Review

Clinical Cancer Research

Emerging Role of MicroRNAs as Liquid Biopsy Biomarkers in Gastrointestinal Cancers

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Abstract

Cancer has emerged as a leading cause of mortality worldwide, claiming more than 8 million lives annually. Gastrointestinal cancers account for about 35% of these mortalities. Recent advances in diagnostic and treatment strategies have reduced mortality among patients with gastrointestinal cancer, yet a significant number of patients still develop late-stage cancer, where treatment options are inadequate. Emerging interests in "liquid biopsies" have encouraged investigators to identify and develop clinically relevant noninvasive genomic and epigenomic signatures that can be exploited as biomarkers capable of detecting premalignant and early-stage cancers. In this context, microRNAs (miRNA), which are small, noncoding RNAs that are frequently dysregulated in cancers, have emerged as promising entities for such diagnostic purposes. Even though the future looks promising, current approaches for detecting miRNAs in blood and other biofluids remain inadequate. This review summarizes existing efforts to exploit circulating miRNAs as cancer biomarkers and evaluates their potential and challenges as liquid biopsy–based biomarkers for gastrointestinal cancers. *Clin Cancer Res; 23(10); 2391–9. ©2017 AACR.*

Introduction

Gastrointestinal cancers occur primarily in the liver, stomach, colorectum, esophagus, and pancreas and account for about 35% of global cancer-related mortalities (1). Recent advances in surgical and endoscopic procedures have significantly improved the survival of patients with early-stage disease. However, the inherently low frequency of some of these cancers, the invasive nature of screening procedures, and the high costs associated with such modalities have resulted in poor compliance for the current generation of screening assays. Although noninvasive screening tests such as fecal immunochemical tests (FIT) are available for screening patients with colorectal cancer, their efficacy remains limited because of low sensitivity and specificity (2) and inability to detect other types of cancers within the gastrointestinal tract. Consequently, inadequate screening modalities for patients with gastrointestinal cancers highlight the imperative need for further research on this important clinically relevant issue.

Within the context of cancer, particularly gastrointestinal malignancies, epigenetic alterations, together with genetic events, have emerged as key drivers of disease development and progression (3). The term "epigenetic" broadly encompasses all heritable changes in gene expression that do not involve a permanent change in the DNA sequence. In cancer, the most well-investigated epigenetic alterations include aberrant DNA methylation, histone modifications, and dysregulated expression of noncoding RNAs (ncRNA; ref. 4). Epigenetic alterations manifest far more frequently than genetic mutations and often appear in early stages of tumorigenesis (5). These alterations are dynamic in nature and potentially reversible, and, hence, have shown promise as attractive substrates for developing disease biomarkers and serve as therapeutic targets in human cancers (5). To date, miRNAs remain the most studied epigenetic alteration in circulation, both as diagnostic and as prognostic cancer biomarkers. In contrast, DNA methylation has been preferentially assessed in tissues, primarily due to the limitation that significant volume of serum/plasma is needed to obtain adequate amounts of DNA for methylation analysis. Furthermore, the assessment of posttranslational histone modifications in the serum is quite limited. Over the last decade, several important studies have evaluated the potential of miRNAs as "liquid biopsy" biomarkers, and, therefore, now is perhaps the appropriate time to objectively assess their true potential as cancer biomarkers.

Among ncRNAs, dysregulated expression of miRNAs has been most widely studied over the last decade, and they appear to be promising diagnostic biomarkers for a variety of human cancers, including gastrointestinal malignancies (6). A large number of these small ncRNAs have been quite well characterized for their biological function in cancer and their ability to regulate the expression of protein-coding genes. From a clinical standpoint, dysregulated expression of miRNAs has been readily detected in a variety of biological fluids in patients with cancer, highlighting the stability of miRNAs in these biofluids and providing a rationale for developing them as liquid biopsy biomarkers. This review summarizes current efforts for implementing specific

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circulatory miRNAs as diagnostic biomarkers for gastrointestinal cancers and discusses how these nucleotides can be incorporated into future cancer therapeutic strategies.

Liquid Biopsies: Novel Frontiers in Cancer Diagnosis

The first interpretation of the term "liquid biopsy" in cancer originated in 2010 when circulating tumor cells (CTC) were proposed as alternatives to conventional breast cancer biopsy for prognosis and evaluation of treatment responses (7). Subsequently, clinical applications of liquid biopsies have diversified from detecting early-stage cancer to monitoring tumor progression, assessing tumor heterogeneity and residual disease, and potentially monitoring therapeutic response to various surgical and chemotherapeutic interventions (8). Figure 1 depicts a theoretical progression of the clinical applicability of liquid biopsies, illustrating various types of liquid biopsy targets, the spectrum of biofluids in which these can be interrogated, and their plausible applications for improving diagnosis, prognosis, personalized therapeutics, and disease monitoring in patients with cancer. These noninvasive but technologically sophisticated applications can be incorporated into existing treatment practices to decrease gastrointestinal cancer–associated mortality.

