REVIEW

Health disparity of prostate cancer: Molecular insights into the role of exosomes

Hamdy EA Ali[1], Shaimaa A. Gad[1], Gagan Deep[2], Hamed I. Ali[1], Zakaria Y. Abd Elmageed[1*]

[1]Department of Pharmaceutical Sciences, Rangel College of Pharmacy, Texas A&M Health Sciences Center, Texas
[2]Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA.

*Corresponding Author: Zakaria Y. Abd Elmageed; e-mail: elmageed@tamhsc.edu

ABSTRACT

Prostate cancer (PCa) is the second leading cause of morbidity among older men in the States. The morbidity and mortality rates of PCa are twice as prevalent in African American (AA) than in Caucasian American (CA) men. Owing to the involvement of multiple factors contribute to such disparities, it remains unclear whether the high incidence and mortality rates of PCa among AA men are associated with genetic and epigenetic factors. The molecular mechanisms underlying these biological factors have yet to be fully elucidated. Exosomes are cell-derived extracellular bodies secreted by normal and tumor cells therefore promoting cell-cell communications. Exosomes are vesicular bodies that transfer different biological materials such as microRNAs, mRNAs, lipids, DNA and proteins to recipient cells. Our goal here is to illustrate the role of exosomes contributing to different biological activities, especially aggressive behavior of cancer cells and poor clinical outcomes of PCa in AA patients. There is a need to discover new biomarkers used in diagnosis and prognosis of PCa. It follows that a special focus on cancer disparities among AA men. This review indicates that more studies are needed to build on these recent findings for future understanding of the role of PCa-associated exosomes in promoting PCa aggressiveness in AA and other cancer disparities. Elucidating exosomal interactions in cancer and other chronic diseases should help to eliminate morbidity and mortality disparities among US minorities.

KEYWORDS: Prostate cancer, Health disparity, Exosomes, microRNAs

Citation: Ali HEA. et al. (2017). Health disparity of prostate cancer: Molecular insights into the role of exosomes. Cancer Health Disparities;1:e1-e13. DOI: 10.9777/chd.2017.10002

The incidence of prostate cancer remains high, biomarkers and molecular targets are an unmet need. Although the efforts in the field of biomedical research are advancing, more integrated research procedures and new strategies are still needed to promote early discovery, and hence reduce the burden while increasing the overall survival of cancer patients. Prostate cancer (PCa) is the second leading cause of death among older men in the United States (Jemal et al., 2008). After diagnosis, systemic therapies have been used as an option for managing the disease; however, chemotherapy is the ultimate solution, especially in castration-resistant PCa (CRPC) patients (Paller and Antonarakis, 2011). Although PCa is a multifactorial disease, androgens and their receptors (AR) represent the main driving forces for promoting PCa at all stages: initiation, progression and metastasis. Therefore, inhibition of AR and its downstream signaling pathways is the mainstream of current therapeutic targeting approaches. AR variant 7 (ARV7) is one of the most common AR variants that has a current clinical applications. ARV7 sequence contains the first three exons of the full length of AR sequence; exons 4-8 are replaced with a "cryptic exon" and, therefore, ARV7 lacks a ligand-binding domain. A recent emerging role of ARV7 in advanced PCa has been documented in PCa cells (Hu et al., 2009), animal models (Guo et al., 2009; Watson et al., 2010) and CRPC patients (Del Re et al., 2017; Djusberg et al., 2017). The challenge in advanced stages of PCa is to develop new therapeutic agents and suppress androgen activity at its AR (wild form or its variants) and/or androgen metabolizing enzymes.

The most common diagnostic tools for PCa are serum prostatic specific antigen (PSA), systematic prostate biopsies under ultrasound guidance and pathological staging with grading according to the Gleason score system (Heidenreich et al., 2014; Mottet et al., 2017). Although PSA is still the gold standard utilized for detection of PCa, it is also elevated in men who have benign prostate hypertrophy, urinary tract infection, prostatitis, and after prostate surgeries and biopsies. Hence, the escalated level of PSA is not specific to PCa; this might lead to overdiagnosis followed by overtreatment accompanied with adding extra-cost on patients with low-risk of PCa (Lee et al., 2013). Other biomarkers have been developed to stratify patients according to their tumor stage, response to treatment, CRPC status, and metastasis. Current biomarkers, such as prostate cancer antigen 3 (PCA3), α-methylacyl coenzyme A racemase (AMACR), TMPRSS2 (transmembrane serine protease isoform 2)-ERG (ETS transcription factor) gene fusion, and PTEN (phosphatase and tensin homolog) gene deletion, are clinically evaluated and some of them awaiting approvals. Accordingly, tremendous efforts have been directed towards the discovery of second-generation PCa biomarkers that can predict poor prognosis of the disease and can assist oncologists for proposing better treatment options for their patients. Owing to tumor heterogeneity, none of the current biomarkers is ideal to account for the wide variety of human samples, state of the disease and other clinical outcomes. As such, it is essential to develop new strategies for early detection and prognostication of PCa that may serve as surrogate endpoints for better evaluation of disease progression and effective treatment regimens thereby increasing patients' survival.

