

# The role of microRNAs in prostate cancer progression

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**Abstract:** Prostate cancer (PCa) is the most common male malignancy and the second highest cause of cancer-related mortality in United States. MicroRNAs (miRNAs) are small non-coding RNAs that represent a new mechanism to regulate mRNA post-transcriptionally. It is involved in diverse physiological and pathophysiological process. Dysregulation of miRNAs has been associated with the multistep progression of PCa from prostatic intraepithelial neoplasia (PIN), localized adenocarcinoma to metastatic castration-resistance PCa (CRPC). Identification of unique miRNA could provide new biomarkers for PCa and develop into therapeutic strategies. In this review, we will summarize a broad spectrum of both tumor suppressive and oncogenic miRNAs, and their mechanisms contribute to prostate carcinogenesis.

**Keywords:** Androgen receptor (AR); castration-resistant prostate cancer (CRPC); epithelial-to-mesenchymal transition (EMT); microRNA (miRNA)



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## Introduction

Prostate cancer (PCa) is a typical hormone-dependent disease (1); however, almost all PCa patients with androgen-ablation therapy ultimately become castration-resistant prostate cancer (CRPC), which contributes to the majority of mortality in PCa. Androgen receptor (AR) is known as a key mitogen for the growth and morphogen for the development of prostate. In PCa, AR activity is critical for the disease progression and becomes hyperactivated in CRPC. Mounting evidences indicate that the development of PCa is highly associated with aberrant AR activity resulted from AR gene amplification and/or mutation, alternative splicing (2-7), the cross talk with growth factor signaling pathways (8-13), and the presence of AR co-activators and AR co-repressors (14-17). In particular, Dicer-mediated maturation of microRNAs (miRNAs) suppresses the expression level of AR co-repressors, NCoR and SMRT, leading to enhance AR transcriptional activity (18). Based on the interaction between Dicer and AR, the correlation between AR and microRNA signaling has been broadly examined to investigate the fundamental role of miRNAs in PCa progression.

miRNAs are a large family of small 20-25 nt single stranded noncoding RNAs, which can interfere with the expression of ~60% protein coding genes by post-transcriptional suppression, target mRNA degradation, or translational inhibition (19). In the past two decades, significant advances have been achieved in miRNA research. miRNAs are found to be highly conserved among the animal phylogeny. Based on their conserved sequences, miRNAs shared an identical seed region of 2-7 nucleotides are grouped into different family. Up to date, 63 miRNA families have been categorized and more than 1,000 miRNAs have been fully characterized in their expression, epigenetic regulation, biogenesis and functions. In general, miRNAs are either derived from non-coding RNA transcripts or located within the introns of protein-coding genes (20,21). Multiple miRNAs can be clustered in close proximity and encoded together as a single polycistronic primary transcript, such as miR-106a-363 (22) and miR-17-92 clusters (23). The transcriptional mechanism of microRNA is similar to that of mRNA. miRNA gene promoters are regulated by transcriptional factors that also regulate protein-coding gene expression. For example, the promoter region of miR-21 can be regulated by AR, activation protein 1 (AP-1) and

signal transducer and activator of transcription 3 (STAT-3) (24,25). Hence elevation of miR-21 in cancer is partially due to aberrant activation of AR and AP-1. Meanwhile, c-Myc is a well-known oncogene that is suggested to regulate an oncogenic miR-17-92 cluster. Overexpression of both c-Myc and miR-17-92 cluster is indicated to enhance the tumor aggressiveness (26). Moreover, the genomic organization of miRNAs reveals that about 52% of miRNA genes are localized at the fragile chromosomal regions, which are susceptible to amplification, deletion and translocation associated with cancer. A recent study indicates that let-7 miRNAs family is located in the genomic regions that are frequently deleted in multiple cancer types including PCa (27). Moreover, the miR-15a/miR-16-1 cluster is located at chromosome 13q14. The frequency of allelic loss at 13q increases from early, advanced to metastatic PCa (28). In addition, aberrant DNA hypermethylation at the CpG island is often observed in the promoter region or transcriptional start site of tumor suppressive miRNAs such as miR-200/-141, miR-205, miR-34, miR-143 and miR-145 associated with PCa (29-32). Thus epigenetic regulation is also a key regulatory mechanism for miRNA gene expression.

