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Recent Progress on Exosomes in the Diagnosis of Prostate Cancer

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Abstract

Prostate cancer (Pca) is the second common cancer in men worldwide. Although prostate-specific antigen (PSA) screen can serve as a diagnostic marker in most of the patients with Pca, its diagnostic specificity is insufficient, and the false positive rate can result in unnecessary biopsy increasing pain and treatment costs in patients. Exosomes are source for mRNA, microRNA, non-encoded RNA, protein, and lipids. In recent years, exosome are used for assessment of tumors and serve as tumor markers for early diagnosis and disease prognosis. This article highlights the application of exosomes in connection with diagnosis, treatment and prognosis of Pca.

Key words prostate cancer, exosome, tumor marker, diagnosis

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Introduction

Prostate cancer (Pca) is the second common cancer in men worldwide. Most of the prostate cancer-related deaths are caused by local advanced or metastatic diseases [1, 2]. At present, there is a lack of treatment strategy for metastatic Pca [3]. The current standard treatment regimen include androgen deprivation, which can control disease progression, but this modality still have not satisfactory effect on CRPC patients [3, 4]. Therefore, it is particularly important to improve early diagnosis of PCa by developing methods that can detect malignant tumors. This will reduce excessive diagnosis and over-treatment for prostate cancer. Exosomes are small extracellular vesicles which participate in the occurrence, development, and metastasis of tumors [5, 6]. In exosomes, messenger RNA (mRNA), micro RNA (miRNAS), non-encoded RNA, protein, lipids, can serve as Pca biomarker[7-11]. Exosome-associated tumor markers in the body fluid such as urine and blood can improve early detection of the disease [12, 13]. This article highlights the research progress on exosome in the diagnosis of prostate cancer, in order to provide a reference value to further explore more high-specific tumor biomarkers in the exosome content.

Diagnostic method currently used in prostate cancer

At present, prostate-specific antigen (PSA) and digital rectal examination (DRE) is generally combined to screen prostate cancer [14, 15]. PSA is especially produced in human prostatic acinus and ductal epithelial cytoplasm. Because of dense prostatic structure, PSA does not enter the blood. During the diseased state, the current prostatic structure is destroyed, and the serum PSA rises in the blood, increasing the risk of Pca [16]. However, the rising PSA level is not distinctive to Pca [17]. Among men with benign prostate hyperplasia (BPH) and prostate inflammation, PSA levels also increase [18]. False positive results of PSA screening, and prostatic puncture will cause overexamination and over-treatment, which undoubtedly increases the psychological pressure and medical expenses for the patients [19]. When the PSA levels exceeds 4.0 ng • ml -1 or DRE result is abnormal, it usually suggestive of Pca, but the standards may change along with the changes in age, race and personal physiological conditions [20]. PSA detection is unable to distinguish between BPH or Pca. Currently, patients with PSA gray areas can be used to distinguish Pca and BPH with the ratio of free PSA /total PSA, but their diagnostic sensitivity and specificity are insufficient, only 75 % and 32 % [21, 22]. Denham et al. assumed that the ratio of free PSA /total PSA did not have advantages in clinically distinguishment for Pca and BPH [22]. The requirements in Australia is very different in assessing the PSA/Total PSA ratio. PCA3 is also a very widely used Pca urine markers. Urine PCA3 test is approved by the US Food and Drug Administration (FDA) in 2012 for detection of Pca. The urine sample testing after DRE is simple and fast.

Introduction to exosomes

The exosomes are cup-shaped or spheroid organelle, with a diameter of 30 ~ 150nm. The exosomes participates in the communication, coagulation, nucleic acid and protein metastasis between the exosomes [23, 24]. Exosomes is inseparable from the process of tumors development, development, and metastasis [25, 26]. There are three ways to enter recipient cell for exosomes: 1) Direct fusion with the cell membrane into recipient cells; 2) Exosomes enter by binding to the surface molecules of the recipient cell membrane; 3) The endocytosis of recipient cells enables exosomes into and secrete exosome contents, thus let exosomes function [27]. **Figure 1** shows that schematic diagram of an exosome.

CircRNA organisms mainly include four models: Splice looping of the exon lariat, Looping of intron pairs, Splice looping of the inner ring daughter lariat, Splice looping of internal ring rope, cyclization driven by RNA-binding proteins or trans factors [17]. Most circRNAs are encoded by known protein-coding genes and fall into three categories based on their structural composition: exon circRNA(EcircRNA), annular intron RNA(ciRNA), and exon-intron circRNA(EIciRNA) [18].

