REVIEW

Interplay between the androgen receptor signaling axis and microRNAs in prostate cancer

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Abstract

The androgen receptor (AR) is a ligand-activated transcription factor that drives prostate cancer. Since therapies that target the AR are the mainstay treatment for men with metastatic disease, it is essential to understand the molecular mechanisms underlying oncogenic AR signaling in the prostate. miRNAs are small, non-coding regulators of gene expression that play a key role in prostate cancer and are increasingly recognized as targets or modulators of the AR signaling axis. In this review, we examine the regulation of AR signaling by miRNAs and vice versa and discuss how this interplay influences prostate cancer growth, metastasis and resistance to therapy. Finally, we explore the potential clinical applications of miRNAs implicated in the regulation of AR signaling in this prevalent hormone-driven disease.

Key Words

- ▶ microRNA
- ▶ androgen receptor
- ▶ prostate cancer
- ▶ biomarkers
- ▶ therapy

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Introduction

The androgen receptor (AR) signaling axis is critical for normal prostatic development and maintenance and is the major driver of prostate cancer (PCa) growth and disease progression. Androgens such as testosterone and its more active metabolite, dihydrotestosterone (DHT), bind to the AR in the cytoplasm of prostate epithelial cells, which elicits its translocation into the nucleus. Ligand-activated AR binds to specific DNA sequences termed AR response elements (AREs) in regulatory regions of target genes, resulting in transcriptional activation or, less commonly, repression (Wang *et al.* 2009). In normal prostate epithelial cells, a key function of AR target genes is to maintain luminal differentiation; by contrast, in malignant prostate cells an aberrant AR transcriptional program promotes cell proliferation and survival (Coutinho *et al.* 2016).

Given the critical role of AR in PCa, androgen deprivation therapy (ADT) is the primary treatment for metastatic disease (Feldman & Feldman 2001). ADT encompasses multiple agents that: (i) decrease circulating androgen levels by inhibiting pituitary signals that stimulate testicular androgen production, i.e. luteinizing hormone-releasing hormone (LHRH) and gonadotropin-releasing hormone (GnRH) agonists; (ii) diminish biosynthesis of androgens, i.e. inhibitors of androgen biosynthetic enzymes; and/or (iii) inhibit AR activity i.e. agents that block androgen binding to the AR, termed AR antagonists. Unfortunately, these treatments inevitably fail, normally after a period of 2–3 years. The resultant disease is termed castration-resistant prostate cancer (CRPC) (Scher *et al.* 2004). AR remains the key driver of most cases of CRPC. Maintenance of AR activity

despite low levels of circulating androgens can be achieved through amplification or mutation of the *AR* gene, alternative splicing to produce constitutively active *AR* splice variants, altered expression of *AR* co-regulators and/or enhanced intra-tumoral androgen production (Coutinho *et al.* 2016).

miRNAs are a class of small non-coding RNAs, approximately 22 nt in length, that post-transcriptionally silence target mRNAs. An estimated 60% of all protein-coding mRNAs are targeted by miRNAs (Friedman *et al.* 2009), highlighting the powerful influence these small transcripts can have on the transcriptome and how it is interpreted by the cell. Primary transcripts of miRNA genes (pri-miRNAs), transcribed primarily by RNA polymerase II, can be encoded by sequences within (intragenic) or outside of (intergenic) protein-coding genes (Lee *et al.* 2004, Rodriguez *et al.* 2004). Following transcription of a miRNA gene (or host gene), pri-miRNAs are processed into precursor miRNAs (pre-miRNAs) that fold into a stem loop structure and are exported from the nucleus (Kim 2005). Within the cytoplasm, pre-miRNAs are cleaved into a miRNA duplex that is then separated; one strand is the final mature form of approximately 22bp and the other strand is degraded (Kim 2005). As part of the RNA-induced silencing complex (RISC) that includes Argonaute (Ago) proteins, miRNAs bind to target mRNAs and promote transcript degradation and/or translational inhibition (Fabian & Sonenberg 2012). miRNAs generally bind to the 3'UTR of their target genes through complementarity with a region called the seed sequence, consisting of nucleotides 2–8 on the 5' to 3' ends of a miRNA sequence (Lewis *et al.* 2005), although an increasing number of studies have also identified functional miRNA recognition sites in the coding and 5'UTR regions of genes (Brummer & Hausser 2014, Zhou & Rigoutsos 2014, Ito *et al.* 2017, Zhang *et al.* 2018).

Dysregulated miRNA expression is a hallmark of cancer development and metastasis (Calin & Croce 2006, Jackson *et al.* 2014). PCa-associated miRNAs were initially reported by Porkka and colleagues who identified differential expression of miRNAs between benign and malignant prostatic tissue (Porkka *et al.* 2007). miRNAs have since been implicated in all aspects of prostate carcinogenesis and progression to metastatic and therapy-resistant disease (Kojima *et al.* 2017, Luu *et al.* 2017). Moreover, since some PCa-associated miRNAs exist stably in the circulation of patients, there is considerable interest in their utility as blood-based biomarkers to improve PCa diagnosis, prognosis and treatment management (Fabris *et al.* 2016, Kanwal *et al.* 2017, Matin *et al.* 2018).

In this review we explore the significance of the interplay between the *AR* signaling axis and miRNAs as it relates to PCa growth and progression. Finally, we speculate on the potential clinical applications of miRNAs implicated in the regulation of *AR*.

Regulation of *AR* by microRNAs

Direct targeting of *AR* by miRNAs

Mechanisms by which miRNAs influence the *AR* signaling axis are depicted in Fig. 1. One prominent feature of this interplay is direct targeting of the *AR* 3'UTR by miRNAs. Indeed, a recent study predicted that the *AR* 3'UTR is likely to be more heavily regulated by miRNAs than all other PCa driver genes and in the top 5% of regulated genes overall (Hamilton *et al.* 2016). This elegant work exploited photoactivatable ribonucleoside-enhanced cross-linking immunoprecipitation of the Argonaute protein (Ago-PAR-CLIP) to identify 147 miRNA seed sides corresponding to 71 miRNA families in the *AR* 3'UTR (Table 1). Importantly, four of the miRNAs identified by Hamilton and colleagues – miR-9, miR-34c, miR-185 and miR-488 – had been previously discovered in two high-throughput screens aimed at identifying *AR*-targeting miRNAs (Östling *et al.* 2011, Kumar *et al.* 2016) (Table 1). In addition to these unbiased approaches, many other studies have characterized specific *AR*-targeting miRNAs in a more directed manner. Interestingly, in addition to classical targeting of the 3'UTR, miRNA regulation of the *AR* via 5'UTR (miR-31) and the coding region (miR-421, miR-449a, miR-449b, miR-646, miR-371, miR-193a and miR-9) has also been reported (Östling *et al.* 2011, Lin *et al.* 2013, Kumar *et al.* 2016). A list of putative *AR*-targeting miRNAs and their putative modes of action is provided in Table 1.