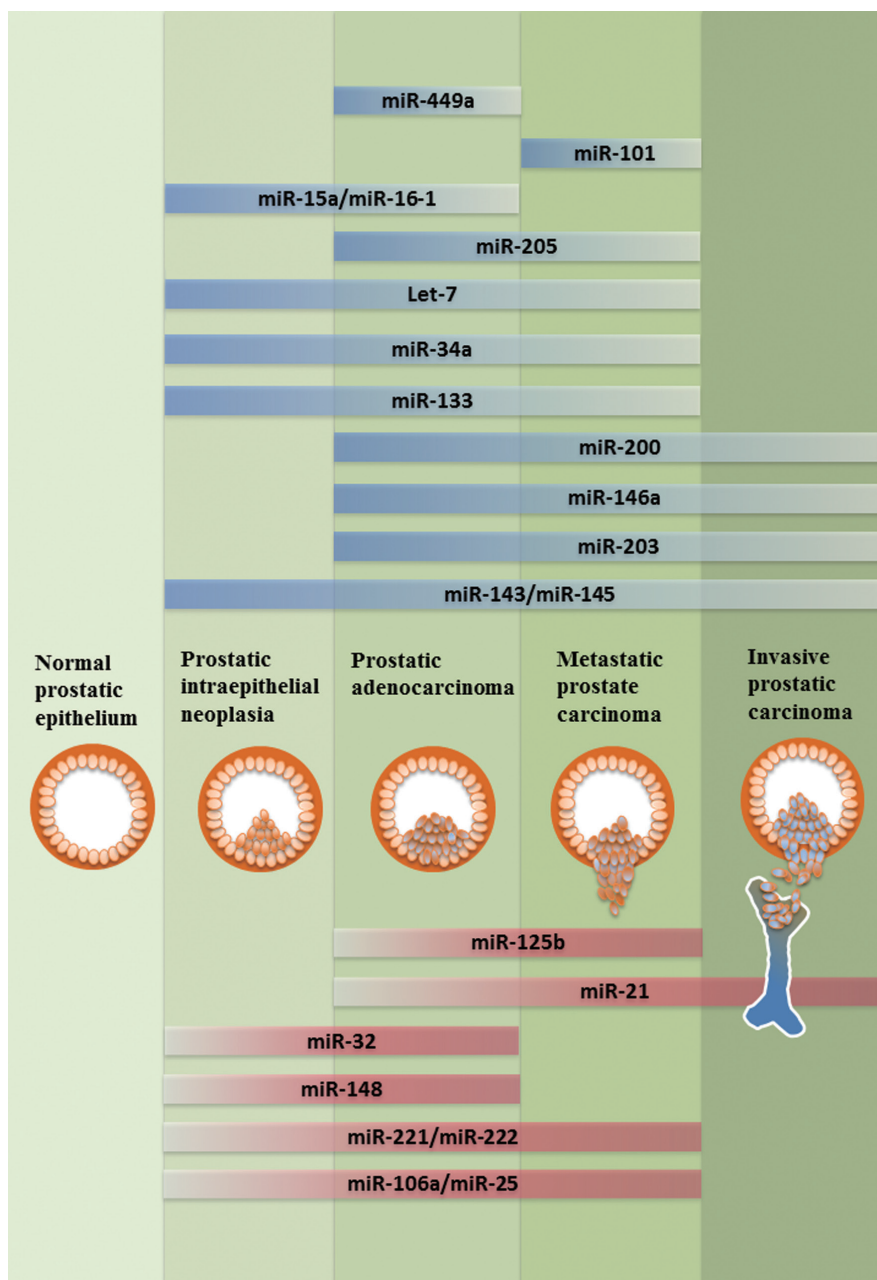
In addition to the regulation of miRNA gene expression, the biosynthetic process of miRNA maturation becomes an emerging area. The biogenesis of miRNAs composes sequential steps of RNase III-mediated endonucleolytic cleavage mechanisms (33,34). In brief, the primary transcripts of miRNAs are transcribed by RNA polymerase II and processed in the nucleus by Drosha and Pasha (DGCR8) into a 70-100 nucleotides-long precursor miRNAs (pre-miRNAs) (35). Pre-miRNAs are exported to the cytoplasm through Exportin 5 and further processed by Dicer, generating a 20-25 nt RNA duplex comprise of a matured guide strand and a complementary passenger strand (miRNA\*). The single stranded matured miRNA is then incorporated into the RNA-induced silencing complex (RISC) associated with Agonout (AGO2), and bound to the complementary sequence on the 3' untranslated region (3' UTR) of target mRNA, leading to mRNA degradation (36-38). Based on their post-transcriptional regulation on a variety of target genes, miRNA is expected to be involved in virtually every biologic process in cell. In cancer, based on their post-transcriptional repression on a variety of oncogenes or tumor suppressor genes, miRNAs are also divided into onco-miRNAs (oncomirs) and tumor suppressors miRNAs (Figure 1). Overall, the importance of microRNAs has become a key to gain more understanding of molecular mechanisms associated with prostatic

carcinogenesis. In this review, we will focus on key unique miRNAs (Tables 1,2) involved in PCa.

