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INVITED REVIEW

Prostate Cancer

MicroRNA expression and function in prostate cancer: a review of current knowledge and opportunities for discovery

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MicroRNAs (miRNAs) are well-conserved noncoding RNAs that broadly regulate gene expression through posttranscriptional silencing of coding genes. Dysregulated miRNA expression in prostate and other cancers implicates their role in cancer biology. Moreover, functional studies provide support for the contribution of miRNAs to several key pathways in cancer initiation and progression. Comparative analyses of miRNA gene expression between malignant and nonmalignant prostate tissues, healthy controls and prostate cancer (PCa) patients, as well as less aggressive versus more aggressive disease indicate that miRNAs may be future diagnostic or prognostic biomarkers in tumor tissue, blood, or urine. Further, miRNAs may be future therapeutics or therapeutic targets. In this review, we examine the miRNAs most commonly observed to be de-regulated in PCa gene expression analyses and review the potential contribution of these miRNAs to important pathways in PCa initiation and progression.

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INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer in men worldwide and it is the fifth leading cause of cancer deaths.¹ Historically, PCa has provided important knowledge to the areas of early diagnosis and treatment of cancer, from highly-sensitive PSA screening and the modern radical prostatectomy, to the current recognition of PCa over-diagnosis and over-treatment of indolent disease. PCa was one of the first solid tumors with a targeted treatment for metastatic disease through androgen deprivation therapy (ADT), which has provided important clinical, molecular, and genetic knowledge of pathways that contribute to cancer progression and tumor cell therapeutic resistance.

The PCa field is again paving new paths in cancer management through the active surveillance of low-risk disease, as opposed to more radical treatments, and through the development and approval of several novel targeted drugs for metastatic disease and the metastatic microenvironment.^{2–4} As more is discovered about PCa biology and as PCa management evolves, our knowledge of microRNA (miRNA) biology and expression has grown. Here, we review the recent discoveries of numerous miRNAs reported to be associated with PCa, and we consider the potential contributions of these miRNAs to PCa biology and cancer management.

PROSTATE CANCER PROGRESSION AND MANAGEMENT

To understand in what ways miRNAs might contribute to PCa biology and how they might be helpful in its management, it is important to understand the natural progression of PCa, how it is managed at each stage, and the potential sources for miRNA analysis (**Figure 1**).

PCa is thought to arise from focally damaged epithelium that is characterized as proliferative inflammatory atrophy (PIA) and/or prostatic intraepithelial neoplasia (PIN).^{5,6} These lesions are considered precancerous and are of insufficient risk alone to warrant treatment. Cancer is suspected upon detection of an elevated serum PSA and/or an abnormal digital rectal exam; it is then confirmed by pathologic diagnosis of a biopsy specimen. Most often, the tumors diagnosed by PSA screening are too small to be clinically imaged. Therefore, individual biopsies are not targeted to a specific lesion; rather, multiple systematic biopsies are used to screen regions suspected to have cancer. There is a significant risk that biopsy needles may miss the tumor and result in a false negative diagnosis. In light of this, there are opportunities to discover new miRNA biomarkers that may improve the specificity of PCa diagnosis and reduce unnecessary repeat biopsy and/or patient anxiety.

Once diagnosed, the risk of PCa progression is assessed pathologically by tumor stage and volume, serum PSA level, and Gleason score. Lower-risk patients (e.g., those with less than three cancer-positive biopsy cores, low PSA (<10 ng ml⁻¹), and/or Gleason score ≤6) are given the option to enter active surveillance (AS) programs in which treatment is delayed until the disease shows the signs of increased risk upon repeat biopsy or elevated serum markers.⁷ Intermediate-risk PCa is characterized by intermediate stage (T2b, T2c) and grade (Gleason 7), and can be successfully treated with surgery or ionizing radiation therapy. High-risk PCa has an elevated stage (T3–T4), high serum PSA (>20 ng ml⁻¹), and/or high grade (Gleason ≥8). Due to the elevated risk for local recurrence or metastasis, treatment includes both local therapies, such as surgery or

radiation, as well as systemic ADT.⁸ One important consideration for the management of localized PCA is the multifocal nature of the disease, where a patient may be inaccurately labeled as having lower-risk disease because a higher-grade lesion was not detected by biopsy. The prevalence of upgrading after repeat biopsy or surgery is significant, as more than 30% of men initially diagnosed with low-risk disease are upgraded.^{9,10} Consequently, there are considerable unmet needs in the localized PCA patient population that could be addressed by the discovery of miRNA biomarkers that are associated with low-risk disease that does not require immediate treatment or high-risk disease that requires surgery or radiation therapy.

There is regrettably no cure for those PCA cases detected later in disease progression or that progress to metastasis after unsuccessful local treatment. Metastatic disease is initially treated with ADT through luteinizing hormone-releasing hormone (LHRH) analogs or anti-androgens, such as flutamide or bicalutamide. As resistance develops to these traditional ADT agents, second-generation agents such as abiraterone and enzalutamide are utilized, as well as bone-targeted radiotherapeutics and taxane-based chemotherapy. These new drugs have resulted in the development of a new type of therapeutically resistant PCA, which the field is just beginning to study.⁴ Here, miRNAs have a potential value as novel biomarkers of disease aggressiveness or therapeutic resistance, as well as therapeutic targets or as oligonucleotide therapeutics.

