirath et al. 2019)</td>
</tr>
<tr>
<td>Fatty acid binding protein 5</td>
<td>Protein</td>
<td>Urine</td>
<td>Mass spectrometry-based proteomics</td>
<td>Upregulation in PCa patients compared to negative control individuals and is associated with high GS</td>
<td>(Fujita et al. 2017)</td>
</tr>
<tr>
<td>Flotillin 2 and Parkinson protein 7</td>
<td>Protein</td>
<td>Urine</td>
<td>ELISA</td>
<td>Upregulation in PCa patients compared to healthy individuals</td>
<td>(Wang et al. 2017)</td>
</tr>
<tr>
<td>Gamma-glutamyltransferase activity</td>
<td>Protein</td>
<td>Serum</td>
<td>Fluorescent probe, γ-glutamyl hydroxymethyl rhodamine green</td>
<td>Upregulation in PCa patients compared to BPH patients</td>
<td>(Kawakami et al. 2017)</td>
</tr>
<tr>
<td>IncRNA-p21</td>
<td>IncRNA</td>
<td>Urine</td>
<td>PCR</td>
<td>Upregulation in PCa patients compared to BPH patients</td>
<td>(Isin et al. 2015)</td>
</tr>
<tr>
<td>miR-19b</td>
<td>miRNA</td>
<td>Urine</td>
<td>PCR</td>
<td>Upregulation in PCa patients compared to healthy individuals</td>
<td>(Bryzgunova et al. 2016)</td>
</tr>
<tr>
<td>miR-21 and miR-375</td>
<td>miRNA</td>
<td>Urine</td>
<td>PCR</td>
<td>Upregulation in PCa patients compared to healthy individuals</td>
<td>(Foj et al. 2017)</td>
</tr>
<tr>
<td>miR-145</td>
<td>miRNA</td>
<td>Urine</td>
<td>PCR</td>
<td>Upregulation in PCa patients compared to BPH patients</td>
<td>(Xu et al. 2017)</td>
</tr>
<tr>
<td>miR-375 and miR-141</td>
<td>miRNA</td>
<td>Serum</td>
<td>PCR</td>
<td>Upregulation in recurrent metastatic PCa patients compared to nonrecurrrent PCa patients</td>
<td>(Bryant et al. 2012)</td>
</tr>
<tr>
<td>miR-375 and miR-1290</td>
<td>miRNA</td>
<td>Plasma</td>
<td>PCR</td>
<td>Upregulation in CRPC patients with 80% mortality rate</td>
<td>(Huang et al. 2015)</td>
</tr>
<tr>
<td>PCA3 and ERG ( EXO160 score)</td>
<td>RNA</td>
<td>Urine</td>
<td>PCR</td>
<td>EXO160 score + standard of care can discriminate between high GS and GS ≤6 PCa (including benign disease)</td>
<td>(Donovan et al. 2015)</td>
</tr>
<tr>
<td>PSA, CD63, GLPK5, SPHM, and PAPP PSA and CD81</td>
<td>Protein</td>
<td>Urine</td>
<td>Mass spectrometry-based proteomics ELISA and flow cytometry</td>
<td>Distinguish between high- and low-grade PCa patients compared to BPH or healthy individuals</td>
<td>(Sequeiros et al. 2017)</td>
</tr>
<tr>
<td>PTEN</td>
<td>Protein</td>
<td>Plasma</td>
<td>Western Blot</td>
<td>Upregulation in PCa patients compared to healthy individuals</td>
<td>(Gabriel et al. 2013)</td>
</tr>
<tr>
<td>Survivin</td>
<td>Protein</td>
<td>Plasma</td>
<td>Western Blot</td>
<td>Upregulation in PCa patients that have relapsed on Taxotere compared to healthy individuals</td>
<td>(Khan et al. 2012)</td>
</tr>
</tbody>
</table>
2017), EZH2 (Dardenne et al. 2016), or MYCN (Lee et al. 2016), which exhibit high correlation with AR or AR signals, involved in PCa lineage plasticity.

