MicroRNAs potential utility in colon cancer: Early detection, prognosis, and chemosensitivity

Michael Hollis, Kavitha Nair, Arpita Vyas, Lakshmi Shankar Chaturvedi, Sahil Gambhir, Dinesh Vyas

Abstract
Over the past decade, research has shown that aberrant expression of microRNA (miRNA) is involved in colorectal cancer development and progression. MicroRNAs are small sequences of non-coding RNA that regulate expression of genes involved in important cellular functions, such as cell differentiation, multiplication, and apoptosis. A specific miRNA may display the effects of a tumor suppressor or oncogene. Altered miRNA expression is found in colorectal cancer (CRC) and patterns of miRNA expression correlate with CRC detection and outcome. Studies also have examined the use of circulating serum miRNA and fecal miRNA expression as non-invasive markers for early detection. Here, we review recent evidence demonstrating the potential role of miRNA in CRC and the implications of its use in the diagnosis, prognosis, and management of CRC.

Key words: Colorectal cancer; MicroRNA; Expression; Serum microRNA; Fecal microRNA; Diagnostic; Prognostic; Therapeutic

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Core tip: Specific microRNA (miRNA) have potential to display the effects of a tumor suppressor or oncogene. Altered miRNA expression is found in colon cancer and patterns of miRNA expression correlate with its detection and outcome.

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INTRODUCTION

Colorectal cancer (CRC) is currently ranked third in the world among the most common cancers in females and second in men[1]. Each year, approximately more than one million people acquire CRC and over half a million people die from CRC[2,3]. The incidence varies greatly worldwide, with differences dependent on lifestyle, environment, and genetics. Epidemiological studies have identified many risk factors for the development of CRC which include age, family history, inflammatory bowel disease, and preventable risk factors such as obesity, excess alcohol, excess red meat and processed meat consumption[4], high-fat diet, cigarette smoke, low socioeconomic status[5], and sedentary lifestyle[6].

The etiology of CRC is most commonly sporadic, but may also be hereditary as in familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPPC). Regardless, the development of CRC involves genetic mutations leading to the progression of normal epithelial cells of the intestinal mucosa from adenoma to carcinoma. Due to this well-established sequential transformation, there are multiple opportunities to interfere with the natural course of the disease. This may take the form of screening, chemoprevention, chemotherapy, surgical resection, or palliative therapy.

Prognosis depends on the cancer stage at the time of diagnosis. The Tumor Node Metastases (TNM) staging system of the American Joint Committee on Cancer/Union for International Cancer Control is the preferred staging system for CRC. Detection of early stage CRC may confer a 90% 5-year survival rate, compared to 12% if distant metastasis has occurred[7,8]. Given that symptoms are often not obvious, detection of CRC relies heavily on screening[9].

Over the past two decades, the introduction of screening programs such as endoscopy, fecal occult blood testing (FOBT), and barium enema have led to improved early detection of CRC, which has shown a reduction in CRC incidence and mortality in many countries[10]. Unfortunately, less than 40% of CRC patients are identified early enough in the disease when management is most efficacious[11]. In addition, adherence to such screening programs is insufficient at nearly 50% in high-risk patients[11]. Endoscopy is invasive and expensive, whereas the less invasive and less expensive FOBT had a sensitivity of only 47%-73% in case control studies[10,12]. Thus, there is a strong necessity for the development of accurate and non-invasive markers for the diagnosis and prognosis of CRC.

Recent studies have implicated microRNA (miRNA) as serving a key part in the CRC progression and outcome. This article focuses on the utility of miRNA in the diagnosis and prognosis of CRC, as well as its role in predicting sensitivity to chemotherapy.

MICRO-RNA

In 1993, the first miRNA known as lin-4 was identified in C. elegans[13,14]. According to a searchable online database, 2588 mature human miRNAs have since been found in humans[15]. miRNAs are small (18-25 nucleotides) RNA polymers, which are expressed as pre-miRNA and enzymatically cleaved by the protein Dicer into the mature form of miRNA[16]. They are estimated to regulate the expression of over 30% of human genes by directly engaging with and influencing the transcription of mRNA molecules, ultimately regulating protein translation[17]. An miRNA can accomplish this through integration with a RNA-induced silencing complex (RISC) and subsequently binding with a target mRNA molecule. The mechanisms by which the miRNA affects the processing of mRNA most commonly involve binding to the mRNA, resulting in either miRNA degradation or inhibition of its translation. miRNA transcripts are degraded if exact base-pairing occurs between miRNA and mRNA and silenced if the base-pairing is imperfect. Other mechanisms include directly binding to DNA open reading frames, epigenetic modifications such as methylation, and targeting mRNA binding proteins. A single miRNA can act on many mRNAs, while a single mRNA can be acted on by multiple miRNAs[18].