In summary, each step of PCA progression, from initiation to therapeutic resistance, is a potential source for miRNA discovery. MiRNA analysis in patients may improve the clinical decision making regarding cancer management or therapeutic outcome. Sources of miRNA may include the direct analysis of miRNA expression in biopsy or surgical tissue or noninvasive sources such as serum, urine, or semen (**Figure 1**). Finally, miRNAs may prove useful as novel therapeutic targets or as gene therapeutic agents in the future treatment of castration-resistant disease. Below, we review the approximately 10 years of miRNA research in PCA thus far.

miRNA BIOGENESIS AND PCA

The biogenesis of miRNAs in cancer has been recently reviewed elsewhere in detail.¹¹ Briefly, miRNAs are initially transcribed by RNA polymerase II as a large primary miRNA (pri-miRNA) transcript (**Figure 2**). In light of this, miRNA expression can be altered in cancer through oncogenic or tumor suppressive pathways or through genetic or epigenetic aberrations commonly observed in cancer including gene amplification, rearrangement, deletion, or promoter silencing. The miRNA stem-loop structure is then recognized by the Microprocessor complex and the hairpin is released following cleavage by the RNase III enzyme, DROSHA. The liberated pre-miRNA is then bound and exported into the cytoplasm by Exportin 5 (XPO5) and RanGTP. In the cytoplasm, the pre-miRNA is further cleaved by the RNase III enzyme, DICER, as guided by the RNA-binding protein (TRBP). The mature miRNA is then loaded into the RNA-induced silencing complex (RISC), which includes the Argonaute proteins and GW182 family proteins. The miRNA and RISC then directly suppress target gene expression through complementary binding of the seed sequence (6–8 nucleotides) to target mRNAs, leading to the inhibition of translation and/or decreased mRNA stability.

Various components of the miRNA biogenesis pathway have been found to be over-expressed, under-expressed, amplified, deleted, and/or mutated in human cancers.¹¹ In both localized and metastatic PCA, genetic deletion or mutation of miRNA biogenesis machinery appears to be rare.^{12,13} Rather, the miRNA biogenesis machinery is

more often overactive in PCA, suggesting a possible driver role of miRNAs. Early studies found the Microprocessor subunit, *DGCR8*, to be over-expressed in a large cohort of primary tumors.¹⁴ Moreover, knockout (KO) of *DGCR8* in a transgenic *PTEN* KO model of PCA inhibited tumor progression.¹⁵ *DDX5*, the DEAD box RNA helicase p68 that associates with the Microprocessor complex, is also over-expressed in localized PCA and enhances androgen receptor (AR)-regulated gene expression.^{16,17} Further, like the *TMPRSS2* gene, fusions of the *DDX5* gene with *ETV4* have been observed in PCA.¹⁸ Elevated expression of XPO5 has also been reported in metastatic versus organ-confined PCA.¹⁹ The RNase III enzyme DICER has also been shown to be over-expressed in both localized and metastatic PCA.^{14,19,20} Notably, however, low DICER expression has been associated with PCA recurrence.²⁰ This may be reflective of studies in *PTEN* KO transgenic mouse models in which complete *Dicer* KO blocked tumor growth and progression, whereas hemizygous loss of *Dicer* resulted in a more locally invasive phenotype.²⁰ Components of the RISC complex have also been reported to be over-expressed in PCA. *AGO1*, encoded by the *EIF2C1* gene, is upregulated in metastatic PCA.¹⁹ Similarly, *AGO2* has been reported to be over-expressed at the RNA and protein level.^{14,21} The genetic loci of *AGO2* are also located on chromosome 8q24, a region commonly amplified in PCA.^{22–24} *TNRC6A*, a component of GW-bodies that contributes to RISC-mediated silencing, has also been reported to be over-expressed in localized PCA.²¹ Collectively, these studies support that miRNA processing and activity may contribute to PCA biology and progression. These early results warrant additional research to define the upstream and downstream mechanisms of deregulated miRNA biogenesis machinery in PCA, their frequency in clinical specimens, and their potential contribution to PCA initiation and progression in cellular and animal models.

miRNA EXPRESSION IN HUMAN PCA

Approximately, 10 years ago, the first miRNA gene expression profiling studies were performed on clinical cancer samples, including prostate carcinoma.²⁵ These studies found that miRNA expression profiles accurately reflected the developmental lineage as well as the differentiation state of tumors. This was further supported by two studies in 2006 and 2008, in which unsupervised clustering analysis of miRNA expression clearly separated different tissue types and several miRNAs were identified to be commonly de-regulated in multiple human cancers.^{26,27} These pioneering studies highlight the potential of miRNA profiling for cancer research, and suggest that miRNAs may be even better than mRNAs for tissue- and tumor-specific molecular classification. Below, we summarize miRNA gene expression studies conducted on both prostate tissues and bodily fluids.

miRNA gene expression profiles in prostate cancer tissues

Initial high-throughput miRNA expression studies sought to identify differences between nonmalignant prostate tissue and PCA. More recent studies have increased in complexity to include additional tumor-versus-tumor analyses for miRNAs associated with stage, grade, biochemical recurrence, metastases, or castration resistance. Many studies have also validated discovery through additional analysis of distinct tissue sample sets or different quantitative techniques. As new technology has been developed, such as RNA sequencing, additional high-throughput data have accumulated. Here, we focus this portion of the review on high-throughput studies to highlight those miRNAs that are most commonly reported to be differentially expressed in genome-wide expression analyses (**Table 1**). While there are numerous additional expression studies that focus on specific miRNAs, they are not, for the most part, included in this review.