Molecular mechanisms underlying health disparities of PCa have not been well determined. The mortality rate of PCa in African Americans (AA) is about twice that of Caucasian Americans (CA) or any other minorities (Hsing et al., 2000). Considering cancer disparity as a multifactorial event, it remains unclear whether the high incidence of mortality rate of PCa among AA men is promulgated principally by genetic, lifestyle, or socioeconomic-related factors. The molecular mechanisms underlying these discrepancies have yet to be fully elucidated. Levels of AME, growth factors, non-coding RNA and other genetic factors are higher in AA men than in other races (Hatcher et al., 2009; Khani et al., 2014). Interestingly, the hormonal level of estrogen but not testosterone significantly differs between AA and CA men (Rohrmann et al., 2007). Our recent study substantiated higher circulating estrogen and selective expression of its receptor (ERβ) in PCa tissues of AA compared to CA men (Abd Elmageed et al., 2013). Presumably, another factor associated with disease aggressiveness in AA men is the overexpression of SPINK1 (serine peptidase inhibitor Kazal type-1) in tissues of AA men (Khani et al., 2014). Other genetic and epigenetic factors may involve in health disparities of PCa. For example, Chaudhary *et al.* (2016) reported that AA PCa cells exhibited reduced endogenous reactive oxygen species, mitochondrial membrane potential and less expression of heat shock proteins compared to CA PCa cells (Chaudhary et al., 2016). Intriguingly, the mitochondrial genome may contribute to the inherited racial disparities *via* the crosstalk between the mitochondria and the nucleus, which may overactivate signaling pathways in AA patients (Choudhury and Singh, 2017). Also, immunohistochemical studies on formalin-fixed paraffin-embedded tissues collected from 169 AA patients have demonstrated that, although PTEN and ERG expression are less frequent in AA patients, nonetheless, PTEN loss associated with poor prognosis of AA compared CA patients (Davenport, 2004). In a more recent study, the gene locus of RGS12 (regulator of G protein signaling 12) on chromosome 4p16.3 was not detected in AA men suggesting the tumor suppressive role of this gene in regulating cancer progression (Wang et al., 2017). In contrast, using three standard models of risk prediction in PCa, genotype and epigenetic data collected from 59,089 men of AA and CA origin exhibited insignificant difference between the two ethnicities suggesting high similarities in their genetics hallmarks (Gusev et al., 2016).

microRNAs (miRs) are small non-coding regulatory RNAs that regulate gene expression at the post-transcriptional level. A growing body of evidence suggests that miRs are involved in the progression of many cancer types including PCa. Profiling of miRs has identified a specific set of miRs that can be utilized in PCa diagnosis and prognosis. The effect of miRs as epigenetic factors contributes disproportionately to PCa that has been recently studied. A group of scientists applied genomic-wide profiling for microRNAs (miRs)-mRNA in 60 primary PCa compared to 16 normal counterparts; they found that miR-106b-25 cluster/MCM7 and miR-32/C9orf5 are highly expressed in PCa compared to controls (Ambs et al., 2008). Using integrative genomic approach, 10 specific miRs were identified along with their target genes, which were differentially expressed in to AA compared to CA men with PCa (Wang et al., 2015). In this regard, EGFR was the more likely signaling pathway used in cancer cells of AA origin. The

same study also suggests that regulation of miR-mRNA crosstalk is the key step to control the oncogenic activities of miR-133a-MCL1, miR-513c-STAT1, miR-96-FOXO3A, miR-145-ITPR2 and miR-34a-PPP2R2A in AA PCa tumor cells. miR-24 was reported to have a possible regulatory role in PCa progression based on the race. In this study, AA PCa cells MDA-PCa-2b treated with 5-AZA-2'-deoxycytidine differentially restored miR-24 compared to CA PCa cells Du-145. Reconstitution of miR-24 in PCa cells reduced cells growth, induced cell death and decreased AR, ETV1, IGF1 and IGFB5 in AA cells (Hashimoto et al., 2017).

The role of exosomes in PCa progression and metastasis

Exosomes are cell-derived extracellular bodies (30-120 nm in size) secreted by normal and tumor cells to promote cell-cell communications. Exosomes acting as a delivery devices or shuttles for various biological molecules (microRNAs, mRNAs, lipids, DNA and proteins) to recipient cells (Mathivanan et al., 2012). Exosomes are detected in most of body fluids including blood, ascetic fluids, lymphatic fluids, urine, saliva, tears and milk. The molecular content of exosomes is dependent on their cell of origin. Therefore, the identification of tissue- or disease-specific exosomal proteins, miRNAs and mRNAs will enable the use of these vesicles as a source of new noninvasive biomarkers and serve as indicators in the diagnosis, prognosis and surveillance of a variety of diseases including cancer.

The crosstalk between exosomes released from cancer cells as well as stromal cells in and around tumor niche, presumably along with other key players, is a critical factor for promoting metastasis. Carrying a message of exosomes from cells of origin 'donor' and delivering it to the recipient/effector cells, depends on three main basic biological steps: biogenesis, release and internalization. During each step, there is a tight control of the quantity, biological contents and specificity of exosomes delivery to their target cells. The process of exosomal biogenesis and release is securely regulated by several factors inside the donor cells comprising endosomal sorting complex required for transport (ESCRT-o, -I, II, III) machinery, Syndecan-syntenin-ALIX, Rab proteins, small integral membrane protein of the lysosome/late endosome (SIMPLE), phospholipase D, and sphingomyelinase (reviewed in (Hessvik and Llorente, 2017)). A growing body of research in exosomes suggests that their release by cancer and cancer-associated cells is a key-step for promoting PCa cell survival, growth, angiogenesis and suppression of immune system (Ge et al., 2012). Interestingly, collecting exosomes from highly metastatic melanoma cells promoted melanoma metastasis from the pre-metastatic site through reprogramming of bone marrow stem cells and silencing of Rab27A decreased exosomal production and reduced melanoma metastasis (Peinado et al., 2012). Our group demonstrated that exosomes are associated with PCa progression by transferring known and uncharacterized set(s) of miRs into recipient stem cells altering network of genes to transform non-malignant into PCa-like cells (Abd Elmageed et al., 2014).

