# Chapter 14
# MicroRNAs in the Development and Progression of Prostate Cancer

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**Abstract** Prostate cancer is one of the cancers with high incidence and mortality rates among US men. Even though prostate-specific antigen screening and other diagnostic approaches have improved patient prognosis, an urgent need for molecularly-based biomarkers of progression persists. MicroRNA (miRNA) dysregulation can have profound consequences as the loss of tumor-suppressive miRNAs enhances the expression of target oncogenes, while the upregulation of oncogenic miRNAs represses the target tumor suppressor genes. The realization of the importance of miRNAs in biological processes has led to a quest to understand the molecular mechanisms regulating miRNAs using a variety of model systems and to entertain the possibility of using miRNA antagonists or mimics for anticancer therapy. The promise of miRNAs as diagnostic and prognostic markers will also need to be realized by using validated datasets and standardized methodologies which will give us a way to compare and verify expression profiles. Here, we discuss the past and current studies which have led us to this point as miRNA-based therapeutics make their way into clinical trials.

**Keywords** MicroRNAs · Prostate cancer · CRPC · Progression · Androgen receptor · Biomarkers · Circulating miRNA · Clinical trials · p53 · Epigenetics

## 1 Introduction

Prostate cancer (CaP) ranks first in incidence and second in cancer-related mortality rates among males in the US [1]. There were 241,740 cases and 28,170 deaths in 2012, which makes it the second deadliest after lung cancer. Androgen deprivation and radiation constitute the first-line therapy against androgen-dependent as well as castration-resistant prostate cancer (CRPC). Even though most patients who fail androgen deprivation therapy (ADT) go on to develop CRPC, the majority of patients

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S. R. Singh, P. Rameshwar (eds.), *MicroRNA in Development and in the Progression of Cancer,* DOI 10.1007/978-1-4899-8065-6_14,

continue to live with indolent CaP. There is an urgent need to predict which patients may fail ADT and which may never progress to CRPC. Existing diagnostic methods and prognostic indicators have improved risk stratification, so that patients who are at higher risk of progression are treated more aggressively, but the problems of overtreatment and subsequent side effects associated with treatment continue to be significant stumbling blocks. By itself, the Gleason score remains the best predictor of recurrence, progression, and death [2]. Additional prognostic modalities such as the Cancer of the Prostate Risk Assessment (CAPRA) score have improved outcomes, but molecularly-based biomarkers have been in short supply. One of the areas in which molecular biomarkers can best help is the characterization of biopsy specimens into indolent and aggressive cancers, and this is where small noncoding RNAs such as microRNAs (miRNAs) enter the picture.

miRNAs are small non-protein-coding RNA molecules that regulate gene expression by binding to the 5′- or 3′-untranslated regions (UTRs) of target transcripts, inhibiting their translation or inducing degradation. miRNA genes are frequently located in fragile sites, minimal regions of loss of heterozygosity, and minimal regions of amplification [3]. Oncogenic miRNAs are associated with regions of amplification, while tumor suppressor miRNAs are associated with frequently deleted chromosomal regions. Single nucleotide polymorphisms in miRNA genes can also be predictive of cancer recurrence and survival [4]. miRNA genes can be mono- or polycistronic, and can be intergenic, intronic, or exonic [5]. When located in exons of protein-coding genes, miRNAs frequently share the promoter of the host gene and are cotranscribed with it. The genes encoding miRNAs are transcribed in much the same way as protein-coding genes are, and produce a primary transcript of several hundred nucleotides in length. After multiple stages of processing in both the cytoplasm and nucleus, the mature 21–23-nucleotide (nt) miRNA is generated. Mature miRNAs are incorporated into RNA-induced silencing complexes (RISCs), and bind with imperfect complementarity to specific seed sequences in their target genes. Recent estimates predict that approximately 30 % of all genes are regulated by miRNAs where each miRNA can target multiple genes, and each messenger RNA (mRNA) can be targeted by multiple miRNAs, generating a complex network of gene regulation which can have a profound impact on cellular programs. These layers of complexity are required since miRNAs can be double-edged swords due to the immense variety of possible downstream effects. miRNAs are well on the way to becoming the central focus of many fields of research in oncology, as evidenced by the exponential growth in number of publications since their discovery a decade ago.

## 2 miRNAs Deregulated in CaP

A comprehensive literature search using the keywords “miRNAs and prostate cancer” reveals > 350 studies which focused on examining the role of miRNAs in CaP. A large number of miRNAs have already been explored in CaP with regard to their biological function, expression profile in cell lines and clinical samples, prognostic/

diagnostic ability, and population dynamics. Results from most of these studies to date have been summarized in Table 14.1. It is interesting to note that most of the miRNAs studied so far are down-modulated in CaP, denoting their role as primarily tumor suppressors, whereas the number of oncogenic miRNAs, commonly referred to as oncomiRs, is only a handful. This makes the list of miRNAs that may potentially be used as markers of progression and recurrence short, since upregulated genes are considered to be more reliable biomarkers compared to downregulated genes. Several miRNAs target the androgen receptor (AR), which is an important survival factor for prostatic tissues, both in benign stages and in neoplastic stages. The list of miRNAs that target the AR is similarly short, which is conducive to developing strategies to modulate AR expression and functions.

