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The Potential of MicroRNAs as Prostate Cancer Biomarkers

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Abstract

Context—Short noncoding RNAs known as microRNAs (miRNAs) control protein expression through the degradation of RNA or the inhibition of protein translation. The miRNAs influence a wide range of biologic processes and are often deregulated in cancer. This family of small RNAs constitutes potentially valuable markers for the diagnosis, prognosis, and therapeutic choices in prostate cancer (PCa) patients, as well as potential drugs (miRNA mimics) or drug targets (anti-miRNAs) in PCa management.

Objective—To review the currently available data on miRNAs as biomarkers in PCa and as possible tools for early detection and prognosis.

Evidence acquisition—A systematic review was performed searching the PubMed database for articles in English using a combination of the following terms: *microRNA*, *miRNA, cancer, prostate cancer, miRNA profiling, diagnosis, prognosis, therapy response,* and *predictive marker*.

Evidence synthesis—We summarize the existing literature regarding the profiling of miRNA in PCa detection, prognosis, and response to therapy. The articles were reviewed with the main goal of finding a common recommendation that could be translated from bench to bedside in future clinical practice.

Conclusions—The miRNAs are important regulators of biologic processes in PCa progression. A common expression profile characterizing each tumor subtype and stage has still not been identified for PCa, probably due to molecular heterogeneity as well as differences in study design and patient selection. Large-scale studies that should provide additional important information are still missing. Further studies, based on common clinical parameters and guidelines, are necessary to validate the translational potential of miRNAs in PCa clinical management. Such common signatures are promising in the field and emerge as potential biomarkers.

Patient summary—The literature shows that microRNAs hold potential as novel biomarkers that could aid prostate cancer management, but additional studies with larger patient cohorts and common guidelines are necessary before clinical implementation.

Keywords

Prostate cancer; microRNA; Cancer biomarker; Diagnosis; Prognosis

1. Introduction

1.1. Prostate cancer: the state of the art

Prostate cancer (PCa) is the most common nonskin cancer worldwide and a leading cause of cancer-related death in the United States and in Europe [1,2]. An estimated 220 800 men

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will be diagnosed with PCa in the United States this year, and nearly 27 500 men will die of this disease [2]. After the introduction of the prostate-specific antigen (PSA) test, the detection of PCa dramatically increased with a peak in the early 1990s. Today, approximately 85% of men newly diagnosed with PCa present with localized early stage tumors [3]. Despite the significant improvement in early detection due to routine PSA testing, medical and scientific communities are still debating its benefits because there is no consensus regarding whether it effectively reduces the risk of death from the disease [4]. PSA levels are prostate but not cancer specific and may fluctuate due to, for example, infections, inflammation, or benign prostatic hyperplasia (BPH), resulting in high false-positive rates. The poor correlation between PSA levels and disease state leads to unnecessary diagnoses and overtreatment of indolent PCa [5].

Due to the molecular heterogeneity of PCa [6], the identification and clinical translation of routinely tested disease- and stage-specific molecular markers is a rational approach to potentially expedite PCa diagnosis, prognosis, and treatment response, opening the way to personalized medicine. Beyond proteins and messenger RNAs (mRNAs), which have shown clinical utility in various clinical scenarios, there is a growing interest in the potential utility of microRNAs (miRNAs) as PCa biomarkers.

1.2. MicroRNAs in cancer

The miRNAs are evolutionarily conserved short (approximately 18–22 nucleotides [nt]) noncoding single-stranded RNA molecules that act as posttranscriptional gene regulators [7]. Initially transcribed in the nucleus by RNA polymerase II or III as long primary transcripts (pri-miRNAs), miRNAs are subsequently processed into 70- to 100-nt precursor RNAs (pre-miRNAs) by the microprocessor complex, consisting of the RNase III enzyme Drosha and its interacting partner DGCR8. This initial cleavage is followed by Exportin-5/RanGTP-mediated pre-miRNA translocation to the cytoplasm for further processing into a 19- to 25-nt duplex by the RNase III endonuclease Dicer and TRBP. The final processing by Dicer is likely to culminate in the assembly of the two strands into the RNA-induced silencing complex (RISC); the key component of the RISC complex is an Argonaute protein. Within RISC, miRNAs negatively regulate translation of target mRNAs by altering their stability either through the binding to their 3′ untranslated region (UTR), or, to a lesser extent, to the 5′ UTR or to the coding sequence. As a result, the miRNAs can directly target mRNA to degradation in the presence of perfect complementarity or induce translational repression through different mechanisms (reviewed in Valinezhad et al [8]). It has also been demonstrated that miRNAs not only repress, but in some cases also may be able to activate gene expression directly or indirectly through the interaction with micro-ribonucleoproteins such as Ago2 and FXR1 [9,10].

Due to the complexity of these regulatory mechanisms, and because each miRNA can modulate the expression of multiple mRNAs and, furthermore, each mRNA may be targeted by several different miRNAs [11], it is not surprising that miRNAs have been shown to be involved in almost all key cellular processes, such as proliferation, differentiation, migration, apoptosis, and stemness maintenance. Alterations in the expression of cancer-related miRNAs can be affected by chromosomal rearrangements, promoter methylation, or

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transcriptional deregulation. Indeed, 20–40% of miRNAs are located near CpG islands, confirming their possible epigenetic silencing, especially demonstrated for urologic diseases [12]. The miRNAs are frequently located within fragile chromosomal sites that exhibit DNA amplifications, deletions, or translocations during tumor progression [13]. Some individual miRNAs have been characterized either as tumor suppressors or oncogenes (onco-miRs), depending on the deregulated downstream targets [14], making them even more interesting and leading to huge numbers of related publications.

A growing body of literature in particular has investigated the potential use of miRNAs as useful biomarkers for cancer diagnosis, prognosis, and therapy [14], including PCa [15], and associated their levels with clinicopathologic parameters [16–18]. Evaluation of the expression levels of specific miRNAs in clinical prostate tissue samples may be used to detect cancer, predict the cancer prognosis and monitor its evolution, and as markers for therapy selection and response.

1.3. MicroRNAs as biomarkers

The ideal early detection biomarker should be capable of identifying potentially aggressive tumors at a point when the cancer is still curable while minimizing detection of indolent disease. The presence of the tumor at the earliest possible stage should be detected by current available techniques including quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), in situ hybridization, enzymatic luminescence miRNA assay, microarrays, or next-generation sequencing [19], which would make the transition to the clinic much easier. The miRNAs are attractive molecular biomarker candidates because they can be reproducibly extracted from a wide range of biologic samples and are generally stable and resistant to various storage conditions [20]. Furthermore, miRNAs can be easily detected and accurately quantified by a variety of widely used standard techniques, such as qRT-PCR, microarray, and small RNA sequencing.

