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MicroRNAs and Drug Resistance in Prostate Cancers

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Abstract

Prostate cancer is the second leading cause of cancer related death in American men. Androgen deprivation therapy (ADT) is used to treat patients with aggressive prostate cancers. After androgen deprivation therapy, prostate cancers slowly progress to an androgen-independent status. Taxanes (e.g., docetaxel) are used as standard treatments for androgen-independent prostate cancers. However, these chemotherapeutic agents will eventually become ineffective due to the development of drug resistance. A microRNA (miRNA) is a small noncoding RNA molecule, which can regulate gene expression at the post-transcription level. miRNAs elicit their effects by binding to the 3'-untranslated region (3'-UTR) of their target mRNAs, leading to the inhibition of translation or the degradation of the mRNAs. miRNAs have received increasing attention as targets for cancer therapy, as they can target multiple signaling pathways related to tumor progression, metastasis, invasion, and chemoresistance. Emerging evidence suggests that aberrant expression of miRNAs can lead to the development of resistant prostate cancers. Here, we discuss the roles of miRNAs in the development of resistant prostate cancers and their involvement in various drug resistant mechanisms including androgen signaling, apoptosis avoidance, multiple drug resistance (MDR) transporters, epithelialmesenchymal transition (EMT), and cancer stem cells (CSCs). In addition, we also discuss strategies for treating resistant prostate cancers by targeting specific miRNAs. Different delivery strategies are also discussed with focus on those that have been successfully used in human clinical trials.

Graphical Abstract

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miRNA; prostate cancers; chemoresistance; cancer stem cells; epithelial-mesenchymal transition (EMT); androgen resistance; apoptosis; MDR transporters; delivery systems

1. INTRODUCTION

Prostate cancer is the second leading cause of cancer related death in men in the United States. It is estimated that 238,590 new cases of prostate cancer were diagnosed in 2013 and that approximately 29,720 men died from this disease in 2013.^{1,2} Although a localized prostate cancer can be effectively treated with less difficulty, the treatment of aggressive and metastatic forms of prostate cancer is a significant challenge. Currently, androgen deprivation therapy (ADT) is used to treat patients with aggressive prostate cancers. Despite temporary response, most patients relapse and eventually progress to androgen-independent prostate cancers (AIPCs).^{3,4} AIPCs are developed because of the perturbation of androgen receptor (AR) signaling. Taxanes (e.g., docetaxel) are used as standard treatments for AIPCs.⁵ However, only half of the patients respond to docetaxel therapy. Even those who initially respond to docetaxel treatment will eventually become resistant.^{6–9}

MicroRNAs (miRNAs) were first discovered in 1993 as endogenous small noncoding RNA molecules in *Caenorhabditis elegans*.¹⁰ miRNAs can regulate gene expression at the post-transcription level. Mature miRNAs of 18–22 nucleotides in length recognize and bind to the 3′-untranslated region (3′-UTR) of target mRNAs, leading to the degradation of target mRNAs or the inhibition of translation. miRNAs are being implicated in a range of physiological processes (e.g., development, cell differentiation, cell cycle control, and metabolism) as well as the pathogenesis of several human diseases, including prostate cancers.^{11,12} Deregulated miRNAs are involved in initiation, progression, and metastasis of prostate cancers. These miRNAs can be classified as oncogenic miRNAs, which can promote cancer progression, and tumor suppressor miRNAs, which can inhibit the progression of prostate cancers. Because of the significant role of miRNAs in prostate cancers, they have been extensively investigated as therapeutic targets for cancer treatment as well as biomarkers for diagnosis. The unique advantage of using miRNAs as targets for cancer therapy lies in the fact that a single miRNA can regulate multiple target genes in the same signaling pathway or even multiple signaling pathways and therefore can be more

effective in treating cancers that are inherently heterogeneous and are resulted from the deregulation of multiple genes.

An increasing number of miRNAs has been discovered in prostate cancer cell lines as well as human tissue samples, indicating the significant functional roles of miRNAs in prostate cancers. Emerging evidence suggests that miRNAs are involved in the development of resistant prostate cancers through the regulation of androgen signaling pathway, apoptosis, drug transporters, epithelialmesenchymal transition (EMT), and cancer stem cells (CSCs) (Figure 1). Here, we discuss the regulatory roles of miRNAs in the above cellular processes and their involvement in the development of resistant prostate cancers. The use of these miRNAs as potential therapeutic targets for treating resistant prostate cancers and the design of appropriate therapeutic approaches are also discussed. Different delivery strategies are also discussed with focus on those that have been successfully used in human clinical trials.

2. ROLE OF MIRNAS IN DIFFERENT DRUG RESISTANCE MECHANISMS

2.1. Androgen Signaling

Early stage localized prostate cancers need androgen for proliferation. Therefore, androgen ablation is a standard treatment for recurred prostate cancers after radiation therapy. However, most prostate cancers eventually obtain the capacities to survive without androgens and develop resistance to hormone therapy. At this stage, prostate cancers are defined as AIPCs or hormone refractory prostate cancers (HRPCs). AR, which is a nuclear hormone receptor, plays a significant role in the development of AIPCs. The role of AR signaling in the progression of prostate cancers to androgen independence has been previously discussed.¹³ Many studies have investigated the interaction between AR signaling and miRNAs as well as the involvement of miRNAs in the progression of AIPCs. Evidence indicates that miRNAs are critical modulators of androgen signaling (Table 1).

2.1.1. Up-Regulation of miRNAs in AIPCs—miR-125b is overexpressed in AIPCs. The overexpression of miR-125b confers the resistance of LNCaP prostate cancer cells to androgen withdrawal. Prostate cancer cells can be sensitized to chemotherapy through the inhibition of miR-125b. The target genes regulated by miR-125b include p53, Puma, and Bak1.^{14–16} Genome-wide expression profile studies showed that miR-221/-222 were also up-regulated in AIPCs as well as bone metastatic tumor specimens.¹⁷ The analysis of human tumor specimens indicated that 90% of the AIPCs had up-regulated miR-221/-222 expression.¹⁸ The overexpression of miR-221/-222 in AIPCs (e.g., LNCaP and LAPC-4) could promote androgen-independent cell growth.¹⁷ miR-221 also promotes androgen resistance through the down-regulation of HECTD2 and Ras-related protein Rab-1A (RAB1A).¹⁹ miR-21 is also overexpressed in AIPCs.^{20–22} A microarray analysis showed that miR-21 was an AR responsive miRNA. AR regulates the transcription of miR-21 by binding to its promoter. Overexpression of miR-21 in AIPCs could make them resistant to androgen ablation. In addition, the reduction of miR-21 could inhibit androgen-induced prostate cancer growth.^{20,21} miR-616 induces androgen-independent growth of prostate cancers by suppressing the expression of tissue factor pathway inhibitor (TFPI-2).²³ Circulating miRNAs are also associated with the progression of AIPCs. A recent study

showed that serum samples from a patient with AIPCs had high levels of miR-375, miR-378*, and miR-141, indicating their roles in the development of AIPCs. These signature circulating miRNAs have the potential to be used as biomarkers for prostate cancer diagnosis.²⁴