Recently, the sources of liquid biopsies have expanded beyond blood to include other body fluids including stool (9), urine (10), and saliva (10), which may directly detect

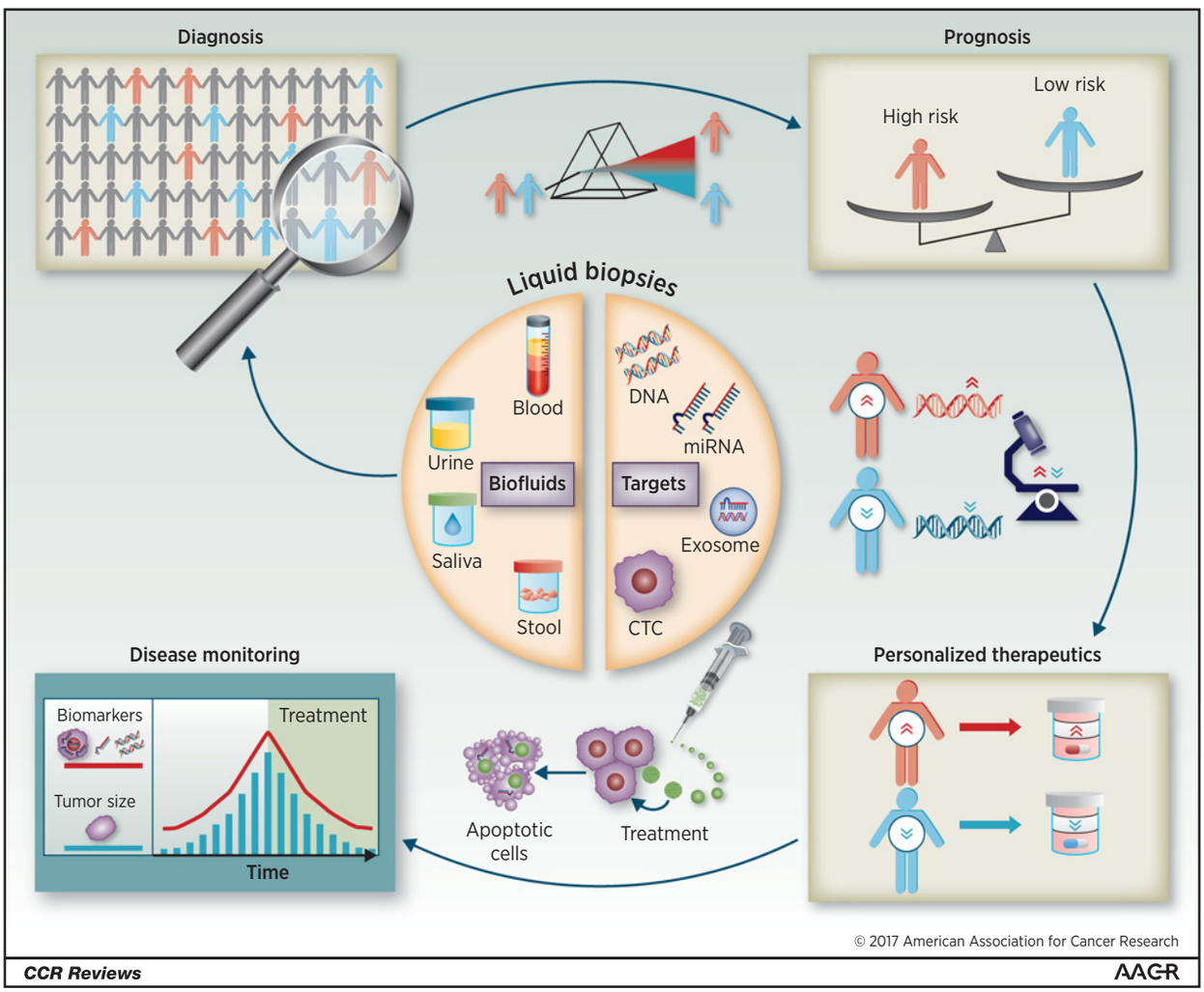

Figure 1.
Clinical applications of liquid biopsies. Liquid biopsies include blood, urine, saliva, and stool. These sources contain cancer-derived subcellular components, such as ctDNAs, circulating tumor miRNAs, CTCs, and exosome-encapsulated DNA and miRNAs. These targets circulate throughout the patient's body and have a number of clinical applications: diagnose cancer at early stages through detection and quantification of these targets; identify aggressive phenotypes and high-risk patients who necessitate intensive treatment; monitor drug efficacy to improve therapy for each patient; and monitor in real time the treatment's effectiveness by correlating these targets with tumor size and stage. Liquid biopsy–based monitoring is potentially more sensitive at following treatment progress than computed tomography and other imaging-based strategies or can be used together to improve the current monitoring protocols.

