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An update on microRNAs as colorectal cancer biomarkers: where are we and what's next?

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Abstract

MicroRNAs (miRNA) are abundant classes of small, endogenous non-coding RNAs, which inhibit the expression of target gene via post-transcriptional regulation. In addition to an important functional role miRNAs play in carcinogenesis, emerging evidence has demonstrated the feasibility of miRNAs as robust cancer biomarkers. In particular, the recent discovery of miRNAs in the body fluids provides an attractive opportunity for the development of non-invasive biomarkers for the diagnosis, prognosis and predictive responses to cancer therapy. Colorectal cancer (CRC) is one of the most common cancers worldwide, and accumulating evidence provides a compelling case for the potential exploitation of miRNAs as CRC-biomarkers. This review summarizes the current state of literature in the field, and primarily focuses on the clinical relevance of miRNAs as potential biomarkers for CRC treatment, and discusses the forthcoming challenges to further advance this exciting field of “academic research” into “bedside clinical care” of patients suffering from CRC.

Keywords

miRNA; biomarker; colorectal cancer; adenoma; blood; feces; diagnosis; prediction; prognosis

Introduction

Colorectal cancer (CRC) is one of the most common and lethal malignancies worldwide[1]. Although the mortality rates have declined over the past decade, especially in developed countries as a result of improved screening and/or treatment options, CRC still remains the third leading cause of cancer-related death in the world, with an estimated incidence of 1.2 million new cases and 608,700 deaths estimated annually[2]. However, CRC is a potentially preventable disease, and early detection form of this disease is almost always curable. In view of these results, we clearly need to establish novel and more effective screening

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programs/strategies that would permit earlier detection of this disease, as well as develop safe and more effective therapies that can help reduce the significant global mortality rates associated with this malignancy. Several CRC screening tests, including fecal occult-blood testing and colonoscopy, have been available for years[3], Although colonoscopy is the current gold standard for early diagnosis of CRC, the invasive nature and the associated high costs have hampered its widespread application as an effective and compliant screening modality. Currently available noninvasive screening methods, such as fecal guaiac-based occult blood tests(FOBT) and fecal immunochemical occult blood tests (FIT), have resulted in somewhat decrease in mortality[7-9]. However, large-scale screening studies have noted the limitation of these tests due to the inherent low accuracy, particularly with regards to the detection of pre-neoplastic lesion, which should be the primary focus for any cancer-screening regimen[5,10,11]. In view of these caveats, there is an urgent need for the development of novel and highly specific non-invasive biomarkers that can help improve early detection of CRC. In contrast, patients with advanced disease frequently receive expensive cytotoxic chemotherapeutic drugs, or targeted monoclonal antibodies- but in both instances the overall benefits derived from these treatments are relatively modest, and leave much to be desired[12]. Hence, alternative strategies are needed to improve the patient outcomes and to identify patients that will benefit truly from treatment with cytotoxic chemotherapies and intensive post-treatment surveillance protocols. Taken together, there is a clear unmet need for developing novel biomarkers for early diagnosis, prognosis, and deciphering predictive responses to chemotherapy in CRC patients. Herein, we focus this article on the clinical utility of these molecular signatures in the context of current patient care with CRC.

MiRNA biogenesis and function

MicroRNAs (miRNAs, or miRs) are a class of small single stranded non coding RNAs (ncRNAs) that are ∼18-25 nucleotides in length, which were first discovered in 1993 as a developmental regulator of *Caenorhabditis elegans*[13,14]. MiRNA genes are transcribed by RNA polymerase II in the nucleus of a cell to produce primary miRNA (pri-miRNA), which consists of a 5′-cap, at least one ∼70-nucleotide hairpin structure and a 3′-poly(A) tail. After transcription, pri-miRNAs are processed to precursor miRNA (pre-miRNA) by Drosha and its cofactor DGCR8 by removing the 5′-cap, the 3′-poly(A) tail and sequences flanking the hairpin structure. Pre-miRNAs are transported to the cytoplasm by exportin 5, and further processed by the ribonuclease III enzyme Dicer, to produce a mature miRNA strand and its complementary miRNA* strand[15,16]. The resulting 18-25 nucleotide long mature miRNA is ultimately integrated into the RNA induced silencing complex (RISC), and negatively regulates the expression of hundreds of target messenger RNAs (mRNAs) by translation inhibition or mRNA degradation, through base-printing to partially complementary sites on the target mRNAs, usually residing within the 3′ untranslated regions[17,18]. Therefore, relatively minor variations of miRNA sequences could have important biological consequences for the cell via regulation of the large number of target mRNAs.

Aberrantly expressed MiRNAs in Colorectal Cancer

Accumulating data have shown that miRNAs are aberrantly expressed in virtually all human cancers, including CRC; in which, these ncRNAs may function either as tumor suppressors (tsmiRs) or as oncogenes (oncomiRs), depending on the downstream target genes or pathways they regulate. The expression status of individual miRNAs may also associate with a variety of clinicopathological and prognostic factors in cancer patients, highlighting the central role miRNAs play in CRC pathogenesis and its progression[19-23].

MiR-143 and miR-145 were the first reported miRNAs associated with CRC development. The expression of these miRNAs was down-regulated in precancerous tissues and neoplastic colorectal tissues compared with normal mucosa[20]. Subsequent functional studies demonstrated that knockdown of expression of these miRNAs induced growth inhibition in CRC cell lines suggesting that miR-143 and miR-145 act as tumor suppressors in this malignancy [24-27].

Mir-17-92a cluster was firstly identified as a critical genomic locus that linked miRNAs to cancer pathogenesis. This cluster, arising from 13q, encodes six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1), and has been frequently implicated by the high incidence of amplification in various types of human cancers. Diosdado et al reported that the increased expression of miR-17-92a seen during the progression of colorectal adenoma to carcinoma is associated with the gain of miR-17-92a locus and increased c-myc transcriptional activity[28]. Moreover, recent evidence further highlights the oncogenic function of miR-17-92a cluster in CRC development[29,30].

The expanded use of high-throughput microarray-based miRNA profiling platforms clearly revolutionized the elucidation of miRNA expression pattern analysis and in unraveling their biological role in various diseases. The first systemic and comprehensive array-based analysis evaluated 389 miRNAs expression levels using 84 CRCs and matched normal colonic tissues. This study identified 37 differentially expressed miRNAs in CRC, including the overexpression of miR-20a, miR-21, miR-106a, miR-181b, and miR-203 in the tumor tissues[31]. Volina and colleagues also performed large scale miRNA profiling using various types of cancerous and normal tissues, and identified a miRNA signature for solid cancers. This study demonstrated that miRNAs, including miR-17-5p, miR-20a, miR-21, miR-24, miR-106, miR-155, miR-203, and miR-223, were dysregulated in colon cancer[19]. In a more recent meta-analysis conducted by Luo et al., the authors consolidated data from 20 tissue-based studies and revealed that up-regulation of miR-31 and down-regulation of miR-145 were most frequently reported miRNAs (both in 8 different studies) in CRC tissues vis-à-vis matched normals[32].

The list of aberrantly expressed miRNAs in CRC tissues is continuously growing, and currently more than several hundred studies have been published on this topic. Furthermore, in many of the publications, a given miRNA biomarker has been published only once, and has not been validated or substantiated by follow-up studies. Therefore, in Table 1, we chose to only list those miRNA biomarkers/studies, which have been validated in two independent articles, hence making the evidence presented in this table more credible and possibly clinically relevant.

Dysregulated miRNA expression in colorectal adenomatous polyps

Colorectal adenomatous polyps, particularly advanced colorectal adenomas (colorectal polyps greater than 1 cm in diameter and/or villous component and/or severe dysplasia), are recognized as critical premalignant lesions for CRC development, and are the primary target lesions for CRC screening. Accumulating evidence indicates that a spectrum of dysregulated miRNAs is associated with tumorigenesis in colorectal adenomas. The following section summarizes some of the latest findings for miRNA expression alterations in colonic adenoma tissues, and a comprehensively tabulated list of such miRNAs is provided in Table 2.

