Review

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miRNAs as novel biomarkers in the management of prostate cancer

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Abstract: microRNAs (miRNAs) are small non-coding RNAs that control gene expression posttranscriptionally and are part of the giant non codifying genoma. Cumulating data suggest that miRNAs are promising potential biomarkers for many diseases, including cancer. Prostate cancer (PCa) detection is currently based in the serum prostate-specific antigen biomarker and digital rectal examination. However, these methods are limited by a low predictive value and the adverse consequences associated with overdiagnosis and overtreatment. New biomarkers that could be used for PCa detection and prognosis are still needed. Recent studies have demonstrated that aberrant expressions of microRNAs are associated with the underlying mechanisms of PCa. This review attempts to extensively summarize the current knowledge of miRNA expression patterns, as well as their targets and involvement in PCa pathogenesis. We focused our review in the value of circulating and urine miRNAs as biomarkers in PCa patients, highlighting the existing discrepancies between different studies, probably associated with the important methodological issues related to their quantitation and normalization. The majority of studies have been performed in serum or plasma, but urine obtained after prostate massage appears as a new way to explore the usefulness of miRNAs. Large screening studies to select a miRNA profile have been completed, but bioinformatics tools appear as a new approach to select miRNAs that are relevant in PCa development. Promising preliminary results were published concerning miR-141, miR-375 and miR-21, but larger and prospective studies using standardized methodology are necessary to define the value of miRNAs in the detection and prognosis of PCa.

Keywords: biomarkers; miRNAs; miR-21; miR-141; miR-375; prostate cancer.

Introduction

New biomarkers have been described during recent years for the management of early prostate cancer (PCa), among them the Prostate Health Index, PCA3 score and the four-kallikrein panel [1–3]. Emerging biomarkers should improve the specificity of prostate specific antigen (PSA) in the detection of PCa, showing a relationship with the aggressiveness of the tumor. Currently, PSA remains the most used biomarker in the detection of PCa despite the controversies around its use as a screening tool. Actually, findings show that the screening based on PSA levels results in a higher incidence of low risk PCa, most of which do require no treatment.

Overdiagnosis and overtreatment has become a major problem in the management of PCa. Because of this fact, active surveillance (AS) has been proposed to palliate these negative effects, delaying any kind of definitive treatment [4]. The selection of patients for AS combines clinical stage, PSA serum levels, Gleason score, the number of positive prostate biopsies and the amount of malignant tissue per core. However, even when these criteria are strictly used, inaccuracies are found [5]. Novel tools are necessary to improve patients selection for AS.

Cumulating data suggest that microRNAs (miRNAs) can be used as potential biomarkers for the detection of PCa, as well as in the selection of patients for AS due to their relationship with the aggressiveness of the tumor. The aim of this paper is to present a systematic review about the role of miRNAs as biomarkers in PCa.

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Biogenesis and function of miRNAs

The involvement of protein coding genes in the development of cancer has been extensively studied in recent years. Although 90% of the human genome is transcribed to messenger RNA (mRNA), only 2% encodes for proteins, so there is a large number of RNAs that encodes for no protein. Recent data suggest that this portion of the genome that does not encode for protein also plays a role in carcinogenesis. These non-coding RNAs include small nucleolar RNA, miRNA, small interfering RNA, piwi-interacting RNA and long non-coding RNA [6], which for example includes *PTENP1*, a pseudogene of the tumor suppressor *PTEN*, and *PCA3*, proposed as a useful biomarker in the detection of PCa [7, 8].

miRNAs are small (17–22 nucleotides) single-stranded non-coding RNAs, that negatively regulate the gene expression through the binding to their corresponding mRNA targets. The first miRNA described was lin-4, identified in 1993 by Lee et al. [9] in the nematode *Caenorhabditis elegans*. Currently, according to the database mirbase.org, 28,645 miRNAs have been identified in different species (miRBase, release 21: June 2014) [10], of which 2588 mature miRNAs have been recognized in humans.

Biogenesis of miRNAs (Figure 1) is a complex process that begins in the cell nucleus with the formation of the double-stranded primary miRNA (pri-miRNA), by the action of RNA polymerase II. Then, the pri-miRNAs are processed by a microprocessor that contains the Drosha RNAse III enzyme and the DGCR8 protein, and allows the formation of the precursor miRNA (pre-miRNA). After that, the pre-miRNA is transferred to the cytoplasm by exportin 5 and Ran GTP cofactor, where the pre-miRNA is processed by Dicer RNAse, forming a double-stranded RNA of approximately 22 nucleotides, which contains two strands of miRNA: one becomes the mature miRNA and the other one the passenger miRNA or 5p miRNA, which is normally degraded. The mature miRNA binds to the Argonaut protein (AGO) and forms the RNA-induced silencing complex (RISC). After being incorporated into the RISC complex, miRNAs can exercise the silencing function on their corresponding mRNA targets.

Extracellular miRNAs are heterogeneous, including miRNAs packaged in microparticles (exosomes,

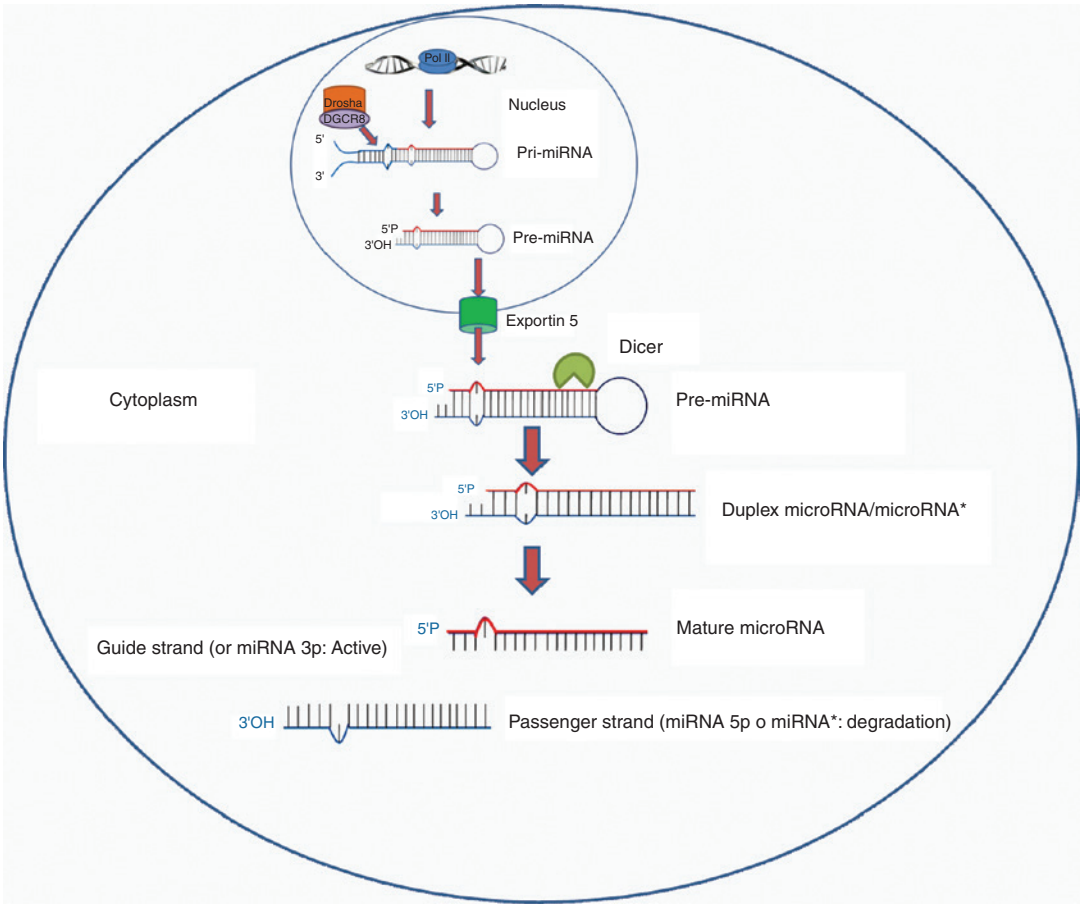

Figure 1: Schematic representation of miRNA biosynthesis.

microvesicles and apoptotic bodies) and miRNAs linked to RNA binding proteins (AGO-2, nucleophosmin-1) and to high-density lipoproteins to prevent their degradation. Apoptotic bodies are the largest microparticles (0.5–2 μm) and are produced from cells during apoptosis. Microvesicles are heterogeneous in size (0.1–1 μm) and they are released from all cell types by different mechanisms. Finally, exosomes are small vesicles (50–100 nm) that originate from the endosome. Results are confusing about the proportion between exosomal and non-exosomal miRNAs. Arroyo et al. [11], using differential centrifugation and size-exclusion chromatography to characterize circulating miRNA complexes in human plasma and serum, concluded that the vesicle associated miRNAs represents the minority, whereas around 90% of miRNAs in the circulation is present in a non-membrane-bound form. Additionally, Dijkstra et al. [12] compared *PCA3* and *TMPRSS2-ERG* expression levels in urinary sediments and exosomes and concluded that in exosomes these biomarkers had lower analytical sensitivity, although exosomes seem to be a more robust source of urinary biomarker. On the other hand, according to Gallo et al. [13] exosome isolation improves the sensitivity of miRNA from human biological fluids, including serum and saliva. Moreover, Cheng et al. [14] showed that in urine the highest proportion of miRNA was extracted from exosomes compared with the cell pellet and cell free supernatant.