## 3 Importance of the miRNA–AR Axis in CaP

Androgen signaling and AR expression levels are critical in the carcinogenesis and survival of CaP, even in hormone-refractory stages. Activation of target genes by AR is required for the normal functioning of the prostate gland as well as for the progression of CaP. The overexpression and aberrant ligand-independent activation of AR have been implicated in the development of CRPC. AR signaling aberrations and miRNAs appear to be closely linked to the progression of CaP, either by regulation of AR signaling by miRNAs or by androgen-dependent regulation of miRNA expression. The disruption of this AR–miRNA axis may contribute to the development of CaP. AR-targeting miRNAs presumably maintain expression of AR at optimal levels. The loss of AR-targeting miRNAs can potentially lead to elevated levels of AR expression and contribute to the development of CRPC. In addition, shortening of AR 3′ UTR resulting from alternative splicing or alternative polyadenylation may lead to the loss of miRNA binding sites, which would potentially disrupt miRNA-dependent repression of AR leading to AR overexpression [6].

A systematic analysis performed to identify potential AR-targeting miRNAs revealed that miRs-135b, -185, -297, -299-3p, -34a, -34c, -371-3p, -421, -449a, -449b, -634, -654-5p, and -9 were found to directly target a longer 3′UTR than previously used by most target prediction algorithms [7]. Of these, miRs-34a and -34c are of interest due to their close association with the tumor suppressor p53. It has been postulated that miRs of the miR-34 family are responsible for most of the functions of p53 [8], even though miR-34-deficient mouse models have failed to corroborate these results [9]. Studies from our laboratory have demonstrated that miRNAs which target the AR indirectly may also play an important role in CaP progression. We showed that miR-let-7c is underexpressed in CRPC and indirectly regulates the expression of AR via modulation of c-Myc, one of the transcription factors binding to AR promoter [10, 11]. In a study which examined the regulation of miRNAs by androgen, 17 miRNAs were >1.5-fold upregulated or downregulated upon dihydrotestosterone (DHT) treatment in CaP cell lines, and 42 after castration in AR-positive xenografts. Only four miRNAs (miRs-10a, -141, -150*, and -1225-5p) were found to be regulated by androgen in both

**Table 14.1** MiRNAs differentially expressed in prostate cancer

| miRNA | Expression | Target | Altered function | References |
|---|---|---|---|---|
| let-7 family | Downregulated | Ras, c-Myc, Cyclin D1, Cdc25A, EZH2, PBX3, Lin28 | Induces apoptosis, inhibits proliferation, regulates AR signaling | [10, 11, 39, 56] |
| miR-1 | Downregulated | SLUG, PNP, FN1, LASP1, PTMA, BRCA1, CHK1, MCM7 | Inhibits invasion, proliferation, EMT, tumorigenesis, prognostic marker | [57–59] |
| miR-7 | Downregulated | ERBB2 | Inhibits cell proliferation, tumor progression | [60] |
| miR-9 | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-10a | Upregulated | HOXA1 | Affects gene expression, cell differentiation | [61] |
| miR-15a | Downregulated | FGF2, FGFR1, CCND1, WNT3A | Reduces tumor-supportive ability of stromal cells | [26, 62] |
| miR-16 | Downregulated | FGF2, FGFR1, CDK1, CDK2, CCND1, WNT3A | Reduces tumor-supportive ability of stromal cells; inhibits proliferation, inhibits growth of metastases | [26, 62, 63] |
| miR-17* | Downregulated | Mitochondrial antioxidant enzymes | Suppresses tumorigenesis | [64] |
| miR-17-3p | Downregulated | Vimentin | Reduces tumor growth; putative tumor suppressor | [65] |
| miR-20 | Upregulated | VEGFA, CDKA1A, NCOA3, HIF1A, CAV1 | High in CaP; biomarker for CaP | [66] |
| miR-20a | Upregulated | CX43 | Promotes proliferation, tumor growth; high in CaP; increased in high Gleason grade | [67, 68] |
| miR-21 | Upregulated | RECK, MARCKS, PDCD4, PTEN, TPM1, SPRY2, TIMP3 | Oncogenic; increases invasiveness; resistance to apoptosis | [17, 18, 34] |
| miR-22 | Downregulated | PTEN | Induces apoptosis and inhibits metastasis | [13, 69, 70] |
| miR-23a/b | Downregulated | Rac1, PRDXIII | Suppresses metastasis and response to hypoxia | [71, 72] |
| miR-24 | Upregulated | FAF1 | Inhibits apoptosis | [16] |
| miR-25 | Upregulated | PTEN | Induces cell proliferation | [16, 73] |
| miR-26a | Up/downregulated | PLAG1, EZH2 | Influences apoptosis, proliferation, invasion | [13, 73, 74] |
| miR-27a | Upregulated | Prohibitin | AR-regulated; induces AR target genes; induces cell growth | [75] |
| miR-27b | Downregulated | Rac1, CYP1B1, NOTCH1 | Inhibits proliferation, regulates hormone metabolism | [13, 71, 74] |
| miR-29a/b | Downregulated | VEGFA, hnRNP-K, DKK1, sFRP2 | Reduces cell proliferation; regulates cell differentiation and immune response | 13, 76] |

