

## MicroRNA Signatures: Novel Biomarker for Colorectal Cancer?

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### Abstract

Aberrant microRNA (miRNA) expression might be of potential use as diagnostic and prognostic biomarker for cancers. We reviewed studies published until March 2011 which assessed expression of miRNAs in colorectal cancer (CRC)/adenoma tissue and normal colorectal mucosa and in plasma of CRC/adenoma patients and healthy controls. Overall, 20 studies that investigated miRNA expression in tissue and 3 studies that investigated miRNA levels in plasma were included. A total of 160 miRNAs were found to be dysregulated in CRC. MiR-20a and miR-31 were found to be significantly upregulated in more than one study, and miR-143 and miR-145 were found to be significantly downregulated in CRC tissue in six or more studies. MiR-92a was significantly upregulated in CRC patients in two of the plasma-based studies and in CRC tissue in one of the tissue-based studies. Our results provide timely and relevant information for miRNAs as potential diagnostic biomarkers for CRC. The expression of miRNAs in plasma may be indicative of presence of CRC. Larger diagnostic studies are needed to evaluate potential use of miRNA expression in early detection and diagnosis of CRC. *Cancer Epidemiol Biomarkers Prev*; 20(7); 1272–86. ©2011 AACR.

### Introduction

Colorectal cancer (CRC) is the third most common malignancy in the world, accounting for more than 1 million cases and 500,000 deaths per year (1). Because of its slow development from premalignant lesions, perspectives to reduce the burden of disease by early detection and treatments are particularly promising for this malignancy.

Although colonoscopy is the most reliable method for early detection of CRC and its precursors available to date, the invasive nature and the cost incurred have hampered its widespread application. The fecal occult blood test (FOBT), which is the most widely used noninvasive screening tool so far, is limited by its low sensitivity, especially with respect to detection of preneoplastic lesions (2). Stool DNA tests may be a promising alternative in the future (3), but widespread application is so far limited by labor-intensive handling and high costs. Thus, there is a pressing need for new noninvasive biomarkers to improve early detection of CRC.

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MicroRNAs (miRNAs) are small noncoding, 19 to 22 nucleotide sequences of RNA that are involved in the regulation of cellular development, differentiation, proliferation, apoptosis, and metabolism (4). Although the natural mechanisms of the dysregulation of miRNAs are still largely unknown, functional studies have indicated deregulation of miRNAs to be involved in the initiation and progression of human cancers (5). Therefore, aberrant miRNAs expression might be of potential use as diagnostic and prognostic biomarker for cancers.

Although functions of miRNAs are far from being fully understood, it is predicted that approximately 30% of protein-encoding genes are controlled by miRNAs (6). During the past few years, a rapidly increasing number of miRNAs have been discovered. Eighteen years after its set up by Lee and Feinbaum in 1993, the miRBase meanwhile contains 15,172 entries representing hairpin precursor miRNAs, expressing 17,341 mature miRNA products, in 142 species, including 1,344 mature miRNAs in humans (7).

miRNAs play important roles in oncogenesis, and the expression level of miRNAs as antioncogenes is frequently reduced in cancers because of chromosomal aberrations (8), transcriptional regulation, or methylation (9, 10). The levels of miRNAs in serum and plasma are remarkably stable, reproducible, and consistent among individuals of the same species (11, 12). Recently, it has been shown that extracellular miRNAs are predominantly floating exosome free (12). It has been shown that the astonishing stability of extracellular miRNA is explained by binding of miRNA to the Ago2 protein, a part of the RNA-induced silencing complex (12). Turchinovich and colleagues hypothesize that extracellular

miRNAs are, in the most part, by-products of dead cells that remain in extracellular space because of the high stability of the Ago2-miRNA complex. If and to what extent miRNA-Ago complexes can be specifically released from cells and confer cell-to-cell communication needs to be investigated. Turchinovich and colleagues show that miRNA remains stable in the extracellular space for at least 1 month, suggesting the possibility of using extracellular miRNA as a biomarker for cancer, tissue/organ damages, and viral infections (12).

Tumor-associated miRNAs have been detected in the serum or plasma from patients suffering from lymphoma, breast, and other cancers (13, 14). Three studies showed that some miRNAs have significantly upregulated or downregulated levels in the plasma of CRC patients, comparing favorably with FOBT for the detection of CRC (15–17). These findings suggest a possible use of miRNAs as novel noninvasive biomarkers for cancer detection.

Several recent articles have reviewed current knowledge about biogenesis and mechanism of action of miRNAs, and the potential role of some miRNAs in the pathogenesis of CRC and their relationship with development, treatment, and prognosis of CRC (18–22). The aim of this article was to assess the differential expression of miRNAs in CRC/adenoma and normal colorectal mucosa and in plasma of CRC patients and healthy controls.

## Methods

A comprehensive literature search was conducted to identify studies assessing dysregulation of miRNAs in blood or tissues of CRC patients. PubMed (-Mar 18, 2011), EMBASE (Elsevier, Amsterdam, the Netherlands, 1980-Mar 18, 2011), and ISI Web of Knowledge (Thomson Scientific Technical Support, New York, 1945-Mar 18, 2011) databases were searched for relevant articles by the following combinations of relevant terms: (colorectal or colon or rectal or rectum) and (cancer or neoplasm or tumor or carcinoma or malignancy or adenoma) and (microRNA or miRNA or miRNA or let-7; for details of the search process see Supplementary Appendix S1). Duplicate publications were deleted. Each title and abstract was checked for relevance. The full text was reviewed if the abstract indicated that the article reported associations between miRNA expression and CRC. The search was limited to studies on humans published in English. Only full-text articles were included because abstracts did not provide enough information for a detailed review. Only studies examining both colorectal cancer/adenoma tissue and normal colorectal mucosa, as well as plasma-based studies with CRC cases and healthy controls were included.

