ORIGINAL RESEARCH

A 5-serum miRNA panel for the early detection of colorectal cancer

Shaohua Guo[1,2]
Jiajin Zhang[2]
Baishi Wang[2]
Bingdong Zhang[1]
Xuning Wang[1]
Liang Huang[1]
Hongyi Liu[2]
Baoqing Jia[1,2]

[1]Chinese PLA Medical School, Beijing, China; [2]General Surgery Department II, Chinese PLA General Hospital, Beijing, China

Objective: The study aimed to screen microRNAs (miRNAs) that can be used for the early detection of colorectal cancer (CRC) based on differential expression of miRNA in serum.

Materials and methods: A three-stage study was designed with a total of 217 CRCs, 168 colorectal adenomas (CRAs), and 190 healthy controls (HCs). A quantitative reverse transcription polymerase chain reaction was performed in three stages. We screened 528 miRNA expression profiles in the sera of 40 patients (CRC n=20, CRA n=10, and HC n=10) for candidate miRNAs, then 210 serum samples (CRC n=90, CRA n=60, and HC n=60) were used for screening of candidate miRNAs. Three hundred and twenty-five independent individual samples (CRC n=107, CRA n=98, and HC n=120) were used to validate the most differentially-expressed miRNAs in the screening stage, and binary logistic regression was used in the validation stage. A receiver operating characteristic curve was drawn to evaluate the diagnostic accuracy.

Results: A 5-serum miRNA panel (miRNA-1246, miRNA-202-3p, miRNA-21-3p, miRNA-1229-3p, and miRNA-532-3p) effectively distinguished CRCs from HCs with 91.6% sensitivity and 91.7% specificity. The area under the curve (AUC) was 0.960 (95% confidence interval [CI]: 0.937–0.983). In addition, the panel also accurately distinguished CRCs from CRAs with 94.4% sensitivity and 84.7% specificity. The AUC was 0.951 (95% CI: 0.922–0.980).

Conclusion: Our 5-serum miRNA panel accurately distinguished CRCs from CRAs and HCs with high sensitivity and specificity. The 5-serum miRNA panel may be a promising prospect for application as a nonintrusive and inexpensive method for the early detection of CRC.

Keywords: colorectal cancer, colorectal adenoma, miRNA

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors. The incidence of CRC among malignant tumors in males and females ranks third and second, respectively. In fact, 1.3 million people are diagnosed with CRC and there are nearly 700,000 deaths annually worldwide; CRC ranks fourth among cancer-related deaths.[1] In China, the number of new cases of CRC in 2015 exceeded 3,763,000 and the number of CRC-specific deaths was approximately 1,910,000.[2] In recent years, with the improvement in living standards and Westernization of lifestyles, the incidence and mortality of CRC have shown a rising trend year after year. Indeed, most patients with CRC are in an advanced stage at the time of diagnosis and have lost curability. The current study showed that the mortality rate of CRC was highly correlated with the stage of disease. The 5-year survival rate for stage I CRC approaches 90%, but is only 12% for stage IV.[3] It is evident that early detection of CRC and appropriate treatment are important. Currently, the common methods for early detection of CRC include the plasma carcinoembryonic antigen (CEA) level, fecal occult blood testing, and fiberoptic colonoscopy; however, the plasma CEA level and fecal occult blood

Correspondence: Baoqing Jia; Hongyi Liu
General Surgery Department II, Chinese PLA General Hospital, No 28, Fuxing Rd, Beijing 100853, China
Tel +86 10 6693 7598; +86 10 6693 7523
Fax +86 10 6693 7533
Email baoqingjia@126.com; liushitou114@163.com

testing are not highly sensitive, and therefore their value is limited.[4–6] Colonoscopy is considered the gold standard for detection of CRC; however, colonoscopy is still limited as an effective approach for early detection due to poor patient compliance, resulting from discomfort during bowel preparation and the examination, as well as the potential complications.[7] Therefore, a new method which is less invasive, highly accurate, and of clinical value for the early detection of CRC is warranted.

MicroRNAs (miRNAs) are a class of noncoding single-stranded RNA molecules (approximately 21–23 nucleotides in length) coded by endogenous genes and specifically bound to the 3′ untranslated region of target mRNAs to inhibit translation and promote degradation.[8] miRNAs participate in a variety of vital biological processes, including cell differentiation, proliferation, and apoptosis. The mechanism of its action may be related to miRNA-related single nucleotide polymorphisms.[9–12] It has been reported that abnormally-expressed miRNA, as an oncogene or tumor suppressor, plays an important role in cancer initiation and progression, including lung, liver, pancreatic, bladder, gastric, ovarian, and colorectal cancers.[13–17] There are a variety of miRNAs in human blood that are usually derived from lysis of apoptotic or necrotic cells and secretion of specific cells.[18] miRNAs remain stable by binding to Argonaute 2 protein[19] or through the autospecific hairpin structure,[20] which prevents degradation by ribonuclease in the blood and facilitates quantitative detection. In 2008, Lawrie et al[21] first reported the quantitative detection of miRNA in serum samples, proving the feasibility for the approach. An increasing number of studies on various diseases, especially on tumors, have focused on circulation miRNA.[22,23] However, in our study, more samples were included and the method of quantitative reverse transcription polymerase chain reaction (qRT-PCR) cost less. The objective of the current study was to identify differentially-expressed miRNAs and to determine the feasibility of their use in the early detection of CRC.

Materials and methods
Study subjects
The study was approved by the Ethics Committee of the Chinese PLA General Hospital, and all patients signed written informed consent forms. The inclusion criteria were as follows: (1) colonoscopy; (2) no inflammatory bowel diseases, hereditary adenomatous polyposis, family history of tumors, and other tumors in the preceding or corresponding periods; (3) colorectal adenomas (CRAs) and CRCs should have pathologic confirmation; and (4) no preoperative chemotherapy for patients with CRC. The patients with CRC were

staged according to the TNM staging system of the American Joint Committee on Cancer.[49] A total of 575 patients were enrolled from the Department of Gastrointestinal Surgical Oncology, Department of Gastroenterology, and Medical Examination Center of the Chinese PLA General Hospital between April 2013 and December 2014. Their general statistical information is presented in Table 1. The collection of all blood samples followed a uniform protocol, ie, a 5-mL vacuum blood collection tube (VACUETTE®; Greiner Bio-One, Frickenhausen, Germany) was used to collect blood, and the sample was allowed to stand for 2 h at room temperature to fully coagulate, and then centrifuged at 1,500 rpm ($2,390 \times g$) at 4°C for 7 min. The centrifugation was completed within 4 h after blood collection. The serum samples collected after centrifugation were stored in a −80°C refrigerator.