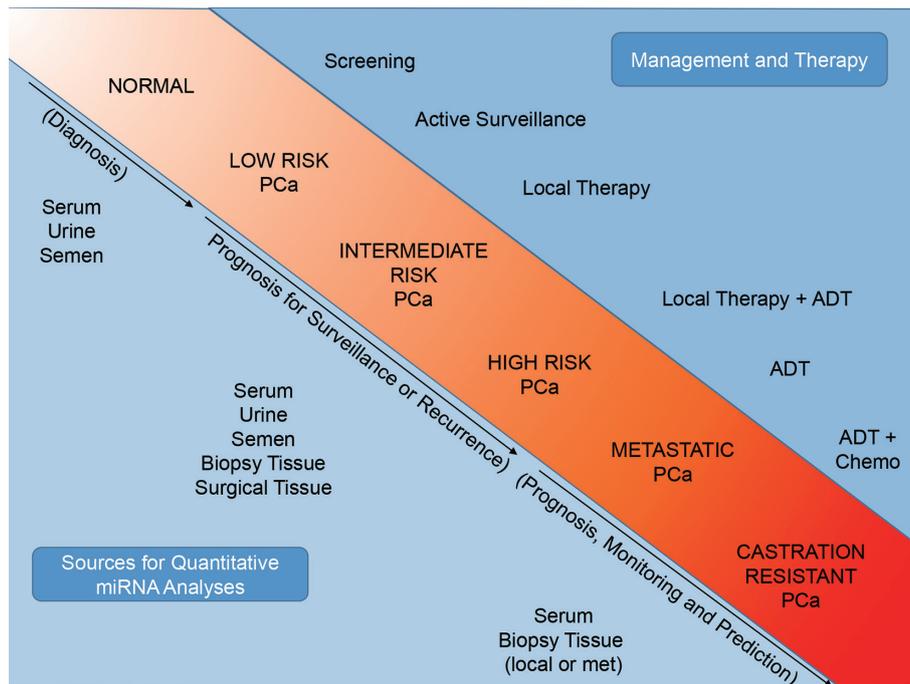


Figure 1: Opportunities and unmet needs in the progression of prostate cancer. The natural progression of prostate cancer from initiation to castration-resistant metastatic disease is highlighted. The upper right quadrant indicates the most common methods for management and therapy as the disease progresses. The lower left quadrant indicates resources for miRNA discovery and biomarker analyses and the potential areas for utility, such as diagnosis or active surveillance.

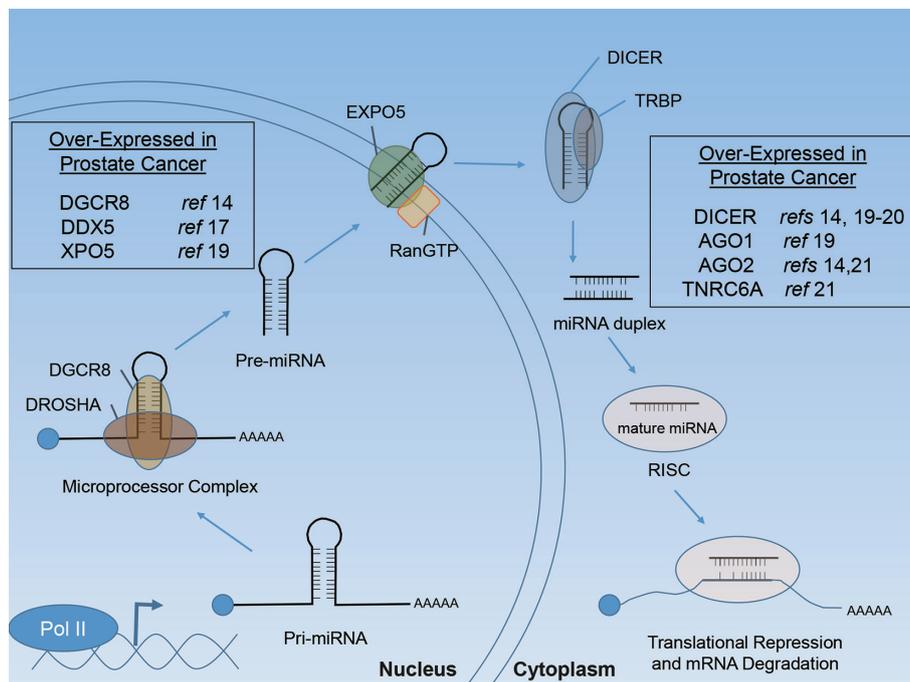


Figure 2: miRNA biogenesis. Schematic representation of miRNA biogenesis initiating with primary miRNA (pri-miRNA) transcription. Boxes indicate miRNA biogenesis components that have been reported to be deregulated in human PCa. References (ref) are indicated.