2.1.2. Down-Regulation of miRNAs in AIPCs—Several tumor suppressive miRNAs are down-regulated in AIPCs, including miR-146a,^{25,26} let-7C,^{27,28} miR-124,²⁹ miR-34a, miR-34c,^{30,31} miR-148a,³² miR-31,³³ miR-200b-3p,³⁴ miR-185,³⁵ and miR-205.³⁶ Down-regulation of these tumor suppressive miRNAs in prostate cancer may lead to cancer progression, increased aggressiveness, and resistance to androgen deprivation therapy. For example, down-regulation of miR-205 is also correlated with the poor therapeutic outcome of prostate cancer patients.³⁶ Restoration of these miRNAs in AIPCs can inhibit cell growth, reduce cell invasion, and prevent metastasis. AR is negatively regulated by several miRNAs including *let-7C*, miR-124, miR-34a, miR-34c, miR-31, miR-183, and miR-205. Down-regulation of these miRNAs results in the overexpression of AR, which is an important risk factor for androgen independence.¹³ These miRNAs also regulate other targets in AIPCs. The targets of miR-146a include Rho-associated, coiled-coil containing protein kinase 1 (ROCK1), epidermal growth factor receptor (EGFR), and matrix metalloproteinase-2 (MMP2). Down-regulation of miR-146a is critical for the overexpression of EGFR in AIPCs.^{25,26} miR-124 can increase p53 levels.²⁹ miR-34a can inhibit prostate-specific antigen (PSA) and Notch-1, thus preventing cell proliferation and decreasing the self-renewal capacity of prostate cancers.^{30,31} miR-148a can inhibit mitogen and stress-activated protein kinase (MSK1).³² miR-31 can inhibit cell-cycle regulators such as E2F1, E2F2, EXO1, FOXM1, and MCM2.³³ These miRNAs (e.g., miR-124 and miR-34a) are down-regulated due to the hypermethylation of their promoters.^{29,30} In addition, *let-7C* is negatively regulated by Lin28.^{27,28} miR-200b-3p expression level is correlated with that of p73 protein, which is related to p53 protein and is considered as a tumor suppressor. The interaction between miR-200b-3p and p73 can also promote the proliferation of AIPCs.³⁴

2.2. Apoptosis Avoidance

Apoptosis is a process of programmed cell death with defined unique morphology and biochemical changes of cells in response to apoptotic signals including death ligands, DNA damage, irradiation, and chemotherapy drugs. The ability to avoid apoptosis is developed during the tumorigenesis and is a hallmark of cancers.³⁷ There are two major apoptotic pathways: extrinsic pathway and intrinsic pathways.^{38,39} These two pathways have considerable cross-talk and may proceed concurrently. The apoptosis defect and dysregulation of apoptotic mediators is an important mechanism leading to the chemoresistance of prostate cancers. Recent evidence has indicated the roles of miRNAs in the regulation of apoptosis and their association with the development of resistant prostate cancers (Table 2 and Figure 2).

Bcl-2 family proteins are important regulators of apoptosis. Overexpression of antiapoptotic proteins (e.g., Bcl-2 and Bcl-XL) or underexpression of pro-apoptotic proteins [e.g., BAX, Puma (p53 up-regulated modulator of apoptosis), and Bim (Bcl-2-interacting mediator of cell death)] has contributed to the resistance of prostate cancers to chemotherapy. Recent

studies have demonstrated the role of miRNAs in the regulation of Bcl-2 family proteins. These studies showed that Bcl-2 was regulated by miR-34a,⁴⁰ miR-34c,⁴¹ miR-205,⁴² and miR-15a/miR-16-1.⁴³ Bcl2L2 was regulated by miR-205,⁴⁴ and Bcl-XL was regulated by miR-574-3p.⁴⁵ Underexpression of these miRNAs results in the overexpression of pro-survival Bcl-2 family proteins. In contrast, miR-32 and 125b regulate pro-apoptotic Bcl-2 family proteins such as *Bcl2L1/Bim*, Puma/BBC3, and Bak1.^{15,16,46} Overexpression of miR-32 or 125b causes down-regulation of above pro-apoptotic proteins. Either overexpression of pro-apoptotic proteins or down-regulation of antiapoptotic proteins can promote cell apoptosis.

P53 is a tumor suppressor protein and a critical mediator of cell cycle and apoptosis in response to different stress signals, including hypoxia, DNA damage, and free radical formation. It initiates apoptosis by activating pro-apoptotic proteins and inhibiting anti-apoptotic proteins. Several miRNAs are involved in the regulation of p53. The up-regulation of miR-125b can inhibit p53 through the up-regulation of mouse double minute 2 homologue (MDM2), which is a negative regulator of p53.^{16,47} miR-29 can up-regulate p53 through the PI3K-AKT-MDM2 pathway. miR-122 activates p53 through the inhibition of cyclin G1/pp2A-MDM2 pathway.⁴⁸ At the same time, p53 also regulates certain miRNAs (e.g., miR-145, miR-34, miR-192, and miR-215) to affect cell survival and proliferation.⁴⁸ For example, the expression of miR-34a is transcriptionally regulated p53. miR-34a is regulating genes controlling cell cycle and apoptosis. The overexpression of miR-34a can inhibit cell growth, induce apoptosis, and sensitize resistant cells to camptothecin, indicating miR-34a as a potential target for p53-defective prostate cancer treatment.⁴⁹ Similar studies were reported by Lodygin et al., who showed that miR-34a was a target of p53. In their studies, miR-34a overexpression in prostate cancers induced cell senescence and cell cycle arrest though targeting cyclin-dependent kinase 6 (CDK6).⁵⁰

miRNAs also regulate apoptosis by targeting E2F transcription factors. E2F6 is a target of miR-31. The over-expression of miR-31 can increase the level of E2F6 and induce apoptosis.⁴⁴ E2F1 are regulated by miR-106b⁴⁶ and miR-20a.⁵¹ Up-regulation of miR-106b or miR-20a may lead to down-expression of E2F1 transcription factors and thus reduce apoptosis.

