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REVIEW ARTICLE

Exosomal microRNAs in liquid biopsies: future biomarkers for prostate cancer

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Abstract Prostate cancer is the second most diagnosed cancer in males in the world. Plasma quantification of prostate-specific antigen substantially improved the early detection of prostate cancer, but still lacks the required specificity. Clinical management of prostate cancer needs advances in the development of new non-invasive biomarkers, ameliorating current diagnosis and prognosis and guiding therapeutic decisions. microRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression at the post-transcriptional level. These miRNAs are expressed in the cells and are also present in cell-derived extracellular vesicles such as exosomes. Exosomes have been shown to act as mediators for cell to cell communication because of the regulatory functions of their content. High levels of exosomes are found in several body fluids from cancer patients and could be a potential source of non-invasive biomarkers. In this review, we summarize the diagnostic and prognostic utility of exosomal miRNAs in prostate cancer.

Keywords Prostate cancer · MiRNAs · Exosome · Biomarker · Liquid biopsies

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Prostate cancer

Prostate cancer is the most commonly diagnosed male malignancy and the second leading cause of cancer-related death in males in the western world [1, 2]. Malignant transformation of prostate epithelial cells and progression to carcinoma are likely to result from a complex series of events under both genetic and environmental influences [3, 4]. Prostate cancer develops mainly in aged men, the inherited risk of prostate cancer is as high as 60% [5] and some predisposing genes have been identified [6–8]. Other risk factors include race, a diet rich in fat, and obesity [3]. A better understanding of the genetic and biologic mechanisms that determine why some prostate carcinomas remain silent while others cause serious, even life-threatening illness are needed [5].

In the early stages, the disease locally confined to the prostate, is hormone or androgen-dependent and can be managed by surgical intervention or radiation treatment [4]. In the case of advanced prostate cancer, androgen deprivation therapy initially reduce tumor burden and circulating prostate-specific antigen (PSA), but unfortunately the disease relapse in most cases [9]. Advanced prostate cancer can present metastasis in the lung, pleura, liver and bone, with a great impact in patient morbidity and

mortality despite aggressive therapy [10]. Currently, prognostic markers are serum levels of PSA, Gleason score and pathological stage [11]. PSA is secreted by prostate cancer cells and can be found in blood, but has a low specificity as biomarker because its level can also be elevated for non-cancerous reasons [12, 13] or even diminished in metastatic disease [14]. These tests do not distinguish exactly the aggressiveness of the tumor or the potential metastatic capacity, so prostate biopsy, an invasive procedure, remains the only definitive diagnostic test for prostate cancer. But the implementation of novel state-of-the-art techniques such as the analysis of exosomal content of microRNAs (miRNAs) might be a promising candidate for the diagnosis and disease stratification of prostate cancer.

