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MicroRNAs as Potential Biomarkers in Human Solid Tumors

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Abstract

MicroRNAs (miRNAs) regulate the expression of approximately 30% of protein-coding genes. Functions of miRNAs are essential to maintain a steady state of cellular machinery. Dysregulations of miRNAs play pivotal roles in the initiation and progression of malignancies. Abnormal miRNA expressions have been found in a variety of human solid tumors. Furthermore, extracellular miRNAs could circulate in body fluids, and hence show great promise for refining diagnosis and prognosis of cancer. Here we review the progress of analysis of microRNAs as a potential approach for diagnosis and prognosis of solid cancer. We will also discuss obstacles in developing miRNAs as circulating biomarkers.

Keywords

MicroRNA; biomarkers; diagnosis; prognosis; circulation; cancer

1. Introduction

1.1. Discovery of circulating miRNAs

Cancer is a leading cause of death worldwide, mainly due to the lack of approaches for its early detection and the development of resistance to chemotherapy. Biomarkers that can be used for the early detection of cancer and precisely identifying early-stage solid tumors after surgery at high risk for recurrence could reduce the mortality [1]. To develop such biomarkers, particularly analyzing tumor-related molecular changes in body fluids, for cancer diagnosis has been investigated for decades. However, little progress has been made.

MicroRNAs (miRNAs) involve in the regulation of several key cellular processes, including cellular development, differentiation, proliferation, cell death, and metabolism. Because a single miRNA can target hundreds of mRNAs, function of dysregulated microRNAs could contribute to the initiation and progression of a variety of malignancies, including solid tumors [2, 3]. Furthermore, miRNA expression profiles have been shown as potential signatures for the classification, diagnosis, and progression of cancer [4–7]. In addition, the tumor-specific mRNA expression profiles are more informative and discriminatory as

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Competing interests

The authors declare that they have no competing interests.

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compared with mRNA profiles, and hence have the potential to be developed as cancer biomarkers.

Various miRNA expression patterns in body fluids have been evaluated [8, 9]. The resulting data implied that miRNAs could be present in the biological fluids, including blood, urine, tears, breast milk, bronchial lavage, colostrum, seminal, amniotic, pleural, peritoneal, and cerebrospinal fluids, et al [8]. Furthermore, miRNAs ubiquitously existing in the biological fluids have functional roles associated with the surrounding tissues. For example, Kosaka et al. [10] found that human breast milk contain large amount of miRNAs capable of transfer to immune cells for the development of an infant's immune system. As presented in Figure 1 and Table 1–2, numerous studies, including our own, have shown the diagnostic and prognostic values of miRNAs in body fluids for a variety of solid cancers [11–13]. For instance, we [14–16] found that plasma miRNA expression profiles could be useful in discriminating non-small cell lung cancer (NSCLC) patients from healthy controls and patients with chronic obstructive pulmonary disease (COPD). Our findings strongly imply the potential of miRNAs as circulating biomarkers for lung cancer diagnosis. Furthermore, by analyzing a panel of four miRNA in sputum of 67 patients with NSCLC and 55 healthy controls, we demonstrated that studying expressions of mir-205, mir-210, and mir-708 in sputum could distinguish lung squamous cell carcinoma (SCC) patients from normal controls [17]. In addition, Park et al. [18] found that mir-125a and mir-200a were present in significantly lower levels in saliva of patients with oral squamous-cell carcinoma, as compared with healthy controls. Moreover, measuring expression levels of mir-126 and mir-152 in urine could indicate the occurrence of bladder cancer with a specificity of 82% and a sensitivity of 72% [19]. Therefore, recent research work in the field of miRNA opened up new perspectives in the development of cancer biomarkers. In this review, we will summarize the development of miRNAs as potential biomarkers that can be tested in clinical specimens, particularly body fluids, for the early detection of solid tumors and predicting prognosis of the patients.

1.2. Functional findings of identified miRNAs as tumor biomarkers

Basically, cancer-related miRNAs function as oncogene (oncomir), tumor suppressor gene (tumor suppressor miRNA), or both oncogene and tumor suppressor [1]. Furthermore, circulating miRNAs have numerous biological effects close by or at a certain distance to affect various types of cells [20]. Circulating miRNAs could be delivered either independent of cell contact or adhesion. Circulating miRNAs could also deliver multiple messages at once and regulate numerous target genes simultaneously [20].

Functional findings of circulating miRNAs in body fluids have recently been described. First, because substantial differences of miRNA expressions exist between platelets and peripheral blood mononuclear cells in healthy subjects [21], miRNAs could have an important function in regulation of hematopoiesis and cellular differentiation [21]. Second, miRNAs could be transported in microvesicles, and thus have important function in gene silencing in recipient cells [22]. Third, as cells could actively secrete endogenous miRNAs through microvesicle, the microvesicle-based miRNA release may play an important role in ceramide dependent secretory machinery [22]. Fourth, because exosomes play essential function in cell-cell communication by transporting miRNAs to surrounding cells, the circulating exosomes-contained miRNAs might have significant functions in transporting genetic information of tumor cells to surrounding cells and supporting tumor growth and progression [23, 24]. Fifth, miRNAs might contribute to manipulating the microvesicles' target cells by regulating their RNA stability and translation [25]. Sixth, circulating miRNAs could involve in adjusting immune responses [26, 27]. Nevertheless, with increased advances in molecular biology, we will have more functional findings of circulating miRNAs in tumorigenesis.

1.3. Mechanisms underlying stability of miRNAs in clinical samples

The development of molecules as biomarkers that can robustly be tested in human specimens heavily depends on their stability and resistance to storage handling. RNase present in body fluids will degrade molecules, particularly mRNAs, and thus affects their stability. For example, blood contains high levels of RNase activity that can rapidly degrade exogenously added mRNAs in just seconds [28]. Therefore, the development of molecular biomarkers has been a great challenge. Notably, endogenous serum miRNAs could remain stable after being subjected to boiling, very low or high pH levels, extended storage time, and multiple freeze-thaw cycles, in which, most RNAs will be degraded [28, 29]. Furthermore, human breast milk miRNA was quite stable even treated with acidic (pH1) solution for one hour and RNase A/T [30]. In addition, miRNAs could be preserved in archived 10-year-old human serum samples [31]. Moreover, miRNAs were also stable in unrefrigerated dried serum blots. Therefore, compared with mRNAs and other longer RNA molecules, miRNAs are fairly steady in fresh and archived clinical specimens [8]. The unique feature of miRNAs in body fluids shows great promise for the discovery of new and more useful biomarkers for cancer diagnosis and prognosis [29, 32, 33].