Although the role of lineage plasticity of CRPC leading to the appearance of NEPC has been delineated (Mu et al. 2017), the role of TDEs in this event remains largely unknown. Several studies have shown that NED phenotypes could be induced by TDEs (Lin et al. 2017, Bhagirath et al. 2019). For example, adipocyte differentiation-related protein (ADRP)-bearing EVs derived from interleukin-6 (IL-6)-stimulated PCa cells induce PCa NED through a paracrine mechanism (Lin et al. 2017). Both proteins and miRNAs of proneural POU-domain transcription factors (such as BRN2 and BRN4), NE drivers suppressed by AR (Bishop et al. 2017, Bhagirath et al. 2019), are detected in TDEs (Bhagirath et al. 2019), which have been suggested to be the underlying mechanism of anti-androgen resistance (Bhagirath et al. 2019). Indeed, treating enzalutamide-resistant PCa cells with exosome biogenesis inhibitor, GW4869, restores drug sensitivity of these resistant cells (Bhagirath et al. 2019). Additionally, miRNAs (such as miR-652 or miR-221) have been shown to regulate the expression of NE markers (such as neuron specific enolase, chromogranin A, or synaptophysin) and induce NED (Zheng et al. 2012, Nam et al. 2018), because TDEs are protective delivery vehicles to transfer miRNAs (such as miR-221) which can regulate NE markers (Wei et al. 2014, Tai et al. 2019). These studies highlight a potent role of TDEs in regulating CRPC lineage plasticity via delivering miRNAs to regulate the expression of NE drivers or NE markers among heterogenous population of PCa cells.

EMT significantly correlates with cancer progression and confers cell plasticity by acquiring cancer stem cell (CSC) phenotypes. Although the origin of CSC still remains controversial, studies have shown that the CSC phenotype can be regained through differentiation or reprogramming processes (Abd Elmageed et al. 2014). We recently showed that TDEs could deliver caveolin-1 to PCa cells that express low levels of this protein by increasing CSC phenotypes that are associated with radio- and chemo-resistance (Lin et al. 2019).

**Effects of TDEs on the immune system**

Several studies have indicated that TDEs facilitate cancer progression by modulating the immune system (Salimu et al. 2017, Poggio et al. 2019). Immune checkpoints such as the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway regulate the immune system in patients with cancer (Nguyen & Ohashi 2015). Given that PD-L1 is a membrane-bound ligand, TDEs derived from metastatic cancer carry PD-L1 on their surface (Chen et al. 2018). Mechanistically, interferon γ stimulation can increase the PD-L1 expression on TDEs, which inhibits cell proliferation, cytokine production, and cytotoxicity of CD8 T cells and subsequently promotes cancer progression (Chen et al. 2018). In a syngeneic model of PCa, ablating EV PD-L1 suppresses PCa growth (Poggio et al. 2019). Systemically introduced EV PD-L1 rescues systemic immunosuppression and growth of PCa which is unable to secrete EV PD-L1 (Poggio et al. 2019).

Mechanically, PD-L1-bearing TDEs promote anti-PCa immunity by suppressing T-cell activation in the draining lymph nodes (Poggio et al. 2019). Likewise, prostaglandin E2-bearing TDEs derived from PCa induce the expression of CD73, an ecto-5-nucleotidase responsible for AMP to adenosine hydrolysis on dendritic cells, leading to adenosine-mediated immunosuppression (Salimu et al. 2017). Also, EVs derived from PCa have been shown to downregulate natural killer group 2D (NKG2D) expression, which is a natural killer (NK) cell activating receptor, on NK cells, thus leading to immune suppression and escape in PCa (Lundholm et al. 2014). Additionally, the interaction between Hsp72-bearing TDEs and myeloid-derived suppressor cells (MDSCs) induces the immunosuppressive function of MDSCs in a signal transducer and activator of transcription 3-dependent manner, while depletion TDEs restore the efficacy of immunotherapy (Chalmin et al. 2010).