Several other pro-apoptotic proteins are also inhibited by miRNAs. Phosphatase and tensin homologue (PTEN) can be inhibited by miR-19b, miR-23b, miR-26a, miR-92a, or miR-21.^{52,53} Programmed cell death 4 (PDCD4) is a target of miR-21.^{53–57} miR-21 can inhibit PDCD4 and results in the resistance of PC3 prostate cancer cells to docetaxel.⁵⁶ Antisense oligonucleotide mediated inhibition of miR-21 in DU145 and PC3 prostate cancer cells has increased their sensitivity to apoptosis.⁵⁴

2.3. MDR Transporters

Overexpression of multiple drug resistance (MDR) transporters, such as P-glycoprotein (Pgp), breast cancer resistance protein (BCRP), and multiple drug resistance proteins (MRPs), is an important reason for chemoresistance.^{58–68} These transporters function as drug efflux pumps, which reduce intracellular accumulation of chemotherapeutic agents. The overexpression of MDR transporters correlates negatively with the sensitivity of

prostate cancers to chemotherapy drugs.⁵⁹ For example, Pgp and other MDR transporters were up-regulated in two paclitaxel-resistant cell lines (DU145-TXR and PC3-TXR), leading to the resistance of these cells to paclitaxel and other chemo-therapeutic agents.⁶¹ Many studies have demonstrated the regulatory roles of miRNAs on various MDR transporters.

Pgp/MDR1 is one of the most important MDR transporters. MDR1 is responsible for the resistance to various chemotherapy drugs including taxol, doxorubicin, etoposide, and vinblastine. The regulation of MDR1 by miRNAs has been demonstrated in multiple studies. miR-9 is involved in the Pgp expression and drug resistance. Inhibition of miR-9 with anti-miR-9 could sensitize brain cancer cells to Temozolomide.⁶⁹ miR-381 and miR-495 are also down-regulated in resistant cancer cells with up-regulated MDR1.⁷⁰ Down-regulation of *let-7* family is associated with the resistance of cancer cells to taxol. *Let-7* enhances cell resistance through the up-regulation of its target IGF2 mRNA-binding protein 1 (IMP-1), which, in turn, can stabilize MDR1.⁷¹ miR-122 can sensitize hepatocellular carcinoma to chemotherapy by the down-regulation of MDR1, MRP, and Bcl-w.⁷²

The role of miR-27a and miR-451 on the modulation of Pgp has been reported in several papers with conflicting results.^{73–77} Some studies indicated that these two miRNAs were down-regulated in resistant cancer cells and were negative regulators of Pgp.^{73–75} Kocalchuk et al. reported that miR-451 was a negative regulator of Pgp. Doxorubicin resistant cells could be sensitized by the transfection of miR-451.⁷³ Similarly, Feng et al. reported that miR-27a and miR-331-5p were down-regulated in leukemia cell lines. Their down-regulation was associated with Pgp overexpression in these cells.⁷⁴ Chen et al. also reported that miR-27a was down-regulated in resistant hepatocellular carcinoma cells.⁷⁵ In contrast, several other studies showed that both miR-27a and miR-451 were overexpressed in resistant cells and caused the overexpression of Pgp.^{76,77} Zhu et al. showed that both miR-451 and miR-27a were up-regulated in MDR cancer cell lines, such as A2780DX5 and KB-V1. The overexpression of these two miRNAs resulted in a high level of Pgp.⁷⁶ Li et al. also showed that both miR-27a and Pgp were up-regulated in drug resistant ovarian cancer cells.⁷⁷ These studies were carried out on different cell lines, indicating the importance of cellular context for miRNA functions. It is worthwhile to further investigate the role of miR-451 and miR-27a in regulating Pgp expression and their effects on drug resistance.

BCRP/ABCG2 is negatively regulated by miR-328 and miR-519.^{78,79} Overexpression of miR-328 could down-regulate BCRP in breast cancer cells and thus increase their sensitivity to mitoxantrone.⁷⁸ However, the expression of BCRP is significantly up-regulated when the miRNA binding site at the 3'-UTR of BCRP mRNA is missing.⁸⁰

MRP1/ABCC1 is up-regulated in VP-16-resistant breast cancer cells (MCF-7/VP).⁸¹ MRP-1 is negatively regulated by miR-326. The down-regulation of miR-326 has been observed in resistant cancer cells with MRP-1 overexpression. MRP4/ABCC4 is another drug efflux transporter that is negatively regulated by miR-124a and miR-506.⁸²

2.4. Epithelial-Mesenchymal Transition

Epithelial–mesenchymal transition (EMT) is a process in which epithelial cells assume a mesenchymal phenotype. During the EMT process, cells demonstrate elongated fibroblast morphology. Cell adhesions between epithelial cells are lost. Actin cytoskeleton is reorganized. Epithelial markers (e.g., E-cadherin) are down-regulated, while mesenchymal markers and matrix metalloproteinases (MMPs) are up-regulated. The EMT process is regulated by multiple transcription factors including N-cadherin, Snail, zinc-finger E-box binding homeo-box 1 (ZEB1), ZEB2, and Slug. EMT process is activated in the initiation of cancer metastasis and during the development of chemoresistance.^{83–88} EMT is associated with the resistance of several different cancers including gemcitabine-resistant pancreatic cancers,⁸⁹ lapatinib-resistant gastric cancers,⁹⁰ and taxol-resistant ovarian cancers.^{91,92} Emerging evidence suggests that miRNAs play critical roles in the regulation of EMT process (Table 3).^{93–96}