**Table 14.1** (continued)

| miRNA | Expression | Target | Altered function | References |
|---|---|---|---|---|
| miR-30b/c | Up/downregulated | BCL-9, MTA1, Snail1, GalNAc | Biomarker, influences metastasis | [13, 77] |
| miR-30d | Downregulated | GRP78 | Induces apoptosis | [78] |
| miR-31 | Downregulated | BCL-2L2, E2F6 | Promotes apoptosis, inhibits proliferation | [79, 80] |
| miR-32 | Upregulated | BTG2, Bim, C9orf5 | AR-regulated; reduces apoptosis; short progression-free survival | [16, 81] |
| miR-34a | Downregulated | c-Myc, BCL-2, SIRT-1, E2F3, MET, CDK4–6, DLL1, CD44 | Suppresses malignancy; inhibits proliferation and survival and metastasis | [16, 82–86] |
| mir-34b | Upregulated | CDK6, CREB, c-Myc, MET | Affects cell cycle and proliferation | [16, 83] |
| miR-34c | Downregulated | CDK6, MET, c-Myc, E2F3 | Inhibits proliferation | [16, 83] |
| miR-92 | Downregulated | Bim | Induces apoptosis | [13, 73, 87] |
| miR-96 | Upregulated | FOXO1, hZIPs | Affects apoptosis | [49, 50] |
| miR-100 | Up/downregulated | PSA, SMARCA5, SMARCD1, Ras, c-Myc | Independent predictor of biochemical recurrence; decreases proliferation | [88, 89] |
| miR-101 | Downregulated | COX-2, EZH2 | Genomic loss during progression; Suppresses growth, invasion | [20, 90] |
| miR-106a | Upregulated | RB1 | Inhibits apoptosis | [73] |
| miR-106b | Upregulated | PTEN | Proto-oncogenic; cooperates with host gene MCM7 in transformation | [69] |
| miR-107 | Downregulated | Granulin | Inhibits proliferation | [73, 91] |
| miR-125a | Up/downregulated | ERBB2, ERBB3 | Cell proliferation, apoptosis | [13, 16, 92] |
| miR-125b | Upregulated | BAK1, p53, PUMA | AR-regulated; inhibits apoptosis, promotes castration resistance | [14, 15, 48] |
| miR-126 | Downregulated | CRK, Spred1, PIK3R2/p85-beta | Inhibits proliferation, invasion, progression | [74, 93] |
| miR-126* | Downregulated | Prostein | Inhibits metastasis | [93, 94] |
| miR-128a | Downregulated | GOLM1, PHB, TROVE2, TMSB10 | Inhibits invasion, progression | [16, 95] |
| miR-130a | Downregulated | CCNB1, ROCK1, GTF2H1, STX6 | Impairs tumor growth; induces cell cycle arrest | [96] |
| miR-132 | Downregulated | TALIN-2, HBEGF | Induces cell death | [97] |
| miR-133a | Downregulated | EGFR, PNP1 | Inhibits proliferation, invasion | [59, 98] |
| miR-133b | Downregulated | FAIM | Inhibits proliferation, metabolic activity | [99] |
| miR-135b | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |

**Table 14.1** (continued)

| miRNA | Expression | Target | Altered function | References |
|---|---|---|---|---|
| miR-141 | Upregulated | Clock, SHP | Activates AR and metastases | [13, 100–102] |
| miR-143 | Downregulated | KRAS, ERK5, MYO6, KLK4, KLK10 | Inhibits progression, proliferation, migration, invasion, enhances docetaxel sensitivity | [36, 103–106] |
| miR-145 | Downregulated | SWAP70, FSCN1, BNIP3, TNFSF10, MYO6, Myc | Inhibits proliferation, invasion, tumorigenesis, migration, induces apoptosis | [31, 35, 36, 103, 107–109] |
| miR-146a | Downregulated | EGFR, MMP-2, ROCK1, CXCR4 | Inhibits invasion, proliferation, metastasis | [110, 111] |
| miR-148a | Up/downregulated | CAND1, MSK1 | Regulated by androgen, inhibits growth, migration, invasion, increases paclitaxel sensitivity | [112, 113] |
| miR-153 | Upregulated | PTEN | Promotes proliferation | [114] |
| miR-181a | Downregulated | GRP78 | Induces apoptosis | [78] |
| miR-181a-1 | Upregulated | RB1, RBAK | Induces tumor progression | [16, 74] |
| miR-182 | Upregulated | hZIP1 | Regulates zinc homoeostasis, inhibits apoptosis | [16, 49, 50] |
| miR-182-5p | Upregulated | Unknown | High in high-grade CaP | [115] |
| miR-183 | Upregulated | hZIP1 | Regulates zinc homoeostasis | [50] |
| miR-185 | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-193b | Downregulated | Unknown | Reduces growth, putative tumor suppressor | [116] |
| miR-194 | Upregulated | DNMT3a, MeCP2 | Induces genomic instability | [117] |
| miR-195 | Up/downregulated | CDK4, GLUT3, WEE1, CDK6, Bcl-2 | Cell cycle, proliferation, apoptosis | [13, 16, 74] |
| miR-200a/b | Up/downregulated | SLUG, PDGF-D, NOTCH1, Lin28B | Regulates EMT, cell growth | [37, 38, 58, 118] |
| miR-200c | Upregulated | SEC23A, JAGGED1 | Induces cell growth and metastasis, inhibits apoptosis | [119, 120] |
| miR-203 | Downregulated | CKAP2, LASP1, BIRC5, WASF1, ASAP1, RUNX2, survivin | Inhibits proliferation, migration, invasion, EMT | [96, 121, 122] |
| miR-204 | Upregulated | PDEF | Increases cell growth | [123] |
| miR-205 | Downregulated | VEGFA, HRAS, KLK2, NCOR2, E2F6, PKCepsilon | Promotes apoptosis, MET, inhibits proliferation | [76, 79, 96, 124–126] |
| miR-210 | Upregulated | EFNA3, MNT, HOXA1, APC, ELK3 | Induces hypoxia, proliferation and migration | [13, 127] |
| miR-214 | Upregulated | EZH2, N-Ras, PTEN | Induces proliferation and cell cycle | [73] |
| miR-218 | Upregulated | RAS, c-Myc, SMARCA5 | Induces cell proliferation | [128] |

