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REVIEW

The function of microRNAs, small but potent molecules, in human prostate cancer

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Prostate cancer is one of the most significant cancers of men all over the world. The microRNAs (miRNAs) possess crucial functions in pathogenesis of the disease and its gain of androgen independency. The miRNAs are small, approximately 18–24 nucleotides, non-coding, endogenously synthesized RNAs that regulate gene expression post-transcriptionally. They are found in viruses, plants, and animal cells. The miRNAs have critical functions in gene expression and their dysregulation may cause tumor formation and progression of several diseases. Here, we have reviewed the most current literature to elucidate the function of miRNAs in human prostate cancer. We believe that this will help investigators not only working in prostate cancer, but also studying the miRNAs in other diseases to delineate the functions of miRNAs implicated in human prostate cancer development and progression.
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Introduction

MicroRNAs (miRNAs) are small, approximately 18–24 nt, non-coding, endogenously synthesized RNAs that regulate gene expression post-transcriptionally. They are found in viruses, plants, and animal cells.[1–3] Today, a total of 10883 miRNAs have been identified and 721 miRNAs belong to *Homo sapiens* (miRBase:Release14.0:-Sept 2009).[4–6] Approximately 30% of total coding RNAs in human are regulated by at least one type of miRNA.[7] The regulations of mRNA transcripts occur by incomplete hybridization of miRNA on 3′UTR of target mRNAs. This hybridization with 'RNA-induced silencing complex' causes degradation of the mRNA, inhibition of the protein synthesis, and/or deadenylation of targeted mRNA.[8,9] Currently, it is known that incomplete hybridization includes a 5′ complete binding on a 5′-seed sequence of 7–8 bp-long-miRNA. After the seed match, a non-hybridized loop sequence and variable hybridization pattern are observed.[7] To discover the true miRNA-mRNA matches, some algorithmic software have been developed to help investigators for finding the exact miRNA-mRNA target list, although these are not always accurate.

The miRNAs have critical functions in gene expression and their dysregulation may cause tumor formation and progression. Today, it is known that tumors possess widespread deregulated miRNA levels. Over-expression or down-regulation of specific miRNAs in different tumor types make them potential therapeutic targets and diagnostic markers. Up-regulated miRNAs inhibiting tumor suppressor genes in tumor cells are commonly termed as oncogenic miRNAs or oncomirs. The miRNAs whose down-regulation promotes tumor progression are tumor suppressor miRNAs. One type of mRNA may possibly be targeted by multiple different miRNAs with variable efficiencies. Conversely, a single miRNA may target more than one mRNA. Thus, to be able to observe a tumorigenic phenotype, some significant changes should occur in microRNome content of the cells.

The initial investigations of miRNA deregulation in tumors were performed through miRNA microarray profiling. Determination of miRNA signature is essential to solve the general alterations and specific expression changes between tumor and normal tissues, hormone refractory samples, metastases, and cultured cells. The miRNA microarray expression studies comparing cancer tissue with normal tissue samples reveal over-expressed and down-regulated individual miRNAs. This deregulated expression of specific miRNAs may be explained by some problems in miRNA mechanism including tendency of miRNA genes to be located in cancer-related genomic regions, such as chromosomal instable regions.

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There is a direct relation between chromosomal instability and miRNA expression. Evidences reveal that in prostate cancer cell lines and xenograft samples, the miRNAs at deleted chromosomal regions show very low expression. The ones located at amplification sites, however, are highly expressed.[10,11] For example, miR-126 and its minor miR-126* is located on the intronic region of vascular endothelial EGF-like 7 gene (Egfl7), which is deleted in most of the prostate tumors. Its absence increases the protein level and its ectopic expression represses the invasiveness and migration of LNCaP cells.[12] The miRNA genes also have promoter under epigenetic regulations. The miR-126 shares the same promoter with Egfl7 gene and epigenetic therapy targeting promoter of this gene also activates the intronic miR-126.[13] The miRNA-processing genes such as *Drosha* and *Dicer* are aberrantly expressed in cancer cells.[14] Absence of transcription factor-inducing miRNAs causes decreased production of precursor miRNAs.[15,16] More complex pathway may be triggered because of mutation on a single miRNA. Microarray profiling can also be helpful to characterize tumors and their subtypes. The aberrantly expressed miRNAs have crucial functions in pathogenesis of cancer, cell survival, apoptosis, invasion, and metastasis.