cancer in associated organs. Likewise, the term "biopsy" has broadened to encompass other subcellular components including circulating tumor DNA (ctDNA; ref. 11), ncRNAs, predominantly miRNAs (12), proteins (13), and extracellular vesicles (14) that can be used as targets for evaluation in gastrointestinal cancer. In this regard, despite the initial enthusiasm for the identification of CTCs and ctDNA in liquid biopsies from patients with cancer, accumulating data indicate that although these targets offer a high degree of cancer specificity, both entities are scarce in circulating biofluids and may be inadequate as clinically applicable diagnostic biomarkers. On average, ctDNA represents less than 1% of the total circulating free DNA found in biofluids, whereas in patients with cancer, the ratio of CTCs to white blood cells is approximately 1:1 million (8). Accordingly, a study that evaluated the ability of ctDNA to identify specific mutations in individuals' primary tumors reported success in only 73% of colorectal, 57% of gastroesophageal, and 48% of pancreatic cancers (15). These results may be considered somewhat disappointing considering that each of these mutations was known *a priori* before screening (16). Consequently, other molecules derived from tumor cells, such as ncRNAs, are far more abundant than ctDNA or CTCs in biofluids, are relatively stable in a variety of biological fluids, and are frequently dysregulated even in the earliest stages of cancer. These characteristics argue in their favor for further development as noninvasive liquid biopsy biomarkers for human cancers.

Circulating miRNAs as cancer diagnostic biomarkers

In 2008, tumor-associated miRNAs (miR-155, miR-210, and miR-21) were first discovered to be upregulated in serum of patients with lymphoma (17). To date, more than hundreds of miRNAs have been identified as potential diagnostic targets in various cancers (6, 18). Circulating miRNAs possess unique features making them likely candidates for development as disease-specific biomarkers. miRNAs are generally stable in blood and other body fluids due to their small size and their ability to escape from RNase-mediated degradation, and nearly 10% of miRNAs are either secreted in membranous nano-sized vesicles called "exosomes," whereas the remaining 90% are stabilized and packaged with other proteins, such as argonaute-2 (Ago2), high-density lipoprotein (HDL), and other RNA-binding proteins (19–21). Furthermore, both exosomal- and Ago2/HDL-attached miRNAs are actively secreted from living cells, whereas the majority of ctDNA is passively released by apoptotic or necrotic cells (8, 21, 22), as illustrated in Fig. 2. A recent study demonstrated that miRNAs offer superior sensitivity and specificity compared with ctDNA for diagnosing colorectal cancers (23). Collectively, miRNAs appear to be promising candidates as liquid biopsy–based cancer biomarkers.

Nevertheless, several obstacles must be overcome before miRNAs can be recognized and adopted as clinically relevant cancer diagnostic biomarkers. In particular, the lack of disease and organ specificity and uncertainty of normalization are among the most critical issues. With the significant body of literature gathered on circulating miRNAs in gastrointestinal cancers and the availability of high-throughput microarray and RNA sequencing profiling from serum and plasma samples from patients with cancer, we are very likely bound to identify robust miRNAs as potential cancer diagnostic markers in the near future. The diagnostic potential of many circulating miRNAs has been assessed in a variety of cancers; those within the gastrointestinal tract are summarized in Table 1, and the key studies are highlighted in the following sections.

Colorectal cancer. Among all gastrointestinal cancers, miRNA-based diagnostic biomarkers largely have been studied in patients with colorectal cancer (18). It is beyond the scope of this article to discuss all reports on this topic, but the more promising miRNA-based diagnostic markers in colorectal cancer have been miR-21, miR-23a, miR-378, and miR-1246, based upon reported AUC values (24–26). More recently, another panel of miRNAs (miR-19a-3p, miR-223-3p, miR-92a-3p, and miR-422a) was derived from pooled serum samples obtained from patients with colorectal cancer and healthy subjects using next-generation sequencing (NGS). Its robustness was confirmed in a collection of 219 specimens, in which these markers successfully distinguished both cancers and adenomas from healthy controls (27). Recent efforts have attempted to translate these findings to liquid biopsy markers for detection of early colorectal neoplasia. In a cohort of 237 patients, circulating levels of miR-21, miR-29a, and miR-125b independently differentiated colorectal neoplasms from healthy controls. However, when combined into a panel, the accuracy of detection improved significantly (28). Collectively, these studies highlight the ability of miRNA biomarkers to identify patients with colorectal cancer and, more importantly, to screen for and detect patients with advanced polyps and early-stage cancer.