## Tumor suppressive miRNAs

### Let-7 family

The *let-7* gene encodes a highly conserved miRNA family of let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i and miR-98, which are significantly down-regulated in localized PCa, compared to adjacent benign tissues (39). The functionality of let-7 has been shown to target oncogenes involved in cell-cycle regulation, cell migration, proliferation, differentiation, and epithelial-to-mesenchymal transition (EMT) progression. In particular, let-7g can inhibit tumor growth via post-transcriptional suppression on RAS oncogene (44). On the other hand, loss of let-7 miRNAs is corresponded with elevated level of Enhancer of Zeste homolog 2 (Ezh2) correlated with PCa progression (45). Ectopic expression of let-7 results in the reduction of Ezh2, accompanied with diminished clonogenic ability and sphere formation in PCa cells (45). Another let-7 target gene is High-mobility group AT-hook 2 (HMGA2) (89) that is highly expressed in PCa compared to adjacent benign tissues. Indeed, HMGA2 was found de-repressed upon let-7 inhibition (43). Meanwhile, co-regulation of HMGA2 and Smad were found to orchestrate an EMT transcriptional network via targeting the promoter of SNAI1 in human hepatocarcinoma cell line (90). These results suggest a possibility that let-7 could inhibit EMT via targeting HMGA2 during PCa progression. Moreover, another study also imply that let-7 can induce cell cycle arrest and xenograft PCa tumor development by suppressing E2F2 and CCND2, which are found to be the direct target of let-7 (43). Lin-28 is a well-identified post-transcriptional suppressor of precursor let-7 maturation (91,92); An inverse correlation between lin28 and let-7 is also found in many cancer cell lines including PC3 (93). Based on these observations, lin28-mediated let-7 biogenesis has become an important mechanism to impact tumorigenesis. Conversely, let-7 can target the lin28 mRNA, suggesting that a reciprocal feedback loop exists between let-7 and lin28 (94-97). In addition, c-Myc is found to be a key factor involved in this interaction. c-Myc acts as a transcriptional activator for lin-28 gene expression and c-Myc is also found to be a target gene of let-7 family in multiple cancer types (40,98,99). Overall, the orchestrated interaction between lin28, let-7 and c-Myc is a complicated network of gene



**Figure 1** Schematic representation of tumor suppressive miRNAs (blue) and oncogenic miRNAs (red) in the prostatic tumorigenesis progression from PIN to metastatic CRPC.

regulation, which is often altered in cancer cells (100). Also, let-7c is shown to antagonize AR expression by targeting c-Myc (101). Overexpression of let-7 leads to AR suppression, accompanied with attenuated cell proliferation, clonogenicity and anchorage-independent growth in PCa cells (39,41). Overall, the let-7 miRNA family exerts tumor suppressor characteristics via targeting multiple oncogenes including RAS,

HMGA2, Ezh2, Lin28 and c-Myc. Therefore, let-7 could be a potential diagnostic biomarker and further developed into a new therapeutic strategy for PCa.