The mechanisms underlying the miRNA's stability can be first explained by the discovery of exosomes [34]. Exosomes contain miRNAs [35]. Inside cells, exosomes are formed through inward budding of endosomal membranes, and therefore, give rise to intracellular multivesicular bodies (MVBs). MVBs fuse with the plasma membrane, thus inducing the release of exosomes to the outer of cells [36]. In addition, exosomes containing miRNAs can be found in various types of body fluids [21, 37]. Importantly, although normal cells within the peripheral circulation can also contribute to exosome population, tumor tissues might be the primary source of circulating exosomes in cancer patients [38]. For instance, the tumor-derived exosomes and an associated miRNA signature were found in peripheral blood of ovarian cancer patients [39]. Furthermore, a similar trend between miRNA signatures in circulating exosomal miRNA and primary lung tumor was found, whereas there was a significant difference in exosomal miRNA levels in peripheral blood between the lung cancer patients and controls [40]. In addition, miRNA containing tumor-derived exosomes can affect biological processes inside the recipient cells [4]. For example, exosomal miRNAs could promote gene silencing similar to cellular miRNA [4]. Furthermore, because messages delivered by the tumor-derived exosomes could be translated by recipient cells and stimulate proliferation of human glioma cells, the tumor-associated molecular changes could be detected in serum exosomes in 28 % glioblastoma patients [41]. Interestingly, let-7, a well-known tumor suppressor miRNA family, is abundant in exosomes produced by a metastatic gastric cancer cells, because exosomal let-7 members might be related to the maintenance of an intercellular tumorigenic and metastatic state [41]. Altogether, exosomes may play a key role in circulating miRNA's stability. Nonetheless, the exosomes-based mechanisms underlying the miRNA's stability remain largely unknown.

Other possibilities also exist for explaining the existence of circulating miRNAs in body fluids. For instance, high density lipoprotein (HDL) transport system can be responsible for transporting circulating miRNAs [42]. For example, native HDL can readily associate with exogenous miRNAs and deliver genetic material to recipient cells with functional targeting capabilities, producing altered gene expression [42]. Furthermore, the RNA-binding protein nucleophosmin may also play an important role in exportation, packaging, and protection of extracellular miRNAs [43]. For instance, almost 90% of the plasma and serum miRNAs are cofractionated with protein complexes rather than encapsulated by vesicles [44]. In addition, the association of Argonaute 2 (Ago2, an effector of the target mRNA silencing by miRNA) with plasma and serum miRNAs could contribute to the stability of circulating miRNAs [45].

1.4. Possible reasons for existence of tumor related miRNAs in serum

Serum is one of the most easily accessible body fluids, thus providing diagnostic material for the development of surrogate cancer biomarkers [25]. Several studies have shown that expression levels of serum miRNAs are reproducibly measurable [28, 29, 46, 47, 25]. Chen et al. [20] recently summarized three possible pathways, by which miRNAs enter body fluids, including serum. First, miRNAs passively outflow from broken cells due to tissue injury, chronic inflammation, cell apoptosis or necrosis, or cells such as platelets. Second, active secretion via microvesicles, including exosomes and shedding vesicles by through membrane-enclosed cell fragments released from a variety of cell types under normal and pathological conditions. Third, active secretion in RNA-binding protein-dependent pathway. For example, RNA-binding protein high-density lipoprotein (HDL) can deliver exogenous miRNAs to recipient cells [42]. Recently, other reports showed that a large parts of circulating miRNAs is not encapsulated in microvesicles, but associated with Argonaute 2 (Ago2) [45] and nucleophasmin 1 (NPM1) [43]. However, the detail mechanisms of how these AGO2- and NPM1-related miRNAs could be released from cells and enter into recipient cells remain unclear.

Regarding the use of miRNAs as serum-based biomarkers, Brase et al. [25] suggest that there are at least two possible reasons for existence of tumor-related miRNAs in serum. First, passive release of miRNAs could happen when tissue is injured [48]. Therefore, the high rate of proliferation and cell lysis in cancerous tissues contribute to the abundance of miRNAs in serum [25]. Second, small (50 nm to 100 nm) particles are shed from the cell plasma membrane into the extracellular space and released into serum [49, 24]; miRNAs that are enclosed in the small particles can be protected from RNase, and hence stably exist in serum [35]. However, recent studies suggested that the cell-free miRNAs could also be derived from normal lysed cells [50, 51]. For example, Kirschner et al. [52] found that expressions of mir-16 and mir-451 were high in red blood cells and proportional to the degree of haemolysis. Furthermore, expressions of 79 miRNA biomarkers in blood cells could be major contributors to circulating miRNAs. Hemolysis can alter the miRNA expression levels up to 50-fold [53]. The observations might be explained by that non-hematopoietic cells, such as intestinal epithelial cells and neuroglial cells are capable to release microvesicles [54, 51]. miRNAs contained in microvesicles derived from the noncancerous cells can be protected against degradation, thus circulate in serum. Therefore, it is imperative to identify tumor-specific miRNA aberrations in serum that can be used as circulating biomarkers for distinguishing cancer and cancer-free diseases.

1.5. Commonly used techniques to analyze miRNA expressions

Major techniques used for the identification and development of miRNAs as biomarkers include microarray, deep sequencing or next-generation sequencing (NGS), and quantitative real-time polymerase chain reaction (qRT-PCR). Microarray has long been used to define miRNA expression profiles and identify cancer-related miRNAs. However, the technique only screen most known miRNAs. NGS has advantages over microarray in characterizing miRNA transcriptome, as it can identify low abundant miRNAs exhibiting expression differences between distinct samples, to an extent that previously could not be effectively detected [55, 56]. Furthermore, NGS allows the successful discovery of novel miRNAs by directly observing and validating the folding potential of flanking sequences [55, 56]. Importantly, NGS offers a highly robust, accurate, and scalable system for rapid and productive investigation of miRNAs on a genome-wide scale [55, 56]. For instance, NGS has recently been used to reveal the differential expression of miRNAs in ovarian cancer [57] and neuroblastoma [58]. However, both microarray and NGS systems are expensive and labor-intensive techniques, requiring a large amount of RNA samples. Furthermore, analysis of the large number of genes including ones whose changes are not associated with

cancer in clinical settings is not practically necessary. Therefore, microarray and NGS profiling data need to be translated into clinical trials by developing an easily performed and co-effective assay that can analyze the cancer-specific miRNAs for cancer diagnosis. In contrast, qRT-PCR is a more easily performed and cost-effective technique compared with the above two platforms. Importantly, the technique has already been widely used in clinical settings for analysis of disease-biomarkers. Therefore, qRT-PCR could be applied to the laboratory settings to examine miRNA-based biomarkers.

