REPRODUCTION
REVIEW

Implications of microRNA dysregulation in the development of prostate cancer

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Abstract

MicroRNAs (miRNAs) are non-coding small RNAs that target mRNA to reduce protein expression. They play fundamental roles in several diseases, including prostate cancer (PCa). A single miRNA can target hundreds of mRNAs and coordinately regulate them, which implicates them in nearly every biological pathway. Hence, miRNAs modulate proliferation, cell cycle, apoptosis, adhesion, migration, invasion and metastasis, most of them constituting crucial hallmarks of cancer. Due to these properties, miRNAs emerged as promising tools for diagnostic, prognosis and management of cancer patients. Moreover, they come out as potential targets for cancer treatment, and several efforts are being made to progress in the field of miRNA-based cancer therapy. In this review, we will summarize the recent information about miRNAs in PCa. We will recapitulate all the miRNAs involved in the androgen pathway and the biology of PCa, focusing in PCa initiation and progression. In particular, we will describe the miRNAs associated with cell proliferation, cell cycle and apoptosis in PCa, as well as invasion, adhesion and metastatic miRNAs. We will revise the recent progress made understanding the role of circulating miRNAs identified in PCa that might be useful for PCa patient stratification. Another key aspect to be discussed in this review is miRNAs' role in PCa therapy, including the miRNAs delivery.

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Introduction

After the discovery of the first microRNA (miRNA) (*see Box 1 for definition*), lin-4 in *Caenorhabditis elegans*, the regulation of multiple biological pathways by miRNAs was investigated. Nowadays, we know that miRNAs regulate post-transcriptional gene expression mostly by imperfect binding of miRNA to the partially complementary sequence in 3′UTR, which represses protein translation, but also by cleavage of mRNA with perfect base-pairing homology through the action of AGO in the RISC complex (Jackson & Standart 2007). Interestingly, it is estimated that miRNAs regulate up to 30% of all protein-coding genes (Jackson & Standart 2007).

miRNAs modulate multiple biological processes such as genome stability, proliferation, cell cycle, apoptosis, adhesion, migration, invasion and inflammation, most of them constituting crucial hallmarks of cancer (Ruan *et al.* 2009).

Functional studies identified several miRNAs with key roles in carcinogenesis, cancer progression and metastasis. Cancer-related miRNAs could be grouped in two main categories: oncomiRs that repress tumor suppressor genes resulting in oncogenic properties and tumor suppressor miRs (tsmiRs), with a role in tumor suppression since they reduce oncogene protein expression (Ruan *et al.* 2009).

Even when individual miRNAs can be oncomiRs or tsmiRs, global levels of miRNAs seems to be suppressed in tumors compared with normal tissues suggesting that miRNA biogenesis could be dysregulated in cancer (Lin & Gregory 2015).

Numerous studies show that miRNA expression profiles could differentiate between normal and tumor tissues to identify tumors of unknown origin and to classify subtypes of the same tumor (Metias *et al.* 2009). Increasing evidence support that miRNAs can be detected in serum in a very stable form, making circulating miRNAs a useful field in active research for diagnosis and follow-up of cancer. miRNA expression could predict clinical outcome and prognosis in some types of tumors or they can be used as markers of disease relapse and metastases (Metias *et al.* 2009). Therefore, miRNAs emerged as promising tools for diagnostic, prognosis and management of cancer patients. Since miRNAs target multiple gene pathways, they come out as potential targets for cancer treatment, and progress is being made in the field of miRNA-based cancer therapy (Metias *et al.* 2009).

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BOX1: miRNAs definition and biogenesis

MicroRNAs (miRNAs) are an abundant class of non-coding small RNAs of 19–25 nucleotides length that target mRNA to reduce protein expression. They play fundamental roles in most biological processes, including several diseases. miRNA biogenesis is a multistep process that starts with transcription of miRNA genes by RNA polymerase II generating a long primary precursor (pri-miRNA). This precursor, which can be thousands of nucleotide long, has a local hairpin structure, which is cleaved by the enzymes Drosha and DGCR8, producing a hairpin ~80 bp long, called pre-miRNA. This pre-miRNA is exported to the cytoplasm by Exportin 5, where it is processed by the enzyme Dicer and bound to Argonaute (AGO) protein, resulting in the RISC complex. The mature miRNA assembled into the RISC complex typically binds to the 3′ untranslated region (UTR) of the target mRNA and usually inhibits protein translation.

In this review, we will summarize the recent information about miRNAs in PCa. We will recapitulate all the miRNAs involved in the androgen pathway and the biology of PCa, focusing in PCa initiation and progression. In particular, we will describe the miRNAs associated with cell proliferation, cell cycle and apoptosis in PCa, as well as invasion, adhesion and metastatic miRNAs. In addition, we will revise the recent progress made understanding the role of circulating miRNAs identified in PCa that might be useful as diagnostic and prognostic tools. Another key aspect to be discussed in this review is the role of miRNAs in PCa therapy, including the miRNAs delivery.

miRNAs in the Androgen Receptor (AR) signaling pathway in PCa

The controversial use of PCa biomarkers, such as PSA (Cary & Cooperberg 2013), established the need for novel clinically useful biomarkers, including miRNAs (*see Box 2 for PCa related terms*). AR signaling axis is persistently activated and plays an important role in the growth and survival of hormone-refractory PCa cells. Both AR itself and its downstream signaling processes remain attractive targets for treating CRPC.

As AR regulates the expression of several miRNAs, and the *AR* mRNA has a long 3′UTR, which is a predicted target of many miRNAs, in this section, we will review the impact of miRNAs linked to reactivation of the AR signaling axis and the relevance of miRNAs in the development of CRPC (Fig. 1).

Androgen-upregulated miRNAs

We will describe *miR-135a*, *miR-32*, *miR-125b*, *miR-27a*, *miR-141* and *miR-21* as the most important miRNAs reported that are induced by AR (Fig. 1).

miR-135a

AR directly binds to AR-binding sites (ARBSs) in the *miR-135a* gene activating its transcription. Overexpression of miR-135a decreased *in vivo* and *in vitro* invasion and migration of PCa cells through ROCK1 and ROCK2 downregulation (Kroiss *et al.* 2015).

miR-32

miR-32 was also reported as a miRNA induced by androgens. The stimulation of LNCaP cells with dihydrotestosterone (DHT) leads to miR-32 overexpression. This miRNA showed putative ARBSs in close proximity to the miRNA genomic loci. miR-32 overexpression significantly induced LNCaP cells growth while reduced apoptosis, which correlates with BTG2-downregulated expression (Jalava *et al.* 2012).

miR-125b

miR-125b is overexpressed in PCa and it is regulated by androgens, but also is an important regulator of AR. Shi *et al* demonstrated that androgen stimulation of LNCaP cells increased miR-125b expression. Transfection of synthetic miR-125b mimic stimulated the androgen-independent growth and downregulated the expression of the proapoptotic regulator Bak1

BOX2: Prostate cancer (PCa)-related terminology

Prostate gland development is initially androgen sensitive. Under constant stimulation of androgen, prostate gland can gradually develop into androgen-sensitive PCa, which is the most common cancer among men (after skin cancer) (https://www.cancer.org/cancer/prostate-cancer.html). For patients who show locally advanced or disseminated PCa, androgen deprivation therapy (ADT) is widely used as a treatment. Most patients initially respond to ADT; however, castration-resistant prostate cancer (CRPC), the terminal stage of PCa, arises when hormone-refractory growth occurs in a castrate androgen-level environment. Bone metastases occur in 80–90% of men with CRPC, which seriously jeopardizes the patient's quality of life and morbidity. Despite several advances in PCa treatment, metastatic CRPC remains an incurable disease. The androgen receptor (AR) is one of the most important nuclear transcription factors from the steroid hormone receptor superfamily of genes. Normal prostate growth and development, PCa carcinogenesis and CRPC progression are dependent on AR expression and function. Currently prostate-specific antigen (PSA) is the most specific marker used to detect PCa and strongly correlates with the risk of harboring PCa. However, PSA has limitations for PCa detection given its low specificity and poor sensitivity. The lack of specificity of PSA for PCa has led to both unnecessary biopsies and overdiagnosis of indolent cancers (Cary & Cooperberg 2013).

