Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

Cancer Epidemiology, Biomarkers & Prevention

Review

MicroRNA Signatures: Novel Biomarker for Colorectal Cancer?

Xiaoya Luo[1], Barbara Burwinkel[2,3], Sha Tao[1], and Hermann Brenner[1]

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.

Authors' Affiliations: [1]Division of Clinical Epidemiology and Aging Research; [2]Molecular Epidemiology Group, German Cancer Research Center; and [3]Divison of Molecular Biology of Breast Cancer, Department of Gynecology and Obstetrics, University Heidelberg, Heidelberg, Germany

Note: Supplementary data for this article are available at Cancer Epidemiology Biomarkers and Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Hermann Brenner, Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany. Phone: 49-6221-421300; Fax: 49-6221-421302; E-mail: h.brenner@dkfz.de

doi: 10.1158/1055-9965.EPI-11-0035

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

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

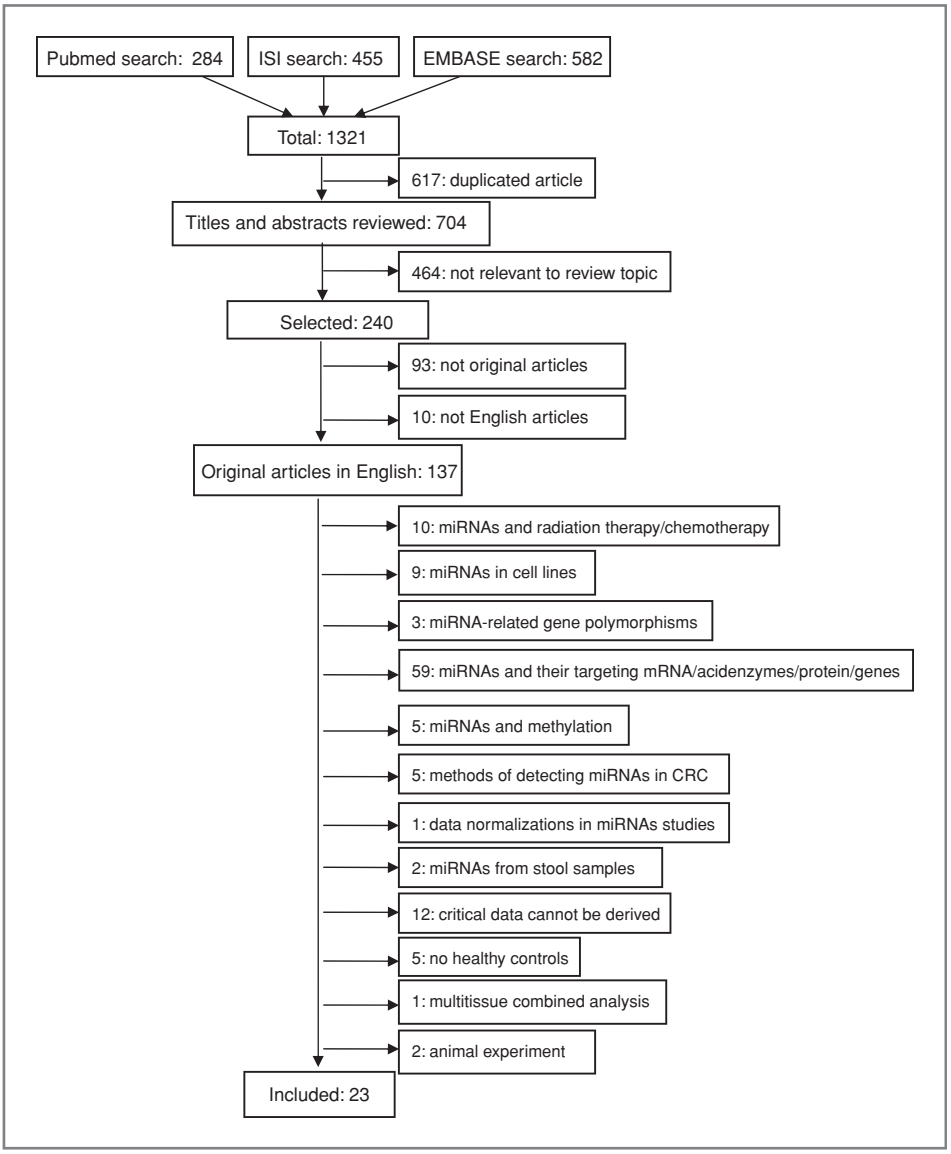

Figure 1. Flow diagram of the literature search process.

