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MicroRNAs in Prostate Cancer: Small RNAs with Big Roles

Ping Mu[1*], **Su Deng**[2,3] **and Xiaozhou Fan**[4]

[1]*Memorial Sloan Kettering Cancer Center, Human Oncology and Pathogenesis Program,1275 York Avenue, New York, NY, 10065, USA*

[2]*Memorial Sloan Kettering Cancer Center, Developmental Biology Program, 1275 York Avenue, New York, NY, 10065, USA*

[3]*Weill Cornell Graduate School of Medical Sciences of Cornell University, Physiology, Biophysics and System Biology Program, 1300 York Avenue, New York, NY, 10065, USA*

[4]*The University of Texas M. D. Anderson Cancer Center, Department of Immunology, Houston, TX 77030*

[*]**Corresponding Author:** Mu P, Memorial Sloan Kettering Cancer Center, Human Oncology and Pathogenesis Program, 1275 York Avenue, New York, NY, 10065, USA, Tel: 646-888-2139; E-mail: mup@mskcc.org

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Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression either by mediating translational repression or reducing the stability of a target mRNA. Deregulated expression of miRNAs is a common feature of human cancers and a growing body of evidence demonstrates the role of miRNAs as either oncogenes or tumor suppressors. Many miRNAs have been reported to be associated with the pathogenesis of primary prostate cancer (PCa) and the development of castration resistant prostate cancer (CRPC). PCa is the most common cancer and second leading cause of cancer death in American men. Although patients with primary PCa can be treated with chemotherapy and hormone therapy, many of them will develop resistance to conventional therapies and progress to a more sever condition called CRPC, which remains one of the most difficult cancers to treat. Since emerging evidence suggests miRNAs' significant roles in the tumorigenesis of primary PCa and CRPC, the potential of using miRNAs as drug targets and biomarkers for primary PCa and CRPC has been gaining more attention. The aim of this review is to summarize recent studies on the involvements and mechanisms of the actions of several miRNAs in the development and progression of primary PCa and CRPC. Additionally, the potentail applications of using miRNAs as biomarkers and drug targets are briefly discussed.

Keywords: MicroRNA; Prostate cancer; Castration resistance; Oncogene; Tumor suppressor; Biomarker

Introduction

MicroRNA and mechanism of action

MicroRNAs (miRNAs) are small non-coding RNAs which are approximately 19-23 nucleotides in length and regulate gene expression at the post-transcriptional level [1]. More than two thousand miRNAs have been identified in human genomes since their initial identification in the nematode *Caenorhabditis elegans* [2,3]. miRNAs have been shown to be important regulators of gene expression [4], impacting cell proliferation, apoptosis, differentiation and many other physiological functions. The biogenesis of miRNA starts from its transcription, by RNA Polymerase II, of a longer precursor called pri-miRNA [5]. This pri-miRNA is then cleaved by the Drosha and DGCR8 complex in the nucleus to generate a 70-nucleotide stem-and-loop precursor called pre-miRNA [6-8]. After being exported to the cytoplasm by Exportin-5 [9], pre-miRNA is then cleaved by the RNAse Dicer to yield a 20 base pair miRNA/miRNA* duplex. This duplex is further loaded onto Argonaute (Ago) proteins to form a RNA-induced silencing complex (RISC) [10].

miRNAs suppress their target genes either by mediating translational repression or reducing the stability of a target mRNA via induction of its deadenylation and degradation [11-16]. miRNAs bind to the 3'-untranslated region (3' UTR) of target mRNAs through partial sequence complementarity [2,3,17] and the critical determinants of binding specificity are the nucleotides from position 2 to position 7, comprising the miRNA "seed sequence" [4,14,18,19]. Thus, miRNAs that have the same "seed sequence" are predicted to have highly overlapping sets of mRNA targets and thus similar biological functions [20].

MicroRNA and carcinogenesis

Deregulated expression of miRNAs is a common feature of human cancers and a growing body of evidence demonstrated the role of miRNAs as either oncogenes or tumor suppressors. miRNAs modulate tumorigenesis through multiple mechanisms. For example, miRNAs have been shown to function as oncogenes by inhibiting key molecules in anti-oncogenic pathways. Deletion of a miRNA that targets and represses oncogenes can lead to the activation of oncogenic pathways [21]. Furthermore, recent reports show that chromosomal translocations can sometimes cause truncation of the 3' UTRs of oncogenes, leading to loss of miRNA-mediated repression and promotion of oncogenic transformation [22]. Several miRNAs have been frequently reported to act as oncogenic miRNAs in various human cancers, especially in human prostate cancer (PCa), including *miR-21*, the *miR-125* family and the *miR-221~222* cluster. Their functions in the carcinogenesis of various human cancers, especially prostate cancer will be discussed in more detail in the next sections.

