

Review Article

MicroRNAs as predictive biomarkers and therapeutic targets in prostate cancer

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Received September 15, 2014; Accepted September 30, 2014; Epub October 2, 2014; Published October 15, 2014

Abstract: Prostatectomy or irradiation is the most common traditional treatments for localized prostate cancer. In the event of recurrence and/or metastasis, androgen ablation therapy has been the mainstay treatment for many years. Although initially effective, the cancer inevitably recurs as androgen-independent PCa, a disease with limited effective treatments. Enhanced predictive biomarkers are needed at the time of diagnosis to better tailor therapies for patients. MicroRNAs are short nucleotide sequences which can complementary bind to and control gene expression at the post-transcriptional level. Recent studies have demonstrated that many miRNAs are variably expressed in cancers vs. normal tissues, including PCa. In this review, we summarize PCa-specific miRNAs that show potential for their utilization as identifiers of aggressive disease and predictors for risk of recurrence. Additionally, we discuss their potential clinical applications as biomarkers and therapeutic targets.

Keywords: miRNA, biomarker, prostate cancer

Introduction

Prostate cancer (PCa) is the second leading cause of cancer-related death for men in the US, with estimated 233,000 new cases and 29,480 deaths from PCa in the United States in 2014. (<http://www.cancer.gov/cancertopics/types/prostate>). The natural course of the disease varies from indolent to highly aggressive cancer that metastasizes and leads to untimely death [1]. Due to the heterogeneity of PCa, identification of disease-specific molecular biomarkers is a rational approach to preemptively assess metastasis and systemic progression of PCa.

MicroRNAs (miRNAs) are small non-coding RNA strands that regulate expression of genes at the post-transcriptional and the translational levels. Growing evidences indicate that miRNAs can serve as ideal biomarkers for cancer diagnosis, prognosis and therapy. Individual miRNAs have been characterized either as tumor suppressors or oncogenes (oncomiRs). Either upregulation or downregulation of miRNAs have been associated with the clinicopathologi-

cal parameters of different cancers, including lung cancer, hepatocellular carcinoma and breast carcinoma [2-4]. Evaluation of the expression levels of specific miRNAs in prostate tissues may be used to detect cancer, predict the cancer prognosis and provide therapeutic targets. A summary of miRNAs, with altered expression, including targets, pathways, and clinical significance, in PCa are included in **Table 1**.

miRNA target prediction and miRNA/mRNA regulation networks

Identifying specific targets of miRNAs has been very challenging due to limited complementarity between miRNAs and messenger RNA (mRNA) transcripts. Functional characterization of miRNAs depends heavily on identification of their specific mRNA binding partners [5]. Many target prediction tools have been developed to discover miRNA targets. Most tools are designed to concurrently reduce the false-positive rate and maximize the accuracy of their findings [6]. For example, TargetScan [7-10] allows the researcher to search by miRNA

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Table 1. Summary of studies on miRNAs expression in PCa

	Chromosome location	upregulate/ downregulate	Target gene	Pathway	Role in Pca	Functions/clinical significants	Reference	
Predicting the risk of recurrence after surgery for PC	miR-200 family	miR-200a, miR-200b, and miR-429: 1p36.33, miR-200c and miR-141: 12p13.31	downregulation	zinc-finger E-box binding homeobox 1 (ZEB1) and ZEB2, SLUG	PDGF-D, TGF-beta	tumor suppression	Inhibit epithelial-to-mesenchymal transition	[36, 85, 86]
	miR-21	17q23.1	upregulation	PDCD4, TPM1, TGFB2, MARCKS HIF-1a and VEGF	TGFb pathway	onco-miRNA	Increase tumor growth, invasion, metastasis, and apoptosis resistance	[106-108]
	miR-100	11q24.1	downregulation	SWI/SNF		tumor suppression	inhibit invasion, cell proliferation	
	miR-221/222	XP11.3	upregulation	p27/kip1, p27	AR-dependent and AR-independent pathways	onco-miRNA	Increase cell proliferation, enhance colony formation, invasion, and cell survival	[112-115]
	miR-145	5q32	downregulation	FSCN1; OCT4, SOX2, KLF4, MAP3K3, MAP4K4, proto-oncogene YES, the core-binding transcription factor CBF3	Akt and Kras, P53, c-MYC	tumor suppressor	inhibit migration, invasion and metastasis	[83, 84]
	miR-143	5q32	downregulation	KRAS, ERK5, CD133, CD44, OCT4, KLF4, c-MYC	EGFR-RAS-MAPK	tumor suppressor	Suppress cell proliferation, migration in vitro, attenuate bone metastatic invasion in vivo, inhibit tumor sphere formation	[79-82]
	miR-133b	6p12.2	downregulation	CXCR4, FGFR1 and FSCN1	EGFR	tumor suppressor	Decrease cell proliferation, migration and invasiveness	[99]
	miR-205	Chr 1 and Chr 12	downregulation	N-chimaerin, ErbB3, E2F1, E2F5, ZEB2, and protein kinase C α , BCL2, PSAP, ARA24, HRAS, PARK7, AR, NR4A2, EPCAM, MED1 (also called TRAP220 and PPARBP)	the MAPK/ERK, Toll-like receptor and IL-6 signaling pathways	tumor suppressor	Counteract EMT, promote cell apoptosis, impair cell growth	[49, 58, 89, 90]
	miR-663	20p11.1	upregulation			onco-miRNA	promotes cell proliferation and invasion, neuroendocrine differentiation	
	miR-23b and miR-27b	9q22.32	downregulation	Rac1 and E-cadherin	PI3-kinase, MAPK, TGF-Beta, Wnt, mTOR, Jak-STAT, toll-like receptor and Notch		suppresses key metastatic processes including cell invasion, migration and anchorage-independent survival without affecting cell proliferation.	
miR-182 and 187	7q32.2/18q12.2	upregulation				promising biomarkers for prostate cancer prognosis		

