Minireview

MicroRNAs in prostate cancer: From function to biomarker discovery

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Impact statement

The primary goal of this article was to review recent literature on miRNA biogenesis and further elaborate on the identity of newly discovered miRNAs and their potential functional significance in the complex biological network associated with prostate tumorigenesis and disease progression and as biomarkers for prostate cancer.

Abstract

MicroRNAs (miRNAs) are a small functional non-coding RNAs that post-transcriptionally regulate gene expression through mRNA degradation or translational repression. miRNAs are key regulatory components of various cellular networks. Current evidence support that multiple mammalian genome-encoded miRNAs impact the cellular biology, including proliferation, apoptosis, differentiation, and tumorigenesis, by targeting specific subsets of mRNAs. This minireview is focused on the current themes underlying the interactions between miRNAs and their mRNA targets and pathways in prostate tumorigenesis and progression, and their potential clinical utility as biomarkers for prostate cancer.

Keywords: Prostate cancer, microRNA, onco-miR, tumor suppressor miRNAs, biomarkers

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Introduction

Behind lung and colorectal cancer, prostate cancer (PC) is the most common and the third leading cause of cancer-related death among American men.[1] According to the 2017 American Cancer Society report, PC is the most common cancer among American males, with about 164,690 newly diagnosed cases and an estimated 29,430 PC-related deaths in 2018.[1,2] Family history, age, and African Ancestry are among the well-established risk factors for PC.[2] Despite significant advances in disease stratification and functional imaging, challenges in the clinical management exist primarily due to the inherent problems associated with inaccurate disease staging/grading by needle biopsy or prostate-specific antigen (PSA).[3] Currently, there are no sensitive and reliable biomarkers that can distinguish patients with less aggressive localized disease from those presenting with an aggressive phenotype.

MicroRNAs (miRNAs) are non-coding RNAs with short sequences (18–22 nucleotides) with the ability to regulate a wide array of cellular functional molecules under physiologic and disease states. MiRNAs repress target-gene expression post-transcriptionally either by inhibiting translation or promoting RNA degradation by binding to the 3′ untranslated region (3′UTR) of mRNA sequences. In cancer, miRNAs target and regulate the expression of multiple target genes, including oncogenic factors and tumor suppressors and their downstream effectors. The stability and existence of circulating free and exosomal miRNAs in biological biofluids (blood, urine, spinal fluids, etc.) made them attractive targets for biomarker discovery and development of non-invasive liquid biopsy clinical tools, including screening, diagnosis, prognosis, monitoring tumor progression, and therapeutic response. Identification and association of unique miRNA signatures with PC tumorigenesis have been established[4,5] and their potential clinical utilities as novel diagnostic and prognostic tools for PC have been explored.[6,7] The purpose of the current review is to highlight the current knowledge related to miRNAs in prostate tumorigenesis and progression and further discuss their potential clinical utility as biomarkers for PC.

PC: Diagnosis and treatment options

Located below the bladder, the prostate gland is a walnut-sized gland that encircles the urethra.[8] Male ejaculate contains about 30% of prostatic fluid.[9] From a histological point of view, the prostate gland is a tubuloalveolar exocrine gland of the male reproductive system.[10] Prostate epithelial cells consist of three types of cells that can be categorized by morphological and functional significance. First, the secretory luminal cells are the most predominant, differentiated, and androgen-dependent cells that are responsible for the secretion of prostatic proteins. Different biomarkers are expressed by these cells, including androgen receptor (AR), cytokeratin 8 and 18, and cell surface marker CD57.[11–13] Second, the basal cells are found between the luminal cells and the basal membrane and represent the second major type of prostate epithelial cell. These cells are characterized by the molecular expression of cytokeratin 5 and 14, as well as CD44 and a low level of AR.[11–14] Third, the neuroendocrine cells are a minor population of prostatic cells that confer growth signal to the luminal cells. They are AR-independent cells and express chromogranin A, serotonin, and various neuropeptides.[15–17]