MiR-21 is one of the most well-established oncogenic miRNAs, and it is frequently overexpressed in colorectal adenoma tissues compared with normal colonic mucosa. Schetter and colleagues demonstrated that the expression of miR-21 was significantly up-regulated in 18 adenoma tissues compared with the matched adjacent normal mucosa, while the expression of miR-21 in adenoma tissues was significantly lower than in CRC tissues[31]. Oberg and coworkers also performed comprehensive analysis using 41 adenoma and 55 normal tissue specimens, and found that miR-31 and miR-135b were overexpressed while miR-1, miR-9, miR-99b and miR-137 were downregulated in the adenoma tissues[33]. In accordance with this study, later studies confirmed that expression of miR-135a and miR-135b was upregulated in adenoma tissues in comparison with normal mucosa[34]. Increased expression or amplification of miR-92a in adenoma specimens have also been demonstrated in other independent studies[28,30]. MiR-21 has been shown to participate in the multistep process of colorectal tumorigenesis, by not only regulation of MAPK pathway, but also by associating with WNT/β-Catenin signaling by targeting PTEN, PDCD and DKK2[35-37]. MiR-135a/b and miR-17-92a cluster can also activate the WNT signaling pathway via suppression of APC or E2F1, respectively [34,38]. These evidences clearly highlight the functional role of a subgroup of miRNAs to be involved in the early phases of multi-step colorectal carcinogenesis via regulation of WNT/β-Catenin, and MAPK signal pathways.

Potential of MiRNAs as a disease biomarker

For any biomarker development endeavor, certain technical steps including sample collection and storage of clinical samples for extended periods of time (months or years) have been identified as potential sources of bias that can confound results[39-42]. In this context, miRNAs have a considerable potential for development as disease biomarkers compared to DNA and mRNAs that have been unsuccessfully exploited for decades. MiRNAs are remarkably small, can exist freely or in exosomal shells, are less vulnerable to RNase-mediated degradation, and can be easily extracted from a wide variety of biological and clinical materials, including archival formalin fixed paraffin embedded (FFPE) tissues and body fluids collected in clinical settings. In particular, RNase present in body fluids tends to degrade such biomolecules, particularly mRNAs, which posed the key impediment and success for developing mRNA-based biomarkers over the years. However, despite the presence of RNase activity in the blood, endogenous miRNAs tend to remain stable for long periods of time and can withstand several repeated freeze-thaw cycles[43-45]. Furthermore, accumulating evidence indicates that cancer cells secrete some miRNAs into systemic

circulation[43,45]. This unique feature of miRNAs is one of the central reasons for the recent explosion of miRNA biomarker studies in the field of cancer research.

MiRNAs as non-invasive early detection biomarkers in CRC

Currently, large array of body fluids are being explored for their feasibility as non-invasive miRNA-based biomarkers for the early detection of various type of cancers. Considering that blood (serum/plasma) is one of the most easily accessible body fluids, it is most frequently used as a diagnostic material for the development of surrogate cancer biomarkers[46]. The first study of serum miRNA profiling in CRC reported 69 serum miRNAs that were differentially expressed in CRC patients, but not in healthy volunteers. Among these, 14 miRNAs were uniquely expressed in CRC patients and the authors suggested that these miRNAs may have high degree of CRC-specificity[44]. A follow-up study performed the first systematic and comprehensive analysis for miRNA biomarkers using plasma samples[47]. In the discovery phase of this study, miRNA profiling using tissue and plasma specimens from CRC and healthy volunteers detected 5 candidate miRNAs, and these markers were validated in a large set of plasma from 90 patients with CRC, 20 patients with gastric cancer, 20 patients with inflammatory bowel disease, and 50 healthy controls. The authors noted that the expression levels of both miR-17-3p and miR-92a were significantly over-expressed in plasma from CRC patients. MiR-92a expression status in plasma could discriminate patients with CRC from controls (sensitivity: 89%, specificity: 70%, AUC: 0.89). Intriguingly, the expression status of these over-expressed miRNAs was lowered following surgical removal of the tumor, indicating that the expression levels of these miRNAs in plasma was a reflection of underlying CRC. Furthermore, miR-92a expression was also capable of distinguishing CRC from other gastrointestinal diseases and tumors in this study, highlighting the specificity of miRNA biomarkers for each disease and suggested that plasma miR-92a expression levels may offer a valuable diagnostic biomarker for noninvasive screening of CRC[47]. The concept and results from this study contributed to significant progress in the CRC blood-based diagnostic marker research. Early diagnosis, including detection of adenomas, the precancerous lesions for CRC, is considered as a key concept for improving patient survival in CRC treatment. Huang and colleagues firstly conducted evaluation of miRNA expression analysis using 216 plasma specimens including specimens from 37 patients with colorectal adenomas[48]. This study successfully demonstrated that plasma miR-29a and 92a could be used to discriminate not only CRC, but also adenoma patients from healthy subjects. Hence the evaluation of plasma miR-29a and -92a status provided a strong rationale as novel non-invasive biomarkers for screening of CRC patients.

Recently, our group also discovered miR-21 as a potential biomarker for the early detection and prognosis in CRC patients using a large cohort of serum and matching tissue samples[49]. The study reported three key findings: First, miR-21 was identified as a novel secretory miRNA using supernatants from cultured CRC cell lines, and serum expression of this miR in CRC patients correlated positively with expression status in tumor tissues. Second, serum miR-21 expression levels could robustly identify not only patients with CRC (Sensitivity: 92%, Specificity: 81%, AUC: 0.92), but also patients with premalignant adenomatous polyps (Sensitivity: 81%, Specificity: 77%, AUC: 0.81). Third, serum

expression status of miR-21 increased in a stage-dependent manner in CRC patients with stage I-IV disease, highlighting its potential use as a novel prognostic marker in CRC patients. Furthermore, we also demonstrated that serum miR-21 expression dropped significantly in post-operative serum samples from patients who underwent curative CRC surgery. Taken together, these data indicate that serum miR-21 is a promising biomarker that specifically reflects the tumor burden in the colon, and that serum miR-21 could be developed as a robust biomarker for early detection and prognosis in CRC patients. A subsequent study confirmed our findings and also reported the potential of plasma miR-21 as a diagnostic marker in CRC [50]. In this study during the discovery step, level of 380 miRNAs were determined using CRC tissues and corresponding normal mucosa from 30 patients, and the 4 most dysregulated miRNAs were then validated in tissue and plasma samples. This study also demonstrated that plasma miR-21 could be used to identify the patients with CRC from healthy volunteers with high sensitivity (90%) and specificity (90%). In line with these studies, another recent study using a large cohort of clinical specimens further reconfirmed that serum miR-21 could be a diagnostic biomarker for CRC patients [51].

In addition to blood, feces have also been widely used as potential substrate for developing non-invasive molecular screening tests for CRC patients. As mentioned earlier, considering the limitations of conventional stool-based screening tests including low sensitivity and specificity for detection of CRC and advanced adenoma, fecal DNA-based testing for CRC has been an area of active investigation since 1990s[52-56]. Although several studies have been reported fecal RNA-based assays[57-59], to date, no optimal method that offers superior detection accuracy compared to conventional screening tests (FOBT/FIT) have thus far been established. In contrast, exfoliated fecal colonocytes or tumor-secreted miRNAs are directly and continuously released from the tumors into intestinal lumen – providing a compelling rationale that a stool-based miRNA test might be better for CRC detection [60-62]. To date, several studies have attempted to identify the potential of fecal miRNAs as screening tools. Ahmed and colleagues evaluated miRNA expression status using fecal specimens from CRC, ulcerative colitis and healthy volunteers, and extended their study to establish a protocol for measuring miRNA levels using fecal specimens[60]. Using an approach to measure miRNA expression in fecal colonocytes, another laboratory extracted total RNA from fecal colonocytes isolated by immunomagnetic beads conjugated with epithelial cell adhesion molecule (EpCAM) monoclonal antibody, and evaluated expression status of 10 miRNAs from 197 CRC patients and 119 healthy volunteers[62]. This study showed that expression of miR-17-92a cluster and miR-135 was significantly higher in CRC patients than in healthy volunteers and suggested miRNA expression profile could be assessed in fecal colonocytes as a potential screening test in patients with CRC. Recently, the same group of investigators extended their research to further optimize their approach in the pursuit to develop a clinically viable assay[63]. In this study, the authors evaluated fecal miRNA expression from the samples extracted from the residual material collected in FOBT kits from CRC patients and healthy volunteers, and demonstrated that the combination of miR-106a status in feces with FOBT could reduce the rates of false negative results compared with FOBT alone to identify CRC patients.

