Clinical and Translational Oncology (2019) 21:126–144
https://doi.org/10.1007/s12094-018-1910-8

REVIEW ARTICLE

The microRNA signatures: aberrantly expressed miRNAs in prostate cancer

N. Sharma[1] · **M. M. Baruah**[1]

Received: 26 December 2017 / Accepted: 18 June 2018 / Published online: 27 June 2018

Abstract
MicroRNAs (miRNAs) are short, non-coding, conserved, oligonucleotides that are regulatory in nature and are often dysregulated in many cancers including prostate cancer. Depending on the level of complementarity between the miRNA and mRNA target, they can either inhibit translation or degrade the target mRNA. MiRNAs expression is specific to the type of cancer, its stage and level of metastasis, making miRNAs potential stage-specific biomarkers of cancer. Recent research has shown that these miRNAs have the potential to be a diagnostic and prognostic non-invasive biomarker for various cancers including prostate cancer. Various miRNAs have been reported as novel biomarkers for prostate cancer therapy. However, there is inconsistency in the data reported and no overlapping expression pattern could be found. In this review, we have highlighted the most consistently reported dysregulated miRNAs in prostate cancer from the existing literature and discussed the currently available data on their role in regulating the hallmarks of prostate cancer. These four most consistently reported dysregulated miRNAs viz. miRNA-141, miRNA-375, miRNA-221 and miRNA-21 need to be further validated in terms of their regulatory potential in regulating various pathways important for prostate cancer management.

Keywords MicroRNA · Profiling · Biomarker · Prostate cancer

Introduction

Prostate cancer (PCa) remains one of the major medical burdens in males. The estimated number of new cases and deaths from PCa in the United States in 2017 is 161,360 and 26,730, respectively [1]. The incidence rate of PCa is lowest in Asian countries and in India it is the sixth most commonly diagnosed cancer among men [2]. Although prostate-specific antigen (PSA) detection in serum has facilitated the early detection of prostate cancer, there are limitations to this as elevated serum PSA is not specific to the malignant disease and it gives a high false-positive and a false-negative rate of approximately 15% [3]. In addition, PSA as a prognostic marker also has detrimental effects as screening detects indolent tumors along with those that can become life threatening over a period of time [4]. Moreover, PCa patients treated for localized prostate cancer show relapse within 5 years [5]. This lack of sensitivity and specificity in serum PSA level calls for a better PCa biomarker. Thus, miRNA profiling in the serum of PCa patients can lead to identification of putative biomarkers for PCa and can be a tool to establish patients with various stages of PCa and, therefore, can be treated accordingly.

MicroRNAs (miRNAs) are a class of non-protein coding endogenous 19–20 nucleotides long small single-stranded RNAs which were originally discovered in *Caenorhabditis elegans* lin four locus and 7 years later in mammals let seven was discovered [6]. These are evolutionarily conserved in nature [7]. MiRNAs are found to have a regulatory role and they regulate around 1–5% of the human genome and at least 30% of the protein coding gene [8]. They negatively regulate target gene expression at the post-transcriptional level either by degrading the target mRNA or inhibiting translation of the mRNA into functional proteins [9]. MiRNA is found to regulate vital functions of the cell including apoptosis, proliferation, cell cycle, differentiation, stem cell maintenance and metabolism [10]. The database miRBase reports

N. Sharma and M. M. Baruah contributed equally to the manuscript.

✉ N. Sharma
neetimohan27@gmail.com

[1] Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Lavale, Mulshi, Pune 412115, India

around 2000 miRNA discovered in the human genome and it is believed that 30% of human genome is under miRNA regulation [11].

Various miRNAs reported have been shown to regulate the hallmarks of cancer and dysregulation of miRNA expression profile is associated with numerous human cancers, including lung, brain, liver, colon, breast, leukemia and prostate cancer [12–14]. Conceptually these miRNAs may function as tumor suppressors and oncogenes [12] depending on the target tumor suppressor gene or oncogenes, respectively. For example, miR-15 and miR-16 act as tumor suppressors by targeting anti-apoptotic gene B cell lymphoma 2 (BCL2) mRNA and miR-17-19 acts as oncogene by targeting two tumor suppressor genes phosphatidylinositol-3,4,5,-triphose-3-phosphatase (PTEN) and retinoblastoma like protein-2 (RB2) [13]. Since miRNAs acts as both tumor suppressors and oncogenes and the fact that miRNAs are diferentially expressed in cancer [14] as compared to normal tissue indicates that miRNAs can be potential clinical targets for cancer therapy.

The development of minimal invasive tests for the detection and monitoring of malignancies can greatly reduce the burden of cancer [15]. One such approach is miRNA as blood-based (circulating) cancer biomarkers as they can be readily detected in small volume of samples using specific and sensitive quantification using real time PCR [15]. MiRNAs can be isolated from the most body fluids, including serum, plasma, urine, saliva, breast milk, tears and semen [16]. They are highly stable in circulation as they are resistant to RNase degradation due to their short sequence [17, 18]. The packaging of miRNAs in lipid vesicles, bound by RNA-binding proteins and associated with high-density lipoprotein, has a wider relevance as miRNA can be transferred between individuals orally [16]. Moreover, being highly conserved between species, it allows the use of animal models for pre-clinical studies [16]. Looking at the redundant role of miRNA, it can be expected to have differential expression pattern in different stages of cancer depending on the requirement of cells and the relationship of this differential expression pattern of miRNAs with PCa hallmarks can have great therapeutic application.

Although several studies have investigated the differential expression pattern of miRNAs in PCa and their association with clinicopathological parameters, unique signatures that can be used as biomarker to detect PCa, its prognosis, therapy selection and response are missing. Thus, in this review, we have identified the most consistently reported dysregulated microRNAs in prostate cancer and have tried to evaluate the genes being regulated by these four microRNAs viz., miRNA-141, miRNA-375, miRNA-221 and miRNA-21 in regulating prostate cancer hallmarks.

MicroRNA biogenesis

The biogenesis of miRNA is a cascade of events which comprises three stages: miRNA transcription, miRNA maturation and finally RISC complex formation (Fig. 1). The first stage, miRNA transcription, initiates in the nucleus with transcription of miRNA by RNA polymerase II [19, 20] which generates 5′ capped and 3′ polyadenylated primary transcripts (pri-miRNA) of variable lengths (around 1–3 kb) [21]. The second stage, miRNA maturation, is catalyzed by a ribonuclease (RNase III), called Drosha which is a large protein of ~ 160 kDa and its cofactor DiGeorge syndrome critical region gene 8 (DGCR8) together forms the microprocessor complex (500–650 kDa) and leads to the cleavage of pri-miRNA into precursor-miRNA (pre-miRNA), a sterm-loop structure, comprising of ~ 70 nucleotides [22–24]. Pre-miRNA is then exported to the cytoplasm by a nuclear export factor called exportin-5 [25], where another RNase III, Dicer along with TAR (HIV) RNA-binding protein cleaves it into an RNA duplex of ~ 22 nucleotide, which is a double-stranded miRNA–mRNA duplex of the mature

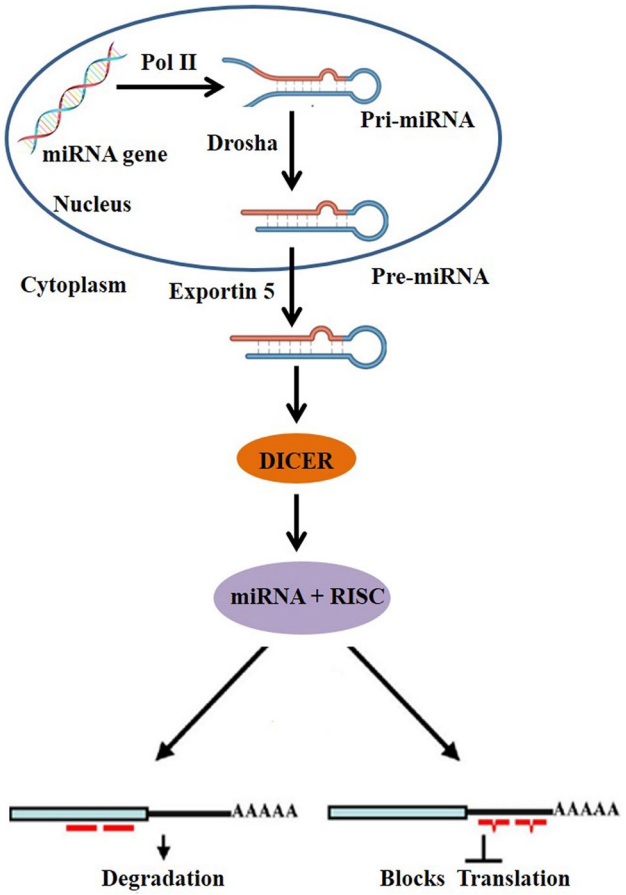

Fig. 1 Schematic representation of microRNA biogenesis

miRNA and its complementary strand [26, 27]. The mature miRNA then associates with argonaute proteins to form an miRNA-protein complex called RNA-induced silencing complex (RISC) or RNA interference (RNAi) complex and the complementary strand gets degraded [28]. Depending on the complementarity between the "seed" sequence of the miRNA and the "seed-match" sequence of target mRNA, the target mRNA is either degraded by Ago1/2 or leads to translational repression [29].

Dysregulated microRNAs in prostate cancer

Circulating miRNAs being abundant in blood, stable, a non-invasive approach for cancer detection and the findings that human blood comprises stably expressed miRNAs [30] have drawn the attention of various studies investigating the potential of miRNAs as blood-based biomarkers. An increasing number of miRNA expression studies investigating differential expression of miRNAs as potential diagnostic, prognostic and predictive tools have been reported, suggesting the potential of miRNAs as novel biomarkers for prostate cancer therapy (summarized in Table 1). The first report on miRNA profiling in prostate cancer was published in 2007 [31]. This study comprised both in vitro and in vivo samples from benign prostatic hyperplasia (BPH) and prostate cancer patients. The expression of 319 miRNAs was analyzed in 6 prostate cancer cell lines, 9 prostate cancer xenografts samples, and 13 clinical prostate tissue samples (4 BPH, 5 untreated prostate carcinomas, and 4 hormone refractory prostate carcinomas) [31]. Expression of only 128 miRNAs (40%) was detectable in array hybridization which was further validated by dot blot hybridization as well as qRT-PCR [31]. Between BPH and carcinoma samples, 51 miRNAs were found to be differentially expressed, 37 miRNAs were found to be downregulated in carcinoma samples and 14 were found to be upregulated [31].

As summarized in Table 1, initial studies have reported various miRNAs as differentially expressed in different stages of prostate cancer; however, data are inconsistent and no overlapping expression pattern has been found. The high variability among the data reported by various groups could be because of various factors such as sample size, sample type and screening methodology. Moreover, not all the differentially expressed miRNAs were validated. By validating clinically relevant miRNA in prostate cancer, one can come up with miRNA targets that have clinical application. Thus, identifying consistently reported differentially expressed microRNAs can widen up new horizons for PCa management. In this review, we have identified a panel of four most consistently reported, differentially expressed miRNAs in prostate cancer. These miRNAs: miR-141, miR-375, miR-221 and miR-21 depicted similar trend in multiple studies with different study structure and exhibited consistent significance.