Androgens and their cognate AR play a pivotal role in prostate gland homeostasis as well as in the development and progression of prostate tumorigenesis.[18] Like other steroid hormone groups of nuclear receptors, the AR is a ligand-dependent transcription factor that regulates expression of androgen-regulated genes. AR amplification and mutations have been implicated in the development and progression of PC.[19,20] During androgen-dependent PC progression, the ratio between the proliferating cells and the dying cells is maintained by an androgen hormone, which stimulates proliferation and inhibits apoptosis.[21] Promiscuous binding and activation of mutant AR by steroid hormones and non-canonical activation by kinases and long non-coding RNAs have been implicated in the development of castration-resistant PC (CRPC).[22]

Treatment options for the early diagnosed indolent disease include surgery and/or radiotherapy but may have negative effects that adversely affect patients' quality of life. Nonetheless, recent changes in the grading system and monitoring of high-risk PC have reduced overtreatment and improved disease management and quality of life. This may be attributed to improved risk stratification and advances in functional imaging (e.g. multiparametric magnetic resonance imaging, mpMRI) and emergence of new biomarkers. As a result, incorporation of monitoring modalities, such as active surveillance, emerged as a preferred approach for the management of less-aggressive indolent disease with PSA level of 10 ng/mL. Despite an initial response to various treatment regimens, including first-line androgen-deprivation therapy,[23] some patients inevitably progress to CRPC via unknown mechanisms.[24] Chemotherapy, vaccines, second-generation hormonal therapeutics, and bone-targeting agents have proven efficacious against metastatic CRPC.

Despite limitations, PSA along with the digital rectal examination remains to be the gold standard approach for PC screening and prognosis.[25] The widespread use of PSA has led to the early detection of PC and a reduction of metastatic disease at diagnosis; however, the overall benefit of monitoring serum PSA after treatment remains controversial. Men without elevated PSA may present with clinically significant PC; about 15% of PC cases were reported in men with very low serum PSA levels.[26] Additionally, the inconclusive reports from two randomized trials on the benefits and impact of PSA screening and subsequent treatment[27,28] prompted the U.S. Preventive Service Task Force (USPSTF) to advise against PSA screening. Accordingly, several new diagnostic markers have been developed for PC screening,[26–29] including serum and urine detection of RNA biomarkers (e.g. PCA3), PC tissue protein antibodies (e.g. EPCA), and the TMPRSS2:ERG gene fusion; however, their sensitivities and specificities remain to be established. Such limitations necessitate the development of a new predictive and reliable biomarker(s) that may serve as surrogate end points to improve distinction between indolent and clinical progression of the disease and/or patient survival.

MicroRNA (miRNA/miR): Biogenesis and nomenclature

MicroRNAs (miRNAs/miRs) were originally discovered in *Caenorhabditis elegans* and were later discovered in eukaryotic and human cells.[30,31] miRNAs are a class of endogenous, short non-coding, evolutionarily conserved RNA molecule.[32,33] This short non-coding RNA molecule regulates its many targets at the post-transcriptional level.[32–36] miRNAs are transcribed by RNA polymerase II and III into a primary miRNA transcript (pri-miRNA). Pri-miRNA is characterized by a secondary hairpin structure. A microprocessor complex cleaved the pri-miRNA.[37,38] The class 2 ribonuclease III enzyme Drosha cuts the 5′and the 3′ arms of the pri-miRNA hairpin, producing 70–110 nucleotides length, known as "precursor miRNA (pre-miRNA)." Following the nuclear processing, exportin 5 (XPO5) export the pre-miRNA to the cytoplasm,[39] where Dicer cleaves the pre-miRNA to generate a ~22-nucleotide miRNA duplex. This duplex binds to the active RNA-induced silencing complex (RISC) that performs gene silencing.

The double miRNA duplex is composed of a functional strand (which is complementary to the target mRNA) and the passenger strand (which is subsequently degraded). According to Chendrimada *et al.*, the mature miRNA functional strand is loaded into the RISC complex and Argonaute 2 (AGO2).[40] RISC-AGO2 complex guides the functional strand to target the 3′UTR of the target miRNA, causing translational inhibition or promoting their degradation. Bartel *et al.* and Hausser *et al.* reported that the specificity of miRNA can be determined by the base pairing between the seed sequence (a 6–8 nucleotide sequence in the 5′ end of the mature miRNA), and the 3′UTR sequence of the target gene[41,42] (Figure 1).