(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[a]	59 healthy[a]	CRC 61 (26–84)	SYBR green qRT-PCR	miR-16	12	2	miR-29a↑	$P < 0.0001$
			37 advanced adenomas[a]		Adenoma 55 (34–75)					miR-92a↑	$P < 0.0001$
					Control 58 (27–81)					miR-29a↑	$P < 0.0001$
										miR-92a↑	$P < 0.0001$
17	Ng et al., 2009	China	130 (6/35/39/50) CRC[b]	75 healthy[b]	CRC 71 (42–91)	Cancer microRNA array and SYBR green qRT-PCR	RNU6B	95	2	miR-17–3p↑	$P < 0.0001$
					Control 69 (45–85)					miR-92↑	$P < 0.0001$

[a]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.
[b]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 seclected 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).

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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 (10/21/ 13/2)	46 MNAT[a]	62.8 (39.9–79.1)	TaqMan MicroRNA Assays	RNU6B	2	2	miR-21↑	$P < 0.0001$
										miR-143↓	$P < 0.0001$
			30 colorectal liver metastases	30 MNAT	59.4 (median; 39.6–82.1)		miR-191			miR-21↑	$P < 0.0001$
							miR-143↓				$P < 0.0001$
24	Chiang et al., 2011	China	107 CRC	107 MNAT	62.1 (17–82)	SYBR green qRT-PCR	RNU6B	1	1	miR-203↓	$P < 0.001$
25	Earle et al., 2010	United States	55 CRC (5/23/ 18/1)	55 MNAT	51.3 (25–85)	TaqMan MicroRNA Assays	$\triangle C_t$ method	24	20	miR-17↑ $P=4.38\times10^{-4}$	miR-135a↑ $P=1.12\times10^{-3}$ / miR-26b↓ $P=8.91\times10^{-9}$
										miR-20a↑ $P=1.52\times10^{-4}$	miR-183↑ $P=2.47\times10^{-16}$ / miR-143↓ $P=2.47\times10^{-8}$
										miR-25↑ $P=3.92\times10^{-7}$	miR-203↑ $P=3.14\times10^{-3}$ / miR-145↓ $P=9.39\times10^{-8}$
										miR-31↑ $P=6.02\times10^{-13}$	miR-223↑ $P=3.69\times10^{-2}$ / miR-191↓ $P=3.71\times10^{-6}$
										miR-92↑ $P=1.96\times10^{-6}$	miR-192↓ $P=3.51\times10^{-16}$
										miR-93↑ $P=3.33\times10^{-5}$	let-7a↓ $P=4.93\times10^{-4}$ / miR-196a↓ $P=7.72\times10^{-4}$
										miR-133b↑ $P=1.36\times10^{-2}$	miR-16↓ $P=1.78\times10^{-3}$ / miR-215↓ $P=2.37\times10^{-13}$
26	Li et al., 2010	China	66 CRC (17/18/22/9)	66 MNAT	-	SYBR green qRT-PCR	RNU6B	1	1	miR-126↓	$P < 0.001$
27	Liu et al., 2010	China	81 CRC	81 MNAT	57.4	qRT-PCR	RNU6B -	1	1	miR-195 ↓	$P < 0.01$
28	Wang et al., 2010	China	3 colonic tumor tissue	3 MNAT	-	microRNA microarray and TaqMan MicroRNA Assays		723	14[b]	miR-18a↑	miR-135b↑ / miR-374a↑
										miR-18b↑ miR-19a ↑ miR-20a* ↑ miR-106b ↑	miR-196b ↑ miR-224 ↑ miR-301b↑ miR-335 ↑ / miR-424↑ 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)	98 MNAT	54.3 (24–83)	TaqMan MicroRNA Assays miR-145↓	5S rRNA $P=0.001$	3	2	miR-31↑	$P=0.001$
30	Diosdado et al., 2009	The Netherlands	55 (30 adenomas 25 adenocar-cinomas)	10 normal mucosa	Case 68.4 (47–88)	TaqMan MicroRNA Assays Control 68	RNU48 miR-18a↑ $p=0.04$	6 miR-20a ↑ $P=0.001$	6	miR-17↑ $P=0.001$	miR-19b-1↑ $P=0.021$
										miR-19a↑ $P < 0.001$	miR-92a-1↑ $P < 0.001$
31	Sarver et al., 2009	United States	80 colon cancer	28 normal colon tissue	-	SYBR green qRT-PCR	RNU6B	735	39	miR-17–3p↑ $P=7.59\times10^{-14}$ / miR-31↑ $P = 0$	miR-552↑ $P=1.72\times10^{-8}$ / miR-584 ↑ $P=0$ / miR-138↓ $P=0$ / miR-139↓ $P=0$