Study design
Our study was divided into three stages (primary screening, screening, and validation). The study design is shown in Figure 1.

Table 1 Clinical characteristics of patients

Variable	Primary screening	Screening	Validation
CRC			
Age, years			
Mean±SD	57.55±13.96	60.27±11.85	59.94±10.95
Gender, n			
Male	12	54	65
Female	8	36	42
Location, n			
Colon	10	48	42
Rectum	10	42	65
TNM stage, n			
0	1	3	6
I	7	16	12
II	6	33	25
III	4	28	43
IV	2	10	21
CRA			
Age, years			
Mean±SD	57.30±8.46	59.07±13.99	58.17±13.34
Gender, n			
Male	9	39	68
Female	1	21	30
Location, n			
Colon	8	11	82
Rectum	2	49	16
HC			
Age, years			
Mean±SD	45.90±12.38	48.88±11.58	47.39±11.82
Gender, n			
Male	6	41	68
Female	4	19	52

Abbreviations: CRC, colorectal cancer; CRA, colorectal adenoma; HC, healthy control.

Primary screening stage
qRT-PCR
n=40 (20 CRC, 10 CRA and 10 HC)

CRC vs CRA

Screening stage
qRT-PCR
n=210 (90 CRC, 60 CRA and 60 HC)

CRC vs HC

Validation stage
qRT-PCR
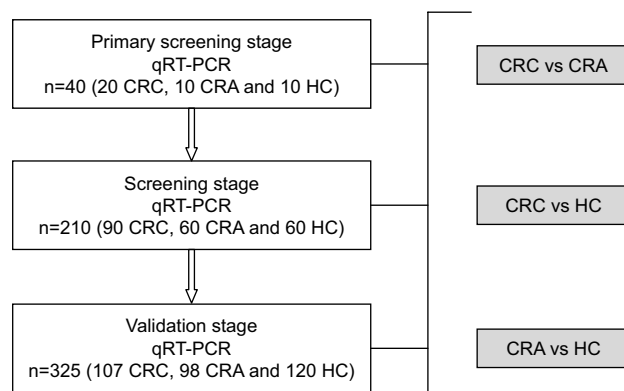n=325 (107 CRC, 98 CRA and 120 HC)

CRA vs HC

Figure 1 Study design.
Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; CRC, colorectal cancer; CRA, colorectal adenoma; HC, healthy control.

Primary screening and screening stages

During the primary screening stage, we screened 528 miRNA expression profiles in 40 serum samples, and CRCs, CRAs, and healthy controls (HCs) were compared in pairs. One hundred and five miRNAs were differentially expressed (Tables S1–S3). The primary screening criteria for differentially-expressed miRNAs were as follows: (1) mean cycle threshold (Ct) <32 and SD within groups <2; (2) 5 Cts less than the negative control (no template control); and (3) the Ct value of the same sample was standardized on the same plate with an external reference (cel-miR-67) to obtain the ΔCt value for comparison between groups and to select the most differentially-expressed miRNAs.

The serum sample size was expanded (n=210) and candidate miRNAs (105 miRNAs in the primary screening stage) satisfying the above three criteria for primary screening were rescreened. The screening criteria were as follows: (1) the results of primary screening and screening were compared, and miRNAs with consistent expression patterns or mean Ct differences between groups >1 were selected; (2) mean Ct <30; and (3) the Ct value of the same sample was standardized on the same plate with an external reference (cel-miR-67) to obtain the ΔCt value for comparison between groups and to select the most differentially-expressed miRNAs. Validation was conducted on miRNAs which satisfied all three screening criteria.

Validation stage

During the screening stage, we identified 10 most significant miRNAs. There were 5 miRNAs in group of HC vs CRA, 9 miRNAs in groups of CRA vs HC, and 9 miRNAs in group of CRC vs HC. But some miRNAs are repetitive (Table S4, Tables 2 and 3). Three hundred and twenty-five serum samples (CRC n=107, CRA n=98, and HC n=120) were used to validate the difference and binary logistic regression was based on data

Table 2 Different expression of microRNAs in individuals with colorectal cancer compared to healthy controls (screening stage)

MicroRNA	Fold-change	p-value
miRNA-1246	2.10	$1.92E^{-6}$
miRNA-21-5p	0.59	0.02
miRNA-19b-3p	0.61	$4.80E^{-4}$
miRNA-105-5p	0.70	<0.01
miRNA-380-5p	0.66	<0.01
miRNA-202-3p	0.75	0.02
miRNA-21-3p	0.57	$2.10E^{-7}$
miRNA-1229-3p	11.88	$1.26E^{-20}$
miRNA-532-3p	5.78	$1.17E^{-23}$

from the validation stage. We validated the 5 miRNAs with the most significant difference (Figure S1). A receiver operating characteristic curve was drawn and a relevant mathematical model was established for detection of CRC.

Extraction of serum total RNA

During extraction of total RNA, 1 µL of cel-miR-67 (20 nM/L; Quantobio, Beijing, China) was added to each sample as an external reference. A total of 0.25 mL of serum was used in the extraction of total RNA following the procedures in the miRNA extraction kit (Biochain, Beijing, China). A Nanodrop2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the quality of total RNA and the OD260-to-OD280 ratio was within 1.8–2.1.