#### *miR-143 and miR-145*

Both miR-143 and miR-145 are derived from the same

**Table 1** Tumor suppressive miRNAs in PCa

miRNA	Target genes	Function	Ref.
Let-7	AR, c-MYC	Suppress cell proliferation, clonogenicity and anchorage-independent growth	(39-41)
	HMGA2	Suppress advanced tumor progression	(42)
	E2F2, CCND2	Induce cell cycle arrest <i>in vitro</i> and suppress tumor development <i>in vivo</i>	(43)
	RAS	Inhibit tumor growth	(44)
	EZH2	Inhibit clonogenicity and sphere formation	(45)
miR-143	KRAS	Suppress cell proliferation, migration <i>in vitro</i> Attenuate bone metastatic invasion <i>in vivo</i>	(46,47)
	ERK5	Arrest cell proliferation and abrogate tumor growth	(48)
	CD133, CD44, OCT4, KLF4, c-MYC	Suppress tumor sphere formation	(49)
miR-145	FSCN1	Inhibit cell proliferation, invasion, migration and arrest cell cycle	(50)
	OCT4, SOX2, KLF4	Suppress tumor stemness by inhibiting sphere formation	(51)
miR-200	ZEB1 ZEB2	Prevent PDGF-D induced EMT	(52)
	SLUG	Inhibit TGF- $\beta$ induced EMT and suppress mesenchymal differentiation	(53,54)
miR-203	CKAP2, LASP1, WASF1, BIRC5, ASAP1	Suppress cell proliferation, promote cell apoptosis and inhibit metastasis dissemination	(55)
	RUNX2	Inhibit tumor invasion destined for bone metastasis	(55,56)
	PARK7, BRCA1	Impair cell growth by promoting apoptosis and cell cycle arrest	(57)
	ZEB2, Bmi, Survivin	Suppress bone metastasis via inhibition of cell motility, invasion and EMT	(56)
miR-205	ErbB3, E2F1, E2F5, PKC $\epsilon$ ,	Counteract EMT by attenuate cell migration and invasion	(58)
	BCL2	Promote cell apoptosis and cell cycle arrest in response to DNA damage	(59)
	PSAP, ARA24, HRAS, PARK7	Induce apoptosis and cell cycle arrest	(57)
miR-34a	AR, NR4A2, EPCAM	Impair cell growth by promoting apoptosis and cell cycle arrest	(60)
	CD44	Inhibit tumor progenitor cells and suppress metastasis	(61)
	AR	Suppress tumor metastasis	(62)
	CDK6	Induce cell-cycle arrest, cell senescence and apoptosis	(32)
	c-MYC	Inhibit cell proliferation and cell invasion	(63)
miR-101	BCL2, SIRT1	Induce cell senescence and apoptosis	(64,65)
	EZH2	Attenuate tumor cell invasiveness	(66,67)
miR-133	EGFR	Reduce cell proliferation, migration and invasiveness	(68)
miR-146a	ROCK1	Suppress cell metastasis to bone marrow endothelium	(69) N.Y.
	EGFR, MMP2	Inhibit cell growth, colony formation and migration <i>in vitro</i> Reduce tumorigenicity and angiogenesis <i>in vivo</i>	(70)
miR-15	FGF-2, FGFR1	Impair the tumor-supportive capability of stromal cells	(71)
miR-16	WNT3A	Attenuate tumor expansion and invasiveness	(72)
	BCL2, CCND1	Induce growth arrest, apoptosis	(72)
miR-449	HDAC1, CCND1	Induce cell cycle arrest and loss of clonogenicity	(73,74)

miR-143/-145 cluster, which are found down-regulated in metastatic PCa samples (29). Both miR-143 and miR-145 share similar functions in tumor suppression.

First, miR-143 is found to exhibit a negative effect on PCa cell proliferation and migration by targeting ERK5 and KRAS, and inactivating subsequent epidermal growth

**Table 2** Oncogenic miRNAs in PCa

miRNA	Target genes	Function	Ref.
miR-21	RECK	Promote tumor invasiveness, support xenograft tumor growth and induce castration-resistance phenotype	(75,76)
	MARCKS, PDCD4, TPM1	Promote cell apoptosis resistance, motility, and invasion	(77)
miR-125b	p53, Puma, BAK1	Promote xenograft tumor growth	(78,79)
	p14ARF	Enhance cell proliferation	(80)
miR-221	ARHI	Enhance cell proliferation, colony formation, invasion, and cell survival	(81,82)
miR-222	p27	Promote cell cycle progression and increase clonogenicity.	(83,84)
		Enhance tumorigenicity <i>in vivo</i>	
miR-32	BTG2, PIK3IP1	Facilitate cell growth by inhibit apoptosis and enhance proliferation	(85,86)
miR-148	CAND1	Facilitate tumor growth by enhance cell proliferation	(87)
miR-106a	CASP7	Facilitate tumor progression	(88)
miR-25			

factor receptor (EGFR)-RAS-MAPK signaling pathway (46,48). On the other hand, miR-145 is shown to inhibit PCa cell proliferation by targeting Fascin homolog 1 (FSCN1) that is an actin bundling protein involved in cell motility, adhesion and cellular interactions during tumorigenesis and metastasis (50). Second, overexpression of both miRNAs in PC3 cells represses fibronectin and enhances E-cadherin expression and both can reverse EMT and further attenuate the tumor invasiveness in an *in vivo* bone metastasis model (47). Third, a recent study indicates that both miR-143 and miR-145 can suppress the stem cell characteristics in PC3 cell lines by inhibiting the stem cell markers or factors including CD133, CD44, Oct4, c-Myc and Klf4 (49). Similarly, some studies of embryonic stem cell (ESCs) indicate that miR-145 has been identified to repress pluripotency by targeting Oct4, Sox2, and Klf4 (51,102). Taken together, both miR-143 and miR-145 can suppress several cancer behaviors of PCa cells from tumor proliferation, invasion/metastasis and stemness.