(Continued on the following page)

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

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
										miR-32 ↑ $P=1.97\times10^{-5}$
										miR-33 ↑ $P=1.76\times10^{-9}$
										miR-96 ↑ $P=7.11\times10^{-14}$
										miR-135b↑ $P=0$
										miR-182 ↑ $P=0$
										miR-182*↑ $P=0$
										miR-183 ↑ $p=0$
										miR-188 ↑ $P=1.20\times10^{-11}$
										miR-224 ↑ $P=0$
										miR-503 ↑ $P=0$
										miR-542–5p↑ $P=7.01\times10^{-11}$ ($P<10^{-16}$ represent as 0)
										HS_287 ↑ $P=3.87\times10^{-8}$
										HS_29 ↑ $P=1.91\times10^{-9}$
										miR-1↓ $P=2.33\times10^{-11}$
										miR-9↓ $P=0$
										miR-9*↓ $P=0$
										miR-10b↓ $P=2.66\times10^{-15}$
										miR-20b↓ $p=1.90\times10^{-7}$
										miR-30a-3p↓ $P=0$
										miR-30a-5p↓ $P=0$
										miR-133a↓ $P=9.55\times10^{-15}$
										miR-137↓ $P=0$
										miR-147↓ $P=0$
										miR-328↓ $P=2.62\times10^{-14}$
										miR-363↓ $P=1.30\times10^{-7}$
										miR-375↓ $P=0$
										miR-378↓ $P=0$
										miR-486↓ $P=6.90\times10^{-9}$
										miR-497↓ $P=0$
										miR-511↓ $P=2.22\times10^{-16}$
										miR-551b↓ $P=0$
										miR-642↓ $P=0$
										miR-650↓ $P=0$
32	Arndt et al., 2009	United States	45 (4/19/20/2) CRC	4 normal colon tissue	CRC 62.8 Control -	TaqMan MicroRNA Assays	A no cDNA control	169	37	miR-18a↑ $P=2.9\times10^{-3}$ miR-19a↑ $P=2.3\times10^{-3}$
										miR-106a↑ $P=5.1\times10^{-4}$ miR-106b↑ $P=1.0\times10^{-4}$
										miR-125a↓ $P=6.40\times10^{-3}$ miR-30a-3p↓ $P=7.30\times10^{-4}$
										miR-19b↑ $P=3.4\times10^{-3}$
										miR-20a↑ $P=2.0\times10^{-3}$
										miR-21↑ $P=6.0\times10^{-6}$
										miR-25↑ $P=1.4\times10^{-2}$
										miR-29a↑ $P=3.6\times10^{-4}$
										miR-29b↑ $P=4.9\times10^{-3}$
										miR-31↑ $P=2.6\times10^{-3}$
										miR-34a↑ $P=1.5\times10^{-2}$
										miR-93↑ $P=3.4\times10^{-3}$
										miR-95↑ $P=1.1\times10^{-2}$
										miR-96↑ $P=8.4\times10^{-3}$
										miR-130b↑ $P=1.2\times10^{-3}$
										miR-17–5p↑ $P=1.5\times10^{-3}$
										miR-181b↑ $P=2.2\times10^{-4}$
										miR-182↑ $P=2.8\times10^{-3}$
										miR-183↑ $P=2.3\times10^{-3}$
										miR-203↑ $P=2.2\times10^{-2}$
										miR-224↑ $P=1.1\times10^{-3}$
										miR-1↓ $P=1.6\times10^{-3}$
										miR-10b↓ $P=2.1\times10^{-2}$
										miR-200a↑ $P=0.036$
										miR-30a-5p↓ $P=1.60\times10^{-5}$
										miR-30c↓ $P=4.00\times10^{-4}$
										miR-133a↓ $P=6.50\times10^{-4}$
										miR-139↓ $P=1.10\times10^{-5}$
										miR-143↓ $P=1.00\times10^{-2}$
										miR-145↓ $P=1.20\times10^{-3}$
										miR-195↓ $P=4.20\times10^{-5}$
										miR-378*↓ $P=1.30\times10^{-5}$
										miR-422a↓ $P=7.30\times10^{-5}$
										miR-422b↓ $P=8.30\times10^{-5}$
										miR-497↓ $P=9.80\times10^{-4}$
33	Chen et al., 2009	China	13 CRC	13 MNAT	57.2	qRT-PCR	-	200	15	miR-31↑ $P=0.016$ miR-145↓ $P=0.048$