miRNA biogenesis, functions and implications in cancer

miRNAs are endogenous, small, from 18 to 25 nucleotides and non-coding RNAs widely found in both animals and plants that regulate post-transcriptionally gene expression. These small RNAs down-regulate gene expression by binding a region in the 3′ untranslated region (3′UTR) of their messenger RNA (mRNA) targets [15–17]. If the miRNA completely binds the sequence of their mRNA, the mRNA degradation is induced, while by contrast when miRNA bind incompletely, translational repression is induced [18]. miRNAs genes are transcribed by RNA polymerase II into long primary miRNAs (pri-miRNAs). These pri-miRNAs are processed in the nucleus into 70–80 nucleotide precursor miRNAs (pre-miRNAs) by the RNase III enzyme Drosha [19] and its cofactor DGCR8. Then pre-miRNA is actively transported from the nucleus to the cytoplasm by Exportin 5/Ran-GTP complex where is processed by the enzyme Dicer in the cytoplasm. Dicer is an RNase III endonuclease that cleaves the pre-miRNA into the mature miRNA that become stably associated with the RNA-induced silenced complex (RISC), forming the miRISC. The miRISC inhibits the target genes by repressing translation initiation, inducing deadenylation of mRNA, and thereby inducing ribosomes to drop off prematurely and promoting mRNA degradation by Argonaute, one of its essential catalytic components [20] (Fig. 1). miRNAs can target hundreds of transcripts, and more than one miRNA can converge on a single target transcript, thus the potential regulatory scenario of miRNAs is enormous. In this regard, miRNAs expression profiles have been found to be tissue type-specific and play important regulatory roles in a variety of biological process, such as cell proliferation, intercellular signaling, cell growth, cell death, cellular differentiation, apoptosis and metabolism control [21]. miRNA expression in tumor has been found to be up or down-regulated compared with normal tissue supporting their complex dual role either as oncogene (oncomir) or tumor suppressor gene [22]. For instance miRNA-125b has been shown to be an oncomir in prostate cancer but can also act as a tumor suppressor in ovarian and breast cancer [23]. Not only miRNAs are deregulated in cancer but also the enzymes involved in their biogenesis and processing. For example, Dicer is up-regulated during prostate cancer progression and its levels correlate with clinical stage, lymph node status and Gleason score [24]. miRNAs can be detected in a small volume samples from most body fluids, including serum, plasma, urine, saliva and are known to circulate in a highly stable cell free form [25]. Their stability, ease detection using a range of techniques, including miRNA cloning, microarray, quantitative PCR and next generation sequencing, make it feasible to identify and confirm abnormal miRNA expression in most human malignances [26]. These characteristics, together with its association with neoplastic disease progression, make miRNA an ideal tumor biomarker either in the tissue or in body fluids [20].

Exosomes and prostasomes

Exosomes are nano-sized (40–100 nm) extracellular vesicles (EV) derived from multivesicular bodies (MVB). Cells use exosomes to exchange of proteins, lipids and nucleic acids [27], therefore are important mediator for cell to cell communication, and indeed are considered to play a fundamental role in many physiological and pathological processes [28]. Exosomes are either released from normal or neoplastic cells and are present in the blood plasma, amniotic fluids, malignant ascites [29], breast milk [30] and other body fluids such as urine [31]. Exosomes contain mRNA, miRNAs and DNA so the transfer of this sort of information and oncogenic signaling to the tumor microenvironment let the modulation of tumor progression, proliferation angiogenic switch, the formation of the metastatic niche [32] and even the suppression of immune responses [33] (Fig. 2).

Several molecules or pathways are involved in the biogenesis of MVBs, such as the ESCRT machinery (endosomal sorting complexes required for transport), certain lipids (such as ceramide) and the tetraspanins [34]. MVBs can be either fused with lysosomes or with the plasma membrane, which allows the release of their content to the extracellular compartment [35]. Exosomes then will interact with recipient target cells via different mechanisms such as plasma membrane fusion and transport (RAB11, RAB27 and RAB35) or adhesion to corresponding receptors [36, 37]. Unfortunately, the mechanism that regulates