The following data were extracted from the eligible studies independently by 2 investigators (Luo X and Tao S) in a standardized manner, and any disagreement was resolved by consensus: authors, publication year,

country, kind of the samples, characteristics of the study population, laboratory methods, miRNAs detected in the study, miRNAs found to be dysregulated in tissue or plasma of CRC/adenoma patients, and the *P* values for the association with CRC.

## Results

A flow diagram of the search process is given in Figure 1. The searches yielded 1,321 entries. Following removal of 617 duplicates, 704 titles and abstracts were assessed and 240 articles seemed to be potentially relevant for inclusion in the review. A total of 217 articles were excluded for the following reasons: not original articles but reviews, comments, or lectures in conferences ( $n = 93$ ), not English articles ( $n = 10$ ), assessments of miRNAs and radiation therapy, chemotherapy ( $n = 10$ ), assessments of miRNAs in cell lines ( $n = 9$ ), assessments of polymorphisms of miRNAs ( $n = 3$ ), assessments of miRNAs' targeting mRNA, acid, enzymes, protein, or genes ( $n = 59$ ), assessments of miRNAs and methylation ( $n = 5$ ), methods of detecting miRNAs in CRC ( $n = 5$ ), data normalizations in miRNAs studies ( $n = 1$ ), assessments of miRNAs from stool samples ( $n = 2$ ), critical data not derivable ( $n = 12$ ), no healthy controls ( $n = 5$ ), multitissue combined analysis ( $n = 1$ ), animal experiments ( $n = 2$ ; Supplementary Appendix S2). Twenty-three studies were included in this review.

Three studies (15–17) investigated miRNAs in the plasma from a total number of 333 patients with CRC, 37 patients with advanced colorectal adenoma, and 166 healthy controls. The other 20 studies (23–42) detected miRNAs in the tissues from altogether 1,126 patients with CRC, 66 colorectal adenoma cases, and 936 normal controls. Overall 114 miRNAs were found upregulated in CRC/adenoma compared with normal tissue/plasma, and 50 miRNAs were found downregulated. Four miRNAs (miR-147, miR-191, miR-203, and miR-215) were found to be upregulated in some studies but downregulated in some other studies (Tables 1–3).

## Methods used in the studies

Most of the studies measured a panel of miRNAs on a small set of samples with the quantitative real-time PCR (qRT-PCR; Refs. 15, 16, 23–27, 29–34, 37, 39, 41, 42; Tables 1 and 2) or miRNA MicroArray, followed by further validations of miRNAs found to be dysregulated in a larger scale set of samples with qRT-PCR (Refs. 17, 28, 35, 36; Tables 1 and 2).

## Dysregulated miRNAs in plasma

The 3 plasma-based studies investigated 3, 12, and 95 miRNAs, respectively (15–17). With one exception (miR-320b), the 3 miRNAs (miR-21, -221, and -222) tested by Pu and colleagues and the 12 miRNAs (miR-17-3p, -25, 29, 92a, 134, 146a, 181d, 191, 221, 222, 223, and 320a) tested by Huang and colleagues were included among the 95 miRNAs assessed in the study by Ng and colleagues