**Table 14.1** (continued)

| miRNA | Expression | Target | Altered function | References |
|---|---|---|---|---|
| miR-221 | Up/downregulated | ARHI, c-Kit, p27kip1 | Oncogenic | [129–133] |
| miR-222 | Up/downregulated | ARHI, c-Kit, p27kip1 | Oncogenic | [80, 129–133] |
| miR-223 | Downregulated | NFI-A | Induces differentiation | [73] |
| miR-224 | Up/downregulated | KLK1, API-5 | Influences progression-free survival | [70, 74, 134] |
| miR-296 | Downregulated | HMGA1 | Inhibits growth and invasion | [135] |
| miR-297 | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-299-3p | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-301a | Downregulated | FOXF2, BBC3, PTEN, COL2A1 | Inhibits proliferation | [132] |
| miR-320 | Downregulated | β-catenin, ETS2 | Inhibits progression | [25, 136, 137] |
| miR-330 | Downregulated | E2F1 | Inhibits growth, induces apoptosis | [138] |
| miR-331-3p | Downregulated | DOHH, ERBB2, KLK4, KLK10, EGFR, HER2 | Inhibits AR and Akt signaling, inhibits proliferation | [21, 60, 106, 139] |
| miR-345 | Upregulated | BAG3 | Induces invasion, metastasis | [13] |
| miR-370 | Upregulated | FOXO1 | Induces proliferation | [140] |
| miR-371-3p | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-373 | Downregulated | CD44 | Suppress CD44 translation, induce invasion | [141] |
| miR-375 | Upregulated | SEC23A | Stimulates proliferation; serum levels predict high risk for progression | [77, 100, 119] |
| miR-421 | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-449a/b | Downregulated | AR, HDAC-1, Cyclin D1 | Induces senescence and growth arrest | [7, 142, 143] |
| miR-488* | Downregulated | AR | Inhibits proliferation, induces apoptosis | [144] |
| miR-521 | Upregulated | CSA | Influences DNA repair | [22] |
| miR-616 | Upregulated | TFPI-2 | Induces castration resistance | [145] |
| miR-634 | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-642-5p | Downregulated | DOHH | Regulates eIF5A activity, inhibits cell proliferation | [139] |
| miR-654-5p | Downregulated | AR | Inhibits androgen-induced proliferation | [7] |
| miR-708 | Downregulated | CD44, Akt2 | Decreases tumorigenicity, regression of established tumors | [146] |

cell lines and xenografts. Of these, miR-141 was found to be expressed more in CaP and CRPC compared to benign prostate hyperplasia [12]. A profiling study analyzed cell lines, xenograft models, and patient samples to establish correlations between AR expression and miRNA signature [13]. AR signaling results in upregulation of miR-125b, which acts as an oncogene in CaP [14]. miR-125b is overexpressed in AR-positive cell lines compared to AR-negative ones and is overexpressed in the majority of CaP patient samples compared to benign prostate tissue [15]. miR-338 has also been shown to be induced by androgen activation in LNCaP cells [16]. AR has been found to induce the oncomiR miR-21 in LNCaP and LAPC-4 cell lines. Elevation of miR-21 was further demonstrated to promote tumor growth and castration resistance in a LNCaP mouse xenograft model [17, 18]. To complicate the role of AR-induced miRNA in CaP, other studies find that AR has anticancer interactions with miRNA. AR is essential to p53-induced apoptosis, which is mediated by miR-34a/c [19], and also induces expression of the antiproliferative miR-101 [20]. Finally, AR signaling was also shown to be indirectly regulated by miR-331-3p in LNCaP cells, suggesting yet another role for miRNA in the AR signaling pathway [21]. Since the only function of miRs is to bind to 3′ UTR and inhibit translation of target genes, androgens may operate via induction of miRs to inhibit repressors of AR function. In concordance, knockdown of DICER in LNCaP cells and in tissues in mice induced the expression of co-repressors, NCoR and SMRT. These studies demonstrate a feedback loop between miRs, co-repressors, and AR and the imperative role of miRs in AR function.