According to Ambros *et al.*,[43] a special annotation system has been adopted for miRNA nomenclature. Briefly, newly identified miRNA genes have a sequential number that is preceded by a prefix "mir/or MiR," and then followed by a

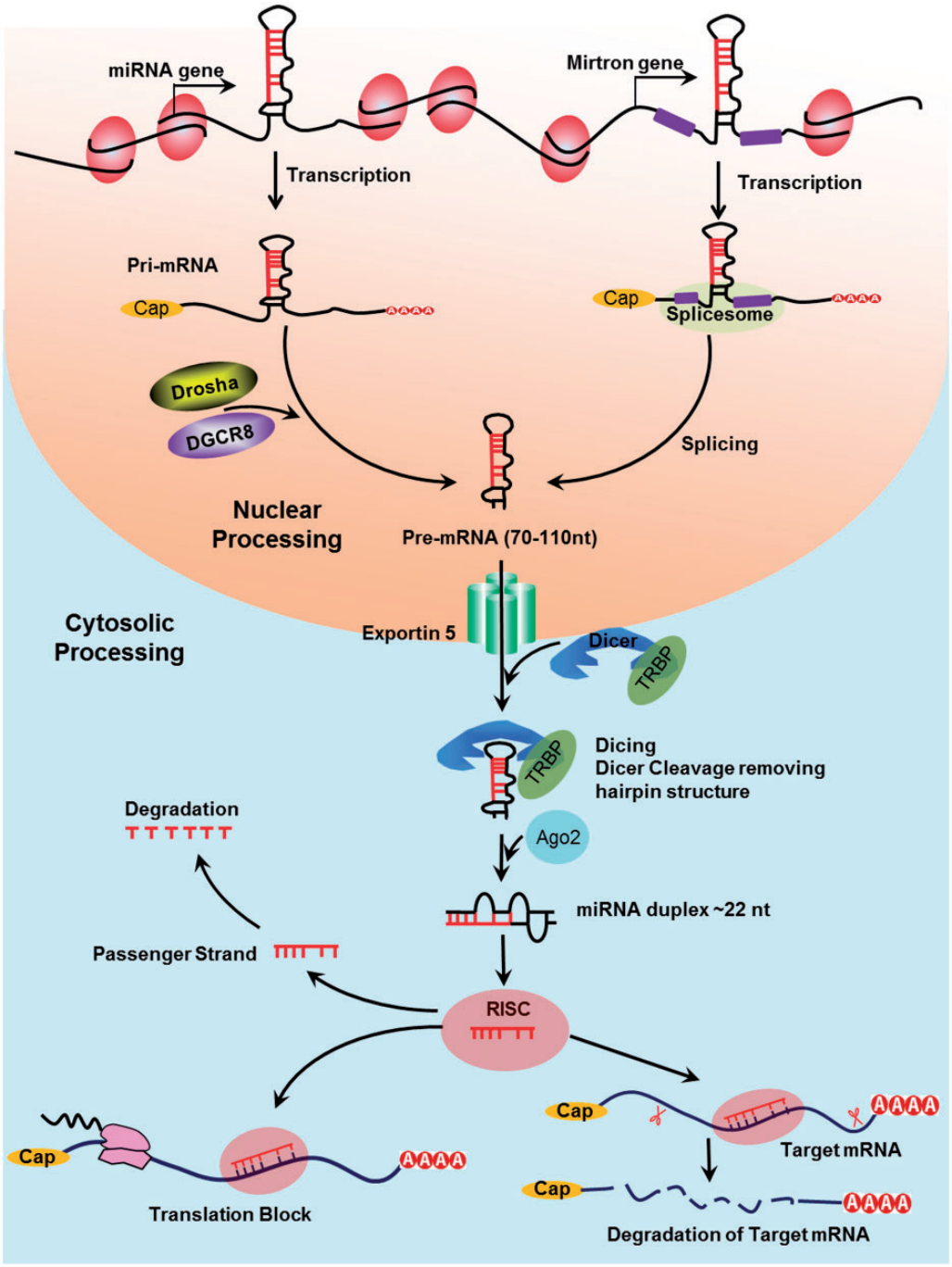

Figure 1. Biogenesis of microRNA: The biogenesis of miRNAs. miRNA is transcribed in the nucleus as a pri-miRNA and then is micro-processed by Drosha, a class 2 ribonuclease III enzyme, and transported to the cytoplasm by exportin 5 (XPO5) where the hairpin structure is removed by Dicer and a single-stranded mature miRNA is produced, which binds to RISC and induces gene silencing.

