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**Cancer Drug Resistance**

**Review**

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# The multifaceted role of extracellular vesicles in prostate cancer-a review

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## Abstract

Prostate cancer is the second most prominent form of cancer in men and confers the highest mortality after lung cancer. The term "extracellular vesicles" refers to minute endosomal-derived membrane microvesicles and it was demonstrated that extracellular vesicles affect the environment in which tumors originate. Extracellular vesicles' involvement is also established in the development of drug resistance, angiogenesis, stemness, and radioresistance in various cancers including prostate cancer. Extracellular vesicles influence the general environment, processes, and growth of prostate cancer and can be a potential area that offers a significant lead in prostate cancer therapy. In this review, we have elaborated on the multifaceted role of extracellular vesicles in various processes involved in the development of prostate cancer, and their multitude of applications in the diagnosis and treatment of prostate cancer through the encapsulation of various bioactives.

**Keywords:** Extracellular vesicles, prostate cancer, stemness, chemoresistance, therapeutic delivery, diagnosis

## INTRODUCTION

One of the most common forms of cancer identified in men is prostate cancer (PCa), which is also one of the deadliest, the second most common type of cancer in men after lung cancer, and the fifth most prevalent

mortality cause in males[1]. In the United States of America alone in 2021, more than 248,000 instances of PCa were identified in men, and just under 34,000 fatalities were attributed to the disease[2]. Over 1.4 million cases worldwide were reported, with more than 300,000 deaths related to PCa[3]. Currently, multiple treatment strategies are used against PCa. These therapies include surgery, radiation therapy, active surveillance, and proton therapy. Apart from these, other strategies including chemotherapy, hormonal therapy, cryosurgery, and HIFU (high-intensity focused ultrasound) are also part of the current treatment regime based on the clinical conditions and outcomes[4-10]. Active surveillance emerged as the management option for the low-risk PCa and various strategies were developed for the same[11]. Urological guidelines proposed various treatment options depending on the stage of PCa. For example, patients with low-risk diseases are offered active surveillance and active treatment such as surgery or radiation therapy. For immediate-risk disease patients, radical prostatectomy, radiotherapeutic therapy, and pelvic lymph node dissection is the treatment option. The same treatment option applies to patients with high-risk localized disease. In addition, radiotherapy and surgery are used as a treatment for locally advanced disease[12]. Hormonal therapy, also known as androgen deprivation therapy (ADT), is a traditional and standard therapy against PCa for over 60 years and shows its effect through the decrease in serum testosterone[13]. Hormonal therapies have also shown combinative strategies with other standard treatment options such as radiotherapy, non-invasive treatments through medications, and hormonal therapy with surgery[14,15]. Hormonal therapy with drugs in prostate cancer provides effective tumor growth and progression control, boosting patient outcomes and quality of life. At present, commonly used medications in hormonal therapy involve drugs such as enzalutamide, abiraterone, goserelin, bicalutamide, *etc.*[16-21].

Following hormonal therapy for a while, the tumor develops into metastatic castration-resistant PCa (mCRPC), which has a low rate of survival and few treatment options[22-24]. The manner in which multilaminar bodies, particularly extracellular vesicles, influence the general environment, the processes, and the expansion of PCa cells is one of the areas that has demonstrated a significant amount of potential when it comes to the treatment of PCa.

The term "extracellular vesicles" was primarily used by Rose Johnstone and her colleagues in 1970 to refer to minute endosomal-derived membrane microvesicles[25,26]. Extracellular vesicles offer several advantages, such as the ease of therapeutic cargo loading (drugs, siRNA), the ability to penetrate through biological barriers, low immunogenicity, ease of cellular uptake, ease of surface modification, *etc.*[27]. However, limitations such as difficulty in obtaining high quantities of pure extracellular vesicles, purification process, and disadvantages pertaining to various isolation techniques limit the application of extracellular vesicles in drug delivery. The advantages and disadvantages associated with extracellular vesicles are graphically depicted in Figure 1[28].

Substantial numbers of researchers concentrated their attention on the roles of extracellular vesicles in the act of tumors. There is a lot of interest in how extracellular vesicles affect the environment in which tumors originate, the role that extracellular vesicles play in the progression of drug resistance in cancer cells in response to anti-cancer medications, and the role that extracellular vesicles can play in mitigating the effects of cancer and perhaps halting the process of cancer growth as well as their potentially intricate role in the delivery of chemotherapeutic agents and other bioactives in the tumor cells. Extracellular vesicles, which initially were considered molecular waste bins, now have shown a multifaceted role in cancer. Extracellular vesicles serve a significant function in angiogenesis through the transport of various pro-angiogenic biomolecules, such as vascular endothelial growth factor (VEGF), microRNAs, and matrix metalloproteinases (MMPs), and also act as key agents in metastasis through their involvement in restructuring metastatic sites to support cancer cell colonization[29-31]; Extracellular vesicles have a key role as