Several miRNAs have been commonly reported to be significantly over-expressed in PCa, when compared to nonmalignant tissue, across several tissue resources and analysis platforms (Table 1), including let-7a, miR-106a and miR-106b, miR-130b, miR-141, miR-148a, members of the miR-17-5p cluster, miR-182, miR-183, the miR-200 family, miR-21, miR-25, miR-32, miR-375, and miR-93.^{14,26,28-39} A

considerably higher number of miRNAs have also been commonly observed to have significantly decreased expression in PCa (Table 1). Some of the most commonly reported under-expressed miRNAs are members of the miR-143/145 family;^{14,29,30,32,33,35,36,38,40} however, new research indicates that this may be reflective of the relative loss of stromal tissue in the tumor microenvironment.⁴¹ This phenomenon

Table 1: Commonly reported miRNAs from gene expression analysis of prostate cancer tissue, serum, and exosomes

miRNAs	Sample types	Up/down	References	miRNAs	Sample types	Up/down	References
let-7a	Tissue	↑	32,35,38	miR-200b	Tissue, serum*, exosomes+	↑*+.	29,35,33,53*,68+
let-7b	Tissue	↓	14,29,30,36	miR-200c	Tissue, serum*	↑*	14,29,35,38,40,53*
let-7c	Tissue, serum*	↓*	29,30,36,50*	miR-205	Tissue, urine [^]	↓ [^]	14,29,30,31,32,33,34,35,36,62 [^]
let-7e	Tissue, serum*	↓*	29,35,50*	miR-20a	Tissue, serum*, exosomes+	↑*+.	26,29,32,33,35,38,57*,68+
miR-1	Tissue	↓	14,33,35	miR-20b	Tissue, serum*	↑*	29,35,39,51*
miR-100	Tissue	↓	29,30,32,36,40	miR-21	Tissue, serum*, exosomes+	↑*+.	26,29,33,35,38,53*,57*,68+
miR-103	Tissue, serum*, exosomes+	↑*+.	35,49*,68+	miR-214	Tissue, urine [^]	↑, ↓ ^{TD,^}	26 ^T ,39 ^T ,35 ^{TD} ,36 ^{TD} ,62 [^]
miR-106a	Tissue, serum*, exosomes+	↑*+.	26,29,38,51*,68+	miR-22	Tissue	↓	30,35,36,38
miR-106b	Tissue	↑	14,29,33,35,38	miR-221	Tissue, serum*	↓↑*	14,30,31,32,33,34,35,36,38,57*
miR-107	Tissue, serum*, urine [^] , exosomes+	↓, ↑* [^] , ⁺	36 ^T ,49*,54 [^] ,68+	miR-221*	Tissue	↓	28,33,34,35,40
miR-125a	Tissue	↓	30,36,38	miR-222	Tissue	↓	30,31,32,33,34,35,36
miR-125b	Tissue	↓	30,32,35,36,38	miR-223	Tissue, serum*	↓*	33,38,51*
miR-126	Tissue	↓	14,33,36	miR-224	Tissue	↓	28,33,34,35
miR-130a	Tissue	↓	29,33,35,36	miR-23b	Tissue	↓	32,34,35,36
miR-130b	Tissue, exosomes+	↑*	31,33,35,68+	miR-24	Tissue, serum*	↓*	32,35,36,38,40,51*
miR-133a	Tissue	↓	14,33,35,36	miR-25	Tissue	↑	14,26,29,32,33,36,38,39
miR-133b	Tissue	↓	33,35,36	miR-26a	Tissue	↓	30,35,36,40
miR-141	Tissue, serum*, exosomes+	↑*+.	29,32,35,53*,54*,55*,56*,68+	miR-26b	Tissue, serum*	↓*	30,34,51*
miR-143	Tissue	↓	29,30,32,33,36,38,40	miR-27b	Tissue	↓	30,33,35,36,38,40
miR-145	Tissue	↓	14,29,30,32,33,35,36,38	miR-29a	Tissue	↓	30,33,35,38,40
miR-145*	Tissue	↓	33,35,40	miR-30a	Tissue	↓	30,35,36,
miR-148a	Tissue	↑	29,33,35,38	miR-30c	Tissue, serum*	↑, ↓ ^{TD,*}	26 ^T , 39 ^T ,30 ^{TD} ,34 ^{TD} ,36 ^{TD} ,51*
miR-149	Tissue	↓	31,33,35	miR-30d	Tissue	↑	29,33,40
miR-150	Tissue	↓	33,35,36	miR-32	Tissue	↑	14,26,33,35,39
miR-152	Tissue	↓	35,36,38,40	miR-346	Tissue, serum*	↑*	36,50*,56*
miR-155	Tissue	↓	33,34,35	miR-34a	Tissue	↓	14,35,36
miR-15b	Tissue	↑	35,38,39	miR-34a*	Tissue	↓	28,35,40
miR-16	Tissue, serum*, exosomes+	↓↑*+.	30,31,36,49*,68+	miR-375	Tissue, serum*, urine [^] , exosomes+	↑* [^] , ⁺	14,29,31,33,35,38,53*,54*,55*,56*,54 [^] , ⁺
miR-17	Tissue	↑	26,29,33,35	miR-425	Tissue	↑	14,33,35
miR-181b	Tissue	↓	31,33,35,36	miR-429	Tissue, serum*	↑*	14,35,53*
miR-182	Tissue	↑	14,28,31,33,35	miR-455	Tissue	↓	33,34,35
miR-182*	Tissue	↑	31,33,35	miR-484	Tissue, urine [^]	↓ [^]	36,40,61 [^]
miR-183	Tissue	↑	31,33,35	miR-574	Tissue, serum*, urine [^]	↑* [^]	34,49*,54 [^]
miR-18a	Tissue	↑	29,35,36,39	miR-92a	Tissue, serum*, exosomes+	↑*+.	29,35,39,49*,68+
miR-191	Tissue	↑	26,29,40	miR-93	Tissue, serum*	↑*	14,29,33,35,40,51*
miR-195	Tissue	↓	30,36,40	miR-95	Tissue	↑	33,35,37
miR-199a	Tissue	↓	30,35,36,38	miR-96	Tissue	↑	31,33,35