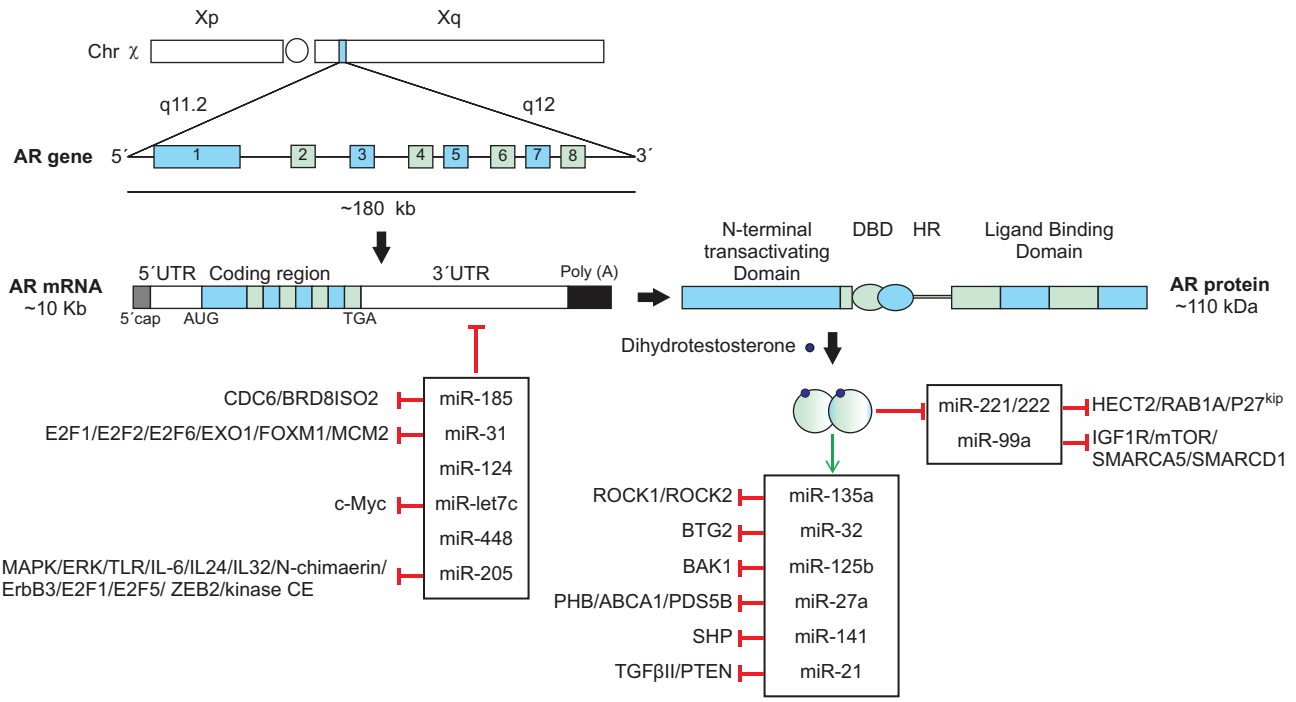

Figure 1 miRNAs involved in AR signaling pathway. miRNAs that target 3′UTR of the *AR* mRNA, including the miRNAs target genes, are indicated (*left panel*). In the right panel, scheme of AR protein structure including miRNAs repressed (red arrow) and induced (green arrow) by AR are indicated. DBD, DNA-binding domain; HR, Hinge region.

(Shi *et al.* 2007). The authors found that AR regulates miR-125b expression through an androgen-responsive element within the promoter region of the miR gene. They also showed that miR-125b is a downstream effector of AR pathway that promotes tumor growth by targeting proapoptotic-genes (Shi *et al.* 2011).

miR-27a

miR-27a is an androgen-regulated oncomiR in PCa, targeting the tumor suppressor and AR corepressor Prohibitin. miR-27a reduced Prohibitin mRNA and protein levels and increased the expression of AR target genes inducing PCa cell growth (Fletcher *et al.* 2012). In addition, Mo *et al* found that miR-27a is directly upregulated by AR and promoted PCa-malignant phenotype by direct regulation of ABCA1 and PDS5B, which facilitates PCa cell proliferation (Mo *et al.* 2013). However, more recently, Wan and coworkers reported to miR-27a as a tsmiR downregulated by PI3K signaling in CRPC cells. Overexpression of miR-27a decreased PCa cell proliferation and migration and induced PCa cell cycle arrest and apoptosis (Wan *et al.* 2016).

miR-141

Waltering *et al* demonstrated that four miRNAs (miR-10a, miR-141, miR-150*, miR-1225-5p) showed similar androgen regulation in both, cell lines and xenografts. miR-141 overexpression enhanced LNCaP cells proliferation while its inhibition by anti-miR-141 suppressed cell growth (Waltering *et al.* 2011). The small heterodimer partner (SHP) also known as NR0B2 is an AR corepressor that represses AR-regulated transcriptional activity. miR-141 targets 3′UTR of *SHP* mRNA resulting in its translational suppression and mRNA degradation causing an increase in AR-regulated transcriptional activity in LNCaP cells (Xiao *et al.* 2012).

miR-21

TGFβ is one of the most important pathways altered in PCa. Mishra and coworkers showed that the coordinated action of miR-21 and AR signaling played a critical role inhibiting TGFβ receptor II (TGFBR2) expression in PCa cells through a positive feedback loop that inhibits growth responses (Mishra *et al.* 2014). AR binds to ARBSs in the *miR-21* promoter and increases its transcription (Ribas *et al.* 2009). miR-21 overexpression enhanced androgen-dependent PCa growth and mediates castration resistance (Ribas *et al.* 2009). miR-21 is overexpressed in different human cancers, and it was described as an oncomiR due to its ability to negatively modulate the expression of the tumor suppressor gene *PTEN* (Talotta *et al.* 2009). However, miR-21 was not differently expressed in carcinomas with respect to normal tissues in 36 samples from PCa patients subjected to radical prostatectomy (Folini *et al.* 2010).

Androgen-downregulated miRNAs

In this section, we will explain miR-221/222 cluster and miR-99a as the major miRNAs that are directly downregulated by AR (Fig. 1).

miR-221/222 cluster

The oncomiRs miR-221 and miR-222 are transcribed from a gene cluster on the X chromosome with identical seed sequences and target genes. Sun and coworkers showed that miR-221/222 cluster was downregulated by androgens and significantly upregulated in CRPC cells. Overexpression of miR-221/222 in LNCaP cells promoted the development of the CRPC phenotype through downregulation of its targets, HECTD2 and RAB1A (Sun *et al.* 2009).

In addition, miR-221/222 cluster was overexpressed in PC3 cells (a model of aggressive prostate carcinoma) compared with LNCaP and 22Rv1 cell lines (models of slowly growing carcinomas). This cluster targeted $p27^{KIP1}$, a tumor suppressor and cell cycle inhibitor, and confers high growth advantage to LNCaP cells and LNCaP-derived tumors in SCID mice (Galardi *et al.* 2007, Mercatelli *et al.* 2008). Consistently, the anti-miR-221/222 treatment of established subcutaneous tumors derived from PC3 cells reduced tumor growth by increasing intratumor $p27^{KIP1}$ protein expression (Mercatelli *et al.* 2008). These studies suggest that miR-221/222 cluster has an oncogenic role in PCa. Nevertheless, the differential expression pattern of miRNA-221/222 associated with PCa progression remains contradictory. Several groups have shown that miR-221/222 was highly expressed in patient-derived primary cell lines and bone metastatic CRPC tumor specimens. They showed a positive association of miR-221 expression with the pathological stage, lymph node metastasis, capsular invasion, organ-confined disease, Gleason score, biochemical recurrence and patient follow-up (Mercatelli *et al.* 2008, Li *et al.* 2012, Sun *et al.* 2013). However, two independent cohort studies using microarrays revealed a progressive reduction of miR-221 in aggressive PCa tumors and their metastasis, which might be used for clinical recurrence prognosis (Spahn *et al.* 2010, Gordanpour *et al.* 2011).

miR-99a

Androgen directly binds to the miR-99a/let7c/125b-2 cluster gene and represses its expression in AR-positive PCa cells. Insulin-like growth factor 1 receptor (IGF1R), mammalian target of rapamycin (mTOR) and chromatin remodeling factors SMARCA5 and SMARCD1 are involved in androgen-induced growth and PCa progression by repression of miR-99a (Sun *et al.* 2011, 2014*a*, Rane *et al.* 2016). Several groups have shown that miR-99a was underexpressed in human prostate tumor tissue, metastatic PCa and CRPC samples compared to normal prostate tissues (Sun *et al.* 2011, 2014*b*, Lin *et al.* 2013, Rane *et al.* 2016). The reduction in miR-99a expression could provide a growth advantage for AR-positive cells under androgen-depleted condition. Moreover, miR-99a overexpression inhibited the growth of PCa cells and decreased the expression of PSA indicating its potential role as tsmiR (Sun *et al.* 2011, 2014*a*). As downregulation of miR-99a requires an active AR, it is expected that the hyperactivated AR frequently seen in CRPC could trigger increased expression of IGF1R and mTOR through transcriptional repression of miR-99a, which may contribute to CRPC progression (Shih *et al.* 2015).