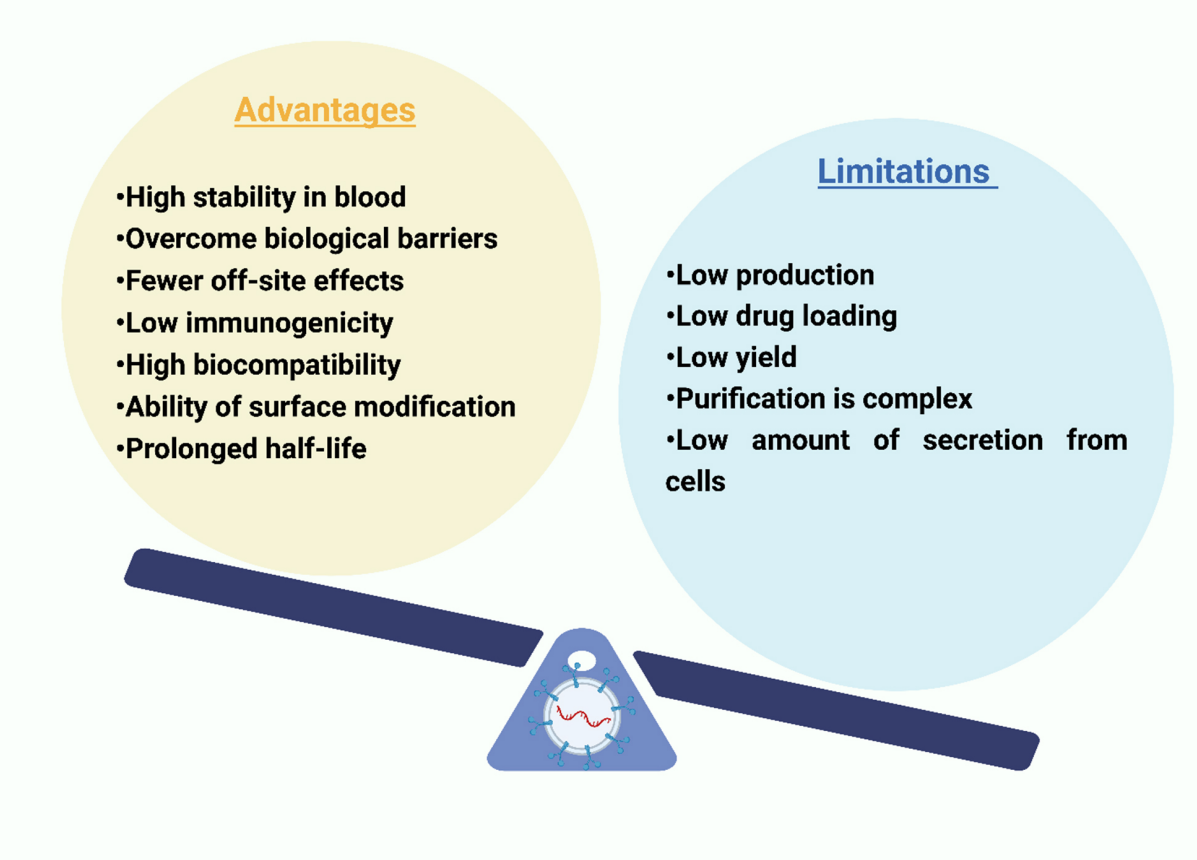


**Figure 1.** Graphical representation of advantages and limitations of extracellular vesicles.

biomarkers in the cancer development through its components including proteins, nucleic acids and also from other biofluids [Table 1][32]. While talking about the exosomal role in PCa, they have been indulged in acting as the key agent in the progression of PCa through numerous mechanisms including changes in the tumor microenvironment and angiogenesis, through the metastasis and cell proliferation, and in the drug resistance in PCa tumor[33-43].

In this review article, we have described the multifaceted functions that extracellular vesicles play in PCa. This review focuses on the pro-tumorigenic role of extracellular vesicles in the key processes engaged in the development of PCa, such as stemness, chemoresistance, radioresistance, angiogenesis, and metastasis. Furthermore, the application of extracellular vesicles in the diagnosis and delivery of therapeutic moieties such as the siRNA, drugs, and phytoconstituents are also elaborated in this review.

## PRO-TUMORIGENIC ROLE OF EXTRACELLULAR VESICLES IN PCa

### Role of extracellular vesicles in stemness of PCa

Stemness refers to the phenomenon that defines the cells' capability of self-renewal and differentiation[61]. It is a characteristic ability shown by adult stem cells to proliferate, and bolster their new generation of daughter cells and also interact with their environment to maintain a balance between quiescence, proliferation, and regeneration[62]. In the case of cancer stem cells (CSCs), this phenomenon acts as a malignant equivalent to normal stem cells. Apart from the common features such as maintenance, sustainability, supporting the microenvironment, and self-renewal, the essential differences found in the

**Table 1. List of biomarkers from extracellular vesicles in prostate cancer**

| Biomarkers from extracellular vesicles | | The role played in prostate cancer | Cell line/ model used | References |
|---|---|---|---|---|
| Proteins | HSP70 | Provides drug resistance, promotes invasion, stemness, and metastasis | PC3 and LNCaP | [44-46] |
| | Caveolin 1 (CAV1) | Progression and metastasis of Prostate cancer; inhibit apoptosis in prostate cancer cells. | LNCaP and PC3 cell lines | [47,48] |
| | Integrin alpha-2 (ITGA2) | Mediates cancer progression and metastasis; possible role in alteration of AR phenotype and development of aggressive prostate cancer | CRPC-derived extracellular vesicles, LNCaP cell lines | [49,50] |
| | Annexin A2 | Enhances the release of IL-6, promoting the proliferation of prostate cancer; migration and adhesion to osteoblasts | DU145, LNCaP, PC-3 | [51-53] |
| | Vimentin | Associated with invasion and metastasis via Src regulation | PC-3M-1E8<br>PC-3M-2B4 | [54] |
| RNA | miR-21 | Promotes growth as well as proliferation following the surgical castration; Also promotes invasion and apoptosis resistance | LNCaP, androgen-dependent PC-3 cell lines, and DU-145 cell lines | [55,56] |
| | TMPRSS2-ERG fusion | Promotes cell proliferation | TMPRSS2-ERG-positive PCa xenograft models | [57] |
| | miRNA-221/222 | Promotes oncogenesis and progression of prostate cancer through p27(Kip1) downregulation | PC-3(aggressive prostate carcinoma model), LNCaP, and 22Rv1 cell line models | [58] |
| | MALAT1 | Through sponging miR-145 promotes cell proliferation, migration, and invasion | LNCaP and CWR22Rv1 cell lines | [59] |
| | HOTAIR (HOX transcript antisense RNA) | Decreasing the inhibitory effect of hepaCAM on MAPK signaling promotes invasion and metastasis. | Samples collected from patients at Dept. of Urology, First Affiliated Hospital of Chongqing Medical University, China | [60] |