(Continued on the following page)

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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

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
										miR-92↑ P=0.049 miR-103↑ P=0.002 miR-142-3p↑ P=0.016 miR-188↑ P=0.001
										miR-200c↑ P=0.033 miR-210↑ P=0.000 miR-125b↓ P=0.010 miR-143↓ P=0.001
										miR-192↓ P=0.008 miR-193b↓ P=0.006 miR-212↓ P=1.88×10^{-9} miR-214↓ P=5.95×10^{-7}
34	Schmitz et al., 2009	Germany	18 SSAs	20 Normal colonic mucosal	SSAs 60.1	TaqMan MicroRNA Assays	RNU48	4	3	miR-21 ↑ P < 0.001
					CRC -	miR-181b ↑	P < 0.001			
			20 CRC	Control -	miR-181b ↑	P < 0.001				
										miR-133↓ P=0.025
35	Motoyama et al., 2008	Japan	69 CRC	69 MNAT	-	MicroRNA microarray and TaqMan MicroRNA Assays	RNU6B	455	8	miR-17–5p ↑ P < 0.05 miR-92 ↑ P < 0.05
										miR-18a ↑ P < 0.05 miR-183 ↑ P < 0.05
										miR-31 ↑ P < 0.05 miR-143↓ P < 0.05
										miR-20a ↑ P < 0.05 miR-145↓ P < 0.05
36	Schetter et al., 2008	United States (test cohort)/ China (validation cohort)	197 primary colon tumor[c]	197 MNAT[c]	Test cohort 64.6 (32–87)	MicroRNA microarray and TaqMan MicroRNA Assays	RNU6B	389	5	miR-20a ↑ P < 0.001
					Validation cohort 55.8 (32–84)	miR-21 ↑	P < 0.001			
										miR-106a ↑ P < 0.001
										miR-181b ↑ P < 0.001
										miR-203 ↑ P < 0.001
		Cooperative Human Tissue Network	18 adenoma	18 MNAT	-	TaqMan MicroRNA Assays		5	1	miR-21 ↑ P=0.006
37	Slaby et al., 2007	Czech Republic	29 CRC (3/11/6/9)	6 Nontumor adjacent tissue	CRC 65 (median; 49–75)	TaqMan MicroRNA Assays	let-7a-1	4	4	miR-21↑ P=0.0001
	Control -	miR-31↑	P=0.0006							
										miR-143↓ P=0.011
										miR-145↓ P=0.003
38	Schepeler et al., 2008	Denmark	49 colon cancers (stage II)	10 normal mucosa	Case 65.7 (36–87)	LNA-based oligonucleotide microarrays and TaqMan MicroRNA Assays	-	315	19[d]	miR-20a↑ miR-302a↑ miR-510↑ miR-527↑ miR-101↓
					Control 67.4 (52–85)		miR-92↑	miR-320↑	miR-512–5p↑	miR-145↓
										miR-191*↑ miR-432*↑ miR-513↑ miR-26b↓ miR-455↓
										miR-200a*↑ miR-492↑ miR-526c↑ miR-30b↓ miR-484↓