Arrows indicate over-expression or under-expression. Primary data or ^TExpression in tissue, *Serum, +Exosomes, [^]Urine. miRNAs: microRNAs

may influence the perceived levels of other miRNAs commonly observed to be over-expressed or under-expressed in tissue specimens. Other miRNAs commonly reported to be significantly down-regulated in PCa tissue include members of the let-7 family, miR-100, miR-125a, miR-125b, miR-130a, miR-133a, miR-199a, miR-205, miR-221, miR-222, miR-24, miR-26a, miR-27b, and miR-29a.^{14,28-38,40,42} The collective contribution of these miRNAs to PCa initiation and progression, as well as their potential as diagnostic and prognostic biomarkers, is still being evaluated.

Several analyses have further compared miRNA expression in higher-risk versus lower-risk tumor tissues, as defined by grade and stage. The most common approach for these studies was to first identify a series of miRNAs differentially expressed between normal tissue and tumor tissue, and then to compare the expression of this

selected list of miRNAs between high-risk and low-risk tumors. Gleason score is a significant indicator of risk and is often a major determinant for treatment in newly diagnosed and AS patients. Several gene expression analyses have reported differences in miRNA expression between lower and higher Gleason score tumors.^{28,37,39,40} However, miRNA expression patterns in tissue that have the ability to differentiate between low and high Gleason score have not yet been identified.^{14,37,39} Several miRNAs have also been reported to be differentially expressed between nonmalignant prostate tissue and low-stage or high-stage PCa.^{28,29} Similarly, there does not appear to be a clear miRNA expression signature that can differentiate between lower- and higher-stage PCa. Further studies directly comparing tumor versus tumor miRNA expression may reveal new signatures associated with stage and/or grade.

Many gene expression studies have also evaluated miRNA expression in the biochemical recurrence (BCR) of PCa. The common approach has been to first identify miRNAs by comparison of nonmalignant prostate tissue to PCa, with later analysis for association with BCR.^{31,32} A common observation between these studies has been elevated miR-96 in samples with BCR. Two other studies have directly compared miRNA gene expression from the tumor tissue of recurrent and nonrecurrent PCa.^{43,44} Both found decreased miR-24-1* expression in recurrent prostate tumors. Down-regulated miR-154 expression has also been commonly found in tumors that biochemically progress.^{32,44}

There have been very few miRNA gene expression studies in metastatic or castration-resistant PCa. Two studies have directly evaluated miRNA expression profiles in nonmalignant prostate tissues versus castration-resistant prostate tumors.^{30,35} Several miRNAs were found to be differentially expressed between normal and castration-resistant tissue, as well as normal and castration-sensitive tissue, though a gene expression signature of castration-sensitive versus castration-resistant was not apparent. In a separate study of metastasis, Peng *et al.* evaluated miRNA expression profiles in primary PCa versus bone metastasis samples.⁴⁵ Martens-Uzunova *et al.* have similarly compared miRNA expression in primary versus lymph node metastatic PCa.³³ Comparative review of these two studies indicates that upregulated miR-210 and decreased miR-125b, miR-27b, and miR-99a are more commonly observed in metastases when compared to primary tumors.

Collectively, these gene expression analyses provide an important insight into miRNA association with disease and disease progression. The last decade has seen several advances in miRNA gene expression analyses and interpretation, as well as improved understanding in tissue selection, processing, storage, and the importance of RNA stability and quality. For example, significant differences in miRNA expression could be expected for laser microdissected versus manually dissected tumors, for tissue isolated by trans-urethral resection of the prostate (TURP) versus radical prostatectomy specimens, or for frozen versus formalin-fixed and paraffin-embedded samples. Differential sensitivities and specificities may also be expected between miRNA hybridization arrays, qRT-PCR libraries, or RNA sequencing platforms. All of these features have contributed to the inconsistencies of miRNA gene expression studies seen thus far in PCa.⁴⁶ Here, we have attempted to focus on the common observations, rather than the inconsistencies, to highlight those miRNAs that have been most commonly observed to be differentially expressed across different tissue sources and gene expression platforms. This does not signify that these miRNAs are more important or significant than others, as there have been numerous focused studies of miRNAs and miRNA biology that are independent of high-throughput gene expression analysis platforms. Nonetheless, we feel these common observations may be valuable and reflective of common biologic events in PCa.