On the other hand, several miRNAs have been implicated as tumor suppressors based on their frequent deletion or reduced expression in multiple human cancers [23]. Several tumor suppressor miRNAs suppress oncogenic pathways by repressing the expression of critical oncogenes. For example, members of the *let-7* family are reported to

repress the *RAS* oncogene in lung cancer [24], decrease the mRNA of *MYC*, and lead to reduced proliferation in Burkitt lymphoma cells [25]. Several miRNAs have been indicated to act as tumor suppressors in PCa, such as the *miR-15a~miR-16-1* cluster, the *miR-34* family and *miR-200* family. Their roles in modulating carcinogenesis of prostate cancer and other human cancers will be discussed more thoroughly later.

Prostate cancer and castration resistance

Prostate cancer is the most common cancer among American men, with 220,800 estimated new cases in 2015. Typically, patients with localized prostate cancer are treated with radical prostatectomy or primary radiation therapy and around 70% of those patients are cured. However, approximately 30% of patients will relapse and require hormone therapy in the form of androgen deprivation, also called "chemical castration". Patients receiving this conventional hormone therapy will typically be treated with agents such as leuprolide, which lowers serum testosterone and is often combined with antiandrogens such as bicalutamide. Although these conventional agents are initially effective, most patients will eventually develop resistance to hormone therapy. This relapsed disease is called castration-resistant prostate cancer (CRPC) and is responsible for almost all prostate cancer deaths in the US (~30,000 annually). This makes prostate cancer the second leading cause of cancer death amongst American men. Although CRPC can be treated with taxane-based chemotherapy, this kind of treatment provides only a moderate survival benefit of 1-2 months [26,27]. Since none of the conventional therapies can provide significant clinical benefit, CRPC remains one of the most difficult cancers to treat.

miRNA	Expression[1]	Role in PCa	Mediated Function	Validated Target Genes	Castration Resistance[2]	Biomarker Application	References
miR-21	Upregulated	Oncogene	Viability, invasion, apoptosis, migration	*MARCKS, TPM1, PDCD4*	Yes	Diagnostic, prognostic, predictive	[38,53,55,57,115]
miR-221~222	Upregulated	Oncogene	Proliferation	*CDKN1B, HECTD2, IRF2, RAB1A, SOCS3*	Yes	Diagnostic, prognostic	[55,65-68]
miR-125	Upregulated	Oncogene	Apoptosis, proliferation	*BAK1, EIF4EBP1, TP53, PUMA, ARF*	Yes		[69,72-77]
miR-32	Upregulated	Oncogene	Apoptosis	*BTG2*	Yes		[40]
miR-148a	Upregulated	Oncogene	Proliferation	*PIK3IP1*			[40]
miR-616	Upregulated	Oncogene	Proliferation	*TFPI-2*	Yes		[123]
miR-34	Downregulated	Tumor suppressor	Proliferation, apoptosis, metastasis, EMT[3]	*Cyclin-E2, CDK6, E2F5, E2F1, E2F3, BCL2, CD44*		Diagnostic	[83,85-89,95]
miR-15~16	Downregulated	Tumor suppressor	Proliferation, invasion, metastasis	*CDK1, CDK2, BCL2, CCND1, WNT3A*		Diagnostic	[46,93-95,115]
miR-141/200	Downregulated	Tumor suppressor	EMT[3]	*ZEB1, ZEB2, SNAIL2*		Diagnostic, prognostic	[53,97-99,102-106]
Let-7	Downregulated	Tumor suppressor	Proliferation, clonogenicity	*MYC*	Yes	Diagnostic, prognostic	[109,116-118]
miR-101	Downregulated	Tumor suppressor	Proliferation, invasion, metastasis	*EZH2*			[119]
miR-126	Downregulated	Tumor suppressor	Viability, invasion, migration	*Prostein*			[120]
miR-146a	Downregulated	Tumor suppressor	Proliferation, invasion, metastasis	*ROCK1*	Yes		[121]
miR-330	Downregulated	Tumor suppressor	Proliferation, apoptosis	*E2F1*			[122]

[1]PCa cohort vs Benign cohort

[2]Involved in the development of Castration Resistant Prostate Cancer (CRPC)

[3]EMT: epithelial mesenchymal transition

Table 1: Summary of studies on deregulated miRNAs in PCa.