(Continued on the following page)

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

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
39	Monzo et al., 2008	Spain	22 (6 is stage I and 16 is stage II)	22 MNAT	69.5 (49–87)	TaqMan MicroRNA Assays	let-7a	156	28[e] (6 stage I vs. MNAT)	miR-17–5p↑, miR-92↑, miR-135b↑, miR-203↑, miR-99a↓ miR-19a↑, miR-95↑, miR-148a↑, miR-224↑, miR-125b↓ miR-20↑, miR-96↑, miR-182↑, miR-339↑, miR-139↓ miR-21↑, miR-103↑, miR-182*↑, miR-145↓ miR-31↑, miR-106a↑, miR-183↑, miR-149↓ miR-34a↑, miR-107↑, miR-200c↑, miR-30a-3p↓, miR-195↓
									64[e] (16 stage I vs. MNAT)	let-7g↑, miR-34a↑, miR-141↑, miR-183↑, miR-221↑ miR-10a↑, miR-92↑, miR-142–3p↑, miR-186↑, miR-222↑ miR-15a↑, miR-95↑, miR-142–5p↑, miR-191↑, miR-224↑ miR-15b↑, miR-96↑, miR-146↑, miR-194↑, miR-301↑ miR-17–3p↑, miR-98↑, miR-147↑, miR-197↑, miR-320↑ miR-17–5p↑, miR-103↑, miR-148a↑, miR-200a↑, miR-324–5p↑ miR-19a↑, miR-105↑, miR-151↑, miR-200b↑, miR-330↑ miR-20↑, miR-106a↑, miR-154*↑, miR-200c↑, miR-338↑ miR-21↑, miR-122a↑, miR-181a↑, miR-210↑, miR-339↑ miR-25↑, miR-128a↑, miR-181b↑, miR-213↑, miR-370↑ miR-27a↑, miR-130b↑, miR-181c↑, miR-215↑, miR-373↑ miR-29a↑, miR-134↑, miR-182↑, miR-216↑, miR-374↑ miR-31↑, miR-135a↑, miR-182*↑, miR-219↑
40	Volinia et al., 2006	United States and Italy	46 colon tumor	8 normal tissue	-	MicroRNA microarray	-	228	22[f]	miR-10a ↑, miR-24–2 ↑, miR-106a ↑, miR-155 ↑, miR-223↑ miR-17-5p↑, miR-29b-2↑, miR-107↑, miR-191 ↑ miR-20a ↑, miR-30c↑, miR-126*↑, miR-203↑, miR-9–3 ↓ miR-21↑, miR-32 ↑, miR-128b ↑, miR-213↑ miR-24-1 ↑, miR-99b ↑, miR-150 ↑, miR-221↑
41	Nakajima et al., 2006	United States and Japan	21 CRC	21 MNAT	-	SYBR green qRT-PCR	5s rRNA RNU6B	5	3	let-7g↑ $P=0.0037$, miR-181b↑ $P=0.0005$, miR-200c↑ $P=0.0001$
42	Xi et al., 2006	Germany	24 CRC (4/4/8/8)	24 MNAT	62 (30–93)	SYBR green qRT-PCR	5S rRNA	10	4	miR-15b↑ $P=0.0278$, miR-191↑ $P=0.0264$, miR-181b↑ $P=0.0002$, miR-200c↑ $P=0.0017$

[a]MNAT: matched nontumor adjacent tissue.
[b]*P* values less than 0.1, details are not available.
[c]84 patients with colon tumors in the U.S. test cohort and 113 patients with colon tumors in the Hong Kong validation cohort.
[d]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%.
[e]SAM and Student's *t* test were done to identify differentially expressed miRNAs with a FDR < 0.1.
[f]SAM and Student's *t* test were done to identify differentially expressed miRNAs with a FDR $\leq$ 0.01.