Effect of the miRNA is dependent on the function of the mRNA with which it interacts. Therefore, elevated activities of miRNAs that reduce tumor suppressor expression facilitate cell proliferation and are known as oncomiRs. Depressed activity of miRNAs functioning as tumor suppressors would also promote oncogenesis, and are known as tsmiRs. Other miRNAs regulate cell migration and invasion, and promote metastasis. MicroRNA genes are also often located within regions of the genome that are especially susceptible to loss of heterozygosity and amplification, at fragile sites, or other areas associated with genetic mutations. Since miRNAs are known to regulate cell differentiation, apoptosis, and proliferations[19], alterations in their expression contribute to human disease, such as colorectal cancer.

MICRO-RNA AND EARLY DETECTION

Successful treatment is best achieved when CRC is detected at its earliest stage. Specifically, detecting CRC and surgically resecting it before it has metastasized is considered the only curative therapy. The most widely used methods for diagnosing early CRC are fecal occult blood tests (FOBT) and colonoscopy. Although these tests have improved the survival rates for CRC, FOBT has a low sensitivity and colonoscopy is both expensive and invasive. In addition, certain foods and medications may lead to false-positive results of FOBT. In recent years, many studies have shown a relationship between miRNA expression and CRC.
Much of this work has suggested that miRNA may serve as a reliable, non-invasive biomarker with high sensitivity for early detection of CRC (Table 1).

Colonicocytes of the gastrointestinal tract are continuously released into the intestinal lumen and can be evaluated in the feces. The first study to identify miRNA in feces showed that fecal samples from CRC patients had elevated miRNA. This data demonstrated a significant correlation with the histopathology of the patients' tumor tissue samples[20]. A recent study by Ahmed et al[20] studied tumor tissue samples from different CRC mouse models and discovered that miR-135 is involved in a complex feedback loop. Mutations of molecular pathways commonly found in CRC involving APC and PTEN/P13K facilitate the overexpression of miR-135b, which itself promotes tumor initiation and progression. Another study showed that miR-135b was elevated in CRC and adenomatous tissue samples in contrast to adjacent tissue without evidence of lesions. Stool samples demonstrated a trend of increasing miR-135b across the adenoma to carcinoma sequence compared to inflammatory bowel disease patients and healthy controls. Stool miR-135b was also shown to drop significantly after surgical resection of the CRC or advanced adenoma. The sensitivity of fecal miR-135b was 78% in CRC, 73% in advanced adenoma, and 65% in any adenoma; the specificity was found to be 68%[21].

Koga et al[22] found that fecal miR-106a was also of value in improving the sensitivity of FOBT screening. The authors extracted fecal RNA from the residuum of the FOBT to analyze for potential miRNA markers. They showed that the sensitivity and specificity of fecal miR-106a was 34.2% and 97.2% compared to FOBT with 60.7% and 98.1%, respectively. Importantly, the addition of fecal miR-106a analysis to the FOBT results demonstrated a sensitivity and specificity of 70.9% and 96.3%, respectively. One quarter of the CRC patients with a false-negative were found to be a true-positive with the addition of the fecal miR-106a analysis, greatly enhancing the sensitivity of the screening test. The authors' use of the FOBT residuum was validated by their earlier study which found no significant difference in the quality or quantity of the miR-106a extracted from FOBT residuum after 5 d if stored at 4 degrees Celsius[23].

The most utilized CRC serum marker is currently carcinoembryonic antigen (CEA), but it has also been found to be elevated in other non-cancerous conditions such as inflammation of the intestines, liver, lung, and pancreas[24]. miRNAs are found circulating in both serum and plasma. They are packaged into microvesicles and exosomes, thus resistant to degradation by RNase[25]. They are also stable in a variety of other conditions such as low/high pH, freeze-thaw cycles, boiling, and long-term storage[26].

In many recent studies, specific plasma miRNAs were found to be either up- or down-regulated in patients with CRC versus controls and also discovered their utility for detecting patients with CRC and adenoma. Kanaan et al[27] identified a group of 8 plasma miRNAs and a group of 3 plasma miRNAs, which could precisely distinguish between patients with colorectal adenoma and patients without colorectal neoplasia. Their panels were also able to distinguish between patients with colorectal adenomas from all stages of CRC. Yong et al[28] also identified a group of 3 plasma miRNAs whose levels were elevated in patients with CRC and were significantly correlated with their level of expression within their respective CRC tissue samples. They also illustrated an increasing trend of plasma levels from the early to late stages of CRC in comparison to control patients. Furthermore, as a biomarker for CRC detection, this triple miRNA panel performed with a sensitivity of 80% and a specificity of 84.4%. Another 20 miRNAs were either up-regulated or down-regulated, in which changes in their serum levels reliably differentiated between patients with stage IV CRC and controls. A follow-up study led to the development of a partial least squares regression model, which was able to correctly assign patients as stages I or II based on the