A notable characteristic of CRPC is its tremendous amount of genetic heterogeneity [28], which dramatically increases the difficulty in developing effective therapies. One of the primary mechanisms of resistance to antiandrogen therapy is elevated androgen receptor (AR)

expression, which is not only required but is also sufficient to restore AR activity and CRPC growth [29]. Based on this finding, several second-generation antiandrogens have been developed, such as enzalutamide and ARN-509 [30,31]. Compared to first-generation antiandrogens, these second-generation antiandrogens have much higher binding affinities to AR and also have significant clinical activities in patients with chemotherapy-refractory CRPC [32,33]. However, despite the exciting clinical success of second-generation antiandrogens, many patients showed only a modest response and some of the initial responders quickly developed resistance again [32,33]. The vast genetic heterogeneity of CRPC and highly variable responses to antiandrogens limit the clinical benefit of hormone therapy, underscoring the importance of developing novel biomarkers and drug targets. Because of the critical roles of miRNAs in regulating PCa carcinogenesis and conferring castration resistance, miRNAs may serve as good candidates for both drug targets and biomarkers in primary PCa and CRPC.

Roles of MicroRNAs in Prostate Cancer and Castration Resistance

Deregulation of miRNAs in PCa

The deregulation of miRNAs has been frequently reported in primary PCa and also CRPC samples [34-36]. Porkka and colleagues have profiled the miRNA expression in 6 PCa cell lines, 9 PCa xenografts samples, 4 benign prostatic hyperplasia (BPH), and 9 PCa tumor samples. In this study they demonstrated 51 individual miRNA have been differentially expressed and the miRNAs expression profile can be used to further cluster the tumor samples into hormone naïve versus hormone refractory PCa [35]. This data not only demonstrates the important role of miRNAs in mediating PCa carcinogenesis, but also suggests that miRNAs can be useful as novel therapeutic targets or biomarkers for diagnosis and classification of PCa. Recently, more than 50 miRNAs have been reported to be involved in PCa carcinogenesis and the functions of many have been identified as well, including *miR-21, miR-32, miR148a, miR-221~222, miR-616, miR-15a~16-1, miR-200, miR-101, miR-126, miR-330, miR-34* family and *miR-125* family. The function of these miRNAs in PCa is summarized in Table 1.

miRNAs and androgen receptor (AR) signaling

As discussed above, the signaling pathway related to androgen receptor (AR) plays crucial role not only in the carcinogenesis of primary PCa, but also in the development of CRPC. A deregulated level of AR expression is one of the primary mechanisms of resistance to antiandrogen therapy, which restores the AR signaling and promotes the growth of CRPC [29]. Another known mechanism of resistance to hormone therapy is mutations in AR. For example, F876L, an identified mutation in AR, can convert antiandrogen enzalutamide into an AR agonist and lead to resistance to therapy [37]. Because of the importance of AR signaling, the interactions of AR and several miRNAs play crucial roles in the carcinogenesis of PCa and development of CRPC.

A good example is *miR-21*, whose promoter has an androgen-response element (ARE). Androgen-induced AR can directly bind to the promoter of *miR-21* and cause the overexpression of *miR-21*, consequently leading to castration-resistant growth of the PCa cell line [38]. Another microRNA with an ARE in its promoter is *miR-125b-1*, which can be directly regulated by AR binding to its promoter region and recruiting chromatin remodelers *EZH2* or *JMJD3*. Repression of *miR-125b-1* by androgen actually protects some target genes, such as the prostate cancer growth factor *IGF1R*, from degradation, which rescues the androgen-induced cell proliferation in LNCaP cells [39]. *miR-141*, one member of the *miR-200* family, is also regulated by AR in both PCa cell lines and xenografts. Up-regulation of AR leads to ectopic overexpression of *miR-141* and enhanced LNCaP cell growth [40]. Two other important targets of *miR-141* are *PTEN* and *CDKN1B* (p27), which are also important mediators of AR-signaling-regulated proliferation and apoptosis [41,42]. The expression level of miR-34a is negatively correlated with AR level and reduced miR-34a expression confers paclitaxel resistance via derepression of its target BCL2 [43]. Rokhlin et al. reported that cooperation between *miR-34a* and *miR-34c* plays a crucial role in AR-dependent p53-mediated apoptosis in PCa cell lines [44]. *miR-221* and *miR-222* are two other miRNAs being down-regulated by AR signaling in PCa. Stable overexpression of *miR-221* in LNCaP cells reduces the transcription of a group of androgen-responsive genes and confers androgen independent growth [45]. Down-regulation of *miR-15a* and *miR-16-1* is also very common in advanced PCa. Restoration of *miR-15a* and *miR-16-1* in the 13q14-defective LNCaP androgen-dependent cell line leads to growth arrest and apoptosis, possibly due to targeting *BCL2* and *CCND1* [46].