Exosomes as a biomarker

Transmembrane serine proteinase 2 (TMPRSS2) in Pca and the erythroblastic virus E26 carcinogen (ERG) fusion gene has been shown to be related to poor cancer prognosis [28]. Three RNAs (ERG, PC3, SP-DEF) in the urine exosomes of patients with the PSA gray area were detected to avoid unnecessary needle biopsy [29]. As a double-edged sword, the exosomes can not only help the clinical detection of disease. It not only induces apoptosis of immune cells but also reduce the function of the human immune system. The FAS ligand in the Pca exosomes induce the apoptosis of CD8 + T cells, inhibit T cell reactions, and completes the immune escape of Pca cells [30]. PCa patients through PKM2 induces CXCL12 in bone marrow mesenchymal stem cells [31], indicating that exosomes may be potential tumor marks of Pca. As a biomarker, the exosome have the following advantages: 1) exosomes released by tumor cells contains tumor-related molecules, which exist in blood [32], urine [33], semen [34], and breast milk [35] and other body fluids; 2) the lipid bilayer structure of exosomes can well maintain the stability of the nucleic acid, and will not be degraded by RNase; 3) exosomes can be extracted from the patient's urine and semen through non-invasive methods; 4) specific protein on the surface of the exosome membrane is conducive to reflect the physiological and pathological functions of its parent cells [36]. For some proteins, mRNAS, miRNAs, and lipids contained in exosomes have been identified as potential Pca biomarkers.

Relationship between exosome content and Pca

mRNA

Two genes kallikrein 3 (KLK3) and androgen receptor splicing variant 7 (AR-V7) encode PSA and AR-V7, respectively. Exosome KLK3 has a strong correlation with serum PSA value. AR-V7 is a splice variant of AR mRNA. The results obtained from the exosome show that among CRPC patients, the expression level of AR-V7 is related to hormone concentrations, as well as the adverse prognosis and the response to Abiraterone and Enzalutamide [37]. The study found that in patients with advanced Pca, AR-V7 mRNA level and AR-V7 /AR-FL ratio value is higher [38]. As a typical Pca biomarker, the expression levels of PSA mRNA and PCA3 mRNA are significantly increased in prostate-specific membrane antigen (PSMA) in urine. Therefore, capturing urine PSMA positive exosome detection PSA and PCA3 mRNA levels can improve the diagnostic efficiency of Pca [6, 39].

miRNA

miRNAs is a single-chain non-encoded RNA with a length of 18 ~ 22 nucleotides. It often blocks gene expression by combining with its target protein encoding mRNA, participating in translation inhibition and degradation of mRNA [7]. There are about 41.72 % of mature miRNA for all miRNA in the exosome [40], miRNA can affect tumorigenesis through oncogene or tumor suppressors, therefore the exosomes miRNA is of great significance to the early

diagnosis and prognosis of tumor. The application of miRNA in the exosome to liquid biopsy for prostate cancer can improve the specificity of PSA test. miR-196A-5p and miR-501-3p expressed downgrade in the urine exosome samples of prostate cancer [41], while miR-145 significantly increased compared to BPH patients (P = 0.018). miR-145 is significantly related to Gleason scores. Compared with the Gleason score ≤ 7 points, patients with Gleason score ≥8 points have increased miR-145 expression. miR-145 and serum PSA can better distinguish between Pca and BPH with PSA indicator alone. In addition, there are researches [42] reported that the combined expression of miRNA-21 and miRNA-375 in the urine exosome in Pca patients has a great advantage to identify prostate cancer. The area under ROC curve (AUC) is 0.863 and 0.805 respectively. miRNA levels are also closely related to the staging of tumors. The exosome miRNA has the potential to predict invasion or local metastasis. Expression of miR-888 in CRPC patients is significantly higher than normal healthy person and BPH patients [43]. The exosome miRNA-1246 expressed disorders in the invasive Pca, and in the invasive prostate cancer, miRNA-1246 is 31 times of the normal person (P = 0. 026), which is 23 times of BPH patients (P = 0.035) [44]. Combined with the detection of PSA and semen exosome miR-142-3p, miR-142-5p, miR-223-3p, patients with Pca and BPH can be accurately divided into 91.7 % sensitivity, and 42.9 % specificity [34, 45]. Thereby the specificity of the PSA screening test is increasing.

