Circulating MicroRNA as Biomarkers: An Update in Prostate Cancer

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Abstract

Prostate cancer (PCa) is the most common non-skin cancer among men. Currently available diagnostic tests for PCa are inadequate in terms of low specificity and poor sensitivity. microRNAs offer a hope to overcome these drawbacks by virtue of their cancer specific expression and high stability. They can readily be detected and quantified in frozen and as well as formalin-fixed paraffin-embedded tissues. Observation of circulating miRNA in serum/plasma samples and other body fluids holds a promise to quickly move from research and provide a biomolecule of clinical relevance and an improvement over presently available biomarkers. This review highlights the potential role of circulating miRNAs as molecular markers for cancer and as targets for therapeutic manipulation. Further, this review summarizes the current understanding of various circulating miRNA with respect to prostate cancer. To conclude, circulating miRNAs are an active area of current investigation and holds promise to serve a wide range of clinical applications and unwrap a new era in cancer diagnosis and therapeutics.

Keywords

miRNA; cancer; Prostate; Circulating miRNA; Biomarker

Introduction

Prostate cancer (PCa) is of increasing significance worldwide due to the increasing aging population. In the U.S. alone, 1 in 6 men will develop prostate cancer in their lifetime; 1 in 30 men will die of this disease. According to the American Cancer Society, PCa is a major health concern for U.S. men resulting in approximately 218,000 new cases and about 32,000 deaths in 2010 (1). The incidence rate of prostate cancer varies widely between the continents; it is least common in South and East Asia, more common in Europe, and most common in the United States. In spite of the low known incidence of prostate cancer in developing countries, its incidence and mortality tends to increase continually (2).

Prostate cancer is a disease of the elderly and tends to develop in men over the age of fifty. During its early development there are no clinical symptom, however if detected at this

stage, surgery is highly curative (3–8). Due to slow-growing propensity of this disease, many men never manifest these symptoms, undergo no therapeutic treatment, and eventually die of other causes. However, in a subset of patients, the disease does progresses causing local symptoms such as urinary retention and symptoms from metastases such as bone pain. Late diagnosis at the time of symptomatic progression is the main reason for mortality from this disease. While surgery has proven to be effective in treating patients with localized disease, the survival rates for patients who have metastatic prostate cancer is low.

Current diagnostic strategies for prostate cancer

Routinely performed tests for early detection of PCa include digital rectal examinations (DREs) and prostate-specific antigen (PSA) testing. PSA testing is non-specific as elevated PSA levels due to benign prostatic hyperplasia (BPH), infection, and/or chronic inflammation may lead to confounding results (9, 10). Low sensitivity (DRE) or low specificity (PSA testing) of these tests restricts its diagnostic value (11). Clinical trials indicate that although PSA screening greatly facilitates the early diagnosis of PCa and helps identify the increased PCa rate, whether this screening procedure significantly lowers the PCa mortality remains a question of debate (12, 13). A recent meta-analysis concluded that routine screening with either a DRE or PSA provides no benefit and does not affect PCa mortality (14). Additional biomarkers have been proposed including PSA derivatives such as total PSA velocity (total PSAV) and different molecular forms (i.e. free PSA, BPSA, pro-PSA, and intact PSA) (15). Other blood based biomarkers such as human glandular kallikrein 2 (hK2), urokinase plasminogen activator (uPA) and its receptor (uPAR), transforming growth factor-beta 1 (TGF- β 1); interleukin-6 (IL-6) and its receptor (IL-6R) have been studied alone or in combination with PSA and have been suggested to help in diagnosis, staging, prognostication, and monitoring of prostate cancer. Still, there is need to develop novel biomarkers that can aid in clinical decision making about the timing of biopsy and the necessity of treatment (16).

microRNAs as the next-generation gene regulators and biomarkers

In the past few years, several studies have emphasized the importance of gene structure and function in regulating disease progression in general and susceptibility and risk factors in particular. The mechanisms of tumor initiation and development and its risk factors could not be explained only by involvement of protein coding genes alone and gene mutations thus emerged the idea of non-coding RNAs and their regulatory function in multiple genes, either as tumor suppressor genes or oncogenes in different cancers including prostate cancer. Among a number of non-coding RNAs, role of microRNAs (miRNAs) has been established to play role in cancer initiation, development, maintenance, and proliferation.

MicroRNAs (miRNAs) are a class of 19–23 nucleotide long, endogenous non-coding RNA molecules that are frequently dysregulated in cancer. These miRNA modulate the activity of transcriptome by binding to the 3'-untranslated regions of target mRNA sequences in various human cancers (17, 18). Oncogenic miRNA also known as oncomirs, are often up-regulated and and they degrade or block the translation of tumor suppressor gene mRNAs. Conversely, down-regulation of tumor suppressor miRNA promotes tumor progression. Although, miRNAs comprises of ~3% of human encoded genes, more than 30% of mRNAs are regulated by miRNAs (19). Currently, there are ~1,424 mature human miRNA sequences listed in the miRNA registry (http://www.mirbase.org/) obtained from various human malignancies and other diseases (14, 20). These miRNAs have been shown to play an important regulatory role in a wide range of biological and pathological processes including cellular proliferation, differentiation, metastasis, or apoptosis (19, 21).