## 4 miRNAs in Radiation Response

Ionizing radiation activates a multitude of survival and death signaling pathways. Previous studies demonstrate that radiation induces changes in a large number of genes, which are involved in DNA repair/synthesis, stress response, and cell cycle control. However, the role of miRNAs and how they integrate into the radiation signaling pathways is largely unknown. About 60% of cancer patients receive radiation treatment, and while it is very effective, some patients may benefit from identification of novel radiosensitizers. It is expected that radiosensitizers should reduce the dose or frequency of radiation treatment and improve disease outcome in patients. In one study, global miRNA profiling was performed to determine important miRNAs in radiation stress response. The study found that of the 330 miRNAs analyzed, 10 miRNAs were significantly downregulated while 5 were significantly upregulated following irradiation in LNCaP and C4-2 CaP cells. They also found that miR-521 played a major role in modulating the radiation sensitivity of CaP cells [22]. Another study performed miRNA array and found that of 132 cancerous miRNAs examined, 10 miRNAs were significantly upregulated by irradiation in LNCaP cells. They also showed that miR-106b induced radioresistance in LNCaP cells by inhibiting the radiation-induced increase in p21 [23]. The identities of the miRNAs found to be modulated by irradiation from both studies are completely different, despite the use of identical experimental systems. One of the possible reasons may be that rapidly occurring changes in miRNA

expression profiles may not be detected in samples collected after longer periods of time after irradiation. One of these studies used samples collected 4 h after irradiation, while another collected samples 24 h after irradiation. These results exemplify the care to be taken when radiation response in miRNA expression profiles is examined and the need for standardization of duration and amount of radiation applied. Another study compared single-dose radiation to fractionated radiation and found that fractionated radiation alters more miRNAs than a single dose. Some miRNAs were altered to a similar extent in both p53-positive and p53-null cells, indicating that p53 may not be the sole determinant of radiation response in CaP cells [24].

## 5 miRNAs in Other Signaling Pathways

miRNAs targeting other important cellular signaling pathways such as Wnt, NF-κB, p53, and cytokine signaling have also been described in CaP. MiR-320 downregulates Wnt signaling by directly targeting β-catenin and suppresses stem cell-like characteristics of CaP cells, possibly reorienting them towards a more differentiated phenotype [25]. The miR-15a-16-1 cluster targets Wnt3a and CCND1 in addition to other oncogenic targets and inhibits proliferation, survival, and invasion of CaP cells [26], indicating that coordinated inhibition of multiple signaling pathways may elicit stronger responses in terms of CaP cell survival. Very few miRNAs targeting either the classical or the alternative NF-κB pathways have been studied in CaP. Recent studies have identified miR-181b as being overexpressed in prostate carcinomas and that it targets CYLD, a known tumor suppressor and inhibitor of classical NF-κB signaling. miR-181b is part of a feedback loop involving STAT3, CYLD, IL-6, and NF-κB and participates in an epigenetic circuit to promote cell transformation [27]. Similarly, miR-21, which is induced by STAT3 and targets PTEN, is involved in induction of NF-κB activation by a positive feedback loop [27]. In addition, miR-21 has been universally reported to be overexpressed in human carcinomas including carcinoma of the prostate [18, 28]. A few recent reports have contradicted the oncogenic role of miR-21 in CaP [29], which goes to show that miRNAs have cell type- and tissue-specific roles in human cancers, and great care should be exercised in extending findings from one human cancer to another.

Several miRNAs have been shown to be downstream of the tumor suppressor p53, which include miR-34 family, miR-192/215, and miR-145—known transcriptional targets of p53. Most of these are downregulated in CaP, which demonstrates their importance in the tumor suppressor network spearheaded by p53. The miR-34 family of miRNAs and miR-145 are also regulated by methylation of promoters [30, 31]. In addition, p53 has been shown to be regulated by a few oncogenic miRNAs, primarily by miR-125b in CaP, which binds to the 3′UTR of p53 and regulates its expression [32]. miR-125b is an androgen-induced miRNA [15], implying that activation of AR signaling represses p53 and its related tumor-suppressive functions.

Cytokine signaling is also modulated by miRNAs in CaP. For example, IL-6 is regulated by the let-7 family, which in turn is regulated by a feedback loop

involving Lin28, STAT3, and NF-κB [33]. IL-6 signaling results in activation of STAT3, which in turn induces the oncomiR, miR-21 [27], which had been shown to be overexpressed in CaP and to mediate proliferation, invasion, and metastasis [17, 18, 34]. The exact mechanisms by which miR-21 operates in CaP are still debated [29] and need to be examined using in vitro and in vivo models. miR-145, which is underexpressed in CaP due to methylation and p53 gene mutation [31], induces expression of pro-apoptotic genes such as TNFSF10 and IL-24 [35]. Epithelial-to-mesenchymal transition (EMT) is defined as the process by which epithelial cells acquire mesenchymal characteristics and transition to an elongated fibroblastic phenotype, thus acquiring attributes such as increased cell motility and invasion. Several miRNAs such as miR-200/200b, miR-143, miR-145, and the let-7 family have been shown to control EMT in CaP [36–39]. These findings collectively indicate that miRNAs occupy a critical niche in cell survival processes and fine-tune the survival and death signals via cross talk with the tumor microenvironment.