Quantitative reverse transcription polymerase chain reaction

During the screening and validation stages, we used a three-step qRT-PCR. PolyA polymerase (NEB, Beijing, China) was used to add an A tail for the miRNA primer and mature miRNA. The reaction system was in a volume of 20 µL at 37°C for 1 h. Then, cRNA was synthetized by reverse transcription. One microliter of universal reverse transcription

Table 3 Different expression of microRNAs in individuals with colorectal cancer compared to colorectal adenoma (screening stage)

MicroRNA	Fold-change	p-value
miRNA-520e	0.55	<0.01
miRNA-1246	1.62	<0.01
miRNA-21-5p	0.55	0.02
miRNA-105-5p	0.76	0.01
miRNA-380-5p	0.59	$9.46E^{-4}$
miRNA-202-3p	0.68	0.01
miRNA-21-3p	0.81	0.02
miRNA-1229-3p	5.50	$5.46E^{-14}$
miRNA-532-3p	3.61	$4.85E^{-13}$

primer (Quantobio) of the tested 528 miRNAs was added to each reaction tube and incubated at 70°C for 5 min, 4°C for 2–10 min, 50°C for 50 min, and 70°C for 15 min. cDNA was obtained. qPCR was performed on an ABI 7900 High Throughput Rapid Real-time Fluorescence Quantitative PCR Amplifier (Applied Biosystems; Thermo Fisher Scientific) at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s and 60°C for 45 s. The melting analysis was added to evaluate the specificity of the PCR products: 95°C for 15 s, 60°C for 15 s, and then 95°C for 15 s. Quantification of the PCR product was evaluated by the level of fluorescence emitted by SYBR Green (SYBR® Premix Ex Taq™ II; TaKaRa, Beijing, China).

Statistical analysis

The ΔCt value was used to represent the relative level of expression of a single miRNA. A two-tailed *t*-test was used between two independent samples to identify significantly-expressed miRNA. Binary logistic regression was used during the validation stage and a receiver operating characteristic curve was drawn to calculate the area under the curve (AUC) and assess the diagnostic value of miRNA for CRC. Based on relevant parameters in binary logistic regression, we established a mathematical diagnosis equation for detection of CRC. SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. Differences were considered significant at $p<0.05$.

Results

Comparison between CRCs and HCs

As compared with HCs, there was a significant difference in 9 miRNAs in the sera of patients with CRC during the screening stage (Table 2). After validation, a panel of 5 miRNAs (miRNA-1246, miRNA-202-3p, miRNA-21-3p, miRNA-1229-3p, and miRNA-532-3p) effectively distinguished CRCs (n=107) from HCs (n=120), with 91.6% sensitivity and 91.7% specificity. The AUC was 0.960 (95% confidence interval [CI]: 0.937–0.983) (Figure 2). Based on relevant parameters in binary logistic regression, a diagnosis discriminant model was established according to the following equation:

$$Y_1 = 1/(1 + e^{-x}),\ x = -5.076 - 1.407\ \Delta Ct\ (miRNA\text{-}1246)$$
$$+ 0.759\ \Delta Ct\ (miRNA\text{-}202\text{-}3p) + 1.328\ \Delta Ct$$
$$(miRNA\text{-}21\text{-}3p) - 0.858\ \Delta Ct\ (miRNA\text{-}1229\text{-}3p)$$
$$+ 0.399\ \Delta Ct\ (miRNA\text{-}532\text{-}3p).$$

The cutoff value was 0.502. When $Y_1<0.502$, the detection result was negative, and determined to be a HC. When $Y_1 \geq 0.502$, the detection result was positive, and determined to be a CRC.