Advances in technology together with the stability of miRNAs in different kind of samples [15] have allowed to identify the role of miRNAs in several diseases [16]. Specific miRNAs profiles of different cancers can be identified using microarrays. qRT-PCR has been generally used to validate the miRNAs selected by microarrays. The development of digital PCR will improve the performance of qRT-PCR, without the use of endogenous or exogenous controls to normalize the results. In contrast, digital PCR is a direct method for quantifying nucleic acids, particularly useful for samples with low abundant miRNAs, and showing a high degree of sensitivity and precision compared to qRT-PCR [17, 18]. Finally, next generation sequencing (NGS) platforms, such as those from Solexa/Illumina and 454 Life Sciences/Roche, have been developed to find novel miRNAs, generating a complete expression profile, distinguishing sequentially similar miRNAs and identifying point mutations.

miRNAs and cancer

In 2002, Calin et al. [19] demonstrated that a miRNA cluster was frequently deleted or downregulated in chronic lymphocytic leukemia. Subsequently, several miRNAs expression and functional studies showed that miRNAs may be associated in cancer initiation and progression. Abnormal expression of miRNAs in cancer is related to different mechanisms, including genomic mutations, epigenetic changes, chromosomal abnormalities and alterations in miRNA biogenesis, i.e. the altered expression of the miRNA processing endoribonuclease Dicer [20].

Currently, different miRNAs have been involved in human carcinogenesis by acting as “oncomirs” or “tumor suppressive miRNAs” [16], inhibiting suppressor tumoral genes or activating oncogenes, respectively. The study of aberrant miRNA expression profiles in cancer is, in fact, an innovative way to identify new biomarkers for cancer detection and prognosis. An exponential growth of studies has been published since the first circulating miRNA profile was reported by Lawrie et al. [21] in 2008 as a diagnostic tool in oncology. The discovery of miRNAs in other body fluids, including urine, saliva and bronchial lavage, has intensified investigations about the use of miRNAs as non-invasive biomarkers in patients with cancer [15].

Interestingly, according to available results, miRNAs signatures are able to classify human cancer origin [22]. The highly tissue specific origin of miRNAs leads to an efficient identification of metastatic cancers of unknown primary origin. Søkilde et al. [23] reported in a series of 208 tumors, representing 15 different histologies including PCa, that miRNA expression profiling on paraffin tissue can efficiently predict the primary origin of the tumor. The authors developed a new miRNAs classification algorithm, obtaining an overall accuracy of 85% (CI: 79%–89%). When the algorithm was applied to an independent test set of 48 metastases, the primary site was correctly identified in 42 cases (88% accuracy; CI, 75%–94%). Additional studies are necessary to confirm this point.

Preanalytical and analytical variables in miRNA measurement

Several preanalytical factors can influence the levels of miRNAs, including procedures of sample collection, specimen acquisition, handling and storage [24]. Differences in circulating miRNAs levels have been observed between plasma and serum. Wang et al. [25] described higher levels of miRNAs in serum than in plasma, suggesting that additional miRNAs would be produced during the coagulation process. Also, the different kind of anticoagulants used can influence the results. Thus, Kim et al. [26] observed that the levels of miR-16 and miR-223

are lower when heparin is used and higher with oxalate, while EDTA provides similar results to serum. Hemolysis is also a factor that affects the analysis of some miRNAs, including miR-16, miR-451, miR-92a and miR-486-5p that are released by erythrocytes [27, 28]. Recommendations to analyze miRNAs in plasma include platelet removal, while serum separator tubes are recommended to work with serum. Hemolysis can be assessed by visual inspection or using a spectrophotometer at 540 nm. Furthermore, it can be checked measuring the ratio between miR-451 and miR-23a, a highly expressed and stable serum miRNA. A ratio higher than five is an indicative of possible hemolysis contamination, while a ratio higher than eight indicates a high risk of hemolysis affecting thus the obtained data [29].

Time between sample extraction and the aliquotation of plasma or serum also affects results, although according to the majority of authors, miRNAs are stable biomarkers. Mitchell et al. [30] reported that miRNA levels remain stable when plasma is subjected to prolonged room temperature incubation or freeze-thawed multiple times. Besides, McDonald et al. [31] showed that miRNAs were stable at room temperature for 24 h and refrigerated or frozen for up to 72 h. However, Rice et al. [32], obtaining plasma at different time points, showed that time to plasma extraction influences ΔCt values in 5 of 11 miRNAs tested. Based on these results, the authors recommend plasma extraction before 12 h.

There are also differences in the results obtained in relation to the method used for isolating total RNA from different body fluids (mirVana, Exiqon miRCURY, miRNeasy, TRIzol) [14, 33–35]. Unfortunately, results published in these studies do not always support similar conclusions. According to McAlexander et al. [33] Exiqon miRCURY Biofluids Kit outperforms other RNA isolation methods tested working with plasma, including Exiqon Cell and Plant kit, TRIzol, mirVana and miRNeasy. In contrast, Kroh et al. [34] reported that Qiagen miRNeasy kit produces 2–3 fold greater RNA than mirVana kit. On the other hand, Cheng et al. [14] compared six commercially available kits for total RNA extraction from the cell pellets, cell-free urine, and exosomes from urine, including miRNeasy, miRNeasy with RNeasy MinElute Cleanup Kit, mirVana, TRIzol reagent with mirVana, miRCURY, and Urine Exosome RNA Isolation kit. miRNeasy kit using the additional RNeasy MinElute was the most efficient reagent to isolate miRNA from exosomes and cell-free urine, according to these authors, while miRCURY was the most efficient in cell pellets.

Evaluation of miRNA requires normalization to minimize data variation that can mask or exaggerate biologically meaningful changes. Variation in the measurement of miRNAs among experiments can be attributed to different sources, such as the RNA isolation, the quantity and quality of RNA, or the efficiency of the reverse transcription and qRT-PCR. Normalization of results is essential to obtain homogeneous results between experiments and convert raw data into valid results. Different strategies have been described to normalize miRNA expression levels, including the normalization to the average Cq value of all measured miRNA assays, the normalization to an endogenous miRNA, and the normalization to an external spike-in oligoRNA added during the RNA extraction. The normalization to the average Cq value, also called the mean centering method, is restricted to studies which assay a large number of miRNAs [36]. One variation of this method is the mean centering restricted method, which has been developed for experiments where a substantial fraction of miRNA data values is missing, using the mean obtained of only the miRNAs expressed across all samples [37].

Several protocols for the normalization using endogenous miRNAs controls have been implemented. The ideal normalizer should be independent of biological variation, disease stage or treatments, showing similar storage stability, extraction properties and quantification efficiency compared to the miRNA target. Stable small non-coding RNAs are often used as reference genes to quantify cellular miRNAs, for instance, the small nucleolar RNAs, like SNORD43, SNORD44, SNORD48 and Mamm U6. Also, some miRNAs have been used as reference genes, like let-7a-1 or miR-130b. Several algorithms, particularly geNorm and NormFinder software, can be used to select the most stable reference material from a list of candidates. GeNorm and NormFinder software are application tools for Microsoft Excel that offer the stability value based on the intra- and intergroup expression variations of the reference genes studied. A low combined intra- and intergroup variation proves high expression stability. Nonetheless, this ideal normalizer, particularly working with plasma/serum or other body fluids, does not exist [38] and the use of endogenous reference genes is controversial.

Actually, there are no validated reference controls for the analysis of the expression of miRNAs in PCa, showing the published studies contradictory results. Sanders et al. [39] proposed SNORD43 as a suitable reference gene for the analysis of circulating miRNAs in patients with urological malignancies, reporting a high stability, although the stability of RNU6-2, RNU1-4, miR-106a, let-7a and SNORD48 was also remarkably high according to these authors. On the other hand, Schaefer et al. [40] recommended the use of miR-130b or the geometric mean of

miR-130b and RNU6-2 to normalize miRNA expression in PCa tissues studies. However, according to Carlsson et al. [41], RNU24 and RNU44 would be suitable control genes for miRNA expression studies in prostate tissues, but not miR-130b, because its stability was not appropriate to be a normalizer control.