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Predicting high risk PCa	let-7b	22q13.31	downregulation	AR, c-MYC, HMGA2, E2F2, CCND2, RAS, EZH2		tumor suppressor	Suppress cell proliferation, Suppress advanced tumor progression, Induce cell cycle arrest in vitro and suppress tumor development in vivo, Inhibit tumor growth	[72-78]
	miR-221	XP11.3	upregulation	SOCS3 and IRF2	JAK/STAT signalling pathway	onco-miRNA	stimulates cell growth and influences cell cycle progression, Enhance cell proliferation, colony formation, invasion, and cell survival	[112, 113]
	miR-203	14q32	downregulation	CKAP2, LASP1, WASF1, BIRC5, ASAP1, RUNX2, PARK7, BRCA1, ZEB2, Bmi, Survivin		tumor suppressor	Suppress cell proliferation, promote cell apoptosis, and inhibit metastasis dissemination, suppress bone metastasis via inhibition of cell motility, invasion and EMT	[58, 87, 88]
	miR-34a	1p36.23	downregulation	CD44, AR, CDK6, BCL2, SIRT1	c-MYC	tumor suppressor	Inhibit tumor progenitor cells and suppress metastasis, induce cell senescence and apoptosis	[91-95]
	miR-101		downregulation	EZH2		tumor suppressor	Attenuate tumor cell invasiveness	[97, 98]
	miR-146a	5q34	downregulation	ROCK1, EGFR, MMP2	EGFR	tumor suppressor	Suppress cell metastasis to bone marrow endothelium, Inhibit cell growth, colony formation and migration in vitro	[100, 101]

name, gene name, or from conserved, or poorly conserved miRNA families across several species. TargetScan is easy to use, but only considers stringent seeds, and ignores many potential targets. RNAhybrid [11, 12] is another tool for the easy, fast and flexible prediction of miRNA targets. RNAhybrid allows the user to find the minimum free energy hybridization of a long and a short RNA. A number of advanced settings are available such as specification of hits per target, helix constraints, maximal internal loop size, maximal bulge loop size and maximum free energy cutoff. PicTar is a combinatorial miRNA target prediction tool and provides search function for miRNA targets on mRNA transcripts. Searches can be performed by miRNA or gene name. PicTar minimizes false positive results by using sequence alignment to eight vertebrate species and the candidate genes of each species are scored to create a combined score for a gene [13-15]. PITA is a miRNA target prediction tool that uses target-site accessibility as its major feature. PITA uses complementarity analysis within seed regions to predict miRNA targets, then compares the free energy to make it accessible to the miRNA [16]. Other available tools include miRanda [17, 18], miTarget [19], MirTif [20], HOCTAR [21] and TargetBoost [22].