Since *miR-21*, *miR-221~222*, *miR-15a~16-1*, *miR-200* family (*miR-141*), *miR-34* family and *miR-125* family are among the most thoroughly studied miRNAs in PCa and their functions are closely regulated by AR signaling, we will discuss their function in PCa in more detail in next section. Moreover, their relationships with AR signaling and related cellular function are summarized in Figure 1. Limited by the scope of this manuscript, other PCa related miRNAs will only be briefly summarized in Table 1 as well.

Oncogenic miRNAs in PCa

miR-21: Among the oncogenic miRNAs in PCa, *miR-21* is one of the most thoroughly studied. The oncogenic role of *miR-21* is strongly suggested by the observation that it is overexpressed in many different human cancers, including diffuse large B cell lymphoma, acute myeloid leukaemia, chronic lymphocytic leukaemia and Hodgkin's lymphoma [47-50]. Overexpression of *miR-21* in a transgenic mouse model leads to a pre-B malignant lymphoid-like phenotype and the tumors regress completely when *miR-21* is inactivated [51]. In addition, overexpression of *miR-21* in another transgenic mouse model (*CAG-miR-21; K- ras*^{LA2}) leads to enhanced tumorigenesis of non-small-cell lung cancer (NSCLC), by repressing multiple negative regulators (*Spry1, Spry2, Btg2, Pdcd4*) of the *Ras/MEK/ERK* pathway. In contrast, genetic deletion of *miR-21* results in impaired Ras-driven tumor development [52].

In prostate cancer, overexpression of *miR-21* has been demonstrated in androgen-independent, more malignant, and metastatic PCa cell lines, including PC-3 and DU-145. Interestingly, its expression is barely detectable in the androgen-dependent LNCaP cell line. In this study, Li et al. also showed that suppression of *miR-21* decreases the motility and invasion of PCa cell lines, driving them to undergo apoptosis through modulating the target genes such as *MARCKS*, *TPM1* and *PDCD4* [53]. Ribas et al. reported that AR directly binds to the *miR-21* promoter *miPPR-21* and leads to *miR-21* overexpression, thus elevating the level of *miR-21* that is required for androgen- dependent PCa growth. More interestingly, overexpression of *miR-21* alone is sufficient to confer castration resistance to

androgen-dependent PCa tumors [38]. *miR-21's* involvement in mediating androgen-independent growth of PCa was shown in yet another study, in which reduced expression of *miR-21* in an androgen-independent PC-3M-MM2 cell line lead to reduced cell viability, migration and invasiveness. The expression and function of *miR-21* in this cell line is dependent on Akt signaling [54].