Although qRT-PCR techniques show great promising in the determination of miRNA expressions in clinical samples, some obstacles still exist. For example, various references genes used for normalizing targeting miRNAs and different sample collection methods produce difficulty to compare miRNA-expression profiles among the experiments carried out at different times. Therefore, previous studies displayed conflicting data in their crossing-comparison of the results [59]. The commonly used internal controls are mir-16 [15], U6 [17], U6B [11], mir-142-3p [38], 18S rRNA [60], mir-638 [61], let-7a [62], mir-1249 [63], mir-295 [64], 5S RNA, RNU38B, RNU43 [21]. Furthermore, synthetic versions of the specific *C. elegans* miRNAs, like cel-mir-39, cel-mir-54, cel-mir-238 were also used as internal control genes [28]. However, none has been widely accepted as a standard control. Altogether, the development of an accurate normalization protocol and empirical validation of stable internal control genes for each type of human specimen is urgently needed to develop miRNAs as robust and reliable cancer biomarkers.

2. miRNAs as Potential Biomarkers for the early Detection of Solid Cancer

2.1. miRNA biomarkers for the early detection of lung cancer

Lung cancers are often diagnosed in a late stage with a concomitant poor prognosis. It is, therefore, crucial to develop biomarkers to identify early stage cancer who can receive effective treatment without delay [65]. Yanaihara et al. [66] analyzed expressions of 352 miRNAs in 144 matched lung tumors and normal lung tissues. Forty-three miRNAs differentially expressed between the tumor and normal lung tissue specimens. Of the 43 miRNAs, 12 miRNAs (mir-17-3p, mir-21, mir-106a, mir-146, mir-155, mir-191, mir-192, mir-203, mir-205, mir-210, mir-212, and mir-214) were suggested to be potential biomarkers for the early detection of NSCLC. Furthermore, a low let-7 expression level was repeatedly observed in lung cancer tissues as compared with normal lung tissues. The finding was consistent with the observations from other studies, in which let-7 could target RAS and HmgA2, and hence inhibit lung cancer growth [67, 68]. Therefore, analysis of let-7 in tissue specimens might provide diagnostic assay for the early detection of lung cancer.

Biomarkers for lung cancer that can be tested in easily accessible body fluids will be more useful in clinical settings, as compared with the ones that can only be analyzed in surgically resected tissues. To this end, we have been the first to analyze expressions of miRNAs in sputum samples, as sputum is one of the most easily accessible and noninvasively obtained biological materials [17]. Furthermore, one of the advantages of sputum over blood specimens is that cancer-associated-genetic changes found in sputum highly indicate that there might be malignancy in the lungs. Our data showed that examining aberrant expression levels of mir-210, mir-708, and mir-205 in sputum could distinguish lung squamous cell carcinoma patients from normal subjects. We also found [12] that analyzing expression levels of sputum mir-21, mir-486, mir-375, and mir-200b could distinguish lung adenocarcinoma patients from normal subjects. Recently, we demonstrated that testing aberrant plasma levels of mir-21, mir-210, mir-126, and mir-486-5p could differentiate NSCLC patients from healthy controls [14]. Chen et al. analyzed serum expressions of miRNAs in 152 NSCLC patients and 75 healthy controls [29]. The results suggested that mir-25 and mir-223 could also be biomarkers for diagnosis of NSCLC. Altogether, these

observations strongly implicated the feasibility of developing miRNAs as noninvasive or circulating biomarkers for the early detection of lung cancer.

2.2. miRNA biomarkers for the early detection of breast cancer

Iorio et al. [69] identified 29 miRNA signatures, whose aberrant expressions measured in surgical specimens could differentiate tumor from normal breast tissues with 100% accuracy. Furthermore, Blenkiron et al. [70] identified 133 miRNAs that displayed aberrant expression levels in breast tumors as compared with the matched normal breast tissues. Interestingly, specific miRNA expression profiles were also identified that could differentiate breast cancer subtypes. For instance, measuring expressions of mir-100, mir-99a, mir-130a, mir-126*, mir-136, mir-146b, mir-15b, mir-107, and mir-103 could discriminate luminal A from luminal B breast cancers [70].

To develop circulating miRNA biomarkers for breast cancer, Roth et al. [71] analyzed serum miRNA expressions in 59 patients with primary breast cancer and 30 subjects with metastatic disease. Analysis of a serum mir-155 expression level could discriminate breast cancer patients from healthy controls, whereas studying serum levels of mir-10b, mir-34a, and mir-155 could discriminate metastatic breast cancer from healthy controls (All $P < 0.001$). Heneghan et al. [60] found that an elevated mir-195 expression level in blood was specifically associated with breast cancer, and could diagnose breast cancer with a sensitivity of 88% and a specificity of 91%. Wu et al. [72] identified that serum expressions of mir-29a and mir-21 were considerably increased in breast cancer patients compared with normal controls ($P < 0.05$). Furthermore, Hu et al. [73] detect serum miRNA expression levels in 48 breast cancer patients and 48 controls, and then validate the findings in additional 76 breast cancer patients and 76 controls. Four miRNAs (mir-16, mir-25, mir-222, mir-324-3p) were identified in the discovery phase that could discriminate breast cancer patients from control with a sensitivity of 91.7% and a specificity 89.6 %. Importantly, the validation of the panel of circulating miRNAs in the second set of cohort yielded 92.1% sensitivity and 93.4% specificity. In conclusion, the serum miRNA signatures could serve as biomarkers for the early detection of breast cancer and discriminate different types of breast cancer.