| miRNA and early detection | Summary                                                                 |
|--------------------------|--------------------------------------------------------------------------|
| miR-135b                 | New article[12,23] Tumor initiation, progression                         |
| miR-106a                 | [24,25] Improves sensitivity of FOBT                                     |
| miR-431                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-15b                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-139-3p               | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-332-3p               | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-331                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-195                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-17                   | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-142-3p               | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-15b                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-532                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR-452                  | [29] Diagnosis of colorectal adenocarcinoma                              |
| miR193a-3p               | [30] Diagnosis of colorectal adenocarcinoma, expression levels in tumor  |
| miR-23a                  | [30] Diagnosis of colorectal adenocarcinoma, expression levels in tumor  |
| miR-338-5p               | [30] Diagnosis of colorectal adenocarcinoma, expression levels in tumor  |
| miR-18a                  | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-20a                  | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-21                   | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-29a                  | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-92a                  | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-106b                 | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-133a                 | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-143                  | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-145                  | [32] Diagnosis of colorectal adenocarcinoma                              |
| miR-1288                 | [33,34] Control for miRNA in colorectal cancer                           |

FOBT: Fecal occult blood testing.
Table 2  Summary of miRNA and prognosis

| miRNA and prognosis | Ref.            | Summary                                      |
|---------------------|-----------------|----------------------------------------------|
| miR-20c             | [35-38]         | Increased expression level predicts worse prognosis |
| miR-378             | [46]            | Potential prognostic biomarker               |
| miR-126             | [47]            | Potential prognostic biomarker               |
| miR-224             | [48,49]         | Potential prognostic biomarker               |
| miR-429             | [50]            | Potential prognostic biomarker               |
| miR-182             | [51]            | Potential prognostic biomarker               |
| mir-32              | [52]            | Potential prognostic biomarker               |
| miR-214             | [53]            | Potential prognostic biomarker               |
| miR-182             | [54]            | Potential prognostic biomarker               |
| miR-92a             | [55]            | Potential prognostic biomarker               |
| miR-124             | [56]            | Potential prognostic biomarker               |
| miR-30b             | [57]            | Potential prognostic biomarker               |
| miR-625             | [58]            | Potential prognostic biomarker               |
| miR-155             | [37]            | Potential prognostic biomarker               |
| miR-210             | [37]            | Potential prognostic biomarker               |
| miR-215             | [59]            | Potential prognostic biomarker               |
| miR-130b            | [60]            | Potential prognostic biomarker               |
| miR-148b            | [61]            | Potential prognostic biomarker               |
| miR-148             | [61]            | Potential prognostic biomarker               |
| miR-16              | [62]            | Potential prognostic biomarker               |
| miR-21              | [10,39-45,47,65-68] | Upregulated in CRC, associated with tumor size, distant metastasis, poor survival and independent biomarker |

CRC: Colorectal cancer.

serum miRNA profile of stage IV CRC patients[a29]. Luo et al.[a30] demonstrated the ability of 9 plasma miRNAs to differentiate between patients with CRC and controls. Gopalan et al.[a31] discovered that expression of miR-1288 was correlated with not only stage, but also location within the gastrointestinal tract; higher miR-1288 expression was found in tumors located more distally within the colon.

In the study of serum miRNAs in CRC, a stable control is needed for the accurate measurement of circulating miRNAs. Hu et al.[a32] found that miR-1228 was steadily expressed among patients with different cancer types, including CRC, and among patients with CRC at different stages. Therefore, they suggest that miR-1228 is the most stable endogenous control for studying circulating miRNAs in CRC.

MICRO RNA AND PROGNOSIS

Although early detection of CRC promotes a reduction in mortality due to the disease, it is frequently diagnosed at a later stage when the prognosis is unfavorable. Current management of CRC relies on clinical and histopathologic factors including, but not limited to, extent of the tumor (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M) or TNM stage, tumor margin involvement, differentiation, and lymphovascular invasion. Increasing evidence supports the use of molecular markers in estimating prognosis and refining clinical management. Many studies demonstrate the utility of miRNAs as prognostic biomarkers (Table 2).

