

Exosomes-based biomarker discovery for diagnosis and prognosis of prostate cancer

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1. ABSTRACT

African American men in the United States have higher incidence and mortality rates due to prostate cancer (PCa) compared to other races. In 2016 alone, nearly 30,000 cases of PCa in African American men were diagnosed and 4,450 men died from PCa. The underlying reasons for this health disparity in PCa are complex and include social, economic, and biologic determinants. To reduce or eliminate this health disparity, we must better understand the biology of the disease in African Americans and then develop novel diagnostic and prognostic biomarkers useful for timely and effective treatment decisions. Recently, there has been remarkable progress in understanding the role of exosomes (vesicles of 30-150 nm diameter) in cancer development and progression. Exosomes are loaded with unique cargo, including proteins, nucleic acids, lipids, and metabolites, that could predict the cells of their origin. Therefore, circulating exosomes in cancer patients are being used as a type of biopsy to identify novel biomarkers for early diagnosis, prognosis, and therapeutics. In this review, we discuss the promising use of exosomes to (a) identify race-related unique biological features of PCa, and (b) discover novel biomarkers for better diagnosis and prognosis of PCa, with the goal of reducing cancer health disparities.

2. INTRODUCTION

In the United States, African Americans have the highest death rate and shortest survival of any racial or ethnic group for most cancers. In 2016, about 190,000 new cancer cases were expected to be diagnosed among African Americans, including 93,990 cases in men and 95,920 cases in women (1). Approximately 69,410 African Americans were estimated to die from cancer in 2016, including 35,660 men and 33,750 women (1). Prostate cancer (PCa) is the most frequently diagnosed cancer in men, and breast cancer is the most common cancer in women. Lung and colon cancers are the second and third most common cancers in African Americans. There has been unsatisfactory improvement in reducing this health disparity for past several decades. The reasons for health disparities in cancer are complex and include social, economic, and biological determinants. However, even after adjusting for socioeconomic factors, biological differences also appear important in faster disease progression and adverse prognosis in African American patients with PCa (2-15). Therefore, further research into tumor biology and development of novel diagnostic and prognostic biomarkers are needed to reduce cancer-related health disparities. In the present review, we describe the potential use of nanovesicles called exosomes, to identify novel biomarkers for PCa diagnosis and prognosis and to

begin to assess their usefulness to reduce cancer-related health disparities.

3. EXOSOMES: A BRIEF HISTORY AND MECHANISM OF THEIR BIOGENESIS

Exosomes are approximately 30-150 nm in diameter, released by all cell types and are present in most of the biological fluids including blood, urine, saliva, milk, and cerebrospinal fluid (16). Exosomes are highly heterogeneous in size and cargo, and generally reflect the physiological state of the cell that produces them. As that of cells, exosomes are surrounded by a lipid bilayer and could contain all known constituents of a cell, including proteins, lipids, RNA, DNA, and metabolites (17). Exosomes are mostly characterized by the presence of specific proteins such as tetraspanins (including CD81, CD63, and CD9); and proteins of the endosomal sorting complex required for transport (ESCRT) complex, flotillin, annexins, heat shock proteins (HSPs), tumor susceptibility gene 101 (TSG101), and various integrins and RAB family GTPases (16, 18-21).

There has been confusion regarding the nomenclature of exosomes and other vesicles released by cells. Lately, some consensus is emerging around use of the broader term extracellular vesicles (EVs) for all types of vesicles, including exosomes. Besides exosomes, other major vesicles being studied are microvesicles, large oncosomes, and apoptotic bodies. These different categories of vesicles mainly differ in terms of their cellular origin and size. For example, exosomes (~30-150 nm) are generated through the endosomal system, while microvesicles (100-1000 nm or more) bud off from the plasma membrane. Identifying the unique molecular features of vesicles is currently an intense area of research.