lncRNA

LncRNA (long-chain non-encoded RNA) can regulate tumor suppression, metastasis, and progress [46]. LncRNA-Myu can be transported by an exosome. It plays a role in the extracellular environment of Pca cells, LncRNA-Myu has raised c-Myc expression by competitive combination of miR-184, thereby inducing the prostate cancer cells, and the knockout of the MyU gene will decline the growth and migration of prostate cancer cells [47]. The level of exosome LncRNA may help diagnose BPH

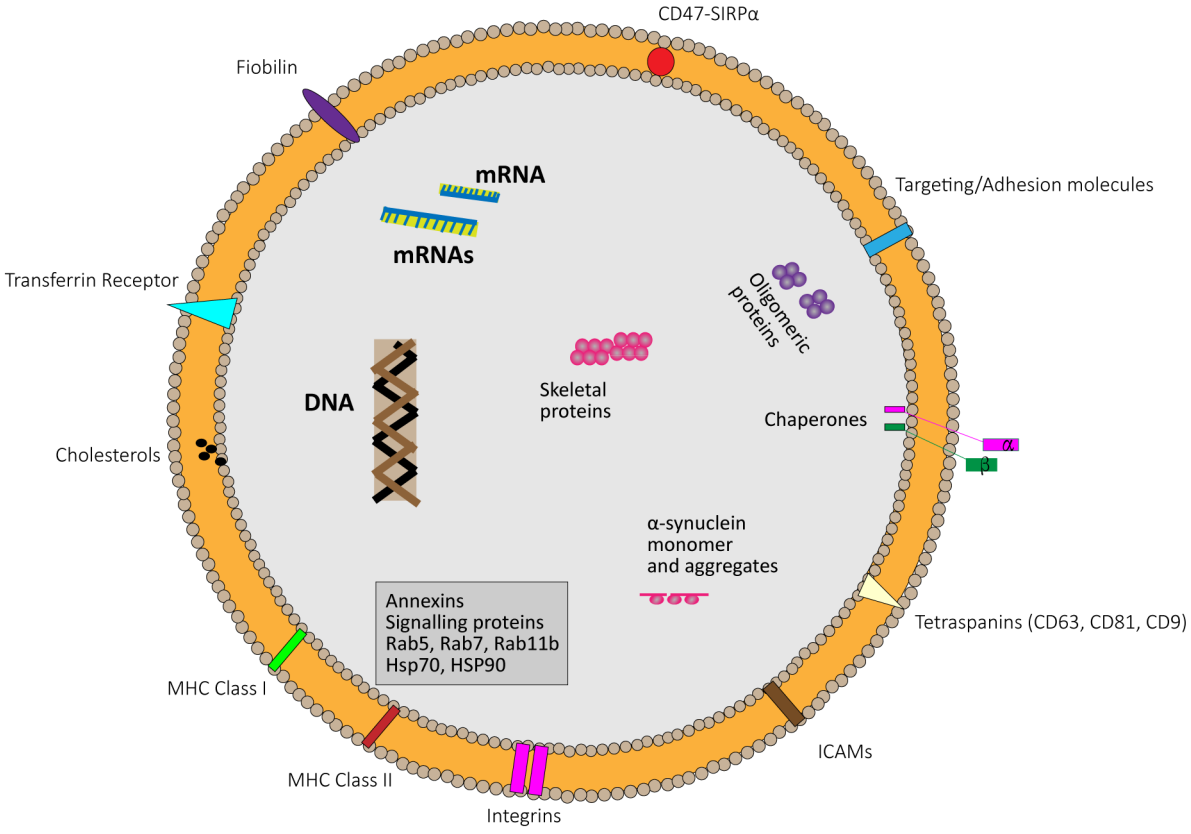

Figure 1. Schematic diagram of an exosome.