The first study to establish a relationship between miRNAs and CRC prognosis found a connection between the levels of miRNA-200c and survival[a33]. Since then, multiple studies have shown that both decreased and increased levels of various miRNAs are associated with poor outcome. A study by Toiyama et al.[a34] demonstrated elevated serum miR-200c in stage IV CRC compared to stages I-III, and showed that it served as a predictor for lymph node metastasis and recurrence. miR-200c was also found to serve as an independent indicator for CRC prognosis. Another study also found elevated miR-200c in the serum and tumor tissue of CRC patients compared to healthy controls[a35]. Serum miR-200c was also compared among CRC patients treated with surgical resection and chemotherapy. The serum miR-200c levels returned to normal levels in those with good prognosis, whereas in those with recurrence or distant metastasis miR-200c either remained elevated or elevated again after a transient decline. Hur et al.[a36] identified elevated expression of miR-200c in liver metastasis tissue compared to the primary CRC tissue. The miR-200c was found to be epigenetically regulated.

Another miRNA with significant potential as a prognostic marker is miR-21. miR-21 is upregulated in six different forms of cancer, including CRC[a37]. Previous studies have shown that tissue samples with elevated miR-21 expression were associated with lymph node and distant metastases; it was also correlated with clinical stage of CRC[a38]. Another study found that serum miR-21 was significantly elevated in CRC patients[a39]. Moreover, serum miR-21 accurately differentiated both adenoma and CRC patients from healthy controls. Elevated serum miR-21 was also
Table 3 Summary of miRNA and chemosensitivity

| Micro RNA | Ref. | Summary |
|-----------|------|---------|
| miR-21    | [65-68] | Increased expression causes decreased chemosensitivity |
| miR-153   | [69] | Up regulation, increased resistance to oxaliplatin and cisplatin |
| miR-19a   | [70] | Up regulation leads to resistance of FOLFOX therapy |
| miR-106a  | [71] | Up regulation leads to resistance of 5-FU and oxalilatin |
| miR-130b  | [71] | Up regulation leads to resistance of 5-FU and oxalilatin |
| miR-484   | [71] | Up regulation leads to resistance of 5-FU and oxalilatin |
| miR-129   | [72] | Down regulation increases resistance to 5-FU, transfection of miRNA into existing cells increase cytotoxic effect 5-FU and oxalilatin |
| miR-15b   | [73] | Down regulation increases resistance to 5-FU |
| miR-1915  | [74] | Down regulation decreases chemotherapy by modulating apoptotic pathway |
| miR-122   | [75] | Down regulation increases resistant to 5-FU |

5-FU: 5-fluorouracil.

associated with tumor size, distant metastasis, and poor survival, and served as an independent biomarker for CRC prognosis. Schetter et al.\(^4^0\) reported a strong association between elevated miR-21 expression and CRC prognosis based on two patient cohorts, one comprised of 84 American CRC patients and the other of 113 Chinese CRC patients. Elevated expression conferred upon each cohort a worse prognosis and was independent of staging and other clinical characteristics. Subsequent studies performed on cohorts of patients from other ethnic populations has validated their findings and suggest that elevated miR-21 serves as a strong prognostic biomarker regardless of ethnicity.\(^4^1\)-\(^4^3\)

Kjaer-Frifeldt et al.\(^4^4\) performed another cohort study demonstrating the use of miR-21 as a prognostic indicator in patients with stage II CRC. The authors showed that miR-21 expression may be combined with traditional characteristics used to stratify patients as either high- or low-risk of disease recurrence. Their thinking was that patients that had high miR-21 expression, and therefore a high-risk of recurrence, would be more likely to benefit from adjuvant chemotherapy. However, Oue et al.\(^4^5\) found that in a cohort of Japanese patients with stage II CRC, high expression of miR-21 was correlated with a poorer response to adjuvant chemotherapy. The authors showed instead that low miR-21 expressing patients had a positive response to adjuvant chemotherapy.

Many other miRNAs expression patterns have been discovered as potential prognostic biomarkers. The expression levels of the following miRNAs have each been reportedly correlated with prognosis in at least one study: miR-378\(^4^6\), miR-126\(^4^7\), miR-224\(^4^8\), miR-429\(^5^0\), miR-182\(^5^1\), miR-32\(^5^2\), miR-214\(^5^3\), miR-182\(^5^4\), miR-92a\(^5^5\), miR-124\(^5^6\), miR-30b\(^5^7\), miR-625\(^5^8\), miR-155 and miR-210\(^5^9\), miR-215\(^6^0\), miR-130b\(^6^0\), miR-148\(^6^1\), and miR-16\(^6^2\). The RNA III endonuclease known as Dicer is involved in the processing of miRNA and its expression is associated with poor prognosis.\(^6^3\). Iliou et al.\(^6^4\) showed that impairment of Dicer function lead to the downregulation of miRNAs associated with the regulation of the stem cell marker, CD44, and epithelial-to-mesenchymal transition-inducing transcription factors. The authors also identified that such changes enhanced tumor initiation and liver metastasis.