The first report on miR-141 as a potential diagnostic marker was reported in the year 2008 by Mitchell et al. [32]. The authors validated six miRNAs including miR-141 in the serum samples of a case control cohort of 25 metastatic PCa patients with 25 age-matched healthy controls [32]. Of all the miRNAs miR-141 showed the greatest differential expression (46-fold overexpressed) in the patient sample and could differentiate between advanced metastatic PCa cases and healthy controls with a specificity of 60% and sensitivity of 100% [32]. Later in the year 2011, contradictory results were reported by Yaman Agaoglu et al., who investigated the expression pattern of three miRNAs, miR-21, miR-141 and miR-221 and found that there was no difference in the expression level of miR-141 when localized, local advance and metastatic PCa patient's plasma samples were compared with healthy controls [33]. However, the expression, miR-21 and miR-221, could differentiate between patients and healthy controls [33]. Also, among the three miRNAs investigated miR-141 was reported to be the strongest discriminator of metastatic PCa from localized/local advanced disease [33]. Another study was carried out in the same year by Brase et al., where they identified 69 miRNAs to be upregulated in the serum of metastatic PCa patients [34]. MiR-141 along with other two miRNAs (miR-375 and miR-200b) showed the highest correlation with clinical parameters [34]. Followed by this in 2012, Selth et al. used transgenic adenocarcinoma of mouse prostate (TRAMP) mouse model of the prostate to discover miRNAs associated with PCa [35]. They identified eight miRNAs based on their serum levels and human homologs were further validated in the sera of 25 human PCa patients with metastatic CRPC and 25 healthy controls [35]. The authors showed that miR-141 along with other three miRNAs (miR-298, miR-346 and miR-375) were consistently upregulated in PCa patients with metastatic CRPC compared to healthy controls [35]. Another study in the year 2012 by Nguyen et al. showed that the expression of miR-141 along with miR-375 and miR-378* could discriminate between metastatic PCa patients and low-risk localized patients [36]. Also the expression of miR-141 was significantly higher in prostate tumor samples compared to normal prostate tissue [36]. Taken together, these studies indicated miR-141 as a marker of metastatic prostate cancer which could differentiate between healthy controls, primary PCa patients and metastatic CRPC patients with high specificity and sensitivity and also correlate with PCa aggressiveness.

MiR-375 was first identified as pancreatic islet-specific miRNA that regulates glucose-induced insulin secretion in murine embryonic β cell line MIN6 [37]. However, various miRNA expression profiling studies revealed dysregulated expression of miR-375 in various malignancies for instance, hepatocellular carcinoma [38], gastric cancer [39], head and

Table 1 Differential expression profile of microRNAs in PCa

Year	Group	Dysregulated microRNAs	Method	Outcome	References
2008	Mitchell et al.	miR-100, miR-125b, miR-141, miR-145, miR-205, and miR-296	qRT-PCR	The study validated 6 miRNAs in a case control cohort of serum samples from 25 metastatic prostate cancer patients and 25 age matched healthy controls. Five out of six showed increased expression in the patient samples compared to controls. Expression of 1 remaining miRNA was undetectable in both the groups. Of all the miRNAs, miR-141 showed the greatest differential expression (46-fold overexpressed) in the patient sample and could differentiate between cases and controls with a specificity of 60% and sensitivity of 100%	[32]
2009	Lodes et al.	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, miR-885-5p	Microarray	Evaluated miRNA expression patterns in five types of human cancers including prostate cancer. 15 miRNAs were found to be upregulated in the serum of prostate cancer patients compared to normal control. However, these miRNAs were not PCa specific and could not differentiate between PCa and other malignancies	[58]
2011	Moltzahn et al.	miR-223, miR-26b, miR-30c, miR-24, miR-874, miR-1274a, miR-1207-5p, miR-93 and miR-106a	qRT-PCR	In this study out of 12, expression of 10 miRNAs (20b, 24, 26b, 30c, 93, 106a, 223, 874, 1207-5p, and 1274a) were substantially different between the healthy and all malignant samples: four were downregulated in cancer group (miR-223, 26b, 30c, and 24) and six were upregulated in the cancer group (miR-20b, 874, 1274a, 1207-5p, 93 and 106a). And the remaining two (miR-19a and 451) were significantly different between the healthy versus high-risk group. MiR-106a and miR-93 showed a linear trend across all groups as well as among cancer patients alone. MiR-24 showed a linear trend among cancer patients but not across all groups. Further, all 12 miRNAs were validated by qRT-PCR: miR-223 was downregulated in all groups, miR-1207, miR-874 showed a stable increase in the cancer patients relative to healthy controls. MiR-24 expression steadily decreased with cancer risk and miR-106a, miR-93 and miR-1274 expression increased with cancer risk. MiR-30c and miR-26b expression were downregulated in low and intermediate risk groups relative to healthy and high-risk groups. MiR-451 expression was increased in a high-risk group	[59]
2011	Agaoglu et al.	miR-21, miR-221, and miR-141	qRT-PCR	In this study miR-21 and miR-221 levels were found to be higher in patients than in healthy controls while there was no difference found for miR-141. All three miRNAs were higher in patients with metastases than in localized advanced disease and miR-141 being the most powerful discriminator of metastatic PCa from the localized advanced disease. MiR-21 is more useful to differentiate PCa patients from healthy controls while miR-141 was a better discriminator of metastatic PCa from localized advanced disease	[33]

Table 1 (continued)

Year	Group	Dysregulated microRNAs	Method	Outcome	References
2011	Zhang et al.	miR-21	qRT-PCR	The study assessed the expression of miR-21 in serum samples of 56 patients (20 localized PCa patients, 20 androgen-dependent prostate cancer patients, 10 HRPC patients and 6 BPH patients) followed by validation with qRT-PCR. MiR-21 expression was found to be higher in ADPC and HRPC patients with PSA >4 ng/ml. Moreover, serum miR-21 levels were higher in patients resistant to docetaxel chemotherapy compared to miR-21 levels in patients sensitive to chemotherapy	[56]
2011	Brase et al.	miR-141, miR-200b, and miR-516-3p)	qRT-PCR	The study screened 667 miRNAs in serum samples from metastatic (7) and localized (14) PCa. 69 miRNAs were found to be elevated in patients with malignant PCA compared to the low-risk group. 5 upregulated miRNAs were selected for further validation. MiR-375, 141 and 200b showed highest correlation with clinical parameters and miR-375 and 141 could discriminate between metastatic and high grade tumors compared to intermediate and low grade tumors	[34]
2011	Mahn et al.	miR-26a, miR-32, miR-195 and miR-let-7i	qRT-PCR	In this study 37 patients with radical prostatectomy, 18 patients with BPH with transurethral resection of the prostate, eight patients with metastatic PCa and 20 healthy controls were involved. The selected miRNAs were further validated in the serum of ten patients with pre-/post prostatectomy and corresponding PCa/adjacent normal prostate tissue. MiR-26a levels could discriminate between early PCa and BPH with a sensitivity of 89.2% and specificity of 55.6%. Levels of miR-26a, miR-195 correlated negatively with surgical margins and miR-195 and miR-let7i correlated negatively with the Gleason score	[60]
2012	Selth et al.	miR-141, miR-298, miR-346 and miR-375	Microarray	The study used TRAMP model to discover PCa associated miRNAs in serum which were further validated in sera of 25 human PCa patient samples with mCRPC and 25 healthy controls. 8 miRNAs (mmu-miR-24, 182, 184, 215, 298, 320, 346 and 887) were chosen for validation based on their levels in serum and their known human homolog. Mmu-141 and 375 were included as additional literature-based candidates for validation. MiR-141, miR-298, miR-346 and miR-375 were consistently elevated in human patients with mCRPC whereas homologs of the remaining 6 miRNAs did not show similar upregulated expression in human sera samples	[35]

Table 1 (continued)

Year	Group	Dysregulated microRNAs	Method	Outcome	References
2012	Bryant et al.	miR-107, miR-130b, miR-141, miR-301a, miR-2110, miR-326, miR-331-3p, miR-432,miR-375, miR-484, miR-574-3p, and miR-625	qRT-PCR	The study evaluated 12 miRNAs which were found to have differential expression in PCa patient samples compared to control (11 upregulated and 1 downregulated). 2 miRNAs (miR-574-3p and miR-107) were confirmed by qRT-PCR. 16 miRNAs are found to have differential expression in metastatic PCa patients when compared to non-metastatic PCa patients. 2 miRNAs (miR-375 and miR-200b) were confirmed by qRT-PCR. The miR-107 expression could differentiate between non-metastatic prostate cancer and normal healthy controls. MiR-375 and miR-200b expression could differentiate between metastatic prostate cancer and non-metastatic cases	[43]
2012	Chen et al.	miRNAs let-7c, let-7e, miR-30c, miR-622, and miR-1285	qRT-PCR	The study identified 5 miRNAs to be differentially expressed that discriminate prostate cancer from BPH and healthy subjects. let-7e, let-7c and miR-30c were downregulated and miR-622 and miR-1285 were upregulated in prostate cancer patients	[61]
2012	Shen et al.	miR-20a, miR-21, miR-145, and miR-221	qRT-PCR	In this study 82 PCa patients were involved and the aggressiveness of PCa cases was categorized by the CAPRA score and D'Amico's criteria. MiR-20a and miR-21 are associated with clinicopathological variables of PCa. MiR-221 independently could not discriminate between PCa aggressiveness but the combination pattern of miR-20a, miR-21, miR-145 and miR-221 significantly distinguish high versus low-risk PCa patients. MiR-141 expression was undetectable for all patients and no further analysis was conducted	[57]
2013	Nguyen et al.	miR-141, miR-375, miR-378*, and miR-409-3p	Microarray	The study included 28 patients with low-risk localized disease, 30 of high-risk localized disease and 26 of metastatic CRPC. Expression of miR-375, miR-378* and miR-141 was upregulated in the serum from metastatic CRPC patients and expression of miR-409-3p was downregulated. Expression of miR-375 was most robust as it was about 35- to 45-fold higher than that of miR-141	[36]
2015	Huang et al.	miR-375 and miR-1290	qRT-PCR	The study screened 192 subjects (23 with CRPC and 169 healthy and non-healthy). Two candidate miRNAs: miR-1290 and miR-375 were identified as prognostic biomarkers in CRPC	[44]

neck cancer [40], esophageal carcinoma [41], melanoma and glioma [42]. MiR-375 as a biomarker for PCa was first reported in the year 2011 by Brase et al. [34]. The authors identified 69 miRNAs which were upregulated in the serum samples of metastatic PCa patients compared to primary PCa patients [34]. Three miRNAs including miR-375 showed the highest correlation with tumor stage and Gleason score [34]. Also, miR-375 expression showed considerable association with lymph-node metastasis; however, it could not discriminate between high-risk PCa patients (Gleason score 8) and intermediate risk PCa patients (Gleason score 7) [34]. MiR-375 also showed higher expression in prostate tumor tissues compared to normal epithelium [34]. A similar study by Bryant et al. in the year 2012 reported differential expression of 16 miRNAs including miR-375, in plasma samples from 16 metastatic PCa patients compared to 55 localized PCa patients [43]. Expression of three miRNAs including miR-375 along with miR-200b could discriminate between metastatic PCa patients and localized PCa patients [43]. Also the expression of miR-375 was found to show correlation with metastatic CRPC in a study by Selth et al. [35]. Similar results were reported by another group in the year 2013 (Nguyen et al.) where they showed that the expression of miR-375 was found to be upregulated in serum from CRPC patients compared to serum from localized PCa patients [43]. Another study in the year 2015 highlighted the potential of miR-375 as prognostic biomarker of PCa where the authors reported significant association of elevated miR-375 levels with shorter overall survival (mortality rate approximately 80%). Thus, miR-375 expression correlates with clinicopathological parameters and can act as a prognostic biomarker of PCa [44].