The miRNAs have been found to express in specific pattern in the course of development in specific tissues. Functional involvement of *lin-4* and *let-7* miRNAs in the developmental process is apparent by their control on the larval developmental timing in *C. elegans.* miRNAs have also shown to be involved in neuronal, muscle, and germline development and embryonic morphogenesis (22). All these studies signify the importance of strictly regulated miRNA homeostasis for normal development. miRNAs also actively participates in stem cell regulation and have specific roles in controlling stem cell differentiation and renewal. Embryonic stem cells have shown to have distinct miRNA signatures. Decreased expression of mir-290~295 clusters and mir-296 was observed during stem cell differentiation, whereas mir-21 and mir-22 were up regulated during the process (23). In somatic tissue stem cells also miRNAs regulate various steps of hematopoiesis, recognizes cell fate during neural development, myogenesis modulation, cardiogenesis, etc (24). miRNAs have also been shown to direct the mammalian immune system and defective miRNA machinery may result in development of severely compromised immune system which can readily lead to immune disorders like autoimmunity and cancer (25). Many miRNAs, including miR-15a/miR-16-1, miR-21, miR-155, miR-200 family, miR-210, miR-205 miR-221 have been found to be differentially expressed in various tumor cells (26–38). Studies focusing on the profiling of these miRNAs on various cancer stages could be exploited as signature for a particular clinical outcome.

Although the origin of these miRNAs in body fluids remain elusive, recent studies have identified distinct miRNA expression in serum and other body fluids of cancer patients and have suggested their usefulness as molecular targets and as potential predictors of preneoplasia to cancer. These circulating miRNA biomarkers could improve on current strategies for cancer detection in cancer patients and can be used as novel noninvasive fingerprints which can readily be used for early diagnosis of various cancers including prostate cancer. Although knowledge on non-coding RNAs is limited, they are essential part in of cells' built-in mechanisms to achieve gene regulation and offer new revenue as next generation of biomarkers.

miRNAs as circulating biomarker

A tumor biomarker should identify the presence of a tumor before it could otherwise be easily detected and the ability of these biomarkers to detect cancers at early stages is a key factor to increase the overall survival. The existence of circulating miRNAs in serum and their possibility to be developed as cancer markers was reported by Lawrie et al. (39). Circulating miRNAs have recently been indicated as practicable and promising biomarkers for non-invasive diagnosis in various tumors (40). In general, the blood based biomarkers, including antigens, enzymes, and lipid components are very useful for prognosis, monitoring the effectiveness of treatments, prediction of recurrence risk, and also to monitor the recurrence of the disease. With respect to tumors, there are very few blood-based biomarkers. Some of the currently available serum/plasma biomarkers, such as carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1) and alphafetoprotein (AFP) offer some promise to analyze tumors systematically without involving the tissue biopsies or surgery (41–43). However, clinical implementation of these biomarkers at a broader level faces some major challenges like their low sensitivities and specificities. The prostate specific antigen (PSA) has been shown to be a sensitive serum marker for prostate cancer but the specificity is inadequate and the test demonstrates a high frequency of falsely elevated values in men with benign prostatic hyperplasia (44).

Utility of minimally invasive serum biomarkers in the form of DNA and RNA fragments in serum for various health conditions is well documented (45). In 1977, Leon et al (46) first observed that concentrations of cell-free circulating DNA (cirDNA) in blood of cancer patients is higher as compared to normal subjects. Since then, various studies have

demonstrated elevated level of cell-free DNA concentration in plasma/serum in various pathological conditions, including cancer, autoimmune disorders, pregnancy and trauma (47–49). Recent discoveries on miRNAs in serum have stirred the demanding biomarker discovery field to identify reliable markers for early disease detection and classification. Serum miRNAs are more resistant to RNase digestion as compared to tissue or cellular miRNAs and shown to be stable, reproducible, and consistent among individuals of same species (49, 50). Presence of miRNAs has been observed in various other body fluids including serum, plasma, urine, saliva and breast milk (51). The expression of these circulating miRNAs in serum, plasma, and other body fluids proclaim enormous potential to be used as novel minimally invasive biomarkers in diagnosing and monitoring human cancers. Recently, some circulating miRNAs in the blood have been successfully revealed as biomarkers for several cancers, cardiovascular disease, brain injury and liver injury.