Today, prostate cancer profiling studies show that most miRNAs have a common deregulation pattern. The profiling studies have been performed by independent laboratories since 2006 and we are among the first showing the widespread deregulation of miRNAs in prostate cancer.[10,11,15–18]

Profiling studies have been reported in cell lines with androgen-independent phenotype (PC3 and DU145) and cell lines with androgen-dependent phenotype (LNCaP, LAPC-4, VCaP, and 22Rv1), tumor xenograft models, and clinical radical prostatectomy samples.

The mechanisms of miRNA targeting and translational inhibition have not yet been fully understood. To be able to understand the true function of miRNAs in prostate cancer pathogenesis, progression, and in therapy, more studies about functions of miRNAs and regulation of both miRNAs and proteins with a function in miRNA maturation are needed.

miRNAs as implicated in prostate cancer pathogenesis

Although the prostate cancer has a high incidence in western countries, the pathogenesis has not yet been clearly defined. Similar to other cancers, prostate cancer occurs because of various genetic and epigenetic changes. Studies investigating the function of miRNAs in the prostate cancer pathogenesis are promising. A schematic diagram of miRNAs, their targets, and interactions with each other is represented in Figure 1.

miRNAs as oncomirs in prostate tumors

Oncomirs are the miRNAs that are amplified or over-expressed in tumors acting as oncogenes. Some miRNAs significantly over-expressed in most of the tumor types are miR-21, miR-291, and miR-17-5p.[19] Depending on the tumor type, various miRNA might have altered expression. All miRNAs should be examined for their potential effect on prostate cancer cell lines.

miR-17-92 cluster contains six-miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1 on chromosome 13) processed from a single transcript by Drosha. These miRNAs are over-expressed in prostate cancer and in some other tumors.[20] The miR-20a in this cluster is over-expressed in prostate cancer samples[21] and its inhibition induces cell death and apoptosis in PC3 cell lines.[22] It is not certain that the over-expression of this cluster alone is sufficient to accelerate prostate

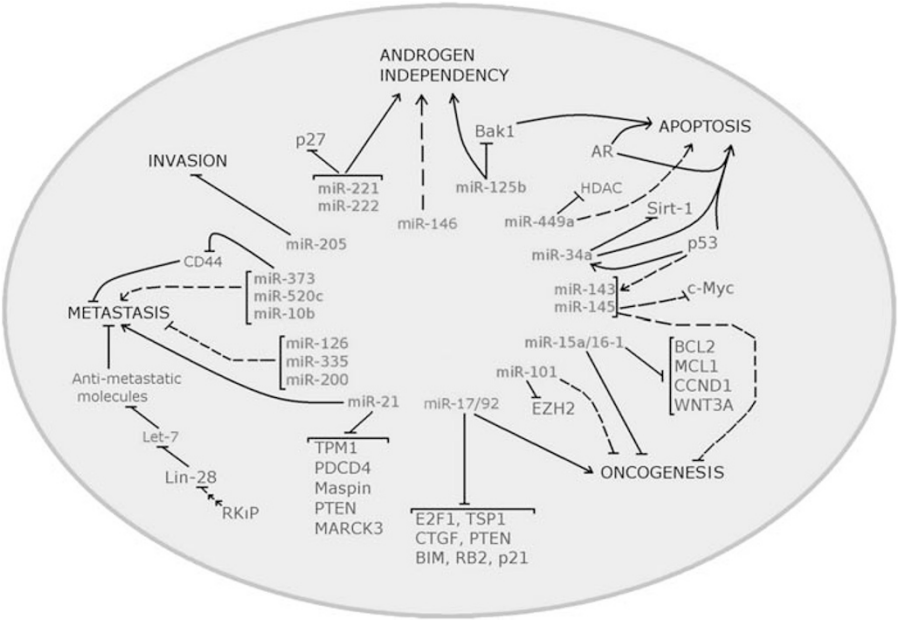


Figure 1 Schematic representation of major miRNAs with interactions as implicated in prostate cancer. Dashed lines indicate that the putative and straight lines are validated interactions.

Table 1 The list of microRNAs and their targets as verified biologically in prostate cancer[a]

microRNA	Target gene
Let-7/miR-98	RAS;[54] MYC;[60] HMGA2[61]
miR-15a-16-1	BCL2[84]
miR-17-92	E2F1;[22] TSP1/THBS1;[85] PTE N;[23] BIM/BCL2L11;[23] RB2/RBL2;[86] p21/CDKN1A[87]
miR-20a	E2F1-3[22]
miR-21	PTEN;[88];TPM1;[30,89] PDCD4;[19,30] MARCKS;[29] MASPIN/SERPINB5[30]
miR-26a	EZH2;[90] SMAD1[91]
miR-29a; miR-29b	MCL1[92]
miR-34a	CDK6[49]
miR-101	EZH2[59]
miR-106a	RB1[21]
miR-125b	HER2;[27] HER3;[27] EIF4EBP1;[18] BAK1[25]
miR-145	CCNA2[46]
miR-146a	ROCK1[47]
miR-205	SIP1/ZEB2;[93] ZEB1;[93] Cepsilon[57]
miR-221; miR-222	p27Kip1/CDKN1B[31,94]
miR-331-3p	CDCA1; KIF23[46]
miR-373; miR-520c	CD44[78]
miR-449a	HDAC1[56]