### *miR-200 family*

During embryogenesis, EMT is established to determine the transition between epithelial and mesenchymal phenotypes at different developmental stages (103,104). However, during prostatic carcinogenesis, EMT has been highly implicated in PCa progression by initiating the tumor invasiveness (105-107). The consequences of EMT result in the suppression of epithelial markers by transcriptional repressors including ZEB1, ZEB2, SNAI1 and SNAI2, which are found to be the target genes of

several tumor suppressive miRNAs including the miR-200 family. The miR-200 family consists of miR-200a, miR-200b, miR-200c, miR-141 and miR-449, which are significantly down-regulated during PCa progression and identified to suppress PCa tumor metastasis particularly via inhibiting EMT. A recent study using PC3 cell line indicates that miR-200 can inhibit the platelet-derived growth factor-D (PDGF-D)-induced acquisition of EMT via targeting of both ZEB1 and ZEB2 (52). Another group studying benign prostate hyperplasia (BPH) also shows that miR-200 can reverse the TGF $\beta$ -induced EMT phenotype in BPH cell line (53). Meanwhile, in kidney epithelial cell line, all miR-200 family members have been shown to suppress TGF $\beta$ -induced EMT via targeting ZEB1, ZEB2 and SNAI2 *in vitro* (108,109); Similar result is also found in unilateral urethral obstruction (UUO) model that miR-200 can protect renal tubular epithelial cells from mesenchymal transition via suppressing ZEB1 and ZEB2 *in vivo* (110). In addition, a regulatory feedback loop has been demonstrated between SNAI2 and the miR-200 family. While miR-200 can target SNAI2 mRNA, SNAI2 protein acts as a repressor to suppress miR-200 gene expression (54). Thus, down-regulation of miR-200 may disrupt the homeostasis between SNAI2 and miR-200. Overall, loss of the miR-200 family in PCa initiates EMT process, which is critical for PCa invasiveness.

### *miR-203*

miR-203 is a well-characterized tumor suppressor and shared the similar anti-metastatic function to miR-200



family (111,112). MiR-203 has been demonstrated to induce MET in PC3 and DU145 cell lines via targeting CKAP2, LASP1, BIRC5, WASF1, ASAP1, and RUNX2, which are critical effectors involved in cell proliferation, migration, invasion and EMT (55). Meanwhile, other study also suggests that miR-203 exhibits its negative effect on multiple steps of the PCa metastatic cascade via targeting on pro-metastatic molecules including ZEB2, Bmi, survivin, and Runx2. As a result, restoration of miR-203 in PC3, VCaP, and MDA-PCa-2b cell lines attenuates the invasiveness of PCa bone metastasis *in vivo* (56). This evidence suggests miR-203 play an important role in the metastatic progression of PCa and that loss of miR-203 may further enhance the invasive characteristics of advanced PCa.

### *miR-205*

Similar to miR-203, miR-205 regulates PCa progression by targeting EMT signaling mechanisms (113). Restoration of miR-205 in PCa cells can induce MET phenotype by up-regulation of E-cadherin, along with attenuated cell invasiveness. In a more detailed study, miR-205 is suggested to attenuate cell invasion and migration via targeting ErbB3, E2F1, E2F5 and protein kinase C $\epsilon$  (PKC $\epsilon$ ) (58). Meanwhile, another study using xenograft model with tail vein injection also demonstrates that miR-205 can inhibit PCa lung metastasis *in vivo* by targeting ZEB1 and vimentin (114). In addition to EMT regulation, miR-205 can also promote PCa cell apoptosis and cell-cycle arrest by targeting the anti-apoptotic gene BCL2 (59). Moreover, miR-205 is able to inhibit tumor cell growth by inducing apoptosis and cell cycle arrest via targeting AR co-regulators (DJ-1, PSAP, ARA24) and MAPK signaling components (57). These accumulating findings indicate that both miR-203 and miR-205 may suppress metastatic progression of PCa by impairing the EMT-induced invasiveness.