Andro-miRs: miRNAs that regulate AR expression and their role in CRPC

Another group of miRNAs highly relevant in PCa are andro-miRs that include all the miRNAs that regulate AR expression. We will give details about the most important reported andro-miRs: miR-185, miR-31, miR-205, Let-7c, miR-488* and miR-124 (Fig. 1).

miR-185

miR-185 is a tsmiR reduced in clinical PCa samples that downregulates AR (Qu *et al.* 2013, Liu *et al.* 2015*a*). Overexpression of miR-185 reduced AR expression and inhibited LNCaP cell proliferation arresting cells at G1 phase and inducing apoptosis. miR-185 suppressed the invasive and migration abilities of cells and inhibited the tumorigenicity in PCa xenografts models. Also, miR-185 tumor suppressor functions could be mediated by downregulation of its target gene CDC6.

A recent study of Jiang and coworkers found that miR-185 attenuated AR function indirectly by binding to the 3′UTR of the AR co-activator BRD8ISO2 (bromodomain containing 8 isoform 2). Additionally, *BRD8ISO2* mRNA expression was inversely correlated with miR-185 expression in clinical specimens (Jiang *et al.* 2016).

miR-31

A complex interaction between the tsmiR miR-31 and AR signaling was reported by Lin and coworkers (Lin *et al.* 2013). miR-31 expression was reduced in primary and metastatic PCa as a result of promoter hypermethylation and its expression was inversely correlated with the aggressiveness of the disease. The expression of AR and miR-31 was inversely correlated in cell lines suggesting that miR-31 and AR could mutually repress each other. Upregulation of miR-31 suppressed AR expression and inhibited PCa growth. Additionally, miR-31 suppressed cell cycle regulators; including E2F1, E2F2, EXO1, FOXM1 and MCM2 (Lin *et al.* 2013). Creighton and coworkers found that miR-31 has anti-proliferative effects inducing apoptosis in PC3 cells through a

dysfunctional p53 pathway. Loss of miR-31 is associated with defects in the p53 pathway in PCa suggesting that patients with p53-deficient cancers might benefit from therapeutic delivery of miR-31 (Creighton *et al.* 2010).

Fuse *et al* found 56 miRNAs significantly downregulated in PCa compared with non-PCa tissues, being the top four downregulated miRNAs: miR-187, miR-205, miR-222 and miR-31. They validated the expression levels of these four miRNAs in PCa specimens (15 PCa tissues and 17 non-PCa tissues) and demonstrated that miR-31 inhibited cell proliferation, invasion and migration in PCa cell lines (Fuse *et al.* 2012).

By methylation status analysis in PCa vs non-PCa tissues, Daniunaite *et al* found that miR-31-5p promoter methylation was predictive of biochemical disease recurrence-free patient survival and increased the prognostic value of clinicopathologic factors (Daniunaite *et al.* 2017).

Bhatnagar *et al* compared miRNA expression profiles of a low aggressive PCa cell line (WPE1-NA22) and a highly malignant cell line (WPE1-NB26). The authors found that miR-205 and miR-31 were significantly downregulated in WPE1-NB26 cells, as well as in other cell lines representing advanced-stage PCa. They identified the antiapoptotic gene *E2F6* as miR-31 target (Bhatnagar *et al.* 2010).

miR-205

miR-205 binds to the 3′UTR of AR and represses its expression. miR-205 levels were significantly lower in CRPC than in hormone-*naive* patients. It was inversely correlated with AR levels in malignant epithelial cells, but there was no correlation in benign epithelium. Moreover, miR-205 expression is inversely correlated with the occurrence of metastases and shortened overall survival of the patients. miR-205 regulated genes involved in crucial pathways for the development of primary tumor, and the progression to incurable CRPC, such as MAPK/ERK, Toll-like receptor and IL-6 signaling pathways (Hagman *et al.* 2013). Boll and coworkers recently demonstrated that miR-130a, miR-203 and miR-205 jointly interfere with important oncogenic pathways in PCa and are downregulated in cancer tissues. miR-205 overexpression in LNCaP cells resemble the effect of androgen deprivation including morphological changes and impeded growth by suppressing several AR coactivators (Boll *et al.* 2013).

Gandellini and coworkers found that the restoring expression of miR-205 in PCa cells resulted in cell rearrangements consistent with a mesenchymal-to-epithelial transition, such as up-regulation of E-cadherin and reduction of cell migration and invasion. miR-205 repressed a group of pro-metastatic genes, including *N-chimerin*, *ErbB3*, *E2F1*, *E2F5*, *ZEB2* and *protein kinase CE*, which are well known to drive EMT (Gandellini *et al.* 2009).

miR-205 overexpression in PCa cells induced apoptosis and cell cycle arrest and impaired cell growth, migration and invasion by targeting the tumor suppressor genes IL24 and IL32 (Majid *et al.* 2010). Altogether these data suggest that miR-205 might possibly act as tsmiR in PCa and could interfere with progression to castration resistance.

Let-7c

Let-7 family is composed of 12 members located on eight different chromosomes. Typically, it correlates with poor prognosis in several cancer types. miR-let-7c represses AR expression in human PCa cells by targeting c-Myc, which in turn decreases cell proliferation, clonogenicity and anchorage-independent growth of human PCa cells. Downregulation of Let-7c in CRPC cells, PCa xenograft models as well as in PCa clinical specimens is inversely correlated with AR expression, whereas the expression of LIN28, a Let-7 repressor, is correlated positively with AR expression. Furthermore, Let- 7c overexpression significantly reduced tumor burden in human PCa xenografts (Nadiminty *et al.* 2012*a*,*b*).

Schubert and coworkers demonstrated that the members of let-7 family were downregulated in the majority of a large, well-characterized high-risk PCa cohort and the expression of let-7a/b/and -c was correlated to clinical outcome parameters of this group. Let-7b and let-7c were associated with clinical failure in PCa patients and functioned partially as independent prognostic marker (Schubert *et al.* 2013).

Gao and coworkers showed that AR serves as a positive regulator of *c-Myc* transcription and both are increased in human CRPC tumor progression (Gao *et al.* 2013). Also, it has been demonstrated that *c-Myc* is a let-7c target gene, but how let-7c negatively regulates *c-Myc* expression in PCa remains unclear (Koscianska *et al.* 2007).

Sun and coworkers reported that AR directly binds to the host gene of the miR-99a/let7c/125b-2 cluster and represses its transcription. Of the twelve mRNA, potential targets of the miR-99a/let7c/125b-2 cluster induced by androgens, nine mRNAs are downregulated by the miRNA cluster, indicating that downregulation of the cluster by androgen protects many of their target mRNAs from degradation and indirectly assist in gene induction (Sun *et al.* 2014*a*).