In addition to tissue-based studies, investigation of circulating miRNAs is a new expanding field in biomarker research because they possess all these characteristics, are detectable in body fluids, and do not require invasive biopsies. Nonetheless, there is still a lack of consensus concerning unequivocal endogenous control (exogenous spike in miRNA or other endogenous normalizers) or contamination from circulating cellular sources (ie, white cells for blood samples). Circulating cell-free miRNAs are extremely resistant to ribonucleases and severe physicochemical conditions, such as freeze-thawing and extreme pH [20], most likely due to their packaging in lipid microvesicles (exosomes and apoptotic bodies) [21] in complexes with RNA-binding proteins, such as Ago2, which protect them from degradation [22]. In addition to plasma and serum, miRNAs have been identified in other body fluids, in particular urine and semen [21,23], which make them even more interesting candidate biomarkers for PCa.

2. Evidence acquisition

We performed a detailed literature search in the PubMed database for articles written in English published up to August 2015 and updated on October 2015, according to the Preferred Reporting Items for Systematic Review and Meta-analysis statement. We used

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AND/OR combinations of the following terms: *microRNA*, *miRNA, prostate cancer, miRNA profiling, diagnosis, prognosis, therapy response,* and *predictive marker*. More than 1300 publications were retrieved, and among them 200 were reviews (Fig. 1). Relevant papers were selected by two authors (L.F. and G.A.C.), and all authors fine-tuned and enhanced the list of papers to be included. We specifically selected papers written in English in which scientific detail and reporting were sufficient to enable our understanding and contained novel findings. Papers presenting larger cohorts of patients and similar screening technologies were preferred, as well as those published in higher impact factor journals. We included and reviewed 67 articles in this comprehensive meta-analysis.

3. Evidence synthesis

3.1. Profiling of microRNAs in prostate cancer tissue

Based on the wide range of biologic processes regulated by miRNAs, their deregulation has been associated with cancer onset, progression, and dissemination. Regarding PCa, although several studies investigated miRNA alteration as potential diagnostic and prognostic tools, unique signatures depicting the differentially expressed miRNAs are still missing. The main problems are linked to differences in study design, underestimated or incomplete information on treatments of the patients, methods of sample collection, presence of contaminating cells, sensitivity and specificity of the profiling platforms used, and in many cases limited patient sample size.

The first attempt to delineate a PCa-specific miRNA expression signature was published in 2006 by Volinia et al [24]. By comparing 56 tumor tissues and 7 normal noncancerous controls, they observed a general upregulation of miRNAs in cancer tissue. In particular, among the 228 miRNAs analyzed through genome-wide microarray analysis, 137 were expressed in at least 90% of the samples, and subsequent computational analyses revealed that 39 miRs were upregulated, whereas 6 were downregulated in PCa samples, compared with normal tissue. A general trend of miRNA upregulation in PCa was partially confirmed by Ambs et al [25], who analyzed up to 329 miRNAs in 60 microdissected PCa tumors and in 16 adjacent normal prostate tissue samples through microarray technology. In this study [25], the results were also validated by qRT-PCR in a random subset of the samples. However, only 8 of 39 miRNAs described as upregulated in the paper by Volinia et al [24] (miR-25, -26a, -32, -92, -93, -181a, and -196a and let-7) were validated in this independent patient set [25].

The seeming global upregulation in miRNA expression in PCa tissue was further supported more recently [18] in a study comparing 19 high-grade PCa, 20 low-grade PCa, and 21 BPH in two different patient sample sets. The authors used high-throughput Illumina genome sequencing in the training sample set while validating the results through qRT-PCR. Six miRNAs were found to be upregulated in PCa, and only one was downregulated, but the signature did not share any common feature with the previous studies by Volinia et al and Ambs et al [18,24,25]. Thus although the overall upregulation in PCa was confirmed, a common diagnostic miRNA signature that could be translated to the clinic was not confirmed. The use of different control samples (BPH vs adjacent normal) and the

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stratification of PCa patients (low and high grade) could at least partially explain the different results.

In contrast, Porkka et al [26] observed a general downregulation of miRNAs expression in PCa by combining data obtained analyzing 6 PCa cell lines, 9 xenografts, and a panel of 13 prostate tissues (4 BPH, 5 hormone-naive carcinomas, and 4 hormone-refractory carcinomas). The authors analyzed up to 319 human miRNAs using a custom miRNA microarray. Among the 51 miRNAs identified as differentially expressed between the clinical samples, 37 were significantly downregulated in PCa tissue. Twenty-two of these 37 miRNAs showed decreased expression in all carcinoma samples (untreated and hormone refractory), whereas 15 were only downregulated in the hormone-refractory carcinomas compared with the BPH samples. In addition to these 37 downregulated miRNAs, 14 miRNAs were upregulated (8 showed increased expression in all carcinoma samples and 6 were upregulated only in hormone-refractory carcinomas). These data clearly contrast with the previous observations that outlined a general miRNA upregulation in PCa [18,24,25]. Nevertheless, the study by Porkka et al is not the only one demonstrating a general miRNA decrease in cancer tissues. The study was in partial agreement with a later one published by Ozen et al [27]. Of the 328 known and 152 novel miRNAs screened using a mirVana miRNA Bioarray (Thermo Fisher Scientific, Wilmington, DE, USA), 85 were detectable in the 16 PCa and 10 adjacent normal tissues analyzed. The authors analyzed samples with a wide range of clinical aggressiveness, from PSA recurrences that occurred in <1 yr to no recurrence after 5 yr. Among the detectable miRNAs, 75 were downregulated, with a tendency of becoming even more significantly decreased in tumors from patients with early PSA recurrence [27]. Nevertheless, while overall the expression of these miRNAs decreased, there was significant variability between cancer linked to the variable percentage of the stromal portion, ranging between 10% and 30%. Three of the 75 miRNAs found in this study were in common with the ones published by Porkka et al (miR-125b, -16, -29, and let-7b). The possible reason for the difference with previous studies could be the difference in the array used. Volinia et al [24] used total RNA extraction, which includes precursor miRNAs, detecting both precursor and mature miRNAs. In contrast, the protocols used by Ozen et al and Porkka et al [26,27] pertained to a purification of small RNAs including mature miRNAs, before analysis, so precursor miRNAs would not be detected. Possible differences in miRNAs stability and processing could explain, at least in part, the differences. Analyses of downstream pathways altered could help in understanding the molecular basis of these different observations, and such comprehensive bioinformatic analyses are useful to be included in the reports of profiling studies.

The high variability between the data obtained from the different groups could be related to several factors. Each study is based on a rather small sample size, and only a few of them have independent validation. Different screening methodology and different sample characteristics (ie, stroma-to-tumor proportion) could also explain the results. Nonetheless, the papers delineate a starting point, and some of the cited papers express common signatures, reported in Table 1. The miRNAs depicted emerge as the most significant ones, found with the same trend in multiple studies with different methodologies. Despite the differences reported throughout the literature, promising miRNA signatures are identified and need to be further confirmed in additional large patient cohorts.

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3.2. Circulating microRNA and prostate cancer diagnosis

In PCa, diagnosis and follow-up monitoring after therapies are some of the major challenges for its clinical management. Although PSA screening improved early detection, its levels poorly correlate with tumor aggressiveness or dissemination, and they are not useful to predict responsiveness or relapses. Diagnostic accuracy, in particular in terms of risk stratification, initial staging, active surveillance, and focal therapy, is one of the main issues in this field. Patients undergo repetitive biopsies, which are not only invasive but also not decisive, even if coupled with PSA and digital rectal examination (DRE). DRE has a low sensitivity, whereas PSA screening is characterized by low specificity; sampling error in the presence of multifocal PCa, for example, could lead to misinterpretation of the results and to the wrong therapy.

