

Review

MicroRNAs and prostate cancer

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MicroRNAs (miRNAs) are a class of small, non-coding, single-stranded RNAs that negatively regulate gene expression by mainly binding to 3' untranslated region (UTR) of target mRNAs at the post-transcriptional level. Recent studies have demonstrated that aberrant expressions of miRNAs are closely associated with the development, invasion, metastasis and prognosis of various cancers including prostate cancer (PCa). The proposed molecular mechanisms that underlie the aberrant expression of miRNAs result from gene changes, epigenetic modification and alteration of Dicer abundance. Although up to 50 miRNAs have been reported to be significantly expressed in human PCa, only a small number of them were experimentally shown to make contribution to the pathogenesis of PCa. The aim of this review is to describe the mechanisms of several known miRNAs, summarize recent studies on the relevance of altered expression of oncogenic miRNAs (e.g. miR-221/-222, miR-21, and miR-125b) and tumor suppressor miRNAs (e.g. miR-101, miR-126*, miR-146a, miR-330, miR-34 cluster, and miR-200 family) for PCa. Additionally, their potential clinical applications and prospects in PCa, such as biomarkers and clinical therapies, are also briefly discussed.

Keywords microRNA; prostate cancer; oncogene; tumor suppressor; biomarker

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Introduction

MicroRNAs (miRNAs) are endogenous small non-coding, single-stranded RNAs that regulate gene expression by affecting the stability or the translation efficiency of target mRNAs. They target at the binding sites located mostly in the 3' untranslated regions (3' UTR) of mRNAs, and in the coding regions or 5' UTR as well [1,2], causing mRNA degradation or translational repression. Considerable efforts have been devoted to explain the importance of miRNAs in both cellular fundamental development and

tumorigenesis at the level of transcription or post-transcription regulation [3–5]. Recent studies have also found that miRNAs are involved in aging by stress [6]. Regulatory effect of miRNAs is mediated by the interaction between miRNAs and their target mRNAs and nearly 30% of gene expression is probably regulated by miRNAs via this interaction [1,7,8]. However, details about the regulatory components involved in the corresponding processes remain largely unclear. Individual miRNA may regulate ~200 targets by partial base pairing to mRNAs [9], whereas a particular target is probably modulated by a few miRNAs via different number and types of binding sites in the 3' UTR of the targets [10,11], suggesting that one miRNA may control numerous biological or pathological signaling pathway by affecting the expressions and functions of their targets. Therefore, regulatory pathways caused by miRNAs are very complicated processes within the cell [12,13]. Efforts are necessary to investigate the expression of miRNAs and novel targets regulated by miRNAs.

Regulations of the growth, differentiation and apoptosis of carcinoma cells controlled by the interaction of miRNAs with their target genes have been investigated recently [3–5]. Hundreds of altered expressions of miRNAs are found to be closely associated with the formation and progression of most kinds of human cancers, and about 50% of miRNA genes are found to be located in cancer-related genomic regions. In these processes, miRNAs may function as oncogenes or tumor suppressors [14], and the former ones are often up-regulated, whereas the latter are down-regulated in cancers. The involvement of miRNAs in human prostate cancer (PCa), which is one of the most commonly diagnosed malignant tumors, and so far the second significant cancer killer of men in America [15], has clearly demonstrated a correlation between miRNAs and their targets with prostate carcinogenesis in recent years [16–18]. Several miRNAs and their targets have been discovered to express abnormally in PCa, leading to the corresponding response in the development, invasion, and metastasis of this disease. The altered expressions of some selected miRNAs are useful as biomarkers for

diagnosis, prognosis, and classification purposes of PCa [19–21]. Thus, understanding of the characteristic miRNA abnormalities, and restoring normal miRNA–mRNA regulation pathways could contribute to the development of novel therapeutic strategies in PCa.

In this paper, we reviewed the involvement of miRNAs in PCa, described the mechanisms of several known miRNAs, and summarized the application and prospect of the miRNAs in this disease.