**MICRO-RNA AND CHEMOSENSITIVITY**

In addition to serving as biomarkers for diagnosis and prognosis, several studies have identified the ability of miRNAs to predict the sensitivity of CRC to chemotherapy. The response to chemotherapy varies between patients, the mechanisms for which are complex and poorly established. The use of miRNAs to predict chemotherapy efficacy allows for a more personalized approach to the treatment of CRC (Table 3).

A previous study has demonstrated that miR-21 expression correlates with poor prognosis and treatment response after 5-fluorouracil (5-FU) treatment\(^6^0\). This suggests that elevated miR-21 plays a role in the resistance to 5-FU. CRC tumor cell exposure to 5-FU facilitates an elevated expression of miR-21, perhaps as a means to overcoming the drug’s cytotoxic effects.\(^6^0\) Recently, Deng et al.\(^6^6\) demonstrated that forced overexpression of miR-21 in CRC cell lines increased resistance to 5-FU, which was reversed with genetic knockdown of miR-21 expression. This was also demonstrated by the effects of the dietary curcumin analog, difluorinated curcumin (CDF), on CRC cell lines resistant to treatment with 5-FU and oxaliplatin\(^6^7\),\(^6^8\). CDF was shown to inhibit growth of these cells when treated with 5-FU and oxaliplatin, while demonstrating a reduced expression of miR-21. The authors suggest that CDF leads to a decline in miR-21 expression, therefore sensitizing the cells to 5-FU and oxaliplatin treatment.

Additional research has recognized several miRNAs whose expression patterns are associated with sensitivity to chemotherapeutic agents. For example, up-regulation of miR-153 was associated with increased resistance to oxaliplatin and cisplatin both in vivo and in vitro.\(^6^9\) Additionally, miR-19a was found to be up-regulated in the serum of resistance-phase advanced CRC and was able to distinguish between patients who respond to FOLFOX therapy and those who are resistant.\(^7^0\). Kjersem et al.\(^7^1\) identified 3 miRNAs (miR-106a, miR-130b, and miR-484) whose up-regulation correlated with a lack of response to 5-FU and oxaliplatin. Interestingly, this lack of response was
not associated with a reduction in progression-free or overall survival.

Down-regulation of other miRNAs has also been associated with chemoresistance. Reduced expression of miR-129\(^{[72]}\) and miR-15b\(^{[73]}\) was found in CRC tissues resistant to 5-FU when compared to normal specimens. Transfection of miR-129 into resistant cells enhanced the cytotoxic effects of 5-FU\(^{[72]}\), miR-1915 was also found to play a role in multi-drug resistant CRC. Overexpression of Bcl-2 has been generally accepted as conferring drug resistance in multiple cancers, including CRC. A study by Xu et al\(^{[74]}\) found reduced levels of miR-1915 and up-regulation of Bcl-2 in a multi-drug resistant CRC cell line. Through transfection of miR-1915, they discovered that increased expression of miR-1915 lead to a reduction in Bcl-2 protein levels and sensitized the cells to multiple chemotherapeutic drugs through modulation of apoptotic pathways.

A recent study by He et al\(^{[75]}\) illustrated the role of miRNAs on the "Warburg effect" and chemoresistance. The "Warburg effect" is the observation that most cancer cells utilize high rates of glycolysis relative to oxidative phosphorylation when compared to normal cells. The study demonstrated that miR-122 directly targets the glycolytic enzyme pyruvate kinase type M2 (PKM2) and consequently, miR-122 is downregulated in 5-FU-resistant CRC cells. The resistance to 5-FU was found to be correlated with the increase in glycolytic glucose metabolism, evidenced by increased glucose consumption and lactate release and increased expression of PKM2, lactate dehydrogenase, and the GLUT-1 glucose transporter. Interestingly, over-expression of miR-122 was shown to suppress PKM2 and significantly improve the cytotoxic effects of 5-FU on these resistant cells.

**CONCLUSION**

The discovery of microRNAs has since bolstered immense popularity in the scientific community and knowledge of their mechanisms continues to expand in the area of colorectal cancer. Certainly, miRNA plays an important part in initiating and fostering the progression of colorectal cancer. Many studies report associations between miRNA expression patterns and the diagnosis, prognosis, and sensitivity to chemotherapy. These studies indicate the utility of miRNA as markers for early CRC detection and their use in directing the management of CRC at all stages. Further research must be conducted to validate these findings and, most importantly, determine if the results provide information that can be adapted in the clinical realm. Additionally, studies illustrate the potential therapeutic utility of miRNAs. More research must be conducted to investigate strategies for the use of miRNAs in CRC treatment such as mechanisms for the delivery of miRNA into cells, enhanced miRNA and mRNA binding, and inhibition of endogenous miRNAs. Research must also continue to develop a more detailed understanding of miRNA biochemical mechanisms and improve precision in the detection, prognosis, and chemosensitivity of colorectal cancer.