In recent years, much effort has been invested in elucidating the possibility of improving patient care by substituting procedures, even if minimally invasive such as DRE or prostate biopsy, with miRNA analysis in patients' serum or plasma. The initial study was published in 2011 by Moltzahn and colleagues, who compared serum miRNA levels of 12 healthy men and 36 patients divided into low-, intermediate-, or high-risk groups based on the Cancer of the Prostate Risk Assessment score [28]. Of the 384 miRNAs tested through multiplex qRT-PCR and analyzed through a microfluidic chip, 10 miRNAs, which were significantly different between healthy and malignant samples (four downregulated and six upregulated), were further validated through single-plex qRT-PCR. Some of them were in agreement with previous studies on prostate tissues (ie, miR-93 was also identified to be upregulated in PCa by Volinia et al [24]), whereas miR-223 and miR-30c were deregulated in the same study but with an inverse trend. A linear correlation between miRNA levels and cancer risk was found for 3 of the 10 deregulated miRNAs; in particular, miR-106 and miR-1274 had a positive correlation; miR-24 had a negative one. Receiver operating characteristic curves generated for individual miRNAs suggested that miR-106a and miR-1274 in particular possess significant diagnostic potential, with an area under the curve (AUC) of 0.928 for both of them. Afterward, Bryant et al analyzed plasma from 78 PCa patients and 28 healthy donors [29]. A signature of 12 miRNAs was found to be deregulated in cancer patients through a qRT-PCR array and then validated in an independent serum cohort. Authors identified two miRNAs (miR-107 and miR-574–3p) able to discriminate significantly the two categories. In particular, miR-107 had an AUC of 0.62 compared with an AUC of 0.79 for PSA. Of note, these miRNAs were also tested in 135 urine samples enriched in prostatic cells following DRE, and they were also found to be highly concentrated in these samples, opening new diagnostic possibilities. Subsequently, Chen et al outlined a panel of five plasma miRNAs (let-7c, let-7e, miR-30c, miR-622, and miR-1285) by analyzing 25 PCa- and 17 BPH-derived samples through the Illumina microarray platform. This signature could discriminate among PCa, BPH, and healthy samples with a high diagnostic performance [30].

In 2011, Yaman Agaoglu et al [31] tested the possible diagnostic utility of three specific PCa-associated miRNAs, miR-21, miR-141, and miR-221, demonstrating that miR-21 and miR-221 were elevated in the plasma of men with localized cancer compared with healthy controls.

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In contrast, a signature composed by miR-141 and miR-375 was validated by more than one group [29,31–34]. These miRNAs seem to be reliable markers of systemic disease because they have been validated to efficiently discriminate men with localized cancer from ones with metastatic disease. The miR-141 and miR-375 have been also associated with tumor stage and Gleason score in patients' sera before undergoing radical prostatectomy (RP), underlying the potential of circulating markers for the early detection of PCa. They have also shown a consistent prognostic value. Interestingly, recently miR-375 purified from plasma exosomes was confirmed as a promising prognostic biomarker in castration-resistant prostate cancer (CRPC) [35]. Authors analyzed plasma exosomal miRNAs from a screening cohort of 23 men and a validation one of 100 men. Of 375 known and 57 putative miRNAs screened through RNA sequencing, miR-375 was validated as a strong predictor of survival, alone or in combination with miR-1290. Patients with high levels of these miRNAs have a significantly shorter overall survival and higher mortality rate.

Even if PCa is a heterogeneous and complex disease, the idea of a panel of markers, including miRNAs, is starting to make headway on the basis of several observations. Table 2 reports the most reliable plasma miRNAs that are consistently deregulated in different papers. Additional studies with longitudinal follow-up will be helpful to delineate the variation of these miRNAs from a localized disease through the development of widespread metastasis, to depict a model for patients' screening and management.

Due to the correlations observed between miRNAs changes in PCa patients and their potential use as circulating biomarkers, looking for their presence as potential biomarkers in urine is a reasonable idea. Although the urine-based miRNA screening is still in its infancy, after the first observations made by Bryant et al [29], in which miR-107 and miR-574-3p levels in urine correlated with their plasma levels and had significant diagnostic potential, some other studies focused on this approach. For instance, miR-34a and miR-148 were significantly downregulated in urine from PCa patients compared with BPH in a paper by Corcoran et al [36] that considered publicly available cohorts. The miRNA deregulation has been observed as clinically significant both in tissues (21 PCa vs 21 BPH) and in the urine of PCa patients ($n = 9$) versus BPH ($n = 8$). Korzeniewski et al [37] analyzed miRNAs in urine from 18 control patients (with PSA <10 ng/ml and negative biopsies) and 72 patients with histologically proven PCa: miR-483-5p, miR-1275, and miR-1290 were the most significantly upregulated in the PCa group compared with controls.

The recent noteworthy work by Haj-Ahmad et al identifies six upregulated miRNAs in urine (miR-234, -1238, -1913, -486-5p, -1825, and -484) that are able to discriminate between PCa patients and BPH [38].

However, this particular field needs further development to delineate a better plasma/serum/urine signature able to discriminate men with potential PCa risk with a quick, easy, and noninvasive test. Urine screening still lacks a consensus signature that could be applied to the routine screening setting and needs further investigation. An important potential problem is the contamination from bladder and kidney epithelia material; the possible use of plasma miRNA test seems to be more developed. Importantly, any potential miRNA-based clinical assay will need to be validated prospectively in an intended use population

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3.3. MicroRNAs as prognostic factors

Expression profiles reported so far for miRNAs as diagnostic factors gave some consistent results, even if differences in methodologies and patient selection are the main pitfalls to reach an unambiguous expression pattern. Thus a global overview should take in consideration the often occurring disagreement of the data reported and the complexity of the clinical management of PCa. Recognized treatment options for early stage PCa include surgery, radiation therapy (RT), and active surveillance. For many men with metastatic or high-risk localized disease, androgen deprivation is a well-established component of the therapy. After RP, the PSA is expected to fall to undetectable levels, thus patients with detectable PSA levels after an RP are thought to have biochemical recurrence (BCR) due to the presence of residual benign prostate tissue or PCa [39]. We considered, in separate sections, the possible outcomes after eradication of primary PCa and the role of miRNAs as potential prognostic factors in these scenarios.