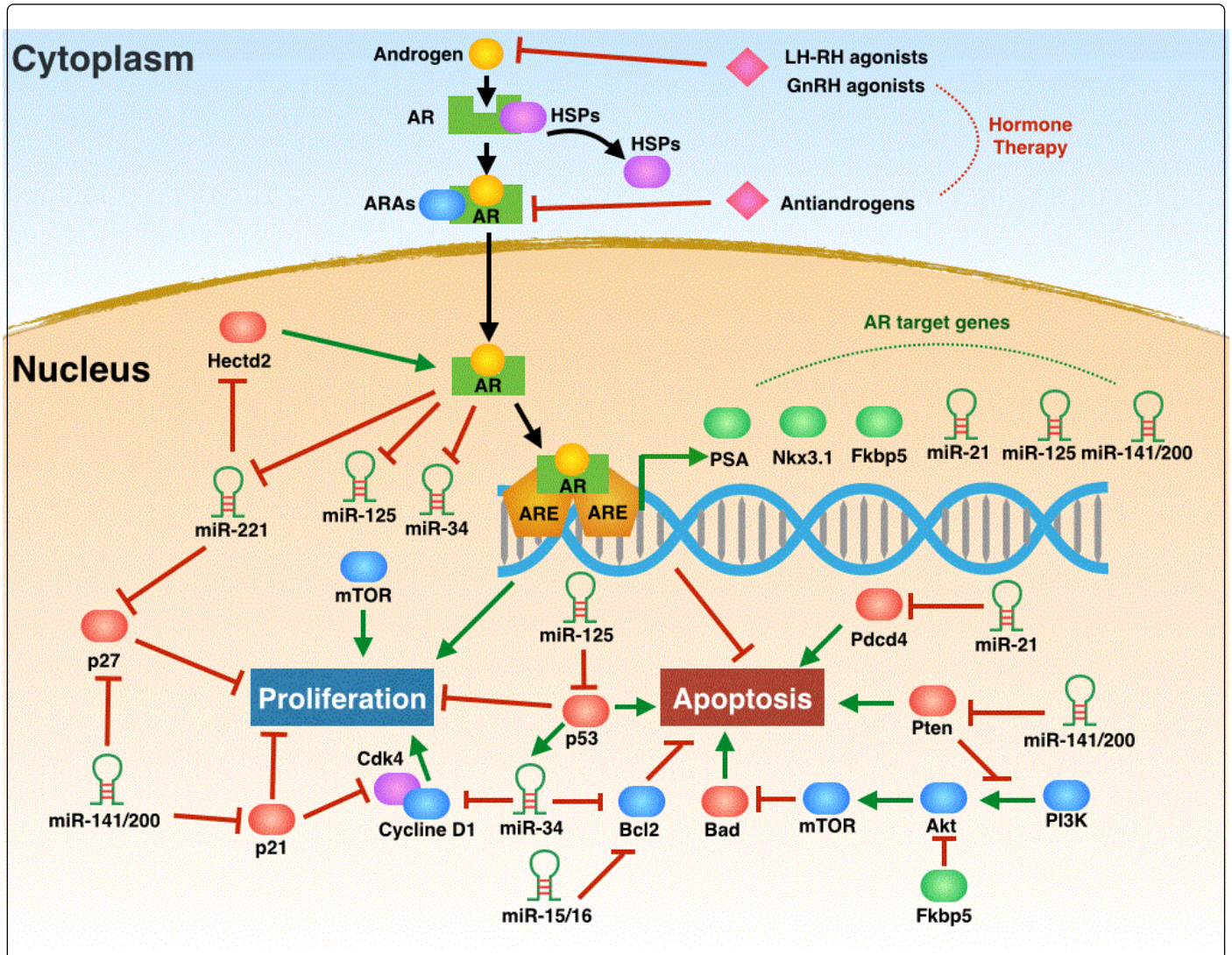

Figure 1: Schematic representation of selected miRNA families and their roles in regulating common signalling pathways in PCa carcinogenesis. AR: Androgen Receptor; ARAs: Androgen Receptor-Associated Coregulators; HSPs: Heat Shock Proteins; LH-RH agonists: Luteinizing Hormone-Releasing Hormone agonists; GnRH agonists: Gonadotropin-Releasing Hormone agonists; ARE: Androgen Response Element; PSA: Prostate-Specific Antigen; Inhibitory regulation is represented as red bars; promoting regulation is represented as green arrow.

Notably, *miR-21's* potential application as both a diagnostic and prognostic biomarker in PCa has also been demonstrated by Yaman Agaoglu et al., who found that the *miR-21* level was not only significantly higher in the blood plasma of 26 patients with localized PCa compared to 20 healthy controls, but also higher in the serum of advanced prostate cancer patients compared to localized PCa patients [55]. Furthermore, serum level of *miR-21* is highest in patients with CRPC compared to patients with androgen-dependent PCa, which suggests its potential to be utilized as a predictive biomarker as well [56]. More recently, high stromal expression of *miR-21* has been proved to be associated with poor biochemical recurrence-free survival after radical prostatectomy in PCa patients with high Gleason score [57].

The *miR-221~222* cluster: Another member of the PCa related oncogenic miRNA is the *miR-221~222* cluster. The *miR-221~222* cluster is frequently overexpressed in a variety of human cancers, including prostate carcinoma, glioblastoma, thyroid papillary carcinoma, non-small cell lung cancer, breast cancer and diffuse large B cell lymphoma [58-63]. Notably, the fact that the ectopic expression of the *miR-221~222* cluster is at a much higher level in the androgen-independent PCa cell lines (DU-145 and PC-3) than it is in androgen-dependent lines (LNCaP and VCaP), strongly indicates the possible role of the *miR-221~222* cluster in conferring androgen-independent growth [58,64]. Sun and colleagues have confirmed the critical role of *miR-221~222* in conferring castration resistance by showing that overexpression of this cluster in the LNCaP cell line greatly attenuated

the dihydrotestosterone (DHT) induced up-regulation of prostate-specific antigen (PSA) and promoted androgen-independent growth of this androgen-dependent PCa cell line. Moreover, this androgen-independent growth is largely dependent on an ectopic level of *miR-221~222* because knock-down of this cluster restored the response to DHT induction of PSA [65]. One of the known mechanisms underlying *miR-221~222* mediated castration resistance is their direct binding to and suppression of the $p27^{Kip1}$ gene [58-66], consequently rescuing PCa cells from cell cycle arrest at the G1 phase. *miR-221~222* has also been reported to suppress *HECTD2* and *RAB1A* genes and the down-regulation of *HECTD2* significantly enhances AR mediated transcription and androgen-independent PCa cell growth [45]. Similar results have been reported in vivo. Mercatelli et al showed that anti-miR-221-222 antagomir treatment in the castration resistant PC-3 xenografting model led to reduced tumor growth by removing the inhibition on p27 [66]. Yang et al., also reported that down-regulation of miR-221 and miR-222 leads to decreased cell proliferation, migration and increased apoptosis in androgen-independent PCa cell line [67].