Esophageal cancer. The primary causes of esophageal cancers include excessive alcohol consumption, tobacco use, and chronic gastroesophageal reflux disease (1). Currently, esophageal cancer is difficult to resect due to its highly aggressive nature and has a low 5-year survival rate of 17% to 19% (29). The flat morphology of early-stage esophageal cancers makes their diagnosis challenging even with endoscopy, emphasizing the need for markers that can facilitate detection of the earliest stages of disease and improve patient survival (30). On the basis of extensive interrogation of miRNAs upregulated in primary esophageal cancers, miR-18a and miR-25 appear as promising diagnostic markers (31, 32). NGS on pooled serum specimens from patients with advanced esophageal cancer identified a panel of miRNAs comprising miR-10a, miR-22, miR-100, miR-148b, miR-223, miR-133a, and miR-127-3p (33). Subsequent validation of this diagnostic panel in two large, independent clinical cohorts yielded an impressive AUC value of 0.93 (33).

Gastric cancer. Historically, *Helicobacter pylori* infections were considered one of the major causes of gastric cancer, but cancer-associated mortality has declined significantly ever since effective antibiotic regimens were implemented to eradicate this pathogen (1). A thorough review of literature on the diagnostic accuracy of miRNA biomarkers for gastric cancer revealed that miR-18a and miR-21 are among the leading candidates that deserve further interrogation and validation (34, 35). Comprehensive RNA sequencing on 20 gastric cancers and healthy controls revealed a panel of miRNAs (miR-1, miR-20a, miR-27a, miR-34, and miR-423-5p) that can differentiate patients with gastric cancer from healthy controls (36). As diffused-type gastric cancers are difficult to detect using endoscopy, miRNA-based liquid biopsy approaches may provide an attractive, noninvasive, and inexpensive option for improved detection of such lesions.

Hepatocellular and biliary cancer. Hepatitis viruses B and C are major contributors to hepatocellular carcinoma development,