Comparison between CRCs and CRAs

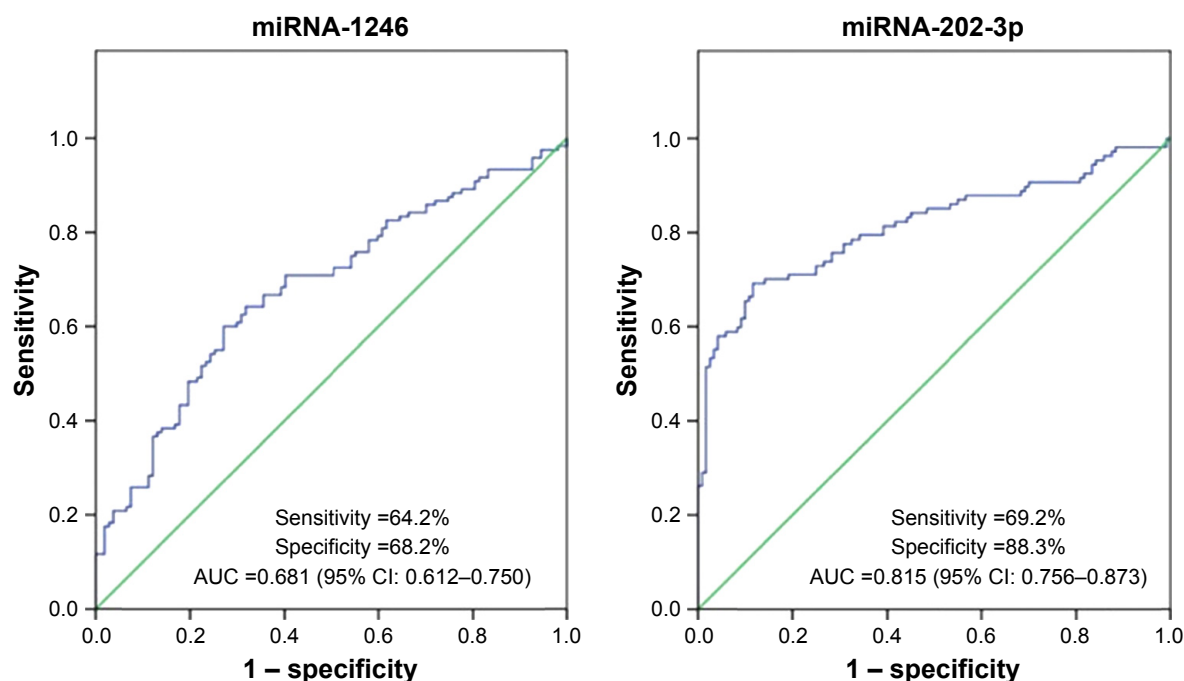As compared with the CRA group, there was a significant difference in 9 miRNAs in the sera of patients with CRCs
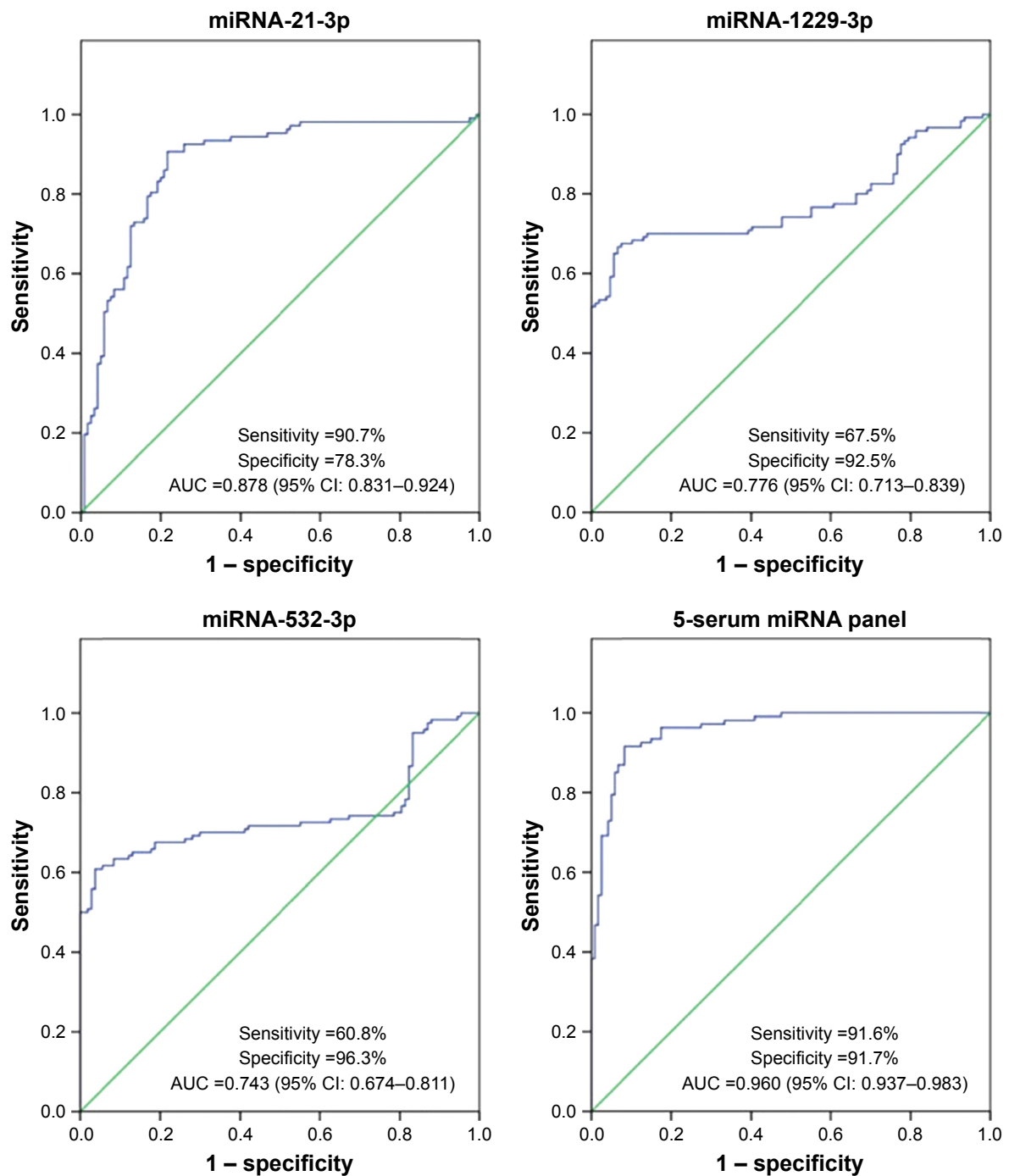

Figure 2 (*Continued*)

Figure 2 Validation stage, colorectal cancers compared to healthy controls.
Notes: Receiver operating characteristic curve analysis of miRNA-1246, miRNA-202-3p, miRNA-21-3p, miRNA-1229-3p, miRNA-532-3p, and the 5-serum miRNA panel.
Abbreviations: AUC, area under the curve; miRNA, microRNA.

during the screening stage (Table 3). After validation, a panel of 5 miRNAs (miRNA-1246, miRNA-202-3p, miRNA-21-3p, miRNA-1229-3p, and miRNA-532-3p) effectively distinguished CRCs (n=107) from CRAs (n=98), with 94.4% sensitivity and 84.7% specificity. The AUC was 0.951 (95% CI: 0.922–0.980) (Figure 3). Based on relevant parameters in binary logistic regression, a diagnosis discriminant model was established according to the following equation:

$$Y_2 = 1/(1 + e^{-x}), x = 1.223 - 1.365\ \Delta Ct\ (\text{miRNA-1246})$$
$$+ 1.924\ \Delta Ct\ (\text{miRNA-202-3p}) - 0.032\ \Delta Ct$$
$$(\text{miRNA-21-3p}) - 0.767\ \Delta Ct\ (\text{miRNA-1229-3p})$$
$$+ 0.131\ \Delta Ct\ (\text{miRNA-532-3p}).$$

The cutoff value was 0.348. When $Y_2 < 0.348$, the detection result was negative, and determined to be a CRA. When $Y_2 \geq 0.348$, the detection result was positive, and determined to be a CRC.