[a]References are given as superscript numbers followed by each microRNA.

tumorigenesis. Its over-expression and homozygous deletion in mouse models cause autoimmunity and premature death of B cells, respectively.[23,24] The miRNAs, originating from the miR-17-92 cluster, target significant genes including E2F1, TSP1, CTGF, PTEN, BIM, RB2, and p21 (Table 1). E2F class contains transcription factors-inducing genes having a function in cell-cycle regulation and apoptosis. Thus, they are strictly regulated both at transcriptional and protein level, post-translational and pre-translational manner. Members of E2F family proteins, E2F1, E2F2, and E2F3, also re-regulate and induce the expression of miR-17-92 cluster, possibly to prevent accumulation of those transcription factors.[22]

The miR-125b deregulation has a significant effect on prostate cancer tumorigenesis and on androgen independency.[25] The miR-125b is up-regulated in clinical samples and androgen-independent cell lines.[18,25,26] Functional studies reveal that miR-125 over-expression is related to progression of androgen independence and cell survival in prostate cancer.[25] Her2 and Her3 are two important proteins over-expressed in prostate cancer and mir-125b inhibits their translation in breast cancer samples.[27] Her2 might have a function in androgen independency in prostate cancer.[28] The increase of miR-125b might be also related with the interaction of HER2-androgen receptor (AR) pathway.[25] The same group has searched for novel targets of miR-125b and suggests Bak1 as a target after *in vitro* expression studies and luciferase assay. Bak1, together with Bax protein, regulate apoptotic signaling in prostate cancer synergistically.[25]

The miR-21 is another oncomir, having function in tumorigenesis, invasion, and metastasis; miR-21 induces invasion and motility of prostate tumor cells. It also takes the function as anti-apoptotic molecule triggering resistance to apoptosis. Possibly, miR-21 does not have a direct negative effect on cellular proliferation.[29] Those effects may be due to targeting and repression of tumor suppressor genes: *TPM1*, *PDCD4*, *maspin*, *PTEN*, and myristoylated alanine-rich protein kinase c substrate (*MARCKS*) by miR-21. Targeting the *PDCD4* is a translational inhibition, as the protein level of *PDCD4* decreased by miR-21, whereas the mRNA level is stable. Down-regulation of *PDCD4* and maspin by mir-21 may promote tumor invasion and metastasis. In addition, maspin protein suppresses the invasion of tumor and inhibits metastatic action, but has no known effect on cell proliferation.[19,30]

Volinia *et al*.[21] reported that miR-106a is over-expressed in prostate cancer and some other solid tumors except breast cancer. Its putative target *RB1* is found to be down-regulated in prostate cancer.

The miR-221 and miR-222 have been earlier reported as up-regulated in several cancers and they are determined as oncomirs because of targeting p27.[31,32] However, some studies claim those miRNAs are down-regulated in stage T2a/b prostatectomy samples and androgen-dependent cell lines.[17,33] The miR-221 and miR-222, transcribed from a cluster on chromosome X, have identical seed regions and possibly common mRNA targets. miR-221 and miR-222 are both increased in prostate tumor cells and almost absent in androgen-dependent cell lines.[31,33] They act as oncomirs as miR-222 targets the p27 (Kip1) and down-regulates its translation.[31,32] Hu *et al*.[34] reported that thrombin down-regulates p27 (Kip1) in TRAMP mice and cancer cell lines, and interestingly miR-222 expression is increased by thrombin. This increase in miR-222 expression suggests the idea that it has an oncogenic function and stimulates tumor proliferation by inhibiting p27 (Kip1) expression.[34] Mercatelli *et al*.[32] also performed *in vitro* studies for identification of oncogenic and triggering androgen-independency functions of miR-221 and miR-222. LNCaP-derived tumors in SCID mice formed tumor by ectopic over-expression of miR-221 and PC3-derived subcutaneous tumor growth was inhibited by anti-miR-221 and anti-miR-222 antagomir treatment. Increased level of p27 was observed in these suppressed tumors.[32]

miRNAs as tumor suppressors in prostate tumors

Tumor suppressor miRNAs are the ones that are down-regulated or deleted from chromosomes. Their reduced expression causes increased level of oncogene expression. The inhibition of proliferation, apoptosis induction,

reduced invasion, and lack of metastatic activities in cancer cells were shown through the *in vitro* and *in vivo* over-expression of tumor suppressor miRNAs by various independent experiments.