The following miRNAs are down-regulated in prostate cancers and are associated with the EMT process: miR-143,⁹⁷ miR-145,^{97,98} miR-29b,⁹⁹ miR-34b,¹⁰⁰ miR-200 family,^{101–103} miR-205,¹⁰¹ miR-203,¹⁰⁴ and miR-23b/27b.¹⁰⁵ The down-regulation of these miRNAs results in high levels of mesenchymal markers and low levels of epithelial markers, which can promote EMT process and increase the aggressiveness of prostate cancers. For example, the down-regulation of miR-143 and miR-145 has contributed to the bone metastasis of prostate cancers.⁹⁷ These miRNAs regulate EMT through the modulation of their targets. miR-145 suppresses EMT through the inhibition of its direct target HEF1.⁹⁸ miR-29b increases E-cadherin expression, while reducing N-cadherin, Twist, and Snail expression.⁹⁹ miR-34b inhibits EMT by reducing mesenchymal markers including vimentin, ZO1, N-cadherin, and Snail, while increasing epithelial markers such as E-cadherin.¹⁰⁰ The levels of miR-200C and miR-205 are reduced in docetaxel-resistant prostate cancer cells. These cells are undergoing EMT with decreased E-cadherin and increased mesenchymal markers. Restoration of miR-200C and miR-205 through transfection can result in the re-expression of E-cadherin.¹⁰¹ miR-1, miR-200, and Slug form a regulatory loop. Slug is a direct repressor of these two miRNAs.¹⁰⁶ The targets for miR-200 family include ZEB1 and ZEB2. Overexpression of miR-200 reverses EMT in PC3 PDGF-D cells through the down-regulation of ZEB1, ZEB2, and Snail.¹⁰³ The treatment with erismodegib (a smoothened inhibitor) could regulate miR-200 and reverse EMT through the inhibition of transcription factors such as Snail, Slug, and ZEB1.¹⁰² miR-203 reduces cancer cell invasion and metastasis through the inhibition of Wnt signaling pathway.¹⁰⁴ miR-23b/27b cluster is down-regulated in metastatic, castration-resistant cancer. They could inhibit Ras-related C3 botulinum toxin substrate 1 (Rac1) activity and increase the level of E-cadherin. The ectopic expression of miR-23B/27B could suppress metastasis of castration-resistant prostate cancers.¹⁰⁵

2.5. Cancer Stem Cells

Cancer stem cells (CSCs), also known as tumor-initiating cells, are a subgroup of less differentiated cancer cells. Although only a small percentage of CSCs are present in tumors, CSCs are critical for cancer progression, metastasis, and chemoresistance. CSCs can self-renew, differentiate, and drive the expansion of cancers. They can be isolated from bulk

tumor cells based on characteristic cell surface markers. CD44 has been used as a marker either alone or in combination with other CSCs markers.^{107–109} CD44⁺ prostate cancer cells demonstrated CSC-like properties such as increased clonogenic potential, tumorigenicity, and metastasis. Most current therapeutic agents are targeting at bulk tumor cells but not at CSCs. The use of these agents can effectively kill bulk tumor cells but are ineffective in the elimination of CSCs. The spared CSCs may cause cancer recurrence and drug resistance. The exact mechanism accounting for the development of drug resistant properties in CSCs is still unclear. The differentiation of CSCs under the selection pressure of chemotherapy drugs may generate drug resistant daughter cells and thus acquire drug resistance. Some CSCs have a high level of expression of MDR drug efflux transporters. For example, MRP1/ABCC1 overexpression in prostate cancers has resulted in the resistance of these cells to arsenic treatment.¹¹⁰ In another study, prostate CSCs showed resistance to several chemotherapeutic agents including cisplatin, doxorubicin, paclitaxel, and methotrexate.¹¹¹

miRNAs have a pivotal role in the regulation of CSCs. For example, miR-34a is down-regulated in CD44⁺, CD133⁺, or $\alpha 2\beta 1$ ⁺ prostate CSCs sorted from prostate cancer xenograft and prostate tumor patient tissues. Overexpression of miR-34a in prostate cancer cells or isolated CD44⁺ CSCs cells can significantly reduce tumor growth and metastasis. miR-34a inhibits prostate CSCs through the inhibition of CD44. Down-regulation of miR-34 in CD44⁺ prostate cancer cells enhances the aggressiveness of tumors. miR-34a is a negative regulator of CD44⁺ prostate CSCs.^{112,113} Similar studies showed multiple miRNAs were down-regulated in prostate CSCs (e.g., miR-34a, *let-7b*, miR-106a, and miR-141), while other miRNAs were up-regulated (e.g., miR-301 and miR-452). The overexpression of *let-7* could inhibit the prostate cancer growth through their effects on CSCs.¹¹⁴ miR-320 has been identified as a negative regulator of CD44⁺ prostate CSCs. miR-320 inhibits β -catenin expression and suppresses prostate CSCs through the inhibition of the Wnt/beta-catenin signaling pathway. Down-regulation of miR-320 enhances CSC properties such as tumor sphere formation, chemoresistance, and tumorigenicity. These CSC properties could be inhibited by the overexpression of miR-320.¹¹⁵ Enhancer of zeste homologue 2 (EZH2) is involved in CSC proliferation and differentiation. It is up-regulated in CD44⁺/CD133⁺ prostate CSCs and is negatively regulated by miR-101.¹¹⁶

2.6. Interrelationship between EMT, CSCs, Chemo-resistance, and miRNAs

Growing evidence suggests the connections among EMT, CSCs, chemoresistance, and miRNAs.^{117–119} EMT cells have CSC-like properties such as self-renewal and mammosphere formation, while CSCs have mesenchymal-like features. EMT cells and CSCs have demonstrated chemo-resistance phenotypes, while a higher percentage of EMT cells and CSCs are identified in drug resistant prostate cancers. In addition, EMT and CSC development shares common signaling pathways, including Wnt, Notch, and hedgehog (Hh) pathways.¹²⁰ EMT process is in parallel with the acquisition of “stemness”. Drug resistant cancers are usually developed through EMT. miRNAs play a pivotal role in the regulation of both EMT process and the CSC proliferation. miRNAs are the common thread connecting EMT, CSCs, and drug resistance (Figure 3). Therefore, targeting specific miRNAs involved in EMT or CSCs will be a promising cancer therapy approach, which could effectively eliminate CSCs and EMT cells and overcome drug resistance.