2.3. miRNA biomarkers for the early detection of prostate cancer

Porkka and colleagues showed that measuring expression levels of 51 miRNAs in surgically resected tissue specimens could distinguish prostate tumors from normal tissues [74]. Of the 51 miRNAs, 22 miRNAs were downregulated in the prostate cancer samples, 15 miRNAs were downregulated in hormone-refractory tumors, and 8 miRNAs were upregulated in all types of prostate cancers. However, six miRNAs (mir-184, mir-198, mir-302c*, mir-345, mir-491, mir-513) were upregulated in hormone-refractory tumours [74]. Furthermore, Ozen et al. [75] confirmed Porkka's data and further found that the downregulated miRNAs could target some oncogenes. Schaefer et al. [76] analyzed miRNA changes in 76 patients who underwent radical prostatectomy. A signature of five miRNAs (mir-96, mir-182, mir-182*, mir-183, mir-375) was defined that had elevated expression levels in tumor tissues compared with noncancerous specimens. In contrast, ten miRNAs (mir-16, mir-31, mir-125b, mir-145, mir-149, mir-181b, mir-184, mir-205, mir-221, and mir-222) displayed lower expression levels in cancer tissues compared with normal prostate tissues. Interestingly, measuring expression levels of two miRNAs (mir-183 and mir-205) could discriminate malignant tissues from noncancerous tissues with 84% sensitivity [76]. Recently, Mitchell et al. [28] found that testing a plasma mir-141 expression level could diagnose prostate cancer with a sensitivity of 60% and a specificity of 100%. The observation was confirmed in an independent study undertaken by Brase and colleagues [77]. Furthermore, Brase et al. first screened 667 miRNAs in serum from seven patients with

metastatic prostate cancers and 14 localized prostate cancers, and then validated the resulting data in an independent cohort; mir-141 and mir-375 were found as the most pronounced markers for high-risk prostate cancer. In addition, Mahn et al. [78] studied 63 prostate cancer patients and 20 healthy controls for miRNA expressions in blood samples. Analysis of an expression level of plasma mir-26a produced a sensitive of 89% and a specificity of 56% for discriminating localized prostate cancer from benign prostate hyperplasia. Therefore, these observations demonstrated the feasibility of developing plasma miRNA profiling as a circulating-based biomarker assay for prostate cancer that has the potential of translation to the clinic.

2.4. miRNA biomarkers for the early detection of colorectal cancer (CRC)

Nagel et al. [79] indicated that high expression levels of both mir-135a and mir-135b in surgical tissues were correlated with a reduced APC expression level in CRC patients. Slaby et al. [80] showed that Dukes' A and B tumors have a lower mir-21 level than Dukes' C tumors. Aberrant mir-143 and mir-145 expressions were correlated to CRC [81]. In addition, Lanza et al. [82] suggested that combined analysis of mRNA and miRNA expressions could identify CRC with microsatellite stability (MSS) in CRC specimens.

To develop blood miRNA biomarkers for CRC, Pu et al. [83] analyzed plasma miRNA expression levels in 103 CRC patients and 37 healthy controls. Plasma mir-221 was found to be a potential biomarker for differentiating CRC patients from controls with 86% sensitivity and 41% specificity [83]. Furthermore, Huang et al. [84] investigated plasma miRNA expression levels in 120 CRC patients and 37 individuals with advanced adenomas. Analysis of abnormal plasma mir-29a and mir-92a expressions displayed diagnostic value for advanced CRC with 73.0% sensitivity and 79.7% specificity [84]. Furthermore, Wang et al. [85] investigated serum miRNA expression levels in patients and found that mir-29a was significantly higher than in colorectal liver metastasis patients than in CRC patients, yielded a sensitivity of 75% and a specificity of 75% in discriminating metastatic from non-metastatic patients.

2.5. miRNA biomarkers for the early detection of pancreatic cancer

Bloomston et al. [86] studied surgically resected specimens from 65 patients with ductal adenocarcinoma of the pancreas and 42 patients with chronic pancreatitis for miRNA expressions. A panel of 18 miRNA signatures was defined that could distinguish amongst pancreatic adenocarcinoma, normal pancreas and chronic pancreatitis. Furthermore, Szafranska et al. [87] demonstrated that mir-196a and 196b expression levels were considerably higher in pancreatic ductal adenocarcinomas compared with normal or inflamed pancreatic tissues. The diagnostic performance of miRNA was reproducibly confirmed in fine-needle aspirate samples [87]. In addition, mir-196 expression was found to be elevated in ductal adenocarcinomas of patients, as compared with healthy subjects [87].

To develop circulating miRNA biomarkers for diagnosis of pancreatic cancer, Wang et al. [88] defined four plasma miRNAs, including miR-21, miR-210, miR-155, and miR-196a, which revealed a sensitivity of 64% and a specificity of 89% for diagnosis of pancreatic adenocarcinoma. Recently, Ho et al. [89] detected plasma miRNA expression levels in a test set consisting of 11 pancreatic patients and 14 controls, and then validated this finding in 11 pancreatic cancer patients and 11 controls. A plasma mir-210 expression level is significantly higher in pancreatic cancer patients as compared with the normal controls. Furthermore, Li et al. [90] measured serum miRNA expression levels of 45 patients with pancreatic ductal adenocarcinoma, 11 preoperative patients with chronic pancreatitis, and 32 healthy controls. Expression levels of mir-200a and mir-200b were significantly elevated in serum of pancreatic cancer and chronic pancreatitis patients, as compared with healthy

controls ($P < 0.0001$), yielding 71.1–84.4% sensitivities and 87.5–96.9% specificities, respectively. Recently, Morimura et al. [91] found that a plasma mir-18a level was considerably higher in pancreatic cancer patients as compared with in healthy controls ($P < 0.0001$). Altogether, circulating miRNAs might be useful plasma biomarkers for the early detection of pancreatic cancer.

2.6. miRNA biomarkers for the early detection of gastric cancer

Analyzing 292 gastric tumor samples for miRNA expressions, Ueda et al. [92] showed that 22 miRNAs were upregulated in gastric tumors compared with normal tissues. Conversely, 13 miRNAs were downregulated in tumor tissues as compared with normal tissues. Combined use of the panel of miRNAs produced 83% sensitivity for diagnosis of gastric cancer. Interestingly, four miRNAs (mir-373*, mir-498, mir-202*, and mir-494) were upregulated in intestinal-type cancer, whereas eight miRNAs (mir-105, mir-100, mir-125b, mir-199a, mir-99a, mir-143, mir-145, and mir-133a) were upregulated in diffuse-type [92]. Therefore, the miRNA signatures could discriminate histological subtypes of gastric cancer. In an independent study, the most highly expressed miRNAs in gastric tumors were mir-20b, mir-20a, mir-17, mir-106a, mir-18a, mir-21, mir-106b, mir-18b, mir-421, mir-340*, mir-19a, and mir-658, as compared with noncancerous tissues [93].