Quantifying miRNAs in plasma/serum or other body fluids using endogenous controls is particularly difficult since invariant reference control has not been found. The use of a spike-in exogenous control appears as a solution to this problem [42]. The addition of an exogenous synthetic miRNA (e.g. the derived *C. elegans* cel-miR-39, cel-miR-54 and cel-miR-238, or the *Arabidopsis thaliana* ath-miR-159a) in the phenolic phase allows to monitorize the efficiency of the reverse transcription and qRT-PCR reactions. Mitchell et al. [30] proposed the combined use of cel-miR-39, cel-miR-54 and cel-miR-238 to quantify circulating miRNAs in plasma. However, Sourvinou et al. [43] concluded that results improve when normalization is based on combined exogenous (cel-miR-39) and endogenous (miR-16) miRNAs controls, because differences in miRNA recovery and differences in cDNA synthesis between samples are compensated. In contrast, McDonald et al. [31] showed that imprecision was higher using the endogenous control miR-16 than using exogenous cel-miR-39, because miR-16 is susceptible to hemolysis. According to these authors, results did not improve when the exogenous controls cel-miR-54 and cel-miR-238 to cel-miR-39 were added. Exogenous miRNAs controls, such as ath-miR-159a, cel-miR-54, cel-miR-238 and particularly cel-miR-39, appear as the optimal way to normalize miRNAs expression in absence of an ideal endogenous control [44, 45], although biological variability should be taken into account to improve miRNAs measurement.

Role of miRNAs in prostate cancer

Altered miRNA expression in PCa has been reported by different authors, showing their deregulation in several processes, including cell proliferation, differentiation and progression [46–48]. The miRNAs involved in PCa pathogenesis and their signaling pathways are shown in Figure 2. Deregulation of the cell cycle leads to aberrant cell proliferation that characterizes cancer. Usually, miRNAs interact with cell cycle proteins, conferring thus the potential to regulate cellular division and cell cycle progression. For example, Lewis et al. [49] reported that miR-888 inhibits the target retinoblastoma-like 1, which

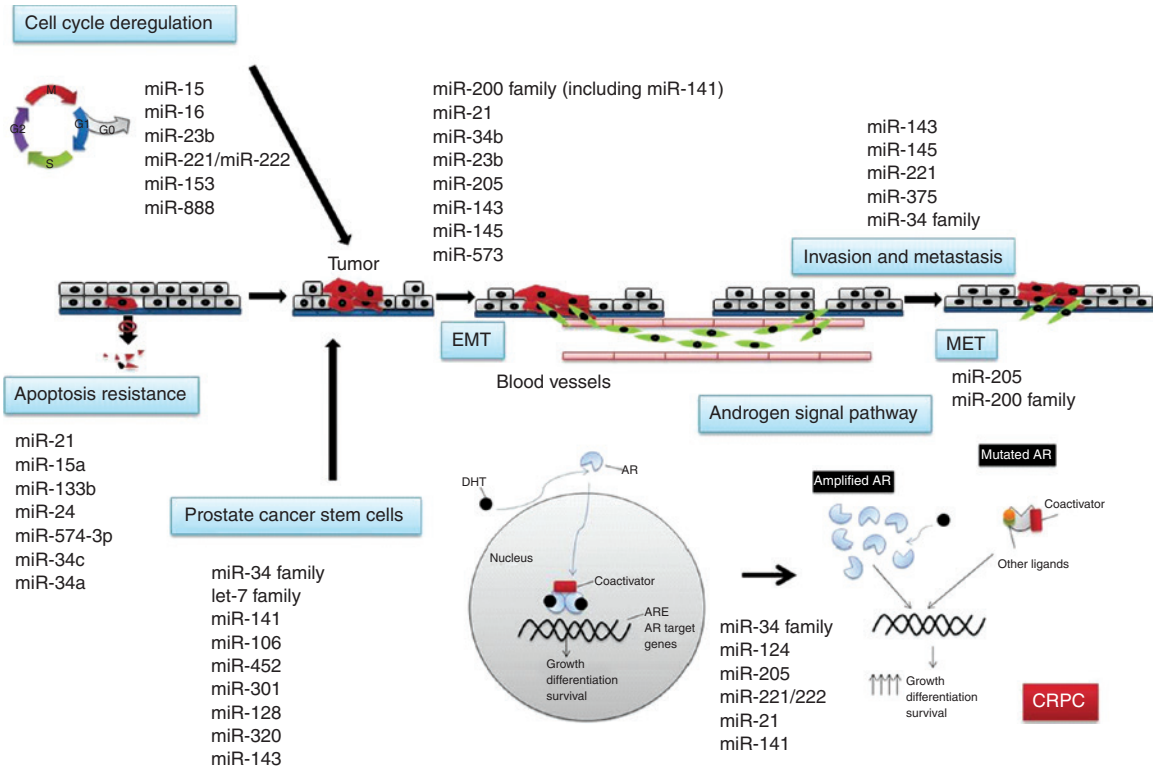

Figure 2: Role of miRNAs in PCa pathogenesis.

blocks first gap phase to synthetic phase cell cycle progression by binding and inhibiting the E2F transcription factors.

Cancer stem cells (CSC) are a subset of cancer cell subpopulations in the tumor mass and play an important role in tumor progression and metastasis in several types of cancers, including PCa. PCa stem cells keep the self-renewal and differentiation properties and can lead to more cancer cells. miRNAs are involved in promoting or inhibiting the stemness of CSCs. Aberrantly expressed miRNAs can cause deregulation of specific signaling pathways that are associated with the functions of CSCs. Particularly, miR-34 family or let-7 family [50, 51] have been involved in the regulation of PCa CSC properties.

miRNAs have been also implicated in the resistance to apoptosis in PCa through different mechanisms. miR-21 is usually upregulated in PCa and directly targets *PTEN*, whose downregulation results in significantly reduced apoptosis in PCa. miR-21 also regulates the myristoylated alanine-rich protein kinase c substrate (*MARCKS*), conferring apoptosis resistance and leading to aberrant proliferation [52, 53] (Figure 3). Another mechanism of regulating apoptosis is through targeting B-cell lymphoma 2 (Bcl-2) family members; for instance some miR-34 family members or the tumor suppressor miR-205, which directly target *Bcl-2* in PCa [54, 55].

The epithelial-mesenchymal transition (EMT) is a biologic process that allows a polarized epithelial cell to submit to multiple biochemical changes to assume a mesenchymal cell phenotype, which confers enhanced migratory capacity, invasiveness and stem cell properties [56]. PCa cells are subjected to EMT, then invade surrounding tissues and colonize several tissues via blood or lymphatic routes to generate metastases. Metastatic cells can then revert through mesenchymal-epithelial transition (MET) to reacquire epithelial characteristics similar to cells in the primary tumor. Some miRNAs are regulators of EMT and MET events through the regulation of the expression of key proteins of these processes. miRNA-200 family members (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) play a critical role in the regulation of the EMT process by targeting and repressing mesenchymal