3.3.1. Biochemical recurrences—The first end point to measure treatment success after RP is the BCR, defined as de novo rising of serum PSA levels. BCR can be potentially predictive of the development of subsequent distant metastases and ultimately death, or alternatively BCR can predate other signs of clinical progression by several years. Different studies attempted to find miRNAs able to stratify patients with different probability to incur biochemical failure. In 2009, Tong and colleagues identified a signature of 16 miRNAs in 40 formalin-fixed paraffin-embedded (FFPE) tumor tissues divided into a group that relapsed within 2 yr or a group that did not relapse within a period of 10 yr after RP [40]. Using these 16 miRNAs, they were able to segregate correctly 75% of the analyzed patients and exclude 85% of patients with no apparent recurrences. While this study describes miR-96 as below the detection limit in BCR, a subsequent study by Schaefer et al found it as the elective biomarker for identification of BCR. In particular, the authors analyzed 76 specimens from RP and matching adjacent normal tissue, 12 of which incur in BCR after surgery. They confirm the role of miR-96 as a predictive BCR biomarker in an independent tumor sample set of 79 patients, demonstrating that tumors with high levels of miR-96 also have a significant decrease in recurrence-free survival [41]. An independent study confirmed in two different cohorts that miR-96 also significantly correlates with overall survival of patients after RP, and it increases concomitantly with the World Health Organization grade of the samples [42].

Other studies failed to confirm these promising data. Mortensen et al later analyzed 35 patients with microdissected prostatectomies, 60% of whom had experienced recurrences; the median follow-up of those without recurrence was 66 mo [43]. Overall, 28 miRNAs were found to be upregulated in patients with recurrence, with miR-449b the most significant one, confirmed in a validation cohort of an additional 163 patients, although this was not identified in the previously cited studies.

More consistently, miR-21 has been proven to be overexpressed in recurrent PCa by multiple groups; for example, Leite et al [44] in 53 localized PCa cases divided into high or low grade, compared with BPH as control [45,46]. Because miR-21 is a known onco-miR

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deregulated in several types of cancer [47], it is not surprisingly that poor recurrence-free survival has been correlated to high levels of miR-21.

More recently, a predictive model for BCR that focused on miRNAs that are potentially predictive for patients who received salvage RT was reported [48]. Besides confirming previous data depicting an 88-miRNA signature, which could distinguish early from late biochemical failure patients, they identified nine miRNAs associated with BCR after salvage RT, and three of them were completely new (miR-1193, miR-4516, and miR-626). In line with previous studies analyzing tissue-specific miRNAs [26,27], Martens-Uzunova et al [49] detected a direct correlation between the downregulation of miRNA expression and tumor progression. They utilized Solexa Illumina Deep Sequencing and cross-validated the results with human miRNA V2 microarray analyses and with qRT-PCR in 87 PCas compared with 15 normal tissues (11 adjacent normal prostate and 4 nonmalignant transurethral prostatic resection). A panel of 25 miRNAs (13 downregulated and 12 upregulated in PCa) significantly correlated with poor clinical parameters, such as high Gleason score. Overall, 13 of these 25 deregulated miRNAs were further analyzed by Larne and colleagues [50] in a cohort of FFPE prostatic tissues derived from 49 PCa patients and 25 men without PCa. From the seven miRNAs differentially expressed in PCa samples, a combination of four miRNAs was chosen on the basis of the best diagnostic discrimination. These four miRNAs, two upregulated (miR-96-5p and miR-183-5p) and two downregulated (miR-145-5p and miR-221-5p), form a miR index quote (named miQ). The miQ was able to discriminate PCa from noncancer samples with high accuracy and able significantly to predict tumor aggressiveness and metastatic status. The predictive value of miQ was further validated in four different cohorts and, despite the differences in size, methodology, and experimental design, the results obtained indicate that miQ is superior to PSA in predicting diagnosis.

The miQ was further found to be a predictor of tumor aggressiveness and in the early organ-confined stages able to predict BCR after RP, indicating that miQ could represent the desired stratifying biomarker. In addition, miQ has higher accuracy in predicting aggressiveness than PSA with an AUC of 0.788, when clinical stage is ≥ T3, and it is associated with the metastatic status and could hence be a useful prognostic marker. Again, one of the miR's part of miQ is miR-221. Low miR-221 levels have not only been associated with PCa diagnosis [25,26,49,50], but recently also with a higher recurrence risk in 59 patients with malignant PCa who experienced BCR compared with 59 controls of matching age, race, pathologic stage, and grade [51].

3.3.2. Castration-resistant prostate cancer—Disease progression upon androgen depletion therapy is referred to as CRPC. It embodies a spectrum of different clinical presentations, ranging from rising PSA levels to manifestation of new metastases and the progression of preexisting tumors.

As for BCR, the oncogenic miR-21 has been associated with CRPC. The upregulation of miR-21 has been reported in tumor specimens [52] as well as in the plasma of patients prone to develop castration-resistant disease [53]. Besides miR-21, widely demonstrated to be a marker of aggressiveness and tumor recurrence, other miRNAs have been associated with the onset of CRPC. Even if the number of specimens analyzed was limited to 17, miR-221

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and -222 were found to be strongly upregulated in the prostatic tissue and bone marrow of CRPC patients when compared with normal prostate tissue [54], whereas miR-23b and miR-27b were downregulated.

Interestingly, most of the miRNAs found to be altered in CRPC are somehow linked to the androgen receptor (AR) pathway. The miRNAs can be regulated by AR while others act upstream regulating the AR at the mRNA or protein level. The miRNAs can be regulated by androgens through direct binding to androgen-responsive elements in the promoter (eg, for miR-21, where overexpression leads to inhibition of the transforming growth factor β pathway associated with chemoresistance) [55]. Direct regulation of AR transcripts by miRNAs has also been demonstrated [56]. In particular, among several potential miRNAs, miR-135b and miR-185 emerged as major AR regulators, both able to regulate AR by targeting its 3′ UTR [57–59]. Downregulation of miR-135b and miR-185 in PCa tissue has been widely demonstrated and could partly explain the common increased expression of the AR in CRPC.

In contrast, indirect regulation has been also observed for miR-141, whereas other miRNAs are deregulated through the reprogramming of downstream AR signaling (miR-221/miR-222) [57].

The formation of distant metastases is the ultimate feature of CRPC. The miR-221 has been highly correlated with metastasis formation [31] and, together with miR-141, could accurately distinguish between men with bone metastases compared with men with localized disease (AUC = 0.755). The miR-221 stands out as one of the most promising prognostic biomarkers for PCa, not only important as a promising diagnostic and prognostic tool [31,50], but also low miR-221 expression was shown to be associated with clinicopathologic factors including the Gleason score and clinical recurrence [58]. PCa patients with localized tumors expressing miR-221 at low levels have a greater risk for recurrence after surgery, most probably regulating proliferation, apoptosis, and invasion by inhibiting IRF2 and SOCS3 [59].

The importance of downregulation of the miR-23b/miR-27b cluster in metastatic CRPC has been pointed out by two different groups, which found it altered by analyzing different cohorts of PCa patients, speculating on their ability to negatively regulate invasion and anchorage-independent growth [60,61]. The miR-23b/27b cluster is under the direct transcriptional control of AR, and its re-expression in CRPC cell lines results in a signi cant attenuation of Rac1 activity and increased levels of the tumor suppressor E-cadherin, pointing out its role in regulating migration and invasion [61].