EVs were first described in the late 1960s, as platelet free plasma containing vesicular material (called 'platelet-dust'). This material could ultracentrifuged into pellets and showed coagulant properties resembling those of platelet factor 3 (22). Later, using electron microscopy, these vesicular structures were observed in fetal bovine serum (called 'microvesicles') and subsequently in tumor shedding (23, 24). Initially, it was supposed that these vesicles were released by outward budding of the cell membrane. However, Johnstone and colleagues proposed that endocytosis followed by formation of multivesicular bodies (MVBs) and their fusion with the plasma membrane led to secretion of vesicles (called 'exosomes') (25). This discovery was based on the observation that reticulocytes release their transferrin receptor, into associated vesicles as a part of their maturation to erythrocytes (25, 26). In 1996, Raposo *et al.* were the first to describe the role of exosomes in antigen presentation while studying immune-modulating activity of B cell-derived vesicles (27). They showed that exosomes derived from both

human and murine B lymphocytes have the ability to induce antigen-specific MHC class II-restricted T cell response and suggested their usefulness in immunotherapy (27). Subsequently, Zitvogel *et al.* revealed that exosomes derived from tumor peptide-pulsed dendritic cells primed specific cytotoxic T cells *in vivo* and eliminated or inhibited the growth of established murine tumors in a T cell-dependent manner (28). This study suggested the application of exosome-based cell-free vaccines as an alternative to dendritic cell adoptive therapy for suppressing cancer growth (28). These studies introduced the notion that exosomes are not solely a waste disposal mechanism, but are also important mediators of intercellular communication. Subsequent studies confirmed and further elaborated the key role of exosomes in intercellular communication in normal growth and development, and in various diseases, including cancer (29-34).

4. MECHANISM OF EXOSOME BIOGENESIS

The ESCRT pathway is so far the best studied pathway of exosome biogenesis. The role of ESCRT machinery in exosome biogenesis was initially deduced from proteomic analyses showing the presence of TSG101 and Alix in exosomes from diverse cell types (35). The ESCRT machinery consists of four multiprotein complexes known as ESCRT-0, I, II, and III, and several accessory proteins including VPS4 and Alix (36). ESCRT-0 has a key role in cargo clustering which is ubiquitin-dependent, ESCRT-I and ESCRT-II trigger bud formation in the early endosomal membrane. ESCRT-III controls vesicle scission and VPS4 ATPase (along with other accessory proteins) play a role in dissociation and recycling of the ESCRT complex. Colombo *et al.* recently reported the ESCRT functions in exosomes biogenesis, composition and secretion by performing an RNA interference screen targeting 23 components of ESCRT machinery in HeLa-CIITA cells (37). Silencing of the ESCRT-0 members HRS and STAM1 or the ESCRT-I component TSG101 reduced the release of exosomes, whereas silencing of VPS4B increased it (37). Although ESCRT seems to play important role in exosome biogenesis, several studies have also noted that ESCRT components could be dispensable for exosome secretion (36, 38, 39).

Several other proteins (including RAB GTPases, syndecan-syntenin, tetraspanins and cortactin) may regulate rate of exosome biogenesis and/or their composition (17, 40-44). Lipids such as BMP (bis-monoacylglycerolphosphate), ceramides, and cholesterol are also important in exosome biogenesis and cargo loading (39, 45). We recently reported that concentrations of secreted exosomes and VEGF loading decreased significantly when PCa cells were cultured under delipidized serum conditions (46). These results were similar when PCa cells were cultured under normoxic or hypoxic conditions, and

suggested the importance of cellular lipids in the biogenesis of exosomes.

5. EXOSOMES UPTAKE AND INTERCELLULAR COMMUNICATION

Exosomes are a key facilitator of intercellular communication via their ability to transfer microRNA (miRNA, small non-coding RNA molecule containing about 22 nucleotides), proteins, and lipids to recipient cells. Cells appear to take up exosomes by a variety of endocytic pathways, including clathrin mediated endocytosis, caveolin mediated endocytosis, macropinocytosis, phagocytosis, lipid raft-mediated endocytosis and membrane fusion (47). There are few examples of cell type specific uptake of exosomes suggesting that exosomes could be targeted to specific cell type/s (48-50). For example, pancreatic adenocarcinoma derived exosomes were shown to be internalized most efficiently by peritoneal exudate cells and less proficiently in granulocytes and T-cells (48). However, there are other studies suggesting that exosomes uptake is quite non-specific (47, 51). Overall, whether or not exosomes uptake is cell type-specific is still an open question.

Exosomes have been implicated in cancer growth and progression, neo-angiogenesis, preparation of pre-metastatic niches, drug resistance, immunosuppression, and cancer relapse (34, 46, 52-55). Although, exosomes have been largely associated with tumor growth and progression, they could also exert antitumor function dependent upon cell type and microenvironment context (56, 57). For example, exosomes from dendritic cells could activate B and T cells and exert anti-cancer effects; on the other hand, cancer cell-derived exosomes could cause immunosuppression via activating Tregs (regulatory T cells) and inhibiting natural killer cells proliferation and cytotoxic functions (17, 58, 59). Similarly, exosomes secreted by human mesenchymal stem cells (hMSCs) have been implicated in both cancer inhibition and promotion (60-65).