We propose that the biological relevance of these *AR*-targeting miRNAs can be prioritized using a set of discrete parameters. First, miRNAs that have been identified in multiple studies using multiple *in vitro* models are likely to have greater biological relevance in PCa. Second, it is known that transfection of cells with miRNA mimics can yield non-physiological miRNA activity; therefore, we prioritize studies that have demonstrated *AR* targeting using both miRNA mimics and inhibitors. Third, given that prostate tumors are 'addicted' to *AR* (Coutinho *et al.* 2016), one would expect oncogenic selection pressure to downregulate biologically relevant *AR*-targeting miRNAs. Indeed, a number of *AR*-targeting miRNAs have been reported to be downregulated in prostate tumors

Figure 1

Key mechanisms by which microRNAs regulate AR expression and activity in prostate cancer, including direct targeting of the AR transcript (1), targeting of AR co-regulators (2) and targeting of factors that influence expression of the AR gene (3).

compared to non-malignant prostate tissues. However, it is worth noting that our own analyses of miRNA expression in The Cancer Genome Atlas (TCGA) and Memorial Sloan-Kettering Cancer Center (MSKCC) (Taylor *et al.* 2010) often contradict the published associations with cancer (Fig. 2A and B). Finally, it would be expected that biologically relevant miRNAs are inversely correlated with AR protein levels in clinical samples. Indeed, our analysis of the TCGA cohort revealed such a correlation for miR-145, miR-205, miR-34a and miR-31 (Fig. 2C). Using these parameters for prioritization and taking into account both published findings and our new analyses, we have generated a list of candidate AR-targeting miRNAs shown in Table 2, which are ranked by predicted relevance in PCa. We propose that downregulation of at least a subset of these miRNAs is a key enabler of enhanced AR expression and activity in this disease.

When considering targeting of AR by miRNAs, it is worth noting that the reference AR 3'UTR sequence is annotated as being 6777 nucleotides in length (NM_000044.4), but other isoforms have been reported. For example, LNCaP cells have been reported to express an AR isoform with the canonical ~6.8kb 3'UTR and another alternatively spliced version that lacks a 3kb region in the 3'UTR (Faber *et al.* 1991). Moreover, Ostling and colleagues delineated a 6680 nucleotide AR 3'UTR in VCaP cells, a sequence that was also identified in LNCaP, LAPC-4, 22Rv1 and MDA-PCa-2b cell line models (Östling *et al.* 2011). In addition to variation in length, five SNPs were identified in the AR 3'UTR in PCa cell lines

(Waltering *et al.* 2006). Interestingly, SNPs have been reported to influence miRNA targeting of multiple genes in PCa (Stegeman *et al.* 2015a,b), although our analyses indicate that none of the AR 3'UTR SNPs occur within conserved miRNA recognition sequences (not shown). Nevertheless, we believe that reported variations in the length and sequence of the AR 3'UTR warrants further consideration in relation to interplay with miRNAs, especially since 3'UTR sequences are often overlooked in genomic and transcriptomic studies.

Direct targeting of AR splice variants by miRNAs

AR splice variants (ARVs) are truncated isoforms of the AR that lack part or all of the C-terminal ligand-binding domain (LBD) (Antonarakis *et al.* 2016). ARV mRNAs were first identified in PCa cell lines (Dehm *et al.* 2008, Guo *et al.* 2009) but have since been detected in patient specimens including primary tumors and metastases, circulating tumor cells and whole blood (Hu *et al.* 2009, Hornberg *et al.* 2011, Antonarakis *et al.* 2014, Liu *et al.* 2016). Recent RNA-seq data indicate the presence of at least 16 distinct ARV mRNA species in primary PCa (Cancer Genome Atlas Research Network 2015) and 23 in metastatic CRPC (Robinson *et al.* 2015). A subset of these ARVs are constitutively active (i.e. they can regulate transcription in the absence of androgen) and are therefore resistant to therapies that target the LBD (Chan *et al.* 2015). Although the relevance of ARVs in driving the growth of CRPC remains to be definitively proven

Table 1 Putative AR- and AR variant-targeting miRNAs.

miRNA	Target	Mechanism of regulation	Mimic and/or inhibitor used	Ago-PAR-CLIP binding site/s in 3'UTR ^a	Readouts	Reference
miR-9	AR	3'UTR and coding region	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)
miR-30b-3p	AR	3'UTR	Mimic and Inhibitor		AR protein and mRNA, AR 3'UTR luciferase reporter (wild-type and mutant)	Kumar <i>et al.</i> (2016)
miR-30c-5p	AR	3'UTR	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type)	Kumar <i>et al.</i> (2016)
miR-30d-5p	AR	3'UTR	Mimic and Inhibitor		AR protein and mRNA, AR 3'UTR luciferase reporter (wild-type and mutant)	Kumar <i>et al.</i> (2016)
miR-31	AR	5'UTR and coding region	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type and mutant)	Lin <i>et al.</i> (2013)
miR-34a	AR	3'UTR	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016)
miR-34c	AR	3'UTR	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)
miR-101-3p	AR and AR-V7	3'UTR	Mimic	Yes	AR protein, AR 3'UTR luciferase reporter (wild-type only)	Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)
miR-124-3p	AR, AR-V7, AR-V4	3'UTR	Mimic	Yes	AR protein, AR 3'UTR luciferase reporter (wild-type and mutated)	Shi <i>et al.</i> (2013), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)
miR-135b	AR and AR-V7	3'UTR	Mimic		AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011)
miR-145	AR	3'UTR	Mimic		AR protein and mRNA	Larne <i>et al.</i> (2015), Hamilton <i>et al.</i> (2016)
miR-149-3p	AR	3'UTR	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type only)	Kumar <i>et al.</i> (2016)
miR-181c-5p	AR-V7	3'UTR	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type and mutated)	Wu <i>et al.</i> (2019)
miR-193a-3p	AR and AR-V7	Coding region	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type only)	Kumar <i>et al.</i> (2016)
miR-205	AR	3'UTR	Mimic	Yes	AR protein, AR luciferase reporter (wild-type)	Hagman <i>et al.</i> (2013), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)
miR-297	AR	3'UTR	Mimic		AR mRNA, AR luciferase reporter (wild-type)	Fang <i>et al.</i> (2016)