The miR-15a/16-1 cluster has two miRNAs functioning as tumor suppressor. They are located at chromosomal region 13q14, which is frequently deleted in most of chronic lymphocytic lymphomas, in 50% of prostate cancers, and some of lymphomas.[35,36] The endogenously down-regulation of this miR-15/16 cluster was found in chronic lymphocytic lymphoma, prostate adenocarcinoma, and pituitary adenomas.[37] Inhibition of miR-15a and miR-16-1 activity causes hyperplasia of prostate in mice and increases *in vitro* cell proliferation and invasion.[36,37] However, increased expression of miRNAs in this cluster provide inhibition of proliferation, induction of apoptotic pathways, and suppression of tumor producing potential of cancer cells both *in vitro* and *in vivo*.[36,37] In addition, increased expression through intra-tumoral delivery of this miR-15a/16-1 cluster for therapeutic purposes has a significant regression potential in prostate cancer xenografts.[36] The validated targets of miR-15a/16-1 cluster are *BCL2*, *MCL1*, *CCND1*, and *WNT3A*.[37]

The miR-145, down-regulated in most of cancer types, is another tumor suppressor miRNA with decreased expression level in prostate cancer cell lines and tumor samples. The miR-145 and miR-143 form a cluster on chromosome 5.[38–40] The direct function of miR-145 in prostate cancer prognosis has not yet been revealed, but its anti-proliferative function was observed in other solid tumors such as colon cancer by repressing insulin receptor substrate-1,[41,42] breast cancer through *RTKN* inhibition,[43] and lung cancer.[44,45] A recent article identified the miR-145 and miR-331-3p as the critical miRNA in aggressive prostate cancer and found that their ectopic expression induces apoptosis and decrease proliferation (our unpublished data).[46] They also identified CCNA2 as potential miR-145 targets in LNCaP and VcaP cells.[46]

Lin *et al*.[47] reported in early studies that miR-146a is down-regulated in androgen-independent prostate cancer samples. *In vitro* insertion of miR-146a into PC3 cell line decreased the proliferation and survival. Endogenously, miR-146a expressing cells have less invasive or metastatic functions.[47] Its lack of expression in androgen-independent state of prostate tumor implies that it may have a function in androgen independency.

The p53, a well-known tumor suppressor, cell-cycle regulator, and activator of DNA-repair genes, induces expression of miR-34a, a commonly reduced miRNA in various cancers that also lack p53 expression.[48,49] Lodygin *et al*.[49] have reported that its lower expression is probably because of irregular CpG methylation on its promoter in prostate carcinoma and some other carcinoma cell lines.[49] The miR-34a transfection into PC3 cell lines provided decreased expression of *SIRT1* in both mRNA and protein levels. *SIRT1*, a sirtuin class III histone deacetylases, is up-regulated in various cancers and its inhibition results in decreased proliferation and colony formation ability of prostate cancer cell lines.[48,50] Dependent to *SIRT1* or not, ectopic expression of miR-34a cause cell-cycle arrest in G1 phase, decrease in cell proliferation and induce apoptosis.[48,49] DNA double-strand breaks-inducing agents also activates DNA-repair-related protein p53 as a survival mechanism. The p53, after DNA breaks recognition induces variety of genes including the ones taking part in apoptosis including miR-34a and -34b/c.[51] Rokhlin *et al*.[51] reported that miR-34a/b/c induce apoptosis only in the presence of AR and p53 activation by DNA double-strand break-inducing agents. The p53 also may induce transcription of miR-145 through p53 response element on its promoter region, in prostate tumor.[52] The p53 represses c-Myc, but the complete mechanism has not yet been identified. Sachdeva *et al*.[52] reported that miR-145 targets c-Myc and inhibits its translation; therefore, miR-145 might involve in p53 pathway and controls tumor cell growth.[52] Very recently, it has been shown that p53 interacts with the Drosha-processing complex through *DDX5*. In this report, a new function of p53 in miRNA processing has been revealed.[53]

Let-7 is one of the first identified and mostly well-known tumor suppressor miRNA in human beings and targets the *RAS* oncogene.[54,55] In different clinical tests and cancer studies, increased expression of Let-7 and some of its isoforms have shown higher survival rate and decreased clonogenicity and radio-resistance *in vitro*.[55]