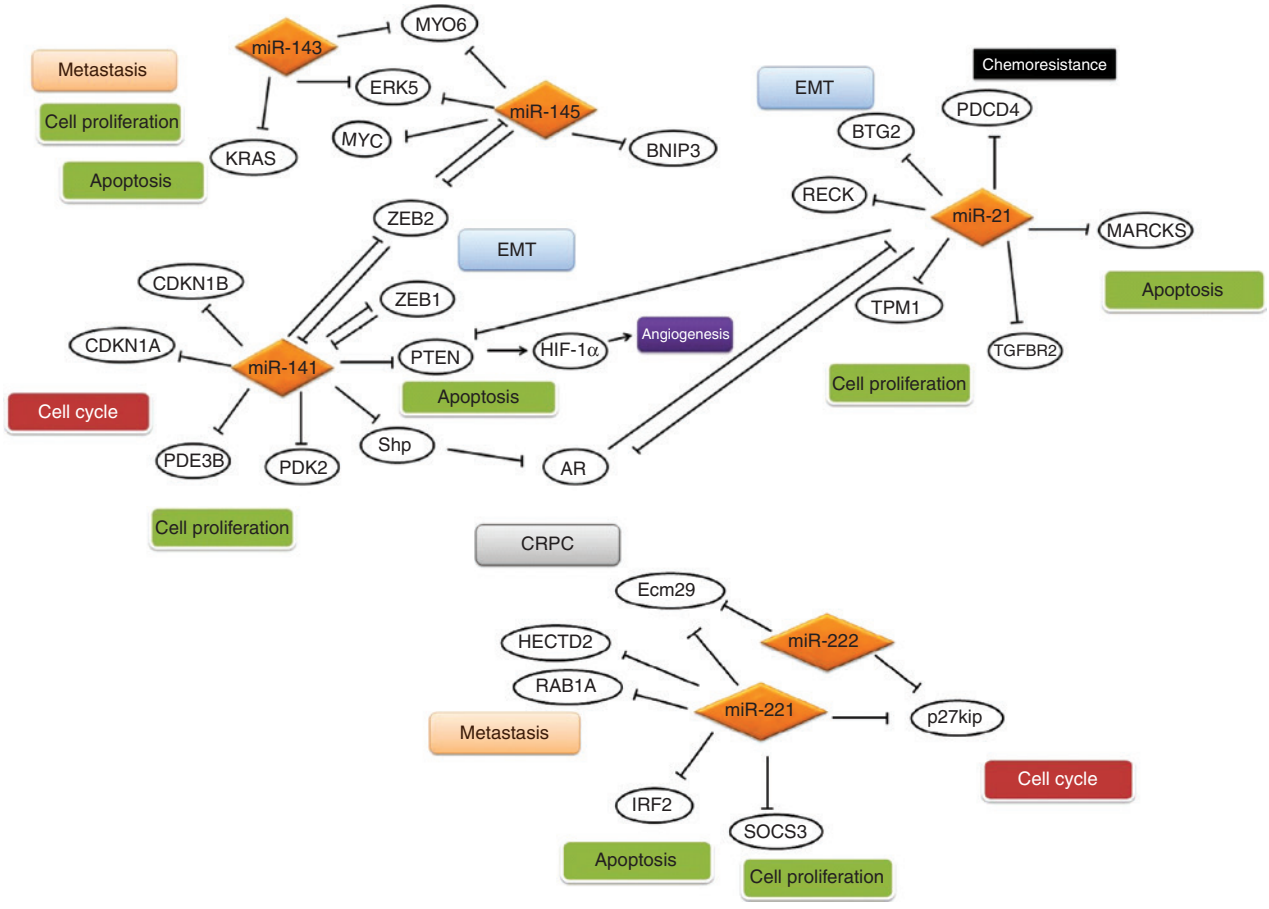

Figure 3: miR-21, miR-141, miR-143, miR-145, miR-221 and miR-222 in PCa pathogenesis: summary of their roles and interactions with targets.

markers, such as vimentin, and up-regulating epithelial markers, such as E-cadherin, by directly targeting *ZEB1* and *ZEB2*, which is a direct target of miR-145, too [57, 58] (Figure 3). Ren et al. [59] reported that *ZEB2* also represses the transcription of miR-145, creating a double-negative feedback loop between *ZEB2* and miR-145, which is a key in the control of EMT and stem cell properties during the bone metastasis of PCa. Besides, upregulation of miR-21 in PCa also plays an important role in EMT (Figure 3) by decreasing the *BTG2* levels, initiating thus the acquisition of luminal markers and EMT [60, 61]. Another invasion-related gene regulated by miR-21 is *RECK*, a matrix metalloproteinase inhibitor. Neutralizing miR-21 represses the matrix metalloproteinase levels and reverses the invasive phenotype [62]. Recently, Wang et al. [63] found that miR-573 expression was significantly lower in metastatic tissues than matched primary PCa. They demonstrated that miR-573 modulates EMT through the direct target of fibroblast growth factor receptor 1 (*FGFR1*) gene, promoting the metastasis of PCa cells, and suggested miR-573 as a potential biomarker and/or therapeutic target for PCa management.

Androgen signaling through the androgen receptor (*AR*) is an important oncogenic pathway for PCa progression. Patients eventually acquire resistance and progress to castration resistant PCa (CRPC). Furthermore, the interactions of miRNAs with AR play a determinant role in the progression from castration sensitive PCa to an incurable CRPC [64]. Several miRNAs (miR-21, miR-31, miR-34 and miR-124) can regulate the androgen receptor expression, and simultaneously, *AR* can regulate the expression of several miRNAs (miR-21, miR-27a, miR-34, miR-125b, miR-221 and let-7) [65]. Goto et al. [66] recently demonstrated that miR-221 and 222 were significantly downregulated in CRPC specimens, even when this cluster was previously described to be upregulated (Figure 3) [67, 68]. This fact shows the dynamic status of miRNAs in the development of PCa, regulating the same miRNA different targets depending on the point of the cancer progression. Loss of the tumor-suppressive miR-221/222 cluster enhanced migration and invasion in PCa cells targeting *Ecm29*, whose precise mechanisms in cancer cells remain unknown but it is involved in cancer cells invasion. Ottman et al. [69], comparing androgen sensitive and resistant PCa cell lines, identified 43 miRNAs that are significantly altered during progression of PCa cells to treatment resistance, suggesting their involvement in the development of CRPC. Specific changes in miRNA expression in CRPC patients could be used as a new predictive tool for monitoring the susceptibility of development of CRPC. Additionally, new therapeutic strategies based on the use of miRNA as drugs or drug targets could be explored in the future for the management of CRPC patients.

Functional enrichment resources for miRNA analysis

In order to understand the effects of miRNAs on the regulation of gene expression it is crucial to predict the targets of miRNAs. In this way, multiple bioinformatic tools have been developed to predict possible targets of miRNAs and their functions or their implication in signal pathways, because in vitro validation of all the possibilities of miRNA-targets regulation would be too costly and time consuming. So it is necessary for scientists to provide some criteria to hierarchize trials, then validate in vitro the predicted networks and discover novel biomarkers related with PCa aggressiveness [70, 71].

The tools to predict miRNAs targets are based on certain assumptions, such as the base complementarity in the 3′UTR, thermodynamic stability, target-site accessibility, and evolutionary conservation of miRNA binding sites. PicTar [72] is a combinatorial miRNA target prediction tool, which allows to search miRNA targets on mRNA transcripts according to the alignments of 3′UTRs matching to miRNA seeds and their thermodynamic stabilities. PicTar minimizes false positive results by using sequence alignment to eight vertebrate species and the candidate genes of each species are scored to create a combined score for a gene. TargetScan [73–75] is a web server similar to PicTar, but it also provides conserved targeting sites in the open reading frames of vertebrate genomes. TargetScan only considers stringent seeds, and ignores many potential targets. The intersection of PicTar and TargetScan predictions is recommended in order to achieve both high sensitivity and high specificity.

miRanda [76, 77] was one of the first miRNA target prediction algorithms and one of the most heavily used for target prediction by multiple interfaces, including microRNA.org and MicroCosm. The algorithm ranks the likelihood of each gene to be a miRNA target and the likelihood of each miRNA to target a gene. miRanda can predict more target sites and detect non-conserved seed match because it allows occasional mismatches and G:U base-pairs.

Enrichment analysis of miRNA targets is a statistical method to identify functional modular changes and to elucidate underlying functional mechanisms. After predicting the genes targeted by selected miRNAs using the aforementioned tools, the next step for enrichment analysis is annotating target genes for their participation

in pathways and processes. Different pathways are commonly used for this step. Gene ontology (GO) [78] annotates genes to biological/cellular/molecular terms in a hierarchically structured way, whereas Kyoto encyclopedia of genes and genomes (KEGG) [79] assigns genes to functional pathways. The final step in enrichment analysis is the statistical testing for over-representation of a biological process in the set of targeted genes. The hypergeometric distribution or Fisher's exact test is used to test for enrichment.

Baglioni et al. [80] using an enrichment analysis strategy developed the miRable method to identify new potentially relevant genes and their interaction networks associate to a specific pathology. miRable combines medical knowledge and gene expression data to find new interesting protein-coding genes and miRNAs involved in the disease. The method predicted some genes that have been already studied and others less known in relation to PCa. The three new predicted genes were estrogen receptor 1 (*ESR1*), miR-548c-3p and miR-494. The role of *ESR1*, a potential candidate target of miR-182, in PCa has not been investigated adequately although a high number of similarities between the *AR* and *ESR1* signaling pathways are known.

Likewise, Zhang et al. [81] identified 39 novel miRNAs candidates as PCa biomarkers analyzing Gene Expression Omnibus datasets for PCa and benign prostatic hyperplasia (BPH) using bioinformatics tools, considering the regulatory power of miRNAs. Results were verified by qRT-PCR concluding that miRNA-648 could be a novel biomarker of PCa. The authors underlined the implication of miRNA-648 in PCa progression, predicting 10 target genes, including eight that have not been previously described in PCa. Using a similar procedure, Feng et al. [82] found the overexpression of miR-31, proposing this biomarker to distinguish PCa tissues from benign tissues.

miRNA expression in PCa tissue

The first miRNA expression study in PCa tissue was reported by Volinia et al. [83] analyzing 228 miRNAs in 56 PCa tissues and six normal prostate tissues. The authors reported that 39 miRNAs were upregulated, while six were downregulated. In contrast, Porkka et al. [84] comparing 319 miRNAs in nine PCa and four BPH tissues showed that eight miRNAs were upregulated and 22 were downregulated. More recently, Carlsson et al. [85], evaluating 667 miRNA, found nine miRNAs that consistently differ between malignant prostate tissues and the adjacent normal tissue from each case. Srivastava et al. [86] showed that miR-205, miR-214, miR-221 and miR-99b were significantly downregulated in PCa tissues compared to adjacent normal tissue. Areas under the curve (AUCs) obtained by receiver operating characteristics were 0.83, 0.92, 0.75 and 0.86, respectively.