(Continued)

Table 1 Continued.

miRNA	Target	Mechanism of regulation	Mimic and/or inhibitor used	Ago-PAR-CLIP binding site/s in 3'UTR ^a	Readouts	Reference
miR-298	AR	3'UTR	Mimic		AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011)
miR-299	AR	3'UTR	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016)
miR-371	AR and AR-V7	Coding region	Mimic		AR protein, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)
miR-421	AR	3'UTR and coding region	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016)
miR-425-5p	AR	3'UTR	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type)	Kumar <i>et al.</i> (2016)
miR-449a	AR	3'UTR and coding region	Lentiviral miRNA overexpression		AR protein and mRNA	Zheng <i>et al.</i> (2015)
miR-449b	AR	3'UTR and coding region	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type)	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)
mir-488	AR and AR-V7	3'UTR	Mimic	Yes	AR protein and mRNA, AR luciferase reporter (wild-type and mutated)	Östling <i>et al.</i> (2011), Sikand <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)
miR-541	AR	3'UTR	Mimic		AR protein and mRNA, AR 3'UTR luciferase reporter (wild-type)	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)
miR-634	AR	3'UTR	Mimic		AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)
miR-635	AR	3'UTR	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type)	Kumar <i>et al.</i> (2016)
miR-646	AR and AR-V7	Coding region	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type)	Kumar <i>et al.</i> (2016)
miR-650	AR	3'UTR	Mimic		AR protein, AR 3'UTR luciferase reporter (wild-type)	Kumar <i>et al.</i> (2016)
miR-654	AR	3'UTR	Mimic		AR protein and mRNA, AR luciferase reporter (wild-type only)	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)

^aAgo-PAR-CLIP, photoactivatable ribonucleoside-enhanced cross-linking immunoprecipitation of the Argonaute protein (Hamilton *et al.* 2016).

(Luo *et al.* 2018), it is worth noting that high expression of certain ARVs, such as AR-V7 and AR-V9, correlates with resistance to AR-targeted therapies and worse survival (Qu *et al.* 2015, Kohli *et al.* 2017, He *et al.* 2018).

Given the clinical relevance of ARVs, it is important to understand how they are regulated by miRNAs in PCa. Of note, ARVs possess 3'UTR sequences distinct from the canonical AR transcript (Hu *et al.* 2011, Shi *et al.* 2015) (Fig. 3A). Recent studies have begun to decipher miRNA

regulation of ARVs. Interestingly, a number of miRNAs regulate both canonical AR and specific ARVs through different target sites. For example, miR-124 targets the AR transcript via a 3'UTR site that is distinct from another functional targeting site in the 3'UTRs of AR-V3, AR-V4 and AR-V7 (Fig. 3B) (Shi *et al.* 2013, Shi *et al.* 2015). Although the complete repertoires of ARV-targeting miRNAs remain to be determined, the identification of miRNAs that target both AR and ARVs via distinct

Figure 2

Expression of putative AR-targeting miRNAs in clinical samples. (A) Expression of putative AR-targeting miRNAs in matched normal and primary prostate cancers from The Cancer Genome Atlas (TCGA). TCGA miRNA expression data were obtained from Genomic Data Commons, which uses miRBase v21 as miRNA reference; miRNA names were derived miRBase v21 and converted where necessary. miRNA abundance (reads-per-million-miRNA-mapped) was calculated by taking the sum of all counts per miRNA (unique MIMAT ID), and the MIMAT ID was then converted to its appropriate miRNA name. Only miRNAs that were expressed in more than 50% of samples were included in this analysis. *P* values were determined using paired *t* tests; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. (B) Expression of putative AR-targeting miRNAs in normal and prostate cancer (primary and metastatic) in the Memorial Sloan-Kettering Cancer Center (MSKCC) cohort. Normalized miRNA expression data for the MSKCC cohort was obtained from cBioPortal (www.cbioportal.org). *P* values were determined using unpaired *t* tests; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. (C) Correlation between putative AR-targeting miRNAs and AR protein levels in the TCGA cohort. AR protein levels are from reverse phase protein array data, obtained from the Broad Institute's GDAC Firehose. RPPA data were available for 145 primary prostate tumors. Only AR-targeting miRNAs that exhibited a significant (*P* < 0.05) negative Pearson correlation with AR protein levels in these 145 tumors are shown.

sequences is intriguing and may suggest co-evolution of miRNAs and alternative AR splicing. Another mechanism by which miRNAs target both AR and ARV transcripts is via shared sequences in coding regions, as exemplified by translational regulation of AR and AR-V7 by miR-646, miR-371-3p and miR-193a-3p (Kumar *et al.* 2016).

Importantly, differences in the 3'UTRs of ARVs and AR suggest that many of the identified AR-targeting miRNAs would not influence ARV expression and vice versa. While this remains to be proven for most miRNAs, a recent study provided proof of concept by showing that miR-181c-5p effectively suppresses AR-V7 expression by

Table 2 Assessing the biological relevance of microRNAs reported to target the androgen receptor.