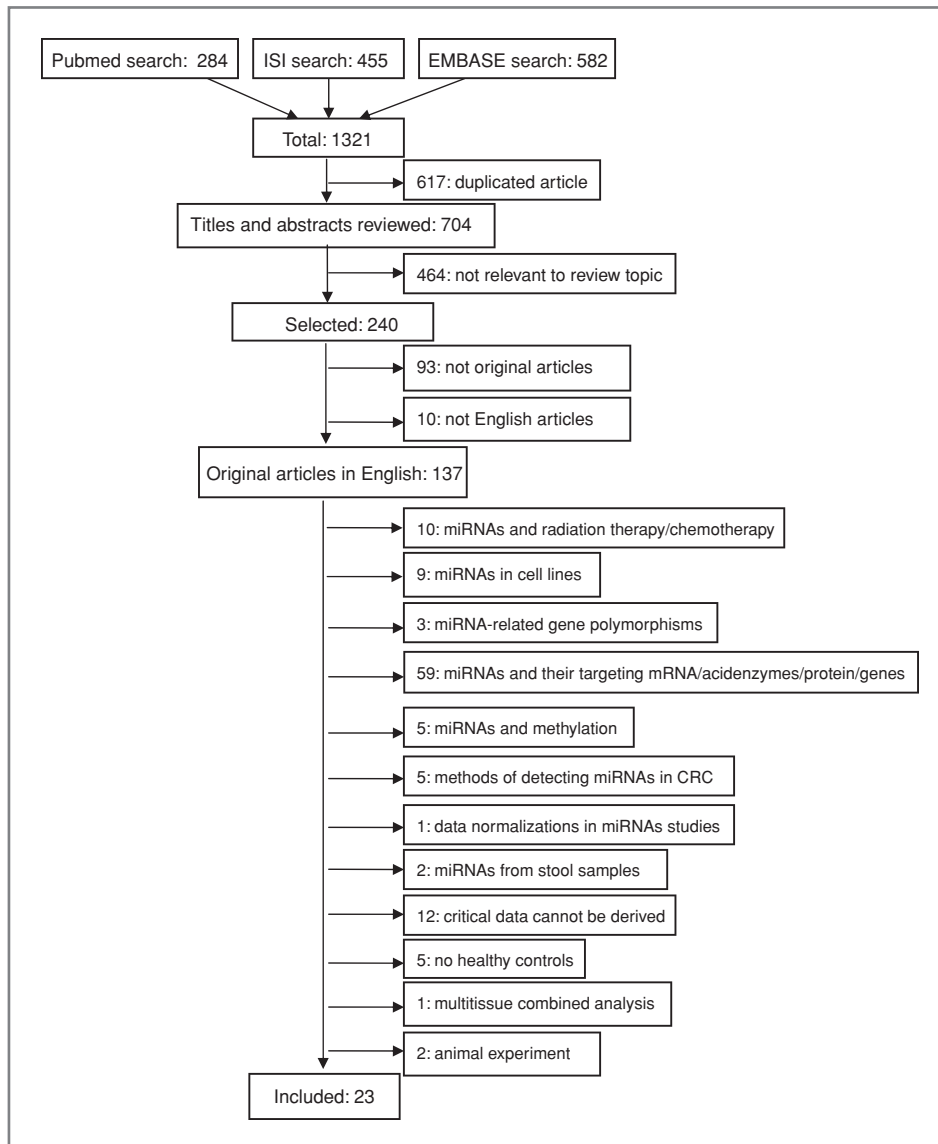


Figure 1. Flow diagram of the literature search process.

(17). The latter study found miR-17-3p and miR-92 (miR-92a, according to the accession number of miRBase) to be significantly upregulated in 130 CRC patients compared with 75 healthy controls ( $P < 0.0001$ ) by using qRT-PCR. In 10 CRC patients, expression of miRNAs was measured before and 7 days after surgical cancer resection, and miR-17-3p and miR-92a seemed to be downregulated after the surgery ( $P = 0.02$ ). Levels of miR-92 of patients with other gastrointestinal diseases were not significantly higher than that in healthy controls [CRC vs. healthy controls  $P < 0.0001$ , gastric cancer vs. healthy controls  $P = 0.4$ , inflammatory bowel disease vs. healthy controls  $P < 0.0001$  (miR-92 was significantly higher in healthy controls)]. Furthermore, expression of miR-92 was not related to other gastrointestinal diseases. Huang and colleagues (16) also found overexpression of miR-92a in plasma of 100 CRC patients and 37 advanced adenoma

patients along with overexpression of miR-29a (both  $P < 0.0001$ ). Analysis of Pu and colleagues (15) showed that expression of miR-221 in plasma was significantly higher in 103 CRC patients than in 32 healthy controls ( $P = 0.0021$ ) from 3 miRNAs.

### Dysregulated miRNAs in tissue

Most of the studies investigated the miRNAs in the tissue samples of CRC patients (23–42). Sample sizes ranged from 3 pairs of tissue (tumor tissue and nontumor tissue) to 215 pairs. The numbers of assessed miRNAs ranged from 1 to 723, and the numbers of miRNAs whose expression was found to differ significantly between tumor tissue and nontumor tissue ranged from 1 to 92. Overall, 164 miRNAs were found to be dysregulated in CRC. In more than one study, miR-20a and miR-31 were found to be significantly upregulated, and miR-143 and

**Table 1.** Studies assessing miRNAs expression in plasma among colorectal tumor patients and healthy controls

Ref. Author(s), year	Study population		Methods	Normalization controls	miR						
	Country	Cases (stage I/II/III/IV)			Control	Age mean (range)	Tested miRs (No.)	Significant miRs (No.)	Difference between cases and controls (P)		
15	Pu et al., 2010	China	103 (7/38/40/18) CRC	32 healthy	CRC 58 (median; 39–84) Control 32 (median; 17–77)	SYBR green qRT-PCR	–	3	1	miR-221↑	P = 0.0021
16	Huang et al., 2010	China	100 (27/25/38/10) CRC <sup>a</sup> 37 advanced adenomas <sup>a</sup>	59 healthy <sup>a</sup>	CRC 61 (26–84) Adenoma 55 (34–75) Control 58 (27–81)	SYBR green qRT-PCR	miR-16	12	2	miR-29a↑ miR-92a↑	P < 0.0001 P < 0.0001
17	Ng et al., 2009	China	130 (6/35/39/50) CRC <sup>b</sup>	75 healthy <sup>b</sup>	CRC 71 (42–91) Control 69 (45–85)	Cancer microRNA array and SYBR green qRT-PCR	RNU6B	95	2	miR-29a↑ miR-92a↑ miR-17-3p↑	P < 0.0001 P < 0.0001 P < 0.0001

<sup>a</sup>3 miRs were selected from 12 miRs on a set of plasma samples (20 cases vs. 20 controls), then validated in 80 CRC, 37 advanced adenomas, and 39 healthy controls.

<sup>b</sup>5 miRs were selected from 95 miRs by comparing miRNA profiles from plasma of 5 CRC patients versus 5 age-matched healthy controls and tissues of 5 CRC patients versus 5 adjacent normal tissues with miRNA Array, then 2 miRs were selected from the validation on a small set of plasma samples (35 cases vs. 20 controls) by qRT-PCR, then a large-scale validation on plasma samples (90 cases vs. 50 controls).