Discussion

The incidence of CRC is increasing year after year. A former study revealed that good prognosis can be achieved for the majority of CRC patients if there is early detection and appropriate treatment.[24] Therefore, early detection becomes key for disease prophylaxis. In recent years, circulating miRNAs have attracted increased attention from investigators, which have become markers for early tumor detection. Chen et al[25] reported that serum miRNA expression profiling of patients with CRC differs from the healthy population, and believed that part of the abnormally-expressed miRNA could be used
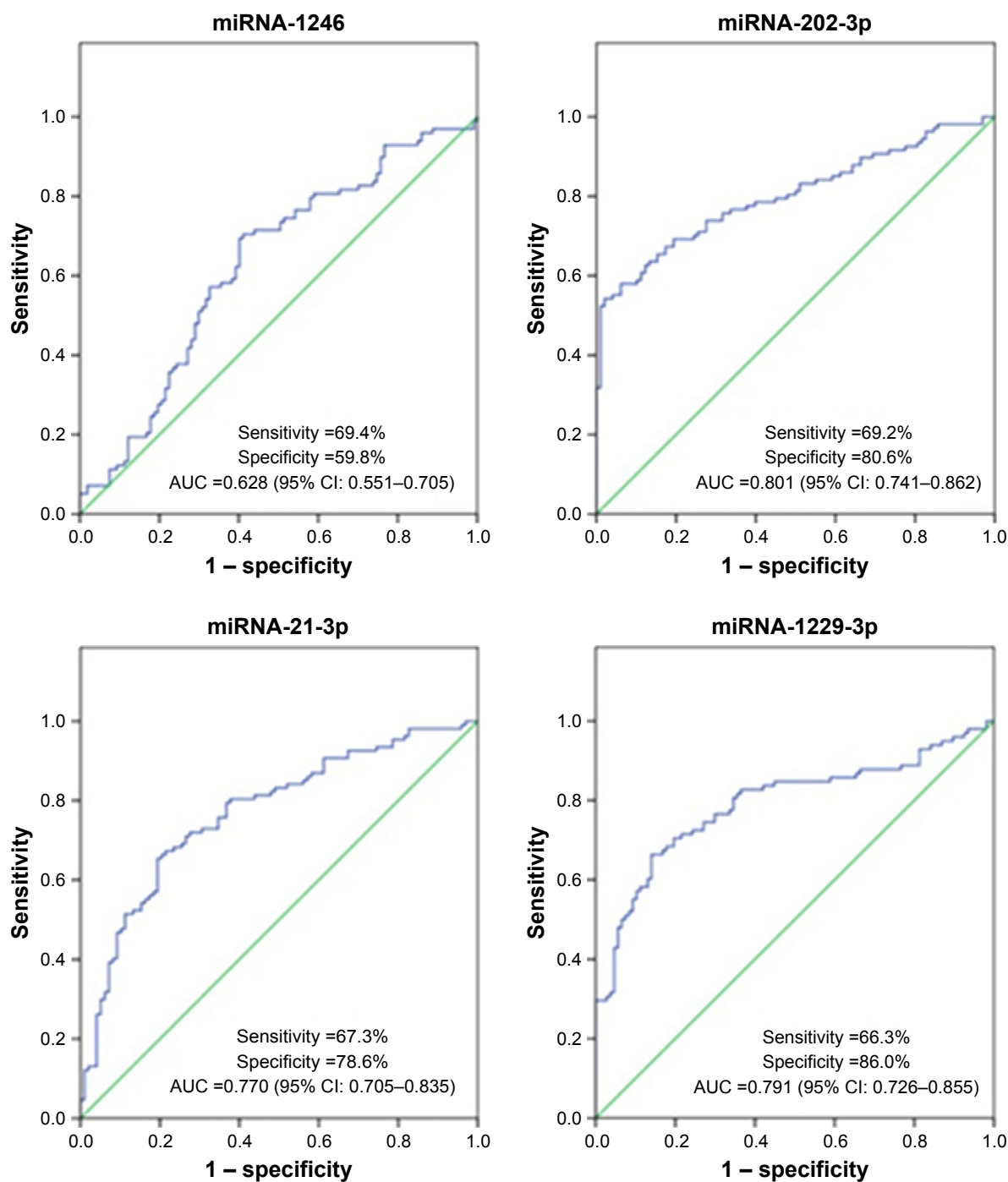

miRNA-1246

Sensitivity =69.4%
Specificity =59.8%
AUC =0.628 (95% CI: 0.551–0.705)

miRNA-202-3p

Sensitivity =69.2%
Specificity =80.6%
AUC =0.801 (95% CI: 0.741–0.862)

miRNA-21-3p

Sensitivity =67.3%
Specificity =78.6%
AUC =0.770 (95% CI: 0.705–0.835)

miRNA-1229-3p

Sensitivity =66.3%
Specificity =86.0%
AUC =0.791 (95% CI: 0.726–0.855)
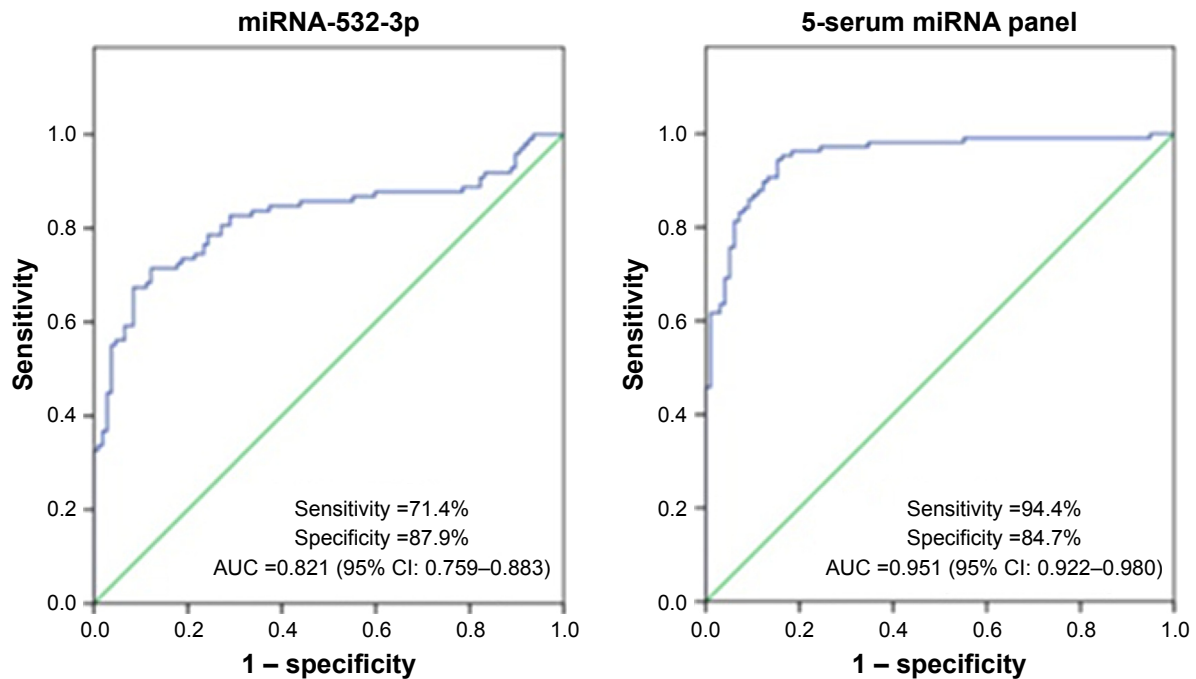
Figure 3 (*Continued*)