In cancer cells, the rate of exosome biogenesis, constitution of their cargo, and their biological effects on recipient cells are also determined by the microenvironment (17, 34, 55, 66). For example, low oxygen conditions (hypoxia) in tumors is correlated with poor prognosis and considered the major reason for treatment failure and disease relapse (67-70). Cancer cells secrete more exosomes under hypoxic conditions (66). A recent study by Li *et al* indicated that exosomes from hypoxic oral squamous cell carcinoma cells (OSCC) have distinct miRNAs – especially higher miR-21 – and promote the migration and invasiveness of normoxic OSCC cells (71). We also recently showed that exosomes released by PCa cells under hypoxic conditions were loaded with 160 proteins compared

to exosomes secreted by PCa cells under normoxia, which had 62 proteins (54). Exosomes secreted under hypoxic conditions promoted the epithelial–mesenchymal transition (EMT), invasiveness, and stemness in naïve PCa cells, and also increased the α -smooth muscle actin expression in naïve normal prostate fibroblasts, indicative of a cancer-associated fibroblast (CAF) phenotype (54). We also found that hypoxic PCa exosomes were loaded with significantly more triglycerides, and hypoxic PCa exosome-induced invasiveness could be inhibited by celecoxib; and our data suggested a role for COX2 enzymatic products in higher PCa clonogenicity and invasiveness (46).

Exosomes also have been implicated in regulation of angiogenesis in tumors (72). The uptake of cancer cell derived exosomes by endothelial cells is known to stimulate angiogenesis (53, 73). Furthermore, exosomes secreted by cancer cells or other tumor microenvironment components could promote metastatic progression. For example, invasiveness and colonization potential of less invasive cancer cells was increased by exosomes from invasive cancer cells via transfer of miR-200 (74). Similarly, tumor exosomes can alter the physiology of both surrounding tumor cells or distant non-tumor cells to allow growth and metastatic spread of cancer cells (75-77). Peinado *et al* reported that exosomes secreted by melanoma tumors expressing a specific tyrosine-kinase receptor (MET) promoted the bone marrow progenitor cells migration to future sites of metastasis (known as 'pre-metastatic niches'), unlike exosomes released by a less aggressive cancer cells without the MET receptor (77). The same group recently reported that exosomes from different types of tumor are presented with unique integrins targeting these exosomes to specific organs, activating specific signaling pathways and initiate pre-metastatic niches preparation at these specific sites (76). Alterations caused by exosomes in these specific organs then attract and guide metastatic tumor cells to these specific sites.

Taken together, the literature indicates that exosomes add another layer of complexity to the tumor microenvironment, and play an important role in intercellular communication that controls tumor growth and progression.

6. EXOSOMES IN TUMOR DIAGNOSIS AND PROGNOSIS

The availability of detailed molecular information within circulating exosomes has created immense interest in their use in cancer diagnosis and prognosis. Because exosomal proteins in biological fluids generally reflect their parent cells' information, their unique composition could assist in the cancer detection. Exosomes are present in almost all kinds of biological fluids, including blood, urine, saliva,

amniotic fluid, cerebrospinal fluid, ascites, and tears (78-81). Exosomes from cancer patients could serve as a biomarker for the diagnosis and prognosis of malignancies (53, 82-88). For example, exosome concentrations were observed to be relatively higher in the blood of patients with prostate, ovarian, breast, and pancreatic cancer (82, 87, 89). Recent studies have enhanced the understanding of exosome contents and their application to diagnose and monitor cancer. Although exosomal lipid and metabolites information could also offer unique insights in the detection and biology of cancer, currently more data is available about proteins and nucleic acids in exosomes (17, 87, 90).

Proteins for example the epidermal growth factor receptor VIII (EGFRvIII) present in exosomes might serve as reliable and sensitive glioblastoma biomarkers (53). Development of sensitive exosomal-based biomarkers from blood or cerebrospinal fluid could be clinically valuable, avoiding risky brain biopsies. Unique exosomal miRs could also be useful in the diagnosis and to monitor the cancer progression (89, 91-95). For example, exosomal miR-21 level was observed higher in the serum of patients with esophageal squamous cell carcinoma and correlated with advanced disease (92). In PCa patients, serum levels of miR-141 discriminated between metastatic and localized disease (94). In addition, Long *et al* reported a miR profile in urine that could be useful in detecting urothelial carcinoma of the bladder (93).