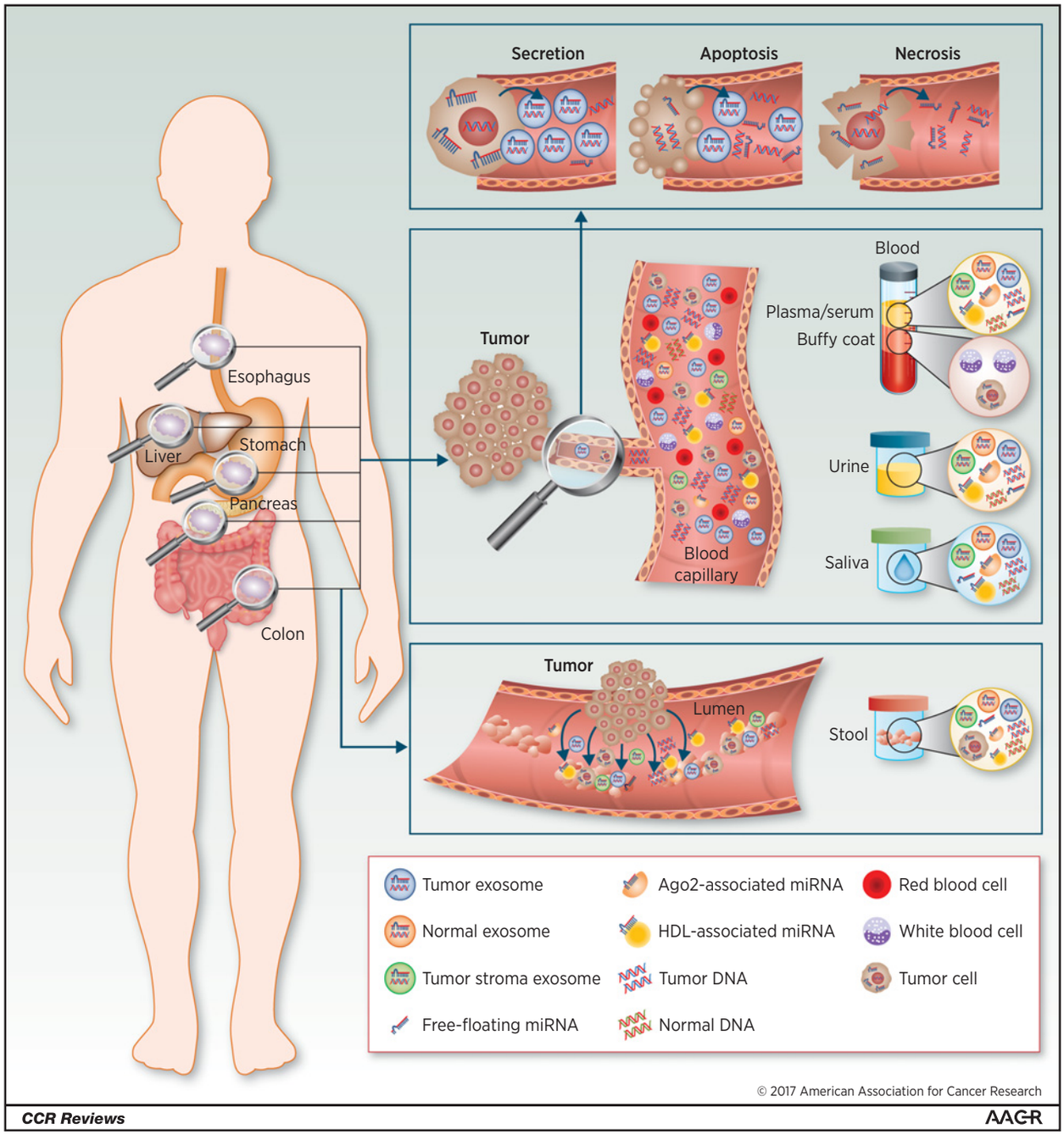

Figure 2.

Screening for gastrointestinal cancers using actively or passively secreted tumor components in liquid biopsies. Gastrointestinal cancers, including esophageal, gastric, liver, pancreatic, and colon, shed subcellular components into the blood stream and/or intestinal lumen. These subcellular components include ctDNAs, circulating tumor miRNAs, CTCs, and exosome-encapsulated DNA/miRNAs. These targets can be detected in biofluids, such as blood, urine, saliva, and feces. Several morphologies of nucleotides are found in biofluids: free-floating DNA/miRNA, Ago2/HDL-associated miRNA, and exosome-encapsulated DNA/miRNA, which are secreted from cancer cells in diverse patterns. Apoptotic or necrotic cells directly shed components extracellularly (passive secretion) as free-floating DNA and miRNAs, whereas living aggressive cancer cells secrete encapsulated DNA and miRNAs in exosomes (active secretion).

whereas other risk factors include cirrhosis, obesity, aflatoxin exposure, and high alcohol consumption (1). A recent large-scale clinical trial developed a plasma-based miRNA panel comprising miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801 (37), which robustly differentiated between two large, independent cohorts of 407 and 390 specimens of hepatocellular

Table 1. Circulating miRNAs as noninvasive diagnostic biomarkers in gastrointestinal cancers