Figure 3 Validation stage, colorectal cancers compared to colorectal adenomas.
Notes: Receiver operating characteristic curve analysis of miRNA-1246, miRNA-202-3p, miRNA-21-3p, miRNA-1229-3p, miRNA-532-3p and the 5-serum miRNA panel.
Abbreviations: AUC, area under the curve; miRNA, microRNA.

for detection of CRC. Ng et al[26] reported that in patients with CRC, the level of miRNA-92a expression is significantly increased not only in cancer tissues, but in plasma as well. After resection of cancerous tissue during surgery, however, plasma miRNA-92a is restored to the normal level and it was confirmed that high miRNA-92a expression is unrelated to inflammatory bowel disease and gastric cancer. Huang et al[27] monitored the levels of miRNA-92a expression in 20 CRC patients before and after surgery. The results indicated an obvious decrease after surgery, which was consistent with the findings of Ng et al,[26] further confirming the value of miRNA92a in detection of CRC. Toiyama et al[28] tested abnormally-expressed miRNA in serum samples of patients with CRCs, CRAs, and HCs, and found that the level of miR-21 expression in sera of patients with CRAs and CRCs before surgery was increased and significantly decreased after surgery. They concluded that miR-21 is a potential marker for the early detection of CRC. Kanaan et al[29] reported that the sensitivity and specificity of distinguishing CRC from the control group based on increased miR-21 expression in plasma were up to 90%; however, miR-92a and miR-21 had poor organ specificity: miRNA-92a was increased in patients with acute leukemia and cervical cancer;[30,31] and miR-21 was also highly expressed in patients with lung, breast, esophageal, and gastric cancers.[32] Therefore, it may be difficult to assess results when a single miRNA is used as a diagnostic marker.

More and more investigators have proposed a combination of several miRNAs to improve diagnostic efficiency. Some studies have also confirmed the feasibility of a panel consisting of multiple circulating miRNAs in the early detection of CRC. Luo et al[33] showed that 9 differentially-expressed miRNAs (miR-18a, miR-20a, miR-21, miR-29a, miR-92a, miR-106b, miR-133a, miR-143, and miR-145) are present in the plasma of patients with CRC. The 9-miRNA panel accurately distinguished patients with CRC from patients with non-CRC, with an AUC of 0.745. Wang et al[34] reported that a panel consisting of miR-409-3p, miR-7, and miR-93 accurately diagnosed CRC with a sensitivity of 82%, specificity of 89%, and AUC of 0.897. Zheng et al[35] believed that a 4-serum miRNA panel (miR-19a-3p, miR-223-3p, miR-92a-3p, and miR-422a) diagnosed early CRC with an AUC up to 0.951. Moreover, the panel accurately identified CRCs, CRAs, and HCs with AUCs of 0.886 and 0.765, respectively. Zhu et al[36] showed that the serum levels of miR-19a-3p, miR-21-5p, and miR-425-5p expression in patients with CRC were higher than normal controls. The panel could be used in detection of CRC with an AUC of 0.830. Nevertheless, there are currently no relevant diagnostic products widely available for clinical use, probably because of the variety of races and samples, and different detection approaches. Therefore, we suggest that additional research data are warranted to provide theoretical support for clinical use.

A total of 575 serum samples were collected in this study, including 217 CRCs, 168 CRAs, and 190 HCs. qRT-PCR was used in all three stages. Through screening and validation, a 5-serum miRNA panel (miRNA-1246, miRNA-202-3p, miRNA-21-3p, miRNA-1229-3p, and miRNA-532-3p) was confirmed to differentiate CRCs from HCs, and CRCs from CRAs. The AUCs were 0.960 and 0.951, respectively, which were superior to previous studies.[33–38] As compared with the CRC group, there was a significant difference in the serum level of expression of 5 miRNAs in the HC and CRA groups. All new diagnostic tests have limitations, especially pertaining to the sample size of the study. A total of 575 serum samples were included in our study, which is larger than other studies, thus ensuring the reliability of the test results. In addition, we adopted a three-step qRT-PCR to avoid the false-negative results in the two-step method caused by stem-loop primers, and adopted a mash-up of long and short primers to ensure high efficiency and accuracy of the amplification. Finally, we also established a mathematical model for CRC detection, which provides great convenience for future clinical application.

Proper standardization is crucial for accurate quantitation of miRNA expression by qRT-PCR. Kanaan et al[39] reported that a panel of 3 plasma miRNAs (miR-431, miR-15b, and miR-139-3p) distinguished stage IV CRC from controls and successfully used U6 as an internal control to standardize miRNA expression. Rice et al[40] recommended using RNU6 and miR-520d-5p as reliable internal controls in a study of plasma miRNA; however, some reports hold the view that U6 is not expressed constantly and easily degraded by RNase in the circulation; the stability is poor.[25,41,42] To date, no uniform standard for internal control has been established. Therefore, it has been proposed to use an external reference for standardization of the level of circulating miRNA expression.[43] Wang et al[44] concluded that the miRNA (cel-miR-39) of *Caenorhabditis elegans* is nonhomologous with humans and has good repeatability and high stability. Therefore, cel-miR-39 can be used for quantification of circulating miRNA by qRT-PCR. Zhu et al[36] reported that the serum levels of miR-19a-3p, miR-21-5p, and miR-425-5p expression in patients with CRC are significantly higher than in the control group. They also used cel-miR-39 as an external reference for standardized calculation of the relative expression of miRNAs. Cel-miR-67 is a miRNA of *C. elegans*. It has no homology with human miRNA and meets the requirement of external reference.[45] We successfully used cel-miR-67 (primer sequence, CAACCTCCTAGAAAGAGTA) as an external reference and obtained stable and repeatable results.