The tumor microenvironment contains a variety of cells such as fibroblasts, cancer-associated fibroblasts (CAFs), endothelial cells, white cells, epithelial cells, and mesenchymal

stem cells as well as soluble growth factors, cytokines, chemokines, and exosomes (Ono et al., 2014). The different biological activities in which exosomes contribute to cell survival, cell proliferation, angiogenesis, immunomodulation and metastasis are depicted in Figure 1. Here, the role of exosomal cargoes in transferring different biologically active materials to exert specific biological effects on different cells in tumor niche (summarized in Table 1) are described. By activating TGF-β/SMAD3 signaling pathway, PCa-associated-exosomes can reprogram fibroblasts into CAFs, which is a critical step needed for cancer progression (Webber et al., 2015). In the same vein, PCa-associated exosomes altered the adipogenic differentiation of mesenchymal stem cells towards CAFs, which gain proangiogenic activities by secreting VEGF-A, HGF and metalloproteinases (Chowdhury et al., 2015). Co-culturing of pancreatic cancer cells with primary pancreatic fibroblasts isolated from wild type C57 mice induced the transformation of fibroblasts to CAFs through exosomal miR-155-TP53INP1 axis (Pang et al., 2015). Exosomes derived from CAFs have proven to transfer miRs to alter the tumor niche and promoting cell proliferation, invasion, epithelial-to-mesenchymal transition to develop chemoresistance in PCa cells (Au Yeung et al., 2016; Li et al., 2016a). Other research groups have reported that exosomes derived from different PCa cells and TRAMP mouse model are enriched with IGF-1R, SRC, FAK, CD9 and G-Protein-Coupled Receptor Kinases (GRK5 & 6) to support tumor progression and migration (DeRita et al., 2017; Soekmadji et al., 2016).

Exosomes released from PCa cells can directly or indireclty through circulation transfer their cargo contents (proteins, mRNA, microRNAs and lipids) into tumor microevironment (TME). TME contains fibroblasts, lymphocytes, macrophage, dentretic cells, extracellular matrix, and growth factors. This will change the signaling pathways in cancer and cancer-associated cells to promote cell proliferation, survival, angiogenesis, metastasis and drug resisiatnce. These molecular events suggest that exosomes could be a determining factor contributing to health disparities among African American men.

The role of exosomes in evasion of immune response in cancer disease is a growing area of research. Exosomes carry immunosuppressive molecules, tumor-associated antigens, costimulatory molecules, major histocompatibility complex (MHC), and intraluminal cytokines (Whiteside, 2013, 2016). PCa-associated exosomes were found to express ligands of NKG2D (natural-killer group 2, member D) and selectively suppress the expression of NKG2D on the surface of NK and CD8+ T cells- case leading to diminish the cytotoxic actions of these receptors (Lundholm et al., 2014). In a group of CRPC patients, the same investigators found a remarkable decrease of NKG2D expression on the surface of NK and CD8+ T cells regarding matched controls. Cancer-associated exosomes upregulate the suppressor activity of Treg and myeloid-derived suppressor to evade the immune response towards cancer cells (Xiang et al., 2009); this could occur by transferring of FasL, TGF-β, galectin-9 and HSP72 by exosomes into recipient cells (Naito et al., 2017).

Exosomes as non-invasive biomarkers in PCa

In 2009, a group of researchers was able to detect the mRNA fusion gene TMRSS2: ERG and PCA-3 in the exosomes isolated from the urine of PCa patients. These findings suggest that circulating exosomes can act as a potential non-invasive biomarker by carrying in their cargoes of enriched mRNA compared to their donor cells (Nilsson et al., 2009). In American cohort, other scientists validated the accuracy of using TMPRSS2: ERG in urinary exosomes versus 21 PCa tissue specimens (Motamedinia et al., 2016). The detected TMPRSS2: ERG in urinary exosomes had 81% sensitivity, 80% specificity and 81% overall accuracy of the fused genes in exosomes collected from urine versus PCa tissues. This was followed by investigating the differential expression of TMPRSS2: ERG in large number of urine samples collected from 39 men with prostate biopsy negative, prostate biopsy 47 biopsy positive, 37 men had radical prostatectomy, and 84 healthy men (44 age-matched 40 young men). Data from ROC analyses showed that TMPRSS2: ERG, ERG, PCA3, BIRC5, and TMPRSS2 genes were able to segregate subjects with prostate biopsy positive from negative ones.