*miR-488**

miR-488* is another miRNA that directly targeted AR by binding to the AR 3′UTR. Although miR-488* expression was not detected in PCa cell lines and no evidence of changes in human PCa specimens was found, the ectopic expression of miR-488* effectively downregulated AR protein expression and inhibited the AR functional activity resulting in retardation of cellular

BOX3: Cell cycle-related terminology

Cyclins are a family of proteins that control cell cycle progression by activating cyclin-dependent kinase (CDK) enzymes. Cyclin D1 (CCND1) is expressed in the early G1 phase and binds to CDK4 and CDK6. Activation of these kinases results in the phosphorylation of retinoblastoma (RB) protein, which leads to E2Fs activation and entering into S-phase. Thus, E2F family of transcription factor proteins are key regulators of the G1/S-phase transition acting as cell cycle checkpoint control. The Cdk inhibitors (CDKI) $p21^{cip/WAF1}$ and $p27^{KIP1}$ prevents the activation of CCNE-CDK2 or CCND1-CDK4/CDK6 complexes, and thus, controls the cell cycle progression at G1. The aberrant expression of CCND1, $p21^{cip/WAF1}$, $p27^{KIP1}$ or E2Fs leads to abnormal cellular proliferation.

growth and increased apoptosis of PCa cells. Hence, miR-488* might function as a tsmiR disrupting the AR signaling pathway (Sikand *et al.* 2011).

miR-124

miR-124 is a tsmiR involved in the control of multiple steps of cancer progression, including tumor cell proliferation, invasion, angiogenesis and metastasis in many cancer types (Shih *et al.* 2015). miR-124 directly targets the AR by binding to its 3′UTR and decreased the AR protein levels and activity, which subsequently leads to an upregulation of p53 and the inhibition of growth and apoptosis of AR-positive PCa cells and xenograft tumors (Shi *et al.* 2007, 2013). miR-124 is significantly downregulated in PCa cells compared to normal cells, and it is negatively correlated with AR in human PCa tissues (Shi *et al.* 2013).

Loss of miR-124 expression in PCa is epigenetically regulated by promoter hypermethylation, and it is associated with elevated AR levels in both cell lines and clinical prostate samples. Also although miR-124 inhibited AR expression, miR-124 was positively regulated by AR, suggesting a negative feedback loop between the AR and miR-124, controlling the progression of CRPC (Shi *et al.* 2013, Chu *et al.* 2015).

miRNAs associated with cell cycle and apoptosis in PCa

miRNAs play a critical role in PCa through modulation of protein expression involved in cell cycle regulation and apoptosis, such as p53, Bcl2/Bcl-xl, E2F, cyclins, CDKs, among others (*see box 3 for related terminology,* Fig. 2).

miRNAs associated to p53

p53 is a tumor suppressor gene that stimulates apoptosis via Noxa and p53 upregulated modulator of apoptosis (PUMA) proapoptotic proteins. Loss of p53 function has a critical role in several cancer types, including PCa. Besides miR-125b is involved in AR signaling pathway and overexpressed in PCa cells and tissues, it was reported that it promoted the growth of PCa xenografts through p53-dependent apoptosis regulation. miR-125b downregulated p53, and its target proapoptotic genes PUMA, $p21^{cip/waf1}$ and Bak1 (Shi *et al.* 2011).

miRNAs associated to Bcl-2/Bcl-xl

Members of the B-cell lymphoma 2 (BCL-2) family regulate the apoptosis intrinsic pathway. BCL-2 is

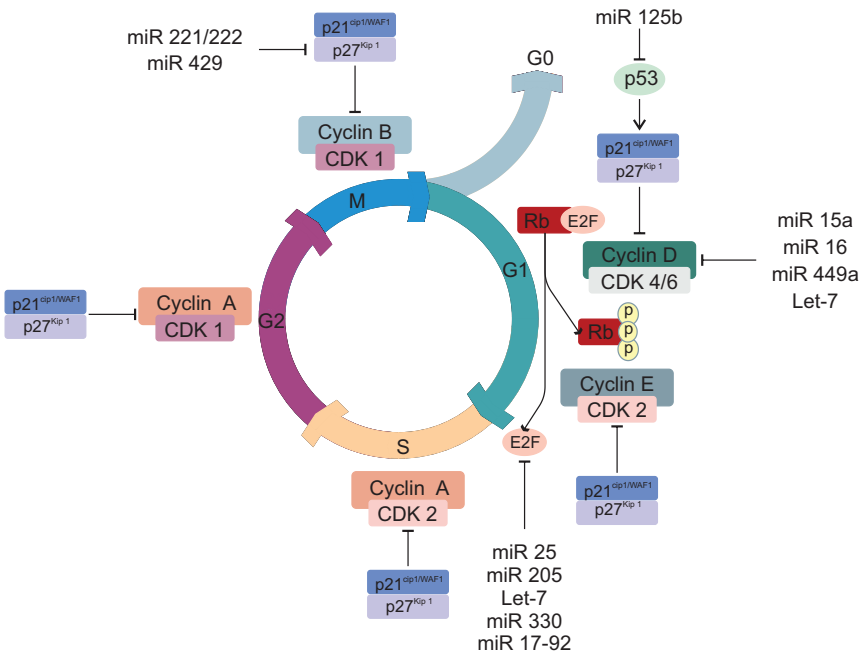

Figure 2 miRNAs associated to cell cycle progression in PCa. miRNAs that target cell cycle proteins are indicated.

directly inhibited by miR-34a, miR-34c, miR-15a and miR-204-5p in PCa, which reduces proliferation, increases apoptosis and sensitivity of multidrug-resistant PCa cells to chemotherapeutic agents. Bcl-2 exerts antiapoptotic effects by inhibiting Noxa and Puma (Kojima *et al.* 2016). Bcl-xl, an antiapoptotic member of the Bcl-2 family, is expressed in variety of cancer types, including PCa. miR-574-3p reduces Bcl-xl in PCa cells, and its expression could be upregulated by genistein, a soy isoflavone with antitumor activity (Chiyomaru *et al.* 2013).

miRNAs possess a major role in chemotherapeutic agent-induced apoptosis modulation in PCa cells. For instance, miR-205 and miR-31 were identified to target Bcl-w (a Bcl-2 family member) and E2F6 respectively (Bhatnagar *et al.* 2010). Downregulation of both miRNAs resulted in apoptosis resistance in highly malignant WPE1-NB26 cells (Bhatnagar *et al.* 2010).

Several miRNAs regulate the extrinsic cell death pathway of apoptosis. miR-24 directly downregulated Fas-associated factor 1 (FAF1), a Fas-binding proapoptotic protein, resulting in a reduction in apoptosis of the hormone-resistant DU 145 PCa cells (Qin *et al.* 2010). In contrast, the proapoptotic protein TRAIL was upregulated by miR-145 (Zaman *et al.* 2010).

miRNAs associated to cell cycle progression

The miR-15a/16 cluster has been reported to be downregulated in 80% of advanced PCa (Aqeilan *et al.* 2010). Downregulation of this cluster resulted in increased BCL-2 activity and upregulation of CCND1, which facilitates G1/S-phase transition and cell survival (Aqeilan *et al.* 2010, Bonci *et al.* 2015). miR-16 overexpression significantly inhibits the growth of prostate tumors through the downregulation of CDK1 and CDK2 (Takeshita *et al.* 2010).

miR-221/222 promotes tumor cell proliferation and represses apoptosis through $p27^{kip1}$ and caspase-10 downregulation in PCa (Galardi *et al.* 2007, Wang *et al.* 2015*a*). $p27^{kip1}$ is also a direct target of miR-429 in PCa. miR-429 expression downregulation leads to $p27^{kip1}$ overexpression, which inhibited cell proliferation through G1 cell cycle arrest (Ouyang *et al.* 2015).

It was published that E2F1-3 bound to *miR-17-92* cluster promoter and activated its transcription, which in turn, downregulated the expression of E2F1-3, thereby producing a negative feedback loop that maintains E2F1-3 expression levels at a relatively constant level (Sylvestre *et al.* 2007). miR-17-92 expression is usually upregulated in PCa cells, resulting in E2F1-3 depletion and consequently apoptosis avoidance. miR-25 and miR-205 have also been shown to downregulate E2F1-3 expression (Ambs *et al.* 2008, Gandellini *et al.* 2009).

miRNA let-7a is a tsmiR downregulated in PCa. Let-7a inhibited cell proliferation and promoted cell cycle arrest at the G1 phase in PCa cells by downregulating the expression of E2F2 and CCND2 (Dong *et al.* 2010).

miRNA-330 induced apoptosis in PCa cells through E2F1-mediated suppression of AKT phosphorylation and suppresses tumor growth (Lee *et al.* 2009).

miR-449a can cause cell cycle arrest, apoptosis and a senescent-like phenotype by hindering the HDAC1 expression (histone deacetylase 1), frequently overexpressed in cancer (Noonan *et al.* 2009). Noonan and coworkers identified miR-449a-mediated growth arrest dependent on the Rb protein. miR-449a was found to suppress phosphorylation by knockdown of CCND1 and target HDAC1 (Noonan *et al.* 2010).

miRNAs associated with invasion/adhesion/EMT/metastasis

During the last years, miRNAs have gained importance in many aspects of metastatic cancer research *(see box 4 for related terminology)*. In this review, we have divided the miRNAs associated with metastasis in two large groups, pro- and antimetastatic (Fig. 3).