Potential Mechanisms of the Aberrant Expression of miRNAs in PCa

As is often the case for aberrant expressions of miRNAs, the common mechanisms include the alteration of miRNAs copy number [22], epigenetic modification of miRNAs, especially the altered DNA methylation triggers the miRNAs silencing [23]. Conversely, miRNAs also regulate target gene expressions by epigenetic mechanism [24]. In addition, mutations of precursor miRNAs are found and remarkably affect miRNAs processing and abundance, when mutations happen in stem or unpaired flanking regions which are critical for DGCR8 binding and Drosha cleavage. However, mutations occurring in the loop region have no influences on the processing of miRNAs [25]. Other factors, including failure in miRNA processing and deregulation in miRNAs promoters, affect the expression of miRNAs as well. These mechanisms also contribute to explain the miRNAs whose expression levels are deregulated in PCa. For example, Dicer, a necessary processing enzyme, is up-regulated in PCa, leading to the deregulation of miRNAs and correlation with clinical stage and Gleason score [26]. Up to now, there are more than 50 miRNAs which are deregulated in PCa [12,27]. We reviewed several aberrantly expressed miRNAs, including some oncogenic miRNAs (miR-221/-222, miR-21, and miR-125b) and some tumor suppressor miRNAs (miR-101, miR-126*, miR-146a, miR-330, miR-34 cluster, and miR-200 family). miRNA-200 family consists of two clusters which are located on chromosome 1 and 12, respectively; the former cluster encodes miR-200b, miR-200a, and miR-429, although the latter encodes miR-200c and miR-141. Vrba *et al.* [14] found that a CpG island near the miRNA is hypermethylated which has negative correlation with the expression of the corresponding miRNA. They have provided evidence that PC3 cells lost expression of miR-200c and miR-141 due to aberrant methylated CpG island near the miR-200c and miR-141, whereas LNCaP and DU145 cells expressed normal level of miR-200c and miR-141 resulting from unchanged CpG island [14]. Almost simultaneously, expression of Dicer was found to be up-regulated in aggressive PCa [26], which may be one of the mechanisms of inducing up-regulation of most of the

miRNAs related to prostate tumors. In addition, the androgen/androgen receptor (AR) signaling is reported to contribute to the regulation of miRNAs in PCa. Studies by Ribas *et al.* [28] showed that androgen-induced AR binds to miR-21 promoter and subsequently results in overexpression of miR-21, which is supposed to be associated to the androgen-dependent (AD) cell growth and castration resistance in PCa. The mechanisms responsible for aberrant expression of miRNAs in PCa are summarized in Fig. 1.

Furthermore, some aberrantly expressed miRNAs are PCa-specific, and the expression levels are remarkably different between benign and malignant PCa. Rokhlin *et al.* [29] found that p53 induced overexpression of miR-34, which was only found in AR positive PCa LNCaP cells, whereas miR-21 was in a high level in androgen-independent (AI) PC3 and DU145 cells and in a low level in LNCaP [30]. It is determined that miRNAs act as oncogenes or tumor suppressors depending on the targets they regulate in PCa.

Nevertheless, these studies generated different sets of miRNAs due to differences in use of samples and analytic methods. Another factor is that computational algorithm methods have been largely used to analyze and identify miRNA targets. This type of computational analysis can not produce precise identification of target genes. It is not uncommon that one target mRNA contains multiple miRNA-binding sites and a miRNA can target multiple mRNAs. Sun *et al.* [12] found a positive correlation between the reduced abundance of target mRNA and the number of miRNA-binding sites and the length of the 3' UTRs. Apparently, experimental validation of each miRNA and its target genes in PCa is greatly needed and would be a huge practical problem [31].

Functions of the Aberrant Expression of miRNAs in PCa

Common approaches to study the functions of miRNAs are gain and loss of miRNAs to verify their impacts in biology. Although more than 50 miRNAs are abnormally expressed, leading to alteration in the expression and activity of their targets in PCa, only several miRNAs have been experimentally determined to be involved in the initiation, progression and metastasis of PCa. These differentially expressed miRNAs function as either oncogenes or tumor suppressors, and target at tumor suppressor genes and oncogenes, respectively [9].