To develop circulating biomarkers for gastric cancer, Zhou et al. [94] investigated plasma miRNA expression patterns of 90 gastric cancer patients and 27 healthy volunteers. Plasma mir-106a and mir-17 levels were significantly higher in the cancer patients than in healthy controls. Combined use of the two miRNAs created 0.741 AUC with 62.96% sensitivity and 80.49% specificity for diagnosis of gastric cancer. Furthermore, Tsujiura et al. [95] detected plasma expressions of five miRNA in 69 gastric cancer patients and 30 healthy controls. Plasma mir-17-5p, mir-21, mir-106a, mir-106b were significantly higher in gastric cancer patients (All $P = 0.05$), whereas let-7a was lower in gastric cancer patients ($P = 0.002$), as compared with the controls. In addition, Liu et al. [96] detected serum miRNA expression levels in ten gastric cancer patients, ten CRC patients, and ten healthy controls in training set; mir-378 was significantly elevated in gastric cancer patients as compared with healthy subjects; the diagnostic performance of the miRNA was successfully confirmed in a different set of cohort, consisting of 40 gastric cancer patients and 41 healthy controls [96]. Moreover, analysis of a plasma mir-378 expression level could yield 87.5% sensitivity and 70.73% specificity in discriminating gastric cancer patients from healthy controls.

2.7. miRNA biomarkers for the early detection of hepatocellular carcinoma (HCC)

Murakami et al. [97] investigated miRNA expressions in 25 pairs of HCC and adjacent noncancerous tissues, and nine additional chronic hepatitis specimens as well. Three miRNAs (mir-18, mir-224, and pre-mir-18) were upregulated, whereas five miRNAs (mir-199a, mir-199a*, mir-200a, mir-125a, and mir-195) were downregulated in the tumor specimens as compared with the normal tissues. Importantly, the genes yield 97.8% accuracy for diagnosis of HCC. Furthermore, Ladeiro et al. [98] found that a high level of mir-224 and low expression levels of mir-122a and mir-422b were common in malignant HCC. In addition, upregulated mir-96 was suggested as an early genetic event in hepatitis C-positive HCC, low level of mir-126 was correlated with alcohol-associated HCC [98].

To develop noninvasive biomarkers for HCC, Li et al. [99] investigated serum miRNA expressions in 513 subjects, including 135 patient with hepatitis B virus (HBV) infection, 48 subjects with hepatitis C virus (HCV) infection, 120 patients with HCC and 210 controls. The findings suggested that plasma mir-25, mir-375, let-7f could clearly separate HCC cases from the normal controls. Furthermore, the use of mir-375 alone produced 96% specificity and 100% sensitivity for diagnosis of HCC. Moreover, Qi et al. [100] investigated serum

miRNA expression levels in a set of 48 HBV patients with HCC, 48 subjects with HBV infection, and 24 healthy controls. Testing a serum mir-122 level could discriminate HCC patients from the controls with 81.6% sensitivity and 83.3% specificity. Altogether, serum miRNAs might serve as novel noninvasive biomarkers for the early detection of HCC.

2.8. miRNA biomarkers for the early detection of glioblastoma

mir-21, mir-221/222, and mir-10b displayed high expression levels in glioblastoma samples, whereas mir-181a, mir-181b, mir-181c, mir-7, mir-128, mir-124, mir-137, mir-451, mir-129, mir-139, and mir-218 had low levels, as compared with normal brain tissues [101–108]. These results imply that the miRNAs expressions could be used as potential diagnostic markers for glioblastoma. However, circulating miRNA biomarkers for the brain cancer remain to be identified and developed.

3. miRNAs as potential prognostic biomarkers for solid tumors

3.1. miRNAs as prognostic biomarkers for lung cancer

A high expression level of mir-155 was associated with a significant shorter survival of lung cancer patients [66]. Furthermore, a low level of let-7a was correlated with poor prognosis in the patients with lung adenocarcinoma [66]. Interestingly, mir-128b was found to be a potential predictive factor of response to therapy in NSCLC [109]. Furthermore, low expression of mir-128b was significantly correlated with the clinical response to therapies with EGFR, because mir-128b directly targeted EGFR in NSCLC cells. In addition, a panel of five miRNAs (let-7a, mir-221, mir-137, mir-372, and mir-182) could be used to detect the risk of relapse of NSCLC [110]. In addition, up-regulations of mir-221 and mir-222 were correlated with aggressive behavior of NSCLC [111]. Moreover, these two miRNAs produced an effect mediated by directly target PTEN and TIMP3, whose functions might involve in cellular migration and metastasis.

To develop serum miRNA signatures to predict survival of lung cancer, Hu et al. [112] used genome-wide serum miRNA expression analysis to investigate the role of serum miRNAs in the prediction of prognosis of patients with lung adenocarcinoma and squamous cell carcinoma. In the discovery stage, 11 serum miRNAs were found to be altered more than five-fold between longer-survival and shorter-survival group. The expression levels of mir-486, mir-30d, mir-1 and mir-499, were significantly associated with an overall survival of the patients. Furthermore, the four miRNAs also functioned as independent biomarkers to predict the poor prognosis after the surgery for the lung cancer patients. Recently, Wang et al. [113] found close correlation between a serum mir-21 expression level and clinicopathological factors of NSCLC patients. The serum expression level of mir-21 was higher in NSCLC patients compared with the healthy controls ($P < 0.01$). In addition, a high serum mir-21 level was significantly correlated with tumor-node metastases stage and lymph node metastasis of NSCLC ($P = 0.016$ and 0.026 , respectively). The 3-year overall survival rate in NSCLC patients with a high serum mir-21 level (39.8%) was considerably shorter than those with a low serum mir-21 expression level (58.2%; $P < 0.001$). Moreover, serum mir-21 expression was an independent prognostic factor in NSCLC ($P = 0.015$, $RR = 2.01$, 95% CI: 1.78–3.26). Thus, a serum mir-21 might be useful as a prognostic marker for NSCLC patients. Boeri et al. [114] investigated miRNAs expression profiles of lung tumors, normal lung tissues, and plasma samples from individuals with variable prognosis identified in a CT screening trial. Several miRNA expression patterns were defined that could distinguish 1) tumors from normal lung tissues; 2) tumor histology and growth rate; 3) clinical outcome; and 4) year of lung cancer CT detection. Interestingly, abnormal miRNA expression levels were found in plasma samples that were collected approximately two years before the onset of disease, at the time of CT detection and in disease-free smokers enrolled

in the screening trial. Therefore, plasma miRNAs could serve as molecular predictors of lung cancer development and aggressiveness, and hence have clinical implication for lung cancer management.