MiR-221 is encoded in tandem located on the X chromosome (Xp11.30) in human, mouse and rat and is highly conserved in vertebrates [45]. MiR-221 is reported to act as an oncomir in various epithelial cancers including prostate cancer [46–49]. MiR-221 as a biomarker of PCa was first reported in the year 2011 by Agaoglu et al. [33]. The authors reported miR-221, along with miR-21, to be elevated in the plasma samples of PCa metastatic patients compared to healthy controls [33]. Also the expression of miR-221 was found to be significantly higher in metastatic PCa cases compared to localized/local advanced PCa [33]. Yet in another study (Selth, 2012) miR-221 in combination with miR-20a, miR-21 and miR-145 could discriminate between intermediate or high-risk PCa patients compared to low-risk PCa as categorized by cancer of the prostate risk assessment (CAPRA) score and D'Amico's criteria [35]. However, there was no significant association between independent expression of miR-221 and PCa aggressiveness by either CAPRA or D'Amino score [35]. Collectively, these studies show that the expression of miR-221 is associated with metastatic PCa and can discriminate between different stages of PCa.

MiR-21 is located in chromosome 17q23.2 in human and is evolutionarily conserved across vertebrate species [50]. The expression of miR-21 is found to be overexpressed in various human tumors and cancer cell lines [51–53], including prostate tumors and it is reported to be an oncogene targeting various tumor suppressor genes [54, 55]. As mentioned above, miR-21 along with miR-221 was reported by Agaoglu et al. in 2011 to be upregulated in metastatic PCa patients compared to healthy controls and could discriminate between metastatic and localized/local advanced PCa cases [33]. Zhang et al. in the same year showed that the expression of miR-21 is upregulated in androgen-dependent PCa (ADPC) and castration-resistant PCa (CRPC) group of patients compared to localized PCa and BPH [56]. Also, serum levels of miR-21 corresponded to that of PSA levels in ADPC and CRPC patients [56]. Moreover, patients who were resistant to chemotherapy had an elevated level of miR-21 compared to responsive group of patients [56]. However, expression of miR-21 could not discriminate between localized PCa and BPH [56]. In a study by Shen et al., expression of miR-21 along with miR-20a was significantly associated with CAPRA score as well as with clinicopathological variables of PCa [57]. Thus, miR-21 expression correlates with CAPRA score and PSA levels and can work as a diagnostic and prognostic marker in PCa.

The molecular basis for dysregulated expression of microRNAs in prostate cancer

The molecular mechanism underlying the dysregulated expression of microRNAs in prostate cancer includes change in copy number of microRNAs [62], epigenetic modifications (DNA methylation and histone modification) [63], upregulated expression of Dicer [64], mutations in the stem regions of pre-miRNAs [65], single nucleotide polymorphisms (SNPs) [66] and androgen receptor (AR) regulated mechanisms [67]. Copy number alterations of miRNAs and their regulatory genes are highly prevalent in cancer and may account partly for the dysregulated microRNA expression profile in many cancers including prostate cancer [62]. It has been seen that in epithelial cancers there is a high frequency of copy number alterations in microRNA containing regions of the genome which correlates with the respective microRNA expression [62]. This copy number alterations correlates with miRNA transcript expression; however, the same does not hold true for many miRNAs. Also microRNA regulation is controlled by transcriptional regulation of the host gene [68]. Since half of the microRNA genes are located in introns of protein coding gene [69], these are more susceptible to epigenetic silencing by aberrant methylation of the CpG island located in the 5′UTR of the host gene [68]. Moreover, as half of the human promoter regions comprises

CpG islands, thus, differential promoter methylation may lead to aberrant expression of microRNAs [70]. Additionally, there exists a bidirectional cross-control mechanism between microRNAs and epigenetic machinery in which microRNA expression can be regulated by the epigenetic system and in return the components of epigenetic machinery are modulated by microRNAs [71]. For instance, miR-29 expression showed inverse correlation and downregulated the expression of DNA methyltransferases (DNMT-3A and -3B) in lung cancer (55). Furthermore, enforced expression of miR-29 leads to decreased global DNA methylation with simultaneous restoration of expression of tumor suppressor genes. Besides these, the position of microRNA in the genome (fragile sites, genomic regions of loss heterozygosity, or cancer-associated regions) [72] and mutations in the microRNA processing machinery also contributes to the microRNA deregulation in cancer [73]. For instance, miR-142 located on chromosome 17 but also found in the breakdown junction of a t(8,17) translocation causes an aggressive B cell leukemia by upregulation of a translocated c-Myc gene which is under the control of an upstream miR-142 promoter [74]. The upregulated expression of Dicer along with other components (EIF2C2, EIF2C1, XPO5, MOV10, HSPCA and TNRC6B) of miR machinery also contributes towards deregulated expression of microRNAs and also correlates with the aggressiveness associated with PCa metastasis and Gleason score [64]. Human Dicer is located in the subtelomeric region of chromosome 14 (14q32.13) and any genomic instability at 14q32 induces Dicer upregulation [64]. In addition, single nucleotide polymorphisms (SNPs) within precursor-microRNAs (pre-miRNAs) also affect microRNA expression levels [75]. For instance, in papillary thyroid carcinoma, an SNP in the pre-miR-146a is reported to decrease mature miRNA expression [76]. Another aspect of regulating the microRNA expression in PCa is androgen-induced androgen-receptor (AR) which binds to the promoter region of microRNA and leads to its over expression followed by androgen-dependent (AD) cell growth and castration resistance in PCa [67].

MicroRNAs and androgen receptor signaling

Prostate cancer cells depend on androgen for its growth and survival [77]. Thus, patients with metastatic prostate cancer are often treated with drugs that block androgen production [78]. These drugs are provided with antiandrogens such as analogs of luteinizing hormone releasing hormones that work as androgen receptor antagonist [79]. Studies carried out by Chen et al. have shown that androgen works in a paracrine manner in normal prostate cells where binding of androgen receptor to its ligand leads to its dimerization and, thereby, it regulates specific genes in the stromal and epithelial prostate cells, regulating proliferation and survival of epithelial cells [78]. However, in prostate cancer cells the paracrine pathway gets converted to the autocrine pathway where androgen directly stimulates proliferation of malignant prostate cells [80]. The postulated mechanisms underlying the reason behind relapse to an unresponsive hormone refractory stage can be divided into four categories, the first category being the mutations such as amplifications or point mutations of the androgen receptor gene which alters the response of the receptor and sensitizes the cancerous cells even to low concentration of androgens [81], also the antagonists start behaving like agonists; the 2nd category includes cases where activation of androgen receptor takes place independently as a consequence of cross talk between AR signaling pathway and other pathways such as epidermal growth factor receptor, Akt pathway, mitogen-activated protein kinase (MAPK) signaling induced by oncogenes [82]; however, the kinases and substrates involved in this mechanism are unknown; the 3rd category includes alterations in the equilibrium between coactivators and corepressors [83] and the 4th category comprises alternating pathways that bypass androgen receptor signaling and progression of the disease which is independent of androgen receptor [84, 85].

Thus, it shows that AR signaling pathways play a crucial role in PCa progression and, hence, the microRNAs that regulate this signaling pathway are of utmost importance. Therefore, in the present review we have compiled the role played by this panel of miRNAs in apoptosis in PCa.

MicroRNA-141 and androgen receptor

Waltering et al. 2011 showed that very few microRNAs are androgen regulated in both cell lines and xenografts of which miR-141 was upregulated in both PCa cases and castration-resistant prostate cancer (CRPC) compared to BPH [86]. The authors analyzed the microRNA expression in LNCaP-derived models, VCaP cell lines, 13 castrated prostate cancer (PCa) xenografts and clinical samples of untreated PCa patients and CRPC, using microarray and q-RT-PCR [86]. They also studied the functional significance of miR-141 in PCa by overexpressing and suppressing miR-141 in cell lines [86]. The forced expression of miR-141 increased the proliferation of LNCaP cell lines in low concentration of dihydrotestosterone (DHT) and the suppression of miR-141 by anti-miR-141 reduced LNCaP proliferation in low androgen medium, indicating the role of miR-141 in enhancing the growth of CRPC cells in the androgen depleted environment [86].

Another study by Xiao et al. [87] showed the correlation of Shp (small heterodimer protein) expression with miR-141 and subsequently its effect on AR signaling pathway in malignant and non-malignant PCa cells. The Shp mRNA and protein were found to be downregulated in PCa cell lines

compared to non-malignant human prostate epithelial cell line (RWPE-1), which was confirmed by real-time PCR, western blot and immunofluorescent staining [87]. The authors also found an upregulated expression of miR-141 in PCa cell lines compared to control. Using miR-141 precursor and anti-miR-141, the authors showed that transfection with anti-miR-141 downregulated the expression of Shp protein in RWPE-1 cell line in a dose-dependent manner and an increase in Shp protein was seen in PCa cell lines after treatment with anti-miR-141 [87]. This suggests that Shp is a target of miR-141 [87]. Thus, the above study showed that Shp, which is a known corepressor and metabolic regulator, is a target of miR-141 and the downregulation of Shp, induced by upregulated miR-141, transcriptionally regulates androgen receptor genes in prostate cells indicating the importance of miR-141 in prostate cancer progression.

MicroRNA-375 and androgen receptor

MiR-375 acts as a tumor suppressor in various cancers viz., esophageal squamous cell carcinoma [88], oral squamous cell carcinoma [89], pancreatic cancer [90], squamous cervical cancer [91], gastric carcinomas [39], head and neck squamous cell carcinomas [92], and melanoma [93]. However, in hormone-dependent cancers, namely prostate cancer and breast cancer, miR-375 is found to be overexpressed and thus is hypothesized to exert an oncogene function [94, 95].

A study by Chu et al. showed that the differential expression pattern of miR-375 is determined by the methylation mediated transcriptional repression of the miR-375 promoter. The AR-positive PCa cells exhibits lower levels of miR-375 and AR-negative PCa cells display a higher level of miR-375 [96]. Androgen receptor negatively regulates the DNA methyltransferases (DNMTs) activity in PCa cells, thereby leading to either hypermethylations or hypomethylation of the miR-375 promoter in AR-negative cells (PC-3) and AR-positive cells (LNCaP), respectively [96]. Also using a demethylating agent such as 5-Aza-dC in AR-positive cells, the lower expression of miR-375 could be reversed, indicating DNA methylation to be a major driving factor in regulating miR-375 expression in prostate cancer cells [96].

The dysregulated expression profile of miR-375 is only relevant to malignant PCa cells such as PC-3 whereas no significant attenuation was observed in benign prostate epithelial cells (RWPE-) [97], indicating a dual role of miR-375 in PCa progression depending on the stage and hormone status [97]. The molecular targets of miR-375 are cyclin D2 (CCND-2) and retinoblastoma 1(RB1) which were seen to be downregulated upon forced expression of miR-375 in PCa cell lines [97].

MicroRNA-375 has been reported to form a complex with miR-93/miR-106b and targets capicua (CIC), which is an HMG box-containing transcriptional repressor [98]. Knock-down of CIC leads to increased expression of cellular retinoic acid binding protein 1 (CRABP1) [99]. CRABP1 is known to have pro-tumorigenic and pro-metastatic activity in mesenchymal tumors [100] and is known to be upregulated in androgen-independent PC-3 cells [101] and CRPC [102].

Another study reported sec23 homolog A, coat complex II component (SEC23A) to be a target of miR-375 [94]. SEC23A works in interaction with N-myc downstream-regulated gene 1 (NDRG1) and the NDRG1 interactions are androgen-regulated driving androgen-dependent PCa to androgen-independent PCa [94]. Further, it has been reported that MHC-I molecules interact with SEC23-24 for their endoplasmic reticulum (ER) to Golgi trafficking, suggesting downregulation of SEC23A by miR-375 which in turn reduces the immunogenicity of PCa cells by reducing the expression of MHC-I [94].