dash (e.g. mir-19 or miR-19). The pre-miRNA is denoted as lowercase "mir," while mature miRNA as capitalized "miR." With lowercase letters, closely related mature miRNA sequences are marked to show their structural similarity (for example, miR-19a and miR-19b). If the mature sequences are expressed from different precursor sequences and genomic loci, an additional number will be added to the miRNA name (for instance, miR-19a1 and miR-19a2). Species annotation should also be mentioned in the miRNA name (for example, has miR-19 in *Homo sapiens* and mmu-miR-19 in *Mus musculus*). If the mature miRNA sequence originated from 3′ or 5′ end, the suffix,-3p′ or, -5p′ it would be denoted, respectively (e.g. miR-19a-3p and miR-19a-5p).[43] A database of miRNA structure and nomenclature is available on the web at http://www.mirbase.org/.[44]

miRNAs silence their targets by binding to the 3′UTR, inducing mRNA degradation or translational repression.[34] Many cellular processes were found to be regulated by miRNAs.[45] The potential role of miRNAs in the pathogenesis of different types of cancer has been reported,[33,45–47] including initiation, propagation, and metastasis.[46,48]

Onco-miRNAs in PC

The process of prostate tumorigenesis is a multifactorial and multistep process, during which different cellular and genetic changes cause normal prostate cells to become malignant.[49,50] MiRs control many cellular processes and their altered signature was reported in many diseases, including cancer.[45,46,51] In cancer, miRNAs may act as "onco-miRNAs" and drive the cells toward cancer progression.[44] The first study that described the relationship between miRNA dysregulation and cancer was led by Calin *et al.*,[52] who reported the dysregulation of miR-15 and miR-16 and their role in the pathogenesis of chronic lymphocytic leukemia.

Several studies reported the role of miRs in PC tumorigenesis.[27,47] For instance, we reported that exosomal oncogenic miR-125b, miR-130b, and miR-155 are implicated in the oncogenic reprogramming of PC-recruited stem cells.[53] Mechanistic studies revealed that exosomal trafficking of miR-125b and miR-155 potentially initiates neoplastic reprogramming in the stem cells by targeted inhibition of the large tumor suppressor homolog2 and programmed cell death protein 4, a neoplastic transformation inhibitor.[53] Moreover, miR-125 was found to act as an oncogene by targeting pro-apoptotic genes in PC.[54] Furthermore, it has been reported that miR-22 contributes to prostate tumorigenesis by targeting PTEN, the loss of which activates PI3K signaling in PC cells.[55] In 2015, Seashole *et al.* described the relationship between the altered signature of miR-9 and PC progression and metastasis.[56] Also, miR-27a was reported to regulate AR processing via targeting prohibition in PC.[57] Additionally, miR-18 was reported to be highly expressed in PC tissue and cell lines and also act as an onco-miRNA by targeting the tumor suppressor serine/threonine-protein kinase 4.[58]

Bone metastasis and skeletal-related events are critical steps in PC progression and portend poorer prognosis than those without metastatic disease.[59] The role of miRs in cancer metastasis has been reported in many studies.[60–62] MiR-96, miR-154, and miR-409–5p were reported to be upregulated in PC and potentially play a crucial role in bone colonization during PC metastasis.[63–65] Likewise, an elevated level of miR-21 induces angiogenesis through AKT and ERK activation in PC.[66] Another study reported that in DU-145, a brain metastatic PC cell line, upregulation of miR-21 contributed to PC via targeting matrix metalloproteinase regulator inhibitor RECK.[67] PC progression was related to upregulation of miR-133b, miR-409, and miR-210 through targeting key molecules that induce fibroblast activations.[68–70] More recently, Bertoli *et al.* described the relationship between altered miR signature and PC metastasis[71] (Figure 2).