miRNA	No of studies reporting association between miRNA and AR	Reference (score: 1 if 2 or more references)	Inverse correlation between AR protein and miRNA (score: 1 if yes, 0 if N/A or no)	Downregulated in cancer compared to normal tissues (score: 0.5 for yes in each of MSKCC and TCGA datasets, 0 if no)	Inverse correlation between AR and miR in PCa tissues (score: 1 if yes, 0 if unknown)	miRNA status in PCa tissues (score: 1 if downregulated, 0 if unknown)	Reported role of miRNA in PCa (score: 1 if tumor suppressor, 0 if unknown)	Rank (sum of score)	References
miR-205	3	Hagman <i>et al.</i> (2013), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	Yes	Downregulated in TCGA and MSKCC	Yes, AR transcript and protein	Downregulated in PCa tissues	Tumor suppressor	6	Gandellini <i>et al.</i> (2009), Hagman <i>et al.</i> (2013), Kumar <i>et al.</i> (2016)
miR-145	2	Larne <i>et al.</i> (2015), Hamilton <i>et al.</i> (2016)	Yes	Downregulated in MSKCC	Yes	Downregulated in PCa metastases	Tumor suppressor	5.5	Larne <i>et al.</i> (2015)
miR-34a	2	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016)	Yes	Downregulated in MSKCC, Upregulated in TCGA	Yes, AR protein	Unknown	Tumor suppressor	4.5	Liu <i>et al.</i> (2011), Östling <i>et al.</i> (2011), Leite <i>et al.</i> (2015)
miR-34c	4	Östling <i>et al.</i> (2011), Fang <i>et al.</i> (2016), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	No	N/A	Yes, AR transcript and protein	Downregulated in PCa tissues	Tumor suppressor	4	Hagman <i>et al.</i> (2010), Östling <i>et al.</i> (2011)
miR-101-3p	2	Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	N/A	Downregulated in MSKCC, Upregulated in TCGA	Yes, AR protein	Unknown	Tumor suppressor	3.5	Cao <i>et al.</i> (2010)
miR-299-3p	2	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016)	No	N/A	Unknown	Unknown	Unknown	2	
miR-421	2	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016)	No	N/A	Unknown	Downregulated in PCa tissues	Tumor suppressor	3	Meng <i>et al.</i> (2016)
miR-124-3p	4	Shi <i>et al.</i> (2013, 2015), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	N/A	N/A	Unknown	Unknown	Tumor suppressor	2	Shi <i>et al.</i> (2015)

(Continued)

Table 2 Continued.

miRNA	No of studies reporting association between miRNA and AR	Reference (score: 1 if 2 or more references)	Inverse correlation between AR protein and miRNA (score: 1 if yes, 0 if N/A or no)	Downregulated in cancer compared to normal tissues (score: 0.5 for yes in each of MSKCC and TCGA datasets, 0 if no)	Inverse correlation between AR and miR in PCa tissues (score: 1 if yes, 0 if unknown)	miRNA status in PCa tissues (score: 1 if downregulated, 0 if unknown)	Reported role of miRNA in PCa (score: 1 if tumor suppressor, 0 if unknown)	Rank (sum of score)	References
miR-185	4	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	No	Upregulated in TCGA	Unknown	Downregulated in PCa tissues	Tumor suppressor	2	Qu <i>et al.</i> (2013)
miR-371	3	Östling <i>et al.</i> (2011), Leite <i>et al.</i> (2015), Kumar <i>et al.</i> (2016)	N/A	N/A	Unknown	Downregulated in PCa tissues	Unknown	2	Leite <i>et al.</i> (2015)
miR-488	4	Östling <i>et al.</i> (2011), Sikand <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	N/A	N/A	Unknown	Unknown	Tumor suppressor	2	Sikand <i>et al.</i> (2011)
miR-449a	2	Östling <i>et al.</i> (2011), Zheng <i>et al.</i> (2015)	N/A	N/A	Unknown	Unknown	Tumor suppressor	2	Noonan <i>et al.</i> (2009), Zheng <i>et al.</i> (2015)
miR-297	2	Östling <i>et al.</i> (2011), Fang <i>et al.</i> (2016)	N/A	N/A	Unknown	Unknown	Unknown	1	N/A
miR-449b	2	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)	N/A	N/A	Unknown	Unknown	Unknown	1	N/A
miR-541	2	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)	No	N/A	Unknown	Unknown	Unknown	1	N/A
miR-634	2	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)	N/A	N/A	Unknown	Unknown	Unknown	1	N/A
miR-654	2	Östling <i>et al.</i> (2011), Kumar <i>et al.</i> (2016)	No	N/A	Unknown	Unknown	Unknown	1	N/A
miR-9	3	Östling <i>et al.</i> (2011), Hamilton <i>et al.</i> (2016), Kumar <i>et al.</i> (2016)	N/A	N/A	Unknown	Upregulated in high grade tumors	OncomiR	1	Seashols-Williams <i>et al.</i> (2016)

Figure 3

Differential 3'UTRs of AR and AR variants in prostate cancer influence their regulation by miRNAs. (A) Graphical representation of 3'UTR sequences for the canonical AR transcript and 6 AR variants. GenBank accession IDs are shown; 3'UTR length was calculated as the number of nucleotides from after the stop codon to the end of the annotated transcript. (B) miR-124 target sites in the canonical AR transcript and AR variants V3, V4 and V7.

targeting its 3'UTR but has no effect on the levels of the prototypical AR (Wu *et al.* 2019). Interestingly, AR-V7 and ARv567es exhibit greater post-transcriptional stability than AR in CRPC bone metastases (Hornberg *et al.* 2011); it is tempting to speculate that this may be (at least partly) explained by differential miRNA targeting.

Indirect modulation of AR expression and activity by miRNAs

As well as direct regulation of AR, miRNAs can indirectly influence the expression and activity of AR via multiple mechanisms (Fig. 1), including targeting AR co-regulators. Approximately 200 AR co-regulators – broadly classified as co-activators and co-repressors – have been identified, comprising a highly complex and potent system for shaping AR function in PCa (Liu *et al.* 2017).

The co-repressor small heterodimer partner (SHP) is downregulated by miR-141, which is frequently elevated in PCa, thereby indirectly contributing to activation of AR's transcriptional activity (Xiao *et al.* 2012). Another AR corepressor, prohibitin (PHB), is targeted by miR-27a, leading to increased expression of AR target genes and PCa cell growth (Fletcher *et al.* 2012). Interestingly, miR-27a is an androgen-regulated miRNA, creating a feedback loop that represents a novel mechanism by which AR enhances its own activity.

Concomitant with enhanced miRNA-mediated targeting of AR co-repressors in PCa is the frequent loss or downregulation of miRNAs that target AR co-activators. For example, miR-137 targets a suite of AR co-activators and is progressively lost with tumor grade due to DNA methylation of the *MIR137* locus (Nilsson *et al.* 2015). Loss of miR-331-3p, which targets the *ERBB2* (HER2)

oncogene, another AR co-activator (Craft *et al.* 1999), also enhances AR signaling in PCa (Epis *et al.* 2009). The AR co-activators p300/CBP-associated factor and bromodomain-containing 8 isoform 2 are targeted by miR-17-5p and miR-185, respectively, leading to reduced AR transcriptional activity in PCa cells (Xiao *et al.* 2012, Jiang *et al.* 2016). Interestingly, miR-185 also directly targets AR, enabling this miRNA to mediate a dual mode of inhibition of the AR signaling axis (Jiang *et al.* 2016).