Similar to *miR-21*, *miR-221* has been reported to be upregulated in the blood plasma in patients with localized PCa compared to healthy individuals, suggesting its potential to be used as a diagnostic biomarker for localized PCa. *miR-221* is also more highly expressed in advanced PCa patients compared to localized PCa patients, suggesting its potential to be a prognostic biomarker as well [55]. *miR-221* is recently reported as an independent predictor for cancer-related death of high-risk PCa patients, by partially regulating *JAK/STAT* signaling pathway [68].

The *miR-125* family: In contrast to *miR-21* and *miR-221~222*, the relationship between the *miR-125* family and carcinogenesis is much more complicated because its members have been reported to function as either tumor suppressor miRNAs or oncogenic miRNAs in different types of tumors [69,70]. The *miR-125* family is highly conserved and consists of three miRNAs: *miR-125a*, *miR-125b-1* and *miR-125-2*. Considering the oncogenic properties of this family, *miR-125b-1* has been reported primarily to promote carcinogenesis in various types of tumors and cell lines. *miR-125b-1* have been previously reported to be overexpressed in pancreatic cancer, oligodendroglia cancer and especially in prostate cancer [70-73]. White et al. has reported that *miR-125b-1* is overexpressed in all the five AR-positive (CWR22R, PC-346C, LNCaP, cds1, cds2) and four AR-negative (DU145, PC-3, pRNS-1-1, RWPE-1) prostate cancer cell lines [74]. Moreover, the level of *miR-125b-1* in PC-3 cell lines is regulated directly by androgen-AR signaling and transfection of synthetic *miR-125b-1* promotes androgen-independent growth of LNCaP cells, partially by targeting one of its target genes, *BAK1*. Since knockdown of *BAK1* alone cannot fully restore the effect of overexpressed *miR-125b-1*, more targets are expected to be involved in mediating *miR-125b-1's* function in PCa [72]. Ozen and colleagues examined the miRNA expression profile as well as their potential targets in PCa tumor samples and found that *EIF4EBP1* is another *miR-125b-1* target involved in mediating PCa carcinogenesis [75]. The other reported *miR-125b-1* targets in PCa are *TP53* and *PUMA* [72-74]. More recently, Amir et al. reported that *miR-125b-1* represses $p14^{ARF}$, which releases the suppression on Mdm2 and inhibits p53. Consequently, this inhibits p21 and Puma, leading to suppression of apoptosis in PCa cell lines. Treatment of PCa cells with a *miR-125b-1* inhibitor can reverse its action by increasing p14, decreasing Mdm2 and inducing apoptosis [76]. Knock-down of *miR-125b-1* using RNA interference in PC-3 prostate cancer cells leads to reduced proliferation, which can be restored by the reintroduction of synthetic mature *miR-125b-1* [77]. Taken together, these results confirm the role of *miR-125b-1* in conferring castration resistance in prostate cancer.

However, the relationship between *miR-125b-1* and AR illustrates a good example of the complexity of miRNA-mediated regulation in PCa carcinogenesis. Sun et al. demonstrated that AR directly binds to the promoter region of *miR-125b-1* and down-regulates its expression to protect multiple AR target genes from degradation, such as *IGF1R*, which plays an important role in carcinogenesis of metastatic CRPC [39]. These results are controversial to the previously demonstrated role of *miR-125b-1* in promoting PCa carcinogenesis and conferring castration resistance growth.

Tumor Suppressor miRNAs in PCa

The *miR-34* family: MicroRNAs belonging to the *miR-34* family, *miR-34a*, *miR-34b* and *miR-34c*, have also been reported to be tumor suppressors and modulators of p53 function in regulating cell cycle arrest and apoptosis [78]. The genomic loci of *miR-34a* or *miR-34b~c* are frequently deleted in various human cancers including pancreatic cancer, neuroblastoma, breast cancer, lung cancer and prostate cancer [79-82]. Ectopic expression of *miR-34a* in the p53 mutant colon cancer cell line or *miR-34a~c* in human lung fibroblast cells significantly induces cell cycle arrest [83,84] by repressing targets involved in cell cycle regulation, including *Cyclin-E2*, *CDK6* and *E2F5* [78,83]. *miR-34a* can also negatively regulate cell cycle machinery by repressing *E2F1* and *E2F3* levels [85]. Moreover, the *miR-34* family may modulate p53-mediated apoptosis by repressing the expression of the anti-apoptotic protein Bcl2 [83,86].