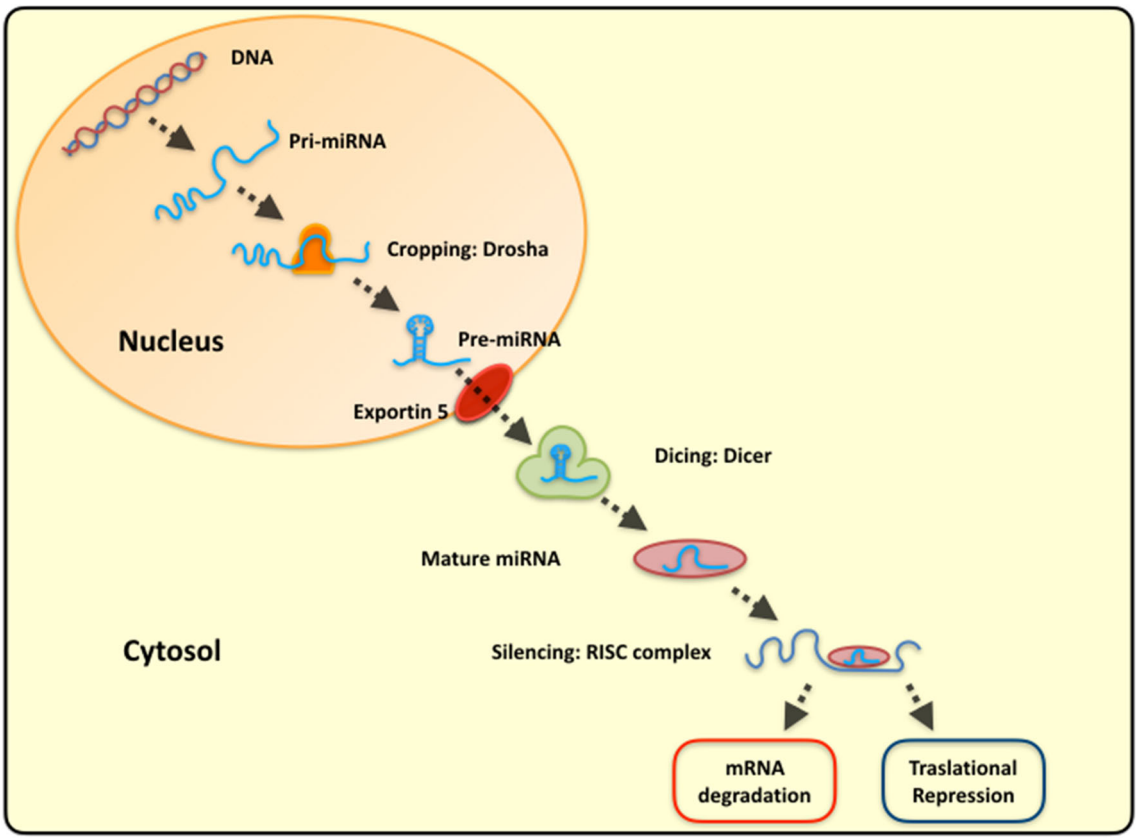

Fig. 1 miRNA biogenesis and mechanism of action

the exosomes release and uptake is still unknown. There are different ways to isolate exosomes either from tissue culture or form body fluids as sucrose density-gradient, ultracentrifugation [38] or by means of antibodies against exosomal markers, such as CD9, CD81, CD63 [39]. Recently, nanomembrane ultrafiltration concentrator and ExoQuick reagent are used as an effective and proven alternative to ultracentrifugation as well as a modified exosome precipitation method offers also a quick and scalable for exosomes isolation [40].

EVs, matching in size to vesicles from the prostate epithelium, now are known as prostasomes, found inside the 'storage vesicles' within prostate epithelial cells [41].

Prostasomes are microvesicles (50–500 nm) present in prostate secretions, produced by prostatic ductal epithelial cells and normal component of seminal fluid [42].

In prostasomes term, there are several populations: one with a small size type equivalent to exosomes, and released by prostate cells because of multivesicular endosomes with the plasma membrane, and other type equivalent to microvesicles with large size and derived by direct shedding of plasma membrane [43].

Prostasomes play a role as antioxidant factors in semen by interacting with polymorphonuclear neutrophils and inhibiting NADPH oxidase activity, and thus can act as antibacterial agents [44]. Interesting component have similarly been found in prostasomes isolated from human semen, such as prostatic acid phosphatase (PAP), PSA, prostate-specific transglutaminase and prostate stem cell antigen (PSCA) which are also markers for prostate cancer [45]. Prostasome have also a peculiar lipid composition with high levels of sphingomyelin, cholesterol, and glycosphingolipids [46] and in addition have also been reported to contain chromosomal DNA, mRNA and miRNA [47]. The protein content on prostasome surface is also very relevant as the presence of complement inhibitory proteins such as CD46 and CD59 that confer resistance to complement dependent cytotoxicity [48]. Prostasomes are not only secret by normal prostatic cells but also by neoplastic cells that export prostasomes to the extracellular environment, participating in tumor proliferation and metastasis [49]. Prostasome levels are reportedly increased in prostate cancer patients and these levels are associated with the disease aggressiveness [50]. The development of future isolation techniques for prostasomes found in biological fluids will let to get better insight in the identification and analysis of the protein, lipid and nucleic acids content of them and the potential utility for the diagnosis and prognosis of prostate cancer.