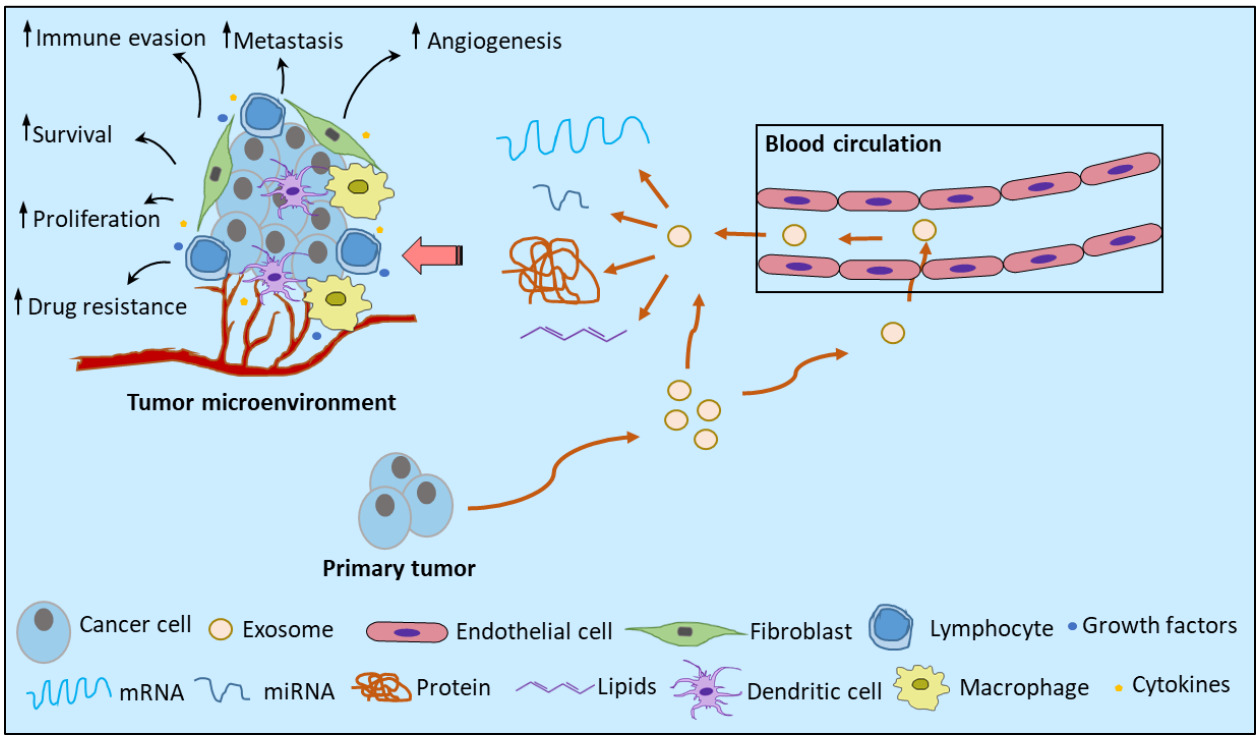

Figure 1. Schematic representation showing suggested biological activities of exosomes in cell survival, cell proliferation, angiogenesis, immunomodulation and metastasis

Table 1. List of exosomes-associate cargoes transferred into recipient cells and used as biomarkers or have a biological impact

Exosomes cargo	Content	Effect/Role	Reference
TGF-β	Protein	Transform fibroblast into cancer associated fibroblast	(Webber et al., 2015)
c-Src, IGF-IR, FAK	Protein	PCa progression	(Del Re et al., 2017)
CD9	Protein	PCa progression	(Soekmadji et al., 2016)
CD33, CD34, CD117, TGFβ1	Protein	Decreased cytotoxic activity of NK towards cancer cells	(Whiteside, 2013)
FasL, PD-L1	Protein	Apoptosis of activated $CD8^+$ Cells	(Andreola et al., 2002; Kim et al., 2005)
Ligands for NKG2D	Protein	Has immunosuppressive effect	(Lundholm et al., 2014)
HSP72	Protein	Has immunosuppressive effect	(Chalmin et al., 2010)
Galectin-9	Protein	Apoptosis of T-Lymphocytes	(Klibi et al., 2009)
CD44	Protein	Transform monocytes into tumor-associated macrophage-like phenotypes	(Baj-Krzyworzeka et al., 2007)
DNA methyltransferase 1 (DNMT1)	Protein	Cisplatin resistance in ovarian cancer cells	(Cao et al., 2017)
AR/ARV7	Protein	Prostate cancer	(Read et al., 2017)
miR-155	microRNA	Transform fibroblast into cancer associated fibroblast by targeting TP53INP1	(Pang et al., 2015)
miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-12 and miR-214	microRNA	Tumor signature of lung adenocarcinoma	(Rabinowits et al., 2009)
miR-21	microRNA	Tumor progression in Esophageal squamous cell carcinoma	(Tanaka et al., 2013)
miR-551b, miR-96, mir-183, miR-182, miR-153, miR-625, miR-141, miR-193b, miR-200c, miR-193a-3p, miR-205, miR-708, miR-365 and miR-34		Tumor signature in PC-3 prostate cancer cell	(Hessvik et al., 2012)
miR-200c and miR-214	microRNA	Tumor stage of ovarian cancer	(Taylor and Gercel-

			Taylor, 2008)
miR- miR-1290 and miR-375	microRNA	prostate cancer prognosis	(Huang et al., 2015)
miR-16, miR-92a, miR- 103, miR-107, miR-97, miR-34b, miR-328, miR-485-3p, miR-486-5p, miR-92b, miR-574-3p, miR-636, miR-640, miR-766 and miR-885-5p	microRNA	Prostate cancer (Stage 3&4)	(Lodes et al., 2009)
PCA3	Long non-coding RNA	Prostate cancer	(Donovan et al., 2015)

In plasma, exosomes were isolated from 67 patients (39 PCa, 8 with recurrence and 20 BPH) in addition to 16 healthy controls. In these exosomes, the expression of survivin was higher in PCa patients compared to BPH and health controls (Khan et al., 2012). The acidic pH of tumor microenvironment increases the secretion of PSA- and CD81-expressing exosomes in PCa cells and as well as in peripheral blood of PCa patients corresponding to BPH and healthy controls (Logozzi et al., 2017). Proteomic analysis has shown that about 64 proteins were detected PC-3-associated exosomes and claudin 3 was the top expressed protein. CLDN3 level was assessed in plasma collected from 69 PCa in addition to BPH and control subjects. The expression of CLDN3 was specific to PCa patients versus other control groups (Worst et al., 2017). Interestingly, in vitro and preclinical studies aimed to investigate whether: 1) PCa-associated exosomes express EGFR (Epidermal growth factor receptor) on their surface and 2) if it has any role in the aggressiveness of cancer cells. The study evidenced that the expression of EGFR in exosomes isolated from the conditioned media of PCa cell lines as well as LNCaP xenograft serum and patient blood (Kharmate et al., 2016). Nuclear translocation of EGFR in other cells was governed by PCa-associated exosomes in an independent fashion of nuclear localization signal. In parallel, AR and its variant ARV7 have shown to be transferred through exosomes into the nucleus of AR-naïve PCa cells (Read et al., 2017). It has been reported that exosomal gamma-glutamyltransferase activity is higher in the serum of PCa versus BPH patients. (Kawakami et al., 2017).

RNA sequencing data demonstrated that miR-1290, miR-1246, and miR-375 were top-listed miRs in exosomes isolated from the plasma procured from 21 CRCP patients. After their validation in 100 CRPC specimens, exosomes-associated miR-1290 and miR-375 had a positive correlation with overall survival of PCa patients (Huang et al., 2015). The level of miR-141 and miR-375 was assessed in exosomes collected from serum and data have shown that it was higher in 78 PCa compared to 28 normal control subjects. There was a positive correlation between miR-141 and miR-375 with metastatic PCa (Bryant et al., 2012). Other miRs are dysregulated in PCa and can segregate in early versus late stages as well as

in localized versus metastatic status (Gallo et al., 2012; Li et al., 2016b; Lodes et al., 2009).