cancer stem cells refer to extra features that include heterogeneity in the cell population, high resistance towards hostile factors like quiescence, chemotherapeutic agents, hypoxia and low nutrient levels[62-67].

Stemness plays an influential role in the development of PCa. The prostate stem cells reside in the basal and luminal layers and are a major target for the oncogenic transformation, which suggests a role in the genesis of PCa[68]. CSCs demonstrated an altered gene expression in exposure to hypoxia, nutrient deficiency, and oxidative stress, rendering them more mobile, invasive, and resilient to further stress. CSCs are anticipated to have endured epithelial-mesenchymal transition + , and the transition to mesenchymal marker expression is frequently one measurement of PCa progression. CSCs are predicted to invade locally and then metastasize[69].

Extracellular vesicles portray a substantial part in the stemness of PCa. They have been shown to be involved in multiple features exhibited by PCa stem cells, such as resistance against hypoxia and tumor progression, promotion of epithelial-to-mesenchymal transition (EMT), and also the transformation of other stem cells into cancer stem cells. Ramteke *et al.* in their work discovered that extracellular vesicles derived from hypoxic PCa cells promote the cancer-associated fibroblast (CAF) phenotype in prostate stromal cells, and also elevate the property of stemness and protruding of naive PCA (PCa) cells. They subjected human PCA PC3 and LNCaP cells to normoxic and hypoxic conditions, respectively, and extracted the extracellular vesicles released in both situations. They observed increased amounts of Annexin II, heat shock proteins (HSP90 and HSP70), and tetraspanins (CD63 and CD81) in hypoxic extracellular vesicles, which led to LNCaP and PC3 cell invasiveness and motility. Aside from that, they discover an increased amount of metalloproteins as well as increased levels of various signaling molecules. Further, in proteome analysis, they found an increased number of proteins in hypoxic extracellular vesicles, which promotes epithelial adheres junction pathway remodeling and proteins, especially in naïve PC3 cells. This study overall suggests how extracellular vesicles promote the stemness of PCa cells, affecting significant

features such as invasiveness and tumor microenvironment, leading to aggressive PCa[44]. In another study, it was found how extracellular vesicles containing PSGR (Prostate-specific G-protein coupled receptor) promote the migration, invasiveness, and stemness of low-aggressive PCa cells. They used transcriptome sequencing to determine the differentially expressed (DE) mRNAs in low invasive cells incubated with overly expressed PC3 extracellular vesicles or negative control (NC) extracellular vesicles. They also discovered that the PSGR was stably overexpressed in PC3 cells. Internalization of PC3 PSGR + extracellular vesicles in LNCaP and RWPE-1 cells greatly promoted cell migration and invasion. After PC3 PSGR + exosome incubation, E-cadherin expression declined, while vimentin, Snail, SOX2, and OCT4a expression elevated in low invasive cells. This resulted in findings indicating that extracellular vesicles released via PCa cells induce invasiveness and stemness[70]. An interesting study was published talking about how extracellular vesicles derived from PCa cells promoted the neoplastic reprogramming of adipose stem cells derived from the patient. They identified that extracellular vesicles obtained from PCa facilitated the transformation of adipose stem cells into neoplastic cells in the patient due to changes in the cell microenvironment. They observed that pASCs (PCa patients derived adipose-stem cells) primed with PCa cell conditioned media (CM) produced prostate-like neoplastic lesions in vivo and replicated aggressive tumors in secondary recipients, in contrast to normal ASCs (adipose-stem cells). The cytogenetic aberrations and mesenchymal-to-epithelial transition of the pASC tumors, along with the expression of epithelial, neoplastic, and vasculogenic markers, were evocative of molecular features of PCa tumor xenografts. This suggests that extracellular vesicles play a tremendous role in not only promoting the stemness, invasiveness, and growth of PCa cells but can also be an agent advocating the transformation of other stem cells into oncogenic cells[71-73].

## Extracellular vesicles in drug resistance and radioresistance of PCa

*Extracellular vesicles and drug resistance*

The development of resistance to treatment drugs has been an extremely difficult obstacle to overcome in PCa treatment[43,74]. The use of chemotherapy is still considered to be one of the more traditional methods for treating advanced PCa[5]. However, it has been demonstrated that several variables, including the heterogeneity of the tumor, epigenetic control by miRNAs, and the combinatorial outcomes of various signaling pathways, including NF-κB/IL-6, Hedgehog, mTOR (mammalian target of rapamycin), Akt/PI3K, MAPK/ERK, and somatostatin receptors, all of these elements result in drug resistance in tumor cells[75-79].