Wang et al[46] reported that upregulation of miRNA-1246 in CRC tissues may cause disease progression. The cause can be miRNA-1246 binding to CCNG2 in CRC cells, which inhibits the expression of cyclin 2 (CycG2). Lai and Friedman[47] detected that miRNA-1246 is significantly increased in the sera of patients with CRC and suggested that miRNA-1246 could be used as a potential biomarker for early detection of CRC. Wang et al[48] showed low expression of miRNA-202-3p in cancer tissues in patients with CRC and demonstrated overexpression of miRNA-202-3p inhibited growth of CRC cells in vitro. However, we have not uncovered any reports that the three miRNAs (miR-21-3p, miR-532-3p, and miR-1229-3p) serve as markers for the early detection of CRC, as reported for the first time in the current study.

Conclusion

Circulating miRNAs can be considered to be important biomarkers for detection of CRC. We found 5 differentially-expressed miRNAs in serum, and as a non-invasive screening approach for CRC, with high sensitivity and specificity, the panel may be used for accurate early detection of CRC, which is a promising prospect for clinical application.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Different expression of microRNAs in individuals with colorectal cancer compared to colorectal adenoma (primary screening stage)

MicroRNA	Fold-change	*p*-value
miR-33	0.265042271	0.001610925
miR-144-3p	0.285667472	0.004198435
let-7d-5p	0.244924368	0.005624263
miR-92b	0.305305096	0.005995937
miR-1179	0.316268149	0.006468747
miR-1229-3p	2.461178616	0.00805813
miR-551a(v2)	0.305288759	0.008684262
miR-376b-3p	0.260034261	0.009699059
miR-146b-3p	0.295102587	0.011135773
miR-34b-3p	0.271451926	0.011544721
miR-19a-5p	0.209116981	0.012224061
miR-520e	0.369872931	0.020855674
miR-143-3P	0.399634302	0.026115223
miR-125a-5p	0.408503948	0.026449147
miR-450b-5p	0.346582118	0.026732608
miR-202-3p	0.409552974	0.02924914
miR-489	0.247163905	0.029727171
miR-877-5p	0.647163939	0.03044741
miR-545-3p	0.359258047	0.030458955
miR-208a	0.340216927	0.030737688
miR-32-5p	0.403988512	0.031181984
miR-181d	0.406456461	0.031941558
miR-135a-3p	0.419868024	0.032504658
miR-105-5p	0.74403822	0.03251836
miR-384	0.399896934	0.03260316
let-7a	0.409143808	0.033506761
miR-1185-5p	0.306196612	0.035229791
miR-532-3p	3.399817361	0.035689427
miR-671-3p	0.358210424	0.036514615
miR-197-3p	0.377791337	0.036633857
miR-1246	1.09930781	0.03688102
miR-325	0.350568779	0.036923277
miR-30b-3p	0.31109897	0.037384379
miR-513a-3p	4.460504751	0.03770905
miR-543	0.413556264	0.04185382
miR-206	0.439335025	0.042124271
miR-15b-3p	0.426465067	0.042831015
miR-760	0.380084283	0.046688543
miR-145-5p	0.400879938	0.047524061
miR-16-2	0.433533813	0.047860264
miR-19b-3p	0.384054717	0.049336907
let-7i-5p	0.433210518	0.049358513
miR-519a/e-3p	0.40297128	0.05001662
miR-627	0.58093362	0.05001731

Table S2 Different expression of microRNAs in individuals with colorectal adenoma compared to healthy controls (primary screening stage)

MicroRNA	Fold-change	*p*-value
miRNA-144-3p	6.11157279	0.000172566
miRNA-671-3p	3.975381731	0.000223319
miRNA-384	4.962448396	0.000546041
miRNA-33	4.647566151	0.000599022
miRNA-676-5p	3.868145332	0.000824278
miRNA-454-3p	4.462812516	0.000899269
miRNA-520e	5.562568511	0.001111901
miRNA-545-3p	4.601334376	0.001149391
miRNA-18a-3p	4.442486832	0.001440373
miRNA-1179	4.15588404	0.001661793
miRNA-376b-3p	2.923586547	0.002050843
let-7d-5p	4.847412982	0.002457329
let-7g-5p	3.682077008	0.00279327
miRNA-144-5p	4.556802996	0.002869924
let-7i-5p	3.45412382	0.003833675
miRNA-1283	2.887384825	0.004153721
let-7a	3.814471808	0.004315298
miRNA-92a-2-5p	3.366292175	0.004637293
miRNA-125a-5p	2.257099315	0.005148163
miRNA-331-5p	2.994388266	0.005623946
miRNA-339-5p	3.144862847	0.005830566
miRNA-190b-5p	3.366488181	0.006189011
miRNA-206	3.51978913	0.006867571
miRNA-192-5p	2.710941535	0.007232893
miRNA-451a	2.827780032	0.007531015
miRNA-21-3p	0.6368149	0.009544443
miRNA-551a(v2)	2.617844312	0.010080582
miRNA-205-5p	2.492757774	0.010250742
miRNA-98-5p	3.008911346	0.010465763
miRNA-500	2.105833248	0.011784554
miRNA-29b-3p	2.501582791	0.012323582
miRNA-33	2.775020059	0.01248712
miRNA-34a-3p	3.358051602	0.01249287
miRNA-342-5p	2.580064799	0.012877499
miRNA-486-5p	2.200055624	0.015583917
miRNA-21-5p	0.42080076	0.0156493
miRNA-151-5p	2.030671394	0.015879556
miRNA-409-5p	2.168444923	0.016020757
miRNA-193a-5p	2.147170268	0.016320118
miRNA-126-5p	2.579005947	0.017259782
miRNA-224-3p	2.937438812	0.018760933
miRNA-34c-3p	2.777459548	0.019721369
miRNA-92b	2.696456388	0.020283018
miRNA-506-3p	2.246626348	0.020706272
miRNA-34c-5p	2.07571691	0.0208559
miRNA-508-3p	2.43041453	0.021277675
miRNA-374a-5p	3.023106701	0.022908037
miRNA-501-3p	1.852612175	0.02378615
miRNA-720	1.856273384	0.025420387
miRNA-301a-3p	2.269381725	0.026782368
miRNA-450a-3p	2.104325529	0.027334903
miRNA-203	2.880692963	0.027476655
miRNA-139-5p	1.917480226	0.029056841
miRNA-125b-5p	1.925013816	0.030014952