MicroRNA-221 and androgen receptor

The expression of miR-221 is upregulated about 6- to 10-fold in LNCaP-derived castration-resistant counterpart androgen-independent (AI LNCaP-abl) cells compared to androgen-dependent LNCaP cells [103]. Upregulating the expression of miR-221 in androgen-dependent (AD), LNCaP cells showed a negative regulation of the AR mediated PSA level and androgen-mediated cell growth; however, it showed no influence on AR expression [103]. Also, transfection of AI LNCaP-abl (CRPC) cells with anti-miR-221 could restore the AR mediated PSA level and androgen-mediated cell growth, indicating the importance of miR-221 in maintaining the CRPC phenotype in PCa [86]. Cyclin-dependent kinase inhibitors, p27/kip1 and p57/kip2, are reported as miR-221 targets [103]. Upregulated expression of miR-221 in PC-3 and LNCaP cell lines and in glioblastoma is associated with upregulated p27/kip1 expression and results in growth inhibition and inability to form colonies in soft agar; however, in AI LNCaP-abl (CRPC) cells upregulated expression of miR-221 had no influence on the p27/kip1 expression, suggesting various other genes which may be involved along with p27/kip1 in maintaining CRPC phenotype in PCa [103].

MiR-221 is also shown to play a role in neuroendocrine (NE) differentiation of prostate cells [104]. NE diferentiation is hypothesized to be a major regulator of CRPC [105], and is associated with various cancerous phenotypes because NE cells do not proliferate; they secretes elevated levels of survival genes viz., Survivin [106] and Bcl-2 [107]; they also secrete certain growth factors and hormones that support the growth of surrounding tumor in a paracrine manner [106]. It has been reported that miR-221 promotes NE differentiation in hormone-sensitive LNCaP cells and sustains the growth

of LNCaP cells in androgen-deprived environment [105]. MiR-221 also induces an S-phase arrest in cells [105]. MiR-221 transfection of LNCaP cells is also associated with NSE mRNA upregulation [105].

MiR-221 is shown to have a direct influence on the sensitivity of PCa cells to androgen. The upregulated expression of miR-221 in PCa cell lines is also consistent in human prostate tumor samples. It has been reported that even in the absence of androgen LNCaP cells could grow when expression of miR-221 was upregulated [108]. Also LNCaP with elevated miR-221 expression rescued LNCaP cells from growth arrest at G1 phase of cell cycle due to androgen depletion, promoting androgen-independent (AI) growth [108]. Upregulated expression of miR-221 also leads to differential expression of various AR-responsive genes such as declined expression of PSA [103] along with other AR sensitive genes viz., polycomb protein enhancer of zeste homolog 2 (EZH2) [26] and cell cycle regulatory gene (cdc20) [109]; however, significant change in expression level was observed in AR and promyelocytic leukemia zing finger protein (PLZF) [110]. It is also reported that upregulated expression of miR-221 is associated with downregulation of various miR-221 targets and, thereby, leads to elevated expression of G2, G2/M phase transition and M-phase cyclins, which eventually sustain AI growth of LNCaP cells [108]. HECT domain containing E3 ubiquitin protein ligase 2 (HECTD2) and Ras-related protein Rab-1A (RAB1A) are reported to be two targets of miR-221 and they are negatively regulated by miR-221 in PCa cells and thus they helped in sustaining the AI growth of PCa cells [108].

MicroRNA-21 and androgen receptor

MiR-21 has been shown to be an AR-regulated microRNA that enhances the androgen-dependent growth of prostate cells and develops the CRPC phenotype [111]. In yet another study, it has been shown that $p57^{Kip21}$ is a target of miR-21 in PCa cells and transfection with miR-21 inhibitors and mimics in MDA-PCa-2b and PC-3 cells upregulated and downregulated $p57^{Kip21}$ mRNA expression, respectively, thereby hampering the tumor suppressive potential of $p57^{Kip21}$ [112].

Thus, the above studies show convincing data indicating miR-141, miR-375, miR-221 and miR-21 as makers of metastatic prostate cancer. MiR-141 is globally upregulated in PCa cases irrespective of the stage and hormone status of the cancer, whereas miR-375 is upregulated in AR-negative PCa cells compared to AR-positive PCa cells and the dysregulated expression profile is only relevant to malignant PCa cells indicating a dual role of miR-375 in PCa progression. Similarly, expression of miR-221 and miR-21 is upregulated in AI PCa cell lines compared to AD PCa counterparts and miR-221 also has a role in regulating neuroendocrine (NE) differentiation of prostate cells which is a regulator of CRPC, thus signifying the role of miR-221 in maintaining the CRPC phenotype in PCa.

MicroRNAs and cell proliferation

Cell proliferation is one of the major factors that mark the beginning of cancer and is the central and key process affected in all malignancies [113]. It is marked by loss of balance between cell loss and cell gain followed by invasion and metastasis [114]. Studies have shown that one way by which microRNAs plays a role in the pathogenesis of cancer is by regulating cell proliferation [7, 115]. For instance, it has been reported that miR-122 plays a role in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) by inhibiting proliferation and growth of malignant tumor cells [116]; miR-19a acts as an oncogene and targets TIA1 (T cell intracytoplasmic antigen) and promotes colorectal cancer proliferation and migration [117]. MiR-144 targets E2F8 (E2F transcription factor 8) gene and inhibits the proliferation of papillary thyroid cancer (PTC), the most common subtype of thyroid cancer [118]; miR-373 suppresses proliferation and invasion in breast carcinoma by inhibiting BCl-2 expression [119]. Similarly in prostate cancer various miRNAs are reported to be tumor suppressor miRs as well as oncomiRs. For instance, miR-181c inhibited cell proliferation in PCa cells viz PC-3 and DU145 by targeting ERK2 (extracellular signal-regulated kinase), a core component of the ERK signaling pathway [120]. Similarly, miR-193a-3p suppressed proliferation in PC-3 and DU145 cells by targeting CyclinD1 and exhibited a significant G1/S phase arrest [121]. MiR-211 also exhibited tumor suppressive effects by inhibiting proliferation of PCa cells by targeting SPARC (secreted protein acidic and rich in cysteine) mRNA [122] which is matricellular glycoprotein and plays a major role in cell proliferation, migration and differentiation, modulating reversible interactions between cells and ECM [123]. Similarly, miR-17 overexpression suppressed LNCaP cells proliferation by downregulating STAT3 expression which is an important transcription factor in the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway that plays a critical role in many biological processes including proliferation [124]. MiR-20b, however, exerted an oncogenic effect in prostate cancer cells by promoting proliferation in VCaP and PC-3 cells by inhibiting PTEN (phosphatase and tensin homolog) expression by directly binding to its 3′-UTR and the proliferating ability of the prostate cancer cells was significantly reduced upon transfection with miR-20b inhibitor [125]. Similarly, miR-671 functions as an oncogene and promotes prostate cell proliferation by inhibiting tumor suppressor gene SOX6 [encoding SRY (sex determining region Y)—box 6] [126]. Thus, miRNAs represent critical regulators of proliferation

in PCa and, thus, in the present review we have compiled the reported data associated with the role played by each of these four most dysregulated microRNAs, viz., miR-141, miR-375, miR-221 and miR-21 in PCa proliferation and also made a comprehensive list of target genes that regulates PCa proliferation.

MicroRNA-141 and proliferation

MiR-141 is reported to have anti-proliferative properties in nasopharyngeal carcinoma, gastric adenocarcinoma cells [127] and hepatocellular carcinoma [128]. In case of PCa miR-141 is shown to interact with kruppel-like-factor-9 (KLF9) and promotes proliferation of PCa cells by upregulating stem cell markers viz., Oct-4, Nanog, SOX-9 and Bmil [103]. Moreover, genes associated with proliferation such as CyclinD1, Cyclin E and c-Myc are reported to be upregulated in miR-mimic transfected PC-3 cells [129]. MiR-141 is also reported to enhance the spheroid forming ability of PC-3 cells when transfected with miR-141 mimics. Thus, miR-141 positively regulates the proliferative and stemness-associated properties in PCa cells [129].

MicroRNA-375 and proliferation

CBX7 (chromobox homolog 7) is reported to be a target of miR-375 [130]. The CBX7 loss is correlated with metastasis in various other cancers such as breast, colon, gastric and pancreatic cancer [131, 132]. Also inhibition of CBX7 by miR-375 leads to abundance of CBX8 in PCa cells and CBX8 is shown to have oncogenic properties in other cancers such as colon [133], esophageal [134] and breast cancer [135]. CBX7 upregulates E-cadherin and knockdown of CBX7 leads to activation of various signaling pathways such as EMT and Wnt/beta-catenin pathway [130]. Thus, increased expression of miR-375 is associated with enhanced proliferation, metastasis and invasion of PCa cells in vitro [130].

MicroRNA-221 and proliferation

MiR-221 positively regulates both cell proliferation and migration and negatively regulates apoptosis of PCa cells [136]. Downregulation of miR-221 is associated with the G0/G1 arrest of cells, indicating a role of miR-221 in cell cycle distribution [136]. Silent information regulator 1 (SIRT1) is one of the putative targets of miR-221 and is reported to act as both oncogene and tumor suppressor depending on the oncogenic pathway specific to the tumor [137]. For instance, in PC-3 cells, increased expression of SIRT1 with downregulated miR-221 expression is associated with inhibition of cell proliferation and migration and increased apoptosis [136], whereas in LNCaP cells, it serves to restrain cell proliferation [138]. Although SIRT1 is not a direct target of miR-221 as shown by the luciferase reporter assay, the biological effects exerted by miR-221 in PCa pathogenesis are in association with SIRT1 [136].

In yet another study, miR-221 has been shown to act as a tumor suppressor, targeting B-cell specific Moloney leukemia virus insertion region homolog 1 (Bmi-1), which is a polycomb ring finger oncogene and downregulation of miR-221 is associated with the promotion of cell proliferation in PCa cells [139].

Another tumor suppressor gene, ARHI, that negatively regulates cell proliferation is reported to be a target of miR-221 and causes cell cycle G0/G1 arrest in PC-3 cells with regulation of genes such as p21, growth arrest and DNA-damage-inducible, alpha (GADD45A) and Hect domain and RLD5 (HERC5) [140]. These genes are reported to function by regulating cell cycle proteins, stimulating DNA excision repair and ISGylation of protein targets, respectively [140].

MicroRNA-21 and proliferation

A recent study has shown that there exists an inverse correlation between miR-21 and phosphatase and tensin homolog deleted on chromosome ten (PTEN) in prostate cancer [141]. PTEN is a tumor suppressor and have both lipid phosphatase and protein phosphatase activity [142] and can inhibit the phosphorylation of downstream PI3K/Akt signaling pathway [141]. Thus, the reduced expression of PTEN is associated with reduced dephosphorylation of PI3K/Akt, enhancing cell proliferation and invasion in prostate cancer cells [141].

Thus, collectively it can be stated that miR-141, miR-375 and miR-21 act as oncogenic miRNAs promoting proliferation of PCa cells and miR-141 also positively regulates the stemness-associated properties of PCa cells. However, there are contradicting data in case miR-221 in PCa cells proliferation and is reported to act both as an oncomiR and a tumor suppressor miRNA regulating PCa cell proliferation. Thus, future studies on comprehensive analysis of miR-221 target genes and regulatory networks will be of great importance to clarify the role of miR-221 in PCa cell proliferation.