Detection of aberrant miRNA expression in bodily fluids

The analysis of miRNAs in bodily fluids has uncovered their potential value as highly stable biomarkers. miRNAs can be detected in bodily fluids as free nucleic acids, within disseminated tumors cells or within exosomes or other microvesicles.⁴⁷ Here, we review the current literature of circulating and secreted miRNAs in PCa. As above, we attempt to highlight those miRNAs most commonly observed to be de-regulated.

Circulating miRNAs

The discovery of circulating miRNAs uncovered their possible value

as noninvasive biomarkers for a variety of indications. Mitchell *et al.* first described circulating miRNAs as blood-based markers for PCa, noting their surprisingly high stability.⁴⁸ miR-141, a miRNA commonly observed to be over-expressed in PCa tissue (**Table 1**), was elevated in the serum of patients with PCa, indicating that circulating miRNA levels may be informative in PCa diagnosis.⁴⁸ Independent miRNA gene expression analyses have also found elevated miR-141 levels in the serum of men with PCa, as well as elevated levels of several other miRNAs including miR-16, miR-92a, miR-103, miR-107, miR-197, miR-34b, miR-328, miR-485-3p, miR-486-5p, miR-92b, miR-574-3p, miR-636, miR-640, miR-766, and miR-885-5p.⁴⁹ A separate miRNA expression analysis found five miRNAs, let-7c, let-7e, miR-30c, miR-622, and miR-1285, as potential serum diagnostic biomarkers for PCa.⁵⁰ In another study, Moltzahn *et al.* found decreased miR-223, miR-26b, miR-30c, and miR-24 and increased miR-20b, miR-874, miR-1274a, miR-1207-5p, miR-93, and miR-106a levels in the sera of men with PCa.⁵¹ Circulating miRNA levels may also be indicative of disease aggressiveness. Differential levels of miR-93, miR-24, and miR-106a have been reported in low- and intermediate-risk PCa patients. RNA sequencing of exosomal miRNAs has also identified miR-1290 and miR-375 as potential prognostic makers for clinical outcome in castration-resistant PCa.⁵² Numerous studies focused on specific miRNAs such as miR-21, miR-221, miR-141, and miR-375 continue to shed light on the potential value of circulating miRNAs as diagnostic, prognostic, or predictive biomarkers.⁵³⁻⁵⁹

The continued study of circulating miRNAs has a great potential to uncover new insights into the function of miRNAs in cancer biology and to capitalize on their potential as biomarkers. As with studies of miRNAs in tissue, a variety of techniques and platforms likely account for the inconsistencies between studies. Nonetheless, several miRNAs have been consistently identified to be elevated in the serum of PCa patients, including miR-375, miR-141, and miR-21 (**Table 1**). Future unbiased analyses of circulating miRNAs in additional patient populations such as newly diagnosed low-risk versus high-risk patients or patients in AS programs may uncover new value for miRNAs as serum biomarkers in early disease management. Further studies of castration-resistant, advanced patients following second-generation ADT will provide further areas for discovery.

miRNAs as urine biomarkers

Urine represents a valuable resource for gene, protein, and RNA biomarkers in urological cancers. While there have been a limited number of miRNA studies in urine, results support that miRNAs are readily detected in urine and that they have promise as next-generation biomarkers (**Table 1**). Using a panel of miRNAs initially identified in the serum of patients with PCa, Bryant *et al.* found elevated miR-107 and miR-574-3p expression levels in the urine of men with PCa when compared to healthy controls.⁵⁴ Importantly, the diagnostic value of these miRNAs appeared to be greater than that of PCA3, an established urine RNA biomarker for PCa diagnosis.⁶⁰ Differential urine levels of miR-1825 and miR-484 have also shown diagnostic potential for men with PCa,⁶¹ as well as miR-205 and miR-214.⁶² The detection of miRNAs in the urine is not limited to cellular RNA. Elevated levels of miR-483-5p can be detected in the cell-free and nonexosome-enriched fraction of urine from patients with PCa.⁶³ While these studies are promising, there is a clear need for stringent assay development and validation for miRNAs as urine biomarkers.⁶⁴ Future studies will clarify their value and reliability. The analysis of miRNA biomarkers might be most helpful for the growing number of men diagnosed with low-risk disease who need accurate, noninvasive

assays to detect or predict progression during AS, without the need for frequent invasive biopsies.