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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 ($P = 0.024$, $T_1 + T_2 + T_3$ vs. T_4) 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

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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						+							+											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)

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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)

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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												–	–							I				3
miR-30a-5p												–	–											2
miR-30b																			–					1
miR-99a																				I				1
miR-101																			–					1
miR-125a													–											1
miR-125b														–						I				2
miR-126							–																	1
miR-133															–									1
miR-133a												–	–											2
miR-137												–												1
miR-138												–												1
miR-139												–	–							I				3
miR-143				–		–				–				–		–		–						6
miR-145						–				–			–	–		–		–	–	I				8
miR-147												–												1
miR-149																				I				1
miR-191						–																		1
miR-192						–								–										2
miR-193b														–										1
miR-195								–					–							I				3
miR-196a						–																		1
miR-203					–																			1

(Continued on the following page)

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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 crossing-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

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

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.

Received January 13, 2011; revised March 26, 2011; accepted April 3, 2011; published OnlineFirst May 6, 2011.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CACancer J Clin 2005;55:74–108.
2. Walsh JM, Terdiman JP. Colorectal cancer screening: scientific review. JAMA 2003;289:1288–96.
3. Collins JF, Lieberman DA, Durbin TE, Weiss DG. Accuracy of screening for fecal occult blood on a single stool sample obtained by digital rectal examination: a comparison with recommended sampling practice. Ann Intern Med 2005;142:81–5.
4. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
5. Cho WC. OncomiRNAs: the discovery and progress of microRNAs in cancers. Mol Cancer 2007;6:60.
6. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?Nat Rev Genet 2008;9:102–14.
7. Release 16, Sep, 2010, http://www.mirbase.org/
8. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857–66.
9. Balaguer F, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR, et al. Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. Cancer Res 2010;70:6609–18.
10. Toyota M, Suzuki H, Sasaki Y, Maruyama R, Imai K, Shinomura Y, et al. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res 2008;68:4123–32.
11. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008;105:10513–8.
12. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular miRNA. Nucleic Acids Res 2011.
13. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008;141:672–5.
14. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 2010;251:499–505.
15. Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, et al. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroenterol Hepatol 2010;25:1674–80.
16. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer 2010;127:118–26.
17. Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009;58:1375–81.
18. Agostini M, Pucciarelli S, Calore F, Bedin C, Enzo M, Nitti D. miRNAs in colon and rectal cancer: A consensus for their true clinical value. Clin Chim Acta 2010;411:1181–6.
19. Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. Mol Cancer 2009;8:102.
20. Wu WK, Law PT, Lee CW, Cho CH, Fan D, Wu K, et al. MicroRNA in colorectal cancer: from benchtop to bedside. Carcinogenesis 2011;32:247–53.
21. Tang JT, Fang JY. MicroRNA regulatory network in human colorectal cancer. Mini Rev Med Chem 2009;9:921–6.
22. Goel A, Boland CR. Recent insights into the pathogenesis of colorectal cancer. Curr Opin Gastroenterol 2010;26:47–52.
23. Kulda V, Pesta M, Topolcan O, Liska V, Treska V, Sutnar A, et al. Relevance of miR-21 and miR-143 expression in tissue samples of colorectal carcinoma and its liver metastases. Cancer Genet Cytogenet 2010;200:154–60.
24. Chiang Y, Song Y, Wang Z, Chen Y, Yue Z, Xu H, et al. Aberrant Expression of miR-203 and Its Clinical Significance in Gastric and Colorectal Cancers. J Gastrointest Surg. 2011;15:63–70.
25. Earle JS, Luthra R, Romans A, Abraham R, Ensor J, Yao H, et al. Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. 2010;12:433–40.
26. Li XM, Wang AM, Zhang J, Yi H. Down-regulation of miR-126 expression in colorectal cancer and its clinical significance. Med Oncol 2010.
27. Liu L, Chen L, Xu Y, Li R, Du X. microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. Biochem Biophys Res Commun 2010;400:236–40.
28. Wang YX, Zhang XY, Zhang BF, Yang CQ, Chen XM, Gao HJ. Initial study of microRNA expression profiles of colonic cancer without lymph node metastasis. J Dig Dis 2010;11:50–4.
29. Wang CJ, Zhou ZG, Wang L, Yang L, Zhou B, Gu J, et al. Clinicopathological significance of microRNA-31, -143 and -145 expression in colorectal cancer. Dis Markers 2009;26:27–34.