MicroRNAs and epithelial mesenchymal transition (EMT)

Epithelial mesenchymal transition is a developmental program with downregulation of epithelial phenotype and upregulation of mesenchymal characteristics [143]. It plays a major role in the metastasis of tumors of epithelial origin [144]. EMT can be both physiological and pathological and plays a key role in embryonic development and many diseases including cancer [145]. MicroRNAs have been reported to regulate EMT in various cancers, for instance,

miR-200 family acts as a suppressor of EMT in various cancer, miR-194 promotes tumorigenesis by positively regulating EMT in colorectal cancer [146]; miR-217 suppressed EMT in gastric cancer by targeting PTPN14 (protein tyrosine phosphatase non-receptor type 14) gene which plays a crucial factor in EMT, metastasis and tumorigenesis [147]; miR-138 acts an a tumor suppressor in breast cancer cells by negatively regulating tumor associated gene Vimentin [148]. In case of prostate cancer, various miRNAs regulated EMT, for instance, miR-200b regulated EMT by increasing epithelial features of PC-3 cells with simultaneous reduction of mesenchymal markers [149]; miR-186 plays a tumor suppressive role in prostate cancer and suppressed EMT by inhibiting Twist1 expression [150]; miR-409-3p/-5p acts as an oncogene and its inhibition reduced the growth of prostate cancer cells with simultaneous induction of MET (mesenchymal–epithelial transition), both in vitro and in vivo [151]; ectopic expression of let-7a downregulated CCR7 (CC chemokines receptor 7) gene expression in PC-3 cells [152]. Mounting evidence supports that miRNAs play a vital role in regulating EMT in prostate cancer and, thus, in the present review we have summarized the role played by each of these four dysregulated microRNAs in regulating EMT in molecular level in prostate cancer from the existing literature.

MicroRNA-141 and EMT

The most potent inducer of EMT, Zeb1, is shown to have a suppressive role on miR-200 family with the most prominent on miR-141 and miR-200c in many cancers such as breast, pancreatic and colorectal cancer [153]. Zeb1 is shown to directly repress transcription of these microRNAs by binding to two conserved sites in their promoter regions [153]. Also, another potent inducer of EMT, TGFβ2, is a putative target of miR-141, exhibiting a regulatory loop between EMT induction and miR-141 expression [153]. However, unlike other members of miR-200 family, which are well known suppressors of EMT, miR-141 is only partial inhibitor of EMT that suppresses Zeb1 and Vimentin but without much effect on other mesenchymal markers such as Zeb2, Snail1, Snail2, Twist and Fibronectin, thus indicating induction of partial MET (mesenchymal–epithelial transition) phenotype [154].

MicroRNA-375 and EMT

Various groups have demonstrated that miR-375 is elevated in prostate cancer [155, 156]; however, there is only a single report showing the association of miR-375 with EMT. The study reported that miR-375 was upregulated in PCa cell lines possessing epithelial phenotype, whereas it was downregulated in cells having mesenchymal phenotypes, indicating miR-375 to be an epithelial marker in prostate cancer cells [157]. The authors have reported that miR-375 acts as a tumor suppressor and also as an inducer of metastasis, in a stage dependent manner [157]. They have also identified yes-associated protein 1 (YAP-1) as the downstream target of miR-375 and have shown that knockdown of YAP-1 in prostate cancer cells leads to downregulation of mesenchymal phenotypes viz., Vimentin and Fibronectin, indicating YAP-1 to be a mesenchymal marker [157]. Additionally, this study also reported that miR-375 is negatively regulated by Zeb-1, which is a key regulator of EMT. Knockdown of Zeb-1 downregulated the expression of miR-375 with the eventual loss of YAP1, indicating YAP1 to be a key downstream target of miR-375 in mediating EMT [157].

MicroRNA-221 and EMT

MiR-221 is a key regulator of EMT in luminal breast cancer cells [158] and there exists a direct correlation between miR-221 expression and E-cadherin repression [159]. Also, it directly targets trichorhinophalangeal 1 (TRPS1), which transcriptionally represses Zeb2 and Dicer, a potent EMT inducer and a key regulator of microRNA maturation, respectively, in pancreatic and breast cancer cells [22, 158]. However, the role of miR-221 in regulation of EMT in prostate cancer is not yet established to the best of our knowledge and more research in the area is needed.

MicroRNA-21 and EMT

Prostate cancer cells are believed to be originated from basal cells but express luminal markers and shows functional properties of basal cells [160–162]. The basal cell compartment expresses B cell translocation gene 2 (BTG2) [163] along with ΔNp63α, an isoform of p63 and is responsible for maintaining stemness and controls differentiation during prostate organogenesis [164, 165]. Loss of ΔNp63α is associated with the acquisition of EMT phenotype [166]. Basal protein (BTG2) is a tumor suppressor and its loss is associated with enhanced extracellular signal-regulated kinase 1 (ERK) signaling in prostate cells and is postulated to be associated with the acquisition of EMT phenotype in PCa cells [167]. The expression of miR-21 is upregulated in androgen-independent RWPE-2, PC-3 and DU145, whereas it is downregulated in androgen-dependent LNCaP and 22Rv1 compared to non-neoplastic RWPE-1 cells and prostate epithelial cells (PrEC) [168]; consistently, the BTG2 expression inversely correlates with miR-21 expression in PCa cells [168]. Studies have shown that miR-21 targets BTG2 and leads towards the phenotypic shift of prostatic basal cells towards luminal phenotype with the acquisition of EMT like features and tumorigenecity [167]. Also, the restoration of BTG2 levels in RWPE-2 cells that expresses

a higher level of mesenchymal markers could shift the EMT phenotype with downregulation of Vimentin, Fibronectin, cytokeratins 8 and 18 (CK8-18) and with upregulation of p63 [167].

Taken together, the above studies indicate that miR-141 and miR-375 works as inhibitor of EMT targeting various genes involved in the EMT process although miR-141 is reported to be a partial inhibitor of EMT. However, miR-21 is reported to exert oncogenic effects by inducing phenotypic shift in PCa cells, thereby acquiring malignant features. The correlation between miR-221 and EMT has not been assessed to the best of our knowledge. Thus, further studies revealing the function of miR-221 in EMT are required.

MicroRNAs and apoptosis

Apoptosis is programmed cell death with certain morphological changes such as cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation [169]. During the transformation of a normal cell to a malignant one, evasion of cell death is one of the major hallmarks and is accomplished by the impaired balance between pro-apoptotic and anti-apoptotic proteins, reduced caspase activity and disrupted death receptor signaling [170]. Dysregulated microRNA expression is associated with tumorigenesis and microRNAs are reported to act as both pro-apoptotic and anti-apoptotic in various cancers [171]. For instance, miR-491 induces apoptosis in colorectal cancer by regulating BCL-XL (BCL2-like 1 isoform) [172], miR-133a suppresses osteosarcoma progression by inducing apoptosis by targeting BCL-XL and Mcl-1 [173], in pancreatic cancer miR-1284 is reported to induce apoptosis by regulating PI3K/Akt pathway [174], in breast cancer cells miR-125b targets Bak1 and is associated with inhibition of apoptosis in Taxol-resistant cancer cells [175]. Similarly in case of prostate cancer, various miRNAs are reported to regulate apoptosis; for instance, miR-218 plays a tumor suppressive role and induces apoptosis by targeting an oncogene TPD52 (tumor protein D52) which is upregulated in prostate cancer [176]; similarly, miR-466 induced apoptosis in metastatic prostate cancer cells (PC-3 and DU145) with simultaneous induction of G0/G1 cell cycle arrest [177]; miR-143 induced apoptosis in LNCaP cells by inhibiting BCl-2 expression [178]; miR-1180 induced apoptosis in PCa cells by targeting TRAF1 (TNF receptor-associated factor 1) and BAG2 [B cell lymphoma 2 (Bcl 2)-associated athanogene 2] [179]. Thus, we see that there exists a correlation between dysregulated miRNA expression and apoptosis. In the present review, we have discussed the current knowledge about each of these four most dysregulated microRNAs, viz., miR-141, miR-375, miR-221 and miR-21 in apoptosis in PCa.

MicroRNA-141 and apoptosis

MiR-141 is shown to regulate cell death pathway in many cancers. For instance, miR-141 overexpression is associated with apoptosis induction in osteosarcoma by targeting ZEB1 and ZEB2 [180]; in pancreatic cancer, miR-141 acts as an apoptosis inducer by targeting mitogen-activated protein kinase isoform 4 (MAP4K4); however, in case of hepatocellular carcinoma downregulation of miR-141 promotes apoptosis by modulating hepatocyte nuclear factor 3β (HNF-3β) [181]. In prostate cancer, miR-141-3p acts as an inhibitor of apoptosis as shown by downregulation of various apoptosis-related mRNAs such as p21, p27, Bax and Caspase-3 upon miR-141-3p mimic treatment and was upregulated on treatment with miR-141-3p inhibitors [103, 182].

MicroRNA-375 and apoptosis

MiR-375 expression is lowest in RWPE-1 cell line and highest in 22Rv1; however, in PC-3 cell line it shows moderate expression [97]. Forced expression of miR-375 could increase the level of apoptosis in PC-3 cells (metastatic prostate cell line); however, in RWPE-1 cells (a benign prostate cell line) there was no change in the level of apoptosis, thus suggesting that deregulated expression of miR-375 is only relevant in malignant prostate cancer [97]. Also, inhibition of miR-375 expression in 22Rv1 could increase the level of apoptosis, suggesting that miR-375 can have an oncogenic phenotype (in 22Rv1) as well as tumor suppressive phenotype (in PC-3) in the same tumor model [97].

MicroRNA-221 and apoptosis

MiR-221 positively regulates apoptosis in androgen-independent PC-3 and DU145 by increasing the caspase 3/7 activity and simultaneous activation of JAK/STAT pathway by negatively regulating suppressor of cytokine signaling 3 (SOCS3) and interferon regulatory factor 2 (IRF2) and, thus, acts as a tumor suppressor [183]. However, another study revealed that miR-221 negatively regulates apoptosis in PCa cells, both PC-3 and LNCaP with a simultaneous increase in proliferation and decrease in Caspase-3 and Caspase-10 activity by inhibiting TNF-α/CHX-induced apoptosis [19]. It was also observed that knocking down of miR-221 was associated with increased expression of Caspase-10 and sensitizing cells to apoptosis [19]. Thus, it shows that there are contradictory data regarding the role of miR-221 being tumor suppressor or an oncogene in prostate cancer.

MicroRNA-21 and apoptosis

MiR-21 is known to play a role in tumorigenesis in various malignancies [184, 185], including prostate cancer [186]

Table 2 Genes regulated by the panel of four differentially expressed microRNAs in PCa

MicroRNAs	Genes regulated in the AR pathway	Genes being regulated in proliferation pathway	Genes being regulated in the EMT pathway	Genes being regulated in the apoptosis pathway	References
MiR-141	Shp	KLF9, cyclinD1, cyclinE, c-Myc	Zeb1, vimentin	p21, p27, bax, caspase-3	[87, 128, 154, 182]
MiR-375	CCND-2, Rb1, CRABP1, SEC23A	CBX7	YAP-1	–	[94, 96, 97, 99, 130, 157]
MiR-221	HECTD2, RAB1A, p27, kip1	SIRT1, Bmi-1, ARHI	–	Caspase-3/7/10, SOCS3, IRF2	[19, 103, 108, 136, 139, 140, 183]
MiR-21	$p57^{Kip21}$	PTEN	BTG2, vimentin, fibronectin, CK8-18, p63	FBXO11	[112, 141, 168, 187]

by targeting various genes that are tumor suppressors in nature [187]. One such tumor suppressor gene is FBXO11 (a member of the F-box subfamily lacking a distinct unifying domain) which has been identified as a target of miR-21 by microarray analysis [187]. FBXO11 targets various proteins that play a role in cell cycle control, apoptosis, metastasis, differentiation and also tags proteins for proteosomal degradation by ubiquitination [187]. In prostate cancer models, FBXO11 acts as an inducer of apoptosis and sensitizes DU145 cells to apoptosis [187]. Thus, miR-21 regulates apoptosis in prostate cancer.