		Sample size			Diagnostic value (%)				
miRNA	**Source**	**Cases**	**Controls**	**AUC**	**Sensitivity**	**Specificity**	**Normalizer**	**Year**	**Reference**
Colorectal cancer									
Single marker									
miR-17-3p	Plasma	90	50	0.72	64	70	U6	2009	(60)
miR-92	Plasma	90	50	0.89	89	70	U6	2009	(60)
miR-21	Serum	186	53	0.93	83	91	cel-miR-39	2013	(26)
miR-23a	Exosome	88	11	0.95	NA	NA	miR-451	2014	(24)
miR-378	Plasma	29	29	0.95	NA	NA	miR-16	2014	(25)
miR-1246	Exosome	88	11	0.95	NA	NA	miR-451	2014	(24)
Panel									
miR-431, 15b, 139-3p	Plasma	45 CRC	26	0.83	91	57	U6	2013	(61)
miR-532-3p, 331, 195, 17, 142-3p, 15b, 532, 652	Plasma	16 Ad	26	0.87	88	64	U6	2013	(61)
miR-19a-3p, 223-3p, 92a-3p, 422a	Serum	117 CRC	102	0.95	84	92	U6	2014	(27)
miR-19a-3p, 223-3p, 92a-3p, 422a	Serum	73 Ad	102	0.77	NA	NA	U6	2014	(27)
miR-21, 31, 92a, 181b, 203, let-7g	Serum	83	59	0.92	96	88	miR-16	2014	(62)
miR-21, 29a, 125b	Serum	160 Ad	77	0.83	NA	NA	cel-miR-39	2015	(28)
Esophageal cancer									
Single marker									
miR-18a	Plasma	106	54	0.94	87	100	mirVana miRNA RP	2013	(31)
miR-1246	Serum	101	46	0.75	71	74	miR-16	2013	(63)
miR-25	Plasma	20	50	0.86	85	86	U6	2014	(32)
Panel									
miR-10a, 22, 100, 148b, 223, 133a, 127-3p	Serum	149	100	0.93	79	96	Serum volume	2010	(33)
miR-21/375 (ratio)	Plasma	50	20	0.82	88	70	mirVana miRNA RP	2011	(64)
miR-25, 100, 193-3p, 194, 223, 337-5p, 483-5p	Serum	63	63	0.83	81	81	let-7d/g/i	2014	(65)
Gastric cancer									
Single marker									
miR-16	Serum	50	47	0.90	79	78	U6	2014	(66)
miR-18a	Plasma	82	65	0.91	81	85	mirVana miRNA RP	2014	(34)
miR-222	Plasma	114	56	0.85	66	88	U6	2014	(67)
miR-21	Serum	50	50	0.91	88	80	U6	2015	(35)
Panel									
miR-106a/let-7a (ratio)	Plasma	69	30	0.88	86	80	U6	2010	(68)
miR-1, 20a, 27a, 34, 423-5p	Serum	142	105	0.88	80	81	miR-16	2011	(36)
miR-223, 21, 218	Plasma	70	70	0.95	84	93	cel-miR-39	2012	(69)
Hepatocellular carcinoma									
Single marker									
miR-21	Serum	101	89	0.87	84	74	miR-181a, 181c	2011	(70)
miR-122	Serum	101	89	0.79	71	69	miR-181a, 181c	2011	(70)
miR-223	Serum	101	89	0.86	80	77	miR-181a, 181c	2011	(70)
miR-18a	Serum	86	45	0.88	86	75	U6	2012	(71)
Panel									
miR-122, 192, 21, 223, 26a, 27a, 801	Plasma	196	66	0.94	83	94	miR-1228	2011	(37)
miR-375, 25, let-7f	Serum	55	100	0.99	98	99	plant miR-168	2011	(72)
miR-23b, 423, 375, 23a, 342-3p	Serum	55	100	0.99	97	99	plant miR-168	2011	(72)
miR-29a, 29c, 133a, 143, 145, 192, 505	Serum	229	108	0.82	75	89	cel-miR-67	2015	(38)
Pancreatic cancer									
Single marker									
miR-200a	Serum	45	32	0.86	84	88	miR-16	2010	(73)
miR-200b	Serum	45	32	0.85	71	97	miR-16	2010	(73)
miR-27a-3p	Whole blood	129	60	0.86	82	79	U6	2013	(74)
miR-1290	Serum	41	19	0.96	88	84	miR-16	2013	(43)
Panel									
miR-16, 196a (with CA19-9)	Plasma	140	68	0.98	92	96	cel-miR-39	2012	(75)
miR-20a, 21, 24, 25, 99a, 185, 191	Serum	95	81	0.99	94	93	Serum volume	2012	(44)
miR-145, 150, 223, 636	Whole blood	86	44	0.83	85	48	ath-miR159a	2014	(76)
miR-26b, 34a, 122, 126*, 145, 150, 223, 505, 636, 885-5p	Whole blood	86	44	0.82	85	55	ath-miR159a	2014	(76)
Biliary cancer									
Single marker									
miR-21	Plasma	94	50	0.93	85	100	miR-16	2013	(40)
miR-126	Serum	31	40	0.87	68	93	cel-miR-39	2015	(77)
miR-1281	Serum	31	40	0.83	55	90	cel-miR-39	2015	(77)
Panel									
miR-6075, 4294, 6880-5p, 6799-5p, 125a-3p, 4530, 6836-3p, 4476	Serum	98	150	0.95	80	98	Microarray-based normalization	2015	(41)

Abbreviations: Ad, adenoma; CRC, colorectal cancer; mirVana miRNA RP, Thermo Fisher Scientific mirVana miRNA reference panel; NA, not available.