Regarding the cell free miRNA in the feces, our research group was among the first to conduct a study analyzing fecal miRNA expression using a much simpler method[61]. We first confirmed the reproducibility of miRNA expression status using the specimens from fresh stool specimens and fecal samples collected in FOBT kits. Subsequently, we developed a one-step fecal miRNA extraction and amplification method (called direct miRNA analysis or DMA) and identified fecal miR-21 and miR-106a as potential candidates for fecal based CRC screening. In support of our data, another laboratory later also demonstrated the feasibility of using fecal miRNA expression as an early detection method of CRC[64]. The interesting feature of this latter study was evaluation of the miRNA status in feces using copy number dysregulation per ng of extracted stool RNA. This study revealed that combined analysis of stool miR-92a and miR-21 resulted in an acceptable sensitivity for the detection of CRC and precancerous advanced adenomas.

However, one of the major concerns for using miRNA expression analysis in body fluids, such as blood and stool, has been inconsistent and possible inadequate selection of internal controls for the normalization of miRNA expression across various published studies. Although many published studies have measured the commonly used reference gene, such as RNU6B, U6, 18S rRNA, and RNU43 as an internal control, these molecules are considered inadequate due to a non-excludable disease-specificity[65]. As a result, currently there is no established housekeeping miRNA gene for normalizing the expression of circulating miRNAs in body fluids [66,67]. Alternative normalization methods using Global means or Quantile normalization which could eliminate the need for endogenous controls are unrealistic, since such measures can only be achieved using large datasets. In these instances, the excessive cost constraints and requirements for large amounts of clinical specimens for such analysis, may limit the “practicality” of this approach. Considering the technical bias such as variability in serum RNA extraction and polymerase chain reaction amplification efficiencies, spike-in synthetics, non-human mature miRNA from *Caenorhabditis elegans* has been used recently for miRNA expression normalization in body fluids[68]. However, this method is not perfect, and can also be influenced by the sample quality. Further investigations for normalization of blood specimens are definitely needed, as we usher into the era on non-invasive diagnostics.

In addition, one of the key-limitations of fecal miRNA analysis is to overcome the complexity of fecal density and volume of sample needed for each assay. Since fecal conditions are more vulnerable to daily changes compared to serum/plasma, standardization of protocols for sample preparation are need to minimize the sample variability. In addition, candidates for the fecal miRNA test are roughly divided into three types: cell free miRNAs from fecal homogenates, exosomal miRNAs from fecal exosomes, and fecal colonocytes miRNAs. Taking into consideration these differences, further investigations and validation using standardized protocol in large cohort are also much required before such markers can be seriously considered for adaptation in the clinic for non-invasive CRC screening. Table 3 lists several non-invasive miRNA biomarkers with potential for clinical application of early detection of CRC.

MiRNA biomarkers for the identification of high-risk stage II patients

Accumulating evidence suggests that adjuvant chemotherapy following surgery significantly improves survival in stage III CRC patients, and is currently a well-accepted, standard treatment for these patients[69]. In contrast, in stage II CRC patients, surgical resection is highly effective; hence treating all stage II patients with adjuvant chemotherapy is still debatable[70-72]. This situation gets muddied further, since a significant proportion of Stage II CRC patients (25-30%) develop tumor recurrence and die because of disease progression. Currently, several clinical risk factors such as inadequately sampled lymph nodes, T4 lesions, intestinal obstruction, perforation, extramural venous invasion, or poorly differentiated histology are generally used to identify these high-risk subgroups in the clinic[71,73-76]. However these parameters are inadequate, highlighting the need for developing more accurate biomarkers for these high-risk populations of stage II CRC patients to improve their survival and spare the others from the toxicity of conventional chemotherapeutic drugs. The use of miRNAs as potential biomarkers using tissue samples in this area has been gradually expanding (Table 4). In one of the earliest studies, Schepeler and colleagues performed a systematic and comprehensive array-based miRNA profiling in stage II CRCs (including 37 microsatellite stable (MSS) and 12 microsatellite instability (MSI) tumor) using 315 miRNA probes[26]. These authors elegantly demonstrated that four miRNAs (miR-142-3p, miR-212, miR-151, and miR-144) could discriminate the microsatellite instability status of these patients with 84% accuracy (Sensitivity: 92%, Specificity: 81%). In addition, the expression level of miR-320 and miR-498 showed a significant correlation with recurrence free survival, and suggested that some of these miRNAs may have prognostic potential in CRC.

Currently, expression status of miR-21 in CRC tissues is the most well recognized miRNA biomarker for identifying high-risk stage II CRC patients. Schetter and coworkers identified miR-21 expression in primary tumor tissues as a prognostic marker in stage II CRC[31]. This group extended their study to evaluate the expression status of 23 inflammatory genes and discovered that combination of inflammatory risk score and miR-21 expression was a better predictor of prognosis than either individually[82]. The largest miR-21 cohort study in stage II CRC patients was reported from a research group in Denmark[83]. This population-based study conducted in situ hybridization using FFPE specimens from 520 stage II colon cancer patients, and showed that increased miR-21 expression levels in tumor tissues correlated significantly with poor recurrence-free cancer specific survival (HR=1.41, 95% CI:1.19-1.67). In another recent study, the investigators utilized 1849 miRNA probes and performed array-based profiling, in 40 paired stage II colorectal tumors and adjacent normal mucosa tissues. The study revealed 35 differentially expressed miRNAs between CRC vs. normal mucosa tissues. In the second phase of this study, expression analysis using quantitative polymerase chain reaction (qPCR) was performed to confirm differential expression of these miRNAs in 138 stage II patients. In this instance, six-miRNA-based classifiers (miR-20a-5p, miR-21-5p, miR-103a-3p, miR-106a-5p, miR-143-5p and miR-215) were used to discriminate high- and low-risk of disease progression in this group. In the final step, the performance of these classifiers were validated using independent cohort of samples (460 stage II patients), and concluded that these six-miRNAs emerged as promising prognostic and predictive tools for disease recurrence in patients with stage II CRC[84].

MiRNA biomarkers for the identification of distant metastasis in advanced CRCs

Distant metastasis is a major cause of death in patients with advanced CRC. Although approximately 70-80% of new CRC cases undergo potentially curative surgery, 40% of these patients develop metachronous metastatic disease[85]. Systemic chemotherapy plays a central role in treating such metastatic CRC (mCRC) patients, and the advances in chemotherapeutic treatments over the last few years have somewhat enabled management of mCRC, if the site of metastasis (especially lung, or liver) is restricted and accessible for local surgical treatment. In view of these clinical challenges, earlier detection of distant metastasis and selective criteria regarding which individuals would benefit the most from invasive treatments is essential for improving their long-term survival.

Although majority of published articles to date have focused on evaluation of miRNA expression in primary tumor specimens (Table 5), Vickers and colleagues were the first to investigate the differential miRNA expression between primary tissues and liver metastatic tissues[86]. This study evaluated 9 previously reported metastasis-associated miRNAs (miR-335, 206, -135a, -146a, -146b, -10b, -21, let-7a, let-7b) using 78 samples including 19 paired primary tumors with corresponding liver metastases. The authors demonstrated that a signature consisting of miR-21, -135a, -206, -335, and let-7a in primary tissues had a specificity of 87% and sensitivity of 76% for detecting the presence of metastasis in CRC. Similarly, another recent study performed miRNA-based profiling of 78 tissue specimens (23 normal colonic mucosa, 31 primary cancerous tissues, and 24 liver metastatic tissues) from 46 CRC patients[87]. It was shown that 3 miRNAs, miR-122, miR-146a and miR-210, were dysregulated in metastatic vs. primary cancer tissues.

One of the key molecular steps in the process of distant metastasis includes epithelial-to-mesenchymal transition (EMT) in primary tumor and mesenchymal-to-epithelial transition (MET) in metastasized location[88-91]. Our group recently demonstrated that miR-200c/141 cluster was up-regulated in metastasized tissue via EMT/MET process when we analyzed 54 paired primary CRC tissues and matched liver metastasis specimens[92]. Furthermore, we investigated the expression of miR-200c in serum specimens from these patients and showed that up-regulated miR-200c in metastatic tissues reflected the expression status in serum specimens[93]. High serum miR-200c expression was significantly correlated with lymph node metastasis and distant metastasis, and was an independent predictor of tumor recurrence and an independent prognostic maker for CRC. In view of these results, it is becoming more apparent that miRNAs play a crucial role in the metastatic process in CRC, and several miRNAs could be used as promising clinical tools for the identification of patients with micrometastasis who and will require intensive monitoring following surgery.