Among all miRNA target prediction tools, there are four main characteristics of miRNA: mRNA target interaction that emerge as common features: seed match, conservation, free energy, and site accessibility [23]. Earlier computational tools focused on identification of functional miRNA binding sites and co-expression of miRNAs and their targets [6, 24]. Sumazin et al. found miRNA interactions which mediate cross-talk between canonical oncogenic pathways by analyzing gene expression data in glioblastoma [25]. Tsang et al. developed a computational method by using gene expression data and find that both positive and negative transcriptional coregulation of a miRNA and its targets are prevalent in the human and mouse genomes [26]. Recently, Afshar et al. developed a new integrative analysis method which can be used to infer certain types of regulatory loops of deregulated miRNA/Transcription factor (TF) interaction. They analyzed mRNA/miRNA expression data from tumor and normal samples and identified several known and novel deregulated loops in PCa [27]. Computational

prediction of miRNA target sites is an efficient tool to understand the molecular mechanisms of miRNA-mediated interactions and plays a key role in miRNA-based therapeutics in clinic.

MiRNAs bind to complementary sequences on target mRNA transcripts, and regulate gene expression post-transcriptionally. TFs are a type of regulator that bind to specific DNA sequences within gene regulatory elements and induce or inhibit transcription. Motifs comprising miRNAs, TFs, and target genes make up transcription networks. The regulation of miRNA transcription involves feedback loops (FBLs) and feed-forward loops (FFLs) in which miRNAs participate together with transcription factors (TFs) [28, 29]. These motifs play crucial roles in regulation of gene expression. For example, O'Donnell found miR-17, E2F1, and c-Myc cluster controls cellular proliferation in cancer [30]. Muniategui et al. reviewed and analyzed the interplay between miRNAs and mRNAs. They grouped different mathematical models and computational prediction methods into: dependency analysis, linear regression (multiple and regularized) and Bayesian methods. They also emphasized that those models combining more heterogeneous experimental data might be more reliable predictors of miRNA-mRNA interactions. Schmeier et al identified 12 TFs which can regulate the expression of several important miRNAs during the differentiation process. They analyzed the transcriptional circuitry of miRNA genes during monocytic differentiation by using time-course expression data for TFs and miRNAs [31].

Predicting the risk of PCa recurrence after treatment

Recently, a study identified 25 differentially expressed miRNAs between the high and low biochemical failure risk groups. This study identified that specific miRNA expression levels could be used as potential predictors of biochemical failure risk at the time of prostatectomy [32].

The miR-200 family contains miR-200a, miR-200b, miR-200c, miR-141, and miR-429. Studies showed that members of the miR-200 family are down-regulated in human cancer cells and play a critical role in the suppression of epithelial-mesenchymal transition (EMT) by repressing the expression of key mRNAs that

are involved in EMT (ZEB1 and ZEB2) [33, 34]. miR-200a over expression reduces PCa cell growth [35]. Kong et al found miR-200 plays an important role in the processes of EMT and mesenchymal-epithelial transition (MET) in PDGF-D over-expressing PCa cells [36]. The loss of miR-200 induces EMT phenotype in LNCaP and PC3 cells, and the re-expression of miR-200 causes reversal of the EMT phenotype to MET phenotype.

miR-21 regulates the expression of multiple mRNA targets associated with tumor invasiveness and micro-vascular proliferation. Positive miR-21 expression is associated with poor biochemical recurrence-free survival and has predictive value for biochemical recurrence risk in patients with PCa after radical prostatectomy [37]. Amankwah et al. found that miR-21 is also related to PCa recurrence after radical prostatectomy and the differential expression of miR-21 in PCa is more prominent in obese than in non-obese cases [38].

Androgen receptor (AR) plays a critical role in cell survival and proliferation in PCa. The TGF β signaling pathway is one of the important pathways that AR cross-talks with. Mishra et al. showed that the AR and miR-21 drive the down-regulation of TGF β R2 by acting through a positive feedback loop that inhibits growth responses in PCa [39].

Using the Cox regression test, Leite et al. compared four miRNAs (miR-100, miR-145, miR-191 and miR-let7c) with risk of PCa biochemical recurrence. They showed that miR-100 and tumor volume were independently related to tumor recurrence [40].

Spahn et al. found that miR-221 was progressively down-regulated in more aggressive PCa and the down-regulation of miR-221 was linked to tumor progression and recurrence [41]. Androgen-dependent PCa typically progresses to castration-resistant prostate cancer (CRPC) after androgen deprivation therapy. Sun et al. found miR-221/222 were significantly increased in CRPC cells and miR-221/222 might be involved in the development or maintenance of the CRPC phenotype [42].