According to numerous research, extracellular vesicles are involved in the development of medication resistance in PCa[42,74,80]. Extracellular vesicles have been discovered as the mechanism underlying resistance against the drug enzalutamide, and in PCa, the emergence of therapy-induced neuroendocrine differentiation stages, as demonstrated by Bhagirath *et al.* in their research[74]. In addition, it was observed that BRN2 and BRN4, which are neural transcription factors, were liberated in PCa extracellular vesicles after the treatment with enzalutamide. These transcription factors are essential for the neuroendocrine remodeling of prostate adenocarcinomas[74,81,82]. Kharaziha *et al.* discovered with the usage of nanoparticle tracking analysis that a greater quantity of extracellular vesicles released by DU145 PCa cells docetaxel-resistant than by DU145 PCa docetaxel-sensitive cells[80]. Extracellular vesicles were shown to be responsible for resistance against docetaxel in docetaxel-susceptible PCa cells (DU145, LNCaP, and 22Rv1)[42]. Following administration of the cells with docetaxel-resistant extracellular vesicles versions of 22Rv1 and DU145 (22Rv1RD and DU145RD, respectively), authors observed the liberation of MDR-1/Pgp, which is a multidrug resistance protein 1/P-glycoprotein and this transporter protein engaged in the efflux of a wide range of exogenous objects, including antineoplastic medications, from extracellular vesicles, played a probable role in imparting resistance to docetaxel-susceptible cells[42]. Another study looked through an interesting pathway to study the chemoresistance in PCa cells. In the study, exosome-derived miR-27a, which portrays a substantial role in chemoresistance in cells of PCa, was examined. When administered

with doxorubicin, cisplatin, and docetaxel in PCa cells, there was a tremendous spike in the levels of the miR-27a; they also co-treated PC3 cells (PCa cells) with primary prostate fibroblasts (PSC27 cells) to analyze tumor treatment resistance mechanisms. The results additionally demonstrate that exosome-derived miR-27a produced by PSC-27 cells enhanced chemoresistance by suppressing P53 gene expression[35]. Although it is not the commonly observed pathway for drug resistance, Saari *et al.* in their research made an interesting discovery. They observed that using two contrasting populations of extracellular vesicles (microvesicle- and exosome-enriched) as paclitaxel carriers in autologous PCa increased the cytotoxicity effect of the paclitaxel, regardless of the fact that cancer cell viability increased without the vesicles, but the overall net cytotoxicity effect remained increased. They also observed that this phenomenon was irrespective of the EV population and cell lines tested[83].

In recent years, various studies demonstrated the role played by the extracellular vesicles in the development of drug resistance in PCa. Shan *et al.* observed that miRNA-423-5p from extracellular vesicles, which was secreted from cancer-associated fibroblasts, was promoting chemoresistance in prostate cancer. They observed that miRNA-423-5p from extracellular vesicles promoted chemoresistance through inhibition of GREM2 via the TGF-β pathway for taxane derivatives while suppressing the TGF-β pathway was able to partially reverse the chemoresistance[84]. In another study, Kato *et al.* utilized the serum extracellular vesicles containing CD44v8-10 mRNA as the diagnostic marker for docetaxel resistance in prostate cancer. The results showed that the levels of CD44v8-10 protein and mRNA in cell lysates and extracellular vesicles were higher in PC-3R cells, which was a docetaxel-resistant cell line compared to normal PC-3 cells. This showed that CD44v8-10 protein had a role in the development of chemoresistance[85]. The role of syntaxin-6-mediated extracellular vesicles in the regulation of enzalutamide resistance in prostate cancer was demonstrated. The authors observed that the enzalutamide-resistant cell lines (CWR-R1, C4-2B, and LNCaP) had a higher amount of extracellular vesicle secretion (about 2-4) times than the enzalutamide-sensitive cells. The observed mechanism underlying was found to be the upregulation of syntaxin-6 accompanied by the increase in colocalization with CD-63 in enzalutamide resistance cell lines. They also observed that knocking down syntaxin-6 with siRNAs resulted in a reduction in cell count and an enhancement in cell death in the presence of enzalutamide[86].

*Extracellular vesicles and radioresistance*

Apart from the drug resistance shown in PCa in the above section, extracellular vesicles are also associated with radioresistance in PCa[87]. In the case of PCa, following the radiation therapy, increased levels of HSP72-containing extracellular vesicles were found in PCa, leading to the conclusion that HSP72-containing extracellular vesicles are a possible patron, leading to pro-inflammatory cytokine production and immune modulation[88]. Other than this, another phenomenon was observed in the cancer stem cells that tend to show more resistance against radiation therapy. This allows the production of extracellular vesicles that will deliver the resistance phenotype to recipient cells[87]. This will allow for the formation of more resistant cancer cells to radiation therapy. Regarding PCa, research has demonstrated the presence of extracellular vesicles released from prostate stem cells; these vesicles have the capacity for autophagy and can also modulate the sensitivity towards radiation therapy[89,90]. All of this suggests a strong negative role of extracellular vesicles not only as a key agent against drug and chemoresistance, but extracellular vesicles also play a quintessential role in the emergence of resistance against radiation therapy in PCa[91]. Radiation therapy, still one of the mainstream therapies for the treatment of cancer across all types, can affect the release of content from extracellular vesicles, which has already been affected by the composition and abundance of the extracellular vesicles, affecting the extracellular vesicle-based intracellular communication.

### Extracellular vesicles in metastasis of PCa

Aside from their influence at metastatic locations, tumor-derived extracellular vesicles have been shown to have a role in the invasion, development, and metastasis of tumors by interacting with cells at isolated, pre-metastatic organ locations[92,93]. This transpires through the establishment of tumor-nurturing microenvironments, a technique known as "pre-metastatic niche generation". The formation requires protracted intercellular communication facilitated by soluble or membrane-bound proteins originating from the original tumor[94-96].