Pro-metastatic miRNAs

These miRNAs are essentially oncomiRs, since they target and downregulate tumor suppressor genes, cell adhesion molecules and pro-apoptosis/anoikis regulators (Fig. 3).

Lin and coworkers demonstrated that the oncomiR miR-30d induced PCa cell proliferation, migration,

Metastasis-related terminology (BOX 4)

The spread of cancer cells from the place where they first formed to another part of the body followed by secondary tumor formation is called metastasis. Metastatic disease is responsible for the majority of cancer-related deaths and PCa is no exception. More than 80% of advanced-stage PCa manifests bone metastasis, and at this point, the disease is nearly incurable. Progression from localized to metastatic disease requires cells to detach from the primary tumor mass, invade the adjacent stroma, intravasate, survive in circulation, extravasate and colonize organs. All these steps are orchestrated by epithelial-to-mesenchymal (EMT) and mesenchymal -to-epithelial (MET) transitions. These are well-characterized events that play a highly relevant role in cancer metastasis. Cells that undergo EMT show shape changes, such as loss of apico-basal polarity and cell adhesion, the repression of E-cadherin (CDH1) expression and increased cell mobility. Accordingly, mesenchymal cell exhibits upregulation of tyrosine kinases, N-cadherin (CDH2), vimentin, fibronectin, zinc-finger domain proteins (SNAI1/SAIL, SNAI2/SLUG, ZEB2/SIP1) and matrix metalloproteinases, as well as basic helix-loop-helix domain protein Twist1 expression. There is a plethora of proteins involved in EMT-MET regulation, from transcription factors (ZEB1/2, SLUG or SNAIL) to cell adhesion molecules, like CDHs or integrins.

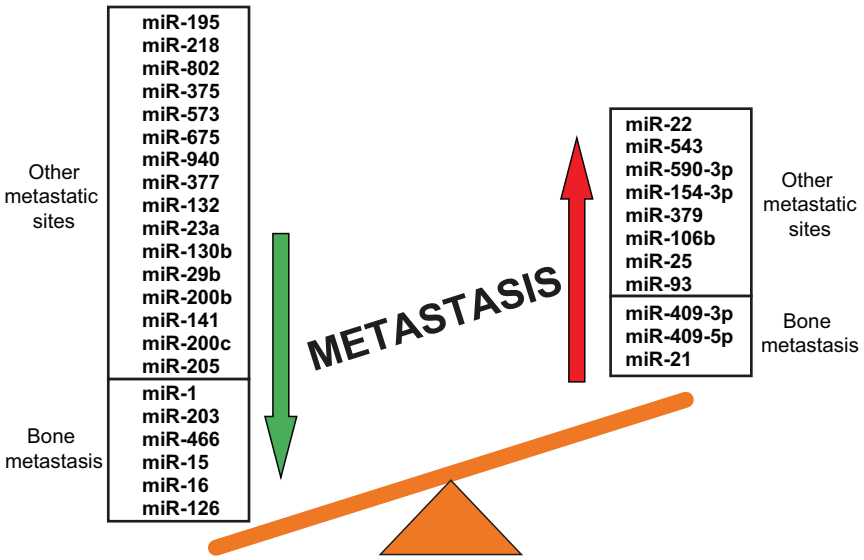

Figure 3 miRNAs associated to PCa metastasis. Antimetastatic and pro-metastatic miRNAs are indicated. Also bone and other organs metastatic miRNAs are highlighted.

invasion and capillary tube formation of endothelial cells. Moreover, it increased angiogenesis in LNCaP and *DU 145*-derived-xenografts downregulating MYPT1 expression. miR-30d was also overexpressed in PCa cells and PCa patient samples. miR-30d expression was associated with clinical features of PCa progression, such as high pre-operative PSA and Gleason score, advanced clinical and pathological stages, biochemical recurrence, reduced overall survival and metastasis. Also, they identified miR-30d/MYPT1 axis as an independent factor to predict biochemical recurrence of PCa patients (Lin *et al.* 2017). In agreement, Kobayashi and coworkers found that miR-30d increased PC3 and LNCaP cell invasion. They also showed that miR-30d was increased in PCa tissue compared to adjacent normal tissue. Furthermore, high expression of miR-30d correlates with shorter time of biochemical recurrence and together with low SOCS1 expression is associated with increased risk of early biochemical recurrence (Kobayashi *et al.* 2012).

The repression of CDH1 observed in PCa EMT is, in part, consequence of miR-22 targeting. Dhar and coworkers reported that overexpression of miR-22 in PCa cells promotes cell invasiveness and migration through CDH1 downregulation (Dhar *et al.* 2017).

Raf kinase inhibitor protein (RKIP) is a tumor and metastasis suppressor gene in PCa (Escara-Wilke *et al.* 2012), and it has been shown to negatively correlate in patient samples with miR-543 expression. RKIP downregulation by miR-543 in PCa cell lines increased invasion and migration *in vitro* and tumor proliferation and VIM/CDH1 ratio *in vivo* (Du *et al.* 2017).

miR-590-3p is overexpressed in PCa samples and its expression is associated to increased cell proliferation, invasion and accelerated growth of xenograft tumors possibly targeting the tumor suppressor inositol polyphosphate-4-phosphatase type II B (INPP4B) (Chen *et al.* 2017).

Ras suppressor protein 1 (*RSU1*), suppressor genes like stromal antigen 2 (*STAG2*) and nitrogen permease regulator-like 2 (NPRL2) are tumor suppressors whose functions range from inhibition of oncogenic ras/MAPK to inhibition of aneuploidy. They are involved in different stages of cancer progression; however, they are all downregulated by miR-409-3p/5p. Upregulation of miR-409-3p/5p increased the invasive and migratory capability of ARCaP cells, while downregulation of these miRNAs decreased PCa bone metastases in mice. Direct miRNA delivery into the murine prostate gland induced tumors with higher expression of EMT markers compared to the control (Josson *et al.* 2014). STAG2 and Mothers Against Decantaplegic homolog 7 (SMAD7) are also targeted by miR-154-3p (Gururajan *et al.* 2014). Inhibition of miR-154-3p increased survival and decreased bone metastasis. Furthermore, the same research group worked with miR-379 and demonstrated that its inhibition resulted in increased CDH1 expression and EMT reversion. These three miRNAs are part of the DLK1-DIO3 miRNA mega-cluster that share similar mRNAs targets and are deregulated in many cancer types (Gururajan *et al.* 2014).

miR-106b-25 cluster was overexpressed in PCa tumors and metastases compared to normal prostate tissue. Increased expression of this miRNA correlated with disease recurrence. Moreover, a combination of high miR-106b and low caspase 7 (CASP7, a direct target of miR-106b) expressions is an independent predictor of early recurrence. 22Rv1 cells overexpressing miR-106b-25 showed enhanced soft agar growth and high adhesion to bone matrix-related extracellular filaments (Hudson *et al.* 2013). This may be interpreted as the acquisition of anoikis resistance and a phenotype that allows for survival and proliferation in the bone microenvironment. Also, it has been shown that hypoxia increases miR-106b-25 expression, accounting in part

for the elevated expression seen on tumor and metastasis (Liang *et al.* 2014).

Antimetastatic miRNAs

These miRNAs are metastasis suppressor genes. They target and downregulate many genes involved in tumor progression and metastatic spread (Fig. 3).

miR-218 overexpression inhibited PC3 and DU 145 cell migration and invasion through downregulation of LIM and SH3 protein 1 (LASP1), a cytoskeletal scaffold protein that plays a crucial role in cytoskeleton organization and cell migration (Nishikawa *et al.* 2014).