(Continued)

Table S2 (*Continued*)

MicroRNA	Fold-change	p-value
miRNA-30e-5p	2.759625506	0.030950566
miRNA-32-5p	2.500353192	0.032146669
miRNA-532-3p	2.179461623	0.032897357
miRNA-146b-3p	2.658376786	0.033550016
miRNA-501-5p	2.632214026	0.034420132
miRNA-324-5p	1.796021101	0.039666032
miRNA-1277-3p	2.355786669	0.041585667
miRNA-769-5p	2.099038895	0.04234743
miRNA-130b-3p	1.868666222	0.04322903
miRNA-505-3p	1.95785877	0.04433078
miRNA-101a-3p	1.830303379	0.044999506
miRNA-373-3p	1.952099513	0.045989963
miRNA-185-5p	1.808758504	0.04761089
miRNA-616-5p	1.794836092	0.047820058
miRNA-508-5p	1.861324636	0.048746558

Table S3 Different expression of microRNAs in individuals with colorectal cancer compared to healthy controls (primary screening stage)

MicroRNA	Fold-change	p-value
miRNA-203	5.665100598	0.000261574
miRNA-1229-3p	3.69033982	0.010024984
miRNA-493-3p	0.287891874	0.011300507
miRNA-450b-5p	0.339407047	0.021402949
miRNA-380-5p	0.60221932	0.021435126
miRNA-448	2.864765252	0.021485785
miRNA-553(v2)	0.333352586	0.027053083
miRNA-18a-3p	2.949312666	0.030434267
miRNA-144-5p	3.317294796	0.033804481
miRNA-191-5p	0.663074233	0.03733962
miRNA-454-3p	2.509424646	0.039062132
miRNA-616-3p	0.285858516	0.040460404
miRNA-21-5p	0.63119403	0.04063326
miRNA-32-3p	0.382740116	0.040787824
miRNA-495-3p	0.277451773	0.040823122
miRNA-21-3p	0.72480618	0.004092244
miRNA-365	2.933616275	0.041040832
miRNA-208a	0.369272549	0.042675424
miRNA-340-5p	2.283193725	0.044386752
miRNA-218-5p	2.315410152	0.046594773
miRNA-520e	2.4124	0.04098
miRNA-1246	2.649032156	0.04880231

Table S4 Different expression of microRNAs in individuals with colorectal adenoma compared to healthy controls (screening stage)

MicroRNA	Fold-change	p-value
miRNA-1246	1.301	0.046
miRNA-19b-3p	0.607	0.001
miRNA-21-3p	0.702	0.007
miR-1229-3p	2.144	0.02
miR-532-3p	1.602	0.008
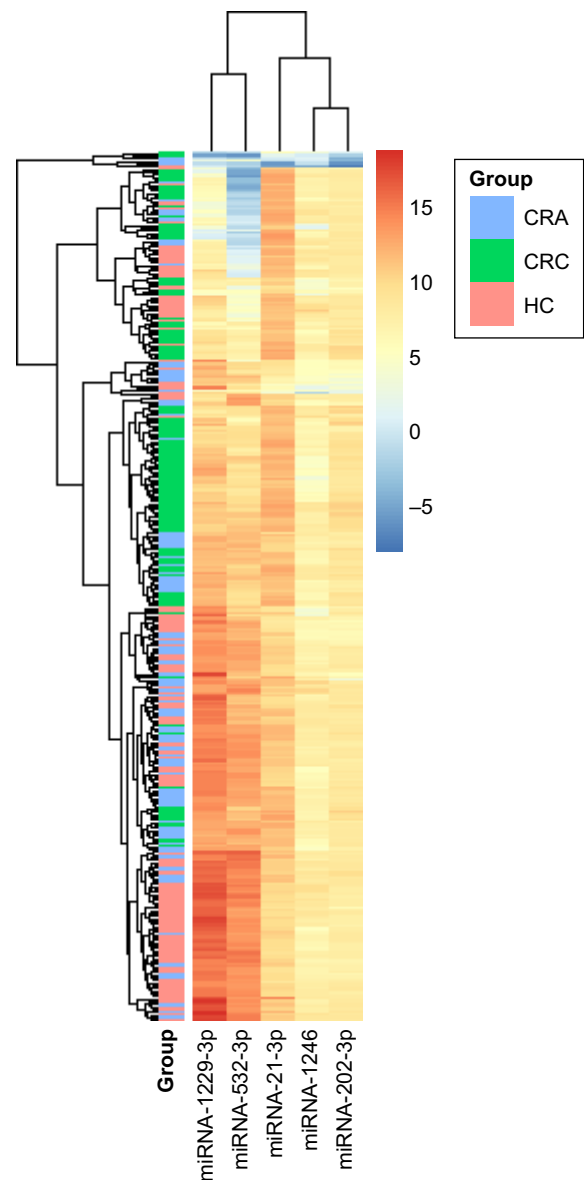
Figure S1 The 5 microRNAs have different expression in the 3 groups.
Note: Created using https://cran.r-project.org/web/packages/pheatmap/index.html.
Abbreviations: CRA, colorectal adenoma; CRC, colorectal cancer; HC, healthy control.