Another mechanism by which miRNAs indirectly influence the AR signaling axis is by targeting factors that regulate AR gene expression. For example, miR-let-7c targets the oncogenic transcription factor Myc, thereby indirectly downregulating AR expression (Nadiminty *et al.* 2012). Similarly, by directly targeting fibronectin type III domain-containing 1, miR-1207-3p reduces fibronectin 1 and subsequently AR expression (Das *et al.* 2016). Todorova and colleagues reported that miR-204 targeting of the DNA methyltransferase DNMT1 results in decreased methylation of, and hence, increased transcription from, the AR gene promoter (Todorova *et al.* 2017). The nuclear matrix protein hnRNPH1, which is upregulated in prostate tumors and promotes the expression of AR and AR-V7, AR transactivation and binding to ARES, is a target of miR-212 (Yang *et al.* 2016); miR-212 is downregulated in prostate tumors and its ectopic expression in PCa cell lines results in decrease in hnRNPH1 and AR expression (Yang *et al.* 2016). In summary, targeting AR co-regulators and upstream regulators of AR expression allows miRNAs to exert an additional layer of regulation on this key oncogenic signaling axis (Fig. 1).

Regulation of microRNAs by the AR signaling axis

Direct regulation of miRNA gene expression by AR

The AR can directly regulate the PCa 'miRNAome' by binding to androgen response elements (AREs) within cis-regulatory regions that regulate miRNA gene expression (Fig. 4). The integration of AR genome-wide DNA-binding profiles ('cistromes') with androgen-regulated transcriptomes has greatly facilitated the identification of direct miRNA target genes (e.g.; Takayama *et al.* 2011, Pasqualini *et al.* 2015). This strategy has been used successfully irrespective of whether miRNAs are encoded in an intragenic or intergenic manner (Pasqualini *et al.* 2015). In general, AR induces oncogenic miRNAs, which act as downstream effectors of AR signaling, and represses tumor suppressor miRNAs (Table 3), although exceptions to this rule exist (see paragraph immediately below). Key oncogenic miRNAs that are directly upregulated by AR's transcriptional activation function include miR-19a, miR-27a, miR-133b and miR-185-5p, all of which can promote PCa cell growth and/or survival (Mo *et al.* 2013, Yao *et al.* 2016), whereas tumor suppressor miRNAs directly repressed by AR include miR-221/222 and miR-421 (Meng *et al.* 2016, Gui *et al.* 2017).

While AR is the major driver of PCa, it also plays an essential role in normal prostate physiology, where it primarily regulates a differentiative rather than proliferative transcriptional program (Coutinho *et al.* 2016). This concept may explain the observation that AR also promotes the expression of miRNAs that inhibit

Figure 4

Key mechanisms by which AR controls the miRNAome in prostate cancer, including direct regulation of miRNA expression (1), regulating epigenetic machinery that modulates expression of miRNAs (2) and regulating the expression and activity of the miRNA biogenesis machinery (3).

Table 3 MicroRNAs directly regulated by the AR signaling axis.

miRNA	Regulation by AR /androgens	miRNA expression in PCa tissues	Proposed function of miRNA	# of studies reporting association between miRNA and AR	References
miR-1	Induced	Downregulated	Tumor suppressor	1	Liu <i>et al.</i> (2015)
miR-19a	Induced	Upregulated (in CRPC)	OncomiR	1	Mo <i>et al.</i> (2013), Lu <i>et al.</i> (2015)
miR-21	Induced	Upregulated	OncomiR	4	Ribas <i>et al.</i> (2009), Murata <i>et al.</i> (2010), Takayama <i>et al.</i> (2011), Waltering <i>et al.</i> (2011)
miR-22	Induced	Downregulated	Tumor suppressor	3	Murata <i>et al.</i> (2010), Waltering <i>et al.</i> (2011), Pasqualini <i>et al.</i> (2015)
miR-27a	Induced	Unknown	OncomiR (Fletcher <i>et al.</i> 2012)/Tumor suppressor (Wan <i>et al.</i> 2016)	3	Murata <i>et al.</i> (2010), Fletcher <i>et al.</i> (2012), Mo <i>et al.</i> (2013)
miR-29a	Induced	Downregulated	Tumor suppressor	3	Ribas <i>et al.</i> (2009), Waltering <i>et al.</i> (2011), Pasqualini <i>et al.</i> (2015)
miR-32	Induced	Upregulated	OncomiR	2	Jalava <i>et al.</i> (2012), Waltering <i>et al.</i> (2011)
miR-99a	Induced (Takayama <i>et al.</i> 2011) / Repressed (Sun <i>et al.</i> 2014)	Downregulated	Tumor suppressor	2	Sun <i>et al.</i> (2011, 2014), Takayama <i>et al.</i> (2011)
miR-125b-2	Induced Murata <i>et al.</i> (2010); Takayama <i>et al.</i> (2011) / Repressed (Sun <i>et al.</i> 2014)	Unknown	OncomiR	3	Shi <i>et al.</i> (2007), Murata <i>et al.</i> (2010), Takayama <i>et al.</i> (2011), Sun <i>et al.</i> (2014)
miR-133b	Induced	Unknown	Unknown	1	Mo <i>et al.</i> (2013)
miR-135a	Induced	Downregulated	Tumor suppressor	1	Kroiss <i>et al.</i> (2015)
miR-148a	Induced	Upregulated	OncomiR	4	Ribas <i>et al.</i> (2009), Murata <i>et al.</i> (2010), Waltering <i>et al.</i> (2011), Jalava <i>et al.</i> (2012)
miR-182-5p	Induced	Upregulated	OncomiR	1	Yao <i>et al.</i> (2016)
miR-193a-3p	Induced	Unknown	OncomiR	2	Waltering <i>et al.</i> (2011), Jia <i>et al.</i> (2017)
miR-203	Induced	Upregulated	Tumor suppressor	1	Jalava <i>et al.</i> (2012), Siu <i>et al.</i> (2016)
miR-221/222	Repressed	Downregulated / Upregulated	OncomiR	3	Takayama <i>et al.</i> (2011), Waltering <i>et al.</i> (2011), Jalava <i>et al.</i> (2012)
miR-421	Repressed	Unknown	Tumor suppressor	1	Meng <i>et al.</i> (2016)
miR-4496	Induced	Unknown	Tumor suppressor	1	Wang <i>et al.</i> (2018)

proliferation (e.g. miR-101, miR-135a and miR-1, which target the oncogenic factors EZH2, ROCK1/2 and SRC respectively; Cao *et al.* 2010, Kroiss *et al.* 2015, Liu *et al.* 2015) and promote epithelial differentiation (e.g. miR-200b, which targets factors that drive epithelial-mesenchymal transition, such as ZEB1; Williams *et al.* 2013).