and Pca [46]. Evidence proves that the expression of LncRNA in different prostate diseases will also be different. The level of LncRNA-SAP30L-AS1 in the BPH group is significantly higher than Pca group (P <0.05) and the control group (P <0.00001), in the Pca group LncRNA-SCHLAP1 level is significantly higher than the BPH group (P <0.0000) and healthy people (P <0.000), and LNCRNA-SCHLAP1 levels will increase with the progress of Pca [48]. It was found thatn exosomal long noncoding RNA HOXD-AS1 promotes prostate cancer metastasis via miR-361-5p/FOXM1 axis [49]. Li Q et al reported that exosomal lncAY927529 enhances prostate cancer cell proliferation and invasion through regulating bone microenvironment [50]. In addition, an experimental study by Ozgur et. provides evidence that H19 might be involved in androgen receptor pathway [51]. And Young X et al. found that the long non-coding RNA PCSEAT exhibits an oncogenic property in prostate cancer and functions as a competing endogenous RNA that associates with EZH2 [52].

cirCRNA

circRNA (circular RNA) is a single-chain closed RNA, without 5' end hats and 3' end Poly (a) tail. It forms a closed-loop structure with covalent bonds, which is more stable than linear RNA [53]. circRNA has an important connection with the proliferation and migration of tumors [54]. In patients with prostate cancer, the expression of circRNA-0044516 in the exosome can be observed significantly, and the lowering of circRNA-0044516 will inhibit the proactive and transfers of prostate cancer cells [55]. There were some studies involving in the exosomal circRNAs and prostate caner recently. It were found that exosome-derived circTFDP2 promotes prostate cancer progression by preventing PARP1 from caspase-3-dependent cleavage [56]. Huang et found that exosomal circKDM4A Induces CUL4B to promote prostate cancer cell malignancy in a miR-338-3p-dependent manner [57]. In addition, it was reported that exosomal circRNA HIPK3 knockdown inhibited cell proliferation and metastasis in prostate cancer by regulating miR-212/BMI-1 pathway [58]. circ0081234 was inhibited is able to reduce prostate cancer tumor growth and metastasis via the miR-1/MAP 3 K1 axis [59].

Proteins

PSA in the exosome may become a new tool for the early screening and diagnosis for Pca. In contrast to patients with BPH, only Pca patients have nanovesicle expressed with PSA and CD81 at the same time [60]. Compared with healthy people, in the plasma of Pca patients, the expression of the exosome CAIX (carbonic anhydrase 9) is elevated [61], because the Pca exosome is acidic, and the acidity of the tumor micro-environment results in increasing the CAIX activity in Pca exosomes, and raising expression levels [62]. The activity of serum exosomes GGT (gamma-glutamyltransferase) may be potential Pca biomarkers. The chemical analysis of immunohistochemicals shows that the expression of GGT1 in cancer tissue is significantly higher than the BPH group (P <0.01), and the activity of serum GGT in Pca is higher than those of patients with BPH (P <0.05) [63]. The horizontal CLDN 3 (closed protein 3) in plasma increases with the increasing tumor grade. CLDN3 is a surface protein of Pca exosome. Gleason 8 and higher levels of tumor patients with exosome surface proteins CLDN 3 are significantly increased compared with BPH or Gleason scores 6 to 7 points (P = 0.029) [64].

The exosome from blood separation in Pca patients, αvβ3 integrin, αvβ3 transferred from exosome to β3 negative receptor cells, which is much related to the disease [65], suggesting that exosome to detect Pca may be a clinical biomarker to track the progress of Pca.

Lipids and fats

The lipids in the exosome may become a potential Pca biomarker. Endosomal sorting transport complex (ESCRT) plays an important role in the formation and release of the exosome [66], while some exosomes are generated by the non-dependent mechanism of ESCRT. Neutral sphingomyelin enzyme can not rely on ESCRT to form an exosome, and ceramides can form exosomes independent of ESCRT, which is one of the key lipids formed by the exosome [67, 68]. On the analysis of the urinary exosome of 15 Pca patients and 13 healthy person, it was found that phosphatidylserine (PS) 18:1/18:1 and Lactose ceramide (LACCER) (D18: 1/16: 0) were the most of significant diference (P <0.001), the latter is also the highest proportion in the samples of Pca patients and health groups, which is 95 %. A specific lipid combination can improve prostate cancer diagnosis 1/16:0. The sensitivity of the combination of PS18:1/18:1 and PS18:0/18:2 and PS18:1/18:1 is 93 %, the specificity is 100 %, and AUC is 0.989 [69]. See Table 1 in the related tumor markers of Pca.

Table 1 shows that a summary on exosome tumor markers in prostate cancer.