The analysis of potential markers for stratification of CRPC patients has also been extended to the alteration of exosomal miRNAs in plasma [35]. Exosomes are cell-derived vesicles present in many biologic fluids such as plasma and urine with diameters between 30 and 100 nm [62]. Plasma exosomal miR-1290 and miR-375 have been found to be promising prognostic biomarkers for CRPC patients, first in a discovery set of 23 specimens and then in a validation set of >100 samples.

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Although prospective validation is needed for a careful evaluation of these candidate miRNAs, the screening of the patients is definitely moving in this direction.

3.4. Role of microRNAs as markers for response to therapy

RT is the gold standard for localized high-risk PCa patients who cannot undergo RP, and testosterone suppression, RT, radium-223, and hormone therapy are the current treatments of choice for men with recurrent PCa that evolves toward a castration-resistant phenotype in most of the cases [63]. For men who are unresponsive to first and even secondary hormone therapy, which comprise competitive AR antagonists (enzalutamide) and steroidogenesis inhibitors (abiraterone), chemotherapy and immunotherapy are the only available options. The possibility of using miRNA measurement for patient-tailored therapy decisions such as the prediction of therapy response, drugs' optimal combination, and disease monitoring in case PSA is not a reliable marker for disease progression are the main focus in this field. To date, an increasing number of studies have been published regarding miRNAs as predictive markers for treatment choice. For instance, the importance of delineating a miRNA signature predictive of RT treatment response (ie, radiosensitivity) a priori would be helpful in managing RT treatment. Although the RT dose is standardized among patients, local recurrences can occur even in the modern era of dose escalation. The first evidence of a miRNA signature altered in response to RT was published in 2008 [64], depicting six miRs (miR-512, -196a, -133b, -143, -145b, and -218) as significantly downregulated in PCa cell lines, both androgen dependent or resistant. In particular, miR-521 was found to be downregulated to a greater extent, and its forced overexpression was able to sensitize cells in vitro to RT, opening the possibility of using miRNAs as therapeutic agents. Subsequently, using the same cellular system, Li et al [65] demonstrated that miR-106b could also be used for targeted therapy because its overexpression is sufficient to override cell cycle arrest in PCa cells following RT, through the regulation of its validated target p21 (*CDKN1A*). To note, this miRNA was previously found upregulated in PCa specimens [25]. More recently, another study confirmed the radiation-protective role of miR-106b in multiple PCa cells, also suggesting two additional miRNAs (miR-890 and miR-740-3p) as potent radiation sensitizers [66].

However, most of the miRNAs described display a limited clinical relevance as markers for radiation-response prediction because their potential role comes from in vitro observation in cell lines, and no clinical data are available from patients who have undergone external RT for PCa.

For more aggressive PCa, after RP and failure of androgen-deprivation therapies, possible alternatives include taxane-based chemotherapy, drugs targeting the AR (enzalutamide), or androgen synthesis (abiraterone acetate) [63]. However, approximately 40–50% of patients with CRPC invariably turn out to be nonresponders, with a median duration of remission of 6–9 mo [67]. As for RT, preliminary studies investigating the role of miRNAs as predictors of chemotherapy response are mostly performed on cell lines. Confirming the important role of miR-21 as an onco-miR, it has also been found to have a fundamental role in controlling the response to taxanes. Indeed, miR-21 is significantly higher in serum from patients with hormone-refractory PCa ($n = 10$) resistant to docetaxel-based chemotherapy when compared

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with those sensitive to chemotherapy (localized PCa $n = 20$, BPH $n = 6$) [68]. Starting from in vitro findings in docetaxel-resistant cell lines, Lin et al validated the association of a signature of six miRNAs (miR-200c, -200b, -146a, -222, -301b, and -20a) in the plasma and sera of 96 CRPC patients divided into responders and nonresponders, where resistant PCa was once considered with a rising PSA or clinical progression after maximal androgen blockade, with a minimum of 4 wk having elapsed between the withdrawal of antiandrogens and the commencement of chemotherapy [69].

A miRNA that seems to be promising for PCa therapy is miR-34a, due to its known correlation with *TP53.* In particular, Kojima et al reported the association between paclitaxel resistance and miR-34a in PCa cells through the modulation of the apoptotic pathway [70]. *TP53* transactivates the promoter region of miR-34a, and its upregulation leads to cell cycle arrest and the downstream modulation of different validated targets (ie, *CDK4, CDK6, CYCD1, E2F3*). The miR-34a has been associated with both apoptotic pathways [36,44,70] and with epithelial-mesenchymal transition in PCa cells [71]. The miR-34 genomic locus is frequently methylated in cancer, leading to downregulation of miR-34a [72]. Overexpression of both miR-34a and miR-34c results in increased p53-mediated apoptosis in response to doxorubicin treatment in PCa cell lines [73]. Nonetheless, miR-34a seems to be the more promising target because its role has been proved in xenograft experiments, where its reintroduction seem to decrease the growth of prostate xenografts [74].

4. Conclusions

The possible use of miRNAs in the clinic as diagnostic or prognostic factors for PCa is based on a growing body of investigations throughout the last decade. Active research is ongoing on the characterization of the miRNA profiles within different tumor subtypes, and there are still controversial results that delay the translation from bench to bedside. Nevertheless, miRNAs represent not only very promising candidate markers for urologic diseases but a potential therapeutic opportunity as well. As depicted in Figure 2 and summarized in Table 1, a consensus already exists regarding potential miRNAs that could be used in clinical practice. Several miRNAs have been found deregulated by different groups using different methodologies, offering encouraging results about the prospective use of miRNAs in the diagnosis, prognosis, and therapy of PCa patients.

Further studies developing guidelines on reporting and assay development are still needed. In particular, differences in study design, in methods of sample collection, and in the profiling platforms used should be minimized. Taken together, all these data underline how intriguing but complicated the miRNA field is, indicating that the use of miRNAs as markers and therapeutic targets is promising but not yet a reality in daily clinical practice.