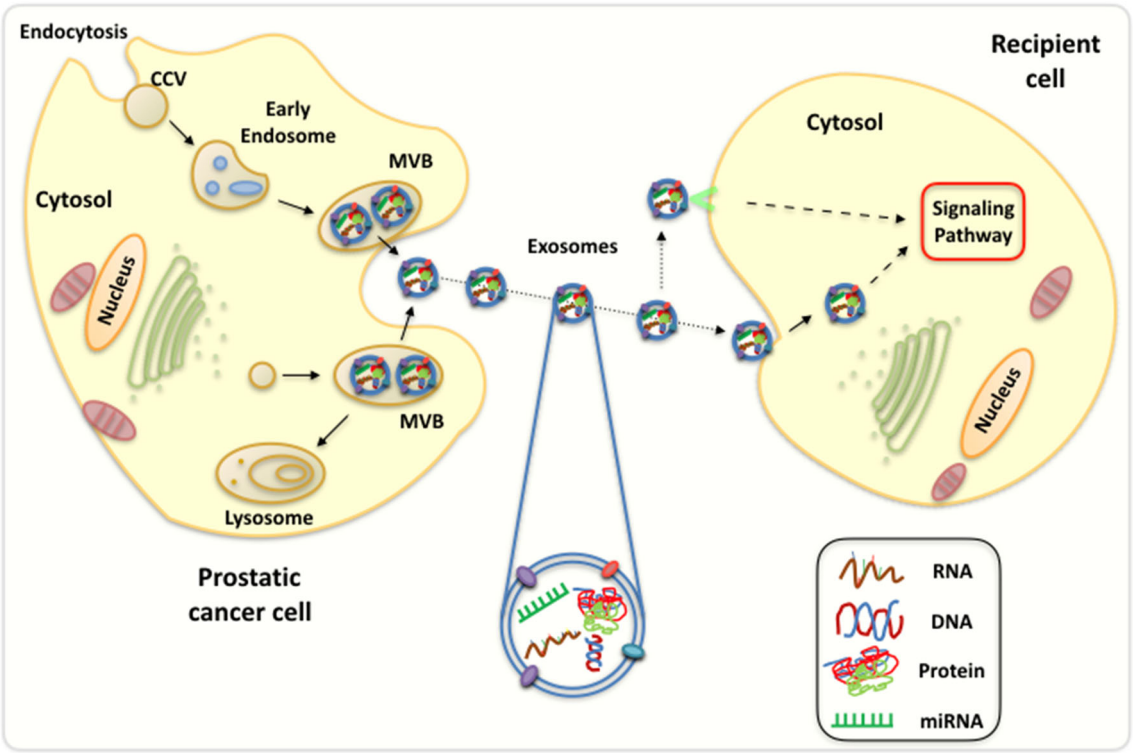

Fig. 2 Exosomes promote cell–cell communication playing an important role in gene regulation due to their ability to transport cancer-promoting material such as miRNAs