**Table 2. Studies assessing miRNAs expression in colorectal tumor tissue and nontumor tissue**

Ref. Author(s), year	Study population		Methods	Normalization controls		miR			
	Country	CRC (stage I/II/III/IV)/ adenoma tissue		Nontumor tissue	Age mean (range)		Tested miRs (No.)	Significant miRs (No.)	Difference between CRC tissue and nontumor tissue
23	Kulda et al., 2010	Czech Republic	46 CRC (102/1/13/2)	TaqMan MicroRNA Assays	RNU6B	2	2	miR-21↑ <i>P</i> < 0.0001	
24	Chiang et al., 2011	China	30 colorectal liver metastases	SYBR green qRT-PCR	miR-191	1	1	miR-143↓ miR-211↓	<i>P</i> < 0.0001 <i>P</i> < 0.0001
			107 CRC		miR-143↓ RNU6B	1	1	miR-203↓	<i>P</i> < 0.0001
25	Earle et al., 2010	United States	55 CRC (525/18/1)	TaqMan MicroRNA Assays	ΔC <sub>t</sub> method	24	20	miR-171 <i>P</i> = 4.38 × 10 <sup>-4</sup>	miR-135a↑ <i>P</i> = 1.12 × 10 <sup>-3</sup> miR-26b↓ <i>P</i> = 8.91 × 10 <sup>-9</sup>
			66 CRC (17/18/22/9)		miR-20a↑ <i>P</i> = 1.52 × 10 <sup>-4</sup> miR-251 <i>P</i> = 3.92 × 10 <sup>-7</sup> miR-311 <i>P</i> = 6.02 × 10 <sup>-13</sup> miR-921 <i>P</i> = 1.96 × 10 <sup>-6</sup> miR-931 <i>P</i> = 3.33 × 10 <sup>-5</sup> miR-139b↑ <i>P</i> = 1.36 × 10 <sup>-2</sup> miR-126↓	1	1	miR-183↓ <i>P</i> = 2.47 × 10 <sup>-16</sup> miR-143↓ <i>P</i> = 2.47 × 10 <sup>-8</sup> miR-145↓ <i>P</i> = 9.39 × 10 <sup>-8</sup> miR-191↓ <i>P</i> = 3.71 × 10 <sup>-6</sup> miR-192↓ <i>P</i> = 3.51 × 10 <sup>-16</sup> miR-196a↓ <i>P</i> = 7.72 × 10 <sup>-4</sup> miR-215↓ <i>P</i> = 2.37 × 10 <sup>-19</sup> <i>P</i> < 0.001	
26	Li et al., 2010	China	81 CRC (3 colonic tumor tissue)	SYBR green qRT-PCR	RNU6B	1	1	miR-188↓ miR-195↓ miR-184↑	miR-196a↓ <i>P</i> = 4.93 × 10 <sup>-4</sup> miR-16↓ <i>P</i> = 1.78 × 10 <sup>-3</sup> <i>P</i> < 0.01
27	Liu et al., 2010	China	81 CRC	qRT-PCR	RNU6B -	1	1	miR-195↓	miR-374a↑ <i>P</i> < 0.01
28	Wang et al., 2010	China	3 colonic tumor tissue	microRNA microarray and TaqMan MicroRNA Assays	RNU6B	723	14 <sup>b</sup>	miR-188↓ miR-19a↓ miR-20a*↑ miR-106b↑	miR-196b↑ miR-378↓ miR-378*↓ (miR-18a, 135b were validated by qRT-PCR)
29	Wang et al., 2009	China	98 (I+II 45/III+IV 53)	TaqMan MicroRNA Assays	5S rRNA	3	2	miR-311	<i>P</i> = 0.001
30	Diosdado et al., 2009	The Netherlands	55 (30 adenomas/25 adenocarcinomas)	TaqMan MicroRNA Assays	RNU48	6	6	miR-171 <i>P</i> = 0.001	miR-196b↑ miR-224↑ miR-301b↓ miR-335↑
			10 normal mucosa		miR-18a↑ <i>P</i> = 0.04	miR-20a↑ <i>P</i> = 0.001	miR-19b-1↑ <i>P</i> = 0.021	miR-92a-1↑ <i>P</i> < 0.001 miR-552↓ <i>P</i> = 7.59 × 10 <sup>-14</sup> miR-139↓ <i>P</i> = 0	
31	Sarver et al., 2009	United States	80 colon cancer	SYBR green qRT-PCR	RNU6B	735	39	miR-19a↑ <i>P</i> < 0.001 miR-17-3p↓ <i>P</i> = 7.59 × 10 <sup>-14</sup> miR-311↓ <i>P</i> = 0	miR-138↓ <i>P</i> = 0 miR-584↑ <i>P</i> = 0 miR-139↓ <i>P</i> = 0

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Table 2. Studies assessing miRNAs expression in colorectal tumor tissue and nontumor tissue (Cont'd)