Therapeutically, significant miRNAs take part in tumorigenesis and apoptosis-specific pathways. Cancer cells possess transcription at high level at certain loci, thus histone deacetylases, neutralizing the positively charged amines on histones, are generally over-expressed in tumors.[56] Noonan *et al*.[56] identified that miR-449a targets histone deacetylases-1 and this miRNA is down-regulated in human prostate cancer samples. miR-449a transfection studies reveal partial triggering apoptosis and cell-cycle arrest in cancer cells.[56]

The miR-205 is expressed lower in prostate cancer cells than it is in normal counterparts. Exogenous expression of miR-205 results in reduced invasion of prostate cancer cells and causes mesenchymal-to-epithelial transition of cells possibly because of translational inhibition of miR-205 of *N-chimaerin*, *ErbB3*, *E2F1*, *E2F5*, *ZEB2*, and protein kinase Cepsilon.[57] The miR-200 family generally triggers epithelial–mesenchymal transition. Kong *et al*.[58] reported that miR-200 family is down-regulated in prostate cancer PC3 PDGF-D cell lines and transfection of miR-200b results in gaining epithelial phenotype and decreased migration and invasion activity.[58]

The epigenetic changes in cancer cells cause silencing of tumor suppressor genes by methylation of their promoters. Enhancer of zeste homolog 2, histone methyltransferase, is up-regulated in most solid tumors including prostate cancer, and its link with miR-101 was reported by Varambally *et al*.[59] Prostate tumor tissues have decreased expression of miR-101, mostly because of genomic loss and increased expression of enhancer of zeste homolog 2 causing cancer progression.[59]

Interactions: miRNA and their targets in prostate cancer

The miRNAs can target genes post-transcriptionally through interacting target mRNA(s). This interaction does not necessarily have to be a perfect match. One miRNA, therefore, can target several mRNAs. Target mRNAs are usually identified bioinformatically and then validated by investigating the transcriptional and/

translational changes after miRNA ectopic expression. For example, it is reported that Let-7/miR-98 targets RAS, MYC, and HMGA2.[54,60,61] The complex interaction in transcriptome and microRNome makes difficult to determine which interaction is more significant biologically. Only a few miRNA targets have been verified biologically in prostate cancer as summarized in Table 1.

miRNAs as potential markers for diagnosis and progression of prostate cancer

Prostate cancer is one of the most common cancers of men all over the world and current therapies such as androgen-ablation therapy do not provide an ultimate cure to patients with metastatic disease. The miRNAs possess crucial functions in pathogenesis of the disease and its gain of androgen independency.

The miRNA profiling studies for prostate cancer are very informative as simultaneous investigation of total microRNome of the samples is possible. However, the microarray expression data should be validated by other quantitative methods such as real-time qPCR. The proliferation, differentiation, metastasis, and apoptosis pathways all include miRNAs and the identification of deregulated ones is critical.[62] Our earlier analysis revealed that miRNAs in prostate tumors are globally down-regulated.[18] Some other experiments propose either widespread down-regulation or up-regulation of miRNA. However, all of the evidences reveal that miRNA expression level is globally deregulated in prostate cancer.[10,11,15–18] The list of miRNAs implicated in prostate cancer so far is given in Table 2. The miRNAs are grouped as up-regulated and down-regulated in prostate cancer in general and in androgen-independent state (Table 2).

Sun *et al*.[15] have reported a computational research on published expression array data to understand the expression profile of miRNAs and their target genes. mRNAs that are targeted by miRNAs, even the predicted ones, have low-expression levels. They also found that mRNAs that have longer 3′UTR are down-regulated with increased level and theoretically higher number of miRNAs that bind to that transcript.[15] Another explanation of this phenomenon could be that genes, which are more regulated or more interacting at protein level, are exposed to higher level of miRNA regulation.[63,64] Some miRNA targets are also up-regulated mainly because of lack or down-regulation of those miRNAs. Chromosomal deletions and epigenetic silencing inhibit certain miRNA genes expression and cause increased expression of their target genes. Ke *et al*.[65] reported the data regarding epigenetic signature of H3K4me3 and H3K27me3 in prostate cancers. Loss or gain of trimethylation pattern on promoters strongly affect the expression of miRNA genes and some other regulatory and structural gene families including the HOX family and desmosomal proteins.[65]

The causes of alterations in miRNA expression are similar to those of mRNAs. These include epigenetic changes, transcription factors, chromosomal aberrations,

Table 2 The list of microRNAs implicated in prostate cancer[a]