Functions of Oncogenic miRNAs in PCa

Many miRNAs have been found to be up-regulated in PCa by miRNA expression profiling analysis. Overexpression of the oncomirs epigenetically silences the apoptosis-related genes and then induces tumor growth and metastasis.

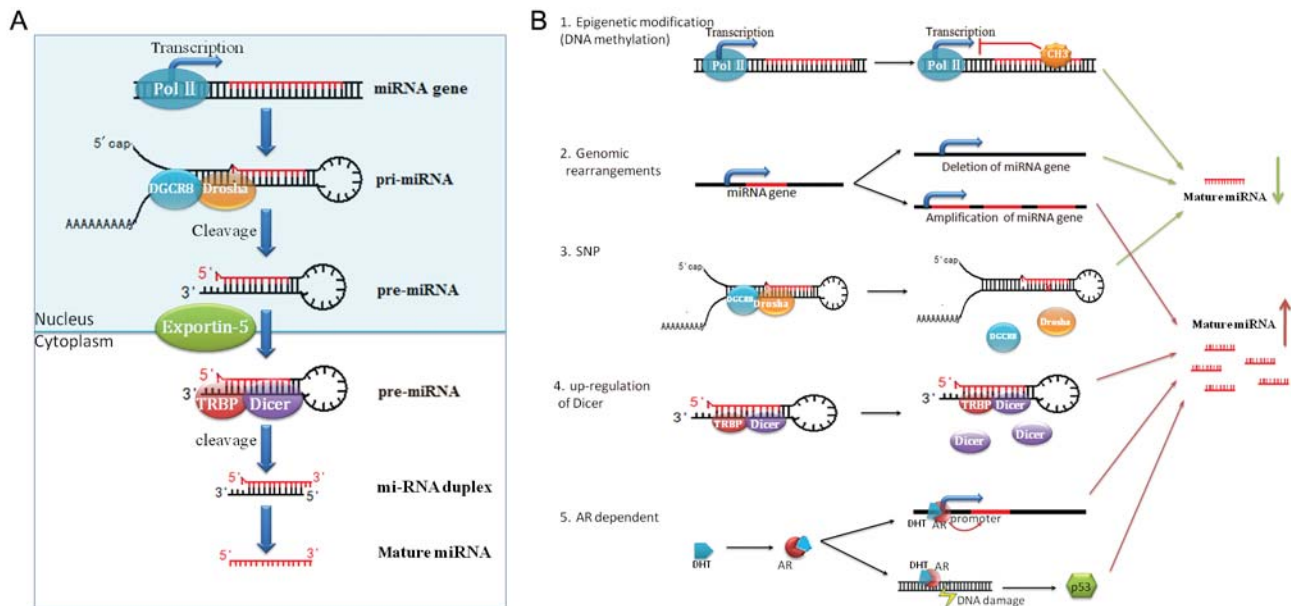


Figure 1 The basic process of miRNA biosynthesis and potential mechanisms of the aberrantly expressed miRNAs in PCA (A) The basic process of miRNA biosynthesis was schemed. (B) Potential mechanisms of the aberrantly expressed miRNAs in PCA were outlined: (1) epigenetic modification, especially DNA methylation near miRNA gene inhibits transcription of miRNA gene; (2) genomic rearrangements, including loss or gain of miRNA gene; (3) single nucleotide polymorphism (SNP) in the pri-miRNA sequence, inhibiting the processing of miRNA by lowering the affinity of Drosha with pri-miRNA; (4) up-regulation of Dicer, increasing the productivity of mature miRNAs; and (5) AR dependent includes: (I) androgen-induced AR binds to miR-21 promoter and subsequently results in overexpression of miR-21 and (II) activation of p53 by DNA damage triggers miR-34a/-34c overexpression in AR positive PCA cells.