References

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013; 49:1374–403. [PubMed: 23485231]
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65:5–29. [PubMed: 25559415]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Cooperberg MR, Broering JM, Litwin MS, et al. CapSURE Investigators. The contemporary management of prostate cancer in the United States: lessons from the cancer of the prostate strategic urologic research endeavor (CapSURE), a national disease registry. J Urol. 2004; 171:1393–401. [PubMed: 15017184]
4. Schroder FH, Hugosson J, Roobol MJ, et al. Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. Lancet. 2014; 384:2027–35. [PubMed: 25108889]
5. Dall'Era MA, Cooperberg MR, Chan JM, et al. Active surveillance for early-stage prostate cancer: review of the current literature. Cancer. 2008; 112:1650–9. [PubMed: 18306379]
6. Fraser M, Berlin A, Bristow RG, van der Kwast T. Genomic, pathological, and clinical heterogeneity as drivers of personalized medicine in prostate cancer. Urol Oncol. 2015; 33:85–94. [PubMed: 24768356]
7. Ambros V. The functions of animal microRNAs. Nature. 2004; 431:350–5. [PubMed: 15372042]
8. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. Int J Genomics. 2014; 2014:970607. [PubMed: 25180174]
9. Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell. 2008; 30:460–71. [PubMed: 18498749]
10. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science. 2007; 318:1931–4. [PubMed: 18048652]
11. Jansson MD, Lund AH. MicroRNA and cancer. Mol Oncol. 2012; 6:590–610. [PubMed: 23102669]
12. Rauhala HE, Jalava SE, Isotalo J, et al. miR-193b is an epigenetically regulated putative tumor suppressor in prostate cancer. Int J Cancer. 2010; 127:1363–72. [PubMed: 20073067]
13. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A. 2004; 101:2999–3004. [PubMed: 14973191]
14. Cho WC. MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. Int J Biochem Cell Biol. 2010; 42:1273–81. [PubMed: 20026422]
15. Kim WT, Kim WJ. MicroRNAs in prostate cancer. Prostate Int. 2013; 1:3–9. [PubMed: 24223395]
16. Fang YX, Gao WQ. Roles of microRNAs during prostatic tumorigenesis and tumor progression. Oncogene. 2014; 33:135–47. [PubMed: 23455326]
17. Kristensen H, Haldrup C, Strand S, et al. Hypermethylation of the GABRE~miR-452~miR-224 promoter in prostate cancer predicts biochemical recurrence after radical prostatectomy. Clin Cancer Res. 2014; 20:2169–81. [PubMed: 24737792]
18. Song C, Chen H, Wang T, Zhang W, Ru G, Lang J. Expression profile analysis of microRNAs in prostate cancer by next-generation sequencing. Prostate. 2015; 75:500–16. [PubMed: 25597612]
19. Lan H, Lu H, Wang X, Jin H. MicroRNAs as potential biomarkers in cancer: opportunities and challenges. BioMed Res Int. 2015; 2015:125094. [PubMed: 25874201]
20. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008; 18:997–1006. [PubMed: 18766170]
21. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011; 8:467–77. [PubMed: 21647195]
22. Schwarzenbach H, Machado da Silva A, Calin G, Pantel K. Data normalization strategies for microRNA quantification. Clin Chem. 2015; 61:1333–42. [PubMed: 26408530]
23. Mall C, Rocke DM, Durbin-Johnson B, Weiss RH. Stability of miRNA in human urine supports its biomarker potential. Biomark Med. 2013; 7:623–31. [PubMed: 23905899]
24. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006; 103:2257–61. [PubMed: 16461460]
25. Ambs S, Prueitt RL, Yi M, et al. Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res. 2008; 68:6162–70. [PubMed: 18676839]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

26. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res. 2007; 67:6130–5. [PubMed: 17616669]
27. Ozen M, Creighton CJ, Ozdemir M, Ittmann M. Widespread deregulation of microRNA expression in human prostate cancer. Oncogene. 2008; 27:1788–93. [PubMed: 17891175]
28. Moltzahn F, Olshen AB, Baehner L, 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. [PubMed: 21098088]
29. Bryant RJ, Pawlowski T, Catto JW, et al. Changes in circulating microRNA levels associated with prostate cancer. Br J Cancer. 2012; 106:768–74. [PubMed: 22240788]
30. Chen ZH, Zhang GL, Li HR, et al. A panel of five circulating microRNAs as potential biomarkers for prostate cancer. Prostate. 2012; 72:1443–52. [PubMed: 22298030]
31. Yaman Agaoglu F, Kovancilar M, Dizdar Y, et al. Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. Tumour Biol. 2011; 32:583–8. [PubMed: 21274675]
32. Brase JC, Johannes M, Schlomm T, et al. Circulating miRNAs are correlated with tumor progression in prostate cancer. Int J Cancer. 2011; 128:608–16. [PubMed: 20473869]
33. Haldrup C, Kosaka N, Ochiya T, et al. Profiling of circulating microRNAs for prostate cancer biomarker discovery. Drug Deliv Transl Res. 2014; 4:19–30. [PubMed: 25786615]
34. Kachakova D, Mitkova A, Popov E, et al. Combinations of serum prostate-specific antigen and plasma expression levels of let-7c, miR-30c, miR-141, and miR-375 as potential better diagnostic biomarkers for prostate cancer. DNA Cell Biol. 2015; 34:189–200. [PubMed: 25521481]
35. Huang X, Yuan T, Liang M, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol. 2015; 67:33–41. [PubMed: 25129854]
36. Corcoran C, Rani S, O'Driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression. Prostate. 2014; 74:1320–34. [PubMed: 25053345]
37. Korzeniewski N, Tosev G, Pahernik S, Hadaschik B, Hohenfellner M, Duensing S. Identification of cell-free microRNAs in the urine of patients with prostate cancer. Urol Oncol. 2015; 33:16, e7–22. [PubMed: 25445383]
38. Haj-Ahmad TA, Abdalla MA, Haj-Ahmad Y. Potential urinary miRNA biomarker candidates for the accurate detection of prostate cancer among benign prostatic hyperplasia patients. J Cancer. 2014; 5:182–91. [PubMed: 24563673]
39. Heidenreich A, Bastian PJ, Bellmunt J, et al. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol. 2014; 65:467–79. [PubMed: 24321502]
40. Tong AW, Fulgham P, Jay C, et al. MicroRNA profile analysis of human prostate cancers. Cancer Gene Ther. 2009; 16:206–16. [PubMed: 18949015]
41. Schaefer A, Jung M, Mollenkopf HJ, et al. Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. Int J Cancer. 2010; 126:1166–76. [PubMed: 19676045]
42. Haflidadottir BS, Larne O, Martin M, et al. Upregulation of miR-96 enhances cellular proliferation of prostate cancer cells through FOXO1. PloS One. 2013; 8:e72400. [PubMed: 23951320]
43. Mortensen MM, Hoyer S, Orntoft TF, Sorensen KD, Dyrskjot L, Borre M. High miR-449b expression in prostate cancer is associated with biochemical recurrence after radical prostatectomy. BMC Cancer. 2014; 14:859. [PubMed: 25416653]
44. Leite KR, Reis ST, Viana N, et al. Controlling RECK miR21 promotes tumor cell invasion and is related to biochemical recurrence in prostate cancer. J Cancer. 2015; 6:292–301. [PubMed: 25663948]
45. Li T, Li RS, Li YH, et al. miR-21 as an independent biochemical recurrence predictor and potential therapeutic target for prostate cancer. J Urol. 2012; 187:1466–72. [PubMed: 22341810]
46. Melbo-Jorgensen C, Ness N, Andersen S, et al. Stromal expression of MiR-21 predicts biochemical failure in prostate cancer patients with Gleason score 6. PloS One. 2014; 9:e113039. [PubMed: 25401698]
47. Selcuklu SD, Donoghue MT, Spillane C. miR-21 as a key regulator of oncogenic processes. Biochem Soc Trans. 2009; 37:918–25. [PubMed: 19614619]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