**REFERENCES**

1. Heavey PM, McKenna D, Rowland IR. Colorectal cancer and the relationship between genes and the environment. *Nutr Cancer* 2004; 48: 124-141 [PMID: 15231447 DOI: 10.1007/s15327914nc4802_2]
2. Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. *Gastroenterology* 2010; 138: 2101-2114.e5 [PMID: 20420949 DOI: 10.1053/j.gastro.2010.01.058]
3. Schetter AJ, Harris CC. Alterations of microRNAs contribute to colon carcinogenesis. *Semin Oncol* 2011; 38: 734-742 [PMID: 22082759 DOI: 10.1053/j.seminoncol.2011.08.009]
4. Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, Norat T. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PLoS One* 2011; 6: e20456 [PMID: 21674008 DOI: 10.1371/journal.pone.0020456]
5. Doubeni CA, Liaymo AO, Major JM, Schootman M, Liang M, Park Y, Graubard BI, Hollenbeck AR, Sinha R. Socioeconomic status and the risk of colorectal cancer: an analysis of more than a half million adults in the National Institutes of Health-AARP Diet and Health Study. *Cancer* 2012; 118: 3636-3644 [PMID: 22898918 DOI: 10.1002/cncr.26677]
6. Hrašovec S, Glavač D. MicroRNAs as Novel Biomarkers in Colorectal Cancer. *Front Genet* 2012; 3: 180 [PMID: 23091478]
7. American Cancer Society. Colorectal Cancer Facts & Figures 2011-2013. Atlanta: American Cancer Society, 2011. Available from: URL: http://www.cancer.org/research/cancerfactsfigures/colorectalcancerfactsfigures2011-2013-page
8. American Cancer Society. Cancer Facts & Figures 2012. Atlanta: American Cancer Society; 2012. Available from: URL: http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2012/
9. Kanaan Z, Rai SN, Eichenberger MR, Roberts H, Keskey B, Pan J, Galanduik S. Plasma miR-21: a potential diagnostic marker of colorectal cancer. *Ann Surg* 2012; 256: 544-551 [PMID: 22683727 DOI: 10.1097/SLA.0b013e318265bd6f]
10. American Cancer Society. Cancer Facts and Figures, 2010. American Cancer Society. Accessed April 13, 2014. Available from: URL: http://www.cancer.org/research/cancerfactsfigures/index
11. Burt RW, Barthel JS, Dunn KB, David DS, Drelichem E, Ford JM, Giardiello FM, Gruber SB, Halverson AL, Hamilton SR, Ismail MK, Jaspersen K, Lazendy AJ, Lynch PM, Martin EW, Mayer RJ, Ness RM, Provenzale D, Rao MS, Shike M, Steinbach G, Terdiman JP, Weinberg D. NCCN clinical practice guidelines in oncology. Colorectal cancer screening. *J Natl Compr Canc Netw* 2010; 8: 8-61 [PMID: 20064249 DOI: 10.1097/MOG.0b013e328233d1f3]
12. Burch JA, Soares-Weiser K, St John DJ, Duffy S, Smith S, Kleijnen J, Westwood M. Diagnostic accuracy of faecal occult blood tests used in screening for colorectal cancer: a systematic review. *J Med Screen* 2007; 14: 132-137 [PMID: 17925085]
13. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* 1993; 75: 855-862 [PMID: 8252622 DOI: 10.1016/0092-8674(93)90503-4]
14. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998; 391: 806-811 [PMID: 9486653 DOI: 10.1038/35988]
15. miRBase, [updated 2013 June; cited 2014 June 6]. Available from: URL: http://www.mirbase.org/index.shtml
16. Kim VN. MicroRNA biogenesis: coordinated cropping and dicing.
Hollis M et al. miRNA in CRC detection and outcome

Nat Rev Mol Cell Biol 2005; 6: 376-385 [PMID: 15852042 DOI: 10.1038/nrm1644]

Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Nat Cell Biol 2005; 7: 120-125 [PMID: 15652477 DOI: 10.1016/j.ncl.2004.12.035]

Ciafrè SA, Galardi S. microRNAs and RNA-binding proteins: a complex network of interactions and reciprocal regulations in cancer. RNA Biol 2013; 10: 935-942 [PMID: 23696003 DOI: 10.4161/ma.24641]

Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Fernandez AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]

Ahmed FE, Jeffreys CD, Vos PW, Blake G, Nuovo GJ, Sinar Pillai S, Ebrahimi F, Salajegheh A, Lam TC, Le TK, Langsford N, Ho YH, Smith RA, Lam AK. Regulation of microRNA-1288 in colorectal cancer: altered expression and its clinicopathological significance. Mol Carcinog 2014; 53 Suppl 1: E36-E44 [PMID: 24069195 DOI: 10.1002/mc.21993]

Hu J, Wang Z, Liao BY, Yu L, Gao X, Lu S, Wang S, Dai Z, Zhang X, Chen Q, Qiu SJ, Wu Y, Zhu H, Fan J, Zhou J, Wang J. Human miR-1228 as a stable endogenous control for the quantification of circulating microRNAs in cancer patients. Int J Cancer 2014; 135: 1187-1194 [PMID: 24488924 DOI: 10.1002/ijc.28577]

Xi Y, Formentini A, Chien M, Weir DB, Russo JJ, Ju J, Kormann M, Ju J. Prognostic Values of microRNAs in Colorectal Cancer. Biomark Insights 2006; 2: 113-121 [PMID: 18079988]

Toiyama Y, Hur K, Tanaka K, Inoue Y, Kusunoki M, Boland CR, Goel A. Serum miR-200c is a novel prognostic and metastasis-predictive biomarker in patients with colorectal cancer. Ann Surg 2014; 259: 735-743 [PMID: 23982750 DOI: 10.1097/01.sla.0000469609]

Chen J, Wang W, Zhang Y, Chen Y, Hu T. Predicting distant metastasis and chemoresistance using plasma miRNAs. Med Oncol 2014; 31: 799 [PMID: 24310813 DOI: 10.1007/s1203-013-0799-x]

Hur K, Toiyama Y, Takahashi M, Balaguer F, Nagasaka T, Koike J, Hemmi H, Koi M, Boland CR, Goel A. MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut 2013; 62: 1315-1326 [PMID: 22735571 DOI: 10.1136/gutjnl-2011-301846]

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Preul RT, Yaniahara N, Lanza G, Scapa A, Vecchione A, Negri M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer disease genes. Proc Natl Acad Sci USA 2006; 103: 2257-2261 [PMID: 16461460 DOI: 10.1073/pnas.0510561103]

Slaby O, Svoboda M, Fabian P, Smerdova T, Knoflickova D, Bednarikova M, Nemutil R, Vyzula R. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology 2007; 72: 397-402 [PMID: 18196926 DOI: 10.1159/000113489]

Toiyama Y, Takahashi M, Hur K, Nagasaka T, Tanaka K, Inoue Y, Kusunoki M, Boland CR, Goel A. Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer. J Natl Cancer Inst 2013; 105: 849-859 [PMID: 23704278 DOI: 10.1093/jnci/djt101]

Schetter AJ, Leung SY, Sohn JH, Zanetti KA, Bowman ED, Yaniahara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM, Harris CC. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 2008; 299: 425-436 [PMID: 18237080 DOI: 10.1001/jama.299.4.425]

Shibuya H, Inunuma H, Shimada R, Horiuchi A, Watanabe T. Clinicopathologic and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. Oncology 2010, 79: 313-320 [PMID: 21412018 DOI: 10.1159/000323283]

Kulda V, Pesta M, Topolcan O, Liska V, Treska V, Sutnar A, Rupert K, Ludvikova K, Babuska V, Holubec L, Cerny R, Goel A. Serum miR-21-3p and miR-21-5p as potential biomarkers of colorectal cancer. PLoS One 2013; 8: e62880 [PMID: 23690963 DOI: 10.1371/journal.pone.0062880]
High miR-21 expression from FFPE tissues is associated with poor survival and response to adjuvant chemotherapy in colon cancer. *Int J Cancer* 2013; 134: 1926-1934 [PMID: 23861214 DOI: 10.1002/ijc.23380]

Li S, Gao J, Gu J, Yuan J, Hua D, Shen L. MicroRNA-215 inhibits relapse of colorectal cancer patients following radical surgery. *Med Oncol* 2013; 30: 549 [PMID: 23532818 DOI: 10.1007/s12032-013-0459-0]

Colangelo T, Fueci A, Votino C, Sabatino L, Pancione M, Laudanna C, Binuschi M, Bigioni M, Maggi CA, Parente D, Forte N, Colantuoni V. MicroRNA-130b promotes tumor development and is associated with poor prognosis in colorectal cancer. *Neoplasia* 2013; 15: 1086-1099 [PMID: 24074333 DOI: 10.1593/neo.13998]