In prostate cancer, doxorubicin (DOX) treatment led to 3-fold increase of *miR-34a* and apoptosis, which can be counterbalanced by knocking down AR. Interestingly, this DOX induced *miR-34* up-regulation does not exist in AR-negative PCa cell lines such as DU-145 and PC-3, or AR-positive LNCaP cells being cultured in androgen free medium. Moreover, the AR-dependent inhibition of p53 suppressed the expression level of both *miR-34a* and *miR-34c*. On the other hand, simultaneous inhibition or forced overexpression of both *miR-34a* and *miR-34c* modulated the apoptosis induced by DOX [44]. It is reported that prostate cancer stem cells (CSCs) are enriched in the CD44+ population and that *miR-34a* is under-expressed in these CSCs isolated from xenograft and primary tumors. Enforced expression of *miR-34a* in these prostate CSCs cells inhibited tumor regeneration and metastasis, while the expression of antagomirs against *miR-34a* promoted carcinogenesis and metastasis. *miR-34a*'s function in modulating prostate CSCs survival is due to its targeting of CD44 because knockdown of CD44 can recapitulate miR-34a overexpression in prostate CSCs [87]. Members of the *miR-34* family have potential to be diagnostic markers since they are significantly overexpressed in the serum of patients with stages 3 and 4 PCa compared to normal control cohorts.

As discussed above, since one miRNA can simultaneously regulate hundreds of target genes and its regulation is frequently modest [11,15], the relationship between miRNAs and their targets can be very complicated. Although many previous in vitro studies have proposed *miR-34* family as key downstream effectors of p53 and that they have crucial roles in regulating PCa carcinogenesis, *miR-34* is not required for p53-mediated cell cycle arrest or apoptosis in a *miR-34*-deficient mouse model. Furthermore, in contrast to previous in vitro studies, genetic ablation of *miR-34* is not sufficient to accelerate tumorigenesis in different in vivo settings [88]. More recently, Cheng et al. reported

that prostate epithelium-specific co-inactivation of both *miR-34* and p53 significantly expands the prostate stem cell compartment and develop adenocarcinomas, while inactivation of either one has no such effect [89]. These results suggest that the function of certain miRNA in carcinogenesis is highly contextual and condition dependent.

The *miR-15a~miR-16-1* cluster: Another notable member of the family of tumor suppressor miRNAs is the *miR-15a~miR-16-1* cluster. The genomic region of the *miR-15a~miR-16-1* cluster (13q14) is deleted in more than half of B cell chronic lymphocytic leukemias (B-CLL) [90]. Deletion of *miR-15a~miR-16-1* promotes the development of clonal lymphocytosis in the peripheral blood and leads to B-CLL in mice [91]. Furthermore, altered expression of *miR-16-1* is associated with the development of autoimmune and B lymphoproliferative disease (B-LPD) in New Zealand black (NZB) mice, a mouse model for human chronic lymphocytic leukemia (CLL) [92]. In prostate cancer, *miR-15a* and *miR-16-1* levels are significantly down-regulated compared to normal prostate. Bonci et al. knocked-down miR-15a and *miR-16-1* by antagomirs and reported an increase in proliferation and invasiveness of untransformed prostate cells. Furthermore, restoration of *miR-15a* and *miR-16-1* in the 13q14-defective LNCaP androgen-dependent cell line leads to growth arrest, apoptosis and marked regression of prostate tumor xenografts [46]. Transfection of synthetic *miR-16-1* alone is sufficient to reduce cell proliferation of various PCa cell lines, such as 22Rv1, DU-145, PC-3M-luc and PPC-1. Injection of synthetic *miR-16-1* in the therapeutic bone metastasis mouse model (PC-3M-luc xenograft model) significantly inhibits the progression of prostate tumors in bone. This antitumorigenic effect of *miR-16-1* is likely due to its targeting of CDK1 and CDK2 associated with cell cycle control and cellular proliferation [93]. Although the *miR-15a~miR-16-1* cluster is also reported to target multiple oncogenes including BCL2, CCND1 (encoding cyclin D1) and WNT3A in prostate cancer [46,94], the identities of the critical targets of these miRNAs remains unclear [21]. Moreover, serum level of miR-16 has been reported to be up-regulated in patients with stages 3 and 4 PCa compared to normal donors, suggesting its potential to be used as a diagnostic marker of PCa [95].