Interestingly, Hellwinkel et al. [87] reported seven miRNAs differentially expressed in normal prostate tissue from patients with PCa compared with normal prostate tissue from patients with negative biopsy. The content of four of these miRNAs (miR-185, miR-16, let-7a and let-7b) in normal prostate tissue remained significantly different comparing patients with PCa and patients with negative biopsy and high PSA levels. The authors suggested that the identification of these cancer-typical miRNA patterns in unsuspicious prostate tissue could lead to the assessment of PCa risk by the evaluation of apparently tumor-free biopsy specimens. Finally, Schaefer et al. [40] identified 15 differently expressed miRNAs comparing 470 miRNAs in matched tumor and adjacent normal tissues obtained after radical prostatectomy. Table 1 shows results regarding miRNAs expression in PCa tissue.

Circulating miRNAs in prostate cancer

In 2008, Mitchell et al. [30] demonstrated that miRNAs originated in human PCa xenografts enter into the circulation and can be identified in plasma. Furthermore, the authors reported that miR-141 was significantly higher in patients with advanced PCa than in the matched controls, showing an AUC of 0.907. This study was the first to report the presence of miRNAs in the serum of PCa patients. More recently, other studies have shown the usefulness of circulating miRNAs in the detection and prognosis of PCa, demonstrating their presence in serum and plasma as well as in exosomes and in blood mononuclear cells.

Several studies have identified specific circulating miRNA signatures for PCa detection and prognosis using microarrays. Differences between panels are substantial and only miR-141, miR-375 and miR-21 appear repeatedly. Therefore, the selected panels differ substantially, probably due to the various types of sample and platforms used, the age of specimens, the small patient size studied, ethnic differences between series, and the different balance between local and advance PCa in every study. These differences are also obvious in the miRNAs suggested as PCa biomarkers by studies based on qRT-PCR. In consequence, while 74 miRNAs have been suggested

Table 1: Expression of miRNAs in tissue.

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Patients
Volinia et al. [83]	228 miRNAs	let-7d, let-7i, miR-101-1 prec, -106a, -124a-1, -135-2, -146, -148, -16-1, -17-5p, -181b-1, -181b-1 prec, -184 prec, -187, -191, -195, -196-1, -196-1, -197, -198, -199a-1, -199a-2, -203, -206, -20a, -21, -214, -223, -25, -26a-1, -27a, -29a, -29b-2, -30c, -32, -34a, -92-2, -93-1, -95	let-7a-2 prec, miR-128a prec, -218-2, -29a prec, -149, -24-1	56 PCa and six normal prostatic tissues
Porkka et al. [84]	319 miRNAs	miR-202, -210, -296, -320, -370, -373*, -498, -503	let-7a, let-7b, let-7c, let-7d, let-7g, miR-16, -23a, 23b, 26a, -92, 99a, -103, -125a, 125b, -143, -145, -195, -199a, -199a*, -221, -222, -497	Nine PCa and four BPH tissues
Schaefer et al. [40]	470 miRNAs	miR-375, -182, -96, -183, -182*	miR-16, -31, -149, -181b, -221, -222, -205, -125b, -145, -184	76 Matched tumor and adjacent normal tissues obtained after radical prostatectomy
Carlsson et al. [41]	667 miRNA	miR622, -30d, -425*, -342-3p	miR126*, -34a*, -195, -26a, -29a*	19 PCa and 19 normal tissues
Hellwinkel et al. [87]	154 miRNAs	–	miR-124a, miR-146a & b, miR-185, miR-16, let-7a, let-7b	31 Normal tissue from PCa patients, 17 normal tissues from patients with negative biopsy and low PSA levels and 14 normal tissues from patients with negative biopsy and high PSA levels
Srivastava et al. [86]	32 miRNAs	miR-367*, -758*, -190*	miR-221*, -205*, -212*, -99b*, -214*, miR-203, -127-3p, -130a, -335, -376, -10a, -589, -422a, -10b, -25, -210, -99a, -429, -92a, -100, -222, -484, -125b, -574-3p, -328, -483-5p, -331-3p, -135a, let-7c	40 Formalin-fixed, paraffin-embedded tissue specimen blocks, comparing PCa tissues to adjacent normal tissue

as circulating biomarker candidates for PCa, only 25 of these miRNAs have been congruently associated with PCa in more than one study (Figure 4). Remarkably, 11 studies suggested the value of miR-141 and miR-375, while 10 studies suggested the utility of miR-21. Discordant results have been published for six miRNAs. Finally, no clinical value for miRNAs was reported in two studies [39, 88].

Chen et al. [89] initially evaluated 1146 miRNAs in the plasma from 25 PCa (15 nonmetastatic, 10 metastatic) and 17 BPH patients, identifying a panel of five miRNAs (miR-622, miR-1285, let-7e, let-7c, and miR-30c) that could differentiate PCa from BPH and from healthy controls with high accuracy, showing AUCs of 0.924 and 0.860, respectively. Additionally, accuracy increased combining miRNAs with routine PSA test. miR-30c was especially useful in patients with PSA ≥4 µg/L, with an AUC of 0.908, whereas the AUC was of 0.509 for patients with PSA <4 µg/L. Similarly, the diagnostic performance of let-7e was better in patients with PSA <4 µg/L, reporting an AUC of 0.969, while the AUC was 0.773 when PSA was ≥4 µg/L.

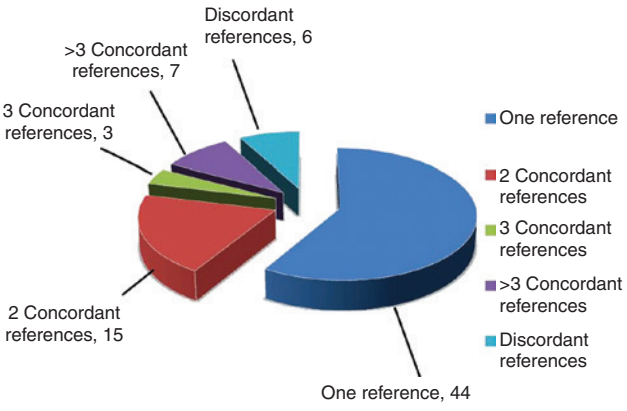

Figure 4: Reported concordant and discordant results on circulating miRNAs.

Diagnostic value of miRNAs in serum was confirmed by Srivastava et al. [90] selecting a signature of three miRNAs (miR-25, miR-101 and miR-628-5p) from a profile analysis of 667 miRNAs. The validation of selected miRNAs demonstrated a good performance, discriminating PCa patients and healthy age matched controls. AUCs were of 0.66, 0.80 and 0.94 for miR-25, miR-101 and miR-628-5p, respectively.

Moltzahn et al. [91] identified in serum a panel of 12 miRNAs from a 384 miRNAs microarray. The validation study found AUCs from 0.812 to 0.928 for miR-106a, miR-1274, miR-93, miR-223, miR-874 and miR-1207, showing a consistent relationship with CAPRA score, a risk assessment score based on patient age, PSA serum level, clinical tumor stage, Gleason score and percentage of positive biopsy cores. The prognostic value of miRNAs was also demonstrated by Nguyen et al. [92], studying the differential expression of 669 miRNAs in 84 serum samples from patients with PCa. The authors showed that miR-375, miR-378* and miR-141 had an increasing trend with disease progression, while miR-409-3p increased in the high-risk group compared with those in the low-risk group, but significantly declined in the metastatic CRPC, which could be in relation with the androgen deprivation therapy.

This study also demonstrated that miR-375 and miR-141 were significantly expressed in prostate tumor tissues, but not in peripheral blood mononuclear cells, suggesting that their increased expression in serum of advanced PCa patients may result from the selective exocytosis of these miRNAs from PCa cells into the circulation system. This hypothesis was confirmed by Cheng et al. [93] validating the significance of a miRNA panel based on the analysis of 365 miRNAs in serum, comparing metastatic CRPC and age-matched healthy controls. A signature composed by miR-141, miR-200a, miR-200c, miR-375 and miR-210 was significantly expressed in metastatic CRPC patients. The authors found that these miRNAs were also overexpressed in PCa tissue as well as in lymph metastases, suggesting that circulating miRNAs could be plausibly cancer cell-derived.

Watahiki et al. [94] analyzed 742 miRNAs in plasma samples from 50 PCa patients. Eight miRNAs were significantly upregulated in metastatic CRPC patients compared with those with localized PCa, while two were downregulated. The authors proposed a panel composed by miR-141, miR-151-3p and miR-16, showing an AUC of 0.944. Also, the authors described that a panel including miR-141, 151-3p, 152 and 423-3p was associated with a poorer outcome and/or a higher Gleason score. Additionally, miR-141 and miR-152 identified patients with a high probability of recurrence after radical prostatectomy.