In a recent study, erismodegib (a smoothened inhibitor) demonstrated the ability to inhibit EMT as well as CSC growth. The treatment with erismodegib may lead to the change of several miRNAs (miR-21, miR-128, and miR-200 family). Erismodegib can increase the levels of PDCD4 and promote apoptosis through the inhibition of miR-21. Erismodegib can also inhibit Bmi-1 through the up-regulation of miR-128. Bmi-1 plays an important role in CSCs. The inhibition of Bmi-1 as well as several other proteins is associated with the inhibition of prostate CSCs. In addition, erismodegib inhibits EMT through the miR-200 family, which can up-regulate E-cadherin and down-regulate N-cadherin, Snail, Slug, and ZEB1.¹⁰² In another study, miR-203 was suppressed during the EMT process through the methylation of the promoter. The deregulation of miR-203 not only promoted EMT but also enhanced stemness of the cancer cells. miR-203 inhibited the invasion and metastasis of cancers by inhibiting the Wnt signaling pathway.¹⁰⁴ Docetaxel-resistant prostate cancer cells are undergoing EMT and have increased CSC populations. miR-200C and miR-205 are key regulators of EMT and CSCs. These two miRNAs are reduced in the above resistant cells. Reintroduction of these miRNAs can reverse EMT. This study has demonstrated the role of EMT and CSCs in docetaxel resistance and their regulation by miR-200C and miR-205.¹⁰¹

3. THERAPEUTIC STRATEGIES

Many miRNAs are aberrantly expressed in resistant prostate cancer cells and cause the resistance of cancer cells through the modulation of their targets. Because of their important roles in the development of chemoresistance, these miRNAs can be used as potential therapeutic targets for overcoming drug resistance. Here, we discuss different therapeutic agents that can be used to target miRNAs for treating resistant prostate cancers.

3.1. Macromolecule Therapeutics

Two categories of macromolecule therapeutics can be utilized to correct the miRNA expression levels in resistant cancers. Antagomirs (also known as anti-miRs)¹²¹ and miRNA sponges (mRNAs with multiple targeting sites for a specific miRNA)^{122,123} are usually used to reduce oncogenic miRNAs that are up-regulated in resistant cancers. An antagomir is a chemically modified synthetic RNA that is complementary to a specific miRNA target and used to silence endogenous miRNAs. The chemical modifications of antagomir are usually used to increase its resistance to nuclease degradation. Tumor suppressive miRNAs are down-regulated in resistant cancers. The levels of tumor suppressive miRNAs can be restored by using synthetic miRNAs (miRNA mimics) or genetic precursors (vector based miRNAs).¹²⁴ The restoration of down-regulated tumor suppressive miRNAs is also called miRNA replacement therapy. Although a variety of therapeutic strategies have been developed and demonstrated therapeutic efficacies, anti-miRs and miRNA mimics are the two most advanced approaches to enter clinical trials.

3.2. Small Molecule Drugs

A growing body of studies have discovered the activities of small molecule drugs in the regulation of miRNAs and demonstrated their potential for overcoming drug resistance in prostate cancers. In our study, we have used the combination of paclitaxel and Hh pathway inhibitor cyclopamine to treat paclitaxel resistant prostate cancers. The combination therapy

has successfully restored the expression of miR-220C and miR-34a in prostate cancer cells, thus effectively reversing chemoresistance and eliminating CSC side population. This study has demonstrated the feasibility of using small molecule agents to overcome drug resistance through the modulation of miRNA expression.¹²⁵ Several chemicals derived from natural products have also been utilized to sensitize cancers by targeting miRNAs.^{126–128} For example, the treatment with 3,3'-diindolylmethane (DIM) and isoflavone increased the expression of miR-200 and *let-7*, which, in turn, reversed EMT process and increased the sensitivity of cancer cells to gemcitabine.¹²⁶ A curcumin analogue, EF24, can effectively induce apoptosis in DU145 prostate cancer cells. It works through the inhibition of miR-21, thus enhancing the expression of miR-21 target genes such as PDCD4 and PTEN.¹²⁹ Similarly, resveratrol can also reduce prostate cancer growth and metastasis by targeting miR-21.²¹ Genistein, a soy isoflavone, has shown anticancer effects on prostate cancer cells through the up-regulation of miR-34a.¹³⁰ The advantages of small molecule drugs are their favorable in vivo stabilities and pharmacokinetics (PK) profiles, thus having less challenge in drug delivery.

3.3. Combination Therapy

The correction of aberrant miRNAs in resistant prostate cancers has been demonstrated to be an effective approach for sensitizing resistant prostate cancer to chemotherapy. For example, the transfection of miR-148 or miR-34a can sensitize paclitaxel resistant PC3 cells to paclitaxel treatment.^{32,40,49} The overexpression of miR-143 has also shown the ability to enhance the sensitivity of prostate cancer cells to docetaxel.¹³¹ Therefore, the combination of small molecule chemotherapeutic agents and miRNA therapeutics can be a promising strategy for treating resistant prostate cancers. The rationale of this combination therapy is that the correction of aberrant miRNA levels can sensitize resistant prostate cancer cells to small molecule drugs. A delivery system that could simultaneously deliver miRNA and small molecule drugs will be of great interest. In the past few years, many delivery systems were investigated for the codelivery of small molecule drugs and gene medicines (including miRNA, siRNA, and DNA) for cancer therapy.^{132–137}

4. DELIVERY STRATEGIES FOR MIRNA THERAPEUTICS

Despite promising results in the development of miRNA therapeutics and successes on in vitro cell based studies, very limited progress has been made at in vivo studies or clinical trials. The major obstacle for miRNA-based therapy is the in vivo delivery of miRNA therapeutics.¹³⁸ Significant efforts have been made for the delivery and targeting of miRNA therapeutics. Various delivery strategies have been successfully developed for miRNA therapeutics. There is still only a limited success with miRNA delivery and targeting and will continue being a great challenge. Although a variety of miRNA therapeutics have been studied, many of them are not suitable for human use. For example, vector-based systems rely on plasmids or viral vectors to deliver and express miRNA in cells. The safety concerns restrict their application in human as a drug candidate even though they can be very useful systems for in vitro cell-based studies. miRNA mimic (a double-strand RNA) is the most promising miRNA therapeutic, which has advanced to clinical trials. For example, MRX34 is the first miRNA mimic that has entered phase I clinical trial as a miRNA replacement

therapy to treat liver cancers.¹³⁹ miRNA inhibitory short single-strand oligonucleotide (e.g., antagomir) is another promising approach, which achieves therapeutic effects through the down-regulation of target miRNA. Therefore, in this section, we will discuss the most promising delivery strategies that have advanced into clinical trials. miRNA therapeutics have large molecular weight and are hydrophilic. They have very poor ability to cross cell membranes, which are composed of lipophilic phospholipids bilayers. In addition, they are unstable when exposed to nucleases in the blood. A successful in vivo delivery approach should be able to (1) protect miRNA therapeutics against serum nucleases, avoid renal clearance, prolong circulation time, and minimize the nonspecific interaction with nontarget cells or organs; (2) enhance the accumulation of miRNA therapeutics in target tissues and facilitate their uptake by target cells and their release inside the cells. Here, we discuss miRNA delivery strategies including (1) chemical modification, (2) lipid-based particles, (3) cyclo-dextrin polymer nanoparticle, and (4) bioconjugates. All of these approaches have been successfully used for in vivo delivery of siRNAs. Because of the similarity between siRNAs and miRNA mimics (both are double-strand RNA), these delivery strategies can be transferred for miRNA delivery.