Ref.	Author(s), year	Study population			Methods	Normalization controls	Tested miRs (No.)	Significant miRs (No.)	miR	Difference between CRC tissue and nontumor tissue
		Country	CRC stage I/II/III/IV/ adenoma tissue	Nontumor tissue						
32	Arndt et al., 2009	United States	45 (4/19/20/2) CRC	4 normal colon tissue	CRC 62.8 Control -	TaqMan MicroRNA Assays miR-19a] $P=2.3 \times 10^{-3}$ miR-30a-3p] $P=1.0 \times 10^{-4}$ miR-106b] $P=7.30 \times 10^{-4}$	169	37	miR-32 ↑ $P=1.97 \times 10^{-5}$ miR-33 ↑ $P=1.76 \times 10^{-9}$ miR-96 ↑ $P=7.11 \times 10^{-14}$ miR-135b] $P=0$ miR-182 ↑ $P=0$ miR-182 ↑ $P=0$ miR-183 ↑ $P=0$ miR-188 ↑ $P=1.20 \times 10^{-11}$ miR-224 ↑ $P=0$ miR-503 ↑ $P=0$ miR-542-5p] $P=7.01 \times 10^{-11}$ $(P < 10^{-16}$ represent as 0) miR-18a] $P=2.9 \times 10^{-3}$ miR-19b] $P=3.4 \times 10^{-3}$ miR-20a] $P=2.0 \times 10^{-3}$ miR-21] $P=6.0 \times 10^{-6}$ miR-25] $P=1.4 \times 10^{-2}$ miR-28a] $P=3.6 \times 10^{-4}$ miR-28b] $P=4.9 \times 10^{-3}$ miR-31] $P=2.6 \times 10^{-3}$ miR-34a] $P=1.5 \times 10^{-2}$ miR-93] $P=3.4 \times 10^{-3}$ miR-95] $P=1.1 \times 10^{-2}$ miR-96] $P=8.4 \times 10^{-3}$ miR-31] $P=0.016$	miR-147] $P=0$ miR-328] $P=2.62 \times 10^{-14}$ miR-363] $P=1.30 \times 10^{-7}$ miR-375] $P=0$ miR-378] $P=0$ miR-486] $P=6.90 \times 10^{-9}$ miR-497] $P=0$ miR-511] $P=2.22 \times 10^{-16}$ miR-551b] $P=0$ miR-642] $P=0$ miR-650] $P=0$ miR-106a] $P=5.1 \times 10^{-4}$ miR-125a] $P=6.40 \times 10^{-3}$ miR-30a-5p] $P=1.60 \times 10^{-5}$ miR-30c] $P=4.00 \times 10^{-4}$ miR-133a] $P=6.50 \times 10^{-4}$ miR-139] $P=1.10 \times 10^{-5}$ miR-143] $P=1.00 \times 10^{-2}$ miR-145] $P=1.20 \times 10^{-3}$ miR-195] $P=4.20 \times 10^{-5}$ miR-378*] $P=1.30 \times 10^{-5}$ miR-422a] $P=7.30 \times 10^{-5}$ miR-422b] $P=8.30 \times 10^{-5}$ miR-497] $P=9.80 \times 10^{-4}$ miR-145] $P=0.048$
33	Chen et al., 2009	China	13 CRC	13 MNAT	57.2	qRT-PCR	-	200	15	miR-1] $P=1.6 \times 10^{-3}$ miR-10b] $P=2.1 \times 10^{-2}$ miR-200a] $P=0.036$

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**Table 2. Studies assessing miRNAs expression in colorectal tumor tissue and nontumor tissue (Cont'd)**

Ref. Author(s), year	Study population			Methods	Normalization controls	Tested miRNAs (No.)	Significant miRNAs (No.)	miR	
	Country	CRC (stage I/II/III/IV)/ adenoma tissue	Nontumor tissue					Age mean (range)	Difference between CRC tissue and nontumor tissue
34 Schmitz et al., 2009	Germany	18 SSAs	20 Normal colonic mucosal	TaqMan MicroRNA Assays	RNU48	4	3	miR-92↓	miR-200c↓
								miR-103↓	miR-193b↓
								miR-102↓	miR-210↑
								miR-142-3p↓	miR-125b↓
35 Motoyama et al., 2008	Japan	69 CRC	69 MNAT	MicroRNA microarray and TaqMan MicroRNA Assays	RNU6B	455	8	miR-183↓	miR-92↑
								miR-31↓	miR-17-5p↑
								miR-20a↑	miR-92↑
								miR-20a↓	miR-92↑
36 Schetter et al., 2008	United States (test cohort)/ China (validation cohort)	197 primary colon tumor <sup>a</sup>	197 MNAT <sup>c</sup>	MicroRNA microarray and TaqMan MicroRNA Assays	RNU6B	389	5	miR-183↓	miR-92↑
								miR-181b↓	miR-17-5p↑
								miR-181b↑	miR-92↑
								miR-181b↓	miR-92↑
37 Slaby et al., 2007	Czech Republic	29 CRC (3/11/6/9)	6 Nontumor adjacent tissue	TaqMan MicroRNA Assays	let-7a-1	4	4	miR-106a↑	miR-512-5p↓
								miR-181b↑	miR-512-5p↓
								miR-203↑	miR-512-5p↓
								miR-21↑	miR-512-5p↓
38 Schepeler et al., 2008	Denmark	49 colon cancers (stage II)	10 normal mucosa	LNA-based oligonucleotide microarrays and TaqMan MicroRNA Assays	miR-92↓	315	19 <sup>d</sup>	miR-143↓	miR-527↑
								miR-145↓	miR-527↑
								miR-20a↓	miR-527↑
								miR-20a↓	miR-527↑

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**Table 2.** Studies assessing miRNAs expression in colorectal tumor tissue and nontumor tissue (Cont'd)