## 6 Epigenetic Regulation of CaP-Related miRNAs

Deregulated expression of miRNAs in cancer cells can result due to genomic abnormalities such as chromosomal rearrangements, genomic amplifications, and deletions of miRNA genes, and also due to altered transcriptional and posttranscriptional control of miRNA expression. In addition, epigenetic changes, such as methylation of CpG islands in the promoter regions of miRNA genes, can alter miRNA expression in cancer cells. An extensive analysis of miRNA genes shows that ~50% are associated with CpG islands suggesting their possible regulation by the DNA methylation machinery. In CaP, miRNA deregulation affects epigenetic reprogramming, blockade of apoptosis, promotion of cell cycle, migration, and invasion and is an alternative mechanism to sustain castration-resistant growth. Although several miRNAs have been reported to be regulated epigenetically in CaP, only a few have been experimentally proven to contribute to the disease. Of the miRNAs that are involved in the epigenetic process in CaP, three distinct types can be described: (1) miRNAs that regulate genes of the epigenetic machinery such as miR-101 and miR-449a, which regulate EZH2 and HDAC1, respectively; (2) miRNAs that are epigenetically regulated, such as miR-1 (MCM7, BRCA1), miR-200c/141 cluster (ZEB1/ZEB2), miR-132 (TALIN-2), miR-205 (ZEB1/ZEB2), miR-126 (DNMT1), miR-193b, miR-196b (target(s) unknown), miR-145 (TNFSF10), miR-34 family (SIRT1/CD44), and miR-21 (multiple targets); (3) miRNAs that are involved in the epigenetic regulation of AR, such as miR-34 family, miR-141, miR-494, and miR-29a/b/c (for a complete review see [40]). In addition, AR-regulated miRNAs such as miR-125b and miR-21 have also been shown to be epigenetically regulated [15, 18]. Thus, epigenetic regulation of miRNAs adds an extra degree of complexity to

the picture which needs to be further elucidated to fully realize the potential of manipulating the epigenetic machinery for therapeutic purposes.

## 7 Circulating miRNAs in CaP

Recent evidence suggests that miRNA profiles from tissue sources as well as circulating body fluids may be good tools for prognostic and diagnostic purposes. miRNA profiles not only can distinguish between tumors of different developmental origin but also possess other prerequisites to be considered useful noninvasive biomarkers. First, they are exceptionally stable in a wide variety of clinical samples such as formalin-fixed paraffin-embedded tissues, blood, serum, and urine [41]. Second, they can be quantitatively measured reliably in small amounts of samples by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) and are amenable to high-throughput strategies [42]. Third, they are resistant to endogenous ribonuclease activity as well as variations in temperature and pH [43]. In addition, they are highly conserved among different species making the use of animals for preclinical studies feasible. A circulating tumor biomarker should also be able to detect a tumor before it can easily be detected by other means, and this is one area in which application of miRNAs has not been explored. The first studies that demonstrated that circulating cell-free miRNA profiles in body fluids are altered in response to different malignancies [44, 45] brought the exciting and limitless possibilities of circulating miRNAs to the fore. Since then, several preclinical studies have analyzed the sensitivity and specificity of cell-free miRNAs as biomarkers. Some of these studies are summarized in Table 14.2.

The mechanisms involved in the release of miRNAs into circulation have been under debate. The theory that miRNAs are passively released into extracellular spaces is being increasingly challenged by recent evidence that shows that miRNAs are released within microvesicles or endosomes and sometimes as Ago2-coupled complexes. These miRNAs may constitute a distinct miRNA profile of that particular tumor type and assist in prognosis. But confounding factors such as contamination of circulating miRNAs by cellular miRNAs and by erythrocyte miRNAs released due to hemolysis still exist. Similarly, even among the limited number of studies which analyzed prognostic indicators of miRNA profiles for CaP, the inconsistencies in sample selection, sample collection, methods of extraction of miRNAs, experimental platforms used, and ignorance of cellular origin make it difficult to effectively compare the results and draw conclusions about the efficacy of a particular miRNA or a panel of miRNAs in risk stratification or prognosis. Large-scale clinical studies with rigorous controls and an internationally established code for sample selection and collection are needed before the promise of miRNAs as circulating biomarkers can be realized in the clinic.