Collectively, the above studies revealed that miR-141 and miR-21 act as oncogenic miRNAs in regulating apoptosis in PCa; however, miR-375 acts as both oncogenic and tumor suppressor miRNA in regulating apoptosis in PCa. Also there are contradictory data reported regarding the role played by miR-221 in apoptosis in PCa. Thus, further research exploring the signaling pathways and functional targets of this panel of miRNAs needs to be conducted.

Conclusion

Dysregulated microRNA profile is associated with prostate cancer aggressiveness and affects various critical cellular processes viz., proliferation, apoptosis, EMT and androgen receptor signaling (Table 2). Although various studies in the last decade have tried to study the relationship between microRNAs and PCa, there is high variability in the data reported. In the present review, we have identified a panel of four consistently dysregulated microRNAs in prostate cancer and have prepared a comprehensive list of their experimentally validated targets both in vitro and in vivo. Thus, validation of these promising candidate microRNAs in larger, prospective cohort will not only define their exact role in prostate cancer progression but may also be used as potential prognostic and diagnostic markers in prostate cancer.

The implication of this panel of four microRNAs in PCa management demands uniformity in the study design, sampling method and profiling platform used. Thus, minimizing the ambiguity associated with miRNA profiling studies and developing a gold standard technique might help in near future to bring this panel of miRNAs from bench to bed side.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent This article does not contain any studies with human participants hence informed consent is not applicable.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67:7–30.
2. George GP, Gangwar R. Genetic variation in microRNA genes and prostate cancer risk in North Indian population. Mol Biol Rep. 2011;38:1609–15.
3. Agnihotri S, Mittal RD, Ahmad S, Mandhani A. Free to total serum prostate specific antigen ratio in symptomatic men does not help in differentiating benign from malignant disease of the prostate. Indian J Urol. 2014;30:28–32.
4. Bangma CH, Roemeling S, Schröder FH. Overdiagnosis and overtreatment of early detected prostate cancer. World J Urol. 2007;25:3–9.
5. Greene KL, Meng MV, Elkin EP, Cooperberg MR, Pasta DJ, Kattan MW, et al. Validation of the Kattan preoperative nomogram for prostate cancer recurrence using a community based cohort: results from cancer of the prostate strategic urological research endeavor (capsure). J Urol. 2004;171:2255–9.
6. Van Rooij E. The art of microRNA research. Circ Res. 2011;108:219–34.

7. Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochim Biophys Acta Mol Cell Res. 2010;1803:1231–43.
8. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
9. Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol. 2005;6:376–85.
10. Rothschild SI. MicroRNA therapies in cancer. Mol Cell Ther. 2014;2:7.
11. Mao L, Oh Y. Does marijuana or crack cocaine cause cancer? J Natl Cancer Inst. 1998;90:1182–4.
12. Zhang B, Pan X, Cobb GP, Anderson TA. MicroRNAs as oncogenes and tumor suppressors. Dev Biol. 2007;302:1–12.
13. Lewis BP, Shih I, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003;115:787–98.
14. Peng Y, Croce CM. The role of microRNAs in human cancer. Signal Transduct Target Ther. 2016;1:15004.
15. Kim Y-K. Extracellular microRNAs as biomarkers in human disease. Chonnam Med J. 2015;51:51–7.
16. Sita-Lumsden A, Dart DA, Waxman J, Bevan CL. Circulating microRNAs as potential new biomarkers for prostate cancer. Br J Cancer. 2013;108:1925–30.
17. Schwarzenbach H, Nishida N, Calin GA, Pantel K. Clinical relevance of circulating cell-free microRNAs in cancer. Nat Rev Clin Oncol. 2014;11:145–56.
18. Aryani A, Denecke B. In vitro application of ribonucleases: comparison of the effects on mRNA and miRNA stability. BMC Res Notes. 2015;8:164.
19. Wang L, Liu C, Li C, Xue J, Zhao S, Zhan P, et al. Effects of microRNA-221/222 on cell proliferation and apoptosis in prostate cancer cells. Gene. 1015;572:252–8.
20. Sun T, Yang M, Chen S, Balk S, Pomerantz M, Brown M, et al. The altered expression of miR-221/-222 and miR-23b/-27b is associated with the development of human castration resistant prostate cancer. Prostate. 2013;2:1093–103.
21. Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, stratagies and challenges. Nat Rev Drug Discov. 2010;9:775–89.
22. Su A, He S, Tian B, Hu W, Zhang Z. MicroRNA-221 mediates the effects of PDGF-BB on migration, proliferation, and the epithelial-mesenchymal transition in pancreatic cancer cells. PLoS One. 2013;8:e71309.
23. Gregory RI, Yan K, Amuthan G. The microprocessor complex mediates the genesis of microRNAs. Nature. 2004;432:235–40.
24. Han J, Lee Y, Yeom K, Kim Y, Jin H, Kim VN. The Drosha—DGCR8 complex in primary microRNA processing. Genes Dev. 2004;18:3016–27.
25. Bohnsack MT, Czaplinski K, Go D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA. 2004;10:185–91.
26. Bohrer LR, Chen S, Hallstrom TC, Huang H. A potential mechanism of androgen-refractory progression of prostate cancer. Endocrinology. 2015;151:5136–45.
27. Russell PJ, Kingsley EA. Human prostate cancer cell lines. Prostate Cancer Methods Protoc. 2003;81:21–39.
28. Lin S, Chang D, Ying S. Asymmetry of intronic pre-miRNA structures in functional RISC assembly. Gene. 2005;356:32–8.
29. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005;123:631–40.
30. Cho WC. Molecular diagnostics for monitoring and predicting therapeutic effect in cancer. Expert Rev Mol Diagn. 2011;11:9–12.
31. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TLJ, Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res. 2007;67:6130–5.
32. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA. 2008;105:10513–8.
33. Agaoglu FY, Kovancilar M, Dizdar Y, Darendeliler E, Holdenrieder S, Dalay N, et al. Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. Tumor Biol. 2011;32:583–8.
34. Brase JC, Johannes M, Schlomm T, Haese A, Steuber T, Beissbarth T, et al. Circulating miRNAs are correlated with tumor progression in prostate cancer. Int J Cancer. 2011;128:608–16.
35. Selth LA, Townley S, Gillis JL, Ochnik AM, Murti K, Macfarlane RJ, et al. Discovery of circulating microRNAs associated with human prostate cancer using a mouse model of disease. Int J Cancer. 2012;131:652–61.
36. Nguyen HCN, Xie W, Yang M, Hsieh C-L, Drouin S, Lee G-SM, et al. Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. Prostate. 2013;73:346–54.
37. Poy MN, Eliasson L, Krutzfeldt J, et al. A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 2004;432:226–30.
38. He X, Chang Y, Meng F, et al. MicroRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth in vitro and in vivo. Oncogene. 2012;31:3357–69.
39. Ding L, Xu Y, Zhang W, et al. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. Cell Res. 2010;20:784–93.
40. Avissar M, Christensen BC, Kelsey KT, Marsit CJ. MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma. Clin Cancer Res. 2009;15:2850–6.
41. Mathé EA, Nguyen GH, Bowman ED, et al. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. Clin Cancer Res. 2009;15:6192–200.
42. Chang C, Shi H, Wang C, et al. Correlation of microRNA-375 downregulation with unfavorable clinical outcome of patients with glioma. Neurosci Lett. 2012;531:204–8.
43. Bryant RJ, Pawlowski T, Catto JWF, Marsden G, Vessella RL, Rhees B, et al. Changes in circulating microRNA levels associated with prostate cancer. Br J Cancer. 2012;106:768–74.
44. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol. 2015;67:33–41.
45. Galardi S, Mercatelli N, Giorda E, et al. MiR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines. J Biol Chem. 2007;282:23716–24.
46. Zhang C, Han LEI, Zhang A, et al. Global changes of mRNA expression reveals an increased activity of the interferon-induced signal transducer and activator of transcription (STAT) pathway by repression of miR-221/222 in glioblastoma U251 cells. Int J Oncol. 2010;36:1503–12.
47. Gramantieri L, Fornari F, Ferracin M, et al. MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. Clin Cancer Res. 2009;15:5073–82.
48. Zhao J, Lin J, Yang H, et al. MicroRNA-221/222 negatively regulates estrogen receptor and is associated with tamoxifen resistance in breast cancer. J Biol Chem. 2008;283:31079–86.
49. Miele F, Costantini A, Spagnoli G, et al. The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice. PLoS One. 2008;3:e4029.
50. Ivan GEM, Krichevsky AM, Gabriely G. MiR-21: a small multifaceted RNA. J Cell Mol Med. 2009;13:39–53.