Exosomes-based pathway in drug resistance of prostate cancer

The development of CRPC is a current major concern in PCa management. Several mechanisms are proposed to understand how CRPC develops including: AR-independent tumor growth, conversion of estrogen to testosterone, activation of androgen metabolizing enzymes, intracrine activation of AR, and exosomes-based pathways. The multifactorial steps in developing resistance in PCa patients suggests the urgent need for adopting a new treatment strategy of targeting multiple signaling pathways contributing to CRPC and improving other methods of disease management. Treatment of CRPC patients with taxanes may lead to the development of resistance. The transfer of exosomal cargoes into different recipient cells could be one of CRPC mechanisms especially exosomes have shown to carry AR, ARV7, growth factors, mRNAs and miRNAs. Using a digital droplet PCR (ddPCR), a recent study suggested that plasma-derived exosomal mRNA of ARV7 is associated with resistance to hormonal therapy in patients with CRPC (Del Re et al., 2017). Along similar lines, exosomes were isolated from the blood of docetaxel-resistant CRPC patients and was shown that their cargo contains *MDR-1, MDR-3* and *PABP4* proteins (Endzelins et al., 2016). Kawakami et al., performed proteomic analysis on exosomes isolated from taxane-resistant PC-3 cells and, interestingly, integrin β4 and vinculin were upregulated (Kawakami et al., 2015). These findings present exosomal integrin β4, vinculin and MDR1 (Kato et al., 2015) as new biomarkers in patients of taxane-resistance. In a recent study, pMet and miR-130b were identified in exosomes isolated from sera of primary and metastatic PCa (Cannistraci et al., 2017). Both factors can significantly differentiate CRPC from early stages and can be used as non-invasive marker for active surveillance and therapy monitoring of CRPC patients. Li et al. investigated the potential networks of exosomes-associated miRNAs derived from chemoresistant PCa cells with their known target genes (Li et al., 2016a). They identified 29 dysregulated miRNAs, in exosome isolated from paclitaxel-resistance PCa cells. The link between miRNAs and their target genes suggests that miR-3176, miR-141-3p, miR-5004-5p, miR-16-5p, miR-3915, miR-488-3p, miR-23c, miR-3673 and miR-3654 are potential targets to AR and PTEN while miR-32-5, miR-141-3p, miR-606, miR-381 and miR-429 are targets to TCF4. In another study conducted on neuorblastoma cells, exosomes-associated miR-21 and miR-155 mediate the crosstalk between neuroblastoma cells and monocytes in cisplatin resistance cells, possibly, through miR-21/TLR8-NF-κB and miR-155/TERF1 signaling pathways (Challagundla et al., 2015).

Concluding remarks

The role of exosomes is emerging as an interesting component in cancer biology but their contribution to cancer disparities and drug resistance remains unclear. Increasing evidence points towards eminent role of exosomal cargoes in transferring cellular messages between cancer cells and their tumor niche. The presence of exosomal protein receptors on the surface of PCa-

associated exosomes may explain, in part, the reasons of PCa disparities among AA men regarding CA men and other minorities. This review justifies the need for more studies to build on these recent findings for future understanding of the role of PCa-associated exosomes in promoting health disproportionate in PCa and other cancer disparities. Adopting such exosomal findings in cancer and other chronic diseases might help to eliminate morbidity and mortality disparities among different minorities. Future studies need to address these questions to fasciltate the development of new generations of therapeutic agents to target tumor exosomes. In parallel, exosomes standardization for their detection and evaluation as well as understanding their biogenesis, release and uptake are of paramount importance. With a growing accessibility to these biological information, new specific and less toxic drugs will be discovered.

Acknowledgements

The authors would like to thank Dr. David Potter, Ph.D., FARVO, for helpful discussions and carefully reading the manuscript. The present study was supported by grant from the NIH/NCI R21CA194750 (Z.Y.A).

Conflict of interest statement

The authors declare that there is no conflict of interest to report it. The funders had no role in study design, writing of the manuscript and decision to publish.

Authors' contributions

Conception and design of the study: ZYA; data collection and preparation: HEAA and SAG; writing the manuscript: HEAA, HIA, SAG, GD and ZYA; reviewing the final version of the manuscript: GA, HIA and ZYA.

REFERENCES

Abd Elmageed, Z.Y., Moroz, K., Srivastav, S.K., Fang, Z., Crawford, B.E., Moparty, K., Thomas, R., and Abdel-Mageed, A.B. (2013). High circulating estrogens and selective expression of ERbeta in prostate tumors of Americans: implications for racial disparity of prostate cancer. Carcinogenesis *34*, 2017-2023.

Abd Elmageed, Z.Y., Yang, Y., Thomas, R., Ranjan, M., Mondal, D., Moroz, K., Fang, Z., Rezk, B.M., Moparty, K., Sikka, S.C., *et al.* (2014). Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. Stem Cells *32*, 983-997.

Ambs, S., Prueitt, R.L., Yi, M., Hudson, R.S., Howe, T.M., Petrocca, F., Wallace, T.A., Liu, C.G., Volinia, S., Calin, G.A., *et al.* (2008). Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res *68*, 6162-6170.

Andreola, G., Rivoltini, L., Castelli, C., Huber, V., Perego, P., Deho, P., Squarcina, P., Accornero, P., Lozupone, F., Lugini, L., *et al.* (2002). Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. J Exp Med *195*, 1303-1316.

Au Yeung, C.L., Co, N.N., Tsuruga, T., Yeung, T.L., Kwan, S.Y., Leung, C.S., Li, Y., Lu, E.S., Kwan, K., Wong, K.K., *et al.* (2016). Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nat Commun *7*, 11150.