Up-regulated microRNAs					
Let-7d[21]	miR-29b[21]	miR-106a[21]	miR-184[21]	miR-200c[11]	miR-373[10]
Let-7i[21]	miR-30c[21,25]	miR-106b[11]	miR-187[21]	miR-202[10]	miR-491[10]
miR-15a-16-1[25]	miR-31[11]	miR-124a[21]	miR-191[21]	miR-203[21]	miR-498[10]
miR-17-92[21,25]	miR-32[11,21]	miR-125b[25,26]	miR-194[17]	miR-206[21]	miR-503[10]
miR-20a[21]	miR-34a[21]	miR-135[21]	miR-195[21]	miR-210[10]	miR-513[10]
miR-21[21,25]	miR-92-2[21]	miR-135b[17]	miR-196[21]	miR-214[21]	miR-96[95]
miR-25[11,21]	miR-93[11,21]	miR-146[21]	miR-197[21]	miR-223[21]	miR-375[95]
miR-26a[11,21]	miR-95[21]	miR-148a[21]	miR-196a[11]	miR-296[10]	
miR-27a[21]	miR-100[25]	miR-181b[21]	miR-198[21]	miR-320[10]	
miR-29a[92]	miR-101[21]	miR-182[11,95]	miR-199a[21]	miR-370[10]	
Down-regulated microRNAs					
Let-7a[10,21]	miR-23a[10]	miR-99a[10]	miR-125b[10,18,95]	miR-199a[10]	miR-490[11]
Let-7b[10]	miR-23b[10]	miR-100[17]	miR-126[13]	miR-205[57,95]	miR-494[11]
Let-7c[10,18]	miR-24[21]	miR-101[59]	miR-128a[21]	miR-218[21]	miR-497[10]
Let-7d[10]	miR-26a[10]	miR-103[10]	miR-143[10]	miR-221[10,11,17,95]	miR-520c[78]
Let-7 g[10]	miR-29a[21]	miR-106a[25]	miR-145[10,11,17,18,95]	miR-222[10,17,95]	miR-520h[11]
miR-1[11]	miR-34a[49]	miR-113a[11]	miR-149[21,95]	miR-373[78]	miR-181b[95]
miR-15a-16-1[10,95]	miR-92[10]	miR-125a[10]	miR-195[10]	miR-449a[56]	miR-31[95]
Androgen-independent state					
Up-regulated miRNAs					
miR-17-92[21]	miR-100[96]	miR-125b[26]	miR-184[10,47]	miR-198[10]	miR-302c*[10]
miR-345[10]	miR-361[47]	miR-424[47]			
Down-regulated miRNAs					
Let-7f[10]	miR-19b[10]	miR-22[10]	miR-26b[10]	miR-27a[10]	miR-27b[10]
miR-29a[10]	miR-29b[10]	miR-30a-5p[10]	miR-30b[10]	miR-30c[10]	miR-100[10]
miR-125b[10]	miR-128b[47]	miR-141[10]	miR-146a[47]	miR-146b[47]	miR-148a[10]
miR-205[10]	miR-221[10,47]	miR-222[10,47]	miR-663[47]		

[a]References are given as superscript numbers followed by each microRNA.

mutations, histone deacetylation, and aberrant post-transcriptional modifications.[16] The miR-127 in tumor cells is silenced with hyper-methylation and its expression is induced with demethylation agents.[66] The main enzymes in miRNA maturation are RNases, Dicer and Drosha, and RNA polymerase II. Their low-expression rate and any mutation or deregulation cause widespread deregulation pattern in miRNA content of the cell. Most of the tumor types possess deregulated miRNA-related proteins at either low or high levels.[67] The transcription factors of coding genes also regulate the transcription of miRNAs. The p53 induces expression of miR-34a and miR-145.[48,48] Similarly, the miR-17-92 cluster is induced by the E2F1-3 transcription factor. The miR-20a, one of the miRNAs in miR-17-92 cluster, targets and inhibits the translation of E2F1-3 mRNA forming a regulatory feedback loop.[16,22] A similar mechanism is present between miR-145 and its embryonic stem cell marker targets. The miR-145 targets *OCT-4* during stem cell differentiation state and *OCT-4* induces the transcription of miR-145 as a triggering mechanism of differentiation.[68] This may indicate a lack of miR-145 in prostate cancer stem cells also and should support the idea that miR-145 ectopic expression may be used in prostate cancer therapy. In prostate cancer and some other types of cells, ARs may have an inductive effect on promoters of certain miRNAs. However, this effect is not well understood. Shi *et al.* identified an miRNA, miR-125b, present in AR positive cells. Ectopic expression of AR in AR(−) cells and addition of androgen factors cause an increase in miR-125b levels. On the other hand, depletion of androgen decreased miR-125b levels in AR(+) cell lines. In addition, at 5′ upstream of miR-125b promoter, there are TATA box and androgen response elements, suggesting the involvement of miRNAs in androgen regulation of gene expression.[16]