### *miR-34a*

In PCa, miR-34a is identified as a tumor suppressor by inhibiting the stemness characteristics of prostate cancer stem cells (CSC) (61). The study demonstrates that miR-34a is down-regulated in the CD44 + PCa cells purified from xenograft tumors; overexpression of miR-34a can attenuate clonogenic expansion, tumor regeneration, and metastasis in CD44 + PCa cells. These results suggest that miR-34a is a negative regulator of prostate CSC and may exert its suppressive effect on PCa progression

before the onset of metastatic CRPC. Moreover, miR-34a appears to be a p53-regulated gene from a study (115) using doxorubicin and camptothecin-induced p53 activation that a significant up-regulation of both miR-34a and miR-34c is shown. However, this p53-mediated miR-34a up-regulation is abolished in both AR-knockdown LNCaP cells and AR-negative cell lines including PC3 and DU145, suggesting miR-34a expression is AR-dependent. On the other hand, AR has been identified as a direct target gene of miR-34a (62); AR activity can be repressed by de-methylation of epigenetically silenced miR-34a promoter in PCa cells. Overall, these findings imply a reciprocal transcriptional regulatory network among miR-34a, p53 and AR in PCa cells. However, whether this network is involved in PCa progression and its clinical significance require further investigation. Another target gene of miR-34a is c-Myc (63). By targeting the c-Myc expression, miR-34a is shown to suppress the signaling cascade of c-Myc-Skp2-Miz1, which leads to RhoA gene expression and subsequent attenuates cell migration and invasion. In addition to PCa stemness and metastasis, miR-34a also affect the PCa tumor growth by inducing cell-cycle arrest, cell senescence and apoptosis via targeting cell-cycle regulatory gene, such as CDK6 (32), and anti-apoptosis genes including Bcl-2 and SIRT1 (64,65). Overall, miR-34a may exert its tumor suppressor role via targeting various signaling molecules at different stages of PCa progression.

### *miR-101*

Ezh2 is a histone methyltransferase that regulates epigenetic silencing and early studies have demonstrated that overexpression of Ezh2 in PCa contributes to the enhanced aggressiveness and metastatic potential of PCa cells (116-119). A study shows an inverse correlation between miR-101 and Ezh2 expression has been observed in human PCa. Meanwhile, miR-101 is found to suppress the expression and function of Ezh2 in PCa cell lines (66) and the overexpression of miR-101 in PC3, DU145 and LNCaP cells also results in the suppression of Ezh2, along with attenuated cell invasion and migration of these PCa cell lines *in vitro* (67,117). These findings clearly indicate the role of miR-101 in the epigenetic regulation critical for PCa progression via targeting Ezh2 expression.

### *miR-133 and miR-146a*

Epidermal growth factor (EGF) and EGFR are known

to be key tumor promoter for PCa (120). A recent study indicates that, under hypoxia condition, EGFR can interrupt the biogenesis of mHESM (miRNAs regulated by hypoxia-dependent EGFR-suppressed maturation) resulted in reducing Dicer binding and abolishing miRNA maturation via targeting AGO2 phosphorylation (121). Implying that EGFR may certainly contribute to the modification of miRNA processing. On the other hand, both miR-133 and miR-146a have been shown to suppress PCa tumor progression via targeting EGFR. Down-regulation of miR-133 has been observed in PC3 and DU-145 cell lines. Ectopic expression of miR-133 can reduce cell proliferation, migration and invasiveness by targeting EGFR (68). Similar to miR-133, expression level of miR-146a is also significantly down-regulated in PCa (122,123). Overexpression of miR-146a has been demonstrated to suppress PCa cell growth, colony formation and migration *in vivo* via targeting EGFR. Additional studies also reveal that miR-146a can inhibit angiogenesis and bone metastasis *in vivo* by suppressing both matrix metalloproteinase-2 (MMP2) and Rho-associated, coiled-coil containing protein kinase 1 (ROCK1) expression (69,70). These findings indicate that loss of both miR-133 and miR-146a in PCa may attribute to enhancement of EGFR signaling, leading to aggressive PCa progression.