Baj-Krzyworzeka, M., Szatanek, R., Weglarczyk, K., Baran, J., and Zembala, M. (2007). Tumour-derived microvesicles modulate biological activity of human monocytes. Immunol Lett *113*, 76-82.

Bryant, R.J., Pawlowski, T., Catto, J.W., Marsden, G., Vessella, R.L., Rhees, B., Kuslich, C., Visakorpi, T., and Hamdy, F.C. (2012). Changes in circulating microRNA levels associated with prostate cancer. Br J Cancer *106*, 768-774.

Cannistraci, A., Federici, G., Addario, A., Di Pace, A.L., Grassi, L., Muto, G., Collura, D., Signore, M., De Salvo, L., Sentinelli, S., *et al.* (2017). C-Met/miR-130b axis as novel mechanism and biomarker for castration resistance state acquisition. Oncogene *36*, 3718-3728.

Cao, Y.L., Zhuang, T., Xing, B.H., Li, N., and Li, Q. (2017). Exosomal DNMT1 mediates cisplatin resistance in ovarian cancer. Cell Biochem Funct.

Challagundla, K.B., Wise, P.M., Neviani, P., Chava, H., Murtadha, M., Xu, T., Kennedy, R., Ivan, C., Zhang, X., Vannini, I., *et al.* (2015). Exosome-mediated transfer of microRNAs within the tumor microenvironment and neuroblastoma resistance to chemotherapy. J Natl Cancer Inst *107*.

Chalmin, F., Ladoire, S., Mignot, G., Vincent, J., Bruchard, M., Remy-Martin, J.P., Boireau, W., Rouleau, A., Simon, B., Lanneau, D., *et al.* (2010). Membrane-associated Hsp72

from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. J Clin Invest *120*, 457-471.

Chaudhary, A.K., Bhat, T.A., Kumar, S., Kumar, A., Kumar, R., Underwood, W., Koochekpour, S., Shourideh, M., Yadav, N., Dhar, S., *et al.* (2016). Mitochondrial dysfunction-mediated apoptosis resistance associates with defective heat shock protein response in African-American men with prostate cancer. Br J Cancer *114*, 1090-1100.

Choudhury, A.R., and Singh, K.K. (2017). Mitochondrial determinants of cancer health disparities. Semin Cancer Biol.

Chowdhury, R., Webber, J.P., Gurney, M., Mason, M.D., Tabi, Z., and Clayton, A. (2015). Cancer exosomes trigger mesenchymal stem cell differentiation into pro-angiogenic and pro-invasive myofibroblasts. Oncotarget *6*, 715-731.

Davenport, R.J. (2004). Culture clash.A growing body of research suggests that yeast have programmed death pathways, yet many researchers are skeptical. Recent studies provide some of the first experimental evidence for why a single-celled organism would commit suicide. Sci Aging Knowledge Environ *2004*, ns9.

Del Re, M., Biasco, E., Crucitta, S., Derosa, L., Rofi, E., Orlandini, C., Miccoli, M., Galli, L., Falcone, A., Jenster, G.W., *et al.* (2017). The Detection of Androgen Receptor Splice Variant 7 in Plasma-derived Exosomal RNA Strongly Predicts Resistance to Hormonal Therapy in Metastatic Prostate Cancer Patients. Eur Urol *71*, 680-687.

DeRita, R.M., Zerlanko, B., Singh, A., Lu, H., Iozzo, R.V., Benovic, J.L., and Languino, L.R. (2017). c-Src, Insulin-Like Growth Factor I Receptor, G-Protein-Coupled Receptor Kinases and Focal Adhesion Kinase are Enriched Into Prostate Cancer Cell Exosomes. J Cell Biochem *118*, 66-73.

Djusberg, E., Jernberg, E., Thysell, E., Golovleva, I., Lundberg, P., Crnalic, S., Widmark, A., Bergh, A., Brattsand, M., and Wikstrom, P. (2017). High levels of the AR-V7 Splice Variant and Co-Amplification of the Golgi Protein Coding YIPF6 in AR Amplified Prostate Cancer Bone Metastases. Prostate *77*, 625-638.

Donovan, M.J., Noerholm, M., Bentink, S., Belzer, S., Skog, J., O'Neill, V., Cochran, J.S., and Brown, G.A. (2015). A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result. Prostate Cancer Prostatic Dis *18*, 370-375.

Endzelins, E., Melne, V., Kalnina, Z., Lietuvietis, V., Riekstina, U., Llorente, A., and Line, A. (2016). Diagnostic, prognostic and predictive value of cell-free miRNAs in prostate cancer: a systematic review. Mol Cancer *15*, 41.

Gallo, A., Tandon, M., Alevizos, I., and Illei, G.G. (2012). The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS One *7*, e30679.

Ge, R., Tan, E., Sharghi-Namini, S., and Asada, H.H. (2012). Exosomes in Cancer Microenvironment and Beyond: have we Overlooked these Extracellular Messengers? Cancer Microenviron *5*, 323-332.

Guo, Z., Yang, X., Sun, F., Jiang, R., Linn, D.E., Chen, H., Chen, H., Kong, X., Melamed, J., Tepper, C.G., *et al.* (2009). A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res *69*, 2305-2313.

Gusev, A., Shi, H., Kichaev, G., Pomerantz, M., Li, F., Long, H.W., Ingles, S.A., Kittles, R.A., Strom, S.S., Rybicki, B.A., *et al.* (2016). Atlas of prostate cancer heritability in European and African-American men pinpoints tissue-specific regulation. Nat Commun *7*, 10979.

Hashimoto, Y., Shiina, M., Kato, T., Yamamura, S., Tanaka, Y., Majid, S., Saini, S., Shahryari, V., Kulkarni, P., Dasgupta, P., *et al.* (2017). The role of miR-24 as a race related genetic factor in prostate cancer. Oncotarget *8*, 16581-16593.