In addition to TDE-mediated immunosuppression, accumulating studies indicate that TDEs act as messengers between cancer cells and immune cells including macrophages, neutrophils, or dendritic cells, which can promote cancer malignancy. For example, integrin αvβ6-bearing TDEs relocate from PCa cells to monocytes, subsequently promoting monocyte-to-macrophage differentiation and polarization toward an M2 phenotype (Lu et al. 2018). Functionally, EV-mediated monocyte M2 polarization was shown to promote PCa progression (Lu et al. 2018). Likewise, TDEs induce N2 polarization of neutrophils that are pro-tumor neutrophils in a NF-κB-dependent manner and subsequently lead to cancer migration (Zhang et al. 2018b). Also, TDE-educated dendritic cells have been shown to increase the production of IL-6 in a toll-like receptor (TLR) 2 and in a TLR4-dependent manner, which then promotes cancer invasion and metastasis via IL-6-elicited matrix metalloproteinase 9 expression (Shen et al. 2017).
Effects of TDEs on the tumor microenvironment

TDEs have been shown to modulate stromal microenvironments, such as those of myofibroblasts, leading to PCa progression (Webber et al. 2015). For example, transforming growth factor β (TGFβ)-bearing TDEs derived from PCa are found to activate the differentiation of myofibroblasts that exhibit proangiogenic and tumor-growth-promoting characteristics (Webber et al. 2015). Consistently, ablating TDE secretion from PCa decreases the differentiation of tumor-promoting stromal myofibroblasts and stroma-supported PCa growth (Webber et al. 2015). Likewise, PCa-TDEs drive the differentiation of mesenchymal stem cells (MSCs) to a myofibroblast-like phenotype and increase the expression of pro-angiogenic factors and metalloproteinase in TDE-treated MSCs (Chowdhury et al. 2015). Functionally, differentiated MSCs exhibit pro-angiogenic functions and facilitate PCa proliferation (Chowdhury et al. 2015).

Because modifying fibroblasts to adopt a cancer-associated fibroblast phenotype promotes PCa progression, TDEs derived from PCa cells in hypoxic conditions are shown to enhance the cancer-associated fibroblast phenotype in prostate stromal cells (Ramteke et al. 2015). Additionally, transferring hyaluronidase 1-bearing TDEs from PCa to prostate stromal fibroblasts enhances the motility of recipient cells in a FAK signal-dependent manner (McAtee et al. 2019). Together, these studies suggest that TDEs and their effects on the characteristics of stromal cells are critical mechanisms in modulating PCa progression.

Effects of tumor microenvironment-derived EVs on PCa

The intercellular communication between the tumor microenvironment and PCa via tumor microenvironment-derived EVs was shown to affect PCa metabolism or progression (Zhao et al. 2016). For example, CAF-derived EVs were found to provide metabolites such as amino acids or tricarboxylic acid-cycle intermediates to PCa (Zhao et al. 2016). Mechanistically, CAF-derived EVs modulate PCa metabolism by increasing glycolysis, glutamine-dependent reductive carboxylation, and lipogenesis (Zhao et al. 2016). Functionally, CAF-derived EVs increase the proliferation of PCa, while significantly decreasing its mitochondrial function (Zhao et al. 2016). Also, miR-409-bearing EVs derived from stromal fibroblasts can be transferred to PCa, subsequently leading to tumorigenesis (Josson et al. 2015).

In contrast, menstrual stem cell-derived EVs inhibit the expression of angiogenic factors, such as VEGF, in PCa, subsequently ablating PCa-induced angiogenesis and its growth (Alcayaga-Miranda et al. 2016). On the other hand, normal prostate-secreted EVs contain tumor-suppressing miRNA, such as miR-143, which suppresses PCa cell proliferation (Kosaka et al. 2012). These studies suggest a potential EV-based PCa treatment strategy.