#### **miR-15a and miR-16-1**

miR-15a and miR-16-1 are in the same cluster; the expression of miR-15a/miR-16-1 is often down-regulated in PCa due to chromosomal deletion at 13q14, which is highly correlated with the progression of PCa (124). A study has demonstrated that miR-15a/miR-16-1 level is inversely correlated with B-cell lymphoma 2 (BCL2), cyclin-D1 (CCND1) and wingless-type 3A (WNT3A) in advanced PCa (72). The same group also found that both CCND1 and WNT3A are putative target genes of miR-15a/miR-16-1. As a result, restoration of miR-15a and miR-16 is shown to arrest cell growth and induce apoptosis and knockdown of miR-15a/miR-16-1 can promote survival, proliferation and invasiveness of PCa xenograft tumor *in vivo*. On the other hand, miR-15a and miR-16-1 also exert tumor suppressive effects by interfering the stromal support in the tumor microenvironment since interaction between tumor cells and the surrounding cellular components is critical for tumor development (125,126). It has been indicated that down-regulation of miR-15a and miR-16 in cancer-associated fibroblasts results in tumor expansion and invasion (72), which is supported by the

reconstitution of both miRNAs in fibroblast can interrupt the stromal support by targeting FGF-2 and FGFR1 (71). All these data indicate that the tumor suppressor role of miR-15a/ miR-16-1 is to suppress cancer cells or interrupt their communication with the microenvironment.

#### **miR-449a**

miR-449a is inversely correlated with the expression of histone deacetylase 1 (HDAC1)-an enzyme critical for epigenetic regulation. It has been indicated that increased expression of miR-449a in PCa cell lines leads to both cell cycle arrest and loss of clonogenicity by targeting HDAC1 (73). In addition, miR-449 can initiate cell cycle arrest and induce cell senescence by targeting cyclinD1 (74).

### **Oncogenic miRNAs**

#### **miR-21**

The recurrence of CRPC is often associated with hyperactivation of AR. Recent studies have suggested that several oncogenic miRNAs is correlated with aberrant AR activation. In particular, miR-21 is an AR-regulated miRNA and its expression level is consistently elevated from androgen-dependent PCa to CRPC (127). Overexpression of miR-21 can support xenograft tumor growth and induce castration-resistant phenotype (75). In addition to androgen response element (ARE), other cis-elements such as AP-1 and STAT-3 are also found in the promoter region of miR-21 (24,25). AP-1 activity is closely associated with CRPC recurrence (128) and STAT-3 is also shown to be involved in PCa metastasis (129). Overall, the highly elevation of miR-21 may be attributed to the aberrant expression of transcriptional activators such as AR and AP-1. The subsequent effect of miR-21 overexpression in turn contributes to the development of prostate tumorigenesis. Several target genes of miR-21 have been shown to suppress tumor progression by inhibiting invasiveness, promoting apoptosis and cell cycle arrest. For example, myristoylated alanine-rich protein kinase c substrate (MARCKS) is a direct target of miR-21, which plays key role in cell motility, membrane trafficking and mitogenesis. Thus, miR-21 promotes the apoptosis resistance, cell motility and invasiveness of PC3 and DU-145 cells partly via targeting MARCKS (77). Meanwhile, a recent study demonstrates that reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is another novel target of miR-21; An

inverse correlation between RECK and miR-21 has been shown from different stages of PCa (76).

### ***miR-125b***

Similar to miR-21, miR-125b is also an AR-induced miRNA. The induction of miR-125b in LNCaP cells inhibits apoptosis and enhances cell proliferation. Mechanistically, miR-125b promotes PCa xenograft tumor growth by targeting major pro-apoptotic genes including p53, Puma and BAK1 (78). Consistent with this observation, miR-125b is shown to modulate the p53 network by interrupting Mdm2 degradation via targeting p14<sup>ARF</sup>, which mediates the Mdm2 sequestration (80). Overall, miR-125b can target the p53-p21 and Puma signaling network, leading to enhanced cell proliferation in both LNCaP and CWR22Rv1 PCa cell lines through p53-dependent and p53-independent manner, respectively.