However, almost all of the studies published to date have focused on the predictive biomarkers for distant metastasis using resected primary tissues or preoperative serum/plasma samples, but there are no reports for the significance of monitoring biomarkers for metachronous metastasis using large cohort serum/plasma samples. In future, further investigations are required for a careful determination of the clinical significance of already published miRNAs as recurrence-monitoring biomarkers, such as CEA and CA19-9.

MiRNAs as predictive biomarkers for response to treatment

Predicting therapeutic response to chemotherapy in CRC patients—Unlike many other human cancers, the treatment options for advanced CRC patients have improved remarkably due to the development of several novel drugs over the last decade[94]. Traditional chemotherapeutic treatment for CRC has been based on 5-fluorouracil (5FU), with the more recent introduction of other cytotoxic agents, such as irinotecan and oxaliplatin. Furthermore, new monoclonal antibodies targeting the vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) have proven to be effective in combination with cytotoxic chemotherapy, or as single agents for the treatment of mCRC[95-97]. Notwithstanding considerable therapeutic advances, the prognosis for the patients with unresectable CRC still remains poor, with the median overall survival of only 18-21 months[98]. One of the best characterized major obstacles in treating CRC patients with unresectable disease CRC is the intrinsic or acquired resistance to chemotherapy. Understanding the molecular mechanisms of drug response through alterations in miRNA expression patterns could provide crucial additional information on patient's potential response, or the lack thereof, to a specific chemotherapeutic regimen prior to initiation of the therapy.

To date, growing evidence suggests that aberrant miRNA expression could play pivotal role in predicting resistance to chemotherapeutic drugs in CRC patients. Using a series of *in vitro* models for CRC, several studies have thus far demonstrated deregulation of several miRNAs to 5-fluorouracil resistance (miR-10b[99], miR-19b[100], miR-20a[101], miR-21[102-104], miR-23a[105],miR-31[106], miR-34[107,108], miR-129[109], miR-140[110], miR-145[111], miR-192/-215[112], miR-497[113]), irinotecan resistance (miR-21[104], miR-451[114]), as well as resistance to oxaliplatin (miR-20a[101], miR-21[104], miR-133a[115], miR-143[116], miR-153[117], miR-203[118], and miR-1915[119]). In Table 6, we summarized the published data related to the potential of predictive biomarker for chemosensitivity using clinical samples in CRC. In clinical settings, Nakajima et al. were the first to demonstrate the significance of miRNA dysregulation in relation to 5FU-based antimetabolite S-1 chemosensitivity, and identified that overexpression of let-7g and miR-181b in CRC tissues was significantly associated with response to S-1 based chemotherapy in CRC[120]. A recent study conducted more systematic and comprehensive analysis focused on response to chemotherapy by performing real time-PCR based profiling of 742 different miRNAs using the 26 cancer tissues with or without response to first-line capecitabine and oxaliplatin (XELOX)/5FU and oxaliplatin (FOLFOX) treatment[121]. In the screening phase of this study, high expression of miR-27b, miR-181b, and miR-625-3p was significantly associated with poor response to first-line treatment. In the second phase, an independent validation of these results was performed on the primary tumor tissues of 94 mCRC patients, and high expression of miR-625-3p was confirmed to be associated with poor response to XELOX/FOLFOX treatment. Our group also performed the screening of 21 candidate miRNAs, and revealed low-expression of miR-148a in primary tissues associated significantly with worse survival and poor therapeutic response to 5-FU and oxaliplatin based chemotherapy in advanced CRC patients[122].

With regards to the predictive miRNA biomarkers for sensitivity to molecularly targeted therapies, a recent study demonstrated that decreased expression of miR-181a in micro-dissected tumor tissues associated with poor progression free survival (PFS) and overall survival (OS) in mCRC patients receiving EGFR targeted therapy[123]. Published around similar time, Cappuzzo and colleagues conducted a study by analyzing 2-different cohorts of mCRC patients treated with anti-EGFR based therapy and performed miRNA arrays for their discovery step. The authors identified dysregulation of a unique miRNA cluster (let-7c/miR-99c/miR-125b), which influenced the PFS and OS of patients with mCRC treated with cetuximab or panitumumab therapy[124].

Although almost all of miRNA biomarker studies for predicting response to chemotherapy are based on the analysis of archival “primary tumor tissues”, one of the major limitations of this tissue-based approach is heterogeneity, which characterizes most advanced cancers. Intra-tumoral heterogeneity might carry different expression profiles in primary tissues, and heterogeneity even exists between metastases within the same patient, such as inter-metastatic heterogeneity. A biopsy or tissue section from one part of a solitary tumor could not represent the overall molecular characteristics of harboring malignant disease. In addition, due to practical and ethical concerns, it is challenging to obtain repeated biopsies, especially metastatic tumors, during disease progression or prior to initiation of chemotherapy. Due to these issues, it has been difficult to characterize the molecular characteristics of growing tumor cells in patients. Therefore, evaluating miRNA expression from serum/plasma in patients with unresectable CRC may be potentially preferable for predicting response to chemotherapy.

Kjersem and colleagues conducted the first systematic and comprehensive array-based analysis by evaluating the expression of 742 miRNAs in plasma samples from 24 metastatic CRC patients (12 responders and 12 non-responders) before oxaliplatin-based chemotherapy[125]. In the validation step, the top differentially expressed 32 miRNAs between responders and non-responders were selected for further analysis using pre-treatment plasma samples from 150 CRC patients. High expression of miR-27b, miR-148a, and niR-326 in pre-treatment plasma was significantly correlated with response to oxaliplatin-based chemotherapy, and associated with poor progression-free survival. Likewsie, Chen et al. utilized a unique approach for dynamic monitoring of serum miRNA levels (miR-155 -200c, and -210) during a 3-year follow up period with adjuvant FOLFOX therapy with cetuximab in 15 patients with CRC patients, and suggested that re-elevation of serum miR-155 levels after surgery and chemotherapy might be a sign of chemoresistance in colon cancer[126]. Recently, as a non-invasive biomarker for response to chemotherapy, analysis of KRAS or BRAF mutations in cell free DNA in plasma/serum is gathering attention in metastatic CRC patients. In view of these results, it is becoming more apparent that miRNAs could be used as non-invasive biomarkers for response to chemotherapy in advanced CRC patients.

Predicting therapeutic response to chemo-radiotherapy or prognosis after treatment in rectal cancer—With regards to the treatment of advanced rectal cancer, the concept of combined modality therapy, which integrates surgery, chemotherapy, and radiotherapy, has been used for improving the odds for surgical operation and lowering local

recurrence rates considerably over the last decades. In particular, preoperative chemo-radiotherapy (CRT) has become the preferred treatment modality for locally advanced rectal adenocarcinoma with a complete pathological response in up to 30% of patients[127,128]. However, at the same time, combined modality therapy including pelvic radiotherapy for rectal cancer can result in a number of acute and late toxicities. In addition, distant recurrence remains the major cause of mortality in patients with preoperative CRT followed by curative resection. Therefore, there is a clinical need to establish molecular biomarkers to predict response to pre-surgical chemo-radiation and/or to predict recurrence after surgery, which may spare the patients from the adverse toxicity and expense, who are poor candidates for such treatments[129,130].

Alteration of miRNA expression associated with clinical response to preoperative CRT and disease outcome in rectal cancer has also been explored in several studies (Table 7). In one of the first studies, an association of miRNA dysregulation and response to CRT in rectal cancer was demonstrated [131]. In this study, the investigators collected the tumor biopsy specimens from 35 patients with rectal cancer before CRT, and in parallel collected tumor biopsies from 31 patients two weeks after starting preoperative CRT. This study revealed that up-regulation of miR-125b and miR-137 after CRT was associated with worse response to CRT. These researchers later extended this study by doing a comprehensive analysis of differentially expressed miRNAs with or without response to CRT, using two sets of biopsy samples (10 responders and 10 non-responders to neoadjuvant CRT for rectal cancer[132]. The authors identified 8 significantly differentially expressed miRNAs in rectal tumors, and highlighted the potential use of miRNA expression status as a predictive biomarker for response to neoadjuvant CRT in rectal cancer. More recently, another study performed genome-wide miRNA profiling on 12 established CRC cell lines that were made resistant to CRT and confirmed the function of four miRNAs (let-7g, miR-132, miR-224, and miR-320a) as potential miRNA targets for resistance to CRT[133]. During the validation step, 128 pre-therapeutic biopsy specimens were used to analyze correlation between expression levels of these miRNAs and disease free survival (DFS). It was quite reassuring to observe that high expression of let-7g was significantly associated with better DFS in this well conducted study.