A similar effect was reported for miR-802 ectopic expression in DU 145 cells through direct downregulation of Flotillin-2 (Flot2). Overexpression of Flot2 canceled the effect of miR-802 and restored DU 145 migratory and invasive capabilities (Wang *et al.* 2017).

Recently, several studies showed that miR-195 is a crucial metastasis suppressor miRNA. Thus, *in vitro* gain- or loss-of-function experiments revealed that miR-195 inhibited proliferation, invasion and migration of PC3, DU 145 and LNCaP cells (Cai *et al.* 2015*a*, Liu *et al.* 2015*b*, Wu *et al.* 2015). Target genes proposed to be responsible of the metastasis suppressor function of miR-195 are the cell motility regulator Fra-1, MMP-1, MMP9, c-Met (Wu *et al.* 2015), RPS6KB1 (Cai *et al.* 2015*a*), BCOX1 and FGF-2 (Liu *et al.* 2015*b*). Besides to its capability to reduce tumor growth, murine models demonstrated that miR-195 repressed angiogenesis and invasion related-genes in LNCaP- and DU 145-derived xenografts (Cai *et al.* 2015*a*) and reduced metastasis in PC3-derived xenografts (Guo *et al.* 2015). More important, clinical studies determined that miR-195 downregulation positively correlates with metastasis in PCa patients (Cai *et al.* 2015*a*) and several features of malignancy, such as high Gleason score (Cai *et al.* 2015*a*), poor biochemical recurrence-free time (Cai *et al.* 2015*a*, Guo *et al.* 2015) and overall survival (Guo *et al.* 2015). Also miR-195 was significantly diminished in metastatic tissues compared to primary PCa tissues (Guo *et al.* 2015, Liu *et al.* 2015*b*).

Overexpression of miR-375 on PC3 cells induced epithelial markers, repressed mesenchymal markers and inhibit invasion *in vitro* (Selth *et al.* 2016). More important, ZEB1 was identified as a transcriptional repressor of miR-375, and in turn, this miRNA target to Yes-associated protein 1 (YAP1), a known oncogene that drives EMT on many cancer models (Selth *et al.* 2016).

miR-573 inhibited migration, invasion and spontaneous PCa metastasis to the lung. miR-573 actions could be through direct downregulation of FGFR1. In addition, GATA3 was found to directly increase miR-573, which in turn, downregulates FGFR1 expression leading to the inhibition of invasion and migration (Wang *et al.* 2015*b*).

Zhu and coworkers reported that TGFBI, a member of the TGFβ signaling pathway that is associated to metastatic spread in many types of cancer, is a direct target of miR-675. Ectopic expression of miR-675 reduced migration and invasion of PCa cells (Zhu *et al.* 2014).

Another study reported that miR-940 inhibited cell migration and invasion, anchored-independent growth and increased CDH1 expression. Migration and invasion enhancer 1 (MIEN1) was found to be a direct target of miR-940 and a candidate that explains miR-940 function over migration and invasion (Rajendiran *et al.* 2014).

We previously discussed the miRNA mega-cluster DLK1-DIO3 for the pro-metastatic function of some of its members. However, there have been reports that identified other members of this cluster as inhibitors of tumor progression and metastasis, such is the case of miR-377. It was shown that miR-377 inhibited migration and invasion of PC3 cells, in part by direct targeting of FZD4, accompanied by epithelial markers increase (Formosa *et al.* 2014).

Re-expression of miR-132, a miRNA hypermethylated in several PCa cell lines, inhibited migration and invasion increasing anoikis in PC3 cells. Formosa and coworkers explained these effects, probably due to the direct target of miR-132, Talin 2 (TLN2), a protein that connects integrins to the actin cytoskeleton (Formosa *et al.* 2013).

miR-23a inhibited migration and invasion in a panel of PCa cell lines, and in an orthoropic PCa model. miR-23a targeted serine/threonine-protein kinase (PAK6), downregulation of which resulted in the inhibition of LIM domain kinase 1 (LIMK1) phosphorylation leading to cytoskeleton remodeling and ultimately inhibition of invasion and migration (Cai *et al.* 2015*b*).

The experimental metastasis *in vivo* model can be used to analyze anoikis resistance, homing to a particular organ and colonization of that organ. One study shown that PC3 cells transfected with miR-130b were unable to form metastasis on *nude* mice after left ventricle injection. This was attributed to miR-130b direct downregulation of MMP2. Silencing of miR-130b led to increased invasion and migration and further MMP2 knockdown antagonized the effect of miR-130b silencing (Chen *et al.* 2015*a*).

Ectopic expression of miR-29b on PC3 cells impaired metastasis formation in SCID mice, increased CDH1 expression and decrease CDH2, TWIST and SNAIL expressions (Ru *et al.* 2012).

The miR-200 family is regarded as a cornerstone of MET-induced miRNAs. Recently, more evidence has been added to support this claim. PC3 cells overexpressing miR-200b injected orthotopically in mice showed reduced angiogenesis and number of metastases due to EMT reversion, evidenced by CDH1 and specific markers of prostate epithelium induction while mesenchymal markers were downregulated (Williams *et al.* 2013).

Another member of the miR-200s family, miR-141, was revealed to be a strong epithelial phenotype inducer by upregulation of CDH1, cytokeratins and many other epithelial markers, but caused partial loss of EMT, evidenced by downregulation of merely ZEB1 and VIM (Liu *et al.* 2017).

Induction of an epithelial phenotype by ectopic expression of miR-200c and miR-205 in PCa cell lines resistant to chemotherapy resulted in EpCAM re-expression (Massoner *et al.* 2014). However, the role of this adhesion molecule in PCa is controversial. EpCAM has been associated to proliferative oncogenic pathways and poor patient overall survival (van der Gun *et al.* 2010).

It was reviewed by several authors that together with the miR-200s family, miR-205 is a major EMT repressor that favor MET (Fenderico *et al.* 2013, Sekhon *et al.* 2016). A novel role of miR-205 in base membrane deposition and cell-ECM interaction was reported (Gandellini *et al.* 2012). miR-205 expression inhibition in RWPE-1 cells was enough to reduce laminin-332 secretion and integrin β4 expression. Thus, loss of miR-205 as PCa progresses may favor metastatic spread by creating discontinuities in the basal membrane (Gandellini *et al.* 2012). Finally, another study reported that miR-205 is directly repressed by HIF-1α upon cancer-associated fibroblast (CAF) stimulation of PCa cells (Gandellini *et al.* 2014).

miRNAs associated to PCa bone metastasis

The bone is a common metastatic site for advanced PCa. Bone lesions following metastatic colonization can aberrantly modify osteoblast and osteoclast proliferation and differentiation causing bone density alterations, pain and, in some cases, render patients bedridden, which greatly lower their quality of life. Advanced PCa is incurable after bone colonization, been responsible for the majority of PCa morbidity and mortality (Weilbaecher *et al.* 2011). miRNAs were also associated to bone metastasis (Fig. 3).

Activation of the EGFR signaling pathways tends to lead to PCa bone metastases. miR-1 was shown to have metastasis suppressor functions in PCa. Chang and coworkers demonstrated a role for EGFR translocation in regulating transcription of miR-1, which directly targets expression of TWIST1. The authors observed decreased miR-1 levels that correlated with enhanced expression of activated EGFR and TWIST1 in a cohort of human PCa specimens. Moreover, EGFR activation caused miR-1 downregulation, TWIST1 induction and subsequent bone metastasis in a mice model (Chang *et al.* 2015).

Inhibition of the EGFR pathway seems to be the obvious therapeutic option. Sadly, therapies against EGFR with tyrosine kinase inhibitors (TKI) have had limited success and led to development of drug resistance. As a study demonstrated, miR-203 downregulation is associated to EGFR activation and TKI resistance. Ectopic expression of miR-203 on the highly metastatic cell line RasB1 (KRAS constitutive overexpression) produced a reduction of bone metastasis and TKI resistance. This is due to miR-203 targets EGFR ligands (AREG, EREG and TGFA) and antiapoptotic proteins (AIP5, BIRC2 and TRIAP1) (Siu *et al.* 2014).