MiR-145 is a tumor suppressor and its expression is regulated by the p53 pathway [43]. MiR-145 has been shown to be down-regulated in

breast cancer and colorectal cancer [44, 45]. Avgeris et al. found that down-regulated miR-145 expression is associated with higher risk for biochemical recurrence and significantly shorter disease-free survival. In addition, the reduction of miR-145 expression in PCa was correlated with higher tumor grade (Gleason scores), advanced clinical stage, larger tumor diameter and higher prostate-specific antigen (PSA) and follow-up PSA levels [46]. MiR-133b locates at chromosome 6p12.2, and is down-regulated in several solid cancers. Li et al. studied miR-133b expression in LNCaP and PC3 PCa cells and clinical PCa samples. They found that miR-133b displays tumor-promoting properties and inhibits apoptosis in LNCaP cells, whereas it acts as a tumor suppressor in PC-3 cells. RB1-inducible coiled-coil 1 (RB1CC1), which is found on chromosome 8q11 and is a target of miR-133b, is an independent prognostic indicator for PCa. They concluded that miR-133b and RB1CC1 might be two independent prognostic factors for biochemical recurrence [47]. Omer et al found that miR-1 and miR-133b were significantly down-regulated in recurrent PCa specimens and might be used as novel biomarkers for prediction of PCa progression [48].

MiR-205 was significantly down-regulated in PCa and re-expression of miR-205 induced apoptosis, cell cycle arrest and EMT [49, 50]. Hagman et al. [51] found that the expression of miR-205 is inversely correlated to the occurrence of metastases and shortened overall survival, and is lower in CRPC patients. Gandellini et al. found replacement of miR-205 in PCa cells can restore basal membrane deposition and 3D organization into normal-like acinar structures by involving Δ Np63 α , which is essential for maintenance of the BM in prostate epithelium [49]. They found that ectopic miR-205 over-expression in PCa cells impaired enhancement of cell invasion, acquisition of stem cell traits, tumorigenicity and metastatic dissemination by counteracting cancer associated fibroblasts induced EMT [52].

Previous studies have shown that miR-663 is associated with viral infection, inflammatory responses, and autoimmune diseases. MiR-663 may also be a potential tumor suppressor in gastric cancer, colorectal carcinoma, PCa, and acute lymphoblastic leukemia. Jiao L et al. [53] showed that miR-663 is upregulated in

CRPC tissues. Overexpression of miR-663 in LNCaP cells promotes cell proliferation and invasion. Jiao et al. showed that miR-663 plays a critical role in the progression of LNCaP cells to androgen independence. They found miR-663 promotes the growth and invasion of LNCaP and LNCaP-AI cells, and the level of miR-663 in the samples from hormone naive PCa and CRPC (consistent nomenclature with rest of paper) patients correlate with tumor grade (Gleason scores) [53].

MiR-23b and miR-27b are two members of the same miR cluster (miR-23b/-27b). Meghan et al. showed that miR-23b and miR-27b are down-regulated in metastatic PCa and CRPC. Overexpression of miR-23b/-27b in aggressive PCa cell lines decreases migration, invasion and anchorage-independent growth. They concluded that miR-23b/-27b is specifically linked to metastasis suppression and may be a useful biomarker for poor prognosis in addition to having therapeutic potential as a target for advanced metastatic PCa [54].

GABRE~miR-452~miR-224 is significantly down-regulated in PCa. Kristensen et al. showed that miR-224 and miR-452 inhibit proliferation, migration, and invasion in PC3 and DU145 cells by regulation of the cell cycle and cellular adhesion and motility. In addition, high GABRE~miR-452~miR-224 promoter methylation was significantly associated with biochemical recurrence in PCa and is a new promising epigenetic candidate biomarker for PCa diagnosis and prognosis [48].

MiR-182 and 187 are another miRNAs most differentially expressed between normal prostate and tumor tissue. Casanova-Salas I et al. found that miR-182 and 187 are promising biomarkers for PCa prognosis to identify patients at risk for progression [55].

Predicting more aggressive/high risk PCa

The treatment of more aggressive/high-risk prostate cancer (HRPCa) is a tremendous challenge for oncologists. The identification of predictive moleculobiological markers allowing earlier assessment of metastasis and systemic progression and avoiding overtreatment is one of the most urgent clinical needs in PCa.

High-motility group AT-hook gene 1 (HMGA1) is a non-histone nuclear binding protein, and is significantly overexpressed in PCa and associated with high grade and advance stage [56]. Schubert et al. showed that HMGA1 is a target of the miRNA let-7b and found correlation of let-7b down-regulation with HMGA1 over-expression in primary PCa samples. They concluded that let-7b is a tumor suppressor miRNA in high-risk PCa and presents a basis for improved individual therapy for high-risk PCa patients [57].