Despite of the promising role of these circulatory miRNAs, little is known about their origin and function in extracellular environment. These extracellular miRNAs have been detected in exosomes isolated from peripheral blood and culture media of several cell lines. However, there are blood/plasma and cells culture studies that suggest that more than 97% of these miRNA amounts are exosome free and emphasize that these miRNAs are predominantly exported in exosome-independent form. It remains indistinct if all extracellular miRNAs are coupled with exosomes and whether the physiologically available amount of extracellular miRNA are adequate for cell-to-cell signaling (52). Moreover, many miRNAs in blood plasma and cell culture are bound to Ago2 protein, a RNA-induced silencing complex (RISC). Other studies suggest that some extracellular miRNAs may bind to other Ago proteins such as Ago1, Ago3 and Ago4 indicating that large amount of circulating miRNAs might be by-products of dead/dying cells which persist due to the stability of the miRNA/Ago2 complex. These Ago2/miRNAs complexes are highly nuclease/protease resistant and this unusual stability of intracellular miRNA supports the hypothesis that large portion of extracellular miRNA might be the by-products of dead/dying cells (52).

Expression of circulatory miRNAs and cancer

The current understanding of miRNA function in human carcinogenesis suggests that they play a dual role and may act either as oncogenic or as tumor suppressor miRNAs (53–55). This dual role of miRNAs is strengthened by the fact that up or down regulated expression pattern of miRNAs in human cancers can be observed as compared to normal tissues (56). A significant reduction or elevation of specific miRNA level in serum/plasma of cancer patients as compared to healthy controls and their correlation with the surgical treatment of the tumor signifies the potential link between miRNA expression levels and primary tumors. Systematically analyzing the expression pattern of various miRNAs in human cancers can also provide significant understanding of origin of an unknown primary carcinoma (57). In one of the pioneer studies considering miRNA levels in serum samples, Lawrie et al. (58) suggested that serum miR-21 levels are associated with relapse-free survival in patients with diffuse large B-cell lymphoma and therefore holds potential to be a diagnostic biomarker for the disease. Heneghan et al (59) showed that cancer-specific miRNAs are detectable and significantly altered (miR-195 and *let*-7a) in the circulation of breast cancer patients, and that increased systemic miR-195 levels in breast cancer patients were also reflected in breast tumors. Further, they also observed a correlation of specific circulating miRNAs with various pathological variables such as nodal status, estrogen receptor status etc. In an attempt to investigate the role of serum miRNA in predicting prognosis of non–small-cell lung cancer (NSCLC), Hu et al. (60) performed genome-wide serum miRNA expression analysis and showed that serum miRNA signatures (miR-486, miR-30d, miR-1 and miR-499) may help in the prediction of overall survival in non-small cell lung cancer.

All these initial findings highlighted the potential of circulating miRNA and their diagnostic importance in cancer. There has been a vast inflow of information on differentially regulated miRNAs in various cancers and to date many miRNAs have been assigned to be up or down regulated in wide range of cancers. Covering this vast body of work is beyond the scope of this review. Therefore we tried to highlight the differentially expressed circulatory miRNA's in various cancers with a special emphasis on prostate cancer.

Altered expression of *let*-7, miR-25 and miR-223 miRNAs in serum has been observed in lung cancer patients while miR-15/16 were found to be down regulated in leukemia patients (27, 61). miR-210, miR-200a and miR-200b have been shown to be overexpressed in patients with pancreatic cancer (62–65). In case of pancreatic ductal adenocarcinoma, a collective analysis of four differentially expressed miRNAs (miR-21, miR-210, miR-155, and miR-196a) in plasma has been suggested to discriminate patients from healthy individuals (64). miR-184 was upregulated in plasma of patients with tongue squamous cell carcinoma (66). *let*-7a miR-21 and miR-195 were observed to be upregulated in the breast cancer (59, 67). In gastric cancer miR-17-5p, miR-21, miR-106a, miR-106b are known to be upregulated (64)while *let*-7a expression was down regulated (68, 69). miR-155 was differentially expressed in the serum of women with hormone-sensitive as compared to hormone-insensitive breast cancer patients (70). The level of miR-500 in the sera of the hepatocellular carcinoma (HCC) patients was highly elevated pre-surgery and which returned to normal level after the surgery, whereas lowered expression of miR-92a to miR-638 in plasma of HCC subjects was observed as compared to controls (71, 72). In colorectal cancer, miR-17-3p and miR-92 levels were elevated significantly in plasma of matched patient samples prior to surgery compared to post surgery (73, 74). Decreased expression of miR-92a in plasma was also observed in acute leukemia patients (75). In ovarian cancer miR-21, -29a, -92, -93, and -126 were significantly overexpressed in serum from cancer patients as compared to healthy controls (76–78). Also, miR-155, miR-210, and miR-21 were found to be elevated in the patients' serum of diffuse large B-cell lymphoma (DLBCL) as compared to healthy controls (58).