The tumor-originated extracellular vesicles are responsible for the extracellular matrix (ECM) remodeling via the accumulation of fibronectin and the promotion of crosslinking by the ECM-modifying enzyme lysyl oxidase (LOX). This is done with the intention of improving bone marrow-derived cell adherence, which is a critical component of the pre-metastatic niche. To improve the adherence of bone-marrow-derived cells, tumor-originated extracellular vesicles remodel the ECM through the aggregation of fibronectin and crosslinking ECM[97-99].

Intriguingly, new research conducted by Henrich *et al.* indicates convincingly that homeostasis of cholesterol in bone marrow myeloid cells is the mediator of communication between bone marrow and PCa cells through extracellular vesicles. They observed that bone marrow myeloid cells absorb PCa extracellular vesicles, leading to an activated NF-κB signaling, improved osteoclast formation *in vitro* and *in vivo*, and lessened myeloid thrombospondin-1 expression[100]. A tailored biomimetic strategy involving myeloid cells *in vitro* and *in vivo* lowering cholesterol levels prevented PCa EV uptake by recipient myeloid cells, eliminated NF-κB activity, maintained thrombospondin-1 expression, decreased osteoclast differentiation, and yielded a 77% reduction in metastatic burden[100]. During early stages of metastasis, the EMT is a pivotal critical step. This state is triggered by tumor-derived extracellular vesicles' autocrine and paracrine signaling and targeting proteins such as transforming growth factor-beta (TGF-β) and catenin, which are EMT-related proteins[101,102]. Extracellular vesicles derived from PCa DU145 and PC3 cells generate a type of TGF-β capable of initiating fibroblast-myofibroblast transformation by activating the TGF-SMAD signaling pathway. This TGF-β is also capable of promoting tumorigenesis and inhibiting immune response[103,104]. McAtee *et al.* showed in their research that prostate tumor cells containing extracellular vesicles have a protein called hyaluronidase 1 (Hyal1) in them. This protein encourages the relocation of prostate stromal cells, which ultimately speeds up the evolution of PCa[105]. Research conducted by Josson *et al.* showed that the microRNA miR-409, which has a crucial part in the EMT in PCa, was observed in stromal-derived extracellular vesicles[106]. Furthermore, integrins are known to play a role in metastasis and progression of cancer, and in PCa, the $\alpha_v\beta_6$ integrin is shown to enhance the ab

ility to migrate[107]. Furthermore, the integrins derived from extracellular vesicles are also known to play a role in angiogenesis[108]. In a research study, the proteins Integrin Subunit Alpha 3 (ITGA3) and Integrin Subunit Beta 1 (ITGB1) were found in extracellular vesicles recovered from LNCaP and PC3 cells, released in the urine of PCa patients, both of them contributing to the diaspora and invasion of both tumors[109]. It is worth noting that Elmageed *et al.* reported that exosome-derived miR-130b, miR-125b, and miR-155 promote mesenchymal-epithelial transition (MET) and neoplastic transformation and stem cells isolated from PCa patients' adipose tissue[71]. Honeywell *et al.* illustrated in their research how miR-105 obtained from tumor-derived extracellular vesicles serves as a tumor suppressor and targets the cyclin-dependent kinase 6 (CDK6), which suppresses the cell proliferation[110]. Another interesting route was discovered, bolstering a pathway for metastasis in PCa. It was also demonstrated that androgen receptors were expressed by PCa cells-derived extracellular vesicles, which, after nuclear localization, led to enhanced cell proliferation[111]. Metastasis of lymph nodes to distant organs can be observed through the probable mediation of extracellular vesicles. Even though a mystery is shrouded suggesting the initiation of this

process by which tumor cells, it is possible that lymph node metastasis could occur through extracellular vesicles. The authors of the research, Maolake *et al.*, revealed that the tumor necrosis factor-α (TNF-α) provides a huge contribution towards metastasizing PCa lymph nodes using LNCaP, DU145, LNCaP-SF, and PC3 cell lines of PCa can be seen through the activation of chemokine (C-C motif) ligand 21/CC chemokine receptor 7 (CCL21/CCR7) axis and this discovery may mediate the influence that extracellular vesicles have on metastasis through the lymphatic pathway[112]. Furthermore, in another research conducted, it was discovered that from the PC3, DU145, CWR-R1 PCa, and LNCaP cells, which were derived from designated metastatic PCa cell lines, integrin alpha 2 subunits (ITGA2) were found packaged in extracellular vesicles. The fact that inhibiting the release of extracellular vesicles to recipient cells in these metastatic PCa cells by knocking down ITGA2 revealed a possible function for ITGA2 in contributing to the development of the illness and the aggressive phenotypes found in PCa. With an elevated Gleason Score of 9, lymph node metastatic tissues were found to have higher ITGA2 expression than lower Gleason Score of 7 PCa tissues, implying that ITGA2 may play a role in enhancing lymph node metastasis[49]. Furthermore, no notable differences were observed in ITGA2 protein expression levels between 24 primary PCa tissues and their matched metastatic lymph node tissues. Regardless of the fact that there were no key differences, this was the case.