Exosomal miRNAs in prostate cancer

miRNAs are expressed not only in cells and present in biological fluids, but can be found also in cell-derived extracellular vesicles such as exosomes [51]. In fact, RNA sequencing analysis of plasma-derived exosomes revealed that miRNAs are the most abundant exosomal RNA species [52]. The miRNA content of extracellular vesicles reflects the miRNA expression profile of the cells they originated from [53]. For example, Brase et al. screened more 60 exosomal miRNAs identifying mir-375 and mir-141 as appropriate markers for prostate cancer [54]. This miRNAs content in exosomes could be considered as a potential novel biomarker for prostate cancer that may be used to diagnose but also to predict the disease stage [55, 56]. This is currently needed because the blood level of the gold standard marker for prostate cancer, PSA, do not always correlate with disease stage and aggressiveness of the malignancy [57]. For example, miR-21 is significantly elevated in the early stage, but not in advanced prostate cancer [58] and miR-16 is up-regulated in plasma of metastatic prostate cancer patients, but down-regulated in primary or metastatic prostate cancer tissues [59]. Additionally, other miRNAs have been reported to be detected in blood exosomes in metastatic prostate cancer patients [60–63]. MiRNAs have identified deregulated in plasma and serum microvesicles in prostate cancer patients compared with healthy control [64] and were also associated with the stage of the disease, the Gleason score and lymph node metastasis. For instance, Lodes et al. found 15 miRNAs (miR-16, -92a, -103, -107, -197, -34b, -328, -485-3p, -486-5p, -92b, -574-3p, -636, -640, -766, -885-5p) over-expressed in serum from stage 3 and 4 prostate cancer patients compared with healthy controls [65]. Furthermore, Mahn et al., found miR-26a, miR-195, and let-7i to be up-regulated in patients with prostate cancer compared with patients affected by benign prostatic hyperplasia [66]. Therefore, the different expression of specific miRNAs in liquid biopsies might be useful for a correct diagnosis. Another source where to investigate neoplastic abnormalities in prostate cancer with clinical value is the urine as its composition reflects the alterations in urogenital system [67].

An investigation of the proteome of urinary exosomes identified 246 proteins differentially expressed in prostate cancer patients compared to healthy male controls being the majority of these proteins up-regulated in exosomes from prostate cancer patients with high sensitivity and specificity [68]. A urinary 3-gene expression assay in exosomes has demonstrated an improved identification of patients with higher-grade prostate cancer among men with elevated PSA reduce the number of unnecessary biopsies