RUNX2 is a transcription factor essential for osteogenesis that is associated to bone metastasis. It has been reported that miR-466 directly targeted RUNX2, which induced downregulation of RUNX-target genes involved in migration, invasion, EMT and metastasis. Overexpression of miR-466 in PC3 orthotopic xenografts greatly reduced the number of spontaneous bone metastases and improved mice overall survival (Colden *et al.* 2017).

A recent study showed that miR-21 upregulation and loss of miR-15/miR-16 cluster can cooperate to promote bone homing, colonization and damage via TGFβ signaling (Bonci *et al.* 2015).

Vascular cell adhesion protein 1 (VCAM-1) is a cell to vascular endothelium adhesion molecule that exhibits aberrant expression in different type of cancers, and it is involved in bone homing and protection from apoptosis. A study suggested that osteoblast-derived Wnt-1-induced secreted protein 1 (WISP-1) promotes migration and VCAM-1 expression by downregulation of miR-126 expression through α5β6 integrin, p38 and FAK signaling (Tai *et al.* 2014).

Circulating miRNAs in body fluids

Circulating miRNAs represent the miRNA population in cell-free portion of body fluids, which attracted tremendous interest in the field of biomarker discovery. Features such as high stability, access by minimally invasive methods and possibility of repeated sampling make them ideal candidates for use as biomarkers (Kosaka *et al.* 2010). Currently, the stratification of PCa patients is guided by the PSA kinetics, clinical stage and tumor grade (Gleason score). Although these parameters are still clinically useful, they have limitations in detecting cases, predicting disease outcomes and guiding clinical management decisions. The most important clinical challenge for the miRNA use is to distinguish men who have a potentially lethal form of PCa from those with an indolent disease. In addition, metastasis is another parameter that might be predicted by circulating miRNAs. Hundreds of reports showing the valuable use of circulating miRNAs are in the literature. Here, we present a table resuming the major studies that identified circulating miRNAs in body fluids from PCa patients (Table 1).

Table 1 Circulating miRNAs from body fluids with potential prognostic value.

Ref#	miRNA up or down relative to control	Sample	Number of cases	Extraction method	Detection method	Remarks
(Kachakova *et al.* 2015)	let-7e Down let-7c Down miR-30c Down miR-622 Up miR-1284 Up	Plasma	158: 54 Healthy 44 BPH 80 PCa	Trizol LS (Invitrogen)	qPCR miScript Reverse Transcription kit (Qiagen)	Cancer diagnosis AUC = let-7c, 0.775; let-7e, 0.804; miR-30c, 0.818; miR-622, 0.791; miR-285, 0.644 Discriminate between PCa and BPH AUC = let-7c, 0.784; let-7e, 0.805; miR-30c, 0.759; miR-622, 0.755; miR-1285, 0.644
(Cheng *et al.* 2013)	miR-141 Up miR-200a Up miR-200c Up miR-210 Up miR-375 Up	Serum	50: 25 Healthy 25 PCa 16: 8 Healthy 8 mCRPC	mirVana PARIS kit (Ambion) miRNeasy RNA isolation kit (Qiagen)	qPCR TaqMan miRNA Assay (Applied Biosystems) Three synthetic *C. elegans* miRNAs	Discrimination between healthy and PCa: miR-141 AUC = 0.899; miR-200a AUC = 0.699; miR-375 AUC = 0.773; miR-200c AUC = 0.721 and miR-210 AUC = 0.678 miR-210 correlated with treatment response as assessed by change in PSA in a cohort of mCRPC patients
(Alhasan *et al.* 2016)	miR-200c Up miR-605 Up miR-135a* Up miR-433 Up miR-106a Up	Commercial serum	28: 10 Healthy 9 VHR PCa 9 LR PCa	mirVana miRNA isolation kit (Ambion)	qPCR TaqMan miRNA Assay (Applied Biosystems)	Discrimination between VHR PCa and control group (healthy + LR PCa): miR-200c AUC = 1.0 miR-433 AUC = 0.98 miR-135a* AUC = 0.98 miR-605 AUC = 0.92 miR-106a AUC = 0.89
(Westermann *et al.* 2014)	miR-26a-1 NA miR-141 Up	Serum	133: 79 BPH 54 PCa	Ambion mirVana Paris Kit, Life Technologies	qPCR miScript SYBR Green PCR Kit (Qiagen)	Both microRNAs failed as diagnostic biomarker. AUC = 0.519 for miR-26a-1 and 0.49 for miR-141 However miR-141 levels were significantly increased in patients with a higher Gleason score ($P = 0.049$)
(Haldrup *et al.* 2014)	Panel 1: miR-562, miR-210, miR-501-3p, miR-375, miR-551b Panel 2: let-7a*, miR-210/ miR-562, miR-616 Panel 3: miR-375, miR-708, miR-1203, miR-200a	Serum	44: 13 BPH 11 LPC 9 N1/M1 11 CRPC	QIAzol / miRNeasy (Qiagen)	qPCR microRNA Ready-to-Use PCR (Exiqon)	Panel 1 was able to identify 84% of all PCa patients; Panel 2 was able to identify 80% of patients with disseminated PCa when compared to BPH patients; Panel 3 was able to identify 75% of disseminated PCa patients when compared to localized PCa patients. All 3 panels showed 100% specificity
(Mihelich *et al.* 2015)	miR-24 miR-106a miR-451 miR-107 miR-93 miR-103 let-7a miR-30c miR-26b miR-100 miR-130b miR-874 miR-223 miR-146a	Serum	150: 50 BPH 100 PCa	miRNeasy (Qiagen)	qPCR Exiqon SYBR green and custom Pick&Mix miRNA PCR plates	miRNA Score able to identify serum miRNAs that could pre-surgically classify patients with low risk or harboring aggressive cancer or BCR (AUC = 0.668)
(Huang *et al.* 2015)	miR-1290 Up miR-375 Up	Plasma	100: CRPC after ADT failure	miRNeasy Micro Kit (Qiagen)	qPCR TaqMan MicroRNA Assays (Life Technologies)	Incorporation of miR 1290/-375 into putative clinical prognostic factors-based models in CRPC stage significantly improved predictive performance with a time dependent AUC increase from 0.66 to 0.73 ($P = 6.57 \times 10^{-6}$)

(Continued)

Table 1 Continued.

Ref#	miRNA up or down relative to control	Sample	Number of cases	Extraction method	Detection method	Remarks
(Watahiki *et al.* 2013)	miR-141 miR-375 miR-151-3p Up miR-126 Up miR-16 Down miR-205 Down	Plasma	50: 25 LPC 25 mCRPC	Trizol LS (Invitrogen)/ miRNeasy mini kit (Qiagen)	qPCR microRNA qPCR panel (Exiqon)	A combination of miR-141, miR-151-3p and miR-16 yielded an AUC of 0.944, slightly below that for PSA (0.964). PSA test and miR-141/151-3p/16 test increased PSA sensitivity by 8%
(Wang *et al.* 2016)	miR-410-5p	Serum	327 57 Healthy 81 BPH 149 PCa 40 other urinary diseases	Trizol LS isolation kit (Thermo Fisher)	qPCR miScript SYBR Green PCR Kit (Qiagen)	Discrimination between PCa and Healthy control or other urinary diseases. AUC=0.8097
(Chen *et al.* 2016)	miR-21miR-152	Plasma	190: 74 Healthy 51 BPH 65 PCa	miRNeasy Mini kit(Qiagen)	qPCR Taqman miRNA assay kits (Applied Biosystems)	No significant differences in expression between PCa patients and healthy controls
(Li *et al.* 2016)	miR-141 Up	Serum (Exosomes)	91 40 Healthy 31 PCa 20 metastatic PCa	miRNeasy Serum/ Plasma kit (Qiagen)	qPCR PrimeScript RT Reagent kit and SYBR Premix Ex Taq kit, Takara Bio Inc	Discriminating metastatic PCa from LPC (AUC=0.8694). Better than PSA (AUC=0.7758)
(Srivastava *et al.* 2013)	miR-205 Down miR-214 Down	Urine	48: 12 Healthy 36 PCa	mirVana miRNA isolation kit (Ambion)	qPCR TaqMan miRNA Assays, Applied Biosytems	Discriminates PCa patients from normal control (AUC=0.7083 for miR-205 and AUC=0.7431 for miR-214). Additionally miR-205 and miR-214 can tougher discriminate PCa patients from healthy individuals with 89% sensitivity and 80% specificity
(Stuopelyte *et al.* 2016)	miR-148a Up miR-375 Up	Urine	300: 62 ASC 23 BPH 215 PCa (143 cohort 1, 72 cohort 2)	miRNeasy Mini Kit (Qiagen)	TaqMan Human MiRNA Assays (Applied Biosystems)	Combined analysis of miR-148a and miR-375 was highly sensitive and specific for PCa in both cohorts (AUC=0.79 and 0.84) and improved the diagnostic power of the PSA test (AUC=0.85, cohort 1), including the 4-10 ng/ml zone (AUC=0.90)
(Lewis *et al.* 2014)	miR-888 Up	EPS urine (after DRE)	56: 24 Healthy 32 PCa	miRNeasy Mini Kit (Qiagen)	qPCR TaqMan miRNA assays (Applied Biosystems)	miR-888 levels were preferentially elevated in PCa patients with high-grade disease
(Casanova-Salas *et al.* 2014)	miR-182 miR-187	Urine (after DRE)	92: 47 negative biopsy 45 Positive biopsy	mirVana miRNA Isolation Kit	qPCR TaqMan miRNA assays (Applied Biosystems)	Combination of miR-187, PCA3 and PSA for the prediction of positive biopsy or diagnosis (AUC=0.711) better than PSA only (AUC=0.615)
(Egidi *et al.* 2013)	miR-9-3p Down miR-19a-3p Up	Urine (after DRE)	79: 38 BPH 41 LPC	Total RNA Extraction Kit (Norgen Biotek Corp)	qPCR mercury LNA Universal RT microRNA PCR SYBR Green master mix (Exiqon)	Combination of KLK3 (gene that encodes PSA) mRNA and miR-19a-3p for the diagnosis of PCa or positive biopsy (AUC=0.880)