Indirect regulation of miRNA expression and/or activity by the AR signaling axis

Indirect regulation of miRNAs by the AR can occur through a variety of mechanisms (Fig. 4). First, AR appears to play an important regulatory role in miRNA biogenesis (Fletcher *et al.* 2012) by upregulating expression of

DICER (Mo *et al.* 2013) and also, at least for certain miRNA transcripts, modulating the activity of Drosha (Fletcher *et al.* 2012). The latter mechanism was elegantly elucidated by Fletcher and colleagues who demonstrated a dual mode by which AR regulates miR-27a: AR induced the expression of the pri-miR-23a27a24-2 cluster and concomitantly accelerated Drosha-mediated processing of this cluster to generate mature miR-27a (Fletcher *et al.* 2012). The biological significance of AR's role in regulating the PCa miRNAome was highlighted by the observation that treatment of LNCaP cells with the AR antagonist enzalutamide results in a ~25% reduction in the number of miRNAs associated with gene 3'UTRs (Hamilton *et al.* 2016).

AR-mediated regulation of the epigenome – more specifically, interplay between the AR and DNA methylation machinery – also modulates miRNA expression. As an example, by negatively regulating DNMT1, AR elicits hypomethylation and activation of the *MIR375* promoter (Chu *et al.* 2014). Similarly, AR appears to regulate the DNA methylation levels of genomic elements that regulate the expression of miR-22 and miR-29a, although the mechanism by which it achieves this is unclear (Pasqualini *et al.* 2015).

Androgen receptor-mediated rewiring of microRNA/mRNA transcriptional networks

The androgen-regulated transcriptome has been examined in many different PCa models and contexts (Nelson *et al.* 2002, Velasco *et al.* 2004, Ngan *et al.* 2009, Massie *et al.* 2011, Pomerantz *et al.* 2015). However, it is often difficult to identify which of these transcripts are directly regulated by AR, largely due to the fact that AR often binds to enhancers that are distal to its target genes (Wang *et al.* 2007). Carefully assessing the temporality of androgen-mediated gene regulation (Massie *et al.* 2011), integrating transcriptomic data with AR cistromes (Pomerantz *et al.* 2015) and/or using more sophisticated transcriptomic techniques such as global run-on sequencing (GRO-seq) (Toropainen *et al.* 2016), which is designed to identify nascent transcription, have improved the detection of *bona fide* AR targets in PCa cell lines and tissues. Nevertheless, accurately differentiating direct vs indirect targets of the AR remains challenging.

We propose that indirect regulation of protein-coding genes by androgens/AR is heavily influenced by the same genes being direct targets of AR-regulated miRNAs. Sun and colleagues explored this concept

by evaluating the AR-repressed miR-99a/let7c/125b-2 cluster and discovered significant enrichment of AR-induced genes that are putative targets of these miRNAs (Sun *et al.* 2014). Importantly, this *in silico* finding was validated for select candidate targets of each miRNA (Sun *et al.* 2014). We have expanded on this earlier work by intersecting TargetScan-predicted targets (Agarwal *et al.* 2015) of AR-induced miRNAs with a panel of androgen-repressed genes, and vice versa (Massie *et al.* 2011) (Table 4). Androgen-repressed genes are significantly over-represented in the putative targets of certain AR-induced miRNAs, whereas androgen-induced genes are over-represented in the targets of certain AR-repressed miRNAs. Our analysis provides strong evidence that protein-coding genes can be regulated by AR signaling via AR-mediated rewiring of miRNA/mRNA transcriptional networks. This phenomenon should be considered when undertaking genomic analysis of AR activity as it may facilitate the elucidation of direct vs indirect transcriptional regulation.

Regulation of AR and miRNA expression by feedback loops

Feedback loops are a common feature of the interplay between AR and miRNAs. For example, the *MIR21* gene is directly activated by the AR; conversely, miR-21 increases expression of AR in PCa cell lines, potentially by targeting *PTEN* – although the precise mechanism remains unclear (Ribas *et al.* 2009, Mishra *et al.* 2014). Since miR-21 potently stimulates prostate tumor growth and metastasis, this feedback loop is likely to play a key role in PCa progression by maintaining expression and activity of 2 oncogenic factors (i.e. AR and miR-21) (Li *et al.* 2009, Reis *et al.* 2012, Bonci *et al.* 2016). Conversely, the AR-repressed miRNAs miR-31 and miR-421 both target the AR directly (Östling *et al.* 2011, Lin *et al.* 2013, Meng *et al.* 2016). In the case of miR-31, this negative feedback loop is shifted in favor of the AR during disease progression, since DNA hypermethylation and subsequent downregulation of miR-31 is a feature of aggressive prostate tumors (Lin *et al.* 2013, Meng *et al.* 2016).

Indirect regulatory loops between miRNAs and the AR also exist. For example, miR-190a is directly repressed via an AR-binding site in its promoter, and in turn it suppresses AR expression and activity by targeting the 3'UTR of *YB1* (Xu *et al.* 2015), a transcription factor that acts to enhance AR gene transcription and also as an AR co-activator (Shiota *et al.* 2011, Xu *et al.* 2015).