TDEs in PCa drug resistance

PCa is an androgen-dependent disease. Read et al. recently showed that the AR can be detected in PCa-TDEs (Read et al. 2017). Transferring AR-bearing TDEs from AR-positive PCa to AR-negative PCa activates the transcription of AR-responsive genes by TDE AR binding to the androgen-responsive promoter region (Read et al. 2017). Moreover, AR-bearing TDEs facilitate the proliferation of recipient cells in the absence of androgen (Read et al. 2017), implying that TDEs regulate resistance to androgen deprivation.

Although anti-androgen agents such as abiraterone or enzalutamide are used to prolong patient survival by inhibiting AR signaling in CRPC (Chen et al. 2009), CRPC usually acquires a resistant phenotype. It was recently shown that TDEs interfere with androgen-deprivation therapies (El-Sayed et al. 2017). TDEs derived from mesenchymal-like PCa cells promote mesenchymal characteristics in recipient epithelial-like PCa cells (El-Sayed et al. 2017). Subsequently, the EMT of recipient PCa cells displays an increased resistance to enzalutamide (El-Sayed et al. 2017). In terms of resistance to either hormonal or anti-androgen therapy, AR-V7, which is resistant to enzalutamide and abiraterone, still functions as a transcription factor to constitutively activate AR signaling (Mostaghel et al. 2014). Recent studies have shown that the AR-V7 protein and RNA can be detected in EVs derived from PCa cells (Read et al. 2017) and metastatic PCa patients (Del Re et al. 2017), respectively. Moreover, AR-V7 protein-bearing EVs promote the proliferation of androgen-responsive cells (Read et al. 2017). Reflecting on the active effect of AR-V7 on AR signaling, AR-V7 RNA-bearing EVs are strongly associated with resistance to hormonal therapy (Del Re et al. 2017). These studies suggest that the AR splice variant in TDEs could impact the response of PCa to hormone therapy.
Although chemotherapy improves the overall survival of hormone-refractory PCa patients, tumors often return and become resistant to chemotherapy (Lohiya et al. 2016). EV-mediated drug efflux by pumping chemotherapeutics out of cancer cells is a novel regulatory mechanism of drug resistance (Soekmadji & Nelson 2015); multidrug resistance-1 (MDR-1) is enriched in TDEs derived from docetaxel-resistant PCa cells but not in those from docetaxel-sensitive PCa cells (Kharazhi et al. 2015). Adding MDR-1-bearing TDEs derived from docetaxel-resistant PCa cells into docetaxel-sensitive PCa cells can confer docetaxel resistance (Corcoran et al. 2012). However, miR-34a-bearing EVs were found to promote the sensitivity of PCa cells to docetaxel by reducing endogenous B-cell Lymphoma 2 (BCL-2) expression (Corcoran et al. 2014). Moreover, TDEs have been shown to modulate the sensitivity of PCa cells to different drugs. For example, camptothecin (CPT)-sensitive DU-145 cells treated with TDEs derived from CPT-resistant DU-145 cells acquire resistance to CPT-induced apoptosis based on reduced PARP cleavage. Conversely, CPT-resistant DU-145 cells become sensitive to CPT treatment after being treated with TDEs from CPT-sensitive DU-145 cells (Panagopoulos et al. 2013). Although the underlying mechanisms remain largely unknown, these studies support the possibility that TDE-mediated intercellular communication between drug resistant cells and drug sensitive cells is critical for determining the therapeutic responses of PCa cells.

In general, EVs have been highlighted as carriers for waste products (Corcoran et al. 2012), implying a critical role of TDEs in leading treatment-induced resistance by exporting drug or drug targets. Apoptosis mediated by 5-fluorouracil is significantly enhanced in PC3 cells in the presence of EV biogenesis inhibitors, such as chloramidine and bisindolylmaleimide-I (Kosgodage et al. 2017), suggesting that TDE release from PCa cells affects drug retention in these cells.