Tumor-suppressor miRs in PC

According to Koturbash *et al.*, miRNA may also act as a tumor suppressor gene.[35] Several studies reported a relationship between miR downregulation and cancer progression.[72–74] In 2015, we showed that dysregulation of miR-212 confers the development of castration-resistant disease via priming hnRNPH1-mediated upregulation of AR and AR-V7.[75] MiR-146b was found to be downregulated in prostate tumor tissue and is considered to be a potential tumor suppressor.[76] Another study showed downregulation of miR-335 in three PC cell lines (LNCaP/DU145/PC3) and also in PC tissues.[77] Zhu *et al.* reported that miR-30a exerts a tumor suppressor effect and inhibits the proliferation and invasion of PC cells via targeting of sine oculis homeobox homolog 1.[78] We recently reported that miRs-595, 4490, -3120–5p, -1299, -21–5p, -3677–3, -let-7b-5p, -5189, 3–121-5p, -4518, -200a-5p, -3682–5p, -3689d, -3149 are downregulated (12–113-fold) in microdissected prostate tumors as compared with adjacent normal glands.[47] Subsequent pathway and target prediction analysis showed that several miRs may serve as potential tumor suppressors.[47]

Additionally, miR-1, miR-29b, and miR-200 were found to be participatory in PC progression via regulation of epithelial–mesenchymal transition signaling pathway (EMT).[79,80] Similarly, downregulation of miR-34b, miR-145, miR-146a, miR-200, and miR-205 inhibit PC migration and invasion via targeting of AKT, ZEB2, ROCK1, SNAI2, and centromere protein F, respectively.[79] Also, downregulation of miR-29b and miR-130b in PC was found to be related to modulation of the extracellular matrix structure via targeting matrix metalloproteinase 2 (MMP2).[80,81] Gandellin *et al.*[82,83] reported that miR-205 downregulation in PC contributed to extracellular matrix structure regulation via targeting laminin-332, integrin-β4, MMP-2in, which in turn inhibits PC progression. Furthermore, downregulation of miR-146a in PC was related to inhibition of angiogenesis via targeting epidermal growth factor receptor pathway.[84] Additionally, miR-1 downregulation promotes PC progression and metastasis via targeting Src and TWIST1.[85,86] In PC, targeted downregulation of loss of miR-154, miR-203, and miR-224 due to stromal antigen 2 (STAG2), EREG, tumor growth factor-alpha (TGF-α), and Tribbles Pseudokinase 1 (TRIB1), respectively, play a regulatory role in EMT and PC progression.[63,87,88]

Overall, the diverse role of microRNAs in prostate tumorigenesis has attracted a surge of interest to gain more understanding of their functional significance in suppression and progression of prostate tumorigenesis. Table 1 summarizes the list of selected miRNAs implicated in various processes of prostate tumorigenesis, including tumor suppressor miRs and onco-miRs implicated in tumor growth, neoplastic reprogramming of stem cells, EMT, CAFs, angiogenesis, extravasation, and colonization of distant organs.

miRNAs as potential circulating biomarkers for PC

Development of a new biomarker strategy for PC screening is urgently needed due to the limitations of the current tools. Early tumor identification and detection are important elements to informed decision-making in the disease management, prognosis, and overall survival of PC. Exosomes are nanovesicles 50 to 150 nm in size formed within the cells as early endosomes containing miRNAs, mRNA, proteins, and lipids and are particularly important in cell–cell communication and modulation of the biology of recipient cells,[89] and indeed are considered to play a