At the moment, the widest study about the clinical usefulness of circulating miRNAs has been performed by Mihelich et al. [95], measuring the levels of 21 miRNAs in 50 BPH patients and 100 PCa patients in stages T1–T2, classified according to the Gleason score. The authors proposed a miRNA signature (let7a, miR-103, miR-451, miR-24, miR-26b, miR-30c, miR-93, miR-106a, miR-223, miR-874, miR-146a, miR-125b, miR-100, miR-107 and miR-130b) to predict with high accuracy the absence of high-grade PCa among the BPH and low-grade PCa patients. Seven of these miRNAs (miR-451, miR-106a, miR-223, miR-107, miR-130b, let-7a and miR-26b) were also significantly lower in PCa patients with biochemical recurrence after radical prostatectomy compared with those without biochemical recurrence. Interestingly, according to a logistic regression analysis, both miRNAs signatures were better predictors of high Gleason score or biochemical recurrence than other presurgical factors, including PSA, age and percentage of positive cores. Similarly, Shen et al. [96] found a plasma miRNA signature composed by miR-20a, miR-21, miR-145 and miR-221 that predicted the aggressiveness of PCa, distinguishing low and high-risk PCa patients according to D'Amico criteria, with an AUC of 0.824. However, the panels proposed for these groups do not overlap in miRNAs composition. On the other hand, downregulated results reported by Mihelich et al. [95] concerning miR-93, miR-106a, miR-107, miR-130b and miR-451 were discordant with those reported in the studies made by Moltzahn et al. [91], Li et al. [97] and Bryant et al. [98], which indicated that these miRNAs were upregulated in PCa patients.

Candidates for AS include patients with a biopsy Gleason score of six or less, PSA lower than 10 μg/L and clinical stages T1 or T2. Misclassification rates are around 20% or higher [99], remaining a severe limitation to identify accurately patients with low risk PCa. Notably, Wang et al. [100] showed that miR-19, miR-345 and miR-519c-5p serum levels independently predicted adverse pathology in PCa patients eligible for AS. The discriminatory ability to distinguish patients with adverse pathology increased from 0.77 to 0.94 when these miRNAs were added to a model including age, PSA, clinical stage and biopsy involvement. Measurement of miRNAs appears as a new way to better select patients for AS.

Contributions using microarrays and RNA-sequencing technologies are summarized in Table 2 [88–94, 98, 100–104], while Table 3 shows contributions based on qRT-PCR [30, 39, 95–97, 105–116].

Table 2: Circulating miRNAs studies in PCa patients using microarrays and RNA-sequencing technologies.

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Body fluid	Patients	Most relevant clinical value
Brase et al. [101]	667 miRs Selected miRs: miR-375, -9*, -141, -200b and -516a-3p	miR-375, -9*, -141, -200b and -516a-3p	–	TaqMan human microRNA array and qRT-PCR	cel-miR-39, cel-miR-54 and cel-miR-238	Serum	Screening set: 7 metastatic PCa and 14 localized PCa 1st Validation set: 45 PCa 2nd Validation set: 71 PCa (48 N1 vs. 23 N0; 29 Gleason ≥8 vs. 42 Gleason 7)	miR-375 and -141 levels correlated with a high Gleason score and lymph-node positive status
Moltzahn et al. [91]	384 miRs 12 miRs selected for validation study	Comparing PCa vs. healthy group: miR-874, -1274a, -1207-5p, -93 and -106a Comparing high risk vs. healthy group: miR-451	Comparing PCa vs. healthy group: miR-223, -26b, -30c and -24	Multiplex qRT-PCR	Median ΔCt	Serum	12 Low-risk PCa, 12 intermediate-risk PCa, and 12 high-risk PCa 12 Healthy male blood donors	AUCs: miR-106a, 0.928; miR-1274, 0.928; miR-93, 0.907; miR-223, 0.876; miR-874, 0.845; miR-1207, 0.812; miR-24: 0.778. miR-93, -106a and -24 differentiate healthy and metastatic groups
Bryant et al. [98]	742 miRs (cMV) 12 Selected miRs	miR-107, -130b, -141, -2110, -301a, -326, -331-3p, -432, -484, -574-3p, and -625*	miR-181a-2*	Exiqon qRT-PCR microarray and qRT-PCR cMV isolated by: exoMir kit	cel-miR-39	Plasma cMV and serum	Set 1 Plasma: 78 PCa and 28 healthy men Set 2 Serum: 119 PCa following radical prostatectomy, 72 without recurrence and 47 with metastasis	miR-141 and -375 are significantly increased in plasma exosomes and cMV in metastatic PCa compared with non-recurrent PCa miR-107 and -574-3p were increased in cMVs of men with non-metastatic PCa vs. healthy men
Chen et al. [89]	1146 miRs Eight selected miRs for validation study	miR-622 and -1285	let-7e, let-7c, and miR-30c	Illumina's Human v2 miRNA microarray and qRT-PCR	U6	Plasma	Screening set: 17 BPH and 25 CaP Validation set: 44 BPH, 54 healthy controls and 80 CaP	Combined miR-622, -1285, -30c, let-7e and let-7c discriminate CaP vs. BPH or healthy controls AUC of 0.924 and 0.860, respectively
Nguyen et al. [92]	669 miRs Selected miRs: -375, -378*, -141, -409-3p	Comparing metastatic vs. low-risk PCa: miR-375, -378*, and -141	Comparing low-risk vs. metastatic PCa: miR-409-3p	TaqMan Human MicroRNA Array and qRT-PCR	U6	Serum	28 Low-risk PCa, 30 high-risk PCa and 26 metastatic castration resistant PCa	miR-375, -378*, and -141 increase with disease progression

Table 2 (continued)

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Body fluid	Patients	Most relevant clinical value
Cheng et al. [93]	365 miRs Selected miRs: -100, -141, -148a, -200a, -200c, -210, -375, -425-5p, -222	miR-141, -200a, -200c, -210, -375	–	TaqMan human microRNA array and qRT-PCR	cel-miR-39, cel-miR-54 and cel-miR-238	Serum	Screening set: 25 metastatic castration resistant PCa and 25 age-matched controls Validation set: 21 metastatic castration resistant PCa and 20 age-matched healthy controls	AUCs: miR-141, 0.842; miR-200a, 0.638; miR-200c, 0.645; miR-210, 0.652; miR-375, 0.660 (validation set) miR-210 levels are related to PSA response in metastatic castration resistant PCa
Watahiki et al. [94]	742 miRs Selected miRs: -141, -152, -375, -16, -21, -126, -151-3p, -200c, -205, -423-3p	Comparing metastatic vs. localized PCa: miR-141, -375, -200c, -126, -21, -151-3p, -152 and -423-3p	Comparing metastatic vs. localized PCa: miR-205 and -16	Exiqon qRT-PCR microarray and qRT-PCR	mir-30e	Plasma	25 Localized PCa and 25 metastatic castration resistant PCa	miR-141, -151-3p, -152 and -423-3p are associated with a poorer outcome and/or a higher Gleason score. Combined miR141, -151-3p, -16 differentiate localized and metastatic PCa AUC: 0.944; sensitivity: 84%; specificity: 96%
Sapre et al. [88]	miRs -16, -20a, -21, -34a, -145, -106b, -182, -205, -221, -222, -331, -375	–	–	TaqMan human microRNA array and qRT-PCR	Geometric mean	Plasma	37 Low-risk PCa and 33 high-risk PCa	No separation between high and low-risk PCa was obtained using a 12 miRs profile. The better AUC was for miR16 (0.62)
Srivastava et al. [90]	667 miRs Selected miRs: -25, 101, -628-5p	–	Comparing PCa vs. healthy controls: miR-25, -101, and -628-5p	TaqMan® array human microRNA and qRT-PCR	miR-223	Serum	Screening study: 12 PCa Confirmation study: 40 PCa and 32 healthy age matched controls	AUCs: miR-25, 0.66; miR-101, 0.80; miR-628-5p, 0.94
Wang et al. [100]	672 miRs 36 Selected miRs for the validation study	miR-19a, -19b	–	Microfluidic-based multiplex qRT-PCR	Median Ct	Serum	Discovery set: 48 low-risk PCa and 48 PCa with pathologic Gleason score ≥7 Validation set: 25 low-risk PCa and 35 PCa with pathologic Gleason score ≥7	miR-19a/b, -345 and -519c-5p independently predict post-surgical pathological upgrade
Lin et al. [102]	46 miRs	–	–	TaqMan human microRNA array and qRT-PCR	Overall mean miRNA expression value	Plasma/serum (14 serum and 26 plasma samples)	97 Metastatic castration resistant PCa patients receiving docetaxel chemotherapy	12 miRNAs are associated with overall survival according to their pre-docetaxel levels or the direction of change in their post-docetaxel levels

Table 2 (continued)