Treatment and prognosis of exosomes and Pca

The exosomes can convey the biological characteristics of various proteins and nucleic acids, so that it may become a carrier that can be used for tumor therapy [70]. Evidence shows that exosomes may have the potential to transport chemotherapy drugs to cancer cells. The serum exosome of Pca can affect the response to docetaxel treatment. CRPC patients use docetaxel as the first choice of antitumor drugs, loaded to the exosomes that source of Pca cells which can increase its cytotoxicity. The exosomes are continuously intake by Pca cells, and the paclitaxel is transported to receptor cells through internal swallowing, resulting in the intracellular release of the drug to enhance drug's ability [71]. High-throughput sequencing of plasma exosomes from Pca patients showed that the expression of exosomes miR-423-5p was downregulated in bone metastasis of Pca [72]. During the metastasis of Pca bone metastasis, the exosome miR-214 can remember the NOTCH1 signal pathway enhanced the differentiation of osteocytes [73], and the high expression of the exosome miR-940 also significantly enhanced the Pca bone metastasis [74]. The high expression of the exosome miR-21-5p of the osteogenesis stem cell source in PC3 can enhance its proliferation, EMT, migration, and invasion capabilities by lowering PHLPP2 protein [75].

Excessive expression of LNCaP cellular exosomes miR-26A can inhibit proliferation, migration, and invasion capabilities of PC-3 cells [76]. Human bone marrow derived stem cells derivative miR-205 high expression help inhibit Pca cells and enhance their apoptosis [77]. With the increasing clinical stage of Pca, the expression level of exosome miR-141 is increased [78], and the exosome miR-141-3P can promote the osteoblast bone metastasis and shorten the time of survival. Exosome of Pca cells MDA-PCA-2B, miR-141-3p can adjust the microenvironment of the bone metastasis and enhance the activity of osteoblasts [79]. The above studies have shown that the detection of exosome contents such as Pca may provide new possibilities for the treatment and prognosis of Pca bone metastasis.

Figure 2 shows that the role on exosomes in prostate cancer.

Summary and future perspectives

PSA combined with the detection of exosome content to improve the specificity and sensitivity of Pca diagnosis, and makeup for

Table 1. Exosome tumor markers in prostate cancer.

Exosome contents	Tumor biomarkers	Expression levels	Exosome source	References
mRNA	KLK3	Increased	plasma	[81]
	AR-V7	Increased	Plasma, urine	[80, 82]
	PSA	Increased	urine	[60]
	PCA3	Increased	urine	[29]
miRNA	miR-196A-5p	Decreased	urine	[83]
	miR-501-3p	Decreased	urine	[83]
	miR-145	Increased	urine	[84]
	miRNA-21	Increased	urine	[42]
	miRNA-375	Increased	urine	[85]
	miR-888	Increased	urine	[43]
	miR-1246	Increased	urine	[44]
	miRNA-142-3p	Increased	seminal fluid	[34, 86]
	miRNA-142-5p	Increased	seminal fluid	[34, 86]
	MiR-223-3p	Increased	seminal fluid	[34, 86]
LncRNA	LncRNA-Myu	Increased	tissue	[47]
	LncRNA-SChLAP1	Increased	plasma	[48]
	LncRNA-SAP30L-AS1	Increased	plasma	[48]
Protein	CAIX	Increased	plasma	[61]
	GGT	Increased	Serum	[63]
	CLDN3	Increased	plasma	[64]
	αvβ3	Increased	blood	[65]
Lipid	LacCer（d18:1/16:0）	Increased	urine	[69]
	PS 18:1/18:1	Increased	urine	[69]
	PS 16:0/18:1	Increased	urine	[69]
	PS 18:0/18:1	Increased	urine	[69]

the deficiency of PSA testing in false positive, diagnosis, and specificity. It can also become a carrier of cancer-targeted drugs, increasing medicinal effects, indicating that the exosome is not only a promising tumor biomarker, but also provide a new mode to treat cancer in the future. At present, the extraction method of exosome is mainly super-speed centrifugal method. It is an advantage that the specimens of various types of body fluids can be applied, but the disadvantage is the cost of the instrument during operation, and requirement of large samples. Exosome sedimentation method (suitable for large samples, simple operation, but long cycle and low purity), immunoaffinity capture method (high purity, high cost, low yield), ExoQuick kits (the operation is simple and efficient but the use cost is high), ultrafiltration method (average purity and affected if blockage of the filter film) [39, 80], has enormous potential but not yet developed a set of high efficiency, good specificity, low cost, and applicable to clinical standard protocols. The standard scheme is necessary to further explore the extraction solution of the exosome. There is currently a new type of micro-current system that can efficiently extract the exosomes. It is convenient, fast, high, and low in cost, however needs to be validated. If these exogenous biomarkers can be integrated into a screening plan, it may be able to improve Pca diagnosis to avoid unnecessary invasive biopsy, help patients reduce pain, reduce psychological pressure, and costs. In summary,