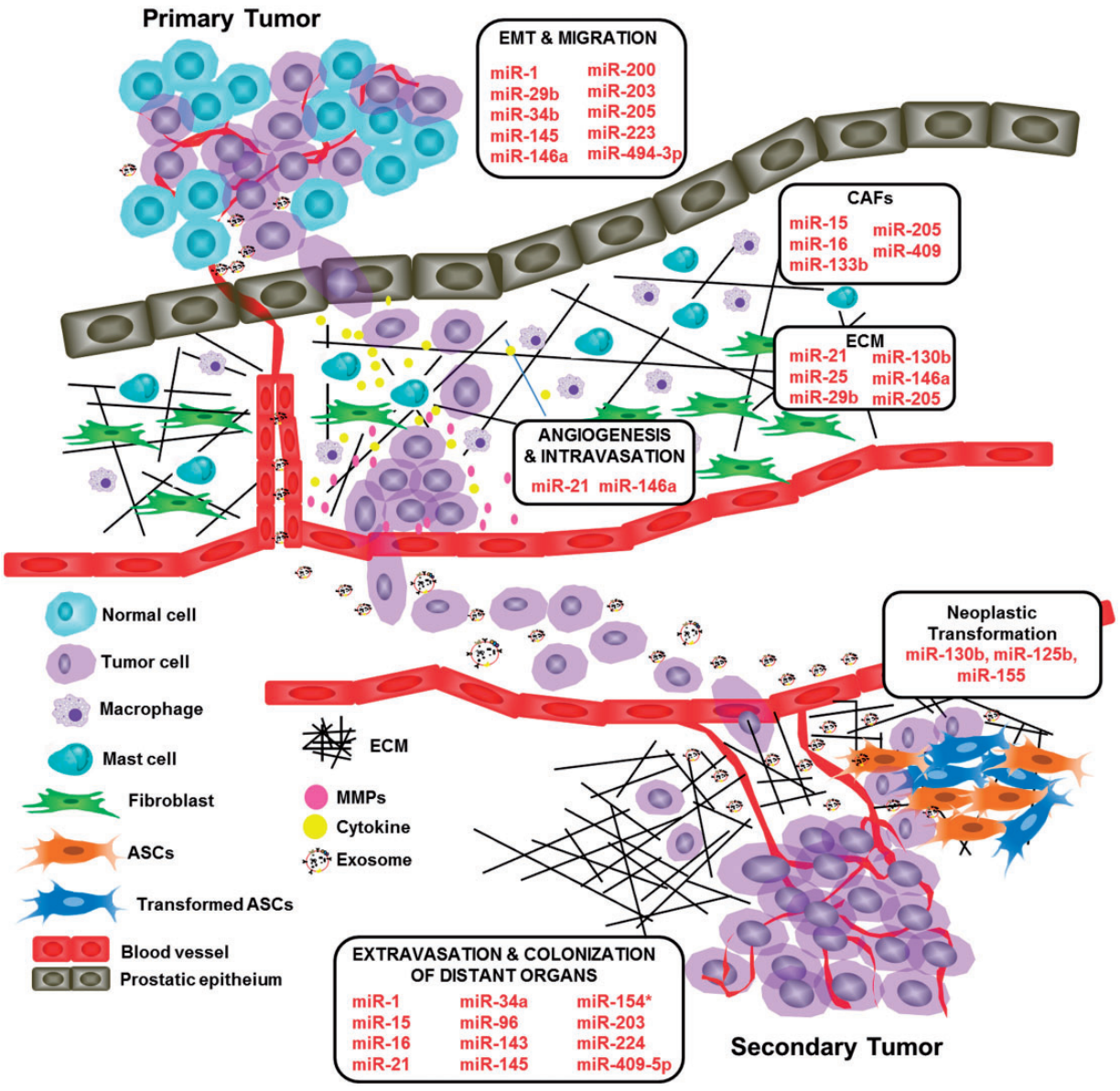

Figure 2. Overview of miRNAs contribution in the metastatic process. The possible miRs function in preventing or promoting PC metastasis through regulation of epithelial-to-mesenchymal transition, regulation of extracellular matrix structure, regulation of anoikis and bone colonization.