Some studies have reported that miR-205 is up-regulated in lung cancer. However, in PCa, miR-205 is down-regulated, and may be associated with a poorer prognosis in PCa [50, 58]. Kalogirou et al. showed that miR-205 to be significantly down-regulated in over 70% of the HRPCa samples. In addition, miR-205 is increasingly down-regulated in lymph node metastases compared to the primary tumor. According to their study, miR-205 is involved in the development and metastasis of PCa, but failed to work as a useful clinical biomarker for HRPCa [59].

Several studies have shown that miR-221 is one of the most strongly and frequently down-regulated miRNAs in primary-PCa [60-62]. Spahn et al. [63] showed that miR-221 is progressively down regulated in aggressive PCa, lymph node-metastases, clinical recurrence and has potential as a biomarker predicting progression and recurrence in high-risk PCa [63]. The same group found that miR-221 regulates proliferation, invasion and apoptosis in PCa, partially via STAT1/STAT3 by activation of the JAK/STAT signaling pathway. miR-221 directly inhibits the expression of oncogenes SOCS3 and IRF2 [64].

Sapre et al. performed the study to determine if miRNA profiling of urine and plasma at radical prostatectomy can distinguish potentially lethal from indolent PCa. They found that the combination of miR-16, miR-21 and miR-222 measured in urine was a better predictor of high-risk disease than any individual miRNA [65].

miRNA in body fluid of patients with PCa

Tumor-specific miRNAs were first discovered in the serum of patients with diffuse large B-cell

lymphoma. Patrick et al. showed the presence of circulating tumor-derived miR-629 and miR-660 were confirmed in blood with 100% sensitivity and specificity by using a xenograft mouse PCa model. Dysregulated miRNA expression is an early event in tumorigenesis, therefore measuring circulating miRNA levels may be useful for early cancer detection, contributing greatly to the success of patient outcome.

Zhang et al. evaluated the possibility of miR-141 as a biomarker for bone-metastatic PCa by measuring the miR-141 level in serum of patients. They found that the serum miR-141 level is positively correlated with alkaline phosphatase (ALP) level in patients with skeletal metastasis [66]. Additionally, Lodes et al. reported that fifteen miRNAs were found to be over-expressed in serum from all stage 3 and 4 PCa patients including miR-16, -92a, -103, -107, -197, -34b, -328, -485-3p, -486-5p, -92b, -574-3p, -636, -640, -766, -885-5p compared to normal controls [67].

Liu et al. analyzed six matched cancer and non-cancerous tissues from non-small cell lung cancer (NSCLC) patients by miRNA microarray and found that the level of serum miR-21 was increased in cancer patients. In addition, over-expression of serum miR-21 was strongly associated with lymph node metastasis and advanced clinical stage of NSCLC [68].

miRNA database and oncomiR

miRNA databases play a critical role for miRNA target predictions. Numerous databases have been developed that facilitate easy and efficient mining of data for miRNAs and their target genes involved in a specific cancer. Different miRNA target prediction algorithms can provide different results, thus researchers have to check across multiple algorithms to get an additional layer of confidence for true positive targets. starBase was developed to facilitate the comprehensive exploration of miRNA-target interaction maps from CLIP-Seq and Degradome-Seq data [69]. This has allowed researchers to search targets predicted by different algorithms, including TargetScan, PicTar, PITA, miRanda and RNA22. The miRBase database is a well-known database that can search published miRNA sequences and annotation. miRBase allows the user to search both hairpin and mature sequences, and entries can also be retrieved by name, keyword, references and

annotation [70]. miRNA databases provide researchers an easy and open access to miRNA sequences and miRNA complex network analysis.

Recent studies showed some miRNAs are up-regulated in PCa, suppressing apoptosis-related genes and leading to increased tumor growth and metastasis. The upregulation of miR-221 and miR-222 have been reported in PCa and involved in the PCa tumorigenesis and metastasis. miR-21 is another potential oncomiR that is overexpressed in several solid tumors including lung, breast and PCa, and plays an important role in tumor growth, invasion and metastasis. The expression level of miR-21 is high in more aggressive PCa cells, such as PC-3 and DU-145 cells, whereas it is low in androgen-dependent LNCaP cells. Additionally, miR-125b is found to be overexpressed in PCa and is essential for androgen independent PCa cell proliferation [71].

Conclusions

Recent studies have suggested that miRNAs play an important role in tumorigenesis, progression, and prognosis of PCa. Since miRNA signatures can reflect differences in molecular changes in PCa at different stages, level, and aggressiveness, they have potential as powerful tools to support the diagnosis and early detection of cases with poor prognosis. Future studies are needed to investigate the role of individual miRNAs in PCa initiation and progression and develop miRNA-based diagnostic and therapeutic strategies.

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