One of the limitations of current studies exploring the potential relevance of miRNAs for predicting response to chemo- or chemo-radiation therapies is the lack of specificity for each treatment option. In order to realize the concept of “individualized/personalized treatment” of CRC patients in the near future, there is clearly an unmet need for the availability of drug-specific, regimen-specific, and treatment-specific biomarkers that can help predict the response to treatment specifically (such as 5FU, irinotecan, oxaliplatin, or radiation). Furthermore, validation using large cohort samples in well-designed prospective clinical studies is needed to confirm the significance of already discovered miRNAs as potential predictive biomarkers for therapeutic response in CRC.

Expert Comment and Five-year view: What's next step and where should we go?

At this moment, unequivocal body of literature exists suggesting a strong potential for the utilization of miRNAs as oncological biomarkers in various human cancers, particularly CRC. This is owing to several inherent advantages of using miRNAs as discussed previously, but most importantly, the stability of miRNAs in various clinical specimens remains at the pinnacle of attention for their development as clinically useful biomarkers. In particular, direct identification of circulating miRNAs would be expected as liquid biopsies that can provide information for diagnosis, prognosis and predictive responses to treatment in CRCs without the need for tumor-tissue biopsies. However, the paradigm of developing liquid biopsies remains very optimistic. In order to realize the fruits of efforts that have been put forth to date, we must collectively and collaboratively address several key issues, before any of these biomarkers can be translated for any meaningful clinical use.

One of the major issues we might need to overcome relates to the methodology for measuring miRNA expression levels. To date, as opposed to ‘absolute quantitation’, almost all published studies have evaluated miRNAs levels by qPCR using ‘relative quantitation’, approach by using cycle threshold (Ct) values. Therefore, the development of absolute quantitative method will be critical for inter-laboratory comparisons and standardization, before such assays can be faithfully used in different laboratories in clinical settings. Although several attempts have been made to establish the method of absolute quantification, especially using blood samples, a recent study elegantly demonstrated the absolute quantities of circulating miRNAs in classical Hodgkin lymphoma using copy number, which was calculated on the basis of known copy numbers of cel-miR-39 spiked-in in plasma[134]. This study showed a high correlation between relative and absolute quantification values for all evaluated miRNAs. This intriguing method allows the absolute value in plasma miRNA to be quantified by eliminating the technical bias of RNA extraction, and has the potential to be implemented in diagnostic laboratories. Furthermore, Droplet Digital PCR, a next generation qPCR method, provides absolute quantification of nucleic acids without the requirement of standard curve, and measures copy number changes of DNA and RNA targets with high sensitivity compared to qPCR methods [135,136]. Combining cel-miR-39 spiking approach and new PCR technology should, or might, help quantification of miRNA levels more close to absolute values, and may allow identification of low-abundance miRNA biomarkers in plasma.

Another major limitation of blood-based miRNA biomarkers for clinical use is the disease specificity. The majority of diagnostic miRNAs identified in CRC have also been recognized to be altered in other types of human cancers, and non-cancerous diseases. For example, miR-92a, which was the firstly reported miRNA as a plasma-based diagnostic marker in CRC, revealed the feasibility of exploiting plasma-based early detection biomarkers in breast cancer and coronary artery disease[137,138]. Recently, several investigators have made an attempt to address this limitation and provided some clues for overcoming this limitation. First major break-through came from studies focusing on the tumor-secreted miRNAs for diagnostic non-invasive biomarkers in CRC, in which the

investigators made direct comparison between tumor tissue and blood expression status to clarify the interaction and origin of circulating miRNAs. Our research group demonstrated for the first time that the miR-21 up-regulation in CRC tissues corresponded with serum levels in large cohort samples, and showed that miR-21 was secreted from CRC cancer cells and confirmed this finding with positive correlation of miR-21 expression between tissue and serum[49]. Ng and colleagues also performed real-time-PCR based miRNA profiling using plasma, corresponding cancer and adjacent normal mucosal tissues from CRC patients, along with plasma from five healthy volunteers in their discovery step[47]. Such an approach aimed at evaluating tissue and matching blood specimens could clarify the correlation of expression status between different types of matched tissue specimens, and may further assist in addressing the issue of tumor specificity in future. Second major breakthrough came with advances in next generation sequencing technologies. Previous studies heavily relied upon the use of microarray-based technologies which often do not include the complete list of growing miRNAs, and hybridization-based non-quantitative techniques can yield data that may fail subsequent validation steps[47,139,140]. Advent of sequencing technologies, such as next-generation sequencing (NGS) for miRNA profiling in biomarker research, now allows the measurement of absolute abundance over dynamic range which was otherwise not possible using conventional microarray technology in the past. In addition, the use of NSG will permit identification of novel miRNAs, miRNA sequence changes, and miRNA variants such as isomiRs[141]. To date, isomiRs are explained to be derived from variable cleavage of Drosha and Dicer through pre-miRNA processing and they differ from each other only by a few nucleotides at the 5- and 3-ends[142-145]. However, more recent evidence suggests that these isomiRs have different physiological roles[146]. Furthermore, it has been suggested that the there is a tissue-dependent preference for isomiRs[143]. Collectively, these new emerging functional roles of isomiRs and the new profiling technologies will allow potential discovery of novel cancer-specific biomarkers. Final potential breakthrough comes from the concept of tumor-derived exosomes. Although previous studies suggested the feasibility of exosomal miRNAs as non-invasive biomarkers in oncology, circulating exosomes can also originate from other organs, and can compound lack of specificity of a given miRNA biomarker. However, Taylor and colleagues have recently proposed an elegant method for isolating circulating exosomes through modification of magnetic activated cell sorting procedure using anti-epithelial cell adhesion molecule (EpCAM), and revealed that the miRNA signature obtained from these tumor-derived (epithelia-derived) exosomes parallels that of the miRNA expression profiles of corresponding tumor tissues in ovarian cancer and lung cancer[147]. Such unique approaches for capturing exosomal miRNAs may be another pivotal step that will assist in enhancing the disease specificity of newly biomarkers in future.

Additional major limitations of miRNA biomarker studies for clinical use is individual variability in the expression of these molecules, due to various confounding factors associated with lifestyle factors. Although recent studies have suggested the potential for influencing miRNA expression profiles as a function of age, sex, and race, there is lack of robust data on the expression patterns of miRNAs as it relates to various life-style associated factors, such as dietary factors, smoking, and alcohol consumption – all of which may have profound effect in modu at ing miRNA expressio n, particularly in the body fluids[148-150].

To realize the actual clinical use of miRNA biomarkers in CRC treatment, further investigations are needed to clarify the effect of these factors on the miRNA expression patterns in body fluids.

Finally, MiRNA research has expanded remarkably over the past few years and accumulating evidence suggest the potential use of miRNA as oncological biomarkers in CRC. Although these results hold tremendous promise, majority of the studies have been based on the analysis of clinical tissues from retrospective patient cohorts and lack of external validation. Therefore, prospective, multicenter, large patient cohort studies are required to prove the clinical significance and promise miRNAs currently hold as diagnostic, prognostic and predictive biomarkers for CRC. Although none of us have a crystal ball to predict what an ideal “cancer assay” will look in the future, but it is logical and very likely that no single biomarker may be sufficient to capture the disease heterogeneity that underlies most CRCs. As a result, it is possible that for clinical purposes, we may end up using a panel of miRNAs rather than a single miRNA, or even combine different types of biomarkers or other available tests, such as CEA, and FOBT – all in an effort to enhance the sensitivity and specificity of these analytical approaches, and making an earnest effort to providing a true “personalized treatment” to every cancer patient in the future.