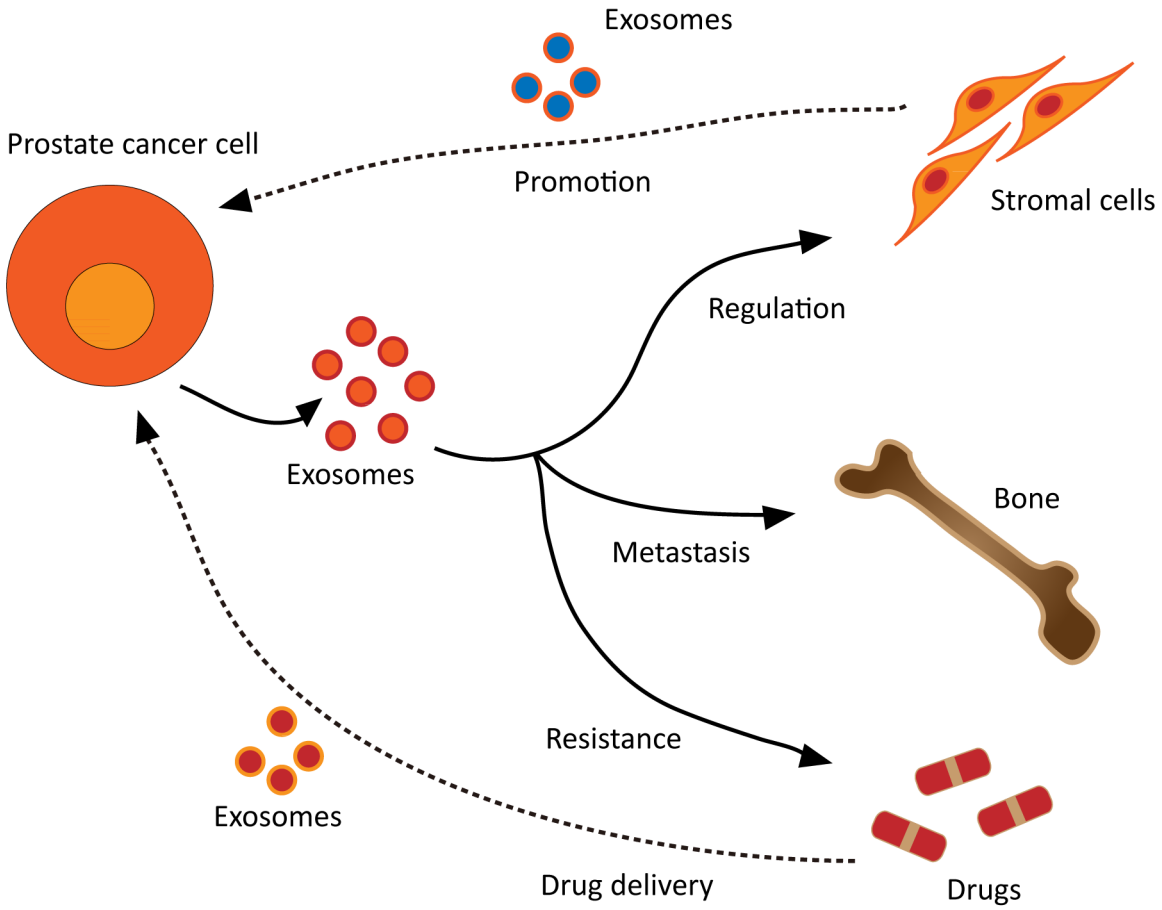

Figure 2. The roles on exosomes in prostate cancer.

it is worth looking for more high-specific tumor biomarkers in the exosome content to improve the diagnostic of Pca.

Acknowledgements

None.

Ethical policy

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutional ethical committee was taken.

Availability of data and materials

All data generated or analysed during this study are included in this publication.

Author contributions

JZW designed the study and was responsible for the writing of the original draft. TLL and MNL edited and approved the final manuscript.

Competing interests

All authors declare no competing interests.

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