### Role in progression and angiogenesis

Extracellular vesicles are released constitutively by tumor cells of diverse sources. These extracellular vesicles play essential roles in the transformation of tumors into malignant forms and the progression of cancers. Extracellular vesicles' effects are mediated by the transfer of cargo, which includes a range of proteins as well as RNA (including miRNAs) and DNA[113,114]. Cancer cells essentially require to sustain their attributes, such as immortality, continued proliferative activity, evasion of immune reactions, angiogenesis, continued invasion and metastasis, and resistance to cell death or apoptosis. The tumor microenvironment supplies this medium, which includes tumor stromal cells such as mesenchymal stromal cells, fibroblasts, pericytes, and immune cells such as T & B lymphocytes. They serve as the growth medium for the tumor cells, which are located within the tumor. The transformation of normal stroma cells into reactive stroma cells, which promotes the growth of cancer cells as well as metastasis, is a feature that is distinctive of the progression of cancer. After being impacted, the stromal cells will employ extracellular vesicles to regulate the microenvironment of the tumor, therefore promoting both the development of the tumor and its ability to metastasize[115-117]. TME extracellular vesicles now perform the role of cellular communicators in addition to assisting with a range of activities. In the instance of PCa, DeRita *et al.* discovered that protein Src was found in PCa cell extracellular vesicles. Through integrin activation, this protein activates focal adhesion kinase, which leads to angiogenesis and metastasis[34]. Eventually, tumor cells and the stromal cells that are in close vicinity will engage in a persistent kind of interaction[115]. By encouraging the differentiation of fibroblasts into a myofibroblast-like phenotype that produces angiogenesis and tumor development, cancer extracellular vesicles that contain TGF- β play critical roles in the generation of stroma that promotes the growth of tumors[30,118]. The TGF-β/SMAD3 cascade has to be activated to produce this effect. The extracellular vesicles that are produced by PCa cells have a high level of latent TGF-β expression. Through focal adhesion kinase via integrin activation, this protein triggers metastasis and angiogenesis[119,120].

The production of extracellular vesicles by PCa cells enables the delivery of sphingomyelin and CD147 into endothelial cells, which facilitates cancer cell migration and endothelial cell pro-angiogenic activity[121-122]. Proteins such as c-Src tyrosine kinase, IGF-R (insulin-like growth factor 1 receptor) and FAK (focal adhesion kinase) all play imperative roles in the formation and advancement of prostate tumors[6,15,34,123-125]. Extracellular vesicles derived from PCa exhibit significant concentrations of these proteins. Angiogenesis is stimulated by the cross-talk that occurs between Src and IGF-1R[34,126]. Extracellular vesicles rich in Src have been shown to have the ability to stimulate angiogenesis in animal models that have prostate tumors. This is

because Src is overexpressed within plasma extracellular vesicles of mice with prostate tumors. Furthermore, Src and IGF1-R affect angiogenesis by activating VEGF and VEGF-C, respectively[127,128]. This is the case for both of these and focal adhesion kinase, which, if activated, leads to metastasis and angiogenesis[34,129]. Cancer extracellular vesicles that contain TGF-β play critical roles in the formation of stroma that is favorable to the development of tumors. These extracellular vesicles encourage the differentiation of fibroblasts into a myofibroblast-like phenotype, which in turn stimulates angiogenesis and tumor expansion[130-131]. The activation of the TGF-β/SMAD3 cascade is the mechanism by which this impact is produced[132]. PCa cells are responsible for producing extracellular vesicles that have a high level of latent TGF-β expression. This latent TGF-β adheres to the exosome surface via proteoglycans, promoting the activation of SMAD3-dependent signaling cascades[133,134]. Figure 2 shows a graphical representation of pro-tumorigenic characteristics of extracellular vesicles in PCa.

## THE ROLE OF EXTRACELLULAR VESICLES IN THE DIAGNOSIS AND THERAPY OF PCa

### Utilization of extracellular vesicles in the of PCa diagnosis-recent advances

On the positive side, extracellular vesicles are widely studied for the diagnosis and therapy of PCa[135,136]. The extracellular vesicles derived from the urine, plasma, and semen[40,137,138] have been utilized for the diagnosis of PCa. Several studies demonstrated the utilization of extracellular vesicles in PCa diagnosis[39,139-141]. A recent clinical investigation revealed that PCa patients may be discriminated from both Benign Prostate Hyperplasia (BPH) and healthy subjects by the expression of PSA on plasmatic extracellular vesicles. In a study, it was demonstrated as a new strategy for differentiating not just PCa from healthy persons but also from benign hypertrophy[142]. In another study, it was found that extracellular vesicles from individuals with PCa exhibited overexpression of CA IX levels, which is related to intraluminal pH, as compared to healthy persons, and demonstrated that the PCa extracellular vesicles are acidic in nature and can be used as a biomarker in PCa[143]. Since the literature depicts the use of extracellular vesicles in the diagnosis of PCa, in this review, we will focus on the latest advances in extracellular vesicles for the diagnosis of PCa. Li *et al.* developed a superparamagnetic conjunctions and molecular beacons (SMC-MB) based platform in which it detected and captured the prostate-specific membrane antigen-positive extracellular vesicles, thereby depicting its efficiency in the diagnosis of PCa[144].

In another study, an economical, easy, and non-invasive method was developed for diagnosing PCa in which the extracellular vesicles containing surface proteins and miRNAs from extracellular vesicles were detected at the same time and permitted the analysis of particular miRNAs and surface proteins through one reaction[145]. A nanoplatform for specific and quick detection, which consists of off-on signal responses and reversible conjunction, was developed in which high-affinity particles of $Fe_3O_4$@$SiO_2$@$TiO_2$ were employed for exosome capture and selectivity was improved through the fluorescence response PMSA aptasensor which aid in the tumor exosome detection. The study concluded that this method was fruitful for quick diagnosis[146]. In another study reported, a microfluidic Raman biochip with immunoassay was developed for the separation and analysis of extracellular vesicles. This chip was able to differentiate the samples of PCa patients and normal samples. Furthermore, this system detected the extracellular vesicles in one hour and thus can be explored as the detection test for PCa[147]. In summary, here we discussed some of the recent advances in the utilization of extracellular vesicles for the diagnosis of PCa. To the best of our knowledge, extracellular vesicles have been extensively utilized for the detection of PCa, and various advances such as the Raman chip and others have also been developed and employed for much rapid, non-invasive and sensitive analysis of the samples. Soon, various additional options will be developed to enhance diagnosis using extracellular vesicles.