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Papers of particular interest, published recently, have been highlighted as:
*** of interest, or**

**** of considerable interest.**

****23 Shetter AJ, et al. *JAMA* 2008; 299(4): 425-36.**

This research group performed the first largest study analyzing miRNA profiles in colon cancer tissues using independent two cohorts, and demonstrated the systematic differences in miRNA expression patterns between colon tumors and paired non-tumor tissues.

****25 Ng EK, et al. *Gut* 2009; 58(10): 1375-81.**

This study showed the first comprehensive analysis in the field of blood-based miRNA biomarkers in CRC., and suggested the feasibility of plasma miRNAs as diagnostic markers in CRC.

***58 Toiyama Y, et al. *J Natl Cancer Inst* 2013; 105(12): 849-59.**

This study is the first miRNA biomarker study that used a large cohort of primary tissues and matching serum samples, and performed the direct comparison between tumor tissue and serum expression status to highlight the specificity of non-invasive detection of these non-coding RNAs in blood.

***71 Koga Y, et al. *Cancer Prev Res (Phila)* 2010; 3(11): 1435-42.**

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Key issues

- MicroRNAs (miRNAs) are a class of small, single stranded, noncoding RNAs (ncRNAs), and ever since their initial discovery in 1993, enough evidence has been gathered that implicates their role in cancer pathogenesis due to their ability to post-transcriptionally regulate the expression of various oncogenes and tumor suppressor genes.
- Dysregulated expression of many miRNAs regulates the expression of hundreds of growth regulatory genes and pathways that are critical in the multistep model of colorectal carcinogenesis, and other human cancers.
- Since the recognition that miRNA expression is stable, immune to RNase-mediated degradation, has allowed exploration of these molecular entities in a variety of biological fluids including archival tissues for biomarker studies.
- Early diagnosis, including detection of premalignant adenomas, is considered as a key concept for improving patient survival in CRC treatment. Emerging evidence suggests promising potential of miRNAs (miR-21, miR-92a, miR-106a, etc.) as potential non-invasive biomarkers for non-invasive screening in blood (plasma/serum) and fecal specimens.
- In stage II CRCs, surgical resection is highly effective; hence treating all patients with stage II CRC with adjuvant chemotherapy is still debatable. However a significant proportion of Stage II CRC patients develop recurrence and the identification of sensitive miRNA biomarkers in such high-risk populations is of key importance.
- Early detection of distant metastasis and selective criteria regarding which individuals would benefit most from invasive treatments is essential for improving long term survival. Several lines of research suggests that miRNAs are intimately involved in the metastatic process in CRC and some of these miRNAs could be used as promising clinical tools for identifying patients with micro-metastasis who will require intensive monitoring after surgery.
- Despite the progress of treatment options for advanced CRC during last decade, major obstacle is intrinsic and acquired resistance to chemotherapy and/or radiotherapy, necessitating the need to develop predictive biomarkers for response to such therapeutic treatments to facilitate personalized treatment of CRC patients. Several studies have identified alterations in miRNA expression (miR-21, miR-145, miR-148, etc.) which could provide crucial additional information on patient's sensitivity to chemotherapy before starting the therapy, and in supporting development of novel therapeutic strategies for enhancing the sensitivity to chemotherapeutic treatments.
- Although miRNA biomarker studies have grown dramatically in numbers in the last few years, future prospective studies are essential in addressing limitations

of retrospective trials, as we usher into the next era of miRNA biomarkers that are optimal for clinical practice.

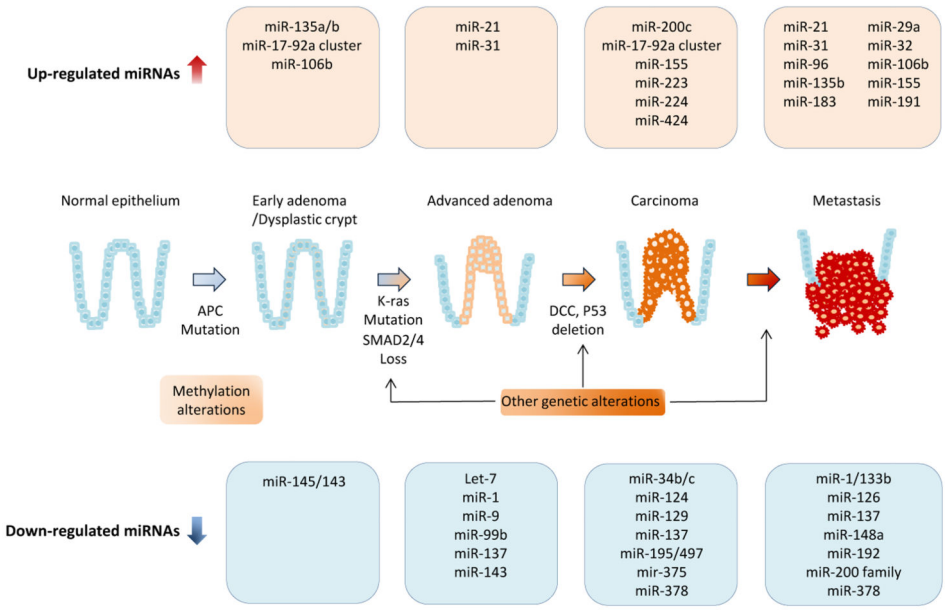

Table 1
A comprehensive list of dysregulated miRNAs in primary colorectal cancer tissues

miRNA	Target gene	Chromosomal location	Reference
Up-regulated miRNA			
miR-16	BMI1, WIP1, CDK6	13q14.2/3q25.33	[151,152]
miR-17	PTEN, DLC1, ZBP1, p130	13q 31.3	[47,84,153,154]
miR-18a	Dicer1, KRAS, PTEN, CTGF, ATM	13q 31.3	[155,156]
miR-20a	PTEN, RUNX1,E2F1,HIF1A	13q 31.3	[25,47,84,153,157]
miR-21	PTEN, PDCD, RECK, JAG1, BCL2, TIMP3, DKK2	17q 23.1	[31,49,84,158-160]
miR-29a	KLF4, DNMT3a, DNMT3b	7q 32.3	[153,161,162]
miR-31	FIH1, RhoBTB1, RASA1	9q 21.3	[49,153,163-165]
miR-32	PTEN	9q 31.3	[19,166-168]
miR-33	Pim1, p53, HMGA2	22q 13.2	[19,168]
miR-92a	PTEN, P63, RECK, DUSP10, ITGA5	13q 31.3/Xq 26.2	[31,47,84,153,169,170]
miR-95	SNX1, SGPP1	4p16.1	[31,47,153,162,171]
miR-96	FOXO1, RECK, RAD51, REV1	7q 32.2	[25,47,162,168,172,173]
miR-106a	PTEN, TIMP2, PDCD4, p130, RUNX3	Xq 26.2	[19,31,47,162]
miR-106b	APC, RBL1, RBL2, CASP8	7q 22.1	[47,84,162]
miR-130b	PPARγ, ITGB1, PTEN	22q 11.21	[162,174-176]
miR-135a	APC, MCL1, c-MYC, HOXA10	3q 21.1	[31,34]
miR-135b	APC, MTSS1, LATS2, β-TrCP, LZTS1	1q 32.1	[25,34,47,173]
miR-155	MLH1, MSH2, MSH6, APC, TP53, INP1	21q 21.3	[177,178]
miR-181b	CBX7, CYLD, AC9	9q 33.3	[31,84,162,179,180]
miR-182	NDRG1, RECK, MTSS1, SMAD4	7q 32.2	[153,168,181,182]
miR-183	IDH2, PDCD4, Dkk3, SMAD4	7q 32.2	[25,84,153,168,183]
miR-191	TIMP3	3p 21.31	[19,180,184]
miR-196b	HOXC8, HOXB7	7p 15.2	[153,185]
miR-200c	ZEB1, ZEB2, BMI1	12p 13.31	[177,180]
miR-203	ZEB2, BMI1, SOCS3, VEGFA	14q 32.33	[31,84,162,186]
miR-223	E2F1, FOXO1, EPB41L3	Xq 12	[31,47,84,186,187]
miR-224	SMAD4, CXR4, PHLPP1, PHLPP2	Xq 23	[47,153,162,168,188]
miR-335	RB1, ROCK1	7q 32.2	[31,86,185]
miR-493	IGF1R	14q 32.2	[153,173]
miR-584	ROCK1	5q 32	[168,173,187]
	Down-regulated miRNA		
let-7a	KRAS, NRAS	9q22.32/11q24.1/22q13.31	[186,187]
miR-1	BDNF, MET, SLUG, CCND2, SDF1	18q 11.2/20q 13.33	[31,50,162,168,173,187,189]
miR-7	YY1, XRCC2	9q 21.32	[190,191]
miR-9	CXCR4, BRCA1, TLN1, ITGB1	1q22/5q14.3/15q26.1	[168,192]
miR-30a	DTL, HIF2α	6q 13	[25,31,162,168]
miR-30b	SIX, KRAS,PIK3D, BCL21	8q 24.22	[187,193,194]
miR-30c	BCL9, SNAI1	1q 34.2/6q 13	[162,187]