### ***miR-221 and miR-222***

Both miR-221 and miR-222 belong to the same miRNA cluster. Overexpression of miR-221/miR-222 has been often found in PCa. The aberrant elevation of miR-221/miR-222 is highly correlated with metastatic CRPC phenotypes. Moreover, an inverse correlation between miR-221/miR-222 expression and p27<sup>Kip1</sup> level has been observed in primary PCa. Several studies demonstrated that miR-221/miR-222 can up-regulate S-phase kinase associated protein 2 (Skp2), cyclin A and cyclin D1 via targeting p27<sup>Kip1</sup> suppression, leading to cell cycle progression at G1-to -S phase, increased clonogenicity *in vitro* and enhanced tumorigenicity *in vivo* (83,84,130). Meanwhile, Ras homolog member I (ARHI), a tumor suppressor identified in ovarian cancer (131), is also identified as the target gene of miR-221/miR-222. Overexpression of ARHI in PC3 cells results in the inhibition of cell proliferation, colony formation, cell invasion and survival (81,82,132), suggesting decreased ARHI mRNA could be an additional mechanism for miR-221/miR-222 contributing to the accelerated tumor growth in PCa. Thus, these data conclude the functional role of miR-221/miR-222 as PCa promoter by targeting tumor suppressor genes such as p27<sup>Kip1</sup> and ARHI.

### ***miR-32***

miR-32 is highly expressed in CRPC specimens compared

to BPH specimens (85). A study demonstrated that miR-32 exerts oncogenic characteristics by targeting on both B-cell translocation gene 2 (BTG-2) and phosphoinositide-3-kinase interacting protein 1 (PIK3IP1), which regulates the inhibition of PI3K, a well-known regulator of cell proliferation, migration and survival (85). An inverse correlation between miR-32 and BTG-2 has been found in the CRPC specimens (85). In addition, numerous studies have identified BTG-2 as a critical target gene of AR-regulated miRNAs including miR-32, miR-148 and miR-21 (85,86). Loss of BTG-2 was implicated in the progression of PCa accompanied by the appearance of EMT markers (133). Meanwhile, a study using LNCaP cells demonstrated that miR-32 facilitates cell growth by inhibiting cell apoptosis and enhancing cell proliferation, respectively. Overall, miR-32 exerts its oncogenic characteristics by targeting on tumor suppressors critical for cell proliferation, survival and migration.

### ***miR-148a***

Similar to miR-32, miR-148 is elevated in advanced PCa compared to primary tumor (134). However, in contrast to the distinctive oncogenic role of miR-32, the role of miR-148a in PCa progression is more controversial. For example, miR-148a was identified as an androgen-responsive miRNA and facilitates LNCaP cell proliferation via targeting cullin-associated and neddylation-dissociated 1 (CAND1) (87). In contrast, miR-148a is shown to be down-regulated in both DU-145 and PC3 cell lines. Furthermore, overexpression of miR-148a in PC3 cells attenuates cell growth, migration, invasion, and enhances the drug sensitivity to Paclitaxel. This phenomenon is paralleled with the effect in MSK1-knockdown PC3 cells. In particular, MSK1 has been identified as the target gene of miR-148a, suggesting miR-148a attenuates the drug-resistance of CRPC cells via targeting MSK1 (135). Apparently, miR-148a represents a unique miRNA with dual function in PCa. Although the exact mechanism remains undetermined, AR may be an important factor involved in miR-148a function or the presence of different target genes of miRNA-148a in PCa cells derived from different origins.

### ***miR-106b/miR-25***

A study (136) using computational approach to identify PTEN-target miRNAs has identified miR-106b/miR-25 as a candidate that is concomitantly overexpressed with



its host gene, minichromosome maintenance protein 7 (MCM7), which result in enhanced cell transformation and initiated prostatic intraepithelial neoplasia (PIN) progression of PCa. This study has a significant finding to show a cooperative expression of an oncomir cluster with its oncogenic host gene, which could simultaneously generate “two-hits” effect on the malignant transformation of normal cells. In addition, a study using LNCaP cell line has demonstrated that miR-106b/miR-25 cluster is associated with PCa progression by targeting caspase 7, apoptosis-related cysteine peptidase (CASP7) mRNA, which is down-regulated in both primary PCa and metastatic lesions (134). Overall, miR-106b/miR-25 cluster is an oncomir cluster and it is often found altered in its expression level between PIN, primary, and metastatic PCa (86,137).

## Conclusions

miRNA represents a new mechanism of regulating gene expression at either post-transcriptional or translational levels. Aberrant alteration of miRNAs has been clearly demonstrated in PCa. However, knowing complex regulatory mechanism and relationship of miRNAs and their multiple target genes have further complicated their functionality during carcinogenesis. Therefore, carefully dissecting the mechanisms and functional role of each miRNA in heterogeneous PCa cells will certainly generate new information that could be applied as biomarker(s) and developed into novel therapeutic strategies.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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