Hatcher, D., Daniels, G., Osman, I., and Lee, P. (2009). Molecular mechanisms involving prostate cancer racial disparity. Am J Transl Res *1*, 235-248.

Heidenreich, A., Bastian, P.J., Bellmunt, J., Bolla, M., Joniau, S., van der Kwast, T., Mason, M., Matveev, V., Wiegel, T., Zattoni, F., *et al.* (2014). EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol *65*, 124-137.

Hessvik, N.P., and Llorente, A. (2017). Current knowledge on exosome biogenesis and release. Cell Mol Life Sci.

Hessvik, N.P., Phuyal, S., Brech, A., Sandvig, K., and Llorente, A. (2012). Profiling of microRNAs in exosomes released from PC-3 prostate cancer cells. Biochim Biophys Acta *1819*, 1154-1163.

Hsing, A.W., Tsao, L., and Devesa, S.S. (2000). International trends and patterns of prostate cancer incidence and mortality. International journal of cancer Journal international du cancer *85*, 60-67.

Hu, R., Dunn, T.A., Wei, S., Isharwal, S., Veltri, R.W., Humphreys, E., Han, M., Partin, A.W., Vessella, R.L., Isaacs, W.B., *et al.* (2009). Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res *69*, 16-22.

Huang, X., Yuan, T., Liang, M., Du, M., Xia, S., Dittmar, R., Wang, D., See, W., Costello, B.A., Quevedo, F., *et al.* (2015). Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol *67*, 33-41.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., and Thun, M.J. (2008). Cancer statistics, 2008. CA Cancer J Clin *58*, 71-96.

Kato, T., Mizutani, K., Kameyama, K., Kawakami, K., Fujita, Y., Nakane, K., Kanimoto, Y., Ehara, H., Ito, H., Seishima, M., *et al.* (2015). Serum exosomal P-glycoprotein is a potential marker to diagnose docetaxel resistance and select a taxoid for patients with prostate cancer. Urol Oncol *33*, 385 e315-320.

Kawakami, K., Fujita, Y., Kato, T., Mizutani, K., Kameyama, K., Tsumoto, H., Miura, Y., Deguchi, T., and Ito, M. (2015). Integrin beta4 and vinculin contained in exosomes are potential markers for progression of prostate cancer associated with taxane-resistance. Int J Oncol *47*, 384-390.

Kawakami, K., Fujita, Y., Matsuda, Y., Arai, T., Horie, K., Kameyama, K., Kato, T., Masunaga, K., Kasuya, Y., Tanaka,

M., *et al.* (2017). Gamma-glutamyltransferase activity in exosomes as a potential marker for prostate cancer. BMC Cancer *17*, 316.

Khan, S., Jutzy, J.M., Valenzuela, M.M., Turay, D., Aspe, J.R., Ashok, A., Mirshahidi, S., Mercola, D., Lilly, M.B., and Wall, N.R. (2012). Plasma-derived exosomal survivin, a plausible biomarker for early detection of prostate cancer. PLoS One *7*, e46737.

Khani, F., Mosquera, J.M., Park, K., Blattner, M., O'Reilly, C., MacDonald, T.Y., Chen, Z., Srivastava, A., Tewari, A.K., Barbieri, C.E., *et al.* (2014). Evidence for molecular differences in prostate cancer between African American and Caucasian men. Clin Cancer Res *20*, 4925-4934.

Kharmate, G., Hosseini-Beheshti, E., Caradec, J., Chin, M.Y., and Tomlinson Guns, E.S. (2016). Epidermal Growth Factor Receptor in Prostate Cancer Derived Exosomes. PLoS One *11*, e0154967.

Kim, J.W., Wieckowski, E., Taylor, D.D., Reichert, T.E., Watkins, S., and Whiteside, T.L. (2005). Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. Clin Cancer Res *11*, 1010-1020.

Klibi, J., Niki, T., Riedel, A., Pioche-Durieu, C., Souquere, S., Rubinstein, E., Le Moulec, S., Guigay, J., Hirashima, M., Guemira, F., *et al.* (2009). Blood diffusion and Th1-suppressive effects of galectin-9-containing exosomes released by Epstein-Barr virus-infected nasopharyngeal carcinoma cells. Blood *113*, 1957-1966.

Lee, Y.J., Park, J.E., Jeon, B.R., Lee, S.M., Kim, S.Y., and Lee, Y.K. (2013). Is prostate-specific antigen effective for population screening of prostate cancer? A systematic review. Ann Lab Med *33*, 233-241.

Li, J., Yang, X., Guan, H., Mizokami, A., Keller, E.T., Xu, X., Liu, X., Tan, J., Hu, L., Lu, Y., *et al.* (2016a). Exosome-derived microRNAs contribute to prostate cancer chemoresistance. Int J Oncol *49*, 838-846.

Li, Z., Ma, Y.Y., Wang, J., Zeng, X.F., Li, R., Kang, W., and Hao, X.K. (2016b). Exosomal microRNA-141 is upregulated in the serum of prostate cancer patients. Onco Targets Ther *9*, 139-148.

Lodes, M.J., Caraballo, M., Suciu, D., Munro, S., Kumar, A., and Anderson, B. (2009). Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One *4*, e6229.

Logozzi, M., Angelini, D.F., Iessi, E., Mizzoni, D., Di Raimo, R., Federici, C., Lugini, L., Borsellino, G., Gentilucci, A., Pierella, F., *et al.* (2017). Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients. Cancer Lett *403*, 318-329.

Lundholm, M., Schroder, M., Nagaeva, O., Baranov, V., Widmark, A., Mincheva-Nilsson, L., and Wikstrom, P. (2014). Prostate tumor-derived exosomes down-regulate NKG2D expression on natural killer cells and CD8+ T cells: mechanism of immune evasion. PLoS One *9*, e108925.

Mathivanan, S., Fahner, C.J., Reid, G.E., and Simpson, R.J. (2012). ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res *40*, D1241-1244.