Table 2. Most promising miRNA biomarkers with diagnostic significance

miRNA	Supporting evidence	Limitations	Source
Colorectal cancer			
miR-21	• One of the most abundant miRNAs • Highly upregulated miRNAs in solid tumors • One of the most studied diagnostic circulating miRNAs • Suitable for early diagnosis	• Not cancer specific • Upregulated by inflammation • Influenced by hemolysis	Serum, plasma
miR-29a	• Unaffected by hemolysis • Suitable for early diagnosis		Serum, plasma
miR-92a	• One of the most abundant miRNAs	• Influenced by hemolysis	Serum, plasma
Gastric cancer			
miR-21	• Same as above	• Upregulated by *H. pylori* infection	Serum, plasma
miR-27a	• Well-established oncogene • Unaffected by hemolysis	• Upregulated by *H. pylori* infection	Serum, plasma
Hepatocellular carcinoma			
miR-21	• Same as above	• Upregulated by hepatitis virus infection	Serum, plasma, exosome
miR-192	• Suitable for early diagnosis	• Upregulated by hepatitis virus infection	Serum, plasma
Pancreatic cancer			
miR-21	• Same as above	• Same as above	Serum, plasma, exosome
miR-223	• Unaffected by hemolysis • Overexpressed in early-stage pancreatic cancer	• Influenced by aspirin	Plasma, whole blood

carcinomas and healthy controls. However, these results could not be replicated in another study, which identified miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505 as diagnostic markers (38). This discrepancy emphasized the need for more carefully designed discovery and validation cohorts for liquid biopsy biomarker discovery.

Biliary cancer is a rare disease and affects 2,000 to 3,000 people each year in the United States (39). Similar to other gastrointestinal cancers, miR-21 appears to be the most promising circulating miRNA-based diagnostic marker for biliary cancer (40). However, a recent study that compared microarray expression profiles in serum from healthy subjects and patients with cancer identified dysregulation of several previously unreported miRNAs including miR-125a-3p, miR-6075, miR-4294, miR-6880-5p, miR-6799-5p, miR-4530, miR-6836-3p, and miR-4476. It is interesting to note that most of these biliary cancer–associated miRNAs are somewhat unique and are not frequently altered in other gastrointestinal cancers (41).

Pancreatic cancer. Pancreatic cancer has the lowest 5-year survival rate—of about 7%—among all gastrointestinal cancers because of the basic biology of the disease, which is further compounded by a dearth of optimal detection methods (42). It cannot be screened by endoscopy, whereas imaging methods include abdominal ultrasonography—the gold standard for detection. These methods have limited detection rates due to the anatomic location of pancreas, particularly for smaller lesions. High-throughput PCR arrays identified serum miR-1290 as a robust circulating diagnostic marker for pancreatic cancer (43). NGS identified an miRNA panel (miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191) that remarkably differentiated patients with pancreatic cancer from healthy controls, with an AUC of 0.99 (44). However, there remains a need for noninvasive liquid biopsy–based biomarkers that will improve survival of patients by detecting precancerous or early-stage pancreatic cancers.

miRNA diagnostics: A panel-based approach

Despite recent discoveries that promote circulating miRNAs to diagnose gastrointestinal cancers, none have led to the implementation of markers for clinical use due to the inadequacy of solitary miRNA biomarkers in clinical testing. A growing interest to combine biomarkers into panels (45) confronts the issue of tumor heterogeneity and low specificity and sensitivity of solitary miRNAs to detect a particular cancer. In this regard, several mathematical models were utilized to evaluate the performance of combinations of miRNAs as cancer diagnostic markers. These strategies include threshold-based methods, decision trees, logistic regression, and support vector machine (46). Although combining markers clearly improved the diagnostic potential of miRNAs, unfortunately one of the limitations is that most miRNA panels reported to date were derived using insufficient sample sizes, and validations were performed inadequately in clinical applications. Furthermore, these biomarker panels failed to exploit the statistical leverage associated with combining multiple markers and instead contributed to vast discrepancies and noise across various studies that selected miRNAs. However, such inadequacies are expected, and, considering the wealth of knowledge gathered on this discipline, future studies will address these concerns and hopefully yield liquid biopsy biomarker panels that can routinely detect gastrointestinal cancers.

Although this article has primarily focused on describing the diagnostic potential of circulating miRNAs, the clinical usefulness of these biomarkers also extends their ability to serve as prognostic and predictive biomarkers for response to chemotherapy, as summarized in Supplementary Tables S1 and S2.

A current perspective on miRNA-based diagnostic cancer biomarkers

Several well-recognized obstacles must be overcome before miRNA biomarkers can realistically transition to the clinic. First, qRT-PCR–based quantification of miRNAs is imperfect due to lack of a consensus endogenous normalizer. Currently, the expression of miRNAs in serum or plasma is commonly normalized using either endogenous internal controls (housekeeping genes) or synthetic spiked-in controls (i.e., cel-miR-39 or ath-miR159a) in a standardized sample volume. Although synthetic spike-in controls are simple and an accurate way to quantify miRNAs, standardization of expression values across multiple cohorts remains challenging. Considering that differences in extraction procedures and storage conditions can affect RNA quality and subsequently influence the outcome of spike-