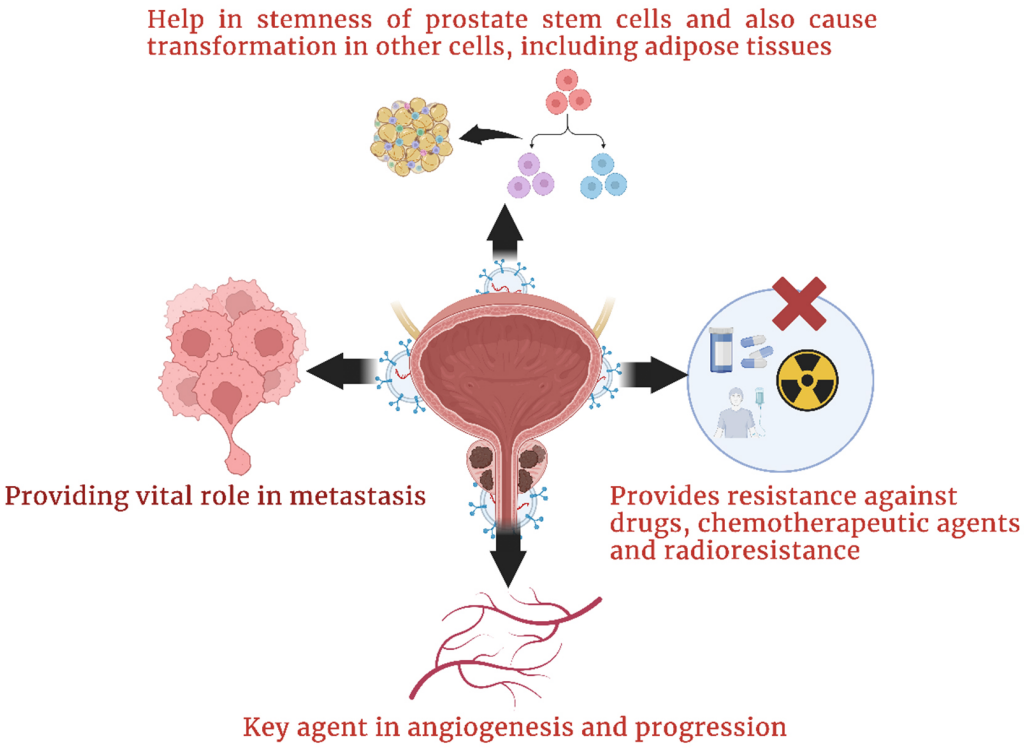


**Figure 2.** Graphical representation of the pro-tumorigenic characteristics of extracellular vesicles in PCa.

## Extracellular vesicles in the PCa therapy

Another such advantage is its application in drug delivery. Owing to their advantages over conventional delivery systems, such as their high ability to overcome biological barriers, enhanced stability, better targeting, unnecessary accumulation in the liver, and low toxicity, extracellular vesicles are widely utilized for the delivery of drugs as well as other therapeutic moieties such as siRNA, phytoconstituents[28,148]. The extracellular vesicles are obtained from various cells, such as tumor cells, immune cells, and mesenchymal stem cells, and are also derived from bovine milk. Of these, the extracellular vesicles derived from mesenchymal stem cells and tumor cells are widely explored for therapeutic purposes[149]. In this section, the role of extracellular vesicles in the delivery of various therapeutic moieties such as the siRNA, chemotherapeutics, and phytoconstituents are discussed [Figure 3].

### *siRNA delivery through extracellular vesicles*

It was shown that due to the underlying mechanisms, extracellular vesicles are found to be the right candidates for the delivery of siRNA[148]. For this reason, extracellular vesicles are utilized for the successful delivery of siRNA for targeting various cancers such as breast cancer[150], colon cancer, gastric cancer, and others, including PCa[151,152]. In the context of PCa, a research study was aimed to determine the efficiency of siRNA in the inhibition of SIRT6 in a PCa model. It was demonstrated that the siRNA via the engineered extracellular vesicles caused the downregulation of the SIRT6 and blocked the metastasis and tumor growth[153]. In another study by Krishn *et al.*, it was demonstrated that delivery of siRNAs targeting ITGB6