48. Bell EH, Kirste S, Fleming JL, et al. A novel miRNA-based predictive model for biochemical failure following post-prostatectomy salvage radiation therapy. PloS One. 2015; 10:e0118745. [PubMed: 25760964]
49. Martens-Uzunova ES, Jalava SE, Dits NF, et al. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. Oncogene. 2012; 31:978–91. [PubMed: 21765474]
50. Larne O, Martens-Uzunova E, Hagman Z, et al. miQ--a novel microRNA based diagnostic and prognostic tool for prostate cancer. Int J Cancer. 2013; 132:2867–75. [PubMed: 23184647]
51. Zheng Q, Peskoe SB, Ribas J, et al. Investigation of miR-21, miR-141, and miR-221 expression levels in prostate adenocarcinoma for associated risk of recurrence after radical prostatectomy. Prostate. 2014; 74:1655–62. [PubMed: 25252191]
52. Ribas J, Ni X, Haffner M, et al. miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. Cancer Res. 2009; 69:7165–9. [PubMed: 19738047]
53. Shen J, Hruby GW, McKiernan JM, et al. Dysregulation of circulating microRNAs and prediction of aggressive prostate cancer. Prostate. 2012; 72:1469–77. [PubMed: 22298119]
54. Sun T, Yang M, Chen S, 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. 2012; 72:1093–103. [PubMed: 22127852]
55. Jalava SE, Urbanucci A, Latonen L, et al. Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer. Oncogene. 2012; 31:4460–71. [PubMed: 22266859]
56. Ostling P, Leivonen SK, Aakula A, et al. Systematic analysis of microRNAs targeting the androgen receptor in prostate cancer cells. Cancer Res. 2011; 71:1956–67. [PubMed: 21343391]
57. ChunJiao S, Huan C, ChaoYang X, GuoMei R. Uncovering the roles of miRNAs and their relationship with androgen receptor in prostate cancer. IUBMB Life. 2014; 66:379–86. [PubMed: 24979663]
58. Spahn M, Kneitz S, Scholz CJ, et al. Expression of microRNA-221 is progressively reduced in aggressive prostate cancer and metastasis and predicts clinical recurrence. Int J Cancer. 2010; 127:394–403. [PubMed: 19585579]
59. Kneitz B, Krebs M, Kalogirou C, 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; 74:2591–603. [PubMed: 24607843]
60. Goto Y, Kojima S, Nishikawa R, et al. The microRNA-23b/27b/24–1 cluster is a disease progression marker and tumor suppressor in prostate cancer. Oncotarget. 2014; 5:7748–59. [PubMed: 25115396]
61. Ishteiwy RA, Ward TM, Dykxhoorn DM, Burnstein KL. The microRNA -23b/-27b cluster suppresses the metastatic phenotype of castration-resistant prostate cancer cells. PloS One. 2012; 7:e52106. [PubMed: 23300597]
62. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: from biogenesis and secretion to biological function. Immunol Lett. 2006; 107:102–8. [PubMed: 17067686]
63. Loblaw DA, Walker-Dilks C, Winquist E, Hotte SJ. Genitourinary Cancer Disease Site Group of Cancer Care Ontario's Program in Evidence-Based Care. Systemic therapy in men with metastatic castration-resistant prostate cancer: a systematic review. Clin Oncol (R Coll Radiol). 2013; 25:406–30. [PubMed: 23587782]
64. Josson S, Sung SY, Lao K, Chung LW, Johnstone PA. Radiation modulation of microRNA in prostate cancer cell lines. Prostate. 2008; 68:1599–606. [PubMed: 18668526]
65. Li B, Shi XB, Nori D, et al. Down-regulation of microRNA 106b is involved in p21-mediated cell cycle arrest in response to radiation in prostate cancer cells. Prostate. 2011; 71:567–74. [PubMed: 20878953]
66. Hatano K, Kumar B, Zhang Y, et al. A functional screen identifies miRNAs that inhibit DNA repair and sensitize prostate cancer cells to ionizing radiation. Nucleic Acids Res. 2015; 43:4075–86. [PubMed: 25845598]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

67. Tannock IF, de Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 2004; 351:1502–12. [PubMed: 15470213]

68. Zhang HL, Yang LF, Zhu Y, 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. [PubMed: 20842666]

69. Lin HM, Castillo L, Mahon KL, et al. Circulating microRNAs are associated with docetaxel chemotherapy outcome in castration-resistant prostate cancer. Br J Cancer. 2014; 110:2462–71. [PubMed: 24714754]

70. Kojima K, Fujita Y, Nozawa Y, Deguchi T, Ito M. MiR-34a attenuates paclitaxel-resistance of hormone-refractory prostate cancer PC3 cells through direct and indirect mechanisms. Prostate. 2010; 70:1501–12. [PubMed: 20687223]

71. Liang J, Li Y, Daniels G, et al. LEF1 targeting EMT in prostate cancer invasion is regulated by miR-34a. Mol Cancer Res. 2015; 13:681–8. [PubMed: 25587085]

72. Cheng CY, Hwang CI, Corney DC, et al. miR-34 cooperates with p53 in suppression of prostate cancer by joint regulation of stem cell compartment. Cell Rep. 2014; 6:1000–7. [PubMed: 24630988]

73. Rokhlin OW, Scheinker VS, Taghiyev AF, Bumcrot D, Glover RA, Cohen MB. MicroRNA-34 mediates AR-dependent p53-induced apoptosis in prostate cancer. Cancer Biol Ther. 2008; 7:1288–96. [PubMed: 18497571]

74. Yamamura S, Saini S, Majid S, et al. MicroRNA-34a suppresses malignant transformation by targeting c-Myc transcriptional complexes in human renal cell carcinoma. Carcinogenesis. 2012; 33:294–300. [PubMed: 22159222]