The miRNAs may have a similar effect on both target mRNA transcripts and proteins. Their expression is affected simultaneously.[9,15] This hypothesis can also be supported by the evidence reported by Ambs *et al*.[11] Their analysis revealed that *Dicer* and *DGCR8*, encoding an essential cofactor for Drosha, are over-expressed in prostate tumors.[11] These findings are consistent with the report of Chiosea *et al*.[14] Higher expression levels of Dicer and DGCR8 may also support the findings about increased abundance of miRNA targets. However, these alone are not sufficient to discard the data regarding the decreased levels of miRNAs, as every miRNA is deregulated by a unique mechanism and over-expression of miRNA maturation components may be a response to down-regulation of miRNAs in cancer. An independent study about the transcriptional targets of *SOX4* transcription factor reveals that it directly regulates Dicer, Argonaute1, and RNA helicase A.[69] *SOX4* is over-expressed in prostate cancer and deregulated in most tumor types;[69–71] therefore, the induction of Dicer is another step in understanding the mechanism of miRNA widespread deregulation.

Prostate cancer cells could invade the surrounding tissues, and mostly observed invasion is the perineural invasion (PNI). The altered expression pattern of miRNAs between the tumor with or without PNI may provide further information regarding the mechanism of invasion and elimination before occurrence. Prueitt *et al*.[72] identified the differentially expressed miRNAs between PNI and non-PNI tumors. The miR-224 is the top over-expressed miRNA in PNI tumors. The putative targets of miR-224 function mostly in mitochondrial function and cellular metabolism pathways and cause decreased expression of metallothionein.[72]

Understanding the differences between miRNA expression profiling studies is important to determine the true characterization of miRNA signatures in prostate cancer. The sample types, number of samples, small RNA isolation efficiency, and the efficiency of hybridization together with the type of array used have a significant influence on the results of the experiments. Cancer is a complex and heterogeneous disease, thus individually and environmentally variable deregulation of miRNAs may occur. Evaluated data about aberrantly expressed miRNAs suggest that most unknown mechanisms in prostate cancer pathogenesis and androgen independency could be related with miRNAs. In addition, understanding the complete picture of miRNA interactions will provide a novel therapeutic target and treatment method. As one miRNA targets tens of different mRNAs, they could be promising anti-cancer targets as well.

miRNA profiling in body fluids

The early detection, diagnosis, and treatment of cancer are necessary before surgical operations or chemo-and/or radio-therapies. Minimally invasive methods and accurate markers of tumors could improve early diagnosis and reduce treatment costs and the mortality rate of the cancers.[73] The miRNAs have been discovered in most of the body fluids including serum/plasma.[73,74] The miRNAs present in plasma are stable against varying conditions and endogenous RNases. Its relation with cancer quite reasonable as different tumor types has unique and altered miRNA expression signatures, and the miRNAs in plasma have high stability.[73] Mouse xenograft models possess differential miRNA content in their plasma relative to normal plasma samples. The distinguishing methods could be qualitative and/or quantitative observation of miRNAs in plasma. However, for both methods, environmental and nutritional changes should be considered as confounding variables in the use of plasma miRNAs as disease biomarkers. One of the candidates is miR-141, a highly expressed miRNA in prostate tumors and its increase in plasma has been detected for human plasma samples from prostate cancer patients. In normal patients, miR-141 is found at levels of normal epithelial cells and other cancer types than prostate cancer.[73] Although the limited studies in the literature are promising, there is a need to broader studies to determine the function of miRNAs in body fluids for prostate cancer diagnosis.

miRNAs having a function in androgen-independent prostate cancer

The most common therapy for metastatic prostate cancer is androgen ablation. However, the tumors ultimately become independent of androgen and become clinically progressive. Although there are several hypotheses in the literature, the true mechanism of this transition has not yet been revealed in all cases. Androgen-independent prostate cancer possesses over-expression of AR or

mutated form of it functioning without androgen induction.[71] To be able to eliminate AR and convert the tumor into androgen-dependent state, targeting AR is a possible strategy. Moreover, altered miRNA expression between androgen-dependent and androgen-independent prostate cancer samples and cell lines could give a clue about the exact mechanism of this transition. DeVere *et al*.[75] identified the differentially expressed miRNAs between androgen-responsive and -independent cell lines including 10 up-regulated and 7 down-regulated miRNAs. One of them is miR-125b, which functions in altering LNCaP cell lines into androgen-independent state. *BAK1*, targeted by miR-125b, takes part in apoptotic pathway.[75] They have also reported that AR positive cell lines possess higher expression of miR-125b than AR negative cell lines. In addition, Sun *et al*.[33] found that miR-221 and miR-222 have increased levels of expression in androgen-independent (or castration-resistant prostate cancer) cell lines, LNCaP-Abl, relative to androgen-dependent cell lines, LNCaP and LAPC-4. Functional studies of miR-221 and/or miR-222 reveal that over-expression in LNCaP and LAPC-4 triggers androgen-independent growth and their inhibition converts LNCaP-Abl into androgen-dependent phenotype.[33] A list of miRNAs reported to be up-regulated and down-regulated in androgen-independent state is given in Table 2.