in control normalized data, spike-in controls may not be suitable for clinical circumstances. Furthermore, the use of spike-in controls is not adequate for analyzing expression of circulating miRNAs contained in exosomes, as it requires an additional step of ultracentrifugation-based purification. Therefore, the current practice remains of involving the use of endogenous controls, such as U6, miR-451, and miR-16, even though several studies have found the expression of these markers to be dysregulated in cancers, making them unsuitable for normalization purposes (47). Alternatively, as the cost associated with RNA sequencing becomes more affordable, RNA-seq–based global normalization procedures such as RPKM (reads per kilobase per million mapped reads) could be used to eliminate the biases associated with endogenous and spike-in controls. Second, the low disease and organ specificity of circulating miRNAs hamper miRNA-based cancer biomarker research. There is a school of thought that changes in circulating miRNAs in patients with cancer often occur holistically and may not truly reflect alterations present in the tumor itself. For example, several cancer-associated circulating miRNAs are also elevated in inflammatory diseases such as colitis and rheumatoid arthritis (48). Well-established oncogenic miRNAs such as miR-21 and miR-155 have been linked to inflammation, and, despite extensive research, the question remains whether the overexpression of these miRNAs is causally linked to cancer or are a consequence of inflammation (49). Similarly, certain oncogenic miRNAs are upregulated in multiple cancer types and, thus, are not organ specific. A significant step to overcome this problem was addressed in a recent NGS-based study where multiple cancer types were compared and 71 organ-specific iso-miRNAs (iso-miR) were identified (50). A follow-up study developed a panel of iso-miRs that adequately identified patients with triple-negative breast cancers (51), highlighting the potential of iso-miRs to identify the organ of origin. In addition, several miRNAs have been identified to undergo RNA editing in cancers, and these miRNAs with edited sequences appear to acquire new biologic functions (52). In melanoma, edited miR-445 enhanced tumor growth and metastasis. Likewise, high-throughput sequencing profiling identified a small population of edited miRNAs in colorectal cancer (52, 53). Collectively, discovery of both iso-miRNAs and edited miRNAs broadens potential candidates for miRNA-based biomarkers and highlights the functional complexity of miRNAs.

Moreover, recent research identified exosomal miRNA populations that are organ and cancer specific (54). For instance, A33 is an epithelial cell–specific antigen found exclusively on the surface of exosomes released from the colon (55). Similarly, a recent report demonstrated that exosomes expressing Glypican-1, a cell surface proteoglycan, are released exclusively from pancreatic cancer cells and not from normal cells (54). Microarray-based comparison between plasma and exosomal miRNA showed significant difference in miRNA contents, indicating that exosomal miRNAs could improve the biomarker potential of conventional serum-based circulating miRNA markers (56). Furthermore, recent technological advancements could make an enormous impact on the development of new screening methods for the detection of cancer exosomes. Recently, a modification was made to the conventional FACS methodology that allows detection and sorting of a specific population of exosomes by labeling surface proteins with fluorescence antibodies (57). This study assessed surface EGFR and CD9 in exosomes isolated from a colorectal cancer cell line as well as plasma-derived human exosomes. Similarly, surface plasmon resonance spectroscopy was used to assess the levels of CD44, CD24, and EpCAM on the breast cancer cell line–derived exosomes (58). These methodologies not only will be utilized for biomarker research in the near future but will also clarify the physiologic and mechanistic roles of cancer exosomes. Adding to our fundamental understanding of exosomal miRNAs, we have now recognized that such miRNAs are frequently taken up by neighboring or distant cells and, subsequently, functionally modulate recipient cells (59). Collectively, comprehensive characterization of epigenome and proteome in cancer-derived exosomes appears to be the focal point of the miRNA-based biomarker field and could transform the conventional school of blood-based molecular cancer diagnostic markers.

Nonetheless, a careful review of literature still supports the notion that a small panel of miRNAs is consistently upregulated in various cancers and detected in the blood of patients with cancer (Table 2). As these miRNA biomarkers have shown validation in multiple independent studies, there is a growing enthusiasm that some of these may likely be ready for clinical applications in the near future.

Conclusions

The field of miRNA-based cancer research has witnessed a remarkable evolution over the last two decades. Although much effort to date has been to identify specific miRNAs and their role in cancer, interest has grown to evaluate their potential as disease biomarkers, as well as recent attempts at exploiting their significance as therapeutic targets. Their small size and stability in a variety of body fluids make them attractive substrates for biomarker development. As this field continues to mature with the identification of more specific subtypes of miRNAs and increase in focus on large-scale, multicenter, comprehensive studies, miRNA-based diagnostic approaches are likely to usher in a new era of personalized medicine for patients with cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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