The *miR-200* family: The *miR-200* family consists five miRNAs belonging to two clusters: the *miR-200a/b/429* cluster (*miR-200a*, *miR-200b*, *miR-429*) and the *miR-200c/141* cluster (*miR-200c* and *miR-141*), which are located on chromosomes 1p36 and 12p13 in humans. The seed sequences of these two clusters only differ by one nucleotide and their target genes largely overlap [96]. The critical role of the *miR-200* family in carcinogenesis was first demonstrated by the feedback loop between its family members and transcription factors of the *ZEB* family (*ZEB1* and *ZEB2*) in controlling the epithelial–mesenchymal transitions (EMT) program. Overexpression of *miR-200b* and *miR-200c* repress *ZEB2* (also termed *ZFHX1B*) and *ZEB1* (also termed *TCF8*) and increase *E-CADHERIN* expression [97,98]. The transcription of *miR-200c* and *miR-141* are regulated by DNA methylation of a CpG island near their transcription start sites. Interestingly, this CpG island is aberrantly methylated in PC-3 prostate cancer cells, which leads to a loss of expression of *miR-200c* and *miR-141*, while this island and correlated *miR-200c/miR-141* expression in LNCaP and DU-145 prostate cancer cells are not changed [99]. Kong et al. also reported that *the miR-200* family is significantly down-regulated in PC-3 cells with platelet-derived growth factor-D (PDGF-D) overexpression and an EMT phenotype, which can be reversed by re- expression of *miR-200b*. Furthermore, transfection of PC-3 PDGF-D cell (which has an EMT phenotype) with *miR-200b* leads to inhibition of cell migration and invasion, due to the suppression of *ZEB1*, *ZEB2* and *SNAIL2* [100]. Small molecules have also been used to target the *miR-200* family. Li et al. treated gemcitabine-resistant pancreatic cancer cells with 3,3'-diindolylmethane (DIM) and isoflavone, resulting in increased expression of *miR-200* and a reversed EMT process [101]. Williams et al. also reported overexpression of *miR-200b* inhibits the EMT, growth and metastasis potential of PC-3 cells [102]. Similar results were also confirmed in another EMT model of DU145-LN4 that decreased expression of *miR-200* family induced and EMT phenotype [103].

Since the level of *miR-141* was found to be correlated with serum PSA level, the *miR-200* family also has promising potential to be used as a diagnostic biomarker for PCa [104]. Serum levels of *miR-141* and *miR-200b* have been found to be significantly higher in advanced PCa patients compared to localized PCa patients, and their levels are elevated with increasing tumor stage as well [105,106]. These results strongly suggest the potential application of using the *miR-200* family as prognostic biomarkers.

Conclusion and Future Challenges

Although the study of miRNAs' function in prostate cancer carcinogenesis only began less than a decade ago, huge progress has been made in demonstrating the critical role of miRNA in primary PCa carcinogenesis, as well as in the development of castration resistant prostate cancer. More than 50 miRNAs have been reported to be involved in PCa carcinogenesis and the functions of many have been identified as well, including *miR-21, miR-32, miR148a, miR-221~222, miR616, miR-15a~16-1, miR-200, miR-101, miR-126, miR-330, miR-34* family and *miR-125* family. Limited by the scope of this review, we only discussed those most thoroughly studied in PCa (others were also summarized in Table-1). As discussed above, these miRNAs play important roles in modulating the progression of PCa tumors and are also involved in the development of castration resistance in CRPC. Because of the high genetic heterogeneity, CRPC is still one of the most difficult cancers to treat with conventional chemotherapy and hormone therapy, underscoring the importance of developing novel biomarkers and drug targets. Therefore, miRNAs' potential for use as treatment targets and biomarkers in PCa has attracted continued attention in recent years. However, there are still many challenges and more work need to be done to address these challenges.

One of the future challenges is effectively identifying the key targets of miRNAs in PCa carcinogenesis, because one miRNA can simultaneously regulate hundreds of target genes and its regulation is frequently modest [11,15]. Many new approaches have been developed in recent years for this purpose, such as HITS-CLIP (high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation) and SILAC (stable isotope labeling with amino acids in cell culture) [11,107,108]. Combined with RNA-Seq and functional library screening, the identification of function relevant miRNA targets in PCa is much more effective. Another challenge in utilizing miRNAs as novel drug targets for PCa is developing antagonist and delivery system with high efficiency and specificity. Various types of chemically modified anti-miRs have been utilized to repress miRNAs, such as locked nucleic acid oligonucleotides (LNAs), polylysine- conjugated peptide nucleic acids (PNAs) and phosphorodiamidate morpholino oligomers (PMOs) [109-112]. However, because the cellular internalization of these hydrophobic, large anti-miRs is often ineffective, a more efficient delivery system needs to be developed. Some attempts have been made to enhance the cellular uptake of anti-

miRs by conjugating the antisense oligonucleotides with cell-penetrating peptides (CPPs), such as Tat, Ant, MPG and more recently, Polymer nanoparticles (NPs) [109,113,114].

Overall, because of the complicated relationship between miRNAs and their targets in different cell contexts, there is still a lot of work to be done to determine the key mechanisms by which miRNAs regulate PCa carcinogenesis and confer castration resistance, and design novel agents and effective delivery approaches for miRNA targeting therapy.

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