Exosomal miRNAs

Exosomes are small extracellular vesicles, 40–100 nm in diameter, that release cellular components including proteins and RNA into the extracellular milieu.⁶⁵ These vesicles contain miRNAs and may be vehicles for transferring miRNAs between cells and tissues; however, it is unclear whether the levels of miRNA in patient exosomes are sufficiently high to be functional.⁶⁶ Nonetheless, exosomes present a separate compartment for miRNA expression analysis in tissue, serum, and urine that may provide additional value beyond total, cellular, or cell-free miRNAs (Table 1). Serum-derived exosomes have been applied to miRNA expression analyses and elevated miR-141 and miR-375 have been observed in men with metastatic PCa.⁵⁴ MiRNAs have also been found in larger extracellular vesicles (>200 nm), indicating that various microvesicles or microvesicle isolation platforms may be informative for miRNA studies.⁶⁷ Indeed, several studies support that miRNAs can be readily detected in PCa-associated exosomes.^{52,68,69}

THE FUNCTION OF miRNAs IN PROSTATE CANCER PROGRESSION

The progression of PCa can be described as a series of common histologic, genetic, and molecular events (Figure 3). There is accumulating evidence that miRNAs can act as upstream or downstream regulators of many of these events. Here, we review literature in which direct studies of miRNA function have been evaluated in PCa cells or models and identify areas for potential discovery.

GSTP1

One of the earliest and most universal events in PCa progression is the loss of GSTP1 expression through CpG island methylation.⁷⁰ The potential role of miRNAs in this early event, either through regulation of GSTP1 itself or the epigenetic regulation of its promoter, has not been well characterized. Aberrant DNA methylation can alter miRNA expression and, likewise, miRNA dysregulation can cause aberrant DNA methylation. Several miRNAs have been identified to regulate the key components of DNA methylation, including the miR-29

family, miR-148a, miR-152, and miR-185.⁷¹ Interestingly, many of these miRNAs are deregulated in PCa (Table 1). Several miRNAs have also been predicted to directly target GSTP1, including miR-133a/b, miR-144, miR-153, and miR-542. However, to our knowledge, there has not yet been direct evidence of miRNA regulation of GSTP1 expression or DNA methylation in prostatic models.⁷²

CDKN1B/p27

Another early event in PCa is the loss of p27/CDKN1B expression, which progressively decreases with increasing tumor grade and stage.⁷³ A well-characterized mechanism of p27 loss is through posttranslational proteasomal degradation,⁷⁴ though decreased expression can occur through additional mechanisms.⁷⁵ The only direct evidence for miRNA regulation of p27 in PCa involves miR-221 and miR-222, which directly suppress expression through interaction sites in the 3' untranslated region (3' UTR).⁷⁶ While miR-221/222 has been commonly reported to be down-regulated in PCa (Table 1), they have also been observed to be upregulated in castration-resistant PCa cells and to enhance PCa cell proliferation.^{76,77} Further studies may uncover additional miRNAs capable of regulating p27 expression either through direct 3' UTR interaction or through indirect regulation of p27 expression or posttranslational stability.

MYC

Another early event in human prostate carcinogenesis is the over-expression of the nuclear Myc protein.⁷⁸ While the MYC gene is located on chromosome 8q24, a region commonly amplified in PCa, MYC protein over-expression does not necessarily correlate with the gain of 8q24.⁷⁸ Thus, there may be alternative mechanisms for elevated MYC over-expression. Several miRNAs have been demonstrated to regulate MYC expression in PCa cells, including miR-34, miR-98, and let-7c,^{79–81} either directly or indirectly through upstream mechanisms. MYC is also known to directly regulate the transcription of several miRNAs commonly deregulated in PCa, including the miR-17-5p cluster.⁷⁹ This cluster was one of the first miRNAs found to be directly regulated by an oncogenic transcription factor.⁸² Several other miRNAs, including miR-145, have been found

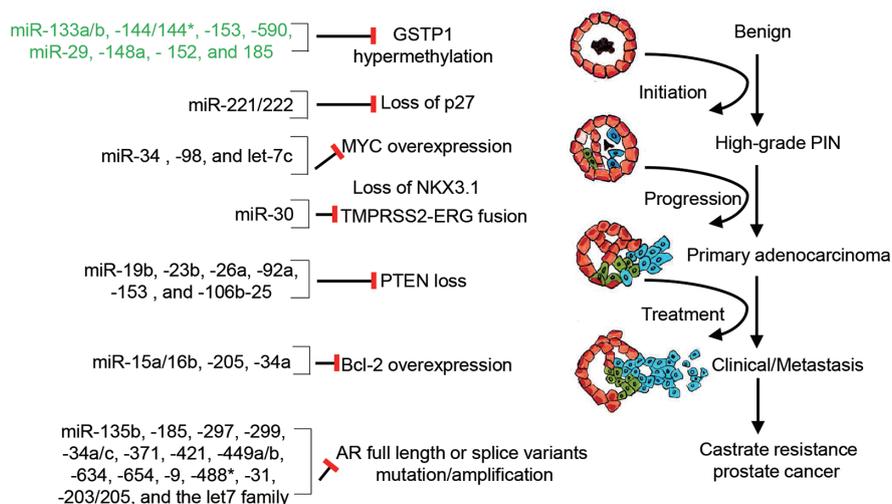


Figure 3: miRNAs in the molecular progression of prostate cancer. Schematic representation of key pathways in the molecular and genetic progression of PCa. miRNAs previously identified to directly regulate each molecule in cellular models of prostate cancer are provided. Those in green font indicate miRNAs that are implicated in regulation, but which have not yet been directly demonstrated in prostate models.

to directly regulate MYC in other cancer models.⁸³ Additional functional studies of MYC-regulated miRNAs and MYC-regulating miRNAs in PCa models may uncover additional contributions to PCa initiation or progression.