ADT, Androgen deprivation therapy; AUC, Area under receiver-operator characteristic curve; BCR, Biochemical recurrence; BPH, Benign prostate hyperplasia; DRE, Digital rectal examination; EPS, Expressed prostatic secretion; HR, High risk; IR, Intermediate risk; LPC, Localize prostate cancer; LR, Low risk; mCRPC, Metastatic castration-resistant prostate cancer; VHR, Very high risk; ASC, asymptomatic control.

PCa therapy using miRNAs

As prostate tumors develop and progress in an androgen-sensitive form, ADT is used as the first treatment either as monotherapy or in combination with other regimens. However, in most of the patients, the disease progresses to CRPC suffering metastases. The available therapeutic approach for the treatment of CRPC is conventional chemotherapy with a survival time lower than 19 months and unpleasant side effects. Here is when the development of new therapeutic approaches such as targeted therapies emerges as an urgent necessity.

Therapeutic strategies based on miRNAs modulation come out as promising approach for treatment of heterogeneous disease such as cancer (Chen *et al.* 2015*b*). Given that miRNAs constitute natural oligonucleotides, usually show less immune response and toxicity than other gene therapy approaches (Chen *et al.* 2015*b*).

miRNA therapeutic strategies could be divided into two main groups: (i) miRNA replacement therapy that involves restoration of miRNAs lost in cancer and (ii) OncomiRs inhibition (Chen *et al.* 2015*b*).

However, one of the main challenges for the use of miRNA-based therapy is the optimization of a proper miRNA delivery system to avoid blood clearance and to optimize their bioavailability in the target tissue and the capability of trapping miRNA in intracellular space (Chen *et al.* 2015*b*). Several miRNA modifications and conjugates were developed to overcome this problem. For local delivery, cholesterol-conjugate, d2′-Omethyl-modified, lentiviral vectors and polyethyleneimine (PEI) were tested (Chen *et al.* 2015*b*). In the case of systemic delivery, some of the miRNA formulations tested were seed-targeting tiny LNAs, cationic liposomes, lentiviral vectors, adeno-associated viruses, silica nanoparticles, PEGylated-PLGA, PLGA-penetratin, PEI, LNP-DP1, cationic DOTMA lipoplexes, neutral lipid and LPH-PEG-GC4 (Chen *et al.* 2015*b*).

For proper delivery of miRNA-based therapy as treatment for PCa, strategies for specific delivery to target tissue should be considered, in regards of primary tumor and metastatic sites. The design of a delivery system to target miRNAs to the bone is even more challenging compared to lung or liver (Chalanqui *et al.* 2016). In the cases where bone metastases are accessible, local administration is a suitable method. However, this approach is faulty as is not a possibility for undetectable micrometastases (Chalanqui *et al.* 2016).

Mercatelli and coworkers showed that intratumor delivery of anti-miR-221/222 significantly reduced tumor growth for as long as 25 days (Mercatelli *et al.* 2008). However, intratumor administration might not be possible for PCa treatment. Nadiminty and coworkers demonstrated that intratumor injection with let-7c-containing lentiviruses reduced tumor size in AR-positive androgen-insensitive PCa xenograft models (C4-2B and PC346C cells) (Nadiminty *et al.* 2012*b*). Another successful study investigated miR-328 effect in tumor growth after local injection of miR-328 mimics into PC3-derived xenografts. It demonstrated an inhibition of tumor growth after treatment (Liu *et al.* 2015*c*).

Several efforts were carried out to introduce miR-34a in targeted therapy for PCa, given its broad tumor suppressor activity in cancer that made possible the development of a liposome-formulated miR-34a mimic (MRX34) and is currently the first phase I clinical trial using miRNA-based therapy for treatment of liver cancer and liver metastasis patients (Bouchie 2013). Liu and coworkers tested the efficacy of miR-34a assembled with the lipid-based delivery agent NLE for systemic treatment of PCa. They demonstrated that intravenous injection with miR-34a reduced tumor growth of PC3- and LAPC9-derived xenografts (Bader 2012). Moreover, miR-34a administration prolonged survival of LAPC9 xenograft mice likely due to diminished metastases to the lung and other tissues (Bader 2012).

Zhang and coworkers overcome the concern about specific delivery of miRNAs to PCa cells by developing an efficient *in vivo* delivery using miR-145 complexed with the polyarginine peptide (R11)-labeled non-toxic SSPEI. This nanocarrier showed less toxicity in PCa and optimal transfection efficacy into PCa cells (Zhang *et al.* 2015). Systemic administration of this compound reduced tumor growth and prolonged survival time in a peritoneal PC3-derived tumor model (Zhang *et al.* 2015).

Finally, Takeshita and coworkers showed that in a model of bone metastases by intracardiac inoculation of PC3 cells, systemic delivery of synthetic miR-16 using atelocollagen-based nonviral vector caused growth retardation of bone metastases (Takeshita *et al.* 2010).

Conclusions

This review summarizes the information published about miRNAs in PCa. miRNAs are promising molecules that might be useful for patient diagnosis, management and therapy. Despite all the information about each miRNA, still we are in the biomarker discovery stage.

We recapitulated all the miRNAs involved in AR signaling pathways (Fig. 1), proliferation and cell cycle (Fig. 2), apoptosis, metastasis (Fig. 3) and circulating miRNAs in body fluids (Table 1). As expected by their ability to modulate complete molecular pathways, most of the miRNAs are implicated in two or more processes, such as miR-205 (metastasis, apoptosis and AR signaling), miR-125b (p53 and AR), miR-21 (AR signaling, bone metastasis), miR-221/222 (AR signaling, cell cycle).

Few studies present one particular miRNA unequivocally as biomarker. This is because most of the studies were carried out in a limited number of patients. In addition, the inconsistency of data has to be

overcome before diagnostic, prognostic and predictive miRNAs are translated to the patients. Although miRNAs are inherently stable molecules, optimization and standardization of miRNA detection methods are required to obtain high-quality and reproducible results.

Currently, the imperative challenge is the selection of the appropriate miRNA for clinical applications. Larger screening studies to select the proper miRNA are urgently needed. Finally, there are few studies that define the value of miRNAs in the management of PCa.

In summary, hundreds of publications come up every year about miRNAs in PCa, future challenge will be to validate them for clinical use.

Declaration of interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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