**Table 14.2** Circulating miRNAs in prostate cancer

| Body fluid | Sample size | Methodology | Findings | References |
|---|---|---|---|---|
| Plasma | 25 patients (metastatic CaP), 25 healthy controls | qRT-PCR (6 miRNAs) | miR-141 levels differentiate CaP patients from healthy controls | [45] |
| | 21 patients (metastatic CaP) | qRT-PCR (miR-141) | miR-141 levels correlated with PSA and with progression | [147] |
| | 51 patients (18 localized, 8 local advanced, 25 metastatic), 20 healthy controls | qRT-PCR (miR-21, miR-221, and miR-141) | miR-21 and miR-221 levels higher in CaP compared to healthy controls; miR-21, miR-221, and miR-141 higher in metastatic vs. localized disease | [148] |
| | 82 patients of stage 2–3; with risk stratification | qRT-PCR (miR-20a, miR-21, miR-145, and miR-221) | miR-20a levels higher in stage 3 compared to lower stages; miR-20a and miR-21 levels higher in high-risk patients; all four could distinguish high risk from low risk | [149] |
| Serum | 6 patients (stages 2–4 CaP), 8 healthy controls | Custom microarray (547 miRNAs) | 15 miRNAs elevated in CaP patients | [150] |
| | 56 patients (20 localized CaP, 20 androgen-dependent CaP, 10 CRPC), 6 BPH controls | qRT-PCR (miR-21) | miR-21 levels higher in CRPC compared to BPH; associated with docetaxel resistance in CRPC | [151] |
| | 29 patients (9 low risk, 11 intermediate risk, 9 high risk), 9 healthy controls | Multiplex qRT-PCR (677 miRNAs) | 10 miRNAs differentially expressed in CaP; 7 miRNAs correlated with risk groups | [152] |
| | 7 high-grade and 14 low-grade patients (profiling); 116 patients of various grades (validation) | qRT-PCR (667 miRNAs) | miR-141, miR-200b and miR-375 elevated in high-grade patients and correlated with clinicopathological parameters | [101] |
| | 45 patients (37 localized, 8 metastatic), 18 BPH controls, 20 healthy controls | qRT-PCR (5 miRNAs) | miR-26a, miR-195, and let-7i levels higher in CaP compared to BPH | [153] |
| | 14 TRAMP mice and 14 healthy controls (profiling); 25 metastatic CaP and 25 healthy controls (validation) | Affymetrix microarray, qRT-PCR (609 murine miRNAs, 10 human miRNAs) | miR-141, miR-298, miR-346, and miR-375 levels higher in CaP | [154] |

**Table 14.2** (continued)

| Body fluid | Sample size | Methodology | Findings | References |
|---|---|---|---|---|
| | 28 low-risk, 30 high-risk localized CaP and 26 metastatic CRPC | TaqMan miRNA microarray, qRT-PCR | miR-375, miR-387*, and miR-141 higher in CRPC compared to low-risk localized CaP; miR-409-3p lower in CRPC | [155] |
| Plasma and serum | 78 patients (various grades, 15 with diagnosed metastases) and 28 healthy controls for profiling; 119 patients (47 recurrent after radical prostatectomy and 72 nonrecurrent) | qRT-PCR (742 miRNAs) | 12 miRNAs altered in CaP compared to healthy controls; 16 miRNAs altered in metastatic vs. localized | [156] |

## 8 miRNAs as Biomarkers, Prognostic Markers, and/or Therapeutic Targets

Numerous studies have described the potential for a particular miRNA or a panel of miRNAs to be used as biomarkers or prognostic markers in CaP. In addition, some studies have attempted to antagonize the functions of miRNAs with a view to using them as therapeutic targets. miR-21, which is one of the few oncomiRs to be described in CaP, is an example of an miRNA that may serve as a biomarker as well as a therapeutic target. miR-21 targets several mRNAs, such as MARCKS [34], RECK [17], and PDCD-4 [46] and is postulated as an independent predictor of biochemical recurrence and as a potential therapeutic target [47]. Similarly, miR-125b, which targets p53 and other molecules in the p53 pathway, such as BAK1 [15, 48], may serve as a biomarker of castration resistance, tumor stage, and perineural invasion as well as a therapeutic target. Another miRNA that is overexpressed in CaP, miR-96 (FOXO1, hZIPs), may be a prognostic marker of biochemical progression and tumor recurrence [49, 50]. Other miRNAs that have been shown to be downregulated in CaP and that are implicated in prognosis are miR-331-3p, miR-146, miR-1, miR-143, miR-145, miR-34 family, miR-200 family, let-7 family, miR-1, etc. This preclinical evidence needs to be corroborated by clinical studies, and although it is patently obvious that miRNAs could help classify CaP progression and recurrence, their potential is still far away from clinical application.

Different approaches are being developed to achieve gain or loss of miRNA functions. Restoring the functions of tumor suppressor miRNAs which have been repressed can be achieved by adeno-associated viruses, lentiviruses, cationic liposomes, or polymer-based nanoparticle formulations [51]. On the other hand, antagonizing functions of oncomiRs can be achieved by introduction of antagomiRs, oligonucleotides which inhibit target pairing competitively [52], or by miRNA "sponges," which

have been designed to carry multiple binding sites for several endogenous miRNAs [53]. Some small molecules such as azobenzene, which blocks miR-21 function, [54] are also being explored as potential inhibitors of miRNA function.