ETS gene fusions

One of the most common genomic alterations in PCa involves the fusion of *TMPRSS2* and *ERG*.⁸⁴ The role of miRNAs in *TMPRSS2/ERG* re-arrangement and downstream signaling has not yet been well defined. Members of the miR-30 family, which have been found to be both over- and under-expressed in PCa (Table 1), directly target *ERG* and *ERG* downstream function in PCa cells.⁸⁵ Two known targets of the *ERG* transcription factor are *EZH2* and *MYC*,^{86,87} which may further contribute to aberrant miRNA expression in PCa. Interestingly, the expression of miR-26a was found to be preferentially decreased in *ERG* rearrangement negative PCa, which was associated with hypermethylation of the miR-26 gene locus.⁸⁸ *EZH2* is a direct target of miR-26a; thus, this miRNA may contribute to *EZH2* over-expression in *ERG* rearrangement negative PCa. Similarly, miR-221 has been observed to be down-regulated in *TMPRSS2-ERG* positive PCa.⁸⁹ Further analyses may uncover new roles for miRNAs in these genetic rearrangements or *ERG* signaling.

PTEN

Genetic mutation and loss of *PTEN* is a frequent event in PCa, leading to aberrant PI3K/AKT signaling.⁹⁰ miRNA-mediated suppression of *PTEN* may provide an alternate route for elevated AKT signaling in PCa. miR-22 and the miR-106b-25 cluster have been reported to be over-expressed in PCa and to directly target *PTEN*, potentiating cellular transformation.⁹¹ Similarly, miR-153 has been reported to be highly expressed in PCa and to directly target and down-regulate *PTEN*, leading to activated AKT signaling.⁹² miR-23b, miR-19b, miR-26a, and miR-92a have also been demonstrated to directly regulate *PTEN* in PCa cells.⁹³ *PTEN* may also be regulated through competition between endogenous RNAs and miRNAs. *PTENP1*, a nonprotein-coding *PTEN* pseudogene, appears to compete with *PTEN* mRNA for miR-19b/20a binding.⁹⁴ Accordingly, loss of *PTENP1* enhances miR-19b/20a-mediated suppression of *PTEN*, thus promoting tumor development.

BCL-2

Another important pathway that is deregulated in advanced metastatic and castration-resistant PCa is the anti-apoptotic protein *BCL-2*,⁹⁵ which is an established direct target of miR-15/16. Knockdown of miR-15/16 in prostate epithelial cells leads to enhanced survival, proliferation, and tumorigenesis in NOD-SCID mice.⁹⁶ miR-205, whose expression is down-regulated in PCa, also regulates *BCL-2* expression.⁹⁷ Further characterization of miRNAs involved in apoptosis and survival may reveal additional mechanisms for the progression and survival of PCa cells.

AR

One of the most studied pathways in PCa progression and therapeutic resistance is the AR signaling pathway. Aberrant AR expression and activity can be attributed to numerous genetic, endocrine, and molecular mechanisms.^{98,99} In addition, several miRNAs have been identified as direct regulators of AR expression or AR activity. These include miR-135b, miR-34c, miR-488*, miR-31, miR-203/205, let-7, and others.¹⁰⁰⁻¹⁰³ Likewise, the expression of miRNAs can be directly regulated by AR transcriptional activity. Several miRNAs, including the commonly over-expressed miR-21 and miR-32 (Table 1), have been shown to be directly regulated by AR at the transcriptional level.^{35,104}

Further characterization of AR-targeting and AR-targeted miRNAs, as well as miRNA-mediated regulation of hormone metabolism, AR cofactors, or mechanisms of aberrant AR splicing, may reveal additional roles for miRNAs in aberrant AR activity in PCa.

In summary, aberrant miRNA expression and activity are commonly observed in clinical PCa, and functional studies clearly implicate miRNAs in PCa initiation and progression. While some mechanisms of miRNA regulation have been relatively well established in PCa biology and several miRNAs have been routinely implicated in PCa development and progression, the field is just beginning to understand and appreciate the role of miRNAs. As the field of PCa research continues to evolve, as well as the study of miRNA expression and function, new areas for discovery will surely be revealed. Some of the most notable areas of unmet need in PCa include the need for diagnostic markers of clinically relevant PCa, biomarkers that can reduce the biopsy frequency of men undergoing AS, and new molecular biomarkers and targets for the management and treatment of locally aggressive and metastatic castration-resistant PCa. The next decade of research holds great promise for continued discovery and translation. The value of these efforts and data will be best realized with systematic and consistent research methods and validation across multiple cohorts and research platforms.

COMPETING INTERESTS

No conflicts of interest to disclose.

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