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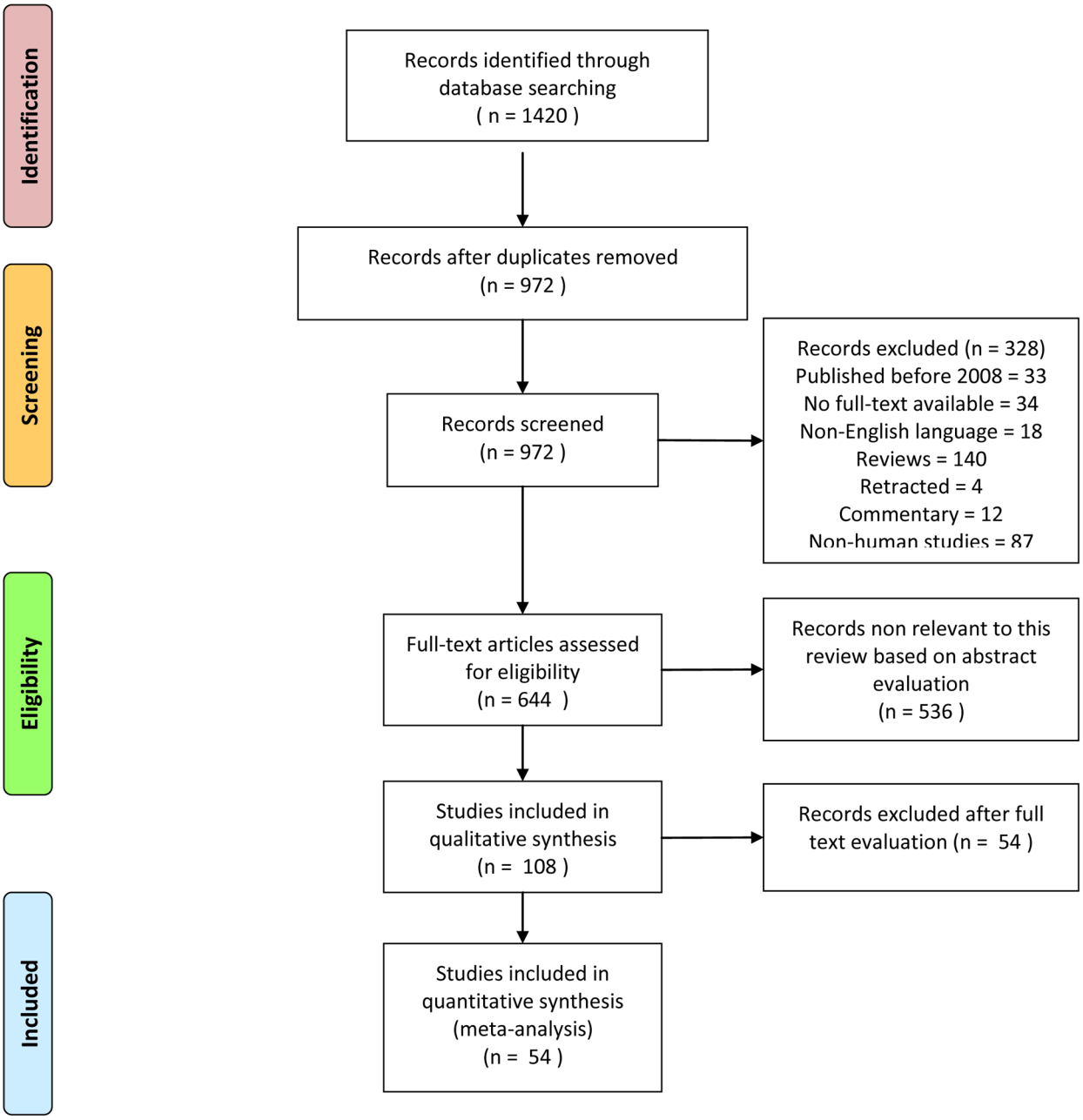

Fig. 1.

Flow diagram for Preferred Reporting Items for Systematic Reviews and Meta-analysis showing the literature selection process resulting in the studies included in the review.

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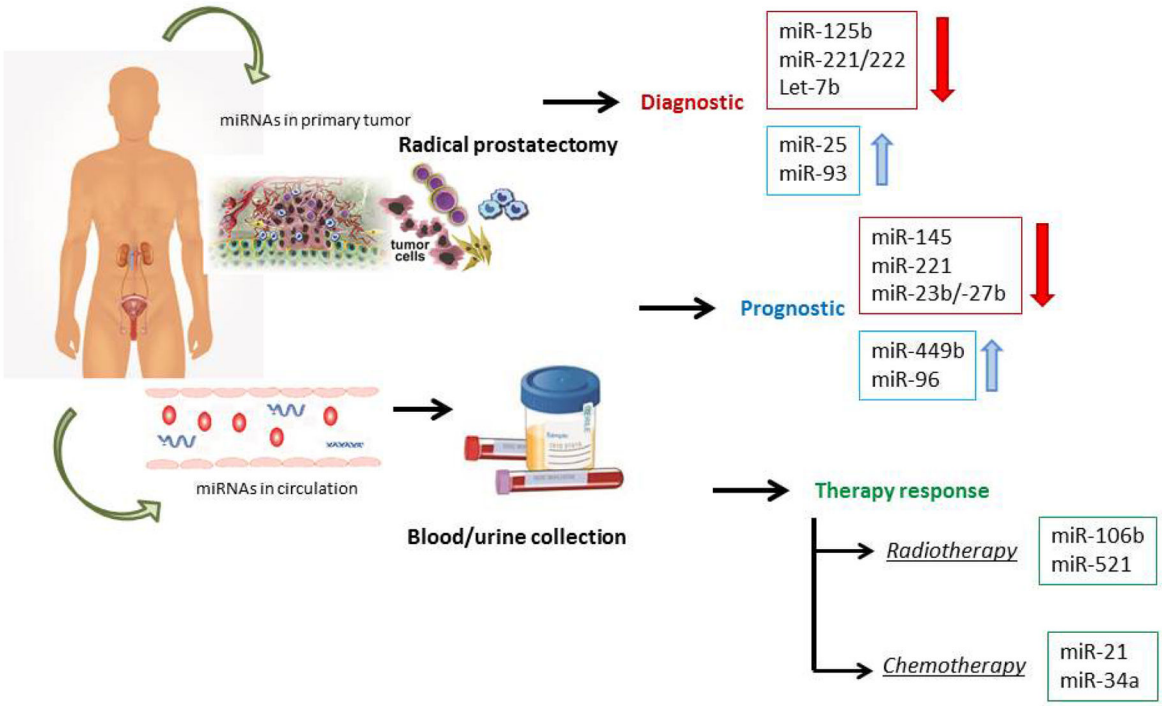

Fig. 2.

Schematic representation depicting the potential source of microRNAs in prostate cancer and their role as prognostic or diagnostic tools or as markers for therapy response. miRNA = microRNA.

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Table 1

MicroRNA (miRNA) expression in prostate cancer tissue: miRNAs consistently altered, including their potential messenger RNA target and affected pathways

	Volinia et al [24]	Ambs et al [25]	Porkka et al [26]	Ozen et al [27]	Martens-Uzunova et al [49]	Larne et al [50]	Putative target	Altered function
Let-7b		Downregulation	Downregulation	Downregulation	Downregulation		Ras, CycD1	Cell cycle, apoptosis
miR-125b			Downregulation	Downregulation			*BAK1*	Apoptosis, AR
miR-145		Downregulation	Downregulation	Downregulation	Downregulation	Downregulation	*MYC, MYO6*	Metastasis, apoptosis
miR-205		Downregulation	Downregulation				*E2F1, ZEB2*	Cell proliferation, EMT
miR-221		Downregulation	Downregulation		Downregulation	Downregulation	p27kip1	Cell cycle
miR-222			Downregulation		Downregulation	Downregulation	p27kip1	Cell cycle
miR-25	Up	Upregulation			Upregulation		*PTEN*	Proliferation
miR-93	Up	Upregulation			Upregulation		*LATS2*	Metastasis

AR = androgen receptor; EMT = epithelial–mesenchymal transition.

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Table 2

MicroRNAs consistently altered in the serum of prostate cancer patients and the related pathways consequently altered

		Trend	Putative targets	Pathway	References
Plasma	miR-141	Up	*SHP*	AR	[31,33,36]
	miR-375	Up	*MYC, CCND2*	Proliferation	[31,33,34,36,37]
	miR-221	Up	p27kip1	Cell cycle, AR	[29,33]
	miR-181a	Down	*LEF1, BCL2*	EMT, apoptosis	[31]
	miR-21	Up	p57kip2, *PTEN*	Proliferation	[33,50,51]

AR = androgen receptor; EMT = epithelial–mesenchymal transition.

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