miR-221 and miR-222, two highly homologous miRNAs whose up-regulation has been reported in several types of human tumors including PCA, were found to be involved in the development and metastasis of PCA. The first indication comes from the finding that the expression of miR-221/-222 is higher in AI PCA cells than in AD PCA cells *in vivo* and *in vitro* [31–33], as indicated that the relatively overexpressed miR-221/-222 was observed in PC-3 cells when compared with LNCaP cells. One of the mechanism by which miR-221 and miR-222 contribute to PCA is due to their binding capabilities to the target mRNA p27^{kip1}, which leads to tumor growth [32–34]. Furthermore, miR-221/-222 contributes to the development or maintenance of castration-resistant prostate cancer (CRPC) by mechanism which affects the response of AR-mediated signaling in AI PCA cells [32]. However, contradictory data are also available regarding the expression level of miR-221/-222 in PCA, and it has been reported that androgen suppressed the production of the miR-221/-222 [35].

miR-21, another potential oncomir, is also overexpressed in solid tumors of lung, breast, colorectal and Pca [31,36], and acts as a key oncogenic regulator that contributes to tumor growth, invasiveness and metastasis [37,38]. Expression profile analysis indicated that overexpressed miR-21 is evident in malignant, AI PC-3 and DU-145 cells, whereas it is in a low level in AD LNCaP cells [30]. The mechanism by which miR-21 acts may be mediated through directly regulating myristoylated alanine-rich protein kinase

c substrate (MARCKS) as well as PDCD4 and TPM1, and subsequently resulting in the elevation of the motility, invasion and apoptosis resistance of PCA cells [30]. This is confirmed by the observation that anti-miR-21 could increase the sensitivity of PCA cells to apoptosis, and negatively regulate the motility and invasion of cancer cells [30]. Another study has recently shown that, in the presence of androgen, AR could bind to a defined miR-21 promoter, miPPR-21, and resulted in overexpression of miR-21 at transcription level, consequently responded for castration resistance [28].

miR-125b is essential for cell proliferation [39] and is found to be overexpressed in PCA as well. Transfection of synthetic mature miR-125b is capable of inducing AI PCA cell growth [25,39], by targeting at the 3' UTR of *BAK1* transcript [16]. However, down-regulation of *BAK1* only by knockdown of *BAK1* using siRNA can not restore the miR-125b-mediated stimulation on cell growth, suggesting that other miR-125b targets exist in PCA [16,25]. A later report defined the *EIF4EBP1*, another specific target of miR-125b in PCA [40]. These evidences verify the hypothesis that an individual miRNA may regulate multiple targets through different pathways.

Functions of Tumor Suppressor miRNAs in PCA

Loss of tumor suppressor miRNAs is another mechanism related to the progression of PCA. EZH2, belonging to a

polycomb family associated with dysplasia of cancers, is a kind of histone methyltransferase which acts as a gene silencer. In prostate tumors, loss of miR-101 is concomitant with the overexpression of EZH2. Genomic loci encoding miR-101 were found to be lost both in clinically localized PCa (37.5%) and in metastatic cancer (66.7%). Overexpression of miR-101 suppressed the cell growth and invasiveness with a low-expression level of EZH2, whereas overexpression of EZH2 rescued the inhibition of cell growth by miR-101 [41].

Another relevant factor for PCa is the natural absence of miR-126*, a product of an intron of the *Egfl7* gene. Ectopic expression of miR-126* inhibited the migration and invasiveness of PCa cells, accompanied by reduction of prostein expression level [17]. Prostein is one of the prostate-specific antigens (PSAs) [42] and contributes to the motility and invasiveness of PCa cells. Evidences have suggested that miR-126* repressed the translation of prostein by targeting at the 3' UTR of prostein mRNA [17].

Lin *et al.* [43] analyzed eight down-regulated and three up-regulated miRNAs in PCa tissues and cell lines, and found that miR-146a, one of the eight down-regulated miRNAs, was dramatically expressed in AD cells and lost in AI cells. Forced expression of miR-146a resulted in remarkably reduced cell migration, invasion, proliferation, anti-apoptosis and metastasis via silencing *ROCK1* and then inhibiting the HA/ROCK1 pathway in PC3 cells, suggesting that miR-146a acts as a tumor suppressor in PCa [43].