Table 4 Intersection between AR regulated miRNA (by binding to ARBS) and AR regulated genes.

miRNA	# of predicted miRNA target genes ^a	Genes in overlap	P value ^b
Overlap between miRNA upregulated by AR and genes downregulated by AR			
miR-19	1338	43	0.027
miR-21	384	13	0.127
miR-22	620	22	0.040
miR-27a	1421	56	0.0001
miR-29a	1264	34	0.245
miR-32	1041	36	0.015
miR-133b	711	18	0.421
miR-135a	847	26	0.108
miR-148a	802	26	0.067
miR-182-5p	1329	44	0.012
miR-193a-3p	286	12	0.041
miR-203	960	25	0.342
miR-4496	5975	168	0.004
Overlap between miRNA downregulated by AR and genes upregulated by AR			
miR-221/222	504	26	0.023
miR-421	450	27	0.003

^aPredicted target genes for each miRNA were downloaded from TargetScan (release 7.2; [Agarwal et al. 2015](#)). ^bP values were calculated using hypergeometric probability tests. Genes in universe = 18,393 i.e. total number of genes in Targetscan database. AR-repressed genes ($n = 436$) and AR-upregulated genes ($n = 625$) are from [Massie et al. \(2011\)](#).

miRNAs as regulators of therapy resistance in prostate cancer

A key feature of CRPC is the acquisition of miRNA activity that promotes cell survival in androgen-depleted conditions. Given the central role of AR in CRPC, it is not surprising that many of these miRNAs act, at least in part, by enhancing AR expression and/or activity. The feedback loop between miR-21 and AR has already been described earlier; in the context of CRPC, it is worth noting that overexpression of miR-21 alone is sufficient to impart androgen-independent growth ([Ribas et al. 2009](#)). Unlike miR-21, miR-221/22 is repressed by AR signaling; however, it plays a similar role in promoting CRPC growth: upon castration, it is immediately upregulated and acts to enhance the expression of key cell cycle genes ([Gui et al. 2017](#)). Another example of a CRPC-relevant miRNA that influences AR is miR-125b, which was found to be elevated in androgen-independent derivatives of the LNCaP model ([Shi et al. 2007](#)). By targeting the AR co-repressor NCoR2, miR-125b enhances AR signaling and thereby promotes androgen-independent growth ([Yang et al. 2012](#)).

Concomitant with gain of oncomiRs in CRPC is loss of miRNAs that inhibit survival, proliferation and other oncogenic properties, including miR-146a, which targets the oncogene Rho-activated protein kinase ([Lin et al. 2007, 2008](#)); let-7c, which targets the oncogenes *Myc* and *Lin28* ([Nadiminty et al. 2012](#)); miR-145-3p, which targets cell cycle-associated genes ([Goto et al. 2017](#)) and the AR-targeting miRNAs miR-30c-5p and 30d-5p ([Kumar et al. 2016](#)).

While the aforementioned miRNAs appear to play important roles in the progression of PCa to a castration-resistant state, it is noteworthy that studies evaluating expression of miRNAs in CRPC tissues are poorly concordant. Nevertheless, by comparing miRNAs putatively associated with CRPC in three published genome-wide miRNA profiling studies ([Jalava et al. 2012, Goto et al. 2015, Goto et al. 2017](#)) and combining this with our own analysis of the Memorial Sloan-Kettering Cancer Center cohort ([Taylor et al. 2010](#)), we have identified a set of miRNAs that are reproducibly dysregulated in CRPC ([Table 5](#)). miRNAs that were not reproducibly altered in these studies may be false positives or alternatively may simply reflect differences in experimental design; for example, one study compared miRNA expression between benign prostatic hyperplasia and CRPC ([Jalava et al. 2012](#)), while the others compare normal prostate tissues or hormone-naïve PCa with CRPC ([Taylor et al. 2010, Goto et al. 2015, 2017](#)). Moreover, only the latest of these employed RNA-seq ([Goto et al. 2017](#)), while the earlier studies used PCR arrays ([Goto et al. 2015](#)) or miRNA microarrays ([Taylor et al. 2010, Jalava et al. 2012](#)).

Clinical potential of AR-associated microRNAs in prostate cancer

AR-associated miRNAs as biomarkers

Circulating miRNAs can be detected in many body fluids – most notably serum, plasma and urine – and have shown

Table 5 Dysregulated miRNAs in CRPC.

miRNA	Up/Down	# of studies reporting differential expression	References ^a
miR-7	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-18a	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-23b	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015), Goto <i>et al.</i> (2017)
miR-24	Downregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-25	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-27a	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-30a	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015)
miR-30b-3p	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-30e-3p	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-95	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-99a	Downregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-100	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-125a-5p	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-125b	Downregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-125b-2	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-130b	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-133a	Downregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-143	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-143	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-145	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-145 ^a	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-150	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-152	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015)
miR-181c	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-182	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-183	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-185	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-196b	Downregulated	3	Taylor <i>et al.</i> (2010), Goto <i>et al.</i> (2015, 2017)
miR-199a-5p	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-205	Downregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-214	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-221	Downregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-222	Downregulated/ Upregulated	4	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2015, 2017)
miR-425	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)
miR-452	Downregulated	3	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012), Goto <i>et al.</i> (2017)
miR-625	Upregulated	2	Taylor <i>et al.</i> (2010), Jalava <i>et al.</i> (2012)

^aThe studies referred to in the above table encompass 32 CRPC samples (18 metastases and 14 localized CRPC), 109 primary prostate tumors, 7 benign prostatic hyperplasia samples and 38 normal prostate tissues. Downregulated miRNA were listed in Table 5 if they were reproducibly altered in >50% of studies i.e. 3 out of 4; upregulated miRNA were listed if they were reproducibly altered in >50% of studies i.e. 2 out of 2 (Goto *et al.* (2015 and 2017) only report miRNAs downregulated in CRPC, and were therefore excluded when identifying upregulated miRNAs). Comparisons were done between benign prostatic hyperplasia vs CRPC (Jalava *et al.* 2012) or both normal/primary vs CRPC (Taylor *et al.* 2010, Goto *et al.* 2015, 2017).

promise as biomarkers for PCa diagnosis and prognosis and predicting therapy response. Since this topic has been comprehensively encapsulated in recent reviews (Fabris *et al.* 2016, Kanwal *et al.* 2017), here we will briefly touch on the relevance of the AR signaling axis to these putative biomarkers. Several PCa-associated circulating miRNAs are known to be regulated by AR, suggesting that – like the classic PCa marker prostate-specific antigen (Catalona *et al.* 1991) – their release into circulation is likely increased in PCa due to elevated AR activity. One prominent example is miR-375, which has consistently been identified as a potential marker of PCa, with higher levels in serum or

plasma being associated with shorter overall survival and metastasis (Mitchell *et al.* 2008, Brase *et al.* 2011, Selth *et al.* 2012, Huang *et al.* 2015). AR indirectly promotes miR-375 transcription by suppressing DNA methylation of its promoter (Chu *et al.* 2014), and we have previously shown that miR-375 levels are positively correlated with androgen signaling in multiple tumor datasets (Selth *et al.* 2017). The release of miR-375 from LNCaP cells into culture medium is stimulated by DHT (Tiryakioglu *et al.* 2013, Gezer *et al.* 2015), suggesting that its levels in the blood of patients would be increased in PCa due to elevated AR activity. Other AR-regulated miRNAs that

have been proposed as potential serum- or plasma-based biomarkers of PCa include miR-21 (Zhang *et al.* 2011), miR-125b (Fredsoe *et al.* 2018), miR-141 (Gonzales *et al.* 2011), miR-19a (Stuopelyte *et al.* 2016), miR-27a (Gao *et al.* 2018) and miR-221/222 (Santos *et al.* 2014).