4.1. Chemical Modification

To increase the stability in serum, a variety of methods have been developed for the chemical modification of RNAs (Figure 4). Both phosphodiester and ribose can be potentially modified. The nonbridging oxygen in the phosphodiester can be replaced with sulfur (phosphorothioate), boron (boranophosphate), or methyl (methylphosphonate) groups. The 2'-position of the ribose can also be modified to 2'-fluoro (2'-F), 2'-O-methyl (2'-OMe), 2'-O-(2-methoxyethyl) (MOE), 2'-O-fluoro- β -D-arabi-nonucleotide (FANA), and locked nucleic acid (LNA). Optimized chemical modifications can increase the resistance to serum nuclease, avoid the activation of innate immune system, and reduce off-target effects.¹⁴⁰ Miravirsin is a good example of a chemically modified miRNA inhibitor, which was developed by Santaris Pharma A/S. Miravirsin is composed of locked nucleic acid (LNAs) RNA interspaced throughout a phosphorothioate oligonucleotide. It is complementary to the 5'-end of miR-122 and can thus hybridize to miR-122. It inhibits Hepatitis C virus (HCV) by blocking the interaction between HCV RNA and miR-122. The chemical modifications in miravirsin can provide nuclease resistance and enhance its affinity to its target. Miravirsin is the first miRNA targeting drug that enters clinical trials and is currently in phase 2 trials for treating patients with HCV.^{141,142} The same platform has also been used by miRagen Therapeutic in the development of three drugs including MGN-9103 (targeting miR-208), MGN-1374 (targeting miR-15 and miR-195), and MGN-4893 (targeting miR-451).¹⁴³ These drugs are being developed in collaboration with Santaris Pharma A/S and are still at the preclinical stage. Chemical modification has also been widely used for siRNAs. For example, siRNA targeting Hepatitis B virus (HBV) has been chemically modified to improve its stability.^{144,145} Although chemical modification can increase the stability of siRNAs or miRNA mimics, a synthetic carrier is usually needed for their in vivo delivery and targeting.^{144,145}

4.2. Lipid-Based Particles

Lipid-based particles (or liposomes) have been used for decades for the delivery of gene medicines including plasmids, antisense oligonucleotides, and siRNAs. A number of lipid-based particle systems have been developed for delivery of siRNAs or miRNA mimics and used in clinical trials (Figure 5A).^{146,147}

Stable nucleic acid-lipid particle (SNALP) is the most popular siRNA delivery system, which was originally developed by Tekira, and has been used as the delivery system for several products in human clinical trials (e.g., TKM-PLK1 and ALN-TTR02).^{148,149} A typical SNALP formulation includes ionizable lipids, shielding lipids, cholesterol, and targeting ligands. (1) Ionizable lipids in SNALP formulation are quite similar to those in Smarticles. The ionization status and thus the charge of the lipids are pH dependent. The positive charge at acidic pH can enhance encapsulation of RNA in the liposome, enhance cellular uptake, and facilitate endosomal escape. These lipids are unionized and neutral at physiological pH, thus avoiding nonspecific interaction and preventing rapid elimination of lipid particles. DlinDMA (1,2-dilinoleyloxy-N,N-dimethyl-3-amino-propane) is the first generation ionizable lipid used in SNALP.¹⁵⁰ The second generation of ionizable lipids including Dlin-KC2-DMA and Dlin-MC3-DMA were discovered through extensive structure-activity relationship (SAR) studies.¹⁵¹ The pK_a of the lipids is critical for siRNA delivery. Optimization of the pK_a of the ionizable lipid head groups could enhance their membrane fusion and endosomal escape properties. In addition, the delivery efficiency is also affected by the structure of lipids including amine head groups, linkers, and hydrophobic tails.¹⁵² ReLNP (rapid eliminated LNP) developed by Alnylam is the third generation of SNALP, which uses degradable lipids derived from Dlin-MC3-DMA. The use of biodegradable lipids can reduce toxicity and improve biocompatibility.¹⁵³ (2) Shielding lipids are PEG-lipid conjugates used to make stealth liposomes. Because of the steric effect of PEG coating, the PEGylation of lipid particles can reduce nonspecific interaction with serum components, avoid uptake by the cells of reticuloendothelial system (RES), minimize the kidney elimination, and increase the circulation time. However, shielding lipids can reduce the cellular uptake of particles in target cells and negatively affect the endosomal release of siRNAs, for PEG can sterically block the interaction of liposomes with cell membranes or endosome membranes. This issue can be addressed by using acid-sensitive shielding lipids, which can be detached from lipid particles inside the endosome, which has an acidic pH environment.^{154,155} (3) Both endogenous and exogenous targeting ligands can be utilized to enhance the delivery of liposomes into target cells or organs. Neutral liposomes often bind to serum proteins and thus are directed by the coated serum proteins to their target cells. For example, lipoprotein Apo-E has been used as a targeting ligand to enhance the uptake of liposomes in hepatocytes.^{156,157} Exogenous ligands can also be used to enhance the delivery of liposomes into target cells. These targeting ligands include antibodies, peptides, and small molecules.