Apart from miRNA, exosomal DNA could also provide information about cancer-specific mutations (96, 97). Whole genome sequencing showed that pancreatic cancer patients serum exosomes contained the entire genomic double-stranded DNA covering all chromosomes (96). Driver mutations linked with pancreatic cancer were also present in this exosomal DNA (96). Importantly, McKiernan *et al* identified a noninvasive 3-gene expression assay in urinary exosome that can discriminate clinically relevant Gleason-grade 7 diseases from low-grade (Gleason grade 6) PCa (98). This is a huge advancement as currently overdiagnosis and unnecessary PCa treatment are immense clinical problems.

Peinado *et al* showed that levels of total MET and phosphorylated MET (Tyr1349) were higher in the exosomes from patients with stage 3 and stage 4 melanoma compared with healthy controls (77). Similarly, increased exosomal levels of macrophage migration inhibitory factor were associated with disease progression among patients with pancreatic cancer (75). Melo *et al* also reported that expression of glypican-1 (GPC-1), a cell surface proteoglycan, was higher on pancreatic cancer-cell-secreted exosomes (82). Further, they detected GPC-1+ exosomes with absolute specificity and sensitivity in the pancreatic cancer patients' serum; and could distinguish healthy people and patients with a

benign pancreatic disease from patients with early- and late-stage pancreatic cancer (82).

Overall, there is evidence that circulating exosomes from patients with several types of cancers could be used in cancer diagnosis (Table 1) and to predict therapeutic outcomes. Such a tool would reduce the need for invasive biopsies. In the future, identification of specific biomarkers on the surface of cancer exosomes could enable their isolation from the heterogeneous population of exosomes in bodily fluids and thereby increase their utility for clinical applications.

7. EXOSOMES AS A THERAPEUTIC TARGET

Since exosomes play an important role at each stage of carcinogenesis, it is plausible to consider cancer exosomes as potential therapeutic targets. In one study, suppression of exosome-mediated intercellular transfer of miRs from cancer cells to endothelial cells decreased angiogenesis and breast cancer metastasis (99). Exosomes derived from cancer cells and tumor stromal cells have also been identified in the development of resistance to chemotherapy (100, 101), suggesting that targeting specific exosomes may improve therapeutic response. Chen *et al* reported that exosomes from drug-resistant breast cancer cells disseminate resistance towards chemotherapy via a horizontal transfer of specific microRNAs (100). In another study, transfer of exosomes derived from fibroblast to breast cancer cells conferred resistance to radiation and chemotherapy therapy (102).

Exosomes can also reduce effectiveness of chemotherapy by removal of chemotherapeutic drugs from the tumor cells (103, 104). For example, cisplatin and doxorubicin were reported in exosomes from cancer cells after chemotherapy (103, 104). Ciravolo *et al* observed that HER-2+ exosomes from breast cancer cells reduced the anti-proliferative effects of trastuzumab (105); thus, removal of these exosomes from circulation might improve response to trastuzumab (106).

Exosome removal from cancer patients could also reduce the immune-suppressive effects mediated by exosomes from cancer cells (107). However, additional studies are warranted to better grasp the broader physiologic effects of systemic exosome depletion. Inhibitors of exosome biogenesis in cancer and/or cancer-associated cells or agents targeting exosome uptake in specific cells could also be an effective adjunct to anti-cancer therapies.

8. PCA-RELATED HEALTH DISPARITIES

African American men in the United States have higher incidence and mortality rates due to PCa than men of other races (1, 3, 108, 109). In 2016 alone,

Table 1. Lead exosomal biomarkers for diagnostic application in different cancers

Exosomal Biomarker	Study Type	Cancer Type	Reference
EGFRvIII	Clinical sample, cell culture	Glioblastoma	(53)
miR21	Clinical sample	Esophageal squamous cell carcinoma	(92)
ERG, PCA3, and SPDEF	Clinical sample	Prostate cancer	(98)
MET	Cell culture, Clinical sample	Melanoma	(77)
Glypican-1	Cell culture, Clinical sample	Pancreatic cancer	(82)

about 30,000 cases of PCa were diagnosed and about 4,450 PCa deaths were expected in African American men (1). For the past several years, PCa has been the number one in terms of incidences and second-leading cause of cancer-related death in African American men after lung cancer (108, 110). In addition, African American men are more frequently diagnosed with advanced PCa at earlier ages, with poorer prognoses and worse overall survival rates than Caucasian men (1, 3, 108-110).