Beside blood/ plasma/ serum, miRNAs (>200 in each fluid type) can also be detected in other body fluids such as saliva, urine, pleural effusion, amniotic fluid, breast milk, tears, seminal fluid, peritoneal fluid, colostrums, cerebrospinal fluid, bronchial lavage (79). The amount of miRNAs detected in urine, CSF, and pleural fluid, were reported to be much lower compared to relatively high concentration found in tears (79). Studies of saliva samples obtained from oral squamous cell carcinoma (OSCC) patients showed miR-31was significantly higher relative to non-cancer patients, and the levels decreased to normal after tumor resection in eight of nine patients (80). Park et al., reported that levels of miR-125a and miR-200a are significantly lower in the saliva of OSCC patients as compared to non-cancer patients (81). A recent study by Yamada et al., (82) demonstrated that miR-96 and miR-183 expression in the urine samples was significantly higher in urothelial carcinoma (UC) and theses miRNAs can distinguish UC patients from non-UC patients with good sensitivity and specificity (miR-96, 71.0% and 89.2%; and miR-183, 74.0% and 77.3%). Although the mechanisms of these miRNAs are not well defined, their discovery in several diseased conditions in various body fluids may also serve as determinants of disease origin and cancer subtype. In addition they may also provide information of tumor environments and can help to identify novel and important oncogenic pathways (83).

Circulating miRNAs and prostate cancer

miRNA signature specific for prostate cancer were identified by miRNA expression profiling from various sources including prostate cancer cell lines, xenografts samples, benign prostatic hyperplasia (BPH), and prostate carcinoma (84). Studies of oligonucleotide

array hybridization miRNA profiling have identified, ~51 miRNAs were differentially expressed between benign and malignant prostate tumors, of which 37 were down-regulated and 14 upregulated. These differentially regulated miRNAs lead to alteration in the expression and activity of their targets in prostate cancer (84). In another elegant study demonstrating the significance of miRNA dysregulation in PCa, Ambs et al., suggested that microRNA expression alters with the development and progression of prostate cancer and some of the cancer-related genes are regulated by microRNAs (85). Although, few miRNAs have been experimentally confirmed to be involved in initiation, progression and metastasis of prostate cancer they are relatively less defined in case of prostate cancer. Nonetheless emerging data clearly suggest that miRNA expression is significantly deregulated in prostate cancer.

Using a mouse xenograft model, Mitchell et al (86) for the first time demonstrated that miRNAs which were originated from the human prostate cancer xenografts enter the circulation even when originated from epithelial cancer types. The differentially expressed miRNAs levels due to xenograft can be easily evaluated. They further demonstrated that miR-141 is highly overexpressed in sera of metastatic prostate cancer patients which can distinguish prostate cancer patients from healthy controls with high sensitivity and accuracy. In concordance with these observations, Lodes et al (87) showed differential modulation of serum miRNAs in PCa patients and a study of plasma samples from prostate cancer patients reported that plasma miR-141 levels can be used to screen for metastatic prostate cancer with high sensitivity. Recently a study by Brace et al (88) also showed that among the circulating miRNAs, miR-375 and miR-141 turned out to be the most pronounced markers for high-risk tumors. Both these miRNAs were further studied in prostate tissue samples and were observed to be significantly upregulated. The investigators concluded that miR-375 and miR-141 expression is enhanced in prostate cancer specimens and their release into the blood is further associated with advanced cancer disease (88). In order to analyse the expression pattern of circulating miRNA in serum with localized prostate cancer (PCA), benign prostate hyperplasia (BPH) and healthy individuals, Mahn et al (19) investigated the expression pattern of selected oncogenic miRNAs in serum and demonstrated that miR-26a level was able to discriminate between PCA and BPH patients (sensitive 89% and 56% specificity). Also, they witnessed that the tissue miRNA levels correlated with preprostatectomy miRNA levels in serum, and serum miRNA decreased after the surgery, indicating a tumor associated release of miRNA (19). Although, there are some recent advancements considering circulating miRNA in prostate cancer, a more robust approach is needed to establish the reliable miRNA based early diagnosis in PCa to effectively reduce the severity of the disease caused due to late diagnosis. A list of identifies circulating miRNA in prostate cancer is provided in Table 1.