51. Kutay H, Bai S, Datta J, et al. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. J Cell Biochem. 2006;99:671–8.
52. Zhang Z, Li Z, Gao C, Chen P, Chen J, Liu W, et al. MiR-21 plays a pivotal role in gastric cancer pathogenesis and progression. Lab Invest. 2008;88:1358–66.
53. Iorio MV, Visone R, Di Leva G, et al. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007;67:8699–707.
54. Yang B, Liu Z, Ning H, Zhang K, Pan D, Ding K, et al. MicroRNA-21 in peripheral blood mononuclear cells as a novel biomarker in the diagnosis and prognosis of prostate cancer. Cancer Biomark. 2016;17:223–30.
55. Yang Y, Guo JX, Shao ZQ. MiR-21 targets and inhibits tumor suppressor gene PTEN to promote prostate cancer cell proliferation and invasion: an experimental study. Asian Pac J Trop Dis. 2017;10:87–91.
56. Zhang HL, Yang LF, Zhu Y, Yao XD, Zhang SL, Dai B, et al. Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. Prostate. 2011;71:326–31.
57. Shen J, Hruby GW, McKiernan JM, Gurvich I, Lipsky MJ, Benson MC, et al. Dysregulation of circulating microRNAs and prediction of aggressive prostate cancer. Prostate. 2012;72:1469–77.
58. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One. 2009;4:e6229.
59. Moltzahn F, Olshen AB, Baehner L, Peek A, Fong L, Stöppler H, et al. Microfluidic-based multiplex qRT-PCR identifies diagnostic and prognostic microRNA signatures in the sera of prostate cancer patients. Cancer Res. 2011;71:550–60.
60. Mahn R, Heukamp LC, Rogenhofer S, Von Ruecker A, Miller SC, Ellinger J. Circulating microRNAs (miRNA) in serum of patients with prostate cancer. Urology. 2011;77:1265.e9–16.
61. Chen ZH, Zhang GL, Li HR, Luo JD, Li ZX, Chen GM, et al. A panel of five circulating microRNAs as potential biomarkers for prostate cancer. Prostate. 2012;72:1443–52.
62. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. MicroRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci. 2006;103:9136–41.
63. Rouhi A, Mager DL, Humphries RK. MiRNAs, epigenetics, and cancer. Mamm Genome. 2008;19:517–25.
64. Chiosea S, Jelezcova E, Chandran U, Acquafondata M, Mchale T, Sobol RW, et al. Up-regulation of dicer, a component of the microRNA machinery, in prostate adenocarcinoma. Am J Pathol. 2006;169:1812–20.
65. Cimmino A, Ph D, Di Leva G, Ph D, Shimizu M, Wojcik SE, et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353:1793–801.
66. Jin Y, Lee CGL. Single nucleotide polymorphisms associated with microRNA regulation. Biomolecules. 2013;3:287–302.
67. Sikand K, Barik S, Shukla GC. MicroRNAs and androgen receptor 3′ untranslated region: a missing link in castration-resistant prostate cancer. Mol Cell Pharmacol. 2012;3:107–13.
68. Grady WM, Parkin RK, Mitchell PS, Lee JH, Kim Y, Tsuchiya KD, et al. Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer. Oncogene. 2008;27:3880–8.
69. Saini HK, Griffiths-jones S, Enright AJ. Genomic analysis of human microRNA transcripts. Proc Natl Acad Sci. 2007;104:17719–24.
70. Vrba L, Jensen TJ, Garbe JC, Heimark RL, Cress AE, Dickinson S, et al. Role for DNA methylation in the regulation of miR-200c and miR-141 expression in normal and cancer cells. PLoS One. 2010;5:e8697.
71. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci. 2007;104:15805–10.
72. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA. 2004;101:2999–3004.
73. Thomson JM, Newman M, Parker JS, Morin-kensicki EM, Wright T, Hammond SM. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev. 2006;20:2202–7.
74. Lagos-quintana M, Rauhut R, Yalcin A, et al. Identification of tissue-specific microRNAs from mouse. Curr Biol. 2002;12:735–9.
75. Manikandan M, Munirajan AK. Single nucleotide polymorphisms in microRNA binding sites of oncogenes: implications in cancer and pharmacogenomics. OMICS. 2014;18:142–54.
76. Jazdzewski K, Murray EL, Franssila K, et al. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci. 2016;105:7269–74.
77. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev. 2004;25:276–308.
78. Isaacs JT, Isaacs WB. Androgen receptor outwits prostate cancer drugs. Nat Med. 2004;10:26–7.
79. Stricker HJ. Luteinizing Hormone-releasing hormone antagonists in prostate cancer. Urology. 2001;58:24–7.
80. Griend DJV, Antonio JD, Gurel B, Antony L, DeMArzo AM, Isaacs JT. Cell-autonomous intracellular androgen receptor signaling drives the growth of human prostate cancer initiating cells. Prostate. 2011;70:90–9.
81. Waltering KK, Helenius MA, Sahu B, Manni V, Linja MJ, Ja OA, et al. Increased expression of androgen receptor sensitizes prostate cancer cells to low levels of androgens. Cancer Res. 2009;69:8141–9.
82. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nat Med. 1999;5:280–5.
83. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004;10:33–9.
84. Raffo AJ, Perlman H, Chen M, Day ML, Streitman JS, Buttyan R. Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. Cancer Res. 1995;55:4438–45.
85. Kinoshita H, Shi Y, Sandefur C, Meisner LF, Chang C, Choon A, et al. Methylation of the androgen receptor minimal promoter silences transcription in human prostate cancer. Cancer Res. 2000;60:3623–30.
86. Waltering KK, Porkka KP, Jalava SE, Urbanucci A, Kohonen PJ, Latonen LM, et al. Androgen regulation of microRNAs in prostate cancer. Prostate. 2011;71:604–14.
87. Xiao J, Gong A, Eischeid AN, Chen D, Deng C, Young CYF, et al. MiR-141 modulates androgen receptor transcriptional activity in human prostate cancer cells through targeting the small heterodimer partner protein. Prostate. 2012;72:1514–22.
88. Komatsu S, Ichikawa D, Takeshita H, Tsujiura M, Morimura R, Nagata H, et al. Circulating microRNAs in plasma of patients with oesophageal squamous cell carcinoma. Br J Cancer. 2011;105:104–11.
89. Lajer CB, Nielsen FC, Norrild B, Borup R, Garnæs E, Rossing M, et al. Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study. Br J Cancer. 2011;104:830–40.

90. Basu A, Alder H, Khiyami A, Leahy P, Croce CM, Haldar S. MicroRNA-375 and microRNA-221: potential noncoding RNAs associated with antiproliferative activity of benzyl isothiocyanate in pancreatic cancer. Genes Cancer. 2011;2:108–19.
91. Wang F, Li Y, Zhou J. MiR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1. Am J Pathol. 2011;179:2580–8.
92. Nohata N, Hanazawa T, Kikkawa N, Mutallip M, Sakurai D, Fujimura L, et al. Tumor suppressive microRNA-375 regulates oncogene AEG-1/MTDH in head and neck squamous cell carcinoma (HNSCC). J Hum Genet. 2011;56:595–601.
93. Mazar J, Khaitan D, Deblasio D, Zhong C, Govindarajan SS, Kopanathi S, et al. Epigenetic regulation of microRNA genes and the role of miR-34b in cell invasion and motility in human melanoma. PLoS One. 2011;6:e24922.
94. Szczyrba J, Nolte E, Wach S, Kremmer E, Hartmann A, Wieland W, et al. Downregulation of sec23a protein by miRNA-375 in prostate carcinoma. Mol Cancer Res. 2011;9:791–801.
95. De Souza P, Simonini R, Breiling A, Gupta N, Malekpour M, Youns M, et al. Epigenetically deregulated microRNA-375 is involved in a positive feedback loop with estrogen receptor α in breast cancer cells. Cancer Res. 2010;70:9175–85.
96. Chu M, Chang Y, Li P, Guo Y. Androgen receptor is negatively correlated with the methylation-mediated transcriptional repression of miR-375 in human prostate cancer cells. Oncol Rep. 2014;31:34–40.
97. Costa-Pinheiro P, Ramalho-Carvalho J, Vieira FQ, Torres-Ferreira J, Oliveira J, Gonçalves CS, et al. MicroRNA-375 plays a dual role in prostate carcinogenesis. Clin Epigenetics. 2015;7:42.
98. Guichet A, Ephrussi A, Casanova J. Relief of gene repression by torso RTK signaling: role of capicua in *Drosophila* terminal and dorsoventral patterning. Genes Dev. 2000;14:224–31.
99. Choi N, Park J, Lee J, Yoe J, Park GY, Kim E, et al. MiR-93/miR-106b/miR-375-CIC-CRABP1: a novel regulatory axis in prostate cancer progression. Oncotarget. 2015;6:23533–47.
100. Kainov Y, Favorskaya I, Delektorskaya V, Chemeris G, Komelkov A, Zhuravskaya A. CRABP1 provides high malignancy of transformed mesenchymal cells and contributes to the pathogenesis of mesenchymal and neuroendocrine tumors. Cell Cycle. 2014;13:1530–9.
101. Russell PJ, Kingsley EA. Human prostate cancer cell lines. Methods Mol Med. 2003;81:21–39.
102. Sirotnak FM, She Y, Khokhar NZ, Hayes P, Gerald W, Scher HI. Microarray analysis of prostate cancer progression to reduced androgen dependence: studies in unique models contrasts early and late molecular events. Mol Carcinog. 2004;41:150–63.
103. Sun T, Wang Q, Balk S, Sun T, Wang Q, Balk S, et al. The role of microRNA-221 and microRNA-222 in androgen-independent prostate cancer cell lines. Cancer Res. 2009;69:3356–63.
104. Zheng C, Yinghao S, Li J. MiR-221 expression affects invasion potential of human prostate carcinoma cell lines by targeting DVL2. Med Oncol. 2012;29:815–22.
105. Hu C, Choo R, Huang J, Ned T. Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. Front Oncol. 2015;5:1–10.
106. Xing N, Qian J, Bostwick D, Bergstralh E, Young CYF. Neuroendocrine cells in human prostate over-express the anti-apoptosis protein survivin. Prostate. 2001;48:7–15.
107. Cadden IS, Johnston BT, Connolly R, Gates D, Tsujimoto Y, Eguchi Y, et al. An investigation into the role of Bcl-2 in neuroendocrine differentiation. Biochem Biophys Res Commun. 2005;326:442–8.
108. Sun T, Wang X, He HH, Sweeney CJ, Liu SX, Brown M, Balk S, Lee GS, Kantoff PW. MiR-221 promotes the development of androgen independence in prostate cancer cells via downregulation of HECTD2 and RAB1A. Oncogene. 2014;33:2790–800.
109. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell. 2009;138:245–56.
110. Feng Jiang, Zhou Wang. Identification and characterization of PLZF as a prostatic androgen-responsive gene. Prostate. 2004;59:426–35.
111. Ribas J, Ni X, Haffner M, Wentzel EA, Hassanzadeh A, Chowdhury WH, et al. MiR-21: an androgen receptor regulated microRNA which promotes hormone dependent and independent prostate cancer growth. Cancer Res. 2010;69:7165–9.
112. Mishra S, Lin C, Huang TH, Bouamar H, Sun L. MicroRNA-21 inhibits p57 Kip2 expression in prostate cancer. Mol Cancer. 2014;13:212.
113. Feitelson MA, Arzumanyan A, Kulathinal RJ, Blain SW, Holcombe RF, Mahajna J, et al. Sustained proliferation in cancer: mechanisms and novel therapeutic targets. Semin Cancer Biol. 2015;35:S25–54.
114. Farber E. Cell proliferation as a major risk factor for cancer: a concept of doubtful. Cancer Res. 1995;55:3759–62.
115. Fang Y, Fullwood MJ. Roles, functions, and mechanisms of long non-coding RNAs in cancer. Genom Proteom Bioinform. 2016;14:42–54.
116. Qiao D, Yang J, Lei X, Mi G, Li S, Li K. Expression of microRNA-122 and microRNA-22 in HBV-related liver cancer and the correlation with clinical features. Eur Rev Med Pharmacol. 2017;21:742–7.
117. Liu Y, Liu R, Yang F, Cheng R, Chen X, Cui S, et al. MiR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. Mol Cancer. 2017;16:53.
118. Sun J, Shi R, Zhao S, Li X, Lu S, Bu H, et al. E2F8, a direct target of miR-144, promotes papillary thyroid cancer progression via regulating cell cycle. J Exp Clin Cancer Res. 2017;36:40.
119. Yunhui Q. Effect of microRNA - 373 on proliferation and invasion of breast cancer cells and its mechanism. Chin J Med Sci. 2017;97:603–7.
120. Su Z, Zhang M, Xu M, et al. MicroRNA181c inhibits prostate cancer cell growth and invasion by targeting multiple ERK signaling pathway components. Prostate. 2018;78:343–52.
121. Liu Y, Xu XIN, Xu X, et al. MicroRNA—193a–3p inhibits cell proliferation in prostate cancer by targeting cyclin D1. Oncol Lett. 2017;14:5121–8.
122. Hao P, Kang BO, Yao G, et al. MicroRNA-211 suppresses prostate cancer proliferation by targeting SPARC. Oncol Lett. 2018;15:4323–9.
123. Shin M, Mizokami A, Kim J, et al. Exogenous SPARC suppresses proliferation and migration of prostate cancer by interacting with integrin β1. Prostate. 2013;73:1159–70.
124. Dai H, Wang C. MiR-17 regulates prostate cancer cell proliferation. Cancer Biother Radiopharm. 2018;33:103–9.
125. Guo JU, Xiao Z, Yu X, Cao R. MiR-20b promotes cellular proliferation and migration by directly regulating phosphatase and tensin homolog in prostate cancer. Oncol Lett. 2017;14:6895–900. https://doi.org/10.3892/ol.2017.7041.
126. Yin Y, et al. MiR-671 promotes prostate cancer cell proliferation by targeting tumor suppressor SOX6. Eur J Pharmacol. 2018;823:65–71.
127. Du Y, Wang L, Wu H, Zhang Y, Wang KAN, Wu D. MicroRNA-141 inhibits migration of gastric cancer by targeting zinc finger E-box-binding homeobox 2. Mol Med Rep. 2015;12:3416–22.
128. Chen CL, Tseng YW, Wu JC, Chen GY, Lin KC, Hwang SM, et al. Suppression of hepatocellular carcinoma by baculovirus-mediated expression of long non-coding RNA PTENP1 and MicroRNA regulation. Biomaterials. 2015;44:71–81.
129. Li J, Li J, Wang H, Li X, Wen B, Wang Y. MiR-141-3p promotes prostate cancer cell proliferation through inhibiting