Smarticles is a proprietary liposomal delivery system from Marina Biotech (Bothell, WA) and has been used for delivery of MRX34, which is the first miRNA mimic that entered clinical trials.¹⁴⁶ MRX34 is a double-strand RNA to treat liver cancers. The ionizable lipid is a key component for enhancing the in vivo delivery of miRNA mimics or siRNAs. Because

of the presence of ionizable lipid, smarticles is anionic at normal physiological pH but becomes cationic at lower pH. This pH-dependent change of surface charge is critical for minimizing nonspecific interaction during circulation in the blood but has efficient cellular uptake and endosomal escape ability in target cells. Smarticles is also used for delivery of PNR2258, which is an anti-Bcl-2 cancer drug developed by ProRNAi Therapeutics and is currently in phase II clinical trials.¹⁴⁷

4.3. Cyclodextrin Polymer Nanoparticle (CDP)

RONDEL (RNAi/oligonucleotide nanoparticle delivery) is the delivery platform for Arrowhead Research's CALAA-01. It is the first targeted delivery of siRNA entering the human trials. RONDEL delivery system has three major components: (1) a water-soluble positive charged cyclodextrin-containing polymer (CDP); (2) a PEG-adamantane conjugate (PEG-AD); and (3) a transferrin decorated PEG-AD (Tf-PEG-AD) (Figure 5B). When the siRNA solution is mixed with RONDEL before, targeted nanoparticles with a size of 50–70 nm are formed through self-assembly.¹⁵⁸ The RONDEL delivery system has several advantages including the simple formulation preparation process, optimal particle size for enhanced permeability, and retention (EPR) effect, and inclusion of tumor targeting ligands (transferrin) for active tumor targeting. However, adverse events were observed among some patients during the phase I studies. Arrowhead Research has decided not to advance the CALAA-01 into phase II trials.¹⁵⁹

4.4. Bioconjugates

Dynamic polyconjugate (DPCs) is a multiple component conjugate composed of an amphiphilic endosomolytic polymer, PEG, targeting ligand (GalNac), and siRNA (Figure 5C).¹⁶⁰ In the first generation of DPC, polymer poly(butyl amino vinyl ether) (PBAVE) is selected as the amphiphilic endosomolytic polymer. Both PEG and targeting ligand (GalNac) are conjugated to the polymer through an acid-sensitive linker. GalNac can enhance the targeting and cellular uptake of DPC by hepatocytes. Membrane distrust PBAVE is masked by attached shielding PEGs during the circulation. After cellular uptake, PEGs are released and the exposed PBAVE can promote endosomal escape. Double-strand RNAs are attached to PBAVE polymer via a degradable disulfide linker, which can be cleaved in the reducing environment of cytoplasm to release RNAs. The small size of DPC (5–20 nm) is also helpful for tumor targeting. A new generation of DPC polymer has also been synthesized through an improved polymerization process to produce homogeneous polymers. In a recent study, siRNA-cholesterol conjugates were coinjected with DPC instead of using siRNA-DPC conjugates. The coinjection approach has also shown effective gene silencing in the liver.¹⁶¹ The coinjection approach is currently used by Arrowhead Research in their flagship pipeline, ARC-520, which is in phase I trials for the treatment of HBV. For ARC-520, a melittin-like peptide is used, which is similar to PBAVE and also has reversibly masked endosomolytic activities.¹⁶²

GalNac-siRNA is a liver-targeted conjugate delivery platform used by Alnylam in several drug candidates (ALN-TTRsc, ALN-AT3, and ALN-PCSSc).¹⁶³ In this delivery system, three targeting ligands (GalNac) are attached to the 3'-terminus of the passenger strand of siRNA via a triantennary spacer. GalNac can bind to the asialoglycoprotein receptor

(ASGPR) overexpressed in hepatocytes and facilitate the cellular uptake through endocytosis (Figure 5D).

In summary, the delivery systems discussed above have all demonstrated in vivo delivery efficacy and have been used in human clinical trials. They are diverse in the working mechanism and have different design features including size, chemical structure, and preparation method. “All roads lead to Rome” is a good summary of the current status of RNA therapeutics delivery approaches. At the same time, we also notice that a successful delivery system usually includes multiple functional elements to overcome various barriers to their in vivo delivery and has a good biocompatibility and safety profile for human use.

5. CONCLUSIONS AND PERSPECTIVES

miRNAs play significant roles in the development of resistant prostate cancers by targeting multiple signaling pathways or mechanisms. Deregulated miRNAs are associated with the occurrence of chemoresistant prostate cancers. Therefore, correction of aberrant miRNAs could be a strategy to overcome resistance in prostate cancer therapy. Many miRNAs related to drug resistance have been discovered, and additional ones are expected to be identified in the future. We are expecting to have a better understanding regarding the mechanisms of drug resistance in prostate cancers. The identification of the most critical miRNA(s) that are associated with drug resistance will be the cornerstone for the design of therapeutics. It is also noticed that, although many miRNAs have differential expression between normal cells and cancer cells, not all these miRNAs are the cause of cancer development and thus cannot be used as therapeutic targets. In addition, most current miRNA targets are discovered in prostate cell lines because they are more accessible than human prostate cancer tissues. These miRNA targets need to be further validated with translational research or clinical studies before they can become clinically useful therapeutic targets. More miRNA targets will be discovered from human prostate cancer tissues, which will be clinically relevant and more valid.

Antagomirs and miRNA mimics are the two most promising miRNA-based therapeutics. miRNA mimics have demonstrated their great potential for treating cancers, especially drug-resistant cancers, by its ability to simultaneously inhibit multiple oncogenes or signaling pathways. Since the development of cancers and their resistance to treatments are usually caused by the aberrant expression of multiple genes and signaling pathways, the use of siRNAs or small molecule inhibitors targeting a single mRNA (or other intracellular target) can merely induce a modest therapeutic response. However, miRNA mimics can restore the level of an underexpressed miRNA, repress multiple oncogenes and pathways regulated by these miRNA, and thus effectively treat resistant cancers. At the same time, we also need to be cautious about the broad effects of miRNA mimics. Since multiple targets or complex signaling networks are regulated by a single miRNA mimic, a nontraditional approach must be used to evaluate their therapeutic effects by monitoring a broad gene expression profile rather than focusing on a single gene or protein. The prediction of side effects might also be a challenge due to the broad inhibition effects of miRNAs.

Off-target effects are side effects caused during the use of RNAi due to the unintended interactions between RNAi molecules and cellular components. Off-target effects can be divided into two categories based on their mechanisms: (1) specific off-target effects (caused by limited degree of complementarity between siRNA and nontargeting mRNAs); and (2) nonspecific off-target effects (toxicities caused by the activation of Toll-like receptor mediated innate immune response or due to the saturation of cellular RNAi machinery).¹⁶⁴ As we discussed before, miRNA inhibition and miRNA replacement are two major approaches of miRNA-based therapeutics. Antagomirs can be designed to reduce the aberrantly up-regulated oncogenic miRNAs by being perfectly complementary to the specific miRNA target by mispairing at the cleavage site of Ago2. Antagomir recognizes and irreversibly binds to specific target miRNA. Antagomirs work similar to siRNAs in silencing target mRNAs. Therefore, off-target effects will be an issue for the use of antagomir or other similar miRNA inhibitors because they may bind to nontarget miRNAs or even mRNAs or induce innate immune responses. Approaches for minimizing off-target effects include the optimization of antagomir sequences and chemical modifications of its backbone and structure. MiRNA replacement therapy refers to the use of miRNA mimics to restore the down-regulated tumor suppressor miRNAs in cancer cells. Unlike siRNA, miRNA mimics are usually present in cells and are considered as “endogenous” molecules. A miRNA mimic has the same sequence as an endogenous miRNA and thus will not cause off-target effects. Since the same miRNAs are also present in normal cells, the introduction of miRNA mimics into normal cells will be well-tolerated. However, the use of miRNA mimics may still cause side effects. Since our knowledge about the expression levels of particular miRNAs is still very limited and their intracellular levels vary among different cells and same cell exposed to different environment, the delivery of too much miRNAs may cause side effects while too less will not be able to achieve sufficient therapeutic effects.

Significant progress has been made in the target identification and the design of miRNA based therapeutics because of the availability of bioinformatics tools and high throughput research methods. The identification of miRNA therapeutic targets and design of miRNA therapeutics is no longer a significant bottleneck. However, it is widely accepted that the delivery of miRNA therapeutics is still a challenge because of their poor in vivo stability, large molecular weight, hydrophilicity, and other in vivo delivery obstacles. Delivery systems discussed in this review have all demonstrated in vivo efficacy and have been pursued in human clinical trials. Although they are diverse in design features, all have demonstrated great potentials in addressing the delivery challenge. Liposomal systems are the most advanced formulation with several products in the development pipeline. Bioconjugate is also a promising system, which has precisely controlled structure and function. These delivery platforms will jump-start the clinical development of miRNA therapeutics, and some of them will finally become available as approved medications. Also, more delivery systems will be developed through the efforts from researchers from different scientific disciplines including chemistry, polymer sciences, engineering, pharmaceutical sciences, and biology. However, it is worth mentioning that most of the current miRNA delivery systems in clinical trials are to deliver therapeutic agents to the liver through either using liver targeting ligands or relying on the passive accumulation of delivery systems in the liver. The delivery into the liver is relatively less difficult because liver is a well-perfused

tissue and has discontinuous endothelium. However, miRNA delivery to hepatocytes remains a challenge as liposomal and polymeric particulates carrying miRNA are likely to be taken up by Kuffer cells before reaching the hepatocytes.¹⁶⁵ The delivery to prostate cancer or other less accessible tissues will be more difficult. Additional efforts are needed to modify the current systems to make them more efficient in delivering miRNA therapeutics into prostate cancers. In addition to the general properties needed for siRNA delivery, additional features such as prolonged circulation for EPR effects and prostate cancer targeting ligands for active targeting are needed.

In conclusion, recent research and development in the understanding of miRNAs has demonstrated their great therapeutic potential for resistant prostate cancers. Further clinical or translational research will lead to the identification of miRNA targets and design of effective miRNA-based therapeutics. The recent progress achieved by using several different delivery systems is inspiring; however, delivery still remains a great challenge.

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Figure 1.
miRNAs regulate prostate cancer drug-resistance through different mechanisms.

Figure 2.
 Aberrant expression of miRNAs targeting apoptotic pathways in prostate cancer leads to avoidance of apoptosis.

Figure 3. Interrelationship between epithelial-mesenchymal transition (EMT), cancer stem cells (CSCs), chemoresistance, and miRNAs. EMT cells, CSCs, and chemoresistant prostate cancer cells are connected and regulated by miRNAs (ref 125).

Figure 4.

(A) RNA molecules can be modified by changing the nonbridging oxygen in the phosphodiester (left) or 2'-position of the ribose (right). (B) The use of a combination of different modifications to improve the in vivo stability of a siRNA (ref 150).

Figure 5.
Types of miRNA delivery systems: (A) lipid particles; (B) cyclodextrin polymer nanoparticles (CDP); (C) dynamic polyconjugate (DPC); and (D) GalNAc-siRNA.

Table 1**Deregulated miRNAs in AIPCs**

miRNA	targets	refs
Up-Regulated		
miR-125b	P53, Puma, BAK1	14–16
miR-221/-222	P27/Kip1, HECTD2, RAB1A	17–19
miR-32	BTG2	22
miR-21	PDCD4, maspin	20,22
miR-148a	PIK3IP1	22
miR-616	TFPI-2	23
miR-375 378 141		24
Down-Regulated		
miR-146a	ROCK1, EGFR, MMP2	22,23
<i>let-7c</i>	AR	24,25
miR-124	AR, p53	29
miR-34a,c	AR, PSA, Notch-1	27,28
miR-148a	MSK1	32
miR-31	AR, E2F1, E2F2, EXO1, FOXM1, MCM2	33
miR-200b-3p		34
miR-185	AR, CDC6	35
miR-205	AR, PSA	36

Table 2**miRNAs Involved in Apoptosis Avoidance**

miRNA	targets	refs
Up-Regulated		
miR-21	PDCD4, TPM1, MARCKS, PTEN	53–57
miR-32	BCL2L11 (Bim)	46
miR-106b	E2F1, p21/WAF1	46
miR-125b	P14(ARF), mdm2, p53, BAK1, Puma	14–16,47
miR-19b,23b,26a,92a	PTEN, PI3K/Akt, Cyclin D1	52
miR-20a	E2F1	51
Down-Regulated		
miR-15a/16-1	BCL2, CCND1, WNT3A	43
miR-31	E2F6	44
miR-205	BCL2	42,44
miR-34a	BCL2, SIRT1	40
miR-34c	BCL2, E2F3	41
miR-574-3p	BCI-XL	45

Table 3**miRNAs Regulate EMT in Prostate Cancers**

miRNA^a	functions	refs
miR-143	enhance E-cadherin; reduce fibronectin	97
miR-145	enhance E-cadherin; reduce fibronectin; inhibit HEF1	97,98
miR-29b	enhance E-cadherin; reduce N-cadherin, Twist, and Snail	99
miR-34b	enhance E-cadherin; reduce vimentin, ZO1, N-cadherin, and Snail	100
miR-200c, 205	enhance E-cadherin; reduce ZEB1, ZEB2, and vimentin	101
miR-200	enhance E-cadherin; reduce N-cadherin, Snail, ZEB1, ZEB2, Slug	102,103
miR-203	reduce N-cadherin and vimentin; modulate Wnt signaling pathway	104
miR-23b/27b	reduce E-cadherin; inhibit Rac1 activity	105

^aThese miRNAs are down-regulated during EMT.