**Potential clinical applications of EVs on PCa**

The isolation of EVs can be minimally invasive, easily obtained, and rapidly profiled, which is a very promising tool in liquid biopsy of biomarker (Tai et al. 2019). Using high-throughput analyses such as genomics, proteomics, and metabolomics to analyze EV contents provides valuable information to identify novel biomarkers and therapeutic targets (Huang et al. 2015, Fujita et al. 2017). Thus, detecting changes in EV bioactive molecule activities provide a new liquid biopsy tool for PCa diagnosis and prognosis.

Noninvasive detection by measuring nucleic acids (such as miRNA or RNA) in urinary EVs from PCa patients allows efficient diagnosis, greatly enhancing therapeutic efficiency. For example, several miRNAs, such as miR-19b, miR-21, miR-145, and miR-375 (Bryzgunova et al. 2016, Foj et al. 2017, Xu et al. 2017), or long non-coding RNAs (lncRNAs), such as IncRNA-p21 (Isin et al. 2015), are upregulated more significantly in PCa patients than in BPH patients or healthy people. Also, several PCa-specific biomarkers, such as prostate cancer antigen 3 (PCA3) and v-ets erythroblastosis virus E26 oncogene homolog (TMPRSS2-ERG) gene fusions, are measured in urinary EVs (Donovan et al. 2015, Motamedinia et al. 2016). Regarding serum EVs, miR-1246 (Bhagirath et al. 2018) are expressed more in PCa than in BPH patients or healthy people. As for the clinical relevance of EV miRNAs in PCa recurrent metastasis, miR-375 or miR-141 was found to be significantly higher in serum EVs from recurrent metastatic PCa patients than in serum from non-recurrent PCa patients based on outcome after radical prostatectomy (Bryant et al. 2012). At the 20-month follow-up, 80% mortality of PCa patients is associated with the high levels of both miR-1290 and miR-375 in plasma EVs, while only 10% mortality of PCa patients is associated with the low levels of both miRs (Huang et al. 2015). Regarding treatment-induced lineage development, serum EV BRN4 and EV BRN2 were upregulated more significantly in CRPC patients with NE features than in CRPC patients with adenocarcinoma features (Bhagirath et al. 2019). To search the potential target(s) of miRNAs capable of inducing NED, ExoCarta database (Keerthikumar et al. 2016) can be used to reveal the expression of candidate miRNAs or their putative targets in EVs. Notably, miRNAs (such as miR-652 or miR-221) that can be detected in EVs (Wei et al. 2014, Hu et al. 2019a) have been reported to modulate the expression of NE markers (such as neuron specific enolase, chromogranin A, or synaptophysin) and induce NED (Zheng et al. 2012, Nam et al. 2018).

In addition, fatty acid binding protein 5 was higher in urinary EVs from PCa patients than in those from healthy controls and was associated with high Gleason Scores (GS) (Fujita et al. 2017). Also, levels of integrin α3 and integrin β1 were found to be higher in urinary EVs from metastatic PCa than in those from BPH (Bijnsdorp et al. 2013). PCa diagnosis biomarkers such as gamma-glutamyltransferase activity (Kawakami et al. 2017), tumor-suppressor protein PTEN (Gabriel et al. 2013), PSA, and CD81 (Logozzi et al. 2017).
2017) were higher in serum or plasmatic EVs from PCA patients than in those from BPH patients or healthy individuals. Plasmatic EV survivin was higher in patients that had relapsed on the chemotherapy agent Taxotere than in healthy people (Khan et al. 2012). Apoptosis protein inhibitors such as survivin were found to be more highly expressed in EVs from African American patients than in those from European American patients (Khan et al. 2017). Moreover, a combination of urinary EV protein biomarkers (including flotillin 2 and Parkinson protein 7) could differentiate between PCA patients and healthy people (Wang et al. 2017); a combination of five proteins (including PSA; CD63 antigen, CD63; putative glycerol kinase 5, GLPK5; N-sulphoglucosamine sulphohydrolase, SPHM; and prostatic acid phosphatase, PAP) was found to be highly expressed in PCA patients with poor prognosis (Sequeiros et al. 2017).