miRNA	Target gene	Chromosomal location	Reference
miR-34b/c	BTG4, MET, CDK4, CDK6, RUNX2	11q 23.1/	[31,195,196]
miR-101	EZH2, COX2, TET2	9q 24.1	[26,187,197]
miR-124a	SLUG, STAT3, EZH2, IL6R	8p23.1/8q12.3/20q13.33	[25,198,199]
miR-126	CXCR4, IRS-1, VEGF	9q34.3	[200-204]
miR-129	BCL2, CDK6, SOX4	7q32.1/11p11.2	[25,109]
miR-133a	LASP1, RFFL, FSCN1	18q 11.2/20q 13.33	[50,115,162,168,205]
miR-133b	CXCR4, MET	6p12.2	[25,189,206]
miR-137	LSD1, FMNL2, CDC42, CDK6	1p 21.3	[168,207]
miR-139	IGF1R, NR5A2	11q 13.4	[162,168,173,208]
miR-143	KRAS, DNMT3A, TLR2, MACC1	5q 32	[84,162,186,187,209]
miR-145	MUC1, KLF4, Fascin1, SOC2, YES1	5q 32	[25,84,162,173,187,210]
miR-148a	BCL2, MMP7, ROCK1	7p 15.2	[122,187,211]
miR-192	BCL2, ZEB2, VEGFA	11q 13.1	[31,186,187,212]
miR-195	BCL2, IKKα, TAB3, E2F3	17p 13.1	[162,173,187,213,214]
miR-212	MnSOD	17p 13.3	[26,215]
miR-215	RB1	1q 41	[31,84,175,186,187,212,216]
miR-218	BMI1, CDK6, DKK2	4p 15.31/5q 34	[217,218]
miR-320a	Rac1, β-catenin, NRP1	8p 21.3	[84,219,220]
miR-342	DNMT1, ID4, BMP7	14q 32.2	[31,187,221]
miR-363	GATA6, SIPR1, PDPN	Xq 26.2	[168,173]
miR-375	PI3K, PDK1, AEG1, IGF1R, YAP	2q 35	[168,187,216,222,223]
miR-378	IGF1R, MYC, VEGFA	5q 32	[168,173,187,216,224]
miR-497	IGF1R, BCL2, RAF1	17p 13.1	[113,162]

Table 2

A comprehensive list of dysregulated miRNAs in colonic adenoma tissues

miRNA	Cases (n)	Controls (n)	Analysis Method	Reference
Up-regulated miRNA				
miR-21	18	18	qPCR	[31]
miR-135a, -135b	20	18	qPCR	[34]
miR-21	-	-	ISH	[159]
miR-17-92a cluster	30	10	qPCR	[28]
miR-21, -181b	19 (SSA)	20	qPCR	[160]
miR-92a	-	-	copy number	[30]
miR-31, -135b	41	55	microarray	[33]
miR-1290	-	-	ISH	[225]
Down-regulated miRNA				
miR-143, -145	65	65	qPCR	[226]
miR-1,-9, -99b, -137	41	55	microarray	[33]
miR-150	-	-	qPCR	[227]

SSA; sessile serrated adenoma, qPCR; quantitative polymerase chain reaction, ISH; in situ hybridization

Table 3

List of Diagnostic miRNA marker using blood or fecal specimen, which reported in Colonic adenoma and CRC

miRNA	Cases (n)	Controls (n)	Adenoma/CRC	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Specimen Type	Dysregulation	Reference
Blood-based biomarker									
miR-17-3p	90	50	CRC	64	70	0.72 (0.63-0.80)	plasma	Up-regulated	[47]
miR-92a	90	50	CRC	89	70	0.89 (0.83-0.94)	plasma	Up-regulated	[47]
miR-29a	100	59	CRC	69	89.1	0.84 (0.79-0.90)	plasma	Up-regulated	[48]
	37	59	Adenoma	62.2	84.7	0.77 (0.67-0.87)	plasma	Up-regulated	
miR-92a	100	59	CRC	84	71.2	0.84 (0.78-0.90)	plasma	Up-regulated	[48]
	37	59	Adenoma	64.9	81.4	0.75 (0.64-0.86)	plasma	Up-regulated	
miR-221	103	37	CRC	86	41	0.61 (0.49-0.72)	plasma	Up-regulated	[228]
miR-21	20	20	CRC	90	90	0.91	plasma	Up-regulated	[50]
miR-601	90	58	CRC	69.2	72.4	0.75 (0.67-0.83)	plasma	Down-regulated	[229]
	43	58	Adenoma	72.1	51.7	0.64 (0.53-0.75)	plasma	Down-regulated	
miR-760	90	58	CRC	80.0	72.4	0.79(0.71-0.86)	plasma	Down-regulated	[229]
	43	58	Adenoma	69.8	62.1	0.68 (0.58-0.79)	plasma	Down-regulated	
miR-7, -93, -409-3p (panel)	22	27	CRC	82	89	0.90(0.75-0.95)	plasma	-	[230]
miR-19a, -19b, -15b (panel)	42	53	CRC	78.6	79.3	0.84 (0.76-0.92)	plasma	Up-regulated	[139]
miR-21	186	53	CRC	91.9	81.1	0.92 (0.87-0.96)	Serum	Up-regulated	[49]
	43	53	Adenoma	81.1	76.7	0.81(0.69-0.91)	Serum	Up-regulated	
miR-21	200	80	CRC	-	-	0.8	Serum	Up-regulated	[51]
	50	80	Adenoma	-	-	0.71	Serum	Up-regulated	
miR-92a	200	80	CRC	-	-	0.77	Serum	Up-regulated	[51]
	50	80	Adenoma	-	-	0.7	Serum	Up-regulated	
Feces-based biomarker									
miR-17-92 cluster	197	119	CRC	69.5	81.5	-	Fecal colonocyte	Up-regulated	[62]
miR-135	197	119	CRC	46.2	95	-	Fecal colonocyte	Up-regulated	[62]
miR-21	19	10	CRN (CRC+adenoma)	-	-	-	Feces	Up-regulated	[61]
miR-106a	19	10	CRN (CRC+adenoma)	-	-	-	Feces	Up-regulated	[61]
miR-144*	35	40	CRC	74.3	87.2	0.83	Feces	Up-regulated	[231]
miR-21	88	101	CRC	55.7	73.3	0.64	Feces	Up-regulated	[64]

miRNA	Cases (n)	Controls (n)	Adenoma/CRC	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Specimen Type	Dysregulation	Reference
miR-92a	88	101	CRC	71.6	73.3	0.78	Feces	Up-regulated	[64]
miR-143	38	13	CRC	-	-	-	Feces	Down-regulated	[232]
miR-145	38	13	CRC	-	-	-	Feces	Down-regulated	[232]
miR-106a	107	117	CRC	34.2	97.2	-	Fecal colonocyte	Up-regulated	[63]

Table 4

List of prognostic markers responsible for poor outcome in stage II CRC patients

miRNA	N	Specimen Type	Dysregulation	Analysis Method	Reference
Tissue-based biomarker					
miR-320	37 (MSS)	Tissue	Down-regulated	qPCR	[26]
miR-498	37 (MSS)	Tissue	Down-regulated	qPCR	[26]
miR-21	42	Tissue	Up-regulated	qPCR	[31,82]
miR-21	197 (Colon:130, Rectum:67)	Tissue (stroma)	Up-regulated	ISH	[233]
miR-29a	59	Tissue	Down-regulated	qPCR	[234]
miR-21	764	Tissue	Up-regulated	ISH	[83]
miR-203	46 (African American)	Tissue	Up-regulated	qPCR	[235]
miR-20a, -21, -103a -106b, -143, -215	138	Tissue	Up-regulated	Microarray, qPCR	[84]
miR-21	145	Tissue	Up-regulated	qPCR	[158]
Blood-based biomarker					
miR-200c	59	Serum	Up-regulated	qPCR	[93]