Besides socioeconomic factors, genetic and epigenetic determinants could also be factors behind the higher burden of cancer in a particular race. For example, single nucleotide polymorphisms (SNPs) in a number of metabolic genes are linked with PCa in African Americans, but not in Caucasians (8). Some of the most studied and promising target genes include cytochrome P450 17 α -hydroxylases-C-17,20-lyase (111), CD14 (112), cytochrome P450 3A4 (113), calcium sensing receptor (9), both overall expression and the number of CAG repeats of androgen receptor (10, 11), and variations at 8q24 site (12). Products of these genes play a role in androgen metabolism, involved in PCa initiation and progression. Several biochemical factors or mechanisms may also be associated with increased PCa incidence and bad prognosis in African Americans. Among these, decrease in serum vitamin D levels (114, 115), insulin-like growth factor 1 (IGF-1), and insulin-like growth factor binding protein 3 (IGFBP3) ratio and serum concentrations (116, 117), decreased serum lycopene levels (118); and an increase in serum low-density lipoprotein (LDL) levels (13) are the key alterations seen in African American men.

Other factors such as DNA methylation, miRNA-mRNA pairing, and dysregulation of miRNAs also have been suggested to underlie ethnic disparities in PCa (14, 119, 120). A recent study pointed out the role of race-specific methylation pattern in the early detection of aggressive PCa (119). This study found that RARB (Retinoic Acid Receptor Beta) methylation was linked with disease outcome in certain subgroups of African American PCa patients with aggressive disease, whereas APC methylation was associated with the risk for PCa progression only in Caucasian men (119). EGFR overexpression has also been associated with PCa in African American patients (15). A total of 18 reciprocal miRNA-mRNA pairs populate the EGFR-PI3K-AKT

signaling pathway in African Americans with PCa, which likely act in concert with overexpressed EGFR and drive disease aggressiveness (14). Among miRNA-mRNA pairs, miR-513c/STAT1, miR-96/FOXO3A, and miR-145/ITPR2 appear to be the key pairs contributing to disparities in PCa among African Americans (14). Yang *et al* defined a novel role for hnRNPH1(heterogeneous nuclear ribonucleoprotein H1) as a putative oncogene, splicing factor, and an auxiliary androgen receptor co-regulator (120). Furthermore, the targeted disruption of the hnRNPH1-AR axis could be used as the therapeutic interventions to improve clinical outcomes in African American patients with advanced PCa (120).

Nonetheless, we have only limited knowledge about key factors and molecular pathways that drive PCa initiation, promotion, and progression in African Americans. For example, only recently it became apparent that the mutation spectrum in patients with PCa is quite different among those of African American versus European ancestry (121). African American patients with PCa has fewer instances of TMPRSS2-ERG gene fusion and mutations in TP53 and PTEN genes, but higher rates of CDC27-OAT gene fusion and mutations in MUC3A and PRIM2 genes, compared to PCa patients of European ancestry (121). However, currently, most diagnostic and treatment decisions are based on studies performed in Caucasian PCa cells or patients. These treatments might not be equally efficacious in African Americans with PCa; in fact, they could even be counterproductive. For example, a recent study suggested the clinical use of zinc chelators to target PCa growth (122). However, African American men with PCa already have extremely low levels of intracellular zinc due to low expression of zinc transporters; thus, they would more likely benefit from zinc supplementation (123). Further research into the unique tumor biology of PCa in African Americans is urgently needed to improve their prognosis and treatment.

9. EXOSOME-BASED NOVEL BIOMARKERS IN AFRICAN AMERICAN MEN WITH PCA

One reason for PCa-related disparity is that currently we lack effective biomarkers to predict PCa aggressiveness and progression in African Americans. For example, African American men with Gleason

score 6 PCa produced significantly lesser prostate specific antigen (PSA) and had significantly lower PSA density than Caucasian men. As a result, active surveillance criteria predictive in Caucasian men were not accurate in African American men (124). Clearly, novel and effective biomarkers are needed for better disease prognosis in African American men.