MicroRNAs as therapeutic target

In addition to biomarkers, miRNA offer profound potential as a new class of therapeutic which can be estimated by the ongoing Phase 2 clinical trials within 10 years of their discovery. The underlying principle of the miRNA based therapeutics is identification and validation of disease associated miRNA targets which can be manipulated for therapeutic purposes. Theoretically the miRNAs based therapeutics follow two basic approaches of miRNA antagonists and miRNA mimics. MiRNA antagonists are used to reduce the level of the miRNAs which show the gain-of-function with respect to the disease whereas; miRNA mimics are used to restore a loss of function also known as 'miRNA replacement therapy'. However, prior to implementation, a thorough examination of theses miRNA based therapeutics needs to be evaluated to assure their efficacy, specificity and toxicity. The miR antagonists are highly chemically-modified miRNAs' that binds with active miRNA with high affinity to form miRNA duplex which is unable to be processed by RISC complex and

is degraded. However, the major drawback faced by the miR antagonists is the possibility of their non-specific binding to other RNAs that could lead to undesired side effects. In contrast miRNA mimics are more likely to be highly specific and well tolerate in normal tissues as they are identical to naturally occurring miRNA and are expected to target the same set of genes. Importance of miRNA replacement therapy is supported by various studies in which they used mimics of tumor suppressor miRNAs that stimulated anti-oncogenic pathway leading to apoptosis and therefore eradication of tumor cells. (89–91).

Some of the miRNA that are already in the process of therapeutic development include antagonist miR-122 for Hepatitis, miR-208/499 in chronic heart failure and miR-195 in post myocardial infarction remodeling which are in the preclinical development or in phase 2 clinical trials. In case of therapeutic development in miRNA replacement based therapies, miR-34 and *let*-7 are in the preclinical developmental stage to target cancer. Therapeutic delivery of *let*-7 (mimics or viruses) has shown to significantly inhibit the tumor growth in human non-small cell lung cancer xenografts and KRAS-G12D transgenic mouse model (92). Similarly, delivery of miR-34 mimic blocked lung and prostate tumor growth in mouse models (91, 93) by enhancing the apoptotic activity of tumor cells and by specific repression of CDK4, Met and BCL2 (91). Remarkably, the delivery of this mimic did not showed any adverse immunological response as no elevation of cytokines or liver and kidney enzymes was observed, suggesting that the therapy is well tolerated.

Challenges for miRNA based biomarkers and therapeutics

microRNAs have only been discovered within the last two decades. Although the assessment of the miRNA expression pattern in various cancers has revealed a plethora of potential miRNA biomarker candidates for cancer risk, diagnosis, prognosis and/or prediction and therapy, development of a reliable and reproducible miRNA clinical test still in infancy. Extensive research is needed and various technical issues are crucial to be addressed prior to the routine clinical use of miRNA as biomarkers. One of the major challenges with the use of miRNAs as biomarkers in clinical practice is their sensitivity and specificity particularly in case of less abundant miRNAs and to distinguish among the members of the same family. Also the exact origin of these miRNAs in circulation needs to be answered as it is still unclear if they are present in the circulation within exosomes and microvesicles, preferentially shed from diseased cells or in the free form. Another challenge is their merit over the currently available patient diagnosis and management as there are only few reports signifying the benefits of miRNA testing over clinical and pathologic information. Further, detailed analysis of preanalytical variables that may affect the clinical implication of miRNAs needs to be exhaustively explored. The currently used methods for discovering and testing biomarkers are difficult, tedious and cumbersome. Once we are able to answer these challenges and miRNA based biomarker fingerprint can be established, miRNA profiling can definitely provide an easier, faster, cheaper and above all accurate assessment.

In terms of miRNA based therapy, an important question which needs to be answered for miRNA based therapy is the targeted delivery of the miRNA containing liposomes. As single miRNA may target 100 of genes, the signaling pathway of each miRNA needs to be recognized before we try to infer what diseases may be diagnosed or cured by their manipulation. Moreover, combination of miRNAs and their dosage for a particular disease condition needs to be established as well. Population based variation in the expression pattern of miRNAs is another major challenge faced by the implementation of miRNA based therapeutics is the need to create adjuvant carrier systems that would increase stability of mRNAs in its microenvironment and enhance uptake of these miRNAs by target tissues. Increasing list of miRNAs and their role in various diseased conditions will surely overcome

these obstacles and will lead to the development of miRNA based therapeutic in very near future.

Acknowledgments

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; 60:277–300. [PubMed: 20610543]

2. Nelen, V. Epidemiology of Prostate Cancer. In: Ramon, J.; Denis, LJ., editors. Prostate Cancer. Berlin Heidelberg: Springer; 2007. p. 1-8.