Tsai HL, Yang JP, Huang CW, Ma CJ, Kuo CH, Lu CY, Jhou SH, Wang JW. Clinical significance of microRNA-148a in patients with early relapse of stage II stage and III colorectal cancer after curative resection. *Transl Res* 2013; 162: 258-268 [PMID: 23933284 DOI: 10.1016/j.trsl.2013.07.009]

Qian J, Jiang B, Li M, Chen J, Fang M. Prognostic significance of microRNA-16 expression in human colorectal cancer. *World J Surg* 2013; 37: 2944-2949 [PMID: 24045965 DOI: 10.1007/s00268-013-2205-4]

Faber C, Horst D, Hubek F, Kirchner T. Overexpression of Dicer predicts poor survival in colorectal cancer. *Eur J Cancer* 2011; 47: 1414-1419 [PMID: 21346667 DOI: 10.1016/j.ejca.2011.01.006]

Blou MS, da Silva Diz V, Carmona FJ, Ramalho-Carvalho J, Heyn H, Villanueva A, Muñoz P, Esteller M. Impaired DICER1 function promotes stemness and metastasis in colon cancer. *Oncogene* 2014; 33: 4003-4015 [PMID: 24096488 DOI: 10.1038/onc.2013.398]

Rossi L, Bonmassar E, Faraoi I. Modification of miR gene expression pattern in human colon cancer cells following exposure to 5-fluorouracil in vitro. *Pharmacol Res* 2007; 56: 248-253 [PMID: 17702597 DOI: 10.1016/j.phrs.2007.07.001]

Deng J, Lei W, Fu JC, Zhang L, Li JH, Xiong JP. Targeting miR-21 enhances the sensitivity of human colon cancer HT-29 cells to chemoradiotherapy in vitro. *Biochem Biophys Res Commun* 2014; 443: 789-795 [PMID: 24275137 DOI: 10.1016/j.bbrc.2013.11.064]

Roy S, Yu Y, Padhye SB, Sarkar FH, Majumdar AP. Difluorinated-curfum (CDF) restores PTEN expression in colon cancer cells by down-regulating miR-21. *PLoS One* 2013; 8: e68543 [PMID: 23894315 DOI: 10.1371/journal.pone.0068543]

Yu S, Sarkar FH, Majumdar AP. Down-regulation of miR-21 induces differentiation of chemoresistant Colon Cancer Cells and Enhances Susceptibility to Therapeutic Regimens. *Transl Oncol* 2013; 6: 180-188 [PMID: 23541709 DOI: 10.1016/j.trsl.2013.07.003]

Zhang L, Pickard K, Jenei V, Bullock MD, Bruce A, Mitter J, Zhou H, Xiao HX, Li Y, Zhou T. miR-378 is an independent prognostic factor and inhibits cell growth and invasion in colorectal cancer. *BMC Cancer* 2014; 14: [PMID: 24555885 DOI: 10.1186/1471-2407-14-109]

Hansen TF, Christensen RD, Andersen RF, Sørensen FB, Johnsson A, Jacobsen A. MicroRNA-126 and epidermal growth factor-like domain 7-angioenic couple of importance in metastatic colorectal cancer. Results from the Nordic ACT trial. *Br J Cancer* 2013; 109: 1243-1251 [PMID: 23922111 DOI: 10.1038/bjc.2013.448]

Zhang GJ, Zhou H, Xiao HX, Li Y, Zhou T. Up-regulation of miR-224 promotes cancer cell proliferation and invasion and predicts relapse of colorectal cancer. *Cancer Cell Int* 2013; 13: 104 [PMID: 24152489 DOI: 10.1186/1475-2867-13-104]

Liao WT, Li TT, Wang ZG, Wang SY, He MR, Ye YP, Qi L, Cui YM, Wu P, Jiao HL, Zhang C, Xie YJ, Wang JX, Ding YQ. microRNA-224 promotes cell proliferation and tumor growth in human colorectal cancer by repressing PHLP1 and PHLP2. *Clin Cancer Res* 2013; 19: 4662-4672 [PMID: 23846336 DOI: 10.1158/1078-0432.CCR-12-0244]

Li J, Du L, Yang Y, Wang C, Liu H, Wang L, Zhang X, Li W, Zheng G, Dong Z. Micro-249 is an independent prognostic factor in colorectal cancer and exerts its anti-apoptotic function by targeting SOX2. *Cancer Lett* 2013; 329: 84-90 [PMID: 23111103 DOI: 10.1016/j.canlet.2012.10.019]

Rapti SM, Kontos CK, Papadopoulou IN, