Oncomir miR-125b Suppresses p14ARF to Modulate p53-Dependent and p53-Independent Apoptosis in Prostate Cancer

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Abstract

MicroRNAs are a class of naturally occurring small non-coding RNAs that target protein-coding mRNAs at the post-transcriptional level and regulate complex patterns of gene expression. Our previous studies demonstrated that in human prostate cancer the miRNA miR-125b is highly expressed, leading to a negative regulation of some tumor suppressor genes. In this study, we further extend our studies by showing that miR-125b represses the protein product of the ink4a/ARF locus, p14ARF, in two prostate cancer cell lines, LNCaP (wild type-p53) and 22Rv1 (both wild type and mutant p53), as well as in the PC-3/46C prostate cancer xenograft model that lentivirally overexpressed miR-125b. Our results highlight that miR-125b modulates the p53 network by hindering the down-regulation of Mdm2, thereby affecting p53 and its target genes p21 and Puma to a degree sufficient to inhibit apoptosis. Conversely, treatment of prostate cancer cells with an inhibitor of miR-125b (anti-miR-125b) resulted in increased expression of p14ARF, decreased level of Mdm2, and induction of apoptosis. In addition, overexpression of miR-125b in p53-deficient PC3 cells induced down-regulation of p14ARF, which leads to increased cell proliferation through a p53-independent manner. Thus, we conclude that miR-125b acts as an oncogene which regulates p14ARF/Mdm2 signaling, stimulating proliferation of prostate cancer cells through a p53-dependent or p53-independent function. This reinforces our belief that miR-125b has potential as a therapeutic target for the management of patients with metastatic prostate cancer.

Introduction

Metastatic prostate cancer (CaP), by progressing to castration-resistant CaP (CRPC), represents a major threat to the life of American men, resulting in estimated 28,170 deaths from this disease in 2012 [1]. Patients with metastatic CaP are customarily treated with androgen deprivation therapy (ADT). Unfortunately, failure of ADT inevitably occurs and the patient’s tumor becomes CRPC. It is known that during CRPC progression CaP cells use a variety of androgen receptor (AR)-dependent and independent pathways to survive and flourish in an androgen-depleted environment [2]. Although several attempts have been made to characterize the molecular signature of CRPC, the precise mechanisms leading to CRPC are not completely understood. In recent years, the discovery of microRNAs (miRNAs) has uncovered a new layer of complexity that governs the mechanisms involved in regulating CRPC [3,4].

MicroRNAs are small non-coding RNAs that function as sequence-specific regulators of gene expression through translational repression and/or transcript cleavage [5]. Studies have shown that miRNAs play key roles in cellular processes of differentiation, proliferation, apoptosis and metabolic homeostasis [6]. Moreover, miRNAs can function as either tumor suppressors or oncogenes, depending on whether they specifically target oncogenes or tumor suppressor genes [7]. In this regard, tumor suppressive miRNAs are usually under-expressed while oncogenic miRNAs tend to be over-expressed in cancer [8]. Studies have shown that miR-125b is oncogenic. Overexpression of miR-125b was reported in colon cancer [9], bladder cancer [10], ovarian cancer [11] and leukemia [12]. We previously reported that clinical CaP tumors express increased levels of miR-125b compared to benign tissues [13]. Additionally, several studies have indicated that miR-125b is highly expressed in CaP, particularly in metastatic and invasive CaP tumors [14,15]. Recently, we investigated the function of miR-125b and observed that overexpression of miR-125b promoted xenograft tumor growth in both intact and castrated mice [16]. Moreover, we demonstrated that miR-125b directly targets several tumor suppressive and proapoptotic genes including p53, Bak1 and Puma [13,16].

The cellular level and activity of p53 is maintained by a complex circuit comprised of p14ARF/Mdm2/p53 [17]. p14ARF was verified to be a potent tumor suppressor both in vitro and in vivo [18] and has been proposed to be the most important member of this surveillance circuit. Expression of p14ARF is induced in response to activated oncogenes such as Ras [19], c-Myc [20], Abl [21] and E2F-1 [22] as well as during replicative senescence [23]. p14ARF mediates the sequestration and subsequent degradation of
the p53-antagonist Mdm2 through the ubiquitin/proteasome pathway, which results in the stabilization (increased half-life) of p53 [17] and the consequent activation of its downstream target genes, such as p21 (cyclin-dependent kinase inhibitor 1A), Puma (p53-upregulated mediator of apoptosis), and Bax (BCL2-associated X protein) [24,25]. Since these molecules are key components in the p53 network, modulation of their expression can disrupt the normal balance between apoptosis and cell proliferation. This observation is further substantiated by our studies showing that inactivation or down-regulation of p53, Puma and Bak1 by miR-125b is associated with CRPC [13,16].

To further elucidate the role of miR-125b in the development of CRPC and its underlying molecular mechanisms, in this study we investigated the involvement of miR-125b in modulating the p53 network by targeting p14ARF, which is supported by our identification of a potential miR-125b binding site in the 3'UTR of p14ARF gene. We expect our studies to provide new insight into the molecular mechanisms related to tumorigenesis and castration resistant growth of CaP and help in facilitating the application of miR-125b as a target for CaP treatment.

Materials and Methods

Antibodies and reagents

For Western blotting analysis, anti-p14ARF (sc-8340), anti-Mdm2 (sc-9635), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); anti-Bak1 (3814), anti-Mcl-1 (4572), anti-Bcl-XL, Mdm2 (sc-965), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); anti-p53 (3154), anti-Bcl-XL, anti-caspase 3 (9662), anti-SMAC (2954) and anti-p21 (DCS60) were purchased from Cell Signaling Technology (Danvers, MA); anti-Puma (PC686), anti-p53 (OP33) from Calbiochem (Billerica, MA); anti-β-actin (clone AC-15) from Sigma (St. Louis, MO). Synthetic miR-125b mimic (miR-125b-5m), miRNA negative control (miR-NC), anti-miR-125b and anti-miRNA negative control (anti-miR-NC) as well as the pMIR-REPORT Luciferase vector were purchased from Ambion (Grand Island, NY). Both p14ARF siRNA (sip14) and Bak1 siRNA (siBak) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Lines and transfection

Human CaP cell lines PC3, 22Rv1 and LNCaP were obtained from the American Type Culture Collection (Manassas, VA). All the cell lines were routinely maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum containing antibiotics and multivitamins. For transient transfection, cells were plated onto 6-well plates one day before the transfection and maintained in serum-containing medium without antibiotics. The following day, cells were transfected with either miRNA or siRNA using lipofectamine 2000 (Invitrogen, Grand Island, NY) according to the manufacturer instructions.

Western blot analysis

Cells were grown to 70–80% confluence and lysed using the cell lysis buffer (Cell Signaling Technology) supplemented with phenylmethylsulfonyl fluoride (1 mmol/L). After 20 min of incubation on ice, lysates were centrifuged at 13,000 RPM for 20 min and protein concentrations in the supernatant were determined using BCA kit (Pierce, Rockford, IL). Total protein (50 μg per sample) in 3× protein sample buffer (50 mmol/L Tris-HCl [pH 6.8], 2% SDS, 10% glycerol, 0.25% β-mercaptoethanol, bromophenol blue (1 mg/mL) were separated on SDS-polyacrylamide gel (Bio-Rad, Hercules, CA), and then transferred to Immobilon PVDF membrane (Millipore, Billerica, MA). After blocking with 5% non-fat dry milk in Tris-buffered saline/0.05% Tween 20 (TBST), the membrane was incubated with a specific primary antibody followed by the horseradish peroxidase-conjugated secondary antibody. Protein bands were detected by enhanced chemiluminescence. The expression level of protein was measured by quantitative densitometric analysis.

Luciferase assay

The human p14ARF 3'UTR sequence containing the putative miR-125b binding site was amplified by PCR from LNCaP cDNA and cloned into the pMIR-REPORT luciferase vector downstream of the luciferase gene. The p14ARF 3'UTR lacking this miR-125b binding site was used as control. The PCR products cloned into the plasmid were verified by DNA sequencing. For the luciferase assay, cells (4×10^3 per well) were seeded into 24-well plates and cultured for 24 hrs. Cells were then co-transfected with reporter plasmids and 100 nM synthetic miR-125b or miRN. The pRL-SV40 Renilla luciferase plasmid (Promega, Madison, WI) was used as an internal control. Two days later, cells were harvested and lysed with passive lysis buffer (Promega). Luciferase activity was measured using a dual luciferase reporter assay (Promega). Luciferase activity was normalized by Renilla luciferase activity.

Co-immunoprecipitation assay

The protein interaction between p14ARF and Mdm2 was detected by co-immunoprecipitation assay. Total protein lysates from miR-125b- or miR-NC-transfected 22Rv1 cells were prepared in the cell lysis buffer. Protein (1.0 mg/0.5 ml) was pre-cleared by mixing with 20 μl of protein A beads and the supernatant was immunoprecipitated at 4°C overnight with a rabbit anti-p14ARF polyclonal antibody or normal rabbit IgG (Cell Signaling Technology). The precipitated proteins were fractionated in a 12% SDS-PAGE gel followed by Western blotting detection of Mdm2 protein using the anti-Mdm2 antibody.

TUNEL assay

TUNEL assay was performed using an in situ cell death detection kit (Roche, Indianapolis, IN) according to the manufacturer’s instruction. Briefly, p53-positive 22Rv1 or p53-null PC3 cells (1×10^5/well) were seeded into individual wells of 4-well chamber slides. After 24 hrs, cells were transfected with 50 nM miR-125b, 50 nM anti-miR-125b and 100 nM sip14, alone or in different combinations. Untreated and irradiated cells were used as negative and positive controls. Medium was removed 72 hrs after the transfection and slides were rinsed twice with PBS, fixed in a fixation solution (4% paraformaldehyde in PBS, pH 7.4) for 1 hr at RT. After fixation, slides were rinsed twice with PBS and incubated in permeabilization solution (0.1% Triton X-100) for 2 min on ice. 50 μl of the TUNEL reaction mixture (50 μl of enzyme solution+450 μl of label solution) was added to each slide. For the negative control, only 50 μl of the label solution was added. DAPI was used as a nuclear counterstain. Slides were incubated in a humidified atmosphere for 60 min at 37°C in the dark. Fluorescence microscopy was performed to visualize cells and acquire digital images using an excitation wavelength in the range of 450–500 nm and emitted in the range of 515–565 nm.

WST-1 assay

Cells (4.5×10^4/well) were plated in 96-well plates in RPMI medium containing 10% FBS. After being cultured for 24 hrs, cells were transfected with 50 nM miR-125b or anti-miR-125b. After five hrs, cells were treated with fresh medium. Tetrazolium-based cell proliferation assay (WST-1, Promega) was carried out according to the manufacturer’s protocol.
Colony assay
22Rv1 (3×10^3/well) and LNCaP (4×10^3/well) were separately plated in six-well plates and transfected with miR-125b or anti-miR-125b at a concentration of 100 nM using lipofectamine 2000. After two weeks, cell colonies were counted after staining in 20% methanol and crystal violet.

Results
miR-125b down-regulates p14ARF in CaP cells
Previous studies demonstrated that the tumor suppressor gene p14ARF is significantly down-regulated in CaP tissues [26]; however, how p14ARF is down-regulated remained poorly understood. Using the TargetScan algorithm, a potential miR-125b binding site was identified in the 3′-UTR of p14ARF mRNA. We thus investigated the effect of miR-125b on the regulation of p14ARF in CaP cells. To do this, LNCaP and 22Rv1 cells were transfected with synthetic miR-125b to elevate the cellular miR-125b abundance, or with anti-miR-125b to repress miR-125b activity. As shown by Western blot and quantitative densitometric analyses, compared to the miR-NC treatment, miR-125b induced reduction of p14ARF expression by 80% in LNCaP cells (Figure 1A, top panel) and 60% in 22Rv1 (Figure 1A, bottom panel) cells.

Figure 1. MiR-125b down-regulates p14ARF in CaP cells. A) Western blot analysis of expression levels of p14ARF in LNCaP (top) and 22Rv1 cells (bottom). Cells grown in 10% FBS media were transfected with 50 nM of miR-125bm or anti-miR-125b (anti-125b) for 72 hrs or treated with 5.0 nM of R1881 androgen for 48 hrs. Then, 50 μg of protein per sample was analyzed. Both miR-negative control (miR-NC) and anti-miR negative control (anti-NC) were used as controls, and β-actin was used as a loading control. B) Western blot analysis of expression levels of p14ARF, mdm2 and p53 in lenti-miR-125b-overexpressed PC-346C xenograft tumor. Both untreated xenograft (untreat.) and lenti-miRNA control vector-infected PC-346C xenograft (vector) were used as controls. In both A and B, the numbers under the gels are the average fold changes of p14 ARF protein from three independent gels relative to the corresponding controls. Fold changes were calculated by scanning the p14ARF bands and normalizing for β-actin bands. C) Luciferase assay of miR-125b binding to the 3′-UTR of p14ARF mRNA in LNCaP cells. The assay was repeated three times with each assay being performed in three wells and similar results were obtained each time. The representative results are shown as a mean ± SD (n = 3).

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Figure 2. MiR-125b regulates the p53 network. A) Western blot analysis of Mdm2 and p53 in miR-125bm-treated LNCaP (top) and 22Rv1 cells (bottom). Cells were transfected with 50 nM of miR-125bm or miR-negative control (miR-NC) for 72 hrs. Equal amounts of protein (50 μg) were used to detect the expression levels of Mdm2, p53, p21 and Puma. B) Western blot analysis of p14ARF, Mdm2 and p53 in p14ARF siRNA (sip14)-treated LNCaP (top) and 22Rv1 cells (bottom). Cells were treated with sip14 and the cellular levels of p14ARF, p53 and Mdm2 were analyzed. β-actin was used as a loading control. C) Co-immunoprecipitation analysis of protein interaction between p14ARF and Mdm2 in 22Rv1 cells. Cells were transfected with miR-125bm and 1.0 mg protein was immunoprecipitated with anti-p14ARF antibody or the rabbit IgG. The resultant immunocomplexes were used to detect the level of Mdm2 by Western blot analysis using anti-Mdm2 antibody. Input: 50 μg protein from total cell lysate. IP: immunoprecipitation. IB: immunoblotting.

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miR-125b Modulates p14ARF Causing Apoptosis in CaP

Studies have established that p14ARF accelerates Mdm2 degradation, resulting in p53 up-regulation [27]. We thus asked: does down-regulation of p14ARF by miR-125b affect the expression of Mdm2 and p53 in CaP cells? To address this issue, LNCaP and 22Rv1 cells were treated with miR-125bm and the levels of Mdm2 and p53 were then examined. Compared with miR-NC, treating LNCaP cells with miR-125bm induced a dramatic increase in Mdm2 expression and a significant reduction of p53 level (Figure 2A, top panel). Similarly, in 22Rv1 cells, miR-125bm treatment also enhanced Mdm2 expression and reduced p53 level (Figure 2A, bottom panel). As expected, miR-125bm-mediated down-regulation of p53 induced significant reduction of two direct p53 effectors, p21 and Puma. Similarly, in the miR-125b-overexpressed PC-346C mouse xenograft tumor [16], and found that the level of p14ARF protein was reduced by 60% in the miR-125b-overexpressed tumor compared to miR-NC control tumor (Figure 1B). To determine whether the putative miR-125b binding site in the 3'-UTR of p14ARF mRNA is responsible for the regulation of p14ARF by miR-125b, luciferase reporter vectors containing the 3'-UTR fragment of p14ARF gene were co-transfected with miR-125bm into LNCaP cells. As shown in Figure 1C, cotransfection resulted in an approximately 50% reduction of the enzyme activity in LNCaP cells. We also performed luciferase assay in 22Rv1 cells and a similar result was observed (data not shown). Taken together, the results shown in Figure 1 validate the regulation of p14ARF by miR-125b in CaP cells.

miR-125b-p14ARF signaling regulates the p53 network

Having determined the regulation of p14ARF/Mdm2/p53 signaling pathway by miR-125b, we next examined the effect of regulation of p14ARF by miR-125b on CaP cell proliferation. To do this, both LNCaP cells and 22Rv1 cells were transfected with synthetic miR-125bm and cell proliferation was determined by WST-1 assay. As shown in Figures 3A and 3B, when compared with the miR-NC treatment, transfection with miR-125bm resulted in a 1.5-fold increase in cell proliferation in both cell lines tested. In addition, we performed clone formation assays. Similar to the WST-1 results, miR-125bm stimulated a 10-fold increase in clonogenic survival of LNCaP cells and 2.5-fold enhancement in 22Rv1 cells, and the addition of anti-miR-125b caused a dramatic reduction in the number of colonies as compared to the untreated and anti-miR-NC cells (data not shown). These data support that downregulation of p14ARF by miR-125bm facilitates growth of CaP cells.

Anti-miR-125b induced apoptosis in CaP cells expressing functional p53

Since miR-125b regulates p14ARF/Mdm2 signaling and subsequently affects the p53 network, we evaluated the effect of downregulation of p14ARF by miR-125b on apoptosis in p53-positive CaP cells. First, we tested the release of mitochondrial SMAC (second mitochondria-derived activator of caspase) and activated caspase 3 (Cas-3) in LNCaP and 22Rv1 cell lines that express functional p53. When compared to miR-NC treatment, miR-125bm caused 10% reduction of SMAC and 40% reduction of activated Cas-3 in LNCaP cells and the reduction was 20% and 30% in 22Rv1 cells, respectively (Figure 4A). These cell lines were also treated with anti-miR-125b. Compared to anti-miR-NC treatment, downregulation of miR-125b activity induced approximately one-fold increase in SMAC and activated Cas-3 (Figure 4A). Since anti-miR-125b...
Figure 4. Inactivation of *miR-125b* induces apoptosis in p53-positive CaP cells. A) Detection of SMAC and activated caspase 3 (Cas-3) in LNCaP (left) and 22Rv1 (right) cells. Cells were transfected with 50 nM miR-125bm or 50 nM anti-miR-125b (anti-125b) for 5 days, and the levels of SMAC and Cas-3 were measured by Western blot analysis. β-actin was used as loading control. The numbers under the gels are the average fold changes of SMAC and Cas-3 from three independent gels relative to the corresponding controls. B) Detection of anti-miR-125b-induced apoptosis in 22Rv1 cells. Cells were transfected using 50 nM anti-miR-125b for 72 hrs and apoptotic cell death was detected using TUNEL assay. The green nuclear fluorescence indicates the apoptotic cleavage of nuclear DNA (left). For quantitation of apoptotic cell death, 400 cells were counted and apoptosis is expressed as % apoptosis (apoptotic cells/400 × 100%). Quantitative analysis was performed three times and result was expressed as mean ± SE.
upregulates SMAC and activated caspase 3, we thus analyzed anti-miR-125b-induced apoptotic cell death by using a TUNEL assay. 22Rv1 cells were transfected with miR-125bm or anti-miR-125b. No apoptotic cell death was observed in miR-125bm-treated 22Rv1 cells. In contrast, treatment of 22Rv1 cells with anti-miR-125b caused 63% of cells to undergo apoptosis (Figure 4B). To validate that miR-125b modulates p53-dependent apoptosis through p14 ARF, 22Rv1 cells were treated with anti-miR-125b, followed by p14ARF silencing. It was found that antisense to p14ARF (sip14) dramatically decreased apoptotic death in miR-125b-inactivated 22Rv1 cells (Figure 4C). As expected, p14ARF silencing stimulated proliferation of these 22Rv1 cells (data not shown). In addition, the expression levels of several pro-apoptotic factors were assessed with Western blot analysis. Indeed, treatment with anti-miR-125b induced an upregulation of p14ARF protein in 22Rv1 (n = 3) (right). Cells treated with irradiation (IR, 6 Gy) were used as a positive control.

Figure 5. Evaluation of miR-125b effect on growth and apoptosis in p53-negative CaP cells. A) Detection of p14ARF and Mdm2 levels in p53-null PC3 cells. Cells were transfected with 50 nM of miR-125bm or anti-miR-125b for 72 hrs. The expression levels of both p14ARF and Mdm2 were analyzed by Western blot assay. β-actin was used as a loading control. B) Detection of anti-miR-125b-induced apoptosis in PC3 cells. Cells were transfected using 50 nM anti-miR-125b for 72 hrs and apoptotic cell death was detected using TUNEL assay. The green nuclear fluorescence indicates the apoptotic cleavage of nuclear DNA (left). For quantitation of apoptotic cell death, 400 cells were counted and apoptosis is expressed as % apoptosis (apoptotic cells/400×100%). Quantitative analysis was performed three times and result was expressed as mean ± SE (n = 3) (right). Cells treated with irradiation (IR, 6 Gy) were used as a positive control. C) miR-125b promotes the growth of p53-null, Bak1-silenced PC3 cells. Cells were treated with 50 nM miR-125bm for 5 days and cell proliferation was measured using WST-1 assay. The results are expressed as the growth inhibition relative to that of miR-NC (mean ± SD, n = 4). Inset: Bak1 expression in Bak1-silenced PC3 cells. D) TUNEL assay of apoptotic death of PC3 cells that were treated with anti-miR-125b followed by p14ARF antisense (sip14). Result was expressed as mean ± SE (n = 3). E) Western blot analyses of p14ARF and Bak1 levels in PC3 cells. Top: PC3 cells were transfected with anti-miR-125; bottom: anti-miR-125-transfected PC3 cells were treated with sip14. Both anti-miR-NC (anti-NC) and scramble siRNA were used as controls.

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cells, while addition of sip14 resulted in obvious downregulation of p14ARF (60%), p53 (30%) and Bak1 (70%), compared to the scramble siRNA treatment (Figure 4D). These data strongly suggest that miR-125b/p14ARF signaling targets the p53 network, regulating p53-dependent proliferation and apoptosis in CaP cells.