3. Blute ML. No proof of inferiority: open radical retropubic prostatectomy remains state-of-the-art surgical technique for localized prostate cancer. J Urol. 2009; 181:2421–2423. [PubMed: 19371882]

4. Zincke H, Oesterling JE, Blute ML, Bergstralh EJ, Myers RP, Barrett DM. Long-term (15 years) results after radical prostatectomy for clinically localized (stage T2c or lower) prostate cancer. J Urol. 1994; 152:1850–1857. [PubMed: 7523733]

5. Zincke H, Bergstralh EJ, Blute ML, et al. Radical prostatectomy for clinically localized prostate cancer: long-term results of 1,143 patients from a single institution. J Clin Oncol. 1994; 12:2254–2263. [PubMed: 7964940]

6. Catalona WJ, Smith DS. Cancer recurrence and survival rates after anatomic radical retropubic prostatectomy for prostate cancer: intermediate-term results. J Urol. 1998; 160:2428–2434. [PubMed: 9817397]

7. Carvalhal GF, Griffin CR, Kan D, Loeb S, Catalona WJ. Reducing blood loss in open radical retropubic prostatectomy with prophylactic periprostatic sutures. BJU Int. 2010; 105:1650–1653. [PubMed: 19888968]

8. Swanson GP, Riggs MW, Earle JD, Haddock MG. Long-term follow-up of radical retropubic prostatectomy for prostate cancer. Eur Urol. 2002; 42:212–216. [PubMed: 12234504]

9. Lilja H, Ulmert D, Vickers AJ. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nat Rev Cancer. 2008; 8:268–278. [PubMed: 18337732]

10. Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med. 1991; 324:1156–1161. [PubMed: 1707140]

11. Backer H. Prostate cancer screening: exploring the debate. Permanente J. 1999; 3:330–340.

12. Schröder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized european study. N Engl J Med. 2009; 360:1320–1328. [PubMed: 19297566]

13. Andriole GL, Grubb RL, Buys SS, et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med. 2009; 360:1310–1319. [PubMed: 19297565]

14. Djulbegovic M, Beyth RJ, Neuberger MM, et al. Screening for prostate cancer: systematic review and meta-analysis of randomised controlled trials. BMJ. 2010; 341:c4543. [PubMed: 20843937]

15. Jansen FH, van Schaik RHN, Kurstjens J, et al. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. Eur Urol. 2010; 57:921–927. [PubMed: 20189711]

16. Shariat SF, Semjonow A, Lilja H, Savage C, Vickers AJ, Bjartell A. Tumor markers in prostate cancer I: Blood-based markers. Acta Oncol. 2011; 50:61–75. [PubMed: 21604943]

17. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136:215–233. [PubMed: 19167326]

18. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–297. [PubMed: 14744438]

19. Mahn R, Heukamp LC, Rogenhofer S, von Ruecker A, Muller SC, Ellinger J. Circulating microRNAs (miRNA) in serum of patients with prostate cancer. Urol. 2011; 77:e1265–e1269.

20. Esquela-Kerscher A, Slack FJ. Oncomirs [mdash] microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6:259–269. [PubMed: 16557279]

21. Brase JC, Wuttig D, Kuner R, Sultmann H. Serum microRNAs as non-invasive biomarkers for cancer. Mol Cancer. 2010; 9:306. [PubMed: 21110877]

22. Stefani G, Slack FJ. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol. 2008; 9:219–230. [PubMed: 18270516]

23. Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific microRNAs. Dev Cell. 2003; 5:351–358. [PubMed: 12919684]

24. Li C, Feng Y, Coukos G, Zhang L. Therapeutic microRNA strategies in human cancer. AAPS J. 2009; 11:747–757. [PubMed: 19876744]

25. Hoefig KP, Heissmeyer V. MicroRNAs grow up in the immune system. Curr Opin Immunol. 2008; 20:281–287. [PubMed: 18554884]

26. Bonci D, Coppola V, Musumeci M, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med. 2008; 14:1271–1277. [PubMed: 18931683]

27. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002; 99:15524–15529. [PubMed: 12434020]

28. 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; 69:7165–7169. [PubMed: 19738047]

29. Selaru FM, Olaru AV, Kan T, et al. MicroRNA-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and tissue inhibitor of metalloproteinase 3. Hepatology. 2009; 49:1595–1601. [PubMed: 19296468]

30. Meng F, Henson R, Wehbe–Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007; 133:647–658. [PubMed: 17681183]

31. Eis PS, Tam W, Sun L, et al. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc Natl Acad Sci U S A. 2005; 102:3627–3632. [PubMed: 15738415]

32. Gironella M, Seux M, Xie M-J, et al. Tumor protein 53- induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. Proc Natl Acad Sci U S A. 2007; 104:16170–16175. [PubMed: 17911264]

33. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol. 2008; 10:593–601. [PubMed: 18376396]

34. Meng F, Henson R, Lang M, et al. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology. 2006; 130:2113–2129. [PubMed: 16762633]

35. Iorio MV, Visone R, Di Leva G, et al. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007; 67:8699–8707. [PubMed: 17875710]

36. Camps C, Buffa FM, Colella S, et al. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in Bbreast cancer. Clinical Cancer Res. 2008; 14:1340–1348. [PubMed: 18316553]