3.2. miRNAs as prognostic biomarkers for breast cancer

Specific miRNA profiles have been found to associate with estrogen receptor (ER) status, proliferation index, tumor stages, and vascular invasion in breast cancer [69]. For example, a low expression level of mir-30 was related with ER and progesterone receptor negative tumors, whereas high levels of mir-213 and mir-203 were correlated with high stage tumors. Furthermore, expression levels of mir-10b and mir-21 were associated with tumor grade, degree of vascular invasion, lymph node metastases, or metastatic potential [115]. In addition, a high expression level of mir-21 was correlated with increased breast cancer invasion and metastatic potential [116, 117], possibly due to that mir-21 directly targeted two tumor suppressor genes related to tumor invasiveness and metastasis. The two tumor suppressors are ropomyosin 1 and PDCD4. Moreover, Upregulation of mir-373 and mir-520c favored the formation of metastases through targeting CD44, a biomarker associated with increased survival in node-negative invasive breast carcinomas [118, 119]. An expression level of mir-10b in primary breast carcinomas was found to correlate with clinical progression of the patients [120]. Interestingly, mir-10b was transactivated by the transcription factor Twist1, and hence promoted tumor invasion. Mir-17-5p could target AIB1 whose expression was increased in breast cancer, and hence promoted cell proliferation [121–124]. Furthermore, low expression levels of mir-335 and mir-126 were correlated with patients with a shorter breast cancer-free survival [125], because mir-335 targeted extracellular matrix protein tenascin C and SOX4, whose upregulation promotes metastases. Mir-145 is an important prognosis biomarker [126], because it directly targets ER- α , and exhibits a pro-apoptotic effect, which is dependent on TP53 activation. TP53 activation can stimulate miR-145 expression, thus establishing a death-promoting loop between miR-145 and TP53.

To develop circulating miRNA prognostic biomarkers for breast cancer, Roth et al. [71] analyzed 89 breast cancer patients' serum samples for miRNA expressions; mir-10b, mir-34a, and mir-155 were associated with the presence of metastases within primary breast cancer (All $P < 0.05$). Furthermore, advanced tumor stage patients had a high mir-34a expression level compared with those with early tumor stages ($P = 0.01$). Recently, Heneghan et al. [127] found that expressions of mir-195 and let-7a in blood samples decreased in cancer patients postoperatively, as compared with control subjects. Therefore, miRNAs could be as noninvasive prognostic biomarkers to predict outcome in breast cancer patients.

3.3. miRNAs as prognostic biomarkers for gastric cancer

Ueda et al. [92] found a panel of signatures whose changes were related to gastric cancer, which include mir-125b, mir-199a, and mir-100. Importantly, low expressions of let-7g, mir-433, and a high expression level of mir-214 were correlated with an unfavorable outcome, such as depth of invasion, lymph node metastasis, and stage of the tumors. Furthermore, Li et al. [128] showed seven miRNAs (mir-10b, mir-21, mir-223, mir-338, let-7a, mir-30a-5p, and mir-126) are stage-independent biomarkers for the prognosis of gastric cancer.

3.4. miRNA as prognostic biomarkers for prostate cancer

Schaefer et al. [76] investigated the relation between expression levels of miRNAs with the Gleason score and the pathological tumor stages of the prostate cancer. Mir-96 expression was correlated with cancer recurrence after radical prostatectomy. Therefore, a high

expression level of mir-96 could be a prognostic biomarker for prostate cancer. Expressions of both mir-375 and mir-141 were elevated in prostate tumors [77]. Furthermore, serum mir-375 and mir-141 levels were correlated with high Gleason score and lymph-node positive status.

Analysis of plasma mir-141 had 78.9% sensitivity in predicting clinical outcome in prostate patients [129]. Furthermore, a serum mir-141 expression level could predicate clinical outcomes of the patients with an odd ration at least 8.3 [129]. In addition, a serum mir-141 expression level also had the highest correlation with temporal changes of PSA (a correlation of $R = 0.77$, $P < 0.001$). Moreover, Mahn et al. [78] found that expression levels of both mir-195 and let-7i in serum were significantly correlated with Gleason score.

3.5. miRNAs as prognostic biomarkers for colorectal cancer (CRC)

A high expression level of mir-21 is associated with higher stages of CRC [80]. Furthermore, a low expression level of mir-133b is inversely correlated to a high level of its target gene, *KRAS*. Interestingly, a low expression level of mir-133 is related with unfavorable outcomes and a shorter overall survival of the patients diagnosed with CRC [130]. An elevated plasma mir-221 level is a significant prognostic factor for a poor overall survival of CRC patients [83]. Cheng et al. [131] investigated miRNA levels in 102 plasma samples from stage IV colon cancer patients, and further validated the results in an independent cohort of 156 plasma samples. They concluded that a high level of mir-141 in plasma was associated with poor prognosis of the cancer patients.

3.6. miRNAs as prognostic biomarkers for pancreatic cancer

Bloomston et al. [86] found that six miRNAs, including mir-452, mir-105, mir-127, mir-518-2, mir-187, and mir-30a-3p, could be used to distinguish long-term patient survivors with node-positive pancreatic cancer from those dying within 24 months. Furthermore, a high level of mir-196a-2 could be used to predict a poor survival of the patients with pancreatic cancer [86]. Recently Morimura et al. [91] investigated plasma miRNA expression from 36 pancreatic cancer patients and 30 healthy controls, concluded that the plasma level of mir-18a was significantly lower in postoperative samples compared with preoperative samples ($P = 0.0077$). Therefore, measuring a plasma mir-18a expression level might potentially provide a prognostic tool in prediction of outcome of pancreatic cancer patients.

3.7. miRNAs as prognostic biomarkers for HCC

Li et al. [132] found that a high expression level of mir-125 was correlated with a favorable overall survival of HCC patients, might due to that the miRNA could inactivate Akt, and hence inhibited cell proliferation. Furthermore, a high expression level of mir-221 was a negative prognostic factor in HCC because it targeted CDKN1B/p27 and CDKN1C/p57 and favored cell cycle control loss [133]. In addition, Jiang et al. [134] investigated more than 200 precursor and mature miRNAs in HCC tissues, adjacent benign liver tissues, and normal liver specimens. The results indicated that altered expressions of 19 miRNAs were associated with outcomes in cancer patients. Interestingly, Yamamoto et al. [135] found that mir-500 levels were significantly reduced after surgery and returned to normal levels. Qi et al. [100] showed that mir-122 in serum was considerably higher in HCC patients than healthy controls ($p < 0.001$). Furthermore, the serum level of mir-122 was considerably reduced in the post-operative serum samples when compared with the pre-operative samples. Therefore, measuring circulating miRNA expression levels could offer a fascinating prognostic tool to monitor therapeutic perspectives for HCC [136].