## 9 miRNAs in Clinical Trials

miRNA therapeutics that are in preclinical development include miR-208/499 in chronic heart failure, miR-195 in post-myocardial infarction remodeling, and let-7 for non-small cell lung cancer. Some of these therapeutics may reach clinical trial stages in the not-so-distant future. There are currently a number of companies which have miRNA therapeutics programs with the most successful being miravirsen (SPC3649), an miR-122 inhibitor (Santaris Pharma), which is in phase II studies for treatment of hepatitis C. Miravirsen was the first miRNA-targeted drug to enter clinical trials. Recently, MGN-4893 (Miragen Therapeutics) that targets miR-451 was given orphan drug status by the US Food and drug Administration (FDA) to treat polycythemia vera, a myeloproliferative disorder characterized by an overabundance of blood cells and platelets in the body.

Although there are currently no clinical trials that use miRNAs as a treatment option for CaP, recent successes by Mirna Therapeutics and researchers at MD Anderson Cancer Center in inhibiting CaP tumor growth, decreasing lung metastasis, and extending survival in mice using liposome-based systemic delivery of miR-34a to suppress the adhesion molecule CD44 are promising. This group hopes to advance miR-34a as a treatment option for CaP patients. Currently, there are a few observational clinical trials to study miRNAs in CaP (www.clinicaltrials.gov) as detailed in Table 14.3.

## 10 Challenges in Using miRNA-Based Therapeutics

miRNAs are naturally occurring molecules, and distinct advantages of using miRNAs as therapeutic agents over currently used conventional drugs are apparent. These include their broad specificity, which would be a disadvantage with other therapies but is a distinct advantage with miRNA-based therapeutics. Consequently, miRNA-based therapeutics can target multiple genes in one or multiple pathways concurrently. In addition, tumor-suppressive miRNAs can be used to cooperatively target one or multiple target genes. Another advantage of using miRNAs as drugs is their small size, which makes them less antigenic than protein- or oligosaccharide-based gene replacement strategies. But as with other kinds of therapeutic agents, there are several challenges associated with using miRNAs as therapeutics; a major hurdle is the mode of delivery. Even though viral-mediated delivery systems (adenoviral, lentiviral) have shown promise in preclinical studies, they are not likely to be extrapolated to human use. Other strategies such as liposome- or nanoparticle- mediated delivery or conjugation to cell-penetrating peptides may be plausible. Adjuvant carrier systems which can in-

**Table 14.3** Current miRNA-related clinical trials in CaP

| Trial title | Study type | Institution | Trial identifier |
|---|---|---|---|
| MicroRNA expression profiles in high-risk prostate cancer | Observational; to study whether miRNA profiles correlate with disease outcome | Würzburg University Hospital, Germany | NCT01220427 |
| Molecular correlates of sensitivity and resistance to therapy in prostate cancer | Observational; to study differences in miRNA profiles in order to discover new biomarkers and drug targets | University of Washington | NCT01050504 |
| Trial of vaccine therapy in curative resected prostate cancer patients using autologous dendritic cells loaded with mRNA from primary prostate cancer tissue, hTERT, and Survivin | Treatment; secondary objective | Rikshospitalet University Hospital, Norway | NCT01197625 |
| Phase II randomized study of combined androgen deprivation comprising Bicalutamide and Goserelin or Leuprolide Acetate with versus without Cixutumumab in patients with newly diagnosed hormone-sensitive metastatic prostate cancer | Biomarker/laboratory analysis, treatment; secondary objective | Saint Anthony's Hospital at Saint Anthony's Health Center, Illinois | NCT01120236 |

crease the stability of miRNAs in the cellular microenvironment and enhance uptake by target tissues need to be invented. Another major hurdle is the concern that delivery of exogenous miRNAs or their mimics may overwhelm the cellular RISC system and interfere with the processing of endogenous miRNAs. Other concerns include off-target effects, toxicity, and possible liver damage [55]. Population-based variation in miRNA expression profiles is another major challenge. Dosage and combinations of miRNAs for each type of cancer need to be established taking into account the gender, race, and environmental conditions of each patient. These are difficult but not insurmountable obstacles, and with the current pace in discovery and application of miRNA-based therapeutics, their resolution would not be too far away in the future.

## 11 Conclusions and Perspectives

Research in the last decade, since the discovery of miRNAs, has suggested that an intimate relationship exists between CaP and miRNA profiles making these discoveries of strong prognostic and therapeutic importance. The field is clearly promising and exciting but further accurate dissection of the mechanistic aspects is absolutely necessary to determine the specific roles of individual miRNAs and collective im-

pact of a particular miRNA profile signature on disease outcome and progression. Once this knowledge is obtained, it would become easier to develop therapeutic approaches to target a specific miRNA or a set of miRNAs to achieve a desired outcome. At the same time, even though several studies have demonstrated the utility of miRNA profiling in predicting clinical outcome, the findings need to be validated and consistency needs to be improved. In conclusion, miRNAs represent valuable prognostic and therapeutic tools which may prove to be essential weapons in the fight against CaP progression, and it is up to the research community to come up with innovative and reliable techniques to utilize them effectively.

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