37. Mathew LK, Simon MC. mir-210: A sensor for hypoxic stress during tumorigenesis. Mol Cell. 2009; 35:737–738. [PubMed: 19782023]

38. 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; 103:2257–2261. [PubMed: 16461460]

39. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008; 141:672–675. [PubMed: 18318758]

40. Yu DC, Li QG, Ding XW, Ding YT. Circulating microRNAs: potential biomarkers for cancer. Int J Mol Sci. 2011; 12:2055–2063. [PubMed: 21673939]

41. Dbouk HA, Tawil A, Nasr F, Kandakarjian L, Abou-Merhi R. Significance of CEA and VEGF as diagnostic markers of colorectal cancer in Lebanese patients. Open Clin Cancer J. 2007; 1:1–5. [PubMed: 18665243]

42. Schneider J, Velcovsky HG, Morr H, Katz N, Neu K, Eigenbrodt E. Comparison of the tumor markers tumor M2-PK, CEA, CYFRA 21-1, NSE and SCC in the diagnosis of lung cancer. Anticancer Res. 2000; 20:5053–5058. [PubMed: 11326667]

43. Huber K, Kirchheimer JC, Ermler D, Bell C, Binder BR. Determination of plasma urokinase-type plasminogen activator antigen in patients with primary liver cancer: characterization as tumor-associated antigen and comparison with alpha-fetoprotein. Cancer Res. 1992; 52:1717–1720. [PubMed: 1372529]

44. Stenman UH, Paus E, Allard WJ, et al. Summary report of the TD-3 workshop: characterization of 83 antibodies against prostate-specific antigen. Tumour Biol. 1999; 20 Suppl 1:1–12. [PubMed: 10628402]

45. Fleischhacker M, Schmidt B. Free circulating nucleic acids in plasma and serum (CNAPS) -- Useful for the detection of lung cancer patients? Cancer Biomark. 2010; 6:211–219. [PubMed: 20660966]

46. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res. 1977; 37:646–650. [PubMed: 837366]

47. Vlassov VV, Laktionov PP, Rykova EY. Circulating nucleic acids as a potential source for cancer biomarkers. Curr Mol Med. 2010; 10:142–165. [PubMed: 20196731]

48. Tong YK, Lo YM. Diagnostic developments involving cell-free (circulating) nucleic acids. Clin Chim Acta. 2006; 363:187–196. [PubMed: 16126188]

49. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008; 105:10513–10518. [PubMed: 18663219]

50. 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]

51. Kosaka N, Izumi H, Sekine K, Ochiya T. microRNA as a new immune-regulatory agent in breast milk. Silence. 2010; 1:7. [PubMed: 20226005]

52. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res. 2011; 39:7223–7233. [PubMed: 21609964]

53. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Developmental Biology. 2007; 302:1–12. [PubMed: 16989803]

54. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435:834–838. [PubMed: 15944708]

55. Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6:259–269. [PubMed: 16557279]

56. Nana-Sinkam SP, Croce CM. MicroRNAs as therapeutic targets in cancer. Transl Res. 2011; 157:216–225. [PubMed: 21420032]

57. Barker EV, Cervigne NK, Reis PP, et al. microRNA evaluation of unknown primary lesions in the head and neck. Mol Cancer. 2009; 8:127. [PubMed: 20028561]

58. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008; 141:672–675. [PubMed: 18318758]

59. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as Novel Minimally Invasive Biomarkers for Breast Cancer. Ann Surg. 2010; 251:499–505. [PubMed: 20134314]

60. Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non–small-cell lung cancer. J Clin Oncol. 2010; 28:1721–1726. [PubMed: 20194856]

61. Heegaard NHH, Schetter AJ, Welsh JA, Yoneda M, Bowman ED, Harris CC. Circulating microRNA expression profiles in early stage non-small cell lung cancer. Int J Cancer. 2011 [Epub ahead of print].

62. Olson P, Lu J, Zhang H, et al. MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. Genes & Dev. 2009; 23:2152–2165. [PubMed: 19759263]

63. Li A, Omura N, Hong S-M, et al. Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels. Cancer Res. 2010; 70:5226–5237. [PubMed: 20551052]

64. Wang J, Chen J, Chang P, et al. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prev Res. 2009; 2:807–813.

65. Ho AS, Huang X, Cao H, et al. Circulating miR-210 as a novel hypoxia marker in pancreatic cancer. Transl Oncol. 2010; 3:109–113. [PubMed: 20360935]

66. Wong T-S, Liu X-B, Wong BY-H, Ng RW-M, Yuen APW, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clin Cancer Res. 2008; 14:2588–2592. [PubMed: 18451220]

67. Wang F, Zheng Z, Guo J, Ding X. Correlation and quantitation of microRNA aberrant expression in tissues and sera from patients with breast tumor. Gynecol Oncol. 2010; 119:586–593. [PubMed: 20801493]