3.8. miRNAs as prognostic biomarkers for glioblastoma

A high expression level of mir-2 and a lower level of mir-128 were found in surgical tissues of glioblastoma, as compared with normal tissues [137, 138]. Furthermore, mir-21 could promote invasiveness by targeting inhibitors of matrix metalloproteinases (e.g., RECK and TIMP3) in glioma cells. Conti et al. [139] found that mir-221/mir-222 expression levels were elevated in grade III or grade IV astrocytomas. Furthermore, mir-221/mir-222 acted as oncogenes in glioblastomas by targeting p27, which triggered a cell cycle arrest in the G1 phase [105]. In addition, Shi et al. [140] indicated that a mir-181a expressions level was inversely correlated with astrocytoma grade, and measuring mir-181b expression could distinguish high from low grades of glioblastomas. Therefore, mir-181a and mir-181b might be important prognostic biomarkers in predicting outcome of glioblastoma. Moreover, Kefas et al. [102] suggested that mir-7 was a pivotal biomarker in determine glioblastoma invasiveness, because it acted as a tumor suppressor by targeting EGFR and inhibiting EGFR/AKT pathway.

4. Conclusions and perspectives

An exciting next decade period can be foreseen in care of cancer patients by developing miRNAs as biomarkers. For example, the circulating miRNA biomarkers could be used to screen the high risk populations to detect early stage of solid tumors. Furthermore, miRNA biomarkers could identify the cancer patients who will have poor prognosis and need more aggressive treatment, while sparing the patients who will not. The cancer mortality will ultimately be reduced. However, substantial work needed to be done before the miRNAs can be used as biomarkers in clinical settings. For instance, tumorigenesis is a complex multistep process resulting from abnormal gene expression consequent to accumulation of epigenetic and genetic abnormalities [1]. The functional targets of some miRNAs and their roles in cellular and molecular pathways remains well determined. Therefore, it is important to uncover the detail mechanisms that drive cancer formation and progression due to the dysfunction of miRNA biogenesis, miRNAs, and their targets. Furthermore, some conditions, such as hypoxia, infection, and cytotoxic treatments could affect the miRNA expressions [141]. It is vital to identify and develop a miRNA signature that not only distinguishes cancer from healthy individuals, but also from the subjects who are affected with the factors to diagnose cancer with high accuracy. In addition, having a comprehensive understanding of the mechanisms by which, miRNAs are protected from degradation and the way miRNAs circulate in body fluids are required. Interestingly, it is recently suggested that plasma levels of myeloid- and lymphoid-expressed some miRNAs tracked closely with changes in corresponding blood counts, and blood cells could be a major contributor to certain circulating miRNAs [52]. Furthermore, perturbations in blood cell counts and hemolysis can alter plasma miRNA levels [53]. A deep understanding of the origin of circulating miRNAs in either healthy or cancer patients or what factors influence levels of circulating miRNA biomarkers are clearly required. Importantly, because circulating miRNA cancer biomarkers are highly expressed in blood cells, caution should be given in interpretation of such results as they may reflect a blood cell-based phenomenon rather than a cancer-specific origin [52, 53]. In addition, the current strategy for develop miRNAs as biomarkers mainly based on measuring their expression levels. Some miRNAs and their target sites have been found to be mutated and altered methylation at miRNA-associated CpG islands in cancer. Therefore, high-throughput mutation and methylation detection approaches may need to be developed that will provide important diagnostic means in the future. Moreover, the numerous miRNAs have demonstrated the potential as cancer biomarkers (Tables 1–2). However, compressive research is needed to determine their clinical values before they could be applied in laboratory settings.

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Abbreviations

miRNA	microRNA
NSCLC	non-small cell lung cancer
COPD	chronic obstructive pulmonary disease
SCC	squamous cell carcinoma
RNase	ribonuclease
MVBs	multivesicular bodies
HDL	high density lipoprotein
Ago2	Argonaute 2
NPM1	nucleophasmin 1
NGS	next-generation sequencing
qRT-PCR	quantitative real-time reverse-transcription polymerase chain reaction
RAS	reticular activating system
HmgA2	high-mobility group AT-hook 2
CRC	colorectal cancer
APC	adenomatous polyposis coli
MSS	microsatellite stability
AUC	area under curve
HCC	hepatocellular carcinoma
HBV	hepatitis B virus
HCV	hepatitis C virus
EGFR	epidermal growth factor receptor
PTEN	phosphatase and tensin homolog
TIMP	metalloproteinases
CT	computed tomography
ER	estrogen receptor
PDCD4	programmed cell death 4
AIB1	amplified in breast 1
SOX4	SRY-related HMG-box 4
PSA	prostate specific antigen
CDKN1	cyclin-dependent kinase inhibitor 1
AKT	protein kinase B

RECK reversion-inducing cysteine-rich protein with Kazal motifs

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Figure 1.

Table 1

Examples of miRNA as diagnostic biomarkers in human cancers

MicroRNA	Cancer type	Reference
mir-17-3p, mir-21, mir-106a, mir-146, mir-155, mir-191, mir-192, mir-203, mir-205, mir-210, mir-212, and mir-214	lung cancer	[66]
Let-7	lung cancer	[68]
mir-210, mir-708, mir-205	lung cancer	[17]
mir-21, mir-210, mir-126, mir-486-5p	lung cancer	[14]
mir-25 and mir-223	lung cancer	[29]
let-7a-2, let-7a-3, let-7d, let-7f-2, let-7f, mir-009-1, mir-010b, mir-021, mir-034, mir-101-1 mir-122a, mir-125a, mir-125b-1, mir-125b2, mir-128b mir-136, mir-143, mir-145, mir-149, mir-155 mir-191, mir-196-1, mir-196-2, mir-202, mir-203, mir-204, mir-206, mir-210, mir-213	breast cancer	[69]
mir-10b, mir-34a, mir-155	breast cancer	[71]
mir-195	breast cancer	[60]
mir-29a, mir-21	breast cancer	[72]
mir-16, mir-25, mir-222, mir-324-3p	breast cancer	[73]
mir-100, mir-99a, mir-130a, mir-126*, mir-136, mir-146b, mir-15b, mir-107, mir-103	breast cancer	[70]
mir-202, mir-210, mir-296, mir-320, mir-370, mir-373*, mir-498, mir-503, mir-184	prostate cancer	[74]
mir-198, mir-302c*, mir-345, mir-491, mir-513		
mir-96, mir-182, mir-182*, mir-183, mir-375 mir-16, mir-31, mir-125b, mir-145, mir-149 mir-181b, mir-184, mir-205, mir-221, mir-222	prostate cancer	[76]
mir-141	prostate cancer	[28]
mir-375, mir-141	prostate cancer	[77]
mir-26a	prostate cancer	[78]
mir-96	colorectal cancer	[142]
mir-135a, mir-135b	colorectal cancer	[79]
mir-21	colorectal cancer	[80]
mir-17-5p, mir-20, mir-25, mir-92-1, mir-92-2, mir-93 and mir-106a	colorectal cancer	[82]
mir-143 and mir-145	colorectal cancer	[81]
mir-221	colorectal cancer	[83]
mir-29a, mir-92a	colorectal cancer	[84]