Ref. Author(s), year	Study population		Methods	Normalization controls	Tested miRNAs (No.)	Significant miRNAs (No.)	miR	Difference between CRC tissue and nontumor tissue
	Country	CRC (stage I/II/III/IV)/ adenoma tissue						
39 Monzo et al., 2008	Spain	22 (6 is stage I and 16 is stage II)	TaqMan MicroRNA Assays	let-7a	156	28 <sup>a</sup> (6 stage I vs. MNAT)	miR-92↓ miR-17-5p↓ miR-135b↓ miR-203↓ miR-99a↓	miR-148a↑ miR-182↓ miR-183↓ miR-200c↓ miR-141↑ miR-183↓ miR-221↑ miR-186↓ miR-191↓ miR-224↓ miR-146↓ miR-147↓ miR-148a↑ miR-151↓ miR-154↓ miR-200c↓ miR-338↓ miR-210↓ miR-339↓ miR-213↓ miR-215↓ miR-373↓ miR-182↓ miR-219↓ miR-155↓ miR-223↓
40 Volinia et al., 2006	United States and Italy	46 colon tumor	MicroRNA microarray	-	228	22 <sup>b</sup>	miR-10a↑ miR-17-5p↓ miR-20a↑ miR-21↑ miR-24-1↑ let-7g↓ P=0.0037 miR-15b↓ P=0.0278 miR-181b↓ P=0.0002	miR-142-3p↑ miR-142-5p↓ miR-191↓ miR-194↓ miR-197↓ miR-200a↓ miR-200b↓ miR-200c↓ miR-338↓ miR-210↓ miR-339↓ miR-181a↓ miR-181b↓ miR-181c↓ miR-182↓ miR-135a↓ miR-24-2↑ miR-106a↑ miR-107↓ miR-203↓ miR-5-3↓ miR-213↓ miR-150↓ miR-200c↓ P=0.0001 P=0.0005 miR-191↑ P=0.0264 miR-200c↓ P=0.0017
41 Nakajima et al., 2006	United States and Japan	21 CRC	SYBR green qRT-PCR	5s rRNA RNU6B	5	3	miR-181b↓ P=0.0005	miR-181b↓ P=0.0005
42 Xi et al., 2006	Germany	24 CRC (4/4/8/8)	SYBR green qRT-PCR	5S rRNA	10	4	miR-15b↓ miR-191↑ miR-181b↓ P=0.0002	miR-15b↓ miR-191↑ miR-181b↓ P=0.0002

<sup>a</sup>MNAT: matched nontumor adjacent tissue.

<sup>b</sup>P values less than 0.1, details are not available.

<sup>c</sup>84 patients with colon tumors in the U.S. test cohort and 113 patients with colon tumors in the Hong Kong validation cohort.

<sup>d</sup>Differentially expressed miRNAs identified by significance of microarray analysis (SAM). The cutoff, delta value, was adjusted so as to set the median false discovery rate (FDR) < 0.0001%.

<sup>e</sup>SAM and Student's t test were done to identify differentially expressed miRNAs with a FDR < 0.1.

<sup>f</sup>SAM and Student's t test were done to identify differentially expressed miRNAs with a FDR ≤ 0.01.



miR-145 were found to be downregulated in CRC tissue in 6 or more studies. Interestingly, miR-203 was found to be upregulated in 5 tissue-based studies, but downregulated in 1 tissue-based study. For further details see Tables 2 and 3.

### Expression of miRNAs in adenomas and different stages of colorectal carcinogenesis

Huang and colleagues (16) found miR-29a and miR-92a to be significantly upregulated in a comparison of plasma samples of 37 patients with advanced adenomas with plasma samples of 59 healthy controls (both  $P < 0.0001$ ).

Wang and colleagues (29) reported miR-31 to be related to tumor node metastasis stage ( $P = 0.026$ , stages I and II vs. III and IV) and tumor invasion ( $P = 0.024$ , T<sub>1</sub> + T<sub>2</sub> + T<sub>3</sub> vs. T<sub>4</sub>) in 98 CRC cases from China.

In a study from Arndt and colleagues (32), among 45 CRC cases from the United States, 6 miRNAs showed significant differential expression between early stage (mostly stage II) and late stage (mostly stage III; miR-31  $P = 1.53 \times 10^{-3}$ , miR-7  $P = 1.96 \times 10^{-2}$ , miR-99b  $P = 3.64 \times 10^{-3}$ , miR-378\*  $P = 3.02 \times 10^{-2}$ , miR-133a  $P = 1.64 \times 10^{-2}$ , and miR-125a  $P = 2.69 \times 10^{-3}$ ).

Schetter and colleagues (35) found miR-21 to be significantly upregulated in the tissue of 18 adenoma patients compared with the paired adjacent nonadenoma tissue ( $P = 0.006$ ). Expression of miR-21 was found to be lower in adenomas than in cancer tissue ( $P < 0.001$ ; ref. 36) and positively related to CRC stage ( $P = 0.032$ ) and poor survival of CRC patients (37).

Diosdado and colleagues (30) found all 6 miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1) in the miR-17-92 cluster to be significantly upregulated in tissue samples from 30 colorectal adenoma patients, 25 adenocarcinomas patients compared with 10 normal controls and all of them were higher expressed in adenocarcinomas than in adenomas (all  $P < 0.001$ ).

In the study of Schmitz and colleagues (34), miR-21 and miR-181b showed significantly higher levels in the tissue of 19 sessile serrated adenomas (SSA) than in 20 normal colonic mucosal specimens.

### Discussion

This article provides an overview of miRNA studies in CRC published to date. We summarized the results of 23 studies which investigated more than 700 miRNAs in 1,525 cases of colorectal tumors/adenomas and 1,102 controls. Among them, 160 miRNAs were found to be significantly dysregulated in at least one study. Overall, samples size was less than 100, between 100 and 200, more than 200 in 11, 9, and 3 studies, respectively. Out of the 164 miRNAs that were found to be dysregulated, miR-31 (upregulated) and miR-145 (downregulated) were most often reported (both in 8 different studies). A total of 107 miRNAs were identified to be dysregulated in only one study.

miRNAs were first reported by Lee and colleagues in 1993, and tumor-associated miRNAs were first described in plasma by Mitchell in 2008 (11). miRNAs were found in extracellular fluids, such as blood plasma and serum and other body fluids, including urine, tear, and saliva (43). It has been shown that the vast majority of circulating miRNAs are Ago-bound and thus protected from RNases (12). This enables miRNAs to serve as stable biomarkers for cancer and other diseases (12). To what extent miRNA-Ago complexes can specifically be released from cells and, possibly, commit cell-to-cell communication needs to be shown (12). The number of studies assessing expression of miRNAs in CRC patients has rapidly increased in recent years but is still rather small. In particular, only 3 studies assessing expression of miRNAs in plasma of CRC patients have been reported, and only 96 miRNAs were investigated in these studies.