Several studies have explored the physicochemical properties of EVs as a prognostic tool for PCA disease progression. For example, it was found that African American patients with PCA exhibited higher amounts of EVs in plasma and serum than European American patients (Khan et al. 2017). Also, circulating prostate microparticles (PMPs), a type of EV, could differentiate between PCA patients with high GS and PCA patients with low GS (Biggs et al. 2016). After being characterized by scanning electron microscopy, EVs from PCA patients were found to be larger in size than those from healthy people (Souza et al. 2018). Recently, an atypical TDE termed large oncosome with diameter ranging 1–10 μm has been identified in clinical PCA tissues (Di Vizio et al. 2012). It appears that Caveolin-1-positive large oncosomes are more abundant in the plasma of PCA patients with high grade than those with low grade, implying the clinical relevance of large oncosome with PCA progression (Morello et al. 2013). Likewise, a recent study suggests an association of αV-integrin-positive large oncosome-like vesicles with high grade (Ciardiello et al. 2019). Notably, large EVs and small EVs exhibit unique DNA, RNA, protein, and lipid profiles or even biophysical properties, suggeting different EV populations with distinct biological functions (Zhang et al. 2018a). Based on a quantitative proteomics analysis, 25% of the protein cargos are unique and differentially expressed in large and nano-sized EVs from PCA cells (Minciacchi et al. 2015). Proteins enriched in large EVs include enzymes which are involved in metabolic processes, suggesting the effects of large EVs on metabolic functions of cancer cells (Minciacchi et al. 2015). Also, the miRNA profiling of EVs derived from tumorigenic RWPE-2 prostate cells indicates that large oncosomes mediate intercellular transfer of functional miRNAs, such as miR-1227-mediated CAF migration (Morello et al. 2013). On the other hand, the concentration of plasma EVs from PCA patients were higher than those from BPH patients or healthy people (Logozzi et al. 2017). Plasma from PCA patients exhibits higher concentrations of prostasomes, a kind of EVs from the prostate, than those from age-matched, healthy people (Tavosidiana et al. 2011). The levels of plasma prostasomes from patients with GS ≥ 7 were higher than those with GS ≤ 6 (Biggs et al. 2016). Moreover, studies that profiled specific proteins or RNA expression in EVs could differentiate between PCA patients with high GS and low GS or benign disease (McKiernan et al. 2016, Park et al. 2016). These studies suggest the promising application of EVs in PCA management.

Conclusions
Prostate carcinogenesis is highly regulated by its surrounding microenvironments. Numerous studies have shown that EV-mediated local and systemic intercellular communication are involved in this process. However, the regulatory mechanism of diverse bioactive molecules loaded into EVs and the in vivo pathologic functions of EVs derived from PCA or its surrounding microenvironments are not fully understood. Nevertheless, these studies have provided a strong rationale of profiling cargo molecules in TDE as a new tool of liquid biopsy for diagnostic and prognostic purposes. In addition, EVs may also be cancer-specific vehicles for delivering cancer therapeutics.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work was supported by grants from the the Ministry of Science and Technology in Taiwan (MOST106-2911-I-002-569 to T K L and T L S).

Acknowledgements
The authors thank Dr Damiana Chiavolini for editorial assistance.

References
The role of exosome in prostate cancer.

Y-L Tai et al. 2023


https://erc.bioscientifica.com
https://doi.org/10.1530/ERC-20-0021 © 2020 Society for Endocrinology Published by Bioscientifica Ltd. Printed in Great Britain

Downloaded from Bioscientifica.com at 09/05/2023 01:18:55AM via free access


Y-L Tai et al. The role of exosome in prostate cancer


Received in final form 3 March 2020
Accepted 23 March 2020
Accepted Manuscript published online 23 March 2020