fundamental role in many physiological and pathological processes.[90] Recently, exosomes have been detected in various body fluids of cancer patients and conditioned media of cultured cells.[91] The PC-derived exosomes are detected in the prostatic secretions, seminal fluid,[92] urine,[93] and blood,[94,95] implicating their clinical utility as "liquid biopsies" in the diagnosis and prognosis of PC. The blood-circulating exosomal miRs have been employed as prognostic markers in many types of tumors.[96] In 2008, a study conducted by Mitchell *et al.* demonstrated that elevated expression of miR-141 can be used as a marker to distinguish between PC patients and normal subjects.[97] Another study done by Lodes *et al.* confirmed Mitchell's and his team's observation.[98] MiR-141 and miR-375 were found to be upregulated in PC patients and their sera levels were associated with disease progression.[99] Huang *et al.* showed that plasma exosomal miR-1290 and miR-375 are promising prognostic biomarkers for CRPC patients.[100] The PC-related miRNA in urinary exosomes revealed significant upregulation of miR-574–3p, miR-141–5p, and miR-21–5p associated with PC.[101] In a genome-wide serum profiling study by Haldrup *et al.*, several new miR markers for PC and disseminated disease were identified in comparison to benign prostatic hyperplasia and localized disease.[102] These and other findings prove the potential clinical utility of miRs as promising biomarkers for PC screening and diagnosis. As with any new biomarker, there are challenges and limitations associated with miRNAs as cancer biomarkers. Before the translation of miRNAs as biomarkers in clinical practice, steps need to be undertaken to remove inaccuracies and inconsistencies due to non-uniform sample choice, handling, and processing, sample preparation and lack of consensus for data normalization. The development and the application of SOPs

Table 1. List of miRNAs in metastatic PC.

Function	mRNA	Target	Reference
Tumor growth	miR-125b	*p53*, *Puma*, and *Bak1*	54,55
	miR-155	*ANX7*	54,73
	miR-27a	MAP2K4	60
Neoplastic transformation in tumor recruited stem cells	miR-125b	p53	53
	miR-130b	TP53INP1	53
	miR-155	p53	53
EMT and migration	miR-1	*Src*, *TWIST1*, *Slug*	79,85,86
	miR-29b	*Snail*	80
	miR-34b	*AKT*	78
	miR-145	*ZEB2*	80
	miR-146a	*EGFR*	84
	miR-200	*Slug*	79
	miR-205	*CENPF*, *ZEB2* and *PKCε*	82,83
	miR-130b	*MMP2*	81
	miR-212	SOX4	75
CAFs (cancer-associated fibroblasts) and angiogenesis	miR-15 and miR-16	*Bcl-2*, *Ccnd1*, *Ccne1*, *Bmi-1* and *Wnt* family members	52
	miR-21	*RECK*	65,67
	miR-133b	*EGFR*	70
	miR-205	*CENPF*, ZEB2 and PKCε	82,83
	miR-409	*RSU1*, and *PHC3*	64,69
Extravasation and colonization of distant organs	miR-1	*Src*, *TWIST1*, *Slug*	79,85,86
	miR-96	AKT1S1	65
	miR-145	*ZEB2*	80
	miR-154*	STAG2	63
	miR-203	SRC, SUZ12, API5 , BIRC2, EREG, TRIAP1 and TGF-α	88
	miR-224	*TRIB1*	87
	miR-409-5p	*STAG2*, *NPRL2*, *RSU1* and *RBL2*	64
Tumor suppressor	miR-9	*E-cadherin* and *SOCS5*	56
	miR-18	*STK4*	58
	miR-22	*E-cadherin*	55
	miR-146b		76
	miR-30a	*SIX1*	78
	miR-335	*eNOS*	77

for whole blood collection to plasma/serum preparation, handling, and banking to RNA extraction and miRNA quantification would keep interlaboratory differences to a minimum and also limit incoherencies among different users. Also, utilization of polymerase chain reaction for miRNA detection may pose a technical challenge, as amplification bias may limit the ability to accurately quantify the relative abundance of specific miRNA targets.[103,104] Another caveat in using miRNAs as a biomarker currently is the high costs associated with detection and validation. Cost-effective techniques, such as next-generation sequencing, allow for a faster and more accurate miR profiling. Overcoming such limitations would increase the potential utility of miRNAs as biomarkers in the clinical management of PC.

Conclusions

As their role, in large part, remains elusive, it is premature to determine whether miRs are tipping the balance towards suppression or progression of the disease. Considering the complexity and heterogeneity of the disease, further studies are required to unravel the functional role of miRs in the underlying mechanisms that govern the stepwise transition from an indolent disease into the aggression castration-resistant phenotype. Finally, additional studies are needed to establish the clinical utility of miRs as a multistep screening strategy for early detection and disease progression.

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