As mentioned above, the microRNA-200 family is a group of potential tumor suppressor miRNAs whose down-regulation in PCa may be resulted from epigenetic modification. Under-expressed miRNA-200 family has been validated to play critical roles in epithelial to mesenchymal transition (EMT), which participates in the migration and invasion of cancers. PC3 cells that have lost expression of miR-200c and miR-141, display a mesenchymal phenotype due to aberrant methylated CpG island near the miR-200c and miR-141, leading to invasion and metastasis of cancer cells [14]. At the post-transcription level, a recent study has shown that miRNA-200 family regulates PDGF-D-mediated EMT, which participates in the migration and invasion of PCa [44].

In addition, miR-330 negatively correlates with the level of E2F1, eventually leading to the activation of apoptosis through suppression of Akt signaling pathway in PCa [45]. Involvement of p53 in miR-34a/miR-34c-mediated apoptosis was also found in AR positive PCa cells [29].

Collectively, these findings proved the involvement of miRNAs in the initiation, progression from AD stage to AI stage, invasion, and metastases of PCa, and evidence is mounting to implicate that the regulation networks in PCa are further complicated due to the discovery of miRNAs. The regulatory effect of miRNAs by binding to their

targets at post-transcription level is mediated either through androgen/AR signaling pathway, prostate-specific proteins or, routinely, sharing common components related with cell cycle checkpoints and survival or through proliferative signaling apoptosis in PCa. More insights into the aspects of gene expression controlled by miRNAs in PCa are being obtained.

Application and Prospect of miRNAs in PCa

miRNA expression profiling studies of multiple human cancers have revealed that miRNAs are aberrantly expressed and strongly tumor- and tissue-specific [46]. Thus, analysis of miRNA expression patterns offers an opportunity for classification, early diagnosis, and potential therapeutic targets discovery of cancers, especially for incurable hormone refractory prostate cancer (HRPC). So based on the achievements, it is conceivable that miRNAs have potential values for clinical applications, such as biomarkers and therapeutic strategy.

The miRNA signatures could accurately distinguish normal and cancer tissues, as well as classify different stages of cancer. In a comparative study between miRNA and mRNA expression, only 1 of 17 carcinomas could be distinguished by mRNA profile patterns whereas 12 of 17 carcinomas were classified by miRNA expression patterns [46]. Since carcinogenesis is a complicated integration of alterations of multiple signal transduction pathways, it is better to study the miRNAs expression profile as tumor-specific biomarkers rather than solely one miRNA. Thus expression patterns of hundreds of miRNAs could improve accuracy of clinical diagnostic result.

At present, PSA measurement is the gold standard for diagnosis of PCa. Yet, PSA testing results in remarkable over-diagnosis and over-treatment for insignificant tumors. Therefore, novel biomarkers are extremely desired due to the limitations of PSA. Given the fact that some miRNAs are specifically expressed in PCa, these miRNAs have received attentions for their potential use in diagnosis, prognosis, and classification of PCa. For instance, the expression level of miR-21 was much higher in AI PCa cells than in AD PCa cells [30]. Therefore, detecting the expression level of miR-21 can be used to discriminate different stages of human PCa. Additionally, optimized high-throughput miRNA expression profiling can be used to discover miRNAs as novel biomarkers possibly due to its high sensitivity by requiring only a picogram quantity of RNA [19]. Mitchell *et al.* [47] has reported that miRNA are remarkably stable both in plasma and in serum as well as in formalin-fixed tissues, so miRNAs have the potential to be blood-based biomarkers for cancer diagnosis. miRNAs originating from PCa tissues enter the circulation