Because of the fact that circulating miRNA is very stable—it remains stable for at least 1 month and even in cell culture media, in which fetal calf serum has been added, miRNA from cattle can be observed (12)—it is astonishing that Ng and colleagues observed a drop down of tumor-associated miRNAs (miR-17-3p and miR-92a) so soon after surgery (7 days). However, this result was based on 10 pairs of samples only. Further studies with repeat longitudinal measurements of miRNAs in larger numbers of patients are needed to clarify occurrence and timing of potential downregulation of miRNAs after surgery.

Nomenclature of miRNAs is also still evolving. As identical mature sequences from distinct precursor sequences/genomic loci and closely related mature sequences are more and more identified, some miRNAs in different studies with different names actually are the same ones according to the accession number of miRBase provided (16, 17).

Most of the studies reported to date had sample sizes of less than 200. Furthermore, not all known miRNAs were investigated; many of the miRNAs investigated in the studies were selected based on previous reports and even when microarrays were applied, not all miRNAs known today were covered. Also, not all miRNAs have been discovered yet.

qRT-PCR is the most sensitive and reproducible method to quantify gene expression, but the accuracy is limited if expression of miRNAs is too low. As a result, some miRNAs with low expression cannot be tested or compared between the patients and healthy controls. A common problem in research on circulating miRNAs is that no consensus housekeeper miRNAs or endogenous controls have been established. The identified studies used several different genes as their endogenous controls, such as RNU6B, 5S rRNA, or let-7a. Davoren and colleagues (44) reported the first systematic assessments of candidate reference genes for miRNA qRT-PCR analysis in breast cancer. Recently, Chang and colleagues (45) reported that miR-16 and miR-345 were identified as the most stably expressed reference genes. Koch and

**Table 3.** Summary of studies reporting significant associations of miRNAs with CRC

Upregulated miRNAs	Ref.																				Number of studies			
	15	16	17	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39		40	41	42
let-7g																				II		+	2	
miR-10a																				II		+	2	
miR-15a																				II			1	
miR-15b																				II			+	2
miR-17						+					+													2
miR-17-3p			+									+								II				3
miR-17-5p												+				+				I/II		+		4
miR-18a										+	+	+				+								4
miR-18b										+														1
miR-19a										+	+	+									I/II			4
miR-19b												+												1
miR-19b-1											+													1
miR-20																					I/II			1
miR-20a						+				+	+					+	+		+			+		7
miR-20a*										+														1
miR-21				+								+			S		C/A	+		I/II		+		7
miR-24-1																						+		1
miR-24-2																						+		1
miR-25						+						+								II				3
miR-27a												+								II				1
miR-29a			+									+								II				3
miR-29b												+												1
miR-29b-2																						+		1
miR-30c																						+		1
miR-31						+				+	+	+	+			+		+		II				8
miR-32											+											+		2
miR-33											+													1
miR-34a												+									I/II			2
miR-92			+			+								+		+				I/II				6
miR-92a			+																			+		2
miR-92a-1										+														1
miR-93						+					+										I/II			2
miR-95											+										I/II			2
miR-96											+	+									I/II			3
miR-98																				II				1
miR-99b																						+		1
miR-103														+						I/II				2
miR-105																				II				1
miR-106a												+					+			I/II		+		4
miR-106b										+		+												2
miR-107																				I		+		2
miR-122a																				II				1
miR-126*																						+		1
miR-128a																				II				1
miR-128b																						+		1
miR-130b												+								II				2
miR-133b						+																		1
miR-134																				II				1
miR-135a						+														II				2
miR-135b										+		+								I				3

(Continued on the following page)

**Table 3.** Summary of studies reporting significant associations of miRNAs with CRC (Cont'd)

Upregulated miRNAs	Ref.																						Number of studies		
	15	16	17	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41		42	
miR-141																					II		1		
miR-142-3p														+								II		2	
miR-142-5p																						II		1	
miR-146																						II		1	
miR-147																						II		1	
miR-148a																						I/II		1	
miR-150																						+		1	
miR-151																						II		1	
miR-154*																						II		1	
miR-155																						+		1	
miR-181a																						II		1	
miR-181b												+		S/C		+						I/II	+	+	6
miR-181c																						II		1	
miR-182											+	+										II		3	
miR-182*											+											I/II		2	
miR-183					+						+	+				+						I/II		5	
miR-186																						II		1	
miR-188											+		+											2	
miR-191																						II	+	+	3
miR-191*																						+		1	
miR-194																						II		1	
miR-196b												+										I		1	
miR-197																						II		1	
miR-200a																						II		2	
miR-200a*																						+		1	
miR-200b																						II		1	
miR-200c																						I/II	+	+	4
miR-203						+							+				+					I	+	5	
miR-210																						II		2	
miR-213																						II	+	2	
miR-215																						II		1	
miR-216																						II		1	
miR-219																						II		1	
miR-221		+												+								II	+	3	
miR-222																						II		1	
miR-223						+																+		2	
miR-224											+	+										I/II		4	
miR-301																						II		1	
miR-301b												+												1	
miR-302a																						+		1	
miR-320																						+	II	2	
miR-324-5p																						II		1	
miR-330																						II		1	
miR-335													+											1	
miR-338																						II		1	
miR-339																						I/II		1	
miR-370																						II		1	
miR-373																						II		1	
miR-374																						II		1	
miR-374a																							+	1	

(Continued on the following page)