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

30. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, Mongera S, Postma C, Meijerink WJ, et al. MiR-17–92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. Br J Cancer 2009;101:707–14.
31. Sarver AL, French AJ, Borralho PM, Thayanithy V, Oberg AL, Silverstein KA, et al. Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states. BMC Cancer 2009;9:401.
32. Arndt GM, Dossey L, Cullen LM, Lai A, Druker R, Eisbacher M, et al. Characterization of global microRNA expression reveals oncogenic potential of miR-145 in metastatic colorectal cancer. BMC Cancer 2009;9:374.
33. Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. Oncogene 2009;28:1385–92.
34. Schmitz KJ, Hey S, Schinwald A, Wohlschlaeger J, Baba HA, Worm K, et al. Differential expression of microRNA 181b and microRNA 21 in hyperplastic polyps and sessile serrated adenomas of the colon. Virchows Arch 2009;455:49–54.
35. Motoyama K, Inoue H, Takatsuno Y, Tanaka F, Mimori K, Uetake H, et al. Over- and under-expressed microRNAs in human colorectal cancer. Int J Oncol 2009;34:1069–75.
36. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 2008;299:425–36.
37. Slaby O, Svoboda M, Fabian P, Smerdova T, Knoflickova D, Bednarikova M, et al. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology 2007;72:397–402.
38. Schepeler T, Reinert JT, Ostenfeld MS, Christensen LL, Silahtaroglu AN, Dyrskjøt L, et al. Diagnostic and prognostic microRNAs in stage II colon cancer. Cancer Res 2008;68:6416–24.
39. Monzo M, Navarro A, Bandres E, Artells R, Moreno I, Gel B, et al. Overlapping expression of microRNAs in human embryonic colon and colorectal cancer. Cell Res 2008;18:823–33.
40. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257–61.
41. Nakajima G, Hayashi K, Xi Y, Kudo K, Uchida K, Takasaki K, et al. Non-coding MicroRNAs hsa-let-7g and hsa-miR-181b are Associated with Chemoresponse to S-1 in Colon Cancer. Cancer Genomics Proteomics 2006;3:317–24.
42. Xi Y, Formentini A, Chien M, Weir DB, Russo JJ, Ju J, et al. Prognostic values of microRNAs in colorectal cancer. Biomark Insights 2006;2:113–21.
43. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997–1006.
44. Davoren PA, McNeill RE, Lowery AJ, Kerin MJ, Miller N. Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. BMC Mol Biol 2008;9:76.
45. Chang KH, Mestdagh P, Vandesompele J, Kerin MJ, Miller N. MicroRNA expression profiling to identify and validate reference genes for relative quantification in colorectal cancer. BMC Cancer 2010;10:173.
46. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods 2010;50:298–301.

Published OnlineFirst May 6, 2011; DOI: 10.1158/1055-9965.EPI-11-0035

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

MicroRNA Signatures: Novel Biomarker for Colorectal Cancer?

Xiaoya Luo, Barbara Burwinkel, Sha Tao, et al.

Cancer Epidemiol Biomarkers Prev 2011;20:1272-1286. Published OnlineFirst May 6, 2011.