MicroRNA	Cancer type	Reference
mir-29a	colorectal cancer	[85]
mir-141	colorectal cancer	[131]
mir-221, mir-181a, mir-155, mir-210, mir-213 mir-181b, mir-222, mir-181b-2, mir-21, mir-181b-1 mir-181c, mir-220, mir-181d, mir-223, mir-100-1/2, mir-125a, mir-143, mir-146 mir-148a, mir-148b, mir-375	pancreatic cancer	[86]
mir-196a, mir196b	pancreatic cancer	[87]
miR-21, miR-210, miR-155, and miR-196a	pancreatic cancer	[88]
mir-210	pancreatic cancer	[89]
mir-200a, mir-200b	pancreatic cancer	[90]
mir-18a	pancreatic cancer	[91]
mir-181d, mir-181a-1(2), mir-181c, mir-181b-1(2), mir-21, mir-25, mir-92-1(2), mir-93, mir-17-5p, mir-106a, mir-20b, mir-135a-1(2), mir-425-5p, mir-106b, mir-20a, mir-19b-1(2), mir-224, mir-18a, mir-135b, mir-19a, mir-345, mir-191, mir-148a, mir-148b, mir-375, mir-29b-1(2), mir-29c, mir-152, mir-218-2, mir-451, mir-30d, mir-30a-5p, mir-30b, mir-30c-1(2), mir-422b mir-373*, mir-498, mir-202*, mir-494 mir-105, mir-100, mir-125b, mir-199a, mir-99a, mir-143, mir-145, mir-133a	gastric cancer	[92]
mir-20b, miR-20a, miR-17, miR-106a, miR-18a, miR-21, miR-106b, miR-18b, miR-421, miR-340* miR-19a and miR-658	gastric cancer	[93]
mir-106a, mir-17	gastric cancer	[94]
mir-17-5p, mir-21, mir-106-a, mir-106-b, let-7-a,	gastric cancer	[95]
mir-378	gastric cancer	[96]
mir-18, mir-224, pre-mir-Pl8	hepatocellular carcinoma	[97]
mir-199a, mir-199a*, mir-200a, mir-125a, mir-195	hepatocellular carcinoma	[98]
mir-224, mir-122a, mir-422b mir-21, mir-10b, mir-222; mir-96; mir-126	hepatocellular carcinoma	[99]
mir-25, mir-375, let-7f	hepatocellular carcinoma	[100]
mir-122	hepatocellular carcinoma	[101]
mir-21,	glioblastoma	[102]
mir-7	glioblastoma	[103]
mir-221, mir-128, mir-181a, mir-181b, mir-181c	glioblastoma	[104]
mir-129, mir-139, mir-10b,	glioblastoma	[105]
mir-221/222	glioblastoma	[106]
mir-218	glioblastoma	[106]

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MicroRNA	Cancer type	Reference
mir-124, mir-137	glioblastoma	[107]
mir-451	glioblastoma	[108]

Table 2

Examples of miRNA as prognostic biomarkers in human cancers

MicroRNA	Cancer type	Reference
mir-155, let-7a	lung cancer	[66]
mir-128b	lung cancer	[109]
let-7a, mir-221, mir-137, mir-372, mir-182	lung cancer	[110]
mir-221 and mir-222	lung cancer	[111]
mir-30, mir-213, mir-203	breast cancer	[69]
mir-10b, mir-21, mir-145, mir-9-3, let-7	breast cancer	[115]
mir-21	breast cancer	[116]
mir-373	breast cancer	[119]
mir-520c	breast cancer	[118]
mir-10b	breast cancer	[120]
mir-17-5p	breast cancer	[121]
mir-335, mir-126	breast cancer	[125]
mir-126	breast cancer	[143]
mir-145	breast cancer	[126]
mir-10b, mir-34a, mir-155	breast cancer	[71]
mir-195, let-7a	breast cancer	[127]
mir-125b, mir-199a, mir-100 let-7g, mir-433, mir-214	gastric cancer	[92]
mir-10b, mir-21, mir-223, mir-338, let-7a, mir-30a-5p, mir-126	gastric cancer	[128]
mir-96	prostate cancer	[76]
mir-375, mir-141	prostate cancer	[77]
mir-141	prostate cancer	[129]
mir-15, mir-195, mir-26a, let-7i	prostate cancer	[78]
mir-21, mir-133b	colorectal cancer	[80]
mir-133	colorectal cancer	[130]
mir-221	colorectal cancer	[83]
mir-141	colorectal cancer	[131]

MicroRNA	Cancer type	Reference
mir-452, mir-105, mir-127, mir-518-2, mir-187, mir-30a-3p, miR-196a-2	pancreatic cancer	[86]
mir-18a	pancreatic cancer	[91]
mir-125	hepatocellular carcinoma	[132]
mir-221	hepatocellular carcinoma	[133]
let-7c, let-7g, mir-26b, mir-29c, mir-30e-3p, mir-31, mir-99a, mir-99b, mir-100, mir-125b, mir-139, mir-148a, mir-150, mir-200c, mir-220, mir-221, mir-345, mir-372, mir-377	hepatocellular carcinoma	[134]
mir-500	hepatocellular carcinoma	[135]
mir-21	glioblastoma	[137]
mir-221/mir222	glioblastoma	[139]
mir-181a, mir-181b	glioblastoma	[140]
mir-7	glioblastoma	[102]
mir-128	glioblastoma	[138]