68. Tsujiura M, Ichikawa D, Komatsu S, et al. Circulating microRNAs in plasma of patients with gastric cancers. Br J Cancer. 2010; 102:1174–1179. [PubMed: 20234369]

69. Liu R, Zhang C, Hu Z, et al. A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. Eur J Cancer. 2011; 47:784–791. [PubMed: 21112772]

70. Zhu W, Qin W, Atasoy U, Sauter E. Circulating microRNAs in breast cancer and healthy subjects. BMC Res Notes. 2009; 2:89. [PubMed: 19454029]

71. Shigoka M, Tsuchida A, Matsudo T, et al. Deregulation of miR-92a expression is implicated in hepatocellular carcinoma development. Pathol Int. 2010; 60:351–357. [PubMed: 20518884]

72. Yamamoto Y, Kosaka N, Tanaka M, et al. MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. Biomarkers. 2009; 14:529–538. [PubMed: 19863192]

73. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer. 2010; 127:118–126. [PubMed: 19876917]

74. Ng EKO, Chong WWS, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut. 2009; 58:1375–1381. [PubMed: 19201770]

75. Tanaka M, Oikawa K, Takanashi M, et al. Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients. PLoS One. 2009; 4:e5532. [PubMed: 19440243]

76. Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol. 2009; 112:55–59. [PubMed: 18954897]

77. Hausler SFM, Keller A, Chandran PA, et al. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer. 2010; 103:693–700. [PubMed: 20683447]

78. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic Oncol. 2008; 110:13–21.

79. Weber JA, Baxter DH, Zhang S, et al. The MicroRNA Spectrum in 12 Body Fluids. Clin Chem. 2010; 56:1733–1741. [PubMed: 20847327]

80. Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. Oral Dis. 2010; 16:360–364. [PubMed: 20233326]

81. Park NJ, Zhou H, Elashoff D, et al. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res. 2009; 15:5473–5477. [PubMed: 19706812]

82. Yamada Y, Enokida H, Kojima S, et al. MiR-96 and miR-183 detection in urine serve as potential tumor markers of urothelial carcinoma: correlation with stage and grade, and comparison with urinary cytology. Cancer Sci. 2011; 102:522–529. [PubMed: 21166959]

83. Chan E, Prado DE, Weidhaas JB. Cancer microRNAs: From subtype profiling to predictors of response to therapy. Trends Mol Med. 2011; 17:235–243. [PubMed: 21354374]

84. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TLJ, Visakorpi T. MicroRNA Expression Profiling in Prostate Cancer. Cancer Res. 2007; 67:6130–6135. [PubMed: 17616669]

85. 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; 68:6162–6170. [PubMed: 18676839]

86. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008; 105:10513–10518. [PubMed: 18663219]

87. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One. 2009; 4:e6229. [PubMed: 19597549]

88. Brase JC, Johannes M, Schlomm T, et al. Circulating miRNAs are correlated with tumor progression in prostate cancer. Int J Cancer. 2011; 128:608–616. [PubMed: 20473869]

89. Kota J, Chivukula RR, O'Donnell KA, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell. 2009; 137:1005–1017. [PubMed: 19524505]

90. Takeshita F, Patrawala L, Osaki M, et al. Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. Mol Ther. 2009; 18:181–187. [PubMed: 19738602]

91. Wiggins JF, Ruffino L, Kelnar K, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Res. 2010; 70:5923–5930. [PubMed: 20570894]

92. Trang P, Medina PP, Wiggins JF, et al. Regression of murine lung tumors by the let-7 microRNA. Oncogene. 2010; 29:1580–1587. [PubMed: 19966857]

93. Liu C, Kelnar K, Liu B, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011; 17:211–215. [PubMed: 21240262]

94. Mostert B, Sieuwerts AM, Martens JW, Sleijfer S. Diagnostic applications of cell-free and circulating tumor cell-associated miRNAs in cancer patients. Expert Rev Mol Diagn. 2011; 11:259–275. [PubMed: 21463236]

95. 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; 71:550–560. [PubMed: 21098088]

Table 1

Circulating miRNAs in prostate cancer patients [adapted from reference (94)]

Sample type	Method	Prostate cancer patients (n)	Healthy donors (n)	Candidate miRNAs (n)	Differentially expressed miRNAs (validated)	Ref.
Serum	qRT-PCR	25	25	6	*miR-141*	(49)
Serum	qRT-PCR	116		2	*miR-141, miR-375*	(88)
Serum	qRT-PCR	27	9	384	*miR-26b, miR-30c miR-24, miR-874 miR-93, miR-1274a miR-106a, miR-1207-5p miR-223, miR-451*	(95)
Serum	qRT-PCR	35	20	4	*miR-26a, miR-195 miR-let7i* (only in benign prostate hyperplasia)	(19)