Biomarker mining from exosomes is an exciting approach, since the cargo of exosomes is well protected by lipid bilayer and they often have a distinctive cargo (e.g. proteins, RNAs, lipids, metabolites) reflecting the cells from which they came (46, 54, 88, 125, 126). Furthermore, compared to the complex environment of plasma, exosomes offer a much cleaner clinical sample devoid of any plasma proteins, facilitating analyses (127). Hence, analyses of exosomes offer a unique opportunity for developing novel protein and/or nucleotide biomarkers for detecting and monitoring PCa.

At present, only a few studies have suggested that exosomes could be useful to develop novel biomarkers aimed at reducing PCa-related health disparities (87, 90). In one such study, Turay *et al* evaluated plasma exosomes from patients with PCa who had different ethnic backgrounds (African American, Caucasian, or Hispanic) (87). Compared to healthy individuals, exosome concentration (measured by acetylcholinesterase activity) was increased ~5 fold in all PCa patients; however, there were no significant differences in exosome concentration across different ethnic groups (87). Mass spectrometry identified 74 unique proteins in the exosomes of healthy individual, 113 unique proteins in the exosomes of PCa patients and 71 overlapping proteins between the two. These unique proteins in the exosomes from healthy individual and PCa patient could lead to the discovery of novel biomarkers (87). This study also identified the unique proteins present in African Americans (22 proteins), Caucasians (13 proteins) and Hispanics (78 proteins) with PCa; in the future, these proteins could be useful to develop race-specific PCa biomarkers. Importantly, several of these have already been associated with various cancers, including PCa. For example, one protein associated with PCa, trinucleotide repeat containing 6B isoform3 (TNR6B), has known interactions with novel loci, single nucleotide polymorphisms (SNPs), onTET2 and SYK with interactions modifying the risk for PCa (128).

Recently, another study evaluated the role of exosome-associated miRNAs as biological determinants for disparities among patients with PCa (90). Exosomes were isolated from the conditioned medium of MDA-PCa-2b (African American origin), PC-3 (Caucasian origin), C4-2B (Caucasian origin), and RWPE-1 (non-tumorigenic) cell lines to study the endogenous expression of miRs and their anticipated target genes. Potential candidates were then validated

in exosomes isolated from the plasma of African American and Caucasians patients with PCa. miR-125b, miR-155, and miR-3613 were upregulated in PCa versus normal RWPE1 cells, and in African American versus Caucasian PCa cells. The expression of tumor suppressor genes, such as Lats2, PDCD4, and SMEK1, were downregulated in PCa cells compared to normal cells. African American patients also had differential expression of PCa exosome-associated onco-miRs compared to Caucasian patients or age-matched normal subjects. Onco-miR-125b and high Gleason score were positively correlated (90). Overall, this study suggested the potential importance of exosome-based miRNAs to understand racial differences in PCa.

However, additional studies are needed before above mentioned or any other putative biomarkers could be used to define risk, presence, or even therapeutic response in PCa. Further, additional studies with larger cohorts of ethnically diverse patients and relevant controls are required to unequivocally identify exosome-based PCa biomarkers to examine health disparities.

10. CONCLUSION AND FUTURE DIRECTION

African American men face a high risk of developing and dying due to PCa; new measures are urgently needed to reduce this cancer burden and health disparity. Circulating exosomes show potential as a type of biopsy, to discover novel biomarkers for early detection and treatment of cancer, since content of exosomes generally reflects the molecular profile of their cell of origin. Tumor exosomes contain vast amounts of molecular information that can be extracted using existing technologies such as proteomic, RNAseq and lipidomic analyses. Therefore, exosomes could reveal unique biological information about disease states in different races useful for making timely treatment decisions. Exosome-based biomarkers could be extremely valuable in managing advanced-stage metastatic PCa, since obtaining biopsies from metastatic sites (such as bones) is difficult and painful. Besides exosomes, circulating tumor cells are also being studied as a source of possible biomarkers. However, these cells are sparse in PCa, and not easy to isolate and characterize in clinical settings. Furthermore, African American men with PCa secrete higher amounts of exosomes, suggesting that depletion of PCa-specific exosomes from blood and/or targeting exosome biogenesis in PCa cells and/or their uptake by other cell type offer attractive therapeutic targets. In this regard, it will also be interesting to study the exosome based interaction between cancer cells and stroma, and effect of such interactions on their intracellular signaling as well as tumor progression. Overall, exosomes show promise as a method to identify race-related unique biological features, and to discover novel biomarkers for better

diagnosis, prognosis, and treatment of PCa with the goal of reducing cancer health disparities.

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