kruppel-like factor-9 expression. Biochem Biophys Res Commun. 2016;482:1381–6.
130. Pickl JMA, Tichy D, Kuryshev VY, Tolstov Y, Schüler J, Reidenbach D, et al. Ago-RIP-Seq identifies polycomb repressive complex I member CBX7 as a major target of miR-375 in prostate cancer progression. Oncotarget. 2016;7:59589–603.
131. Kempkensteffen SHC, Krause FCH, Schostak MSM, Weikert KMS. Expression parameters of the polycomb group urothelial carcinoma of the bladder and their prognostic relevance. Tumor Biol. 2008;29:323–9.
132. Mansueto G, Forzati F, Ferraro A, Pallante P, Bianco M, Esposito F, et al. Identification of a new pathway for tumor progression: microRNA-181b up-regulation and CBX7 down-regulation by HMGA1 protein. Genes Cancer. 2010;46:2304–13.
133. Pallante P, Terracciano L, Carafa V, Schneider S, Zlobec I, Lugli A, et al. The loss of the CBX7 gene expression represents an adverse prognostic marker for survival of colon carcinoma patients. Eur J Cancer. 2010;46:2304–13.
134. Xiao W, Qu C, Qin J, Xing F, Sun Y, Li Z, Qiu J. CBX8, a novel DNA repair protein, promotes tumorigenesis in human esophageal carcinoma. Int J Clin Exp Pathol. 2014;7:4817.
135. Hyup S, Um S, Kim E. CBX8 suppresses sirtinol-induced premature senescence in human breast cancer cells via cooperation with SIRT1. Cancer Lett. 2013;335:397–403.
136. Yang X, Yang Y, Gan R, Zhao L, Li W, Zhou H, et al. Downregulation of miR-221 and miR-222 restrain prostate cancer cell proliferation and migration that is partly mediated by activation of SIRT1. PLoS One. 2014;9:e98833.
137. Lin Z, Fang D. The roles of SIRT1 in cancer. Genes Cancer. 2013;4:97–104.
138. Fu M, Liu M, Sauve AA, Jiao X, Zhang X, Wu X, et al. Hormonal control of androgen receptor function through SIRT1. Mol Cell Biol. 2006;26:8122–35.
139. Xuan H, Xue W, Pan J, Sha J, Dong B, Huang Y. Downregulation of miR 221, 30d, and 15a contributes to pathogenesis of prostate cancer by targeting Bmi 1. Biochemistry. 2015;80:276–83.
140. Chen Y, Zaman MS, Deng G, Majid S, Saini S, Liu J. MicroRNAs 221/222 and genistein-mediated regulation of ARHI tumor suppressor gene in prostate cancer. Cancer Prev Res. 2011;4:76–87.
141. Yang Y, Guo J, Shao Z. MiR-21 targets and inhibits tumor suppressor gene PTEN to promote prostate cancer cell proliferation and invasion: an experimental study. Asian Pac J Trop Med. 2016;10:87–91.
142. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol. 2012;13:283–96.
143. Nistico P, Bissell MJ, Radisky DC. Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. Cold Spring Harb Perspect Biol. 2012;4:a011908.
144. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell. 2008;14:818–29.
145. Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3′ kinase/AKT pathways. Oncogene. 2005;24:7443–54.
146. Cai H. MicroRNA-194 modulates epithelial—mesenchymal transition in human colorectal cancer metastasis. Onco Targets Ther. 2017;10:1269–78.
147. Liu Y, Sun X, Cao X, et al. MicroRNA-217 suppressed epithelial-to- mesenchymal transition in gastric cancer metastasis through targeting PTPN14. Eur Rev Med Pharmacol Sci. 2017;21:1759–67.
148. Zhang J, Liu D, Feng Z, et al. MicroRNA-138 modulates metastasis and EMT in breast cancer cells by targeting vimentin. Biomed Pharmacother. 2016;77:135–41.
149. Williams LV, et al. MiR-200b inhibits prostate cancer EMT, growth and metastasis. PloS One. 2013;8:e83991.
150. Zhao X, Wang Y, Deng R, et al. MiR186 suppresses prostate cancer progression by targeting Twist1. Oncotarget. 2016;7:33136–51.
151. Josson S, Gururajan M, Hu P. MiR-409-3p/-5p promotes tumorigenesis, epithelial-to-mesenchymal transition, and bone metastasis of human prostate cancer metastasis of human prostate cancer. Clin Cancer Res. 2014;20:4636–46.
152. Tang G, et al. MiRNALet-7a mediates prostate cancer PC-3 cell invasion, migration by inducing epithelial-mesenchymal transition through CCR7/MAPK pathway. J Cell Biochem. 2018;119:3725–31.
153. Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E. Simone spaderna TB. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep. 2008;9:582–9.
154. Liu C, Liu R, Zhang D, Deng Q, Liu B, Chao H, et al. MicroRNA-141 suppresses prostate cancer stem cells and metastasis by targeting a cohort of pro-metastasis genes. Nat Commun. 2017;8:1–14.
155. Ambs S, Prueitt RL, Yi M, Hudson RS, Howe TM, Wallace TA, et al. Genomic profiling of microRNA and mRNA reveals deregulated microRNA expression in prostate cancer. Cancer Res. 2009;68:6162–70.
156. Martens-uzunova ES, Jalava SE, Dits NF, Van Leenders GJ, Møller S, Trapman J. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. Oncogene. 2012;31:978–91.
157. Selth LA, Das R, Townley SL, Coutinho I, Hanson AR, Centenera MM, et al. A ZEB1-miR-375-YAP1 pathway regulates epithelial plasticity in prostate cancer. Oncogene. 2016;36:24–34.
158. Hong J, Sun J, Huang T. Increased expression of TRPS1 affects tumor progression and correlates with patients' prognosis of colon cancer. Biomed Res Int. 2013;2013:454085.
159. Zavadil J, Böttinger EP. TGF-β and epithelial-to-mesenchymal transitions. Oncogene. 2005;24:5764–74.
160. Bonkhoff H, Stein U, Remberger K. The proliferative function of basal cells in the normal and hyperplastic human prostate. Prostate. 1994;24:114–8.
161. Lawson DA, Witte ON. Stem cells in prostate cancer initiation and progression. J Clin Invest. 2007;117:2044–50.
162. Mcdonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LWK, Hsieh J, et al. Advances in brief expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. Cancer Res. 1992;52:6940–4.
163. Melamed J, Kernizan S, Walden PD. Expression of B-cell translocation gene 2 protein in normal human tissues. Tissue Cell. 2002;34:28–32.
164. Signoretti S, Pires MM, Lindauer M, Horner JW, Grisanzio C, Dhar S, et al. p63 regulates commitment to the prostate cell lineage. Proc Natl Acad Sci USA. 2005;102:11355–60.
165. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, et al. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol. 2006;157:1769–75.
166. Barbieri CE, Tang LJ, Brown KA, Pietenpol JA. Loss of p63 leads to increased cell migration and up-regulation of genes involved in invasion and metastasis. Cancer Res. 2006;66:7589–98.
167. Boiko AD, Porteous S, Razorenova OV, Krivokrysenko VI, Williams BR, Gudkov AV. A systematic search for downstream mediators of tumor suppressor function of p53 reveals a major

role of BTG2 in suppression of Ras-induced transformation. Genes Dev. 2006;20:236–52.
168. Coppola V, Musumeci M, Patrizii M, Cannistraci A, Addario A. BTG2 loss and miR-21 upregulation contribute to prostate cell transformation by inducing luminal markers expression and epithelial—mesenchymal transition. Oncogene. 2013;32:1843–53.
169. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis. 2000;21:485–95.
170. Wong RSY. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res. 2011;30:87.
171. Maralani M, Neagoe IB. Micrornas as regulators of apoptosis mechanisms in cancer. Clujul Med. 2016;89:50–5.
172. Nakano H, Miyazawa T, Kinoshita K, Yamada Y, Yoshida T. Functional screening identifies a microRNA, miR-491 that induces apoptosis by targeting Bcl-X L in colorectal cancer cells. Int J Cancer. 2010;127:1072–80.
173. Ji F, Zhang H, Wang Y, Li M, Xu W, Kang Y, et al. MicroRNA-133a, downregulated in osteosarcoma, suppresses proliferation and promotes apoptosis by targeting Bcl-xL and Mcl-1. Bone. 2013;56:220–6.
174. Hamada S, Masamune A, Miura S, Satoh K, Shimosegawa T. MiR-365 induces gemcitabine resistance in pancreatic cancer cells by targeting the adaptor protein SHC1 and pro-apoptotic regulator BAX. Cell Signal. 2014;26:179–85.
175. Zhou M, Liu Z, Zhao Y, Ding Y, Liu H, Xi Y, et al. MicroRNA-125b confers the resistance of breast cancer cells to paclitaxel through suppression of pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) expression. J Biol Chem. 2010;285:21496–507.
176. Han G, Fan M, Zhang X. MicroRNA-218 inhibits prostate cancer cell growth and promotes apoptosis by repressing TPD52 expression. Biochem Biophys Res Commun. 2014;456:804–9.
177. Colden M, Dar AA, Saini S, et al. MicroRNA-466 inhibits tumor growth and bone metastasis in prostate cancer by direct regulation of osteogenic transcription factor RUNX2. Cell Death Dis. 2017;8:e2572.
178. Luo Y, Qiu M. MiR-143 induces the apoptosis of prostate cancer LNCap cells by suppressing Bcl-2 expression. Med Sci Monit. 2017;23:359–65.
179. Zhu D, Gao W, Zhang Z. MicroRNA-1180 is associated with growth and apoptosis in prostate cancer via TNF receptor associated factor 1 expression regulation and nuclear factor-κB signaling pathway activation. Oncol Lett. 2018;15:4775–80.
180. Xu H, Mei Q, Shi L, Lu J, Zhao J, Fu Q. Tumor-suppressing effects of miR451 in human osteosarcoma. Cell Biochem Biophys. 2014;69:163–8.
181. Zhao G, Wang B, Liu Y, Zhang J, Deng S, Qin Q, et al. MiRNA-141, downregulated in pancreatic cancer, inhibits cell proliferation and invasion by directly targeting MAP4K4. Mol Cancer Ther. 2013;12:2569–81.
182. Lynch SM, Neill KMO, Mckenna MM, Walsh CP. Regulation of miR-200c and miR-141 by methylation in prostate cancer. Prostate. 2016;76:1146–59.
183. Kneitz B, Krebs M, Kalogirou C, Schubert M, Joniau S, Van Poppel H, et al. Survival in patients with high-risk prostate cancer is predicted by miR-221, which regulates proliferation, apoptosis, and invasion of prostate cancer cells by inhibiting IRF2 and SOCS3. Cancer Res. 2014;74:2591–604.
184. Hatley ME, Patrick DM, Garcia MR, Richardson JA, Duby RB, Van Rooij E, Olson EN. Modulation of K-ras-dependent lung tumorigenesis by microRNA-21. Cancer Cell. 2011;18:282–93.
185. Si M, Zhu S, Wu H, Lu Z, Wu F, Mo Y. MiR-21—mediated tumor growth. Oncogene. 2007;26:2799–803.
186. Folini M, Gandellini P, Longoni N, Profumo V, Callari M, Pennati M, et al. MiR-21: an oncomir on strike in prostate cancer. Mol Cancer. 2010;9:1–12.
187. Yang CH, Pfeffer SR, Sims M, Yue J, Wang Y, Linga VG, et al. The oncogenic microRNA-21 inhibits the tumor suppressive activity of FBXO11 to promote tumorigenesis. J Biol Chem. 2015;290:6037–46.