Potentially the most useful application of circulating miRNAs would be in predicting response to therapies for CRPC. Given that an important subset of circulating miRNAs are AR regulated, it is conceivable that one or more of these may be useful in predicting response to AR-targeted therapies. Supporting this concept, elevated circulating miR-141 could predict clinical progression in a small cohort of CRPC patients with a sensitivity of 78.9% and specificity of 68.8%, although the therapies they received were mixed (chemotherapy, hormone therapy, or novel agents such as vaccines and kinase inhibitors) (Gonzales *et al.* 2011). Additionally, high levels of miR-221 in peripheral blood are predictive of early CRPC development (Santos *et al.* 2014). These examples suggest that circulating miRNAs could be useful additions to the biomarker armamentarium, although robust retrospective and prospective validation studies are required to move this field forward (Tavallaie *et al.* 2015, Wang *et al.* 2016).

Exploiting AR-modulating miRNAs as a therapeutic strategy

miRNA-based therapies are being actively pursued for a multitude of diseases, as eloquently described in several recent reviews (Hong *et al.* 2016, Van der Ree *et al.* 2016, 2017). In cancer, such therapies are focused on increasing levels of tumor suppressor miRNAs by delivery of miRNA mimics or decreasing levels of oncogenic miRNAs by delivery of antisense oligonucleotides (termed antimiRs or antagomiRs) (D'Angelo *et al.* 2016). Since a single miRNA can regulate multiple cancer-related pathways, miRNA-based therapies possess considerable promise. However, like other nucleic acid-based strategies, issues related to stability in biofluids, delivery to the tissue of interest and toxicity of mimics and antimiRs have hindered their clinical development (Rupaimoole & Slack 2017). While recent advances in formulation and delivery have led to miRNA-based therapies being tested in a range of clinical studies (Matin *et al.* 2016), it is worth noting that none are yet used in the treatment of patients.

With these caveats in mind, the application of a miRNA-based therapy in PCa remains a distant goal. Nevertheless, it is clear that opportunities in this area exist, especially given the importance of the interplay between miRNAs and the AR. For example, miR-34a reduces PCa

cell viability, at least in part by targeting AR (Östling *et al.* 2011), but it also downregulates the expression of >30 oncogenes across multiple oncogenic pathways (Beg *et al.* 2017). Cancer therapies based on master tumor suppressor miRNAs such as miR-34 are attractive because they would be expected to have high efficacy and at the same time reduce opportunities for resistance to develop. In pre-clinical studies, systemic delivery of a miR-34a mimic to mice harboring orthotopic PCa xenografts led to decreased tumor growth and metastasis (Liu *et al.* 2011). A liposomal formulation of miR-34a, termed MRX34, was evaluated in a Phase 1 clinical trial of patients with primary liver cancer, advanced solid tumors and hematological malignancies. Unfortunately, this trial was terminated due to immune-related serious adverse side effects (Hong *et al.* 2016, Van Roosbroeck & Calin 2017). Nevertheless, the promising anti-tumor activity of MRX34 observed in a subset of patients (Beg *et al.* 2017) provides incentive to continue investigating miRNA-based therapies for cancer. Indeed, new strategies to more precisely deliver miRNA-based payloads to solid tumors are already showing promise: exciting findings from a recent phase I clinical trial in mesothelioma demonstrated that miR-16-loaded, EGFR-targeted 'minicells' demonstrated an acceptable safety profile and early signs of activity (van Zandwijk *et al.* 2017).

As an alternative to therapies based on miRNA mimics or antimiRs, other strategies to regulate miRNA expression can be envisioned. Indeed, epigenetic therapies are being intensively investigated for a multitude of diseases, and we propose that an important aspect of their activity relates to miRNA biology. As specific examples associated with the AR signaling axis, re-activation of miR-124 using DNA demethylating agents or miR-320a with the histone deacetylase inhibitor OBP-801 lead to decreased AR expression and activity in PCa (Shi *et al.* 2013, Sato *et al.* 2016).

Summary

miRNAs and the AR are involved in an intricate dance mediated by direct interactions or complex indirect mechanisms involving transcription factors, co-regulators and epigenetic machinery (Figs 1 and 4). Despite the complexity of this interplay, one overarching theme is that miRNAs play a major role in enhancing and maintaining AR activity throughout the course of PCa progression; miRNAs that antagonize AR expression and activity are frequently lost, whereas miRNAs that facilitate

AR signaling are frequently gained. This phenomenon may be particularly relevant in CRPC, where miRNAs can play key roles in establishing and maintaining resistance to AR-targeted therapies. With this in mind, and given the continued focus on targeting AR in PCa, we propose that exploitation of miRNAs – potentially as biomarkers or therapeutic targets – warrants further consideration as a strategy to manage and/or treat this common disease. Finally, since AR is a key player and being investigated as a target in other solid tumors, including breast, bladder and hepatocellular, the value of understanding its interplay with miRNAs is increased; indeed, recent studies indicate that such interplay may be crucial to the progression of these other cancer types (Xiong *et al.* 2017, Yang *et al.* 2018, Xiao *et al.* 2019).

Declaration of interest

Wayne Tilley is an Advisory Editor of Endocrine-Related Cancer. Wayne Tilley was not involved in the review or editorial process for this paper, on which he is listed as an author. Luke Selth is an Advisory Editor of Endocrine-Related Cancer. Luke Selth was not involved in the review or editorial process for this paper, on which he is listed as an author. The other authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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