**Table 3.** Summary of studies reporting significant associations of miRNAs with CRC (Cont'd)

Upregulated miRNAs	Ref.																						Number of studies	
	15	16	17	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41		42
miR-424									+															1
miR-432*																				+				1
miR-492																				+				1
miR-503												+												1
miR-510																				+				1
miR-512-5p																				+				1
miR-513																				+				1
miR-526c																				+				1
miR-527																				+				1
miR-542-5p												+												1
miR-552												+												1
miR-584												+												1
HS_287												+												1
HS_29												+												1
Downregulated miRNAs	Ref.																						Number of studies	
	15	16	17	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41		42
let-7a						-																		1
miR-1												-												2
miR-9												-												1
miR-9*												-												1
miR-9-3																						-		1
miR-10b												-	-											2
miR-16						-																		1
miR-20b												-												1
miR-26b						-															-			2
miR-30a-3p												-	-											3
miR-30a-5p												-	-											2
miR-30b																					-			1
miR-99a																								1
miR-101																					-			1
miR-125a													-											1
miR-125b															-									2
miR-126										-														1
miR-133																-								1
miR-133a												-	-											2
miR-137												-												1
miR-138												-												1
miR-139												-	-											3
miR-143				-		-								-			-				-			6
miR-145						-							-	-			-				-	-		8
miR-147												-												1
miR-149																								1
miR-191																								1
miR-192																								2
miR-193b																								1
miR-195																								3
miR-196a																								1
miR-203																								1

(Continued on the following page)

**Table 3.** Summary of studies reporting significant associations of miRNAs with CRC (Cont'd)

Downregulated miRNAs	Ref.																				Number of studies			
	15	16	17	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39		40	41	42
miR-212														–										1
miR-214														–										1
miR-215						–																		1
miR-328												–												1
miR-363												–												1
miR-375												–												1
miR-378									–			–												2
miR-378*								–				–												2
miR-422a												–												1
miR-422b												–												1
miR-455																				–				1
miR-484																			–					1
miR-486												–												1
miR-497												–	–											2
miR-511												–												1
miR-551b												–												1
miR-642												–												1
miR-650												–												1

C: colorectal cancer.

A: colorectal adenoma.

S: sessile serrated adenoma.

I: stage I of CRC.

II: stage II of CRC.

colleagues (46) used miR-16 and miR-223 as endogenous controls and described an optimized method for the circulating miRNAs detection as well as the data analysis. However, there are still very few reports that detailed a robust identification and validation strategy for suitable reference genes for normalization. So, looking for definitely stably expressed reference genes as an appropriate normalization is important and critical for the accurate quantification of RNA levels with qRT-PCR. Most likely not one endogenous control/control set will fit for all studies (tissues, body fluids). Furthermore, if we take the different stability of different RNA species (mRNA, rRNA, and miRNA), especially in serum or plasma into consideration, it will be critical to find a suitable endogenous miRNA control/control set.

Organ and disease specificity is another important issue when investigating miRNAs as biomarker for certain diseases. It may often be difficult to discriminate whether the dysregulated miRNAs are only related to the CRC or are a common phenomenon in the histologic progression to cancer or the immune response. Ng and colleagues (16) also examined the plasma from patients with inflammatory bowel disease and gastric cancer, and miR-92a overexpressed in plasma was not correlated with those diseases. With regard to the other miRNAs, many were found expressed at high levels in different solid tumors (40).

In this article, we aimed for a timely general overview of the rapidly increasing number of the often rather heterogeneous studies about dysregulated miRNAs in CRC patients. Although our review searched 3 databases, PubMed, EMBASE, and ISI Web of knowledge and extensive checks for completeness by cross-referencing were employed, we cannot exclude that we might have missed a relevant study.

The search for noninvasive screening methodologies for CRC screening is subject to ongoing intensive research. There are many challenges to the development and implementation of a miRNA blood test to detect CRC in a clinically useful way, just as with any diagnostic biomarkers. In spite of the challenges, there are encouraging indications that circulating miRNAs have potential as a diagnostic biomarker for CRC and other cancers. For potential use in CRC screening, the ability of tests to detect CRC in an early stage or even in a precancerous stage will be most crucial.

With respect to potential application for screening, the plasma-based studies are of particular interest. Although first results seem promising, especially with respect to miR-92a which was found to be significantly elevated in CRC patients in both studies investigating this marker, further studies are needed which should investigate a much larger number of miRNAs and which should ideally

be conducted in screening settings among the target population of screening. The sensitivity for detecting early stages of CRC and advanced adenomas, the most common precursors of CRC, are of particular relevance in this context. These outcomes have only been specifically addressed in one study (16) so far. Furthermore, combinations of multiple miRNAs and combinations of miRNAs with other noninvasive markers deserve increased attention. Ideally, studies evaluating potential use in CRC screening should be conducted in a true screening setting with availability of a gold standard diagnostic test, for example, among participants of screening colonoscopy. Studies are needed that include reasonable numbers of patients with CRC at various stages, advanced adenomas, and other adenomas as well as adenoma-free patients, and additional factors that might affect expression of miRNAs, including medical history, and lifestyle factors should receive careful additional attention.

Most likely, a signature of miRNAs rather than a single miRNA alone, possibly in combination with other blood

or stool-based biomarkers or other available tests, such as FOBT, will be needed to reach enough sensitivity and specificity for an early detection test. If successful, such a noninvasive test might lead to higher compliance in CRC screening rates, earlier detection of CRC, and an overall reduction in cancer burden.

### Conclusion

The expression of miRNAs in plasma may be indicative of presence of CRC. But phased validation with large-scale samples is needed. Further studies should also focus on early stages and adenoma cases. Furthermore, practical values and costs also need to be considered.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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