Table 1 Summary of studies on aberrant miRNAs expression in PCa

miRNA	Expression	PCa cases (<i>n</i>)	Validated direct target(s)	Related functions	Roles in PCa	References
miR-21 ^a	↑	Cell lines (4)	MARCK, PDCD4,TPM1	Motility, invasion, apoptosis resistance	Oncomir	[30]
miR-125b	↑ ^b	PCa tissues(10) Benign prostate tissue (2) Cell lines (9)	BAK1 EIF4EBP1	Apoptosis proliferation	Oncomir	[16] [40]
miR-220/-221 ^a	↑	Cell lines (2)	p27		Oncomir	[32]
miR-101	↓	Located PCa tissues (16) Metastatic PCa tissues (33)	EZH2	Metastasis	Tumor suppressor	[41]
miR-34 family ^{a,c}	↓	Cell lines (4)		Apoptosis	Tumor suppressor	[29]
miR-126*	↓	Cell lines (1)	Prostein	Motility, invasion	Tumor suppressor	[17]
miR-146a	↓	Cell lines (5) PCa tissues (60)	ROCK1	Motility	Tumor suppressor	[43]
miR-200 family						
miR-200c	↓	Cell lines (4)		EMT	Tumor suppressor	[14]
miR-200b	↓	not available	PDGF-D	EMT	Tumor suppressor	[44]
miR-141	↑ ^b	PCa serum (25) Normal serum (25)				[47]
miR-330	↓	Cell lines (4) Not available	E2F1	EMT Apoptosis	Tumor suppressor Tumor suppressor	[14] [45]

↑, up-regulated; ↓, down-regulated; EMT, epithelial mesenchymal transition.

^aThose miRNAs have close relationship with AR during pathogenesis of PCa.

^bExpression levels of miR-125b and miR-141 are up-regulated in sera of patients with metastatic PCa.

^cmiR-34 family, down-stream targets of p53, mediates apoptosis of PCa. The direct target for miR-34 is not available.

where they are maintained at a very constant level. The expression levels of miR-125b and miR-141 have 6.35-fold and 65-fold increase, respectively, in the sera of patients with metastatic PCa when compared with those in healthy people. Thus, allying miRNAs with PSA as biomarkers can increase the accuracy of diagnosis of PCa.

Analysis of miRNA expression profiles and the discovery of their targets in cancer could lead to the development of novel therapeutic options. Inhibiting the expression of oncogenic miRNAs and elevating the expression of miRNAs that act as tumor suppressor are effective approaches for the therapeutic purpose. Re-introducing miRNAs (that function as tumor suppressors) into tumor cells may inhibit the tumor growth, even revert the tumor cells into normal ones. For example, Friedman *et al.* [24] found that overexpression of miR-101 significantly attenuated the invasion of DU145 PCa cells. miR-125b was believed to promote the growth of AI cells. Transfection of anti-miR-125b into cells inhibited the cell growth via facilitating cell apoptosis [16].

Epigenetic modification contributes to the regulation of miRNAs expression and sequentially affects target activities in cancer. This may provide another option for targeted therapy by developing epigenetic drugs. The agent 5-aza-29-deoxycytidine (5-Adc), an inhibitor of methyltransferases, has been used for this purpose. Treatment of 5-Adc led to the increase of miR-200 in PCa cells [14].

Although these novel therapeutic strategies have only been tested at cell level, and have not been used in clinical treatment, compelling evidence gives promise that miRNAs can be used in monotherapy or in combination therapy with conventional medical treatments.

Conclusion

Studies on the relationship between miRNAs and PCa only started from recent years [9]. As the progression of PCa is rather a multiple combination with changes of expression of oncogenes and tumor suppressors, discovery of miRNAs goes a step forward to provide a novel regulatory mechanism for PCa. There are more than 50 miRNAs reported to be involved in PCa up to now; however, functions of less than 10 miRNAs and only one or more of their target miRNAs have been identified. Thus, there are still lots of work to be done to clarify the complicated interaction between the multiple miRNAs and multiple target mRNAs. In addition, some prostate-specific miRNAs are AD, which may be another reason that contributes to these complicated interactions. The mechanism that miRNAs are regulated through androgen signaling may play roles in the transition to AI PCa. We anticipate that the issues discussed above will be clarified by further researches on miRNAs in PCa in the near future (Table 1).

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