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Body fluid	Patients	Most relevant clinical value
Haldrup et al. [103]	732 miRs	Comparing PCa vs. BPH: miR-17*, -200b*, -210, -297, -375, -501-3p, -551b and -562	–	Exiqon's microRNA ready-to-use PCR panels and qRT-PCR	miR-320a and UniSp3	Serum	13 BPH, 11 localized PCa, nine lymph node or distant metastases PCa, and 11 castration resistant PCa	Combined miR-562, -210, -501-3p, -375 and -551b offer a sensitivity of 84% and 100% specificity (AUC: 0.919)
Huang et al. [104]	miR-30a/e-5p, -99a-5p, let-7c, -1246, -1290, -16-5p, -125a-5p, -375	miR-1290 and -375	–	RNA sequencing and qRT-PCR Exosomes isolation: ExoQuick exosome precipitation solution	miR-30a-5p, -30e-5p and geometric mean of both	Plasma exosomes	Screening set: 23 PCa at the time of androgen deprivation therapy failure Follow-up set: 100 PCa at the time of androgen deprivation therapy failure	Association of miR-1290, -1246, and -375 with overall survival Higher levels of miR-1290 and -375 were significantly associated with poor overall survival in the follow-up cohort.

miRNAs in urine in prostate cancer

Several authors have demonstrated the role of urinary biomarkers in the management of PCa. In theory, urine is a potential way to obtain new PCa biomarkers, because of the anatomical localization of the prostate in relation to the urethra, obtaining exfoliated cancer cells or secreted products through the prostatic ductal system. According to Weber et al. [15], the concentration and number of detectable miRNAs in urine is lower than in plasma, suggesting that they are cleared in the kidneys or destroyed in the urine. However, the enrichment of urine with prostatic cells performing a prostate massage is a way to increase the presence of prostate biomarkers, improving test accuracy [117]. Additionally, the protein content is lower in urine than in serum and plasma, reducing interferences in the miRNAs isolation [118]. Nevertheless, the measurement of miRNAs in urine has the inconvenience derived of the low nucleic acid concentration in this fluid. Methods to obtain RNA based on the enrichment of short RNAs and preamplification before qRT-PCR could be necessary to adequately measure miRNAs in urine.

Most studies used the urine pellet, but total urine and the cell-free fraction are also used, generally after digital rectal examination. According to Cheng et al. [14], miRNAs were found to be significantly enriched and intact in urine-derived exosomes compared with cell-free urine. In fact, exosomes have been found as a robust source of urinary biomarkers, showing the presence after digital rectal examination of high levels of *PCA3* and *TMPRSS2-ERG* in urinary exosomes [12]. However, no studies are currently available about miRNAs in urinary exosomes in PCa, even when they have been widely explored in benign nephropathies [119, 120]. Table 4 presents published results about miRNAs in urine, showing AUCs between 0.639 and 0.769 [49, 86, 88, 98, 121–128]. Unfortunately, there is not a consensus in miRNAs selected for these studies neither congruence with results published in blood. On the other hand, miRNAs were not associated with biochemical recurrence [123], PSA serum levels [126] or Gleason score [122, 126], although according to Lewis et al. [49], miR-888 measured in supernatant, but not in pellet, is associated with high-grade PCa. Finally, the value of miRNAs in the detection of PCa could increase in association with other tests. Egidi et al. [127] showed an AUC of 0.880 combining human kallikrein 3 mRNA with miR-19a-3p, while Yun et al. [128] reported AUCs of 0.761 and 0.738 for hsv1-miR-H18 and hsv2-miR-H9-5p, respectively, for patients in the PSA gray area. These promising preliminary results are worthy of larger studies.

Table 3: Circulating miRNAs studies in PCa patients based on qRT-PCR.

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Body fluid	Patients	Most relevant clinical value
Mitchell et al. [30]	miR-100, -125b, -141, -143, -205, and -296	miR-141	–	qRT-PCR	cel-miR-39, cel-miR-54 and cel-miR-238	Serum	25 Metastatic PCa and 25 matched healthy donors	miR-141 AUC: 0.907 (PCa vs. healthy)
Zhang et al. [105]	miR-21	miR-21	–	qRT-PCR	U6	Serum	20 Localized PCa, 20 androgen-dependent PCa, 10 hormone-refractory PCa and six BPH	Elevated levels of miR-21 in hormone-refractory PCa
Yaman Agaoglu et al. [106]	miR-21, -141, and -221	miR-21 and -221	–	qRT-PCR	RNU1A	Plasma	51 PCa (25 with metastasis) and 20 healthy individuals	AUCs: miR-21, 0.88; miR-221, 0.83 miR-21 and -221 did not reach the power of PSA to discriminate metastatic vs. localized PCa
Mahn et al. [107]	miR-26a, -32, -195, -let7i	Comparing PCa vs. BPH: miR-26a, -195, and -let7i	–	qRT-PCR	cel-miR-39	Serum	20 Healthy controls, 18 BPH, 37 localized PCa, eight metastatic PCa	miR-26a differenciates PCa and BPH with 89% sensitivity and 56% specificity AUC: 0.703. Combined miR-26a, miR-32, miR-195, miR-let7i showed a sensitivity of 78% and specificity of 67%. AUC 0.758 (PCa vs. BPH).
Gonzales et al. [108]	miR-141	miR-141	–	qRT-PCR	Not available	Plasma	21 Patients with CaP	Longitudinal changes of miR-141 correlates with clinical course
Selth et al. [109]	miR-141, mmu-miR-298, mmu-miR-346 and mmu-miR-375	miR-141, -298 and -375	–	qRT-PCR	cel-miR-39	Serum	25 Metastatic castration-resistant PCa and 25 healthy men	Levels of miR-141, -298 and -375 increased in PCa
Shen et al. [96]	–	Comparing intermediate vs. high risk PCa: miR-21 and -145	–	qRT-PCR	Specific RNA oligonucleotides were used to generate standard curves	Plasma	82 PCa (38 low risk, 27 intermediate risk, 17 high risk according to D'Amico criteria)	Combined miR-20a, -21, -145 and miR-221 distinguish low and high risk PCa (AUC: 0.824)
Sanders et al. [39]	miR-21	–	–	qRT-PCR	cel-miR-39 SNORD43	Serum	24 PCa, 24 bladder cancer, 24 renal cell carcinoma, 48 control subjects	miR-21 was similar in cancer patients and healthy controls
Westermann et al. [110]	miR-26a-1 and -141	–	–	qRT-PCR	RNU1-4 and SNORD43	Serum	54 PCa and 79 non-malignant	miR-26a-1 and -141 are not helpful to detect PCa. miR-141 increased in patients with Gleason score 9
Singh et al. [111]	43 miRs	miR-125b, and -222	miR-103	qRT-PCR	Overall mean miRNA expression value	Serum	62 Non progressors PCa and 31 progressors PCa	miR-103, -125b, and -222 predict progression risk in PCa

Table 3 (continued)

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Body fluid	Patients	Most relevant clinical value
Santos et al. [112]	miR-7, -145, -199b, -200b, -221, -222, -1233	Comparing early vs. late castration-resistant PCa: miR-7 and -221	–	qRT-PCR	RNU44	Peripheral whole-blood	45 PCa	miR-7 and -221 predict castration-resistant PCa development
Kotb et al. [113]	miR-21 and -221	miR-21 and -221	–	qRT-PCR	SNORD47	Serum	10 PCa and 10 BPH	miR-21 and -221 predict PCa (sensitivity 90% and 80%; specificity 90% and 80%, respectively)
Mihelich et al. [95]	21 miRs	–	Comparing high vs. low-risk PCa or vs. BPH: let-7a, miR-24, -26b, -30c, -93, -103, -106a, -107, -130b, -146a, -223, and -451	qRT-PCR	Sp3, Sp6, cel-miR-39 and total RNA input normalization	Serum	100 No treated PCa (50 low-grade, 50 high-grade) and 50 BPH	Combined let-7a, miR-103, -451, -24, -26b, -30c, -93, -106a, -223, -874, -146a, -125b, -100, -107 and -130b distinguish low-grade PCa and BPH vs. high-grade PCa patients Combined miR-451, -106a, -223, -107, -130b, let-7a and miR-26b correlates with BCR and predicts disease-free survival
Li et al. [97]	miR-16, -20a, -21, -96, -107, -141, -145, -183, -221, -375, -409 and -574	miR-375, -21, and -574	–	qRT-PCR	Results expressed as average Ct values For exosomes isolation: total exosome rna and protein isolation kit	Serum exosomes	Eight metastatic PCa, six PCa treated with radical prostatectomy, 10 healthy controls.	miR-375, -21 and -574 discriminate between groups
Kelly et al. [114]	miR-16, -21, -34a, -141, -143, -145, -155, -125b, -221, -375, -425 and let-7a	miR-141, -145 and -155	let-7a	qRT-PCR	miR-16 and -425	Whole blood	75 PCa and 27 patients with negative biopsy	Combined let-7a, miR-141, -145 and -155 offers an AUC of 0.783 and a PPV of 80%
Kachakova et al. [115]	let-7c, miR-30c, miR-141, and miR-375	–	Comparing PCa vs. BPH: miR-375	qRT-PCR	U6	Plasma	59 PCa,16 BPH and 11 young asymptomatic men	miR-375 AUC of 0.809. Combined let-7c, miR-30c, -141, -375 and PSA offers an AUC of 0.877 (sensitivity: 86.8%, specificity 81.8%)
Huang et al. [116]	miR-21	Comparing PCa patients with recurrence or metastasis vs. PCa patients without them and to healthy controls: miR-21	–	qRT-PCR	U6	Peripheral blood mono-nuclear cells	75 PCa and 75 healthy subjects	miR-21 AUC of 0.9 (sensitivity 87.5%, specificity 85.7%) miR-21 was associated with tumor differentiation, clinical stage, and lymph node metastasis

Table 4: Urine miRNAs studies in PCa patients.