Motamedinia, P., Scott, A.N., Bate, K.L., Sadeghi, N., Salazar, G., Shapiro, E., Ahn, J., Lipsky, M., Lin, J., Hruby, G.W., *et al.* (2016). Urine Exosomes for Non-Invasive Assessment of Gene Expression and Mutations of Prostate Cancer. PLoS One *11*, e0154507.

Mottet, N., Bellmunt, J., Bolla, M., Briers, E., Cumberbatch, M.G., De Santis, M., Fossati, N., Gross, T., Henry, A.M., Joniau, S., *et al.* (2017). EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. Eur Urol *71*, 618-629.

Naito, Y., Yoshioka, Y., Yamamoto, Y., and Ochiya, T. (2017). How cancer cells dictate their microenvironment: present roles of extracellular vesicles. Cell Mol Life Sci *74*, 697-713.

Nilsson, J., Skog, J., Nordstrand, A., Baranov, V., Mincheva-Nilsson, L., Breakefield, X.O., and Widmark, A. (2009). Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br J Cancer *100*, 1603-1607.

Ono, M., Kosaka, N., Tominaga, N., Yoshioka, Y., Takeshita, F., Takahashi, R.U., Yoshida, M., Tsuda, H., Tamura, K., and Ochiya, T. (2014). Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Sci Signal *7*, ra63.

Paller, C.J., and Antonarakis, E.S. (2011). Cabazitaxel: a novel second-line treatment for metastatic castration-resistant prostate cancer. Drug Des Devel Ther *5*, 117-124.

Pang, W., Su, J., Wang, Y., Feng, H., Dai, X., Yuan, Y., Chen, X., and Yao, W. (2015). Pancreatic cancer-secreted miR-155 implicates in the conversion from normal fibroblasts to cancer-associated fibroblasts. Cancer Sci *106*, 1362-1369.

Peinado, H., Aleckovic, M., Lavotshkin, S., Matei, I., Costa-Silva, B., Moreno-Bueno, G., Hergueta-Redondo, M., Williams, C., Garcia-Santos, G., Ghajar, C., *et al.* (2012). Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med *18*, 883-891.

Rabinowits, G., Gercel-Taylor, C., Day, J.M., Taylor, D.D., and Kloecker, G.H. (2009). Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer *10*, 42-46.

Read, J., Ingram, A., Al Saleh, H.A., Platko, K., Gabriel, K., Kapoor, A., Pinthus, J., Majeed, F., Qureshi, T., and Al-Nedawi, K. (2017). Nuclear transportation of exogenous epidermal growth factor receptor and androgen receptor via extracellular vesicles. Eur J Cancer *70*, 62-74.

Rohrmann, S., Nelson, W.G., Rifai, N., Brown, T.R., Dobs, A., Kanarek, N., Yager, J.D., and Platz, E.A. (2007). Serum estrogen, but not testosterone, levels differ between black and white men in a nationally representative sample of Americans. J Clin Endocrinol Metab *92*, 2519-2525.

Soekmadji, C., Riches, J.D., Russell, P.J., Ruelcke, J.E., McPherson, S., Wang, C., Hovens, C.M., Corcoran, N.M., The Australian Prostate Cancer Collaboration, B., Hill, M.M., *et al.* (2016). Modulation of paracrine signaling by CD9 positive small extracellular vesicles mediates cellular growth of androgen deprived prostate cancer. Oncotarget.

Tanaka, Y., Kamohara, H., Kinoshita, K., Kurashige, J., Ishimoto, T., Iwatsuki, M., Watanabe, M., and Baba, H. (2013). Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma. Cancer *119*, 1159-1167.

Taylor, D.D., and Gercel-Taylor, C. (2008). MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol *110*, 13-21.

Wang, B.D., Ceniccola, K., Yang, Q., Andrawis, R., Patel, V., Ji, Y., Rhim, J., Olender, J., Popratiloff, A., Latham, P., *et al.* (2015). Identification and Functional Validation of Reciprocal microRNA-mRNA Pairings in African American Prostate Cancer Disparities. Clin Cancer Res *21*, 4970-4984.

Wang, Y., Wang, J., Zhang, L., Karatas, O.F., Shao, L., Zhang, Y., Castro, P., Creighton, C.J., and Ittmann, M. (2017). RGS12 Is a Novel Tumor-Suppressor Gene in African American Prostate Cancer That Represses AKT and MNX1 Expression. Cancer Res.

Watson, P.A., Chen, Y.F., Balbas, M.D., Wongvipat, J., Socci, N.D., Viale, A., Kim, K., and Sawyers, C.L. (2010). Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. Proc Natl Acad Sci U S A *107*, 16759-16765.

Webber, J.P., Spary, L.K., Sanders, A.J., Chowdhury, R., Jiang, W.G., Steadman, R., Wymant, J., Jones, A.T., Kynaston, H., Mason, M.D., *et al.* (2015). Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes. Oncogene *34*, 290-302.

Whiteside, T.L. (2013). Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes). Biochem Soc Trans *41*, 245-251.

Whiteside, T.L. (2016). Tumor-Derived Exosomes and Their Role in Tumor-Induced Immune Suppression. Vaccines (Basel) *4*.

Worst, T.S., von Hardenberg, J., Gross, J.C., Erben, P., Schnolzer, M., Hausser, I., Bugert, P., Michel, M.S., and Boutros, M. (2017). Database-augmented Mass Spectrometry Analysis of Exosomes Identifies Claudin 3 as a Putative Prostate Cancer Biomarker. Mol Cell Proteomics *16*, 998-1008.

Xiang, X., Poliakov, A., Liu, C., Liu, Y., Deng, Z.B., Wang, J., Cheng, Z., Shah, S.V., Wang, G.J., Zhang, L., *et al.* (2009). Induction of myeloid-derived suppressor cells by tumor exosomes. Int J Cancer *124*, 2621-2633.