MSS; Microsatellite stable, qPCR; quantitative polymerase chain reaction, ISH; in situ hybridization

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Table 5

List of distant metastasis associated miRNAs in CRC

miRNA	N	Specimen Type	Dysregulated	Type of predictive metastasis	Reference
Tissue-based biomarker					
miR-21	156	Tissues (primary)	Up-regulated in primary tissue	Hepatic metastasis	[178]
miR-125b*, - 139-3p, -150*, 1179	-	Tissues (primary)	Up-regulated in primary tissue	Hepatic metastasis	[236]
let-7a, miR-21, -135a, -335	38	Tissues (primary, metastasis)	Up regulated in metastatic tissue	Hepatic metastasis	[86]
miR-206	38	Tissues (primary, metastasis)	Down regulated in metastatic tissue	Hepatic metastasis	[86]
miR-372	144	Tissues (primary)	Up-regulated in primary tissue	Hepatic metastasis	[237]
miR-22	86	Tissues (primary)	Down-regulated in primary tissue	Hepatic metastasis	[238]
miR-200c, -141	108	Tissues (primary, metastasis)	Up-regulated in metastatic tissue	Hepatic metastasis	[92]
miR-96, -135b	52	Tissue (primary)	Up-regulated in primary tissue	Hepatic metastasis	[172]
miR-144	137	Tissue (primary)	Down-regulated in primary tissue	Hepatic metastasis (Metachronous)	[239]
miR-199a-3p	92	Tissue (primary) Tissues (primary,	Up-regulated in primary tissue	Hepatic metastasis	[240]
miR-122	12	metastasis)	Up regulated in metastatic tissue	Hepatic metastasis	[241]
miR-625	96	Tissue (primary)	Down-regulated in primary tissue	Hepatic metastasis	[242]
miR-146, -201	55	Tissues (primary, metastasis)	Up-/Down-regulated in metastatic tissue	Hepatic metastasis	[87]
miR-191	136	Tissue (primary)	Up-regulated in primary tissue	Hepatic metastasis	[243]
miR-340	19	Bone Marrow DTC	Down-regulated in Bone Marrow DTC	Hepatic metastasis	[244]
miR-214	99	Tissue (primary)	Down-regulated in primary tissue	Hepatic metastasis	[245]
miR-185	50	Tissues (primary)	Up-regulated in primary tissue	Distant metastasis	[246]
miR-133b	50	Tissues (primary)	Down-regulated in primary tissue	Distant metastasis	[246]
miR-9	25	Tissues (primary)	Up-regulated in primary tissue	Distant metastasis	[247]
miR-139	35	Tissue (primary)	Down-regulated in primary tissue	Distant metastasis	[86,208]
miR-103, 107	99	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[86,248]
miR-92a	82	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[170,238]
miR-137	102	Tissue (primary)	Down-regulated in primary tissue	Distant metastasis	[92,249]
miR-155	-	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[170,250]
miR-32	35	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[167,172]
miR-183	94	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[183,239]
miR-138	187	Tissue (primary)	Down-regulated in primary tissue	Distant metastasis	[249,251]

miRNA	N	Specimen Type	Dysregulated	Type of predictive metastasis	Reference
miR-29a	85	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[161,240]
miR-25	186	Tissue (primary)	Up-regulated in primary tissue	Distant metastasis	[241,252]
miR-126	92	Tissue (primary)	Down-regulated in primary tissue	Distant metastasis	[200,251]
Blood-based biomarker					
miR-21, 141	102	Plasma	Up-regulated	Distant metastasis	[244,253]
miR-29a	40	Serum	Up-regulated	Hepatic metastasis	[200,254]
miR-21	186	Serum	Up-regulated	Distant metastasis	[49,245]
miR-200c	182	Serum	Up-regulated	Distant metastasis	[93]
miR-155, -200c, -210	15	Serum	Up-regulated	Distant metastasis/Local recurrence	[126,253]

DTC; Disseminated tumor cell

Table 6

List of miRNA biomarker for resistance to chemotherapy in CRC

miRNA	N (Stage)	Specimen	Treatment	Neoadjuvant/Adjuvant/ Palliative	Dysregulation and response/prognosis	Reference
Tissue-based biomarker						
let-7g, miR-181b	46	Tissue	S-1 +/- CDDP	Palliative	Up-regulated in poor response	[120]
miR-21	56(II/III)	Tissue (primary)	5FU-based chemotherapy	Adjuvant	Up-regulated in poor prognosis	[31]
miR-150	352 (II/III)	Tissue (primary)	5FU +/- LV/levamisole/CDDP	Adjuvant	Down-regulated in poor prognosis	[227]
miR-21	42 (II/III)	Tissue (primary)	FOLFOX4	Neoadjuvant	Up-regulated in poor response	[255]
miR-126	89(IV)	Tissue (primary)	XELOX	Palliative	Down-regulated in poor response	[121]
miR-148a	273 (II/III/IV)	Tissue (primary)	5FU/5FU+oxaliplatin	Adjuvant/Palliative	Down-regulated in poor prognosis	[122]
miR-625-3p	94 (IV)	Tissue (primary)	(XELOX or FOLFOX)	Palliative	Up-regulated in poor response/prognosis	[121]
miR-215	125(II/III)	Tissue (primary)	5FU-based chemotherapy	Adjuvant	Down-regulated in poor prognosis	[256]
miR-21	301(II/III/IV)	Tissue (primary)	5FU-based chemotherapy	Adjuvant/Palliative	Up-regulated in poor prognosis	[158]
miR-200b, -200c, -141, -429	127(I/II/III)	Tissue (primary)	Fluoropyrimidines	Adjuvant	Down-regulated in poor prognosis	[257]
miR-181a	80(IV)	Tissue (primary)	anti-EGFR	Palliative	Down-regulated in poor prognosis	[123]
let-7c, miR-99a, -125b	183(IV)	Tissue (primary)	anti-EGFR	Palliative	Down-regulated in poor prognosis	[124]
Blood-based biomarker						
miR-106a, -130b, -484, -148, -27b, -326	150 (IV)	Plasma	5FU+Oxaliplatin	Adjuvant	Up-regulated in poor response or prognosis	[125]
miR-20a, -130, -145,-216, -372	253	Plasma	-	-	-	[258]
miR-19a	72 (IV)	Serum	FOLFOX	Palliative	Up-regulated in poor response	[259]

5FU;5-fluorouracil, CDDP; cisplatin, LV; leucovorin, FOLFOX; 5FU/LV/oxaliplatin, XELOX; capecitabine/oxaliplatin, EGFR; epidermal growth factor receptor

Table 7

List of miRNA biomarker for response to chemoradiotherapy in Rectal cancer

miRNA	N (Stage)	Neoadjuvant Treatment (Chemotherapy/Radiation)	Specimen Type	Dysregulation	Primary Endpoint	Reference
miR-125b, -137	35(II/III)	Capecitabine 50.4Gy	Pre- and post-therapeutic Tissue	Up-regulated expression change after treatment in poor response	Response	[131]
miR-145	40	5FU 50.4Gy	Pre- and post-therapeutic Tissue	Down-regulated of post-treatment tissue in poor response	Response	[260]
miR-215,-190, -29b-2 let-7e, miR-196b, -450a, -450b-5p, -99a	20 (II/III)	5FU or Capecitabine 50.6 Gy	Pre-therapeutic Tissue	Up-regulated in poor response Down-regulated in poor response	Response	[132]
miR-1183, -483-5p, -622, -125a-3p, -1224-5p, -188-5p, -1471, -671-5p, -1909*, -630, -765	38(III)	Capecitabine + l-OHP	Pre-therapeutic Tissue	Up-regulated in poor response	Response	[261]
miR-1274b, -720		45 Gy		Down-regulated in poor response		
Let-7g	128 (II-IV)	5FU with/without l-OHP 50.4 Gy	Pre-therapeutic Tissue	Down-regulated in poor prognosis	prognosis (DFS)	[133]
miR-16,-153, -519c, -561,-590	12	-	Pre-therapeutic Tissue	-	Response	[262]

5FU;5-fluorouracil, l-OHP; oxaliplatin, DFS: Disease Free Survival