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Urine fraction	Patients	Most relevant clinical value
Ahumada-Tamayo et al. [121]	373 miRs	miR-196b, -574-3p, let-7b, -7c, -7d, -7e, -7g, miR-200b, -149, -20b, -17, -184, -20a, -106a, -671-3p, -148a, -429, -31, -100	miR-150, -328	qRT-PCR	Endogenous control	Urine pellet obtained after prostate massage	Nine PCa and nine BPH	Differential expression of 21 miRNAs between PCa and BPH
Bryant et al. [98]	miR-107, -141, -200b, -375, -574-3p	Comparing Pca vs. controls: miR-107 and -574-3p	–	qRT-PCR	RNU44 RNU48	Urine pellet after DRE	17 Controls, 70 localized PCa, 48 advanced PCa	miR-107, AUC: 0.74 and -574-3p, AUC: 0.66 detect PCa
Srivastava et al. [86]	miR-205, -214, -221 and -99b	–	miR-205 and -214	qRT-PCR	RNU48	Total urine samples	36 PCa and 12 age and ethnicity matched healthy donors	miR-205, AUC: 0.708 miR-214, AUC: 0.743 Combined miR-205 and miR-214 improve detection of PCa: 89% sensitivity and 80% specificity
Korzeniewski et al. [122]	miR-483-5p, -1275, and -1290	Comparing PCa vs. negative biopsy patients: miR-483-5p	–	qRT-PCR	cel-miR-39	Cell-free fraction of freely voided urine	18 Negative biopsy and 71 PCa	miR-483-5p, AUC: 0.694 3-signature miRNA panel did not improve the detection of PCa over PSA level miR-483-5p is not associated with Gleason score
Corcoran et al. [123]	miR-598, miR-34a, miR-146a, miR-148a	–	Comparing PCa vs. BPH: miR-148a	NA	NA	Publically available dataset	Nine PCa and eight BPH Publically available dataset	miR-598, miR-148a, and miR-146a are not associated with biochemical recurrence
Sapre et al. [88]	miR-21, -20a, -375, -331, -205, -218, -16, -222, -221, -34a, -106b, -182, -145 Selected miRs: -16, -21, -222.	Comparing high risk vs. indolent PCa group in the discovery set: miR-16, -20a, -21, -34a, -145, -106b, -182, -205, -221, -222, -331 and -375 miR-16, -21, -222 were upregulated in the discovery set using Taqman array and qRT-PCR, but failed in the validation set using qRT-PCR	–	TaqMan human microRNA array and qRT-PCR	Geometric mean	Total urine obtained after DRE	Discovery set: 33 patients prior to RP (17 indolent PCa and 16 high risk). Validation set: 22 with high risk PCa and 14 indolent PCa	Combined miR-16, -21, -222, AUC: 0.75 predict high-risk PCa. However, in the validation set: AUC 0.35

Table 4 (continued)

References	miRNAs analyzed	Upregulated miRNAs	Downregulated miRNAs	Methodology	qRT-PCR Normalizer	Urine fraction	Patients	Most relevant clinical value
Haj-Ahmad et al. [124]	894 miRNAs assayed Selected miRs: miR-1234, -1238, -1913, -486-5p, -1825, -484 and -483-5p	Comparing PCa vs. healthy: miR-1825	Comparing PCa vs. healthy and BPH vs. healthy: miR-484	LC Science's µParaflo™ miRNA microarray and qRT-PCR	5S rRNA	Urine samples collected with Norgen's urine preservative solution	Eight PCa, 12 BPH and 10 healthy males	Comparing PCa vs. BPH miR-1825 sensitivity 60%; specificity 69% miR-484 sensitivity: 80%; specificity: 19% Combined miR-1825 and 484 sensitivity 45%; specificity 75%
Casanova-Salas et al. [125]	miR-182 and 187	–	miR-187	qRT-PCR	RNU44 and RNU48	Urine pellet obtained after DRE	45 Negative biopsy and 47 PCa	miR-187 provided an independent predictive value for PCa Combined PSA, PCA3 and miR-187 provided 88.6% sensitivity and 50% specificity (AUC 0.711)
Lewis et al. [49]	50 miRNAs in the supernatant of EPS miR-888, -200b, let-7c, in the pellet fraction of EPS	Comparing high vs. low-risk PCa in the supernatant of EPS: miR-888, -34a, -205	Comparing high vs. low-risk PCa in the supernatant of EPS: let-7c, -7d and miR-200b, 92a, -99a, -141, -375	qRT-PCR	Supernatant of EPS: cel-miR39 Pellet fraction of EPS: RNU44 and RNU48	Supernatant and pellet fractions of EPS (urine) obtained after DRE	24 Non-cancer subjects, 25 PCa Gleason 6–7, one PCa Gleason 8, six PCa Gleason 9–10	miR-888 in the supernatant EPS is associated with high-grade PCa Pellets of EPS failed to correlate with clinical grade or detect PCa
Stephan et al. [126]	miR-183, -205	–	–	qRT-PCR	miR-130b, total RNA and PSA mRNA normalization	Urine pellet after DRE	38 PCa and 38 no evidence of malignancy	miR-183 and miR-205 failed to detect early and aggressive PCa miRNAs are not associated with Gleason score and serum PSA
Egidi et al. [127]	miR-9-3p and miR-19a-3p	–	Comparing Pca vs. BPH: miR-9-3p and miR-19a-3p	qRT-PCR	miR-191	Urine pellet after DRE	41 PCa and 38 age-matched subjects with BPH	miR-19a-3p, AUC: 0.723 miR-9-3p, AUC: 0.769 Highest AUC (0.880) was reached combining KLK3 with miR-19a-3p
Yun et al. [128]	1205 Human and 144 viral miRs (discovery set) Selected miRs: hsa-miR-615-3p, ebv-miRBART4, hsv1-miR-H18, hsv2-miR-H9-5p, and hsa-miR-4316. (validation set 1) hsv1-miR-H18 and hsv2-miR-H9-5p (validation set 2 and 3)	Comparing PCa vs. BPH controls. (validation set 1): hsa-miR-615-3p, hsv1-miR-H18, hsv2-miR-H9-5p, and hsa-miR-4316 Comparing PCa vs. BPH controls (validation set 2 and 3): hsv1-miR-H18 and hsv2-miR-H9-5p	–	Agilent human miRNA microarray and qRT-PCR	Synthesized RNA oligonucleotides were used to generate standard curves. Normalization using total RNA concentration	Supernatant urine	Discovery set: 14 PCa and 5 BPH Validation set 1: 173 PCa, 108 BPH Validation set 2: 191 PCa, 143 BPH Validation set 3: 46 PCa, 104 negative biopsy	hsv1-miR-H18 and hsv2-miR-H9-5p, AUCs: 0.639 and 0.760, respectively. AUC for PSA: 0.71 hsv1-miR-H18 and hsv2-miR-H9-5p, AUCs: 0.761 and 0.738, respectively, in the PSA gray area

Conclusions

Cumulative results underlined the role of deregulated miRNAs in the development of PCa. This is a complex process that includes the dynamic participation of several miRNAs in the successive PCa steps. Multiple studies showed the clinical usefulness of miRNAs in the management of PCa; however, results differ regarding their accuracy and the relationship with the aggressiveness of the tumor. Besides, results offer a large variation in the miRNAs included in selected profile. Contradictory results could be associated with important methodological issues related to their quantitation and normalization. In fact, even if miRNAs appear as inherently stable molecules, optimization and standardization of methods are necessary to obtain high quality and reproducible results. The best published results were obtained using miR-141, miR-375 and miR-21. In fact, the selection of the most appropriate miRNAs is a key challenge to obtain outstanding results. Large screening studies to select a miRNA profile result laborious and expensive, whereas bioinformatics tools offer a new approach to select miRNAs that are relevant in PCa development. Currently, the majority of studies concerning the evaluation of the clinical usefulness of miRNAs have been performed in serum or plasma, but urine obtained after prostate massage appears as a new way to describe new PCa biomarkers. Therefore, new efforts are necessary to define the value of miRNAs in the management of PCa.

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