miRNAs modulating tumor progression and metastasis in prostate cancer

In addition to oncogenic and tumor-suppressive functions of miRNAs', they also take part in metastatic pathways. There is not a fully determined pathway of miRNAs with metastatic and anti-metastatic properties. In clinical breast cancer metastasis samples, up-regulation of miR-373 was reported by Huang *et al*.[76] miR-10b, miR-373, and miR-520c stimulation on cancer cells' migration and invasion was reported by Negrini *et al*.[76,77] They also revealed that *in vitro* and *in vivo* increase of miR-373 and miR-520c promotes the cell migration and invasion, targeting and inhibiting the translation of CD44. All these data indicates their metastatic function in breast cancer samples.[76] Similar study in prostate cancer samples proved the binding of miR-373 and miR-520c on 3′UTR of CD44 mRNA and inhibition of its translation after co-transfection. However, exogenous expression of miR-373 itself increased mRNA level of standard form of CD44, but decreased mRNA level of its splice isoform in prostate cancer cell lines. Evidence suggests that their *in vitro* increase also promotes invasion of prostate cancer cells. However, miR-373 and miR-520c levels were observed down-regulated in prostate cancer tissues.[78] This may occur depending on the type and stage of tissues. To better understand this phenomenon, we suggest that androgen-dependent and -independent tissues should be investigated for miR-373 and miR-520c expression levels.

Metastasis mechanisms include various other proteins and miRNAs. Raf kinase inhibitory protein suppresses prostate cancer and breast cancer metastasis through a cascade including *MAPK*, *Myc*, *LIN28*, and let-7. The increased expression of Raf kinase inhibitory protein causes a molecular cascade resulting in Lin-28 suppression. Lin-28 is an anti-pre-Let7 molecule, blocking maturation of let-7 pre-miRNA. Inhibition of Lin28 causes increased production of mature let-7 whose targeting molecules have anti-metastasis functions.[79] A recent study has reported the differentially expressed miRNAs in high-grade localized prostate cancer with respect to bone metastasis, which has identified that miR-let7c, miR-100, and miR-218 have higher expression levels in high-grade carcinoma than metastatic carcinoma.[80] This data is consistent with the findings of Dangi-Garimella *et al*.[79] In breast cancer, miR-126, miR-335, and miR-200 were reported as metastasis suppressors.[81] The miR-126 expression level was reported as low in prostate cancer samples,[13] suggesting an anti-metastatic function in prostate cancer as well.

miRNAs as implicated in prostate cancer therapy and future potentials

Owing to the power of miRNAs in regulating several genes in post-transcriptional level, they may be used in anti-cancer therapy. To be able to achieve this, more should be found about alteration of their expression, their true target genes and the mechanism of their action. Recently, a team from China presented the data on transfer of mir-122 gene by adenoviruses.[82] Although the usage of viruses in gene therapy is already controversial, the success of experiments and identification of anti-tumor activities of miRNAs is crucial. The anti-tumor therapies through miRNAs are not restricted to their ectopic expression. The immune-regulatory function of miRNAs can also be a promising strategy for cancer immunotherapy. Okada *et al*.[83] reviewed the recent developments in this strategy and revealed the possible miRNA targets. In addition, cancer therapy by implementing several miRNAs at the same time, or respectively, can be a better strategy against different cancer types.

Conclusion

The miRNA expression profiling studies on cancer prognosis will be continued, as novel and candidate miRNAs are being identified and most of the identified miRNAs' functions are still unresolved. Current observations suggest that miRNAs are one of the largest and most significant classes of gene regulators implicated in cancer-related processes. The miRNAs are promising biomarker molecules because of their differential expression and relatively easy detection. With today's methods, miRNA detections from clinical samples of prostate cancer are not ready to use as a clinical marker, as considering most of the solid tumors, prostate cancer tissue is heterogeneous.[16] As we understand more about the functions and mechanisms of miRNA maturation, miRNA targeting, and alteration in miRNA content, novel treatment strategies and detection methods will be developed.

Conflict of interest

The authors declare no conflict of interest.

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