Table 1 miRNAs deregulated in prostate cancer compared to healthy controls

MiRNAs deregulated in prostate cancer	Source	Potential target genes	References
Let-7i, mir-16, mir-24, mir-26a, mir-26b, mir-34b, mir-92b, mir-93, mir-103, mir-106a, mir-141, mir-195, mir-197, mir-223, mir-298, mir-328, mir-346, mir-375, mir-1290	Serum	MAPK, p53, WNT5A EZH2,LARP1,AKT, SOX2,PDCD10,SPAG9,SOCS5, MBNL1, MTPN, E2F2, MYC, MCM7, BCL2, PLAG1, ACSL3, HMGA1, EGF2, BCOX1, AKT,ITGA3/ ITGB1,p21	Hessvik et al. [61], Moltzahn et al. [72], Lodes et al. [73]
Let-7e, let-7c, mir-20a, mir-21, mir-30c, mir-130b, mir-145, mir-1811a-2*, mir-221, mir-301a, mir-326, mir331-3p, mir-432, mir-574-3p, mir-622, mir-625*, mir-1285, mir-2110, mir-141, mir-1290	Plasma	HMGA2, IGF1R, AR, ABL2, PDCD4, TGFβ, BCL9, MMP2, SOX2, SENP1, Bmi1, SIRT1, IRF2, RAB1A, HECTD2, NDRG2, DOHH, ERBB-2, WNT5A, EZH2, LARP1	Shen et al. [74], Huang et al. [52]
mir-107, mir-574-3p, mir-141-5p, mir-21-5p, mir-34a, mir-483-5p	Urine	WNT5A, EZH2, LARP1, PDCD4, p57Kip2, SIRT1, CD44, WNT/TCF7, AR, Notch-1, c-Myc	Nina Pettersen Hessvik et al. [61], Samsonov et al. [71]
mir-141, mir-21	Saliva	MAPK, WNT5A, EZH2, LARP1, PDCD4, FBXO11, p57Kip2, TGFBR2, MARCKS	Hizir et al. [58]
mir-141,mir-9, mir-200b,mir-21,mir-221,mir-16,mir-92a, mir-103,mir-107, mir-197, mir-92b,mir-574-3p,mir-885-5p,mir-298,mir-26a,mir-1274a,mir-106a,mir-26b,mir-30b,c,d, mir-24, let-7a,c,e,i, miR-1285,mir-20a,mir-107,mir-130b,mir-301a,mir-331-3p,mir-625,mir-485-3p,mir-874,mir-155,mir-181a-2, mir-326, mir-762, mir-185, mir-151 and mir-149	Metastatic cell line (PC3)	IGFR1, TCR, GH, STAT, MAPK, PRLR, TGFβ, BCL2, ERG, PDGF-D, Bmi, TGFBR2, p57kip2, MARCKS, Bmi, SIRT1, IRF2, SOCS3, HECTD2, RAB14, DVL2, PDCD10, PI3 K, AKT3, WNT5A, ULK2, BCL9, CDKN1B/p27, E2F2, CCND2, AR, ABL2, CX43, MMP2, NDRG2, DOHH, ERBB-2, ANXA7, DAX1, SREBP, CASZ1, IL1RAPL1, SOX17, N4BP1, ARHGDIA	Hessvik et al. [75], Alireza Ahadi et al. [51]
Let-7a, b,c, mir-149, mir-762, mir-30b-3p, mir-20a,b, mir-17-5p, mir-18a-5p, mir-106-5p, mir-93-5p,	Metastatic cell line (VCaP)	KRAS, E2F2, CCND2, IGF1R, RPS2, AR, c-MYC, ABL2, CX43, TIMP3, p300/CBP, RE-1, KEGG	Alireza Ahadi et al. [51]
Let-7a, b,c,d,e,i, mir-17, mir-18a, mir-20a, mir-93, mir-106b, mir-149	Metastatic cell line (LNCaP)	KRAS, E2F2, CCND2, IGF1R, RPS2, AR, c-MYC, BPX3, ABL2, CX43, TIMP3, p300/CBP, RE-1, PTEN, ZBTB4, p21, CASP7, SDC-1	Alireza Ahadi et al. [51]

[69]. In a proof-of-concept study analyzing the transcriptome in tumor exosomes isolated from the urine of patients with prostate cancer, revealed biomarkers, with potential for monitoring cancer patients. If it could expand to include not only mRNAs but also miRNAs it will help to classify the tumor phenotype, its severity and the tumor response to treatment [70]. Additional studies have demonstrated alteration of certain specifics miRNAs, such as mir-107, mir-574-3p and mir-483-5p, found in the urine of men with prostate cancer compared with healthy controls [70]. In metastatic prostate cancer, miR-141 is enriched in exosomes found in cells obtained by urine sediments, as well as in parallel tissue samples, suggesting the diagnostic and prognostic potential of miR-141 for prostate cancer [71]. As shown in Table 1, there are several deregulated miRNAs in different liquid biopsy (serum, plasma, urine, saliva and cells) of prostate cancer. Regarding other important factors, it has been observed that the miRNAs content of exosomes plays a role in docetaxel resistance. mir-34a that was significantly decreased in prostate cancer versus normal tissues as well as in urine, regulates BCL-2 and may in part, regulate the response to docetaxel [76, 77].

Conclusion

There is still limited knowledge about the biological roles of exosomal miRNAs in prostate cancer. The development of new exosome isolations methods and the incorporation of high-throughput technologies as next generation sequencing (NGS) for miRNA analysis will change dramatically the scenario. The scientific community will advance in the use of plasma or urine exosomal miRNAs as source for new prostate cancer biomarkers substituting progressively invasive procedures as biopsy or serum PSA. This challenge of blood-based assays may represent the needed association between basic and clinical research, driving definitively the outbreak of personalized medicine in prostate cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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