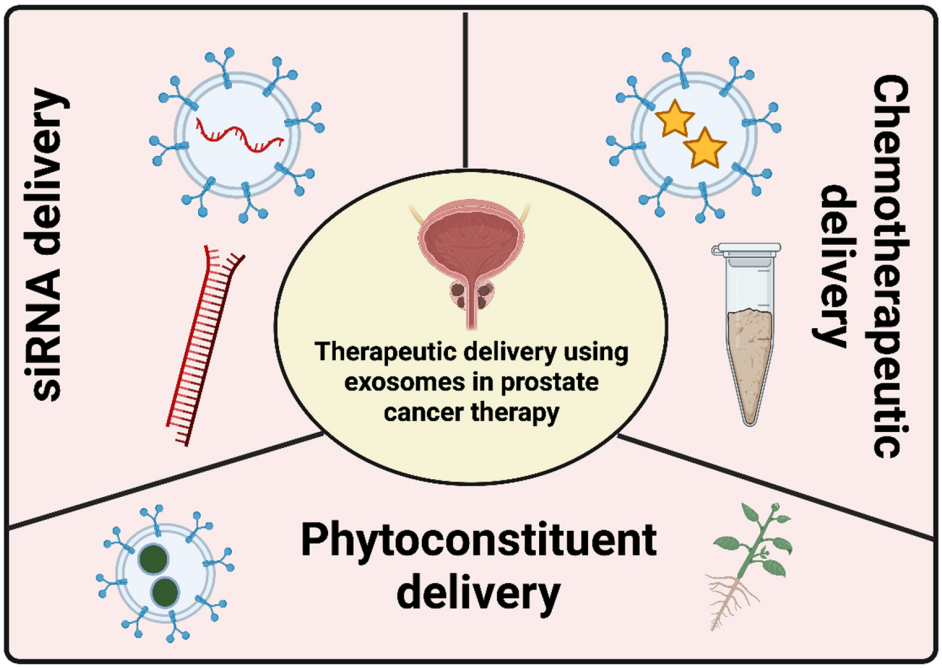


**Figure 3.** The application of extracellular vesicles in the therapy PCa.

into the PCa cells (PC3) led to the downregulation of β6 subunit expression and also led to cell adhesion and migration of PCa cells on a specific substrate of αVβ6[154]. In an *in vivo* study on mice bearing subcutaneous prostate carcinoma, the extracellular vesicles modified with polyethyleneimine showed strong inhibition of tumor growth, which is due to the significant knockdown of the target gene[155]. Even though siRNA delivery through extracellular vesicles has been researched widely in recent times, in our opinion, the extracellular vesicles can be utilized to a greater extent for the targeted therapy of PCa due to their effectiveness in inhibiting the target genes as well as the advantages it offers.

*Drug delivery through extracellular vesicles*

The extracellular vesicles are also used to carry drugs to the delivery site [Figure 3]. Followed by its isolation, the drugs are conjugated with them, which can reduce the toxicity and biocompatibility issues[156]. Saari *et al.* isolated the extracellular vesicles from PCa cells (PC-3 and LNCaP) through a process called differential centrifugation. An increased cytotoxic effect was observed when the paclitaxel was loaded into the extracellular vesicles derived from autologous PCa cells. Furthermore, endocytosis was the key pathway involved in the delivery of paclitaxel to the parental cells, thereby demonstrating the effectiveness of extracellular vesicles as carriers for drug delivery[83]. In another study, the urinary-derived exosomal system containing doxorubicin Exo-PMA/Fe-HSA@DOX nano vectors was explored for synergistic chemodynamic/low-dose chemotherapy of PCa. The nanosystem was shown to cause substantial internalization *in vitro* and to suppress the EGFR/AKT/NF-B pathways in cells[157]. Not just drugs, but a combination of both drugs and siRNAs have also been delivered for PCa therapy. For example, folate-conjugated extracellular vesicles (Co-Exo-FA) were developed which are obtained from nano-complex

loaded macrophages. Docetaxel and PLK1 siRNA were loaded in this system. It was shown that the system blocked the PLK1 gene in addition to its effect on tumor growth and reduced toxicity, which demonstrated the synergistic effect of drug and siRNA combination against castrate-resistance PCa[158].

*Phytoconstituent delivery through extracellular vesicles*

It has been reported that extracellular vesicles aid in the delivery of phytochemicals across biological barriers and have also been used against cancer[159]. However, in PCa, there are very few phytochemicals delivered through the exosome in our knowledge. Overall, extracellular vesicles are utilized to transport various molecules to PCa patients, and still, a lot of molecules such as various siRNAs, drugs, and phytochemicals can be loaded into extracellular vesicles for the targeted inhibition of various genes associated with PCa, tumor growth and to target the various steps involving in the emergence of PCa. In our opinion, this area remains much unexplored.

## CONCLUSION AND FUTURE PERSPECTIVE

Extracellular vesicles show a significant role when it comes to the entire scenario involving PCa. Once considered garbage bags of cells, extracellular vesicles are now being researched for their multifarious roles in cancer development. In the case of PCa, they have shown a dual persona: a negative aspect enables extracellular vesicles to contribute to proliferation and metastases while conferring resistance against the majority of chemotherapeutic agents and therapies, including radiation therapy. However, they also demonstrate a beneficial side, serving as diagnostic biomarkers and delivery vesicles for a vast array of agents, including nucleic acids, diagnostic agents, pharmaceuticals, and many more. This review aims to comprehensively cover both aspects of extracellular vesicles in PCa, encompassing their negative roles, such as involvement in angiogenesis, metastasis, proliferation, stemness, and resistance to multiple agents, alongside their roles as diagnostic agents and delivery agents for various pharmaceuticals.

There is a promising future for extracellular vesicles in treating PCa. While extracellular vesicles have shown tremendous progress as delivery vesicles for multiple agents, there is a limited number of studies focused on the delivery of phytochemicals in PCa using extracellular vesicles. Additionally, further studies can be conducted to explore how their role in drug resistance can be utilized positively while delivering antineoplastic agents in PCa. PCa has been a major threat in the male population, but future studies and the utilization of extracellular vesicles are expected to play a significant role in its treatment and mitigating its effects worldwide.

## DECLARATIONS

### Author contributions

Writing original draft: Jain DP
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All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

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