miR-125b/p14ARF signaling mediates p53-independent growth inhibition

In the above experiments, we validated that miR-125b/p14ARF signaling is involved in p53-dependent mechanisms in CaP cells. However, studies demonstrated that inactivation of p53 function occurs in a portion of patients with metastatic CaP [28,29]. Does miR-125b/p14ARF signaling regulate cell growth and apoptosis in these p53-deficient CaPs? We used p53-null PC3 CaP cells to address this issue. We examined the influence of altered miR-125b activity on the expression levels of p14ARF and Mdm2 proteins. Similar to that in p53-functional LNCaP and 22Rv1 cells, miR-125bm transfection decreased expression of p14ARF by 36% and increased Mdm2 by 43% in PC3 cells while anti-miR-125b induce an obvious upregulation of p14ARF and a slight repression of Mdm2 (Figure 5A). We next tested whether miR-125b affects the proliferation and apoptosis of PC3 cells. To this end, PC3 cells were treated with anti-miR-125b and apoptotic cells was detected with the TUNEL assay. It was found that treatment with anti-miR-125b caused 50% of these cells to undergo apoptosis (Figure 5B). Since Bak1 was reported to mediate p14ARF-induced apoptosis in many biological processes [5], we identified miR-125b as a direct negative regulator of p14ARF. Our study validated that miR-125b can directly repress the p14ARF protein expression through its interaction with the binding site in the 3′-UTR of the human p14ARF mRNA, thereby inhibiting p14ARF function in CaP cells. Moreover, we observed that miR-125b inhibits interaction between p14ARF and Mdm2, with the downstream consequence of modulating the p53 network. Our report is the first to identify miR-125b as a direct regulator of p14ARF in CaP cells. Our data showed that the negative regulation of p14ARF by miR-125b is physiologically relevant to cellular function, as an increase in miR-125b level stimulates cell proliferation and represses intrinsic apoptosis both in androgen-dependent LNCaP cells and CRPC 22Rv1 cells. The point is underscored by the fact that increasing miR-125b in LNCaP cells results in an 80% reduction in p14ARF, while the reduction is 60% in 22Rv1 CRPC cells; when miR-125b is elevated through treatment of these cells with R1881, the reduction of p14ARF in LNCaP again is 80%, while it is 20% in 22Rv1 cells. Additionally, when the reverse is carried out by using anti-miR-125b to counter the activity of endogenous miR-125b in the two CaP cell lines, the increase in p14ARF is 40% and 30%, respectively. Thus, the downregulation of p14ARF by overexpressed miR-125b and subsequent repression of p53 activity are involved in prostatic tumorigenesis and progression.

The tumor suppressor p53 is an important transcription factor that safeguards the cell against tumorigenesis by maintaining a fine balance between apoptosis and cell proliferation [32]. Increasing evidence has shown that the p14ARF/Mdm2/p53 pathway is essential for maintaining and regulating p53 expression and function, and an alteration of components in the pathway, like downregulation of p14ARF or upregulation of Mdm2, can significantly alter p53 intracellular level and activity [33]. In this

Figure 6. Schematic model of miR-125b-controlled oncopathway in CaP cells. In CaP cancer cells, p14ARF facilitates apoptosis in a p53-dependent (left) and p53-independent (right) manner [30]. Since miR-125b directly targets p14ARF and other pro-apoptotic molecules, deregulation of miR-125b can modulate proliferation and apoptosis in both p53-positive and p53-deficient CaP cells. Black arrows represent upregulated molecules and white arrows represent downregulated molecules. Broken arrow indicates undefined upregulation of Bak1 activity by p14ARF.

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miR-125b Modulates p14ARF Causing Apoptosis in CaP

In the last decade, considerable new molecular information has underlined the mechanisms of response and resistance of metastatic CaP to different interventions. The body of work has led to FDA approval of five new therapies for CRPC (docetaxel, cabazitaxel, Provenge, abiraterone acetate, and MDV3100). Regrettably, each improve survival by only approximately four to five months [37,38]. The latter two agents, abiraterone acetate and MDV3100, underscore that while the AR is critical to the process of controlling CaP, targeting it alone will not be sufficient. We believe that the data presented in this paper and in our previous publications [13,16] offer hope that lowering miR-125b in patients with metastatic CaP will attack not a single pathway, but a complicated oncopathway. Modulation of the oncopathway will be both a treatment in itself as well as augmenting presently used interventions. Our ongoing studies are aimed at proving this hypothesis.

In summary, we observed that overexpression of miR-125b negatively regulates the expression of the tumor suppressor protein p14ARF and aberrant expression of miR-125b promotes cell proliferation potential and inhibits apoptosis. Interestingly, inactivation of miR-125b using anti-miR-125b affects apoptosis involving both p53-dependent and p53-independent pathways. Therefore, our data presented in this study suggest that oncomir miR-125b has a great potential in the design of combination therapy for CaP treatment.

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Author Contributions

Conceived and designed the experiments: SA XBS HJK RWD. Performed the experiments: SA XBS LX AHM. Analyzed the data: SA XBS HJK RWD. Wrote the paper: SA XBS HJK RWD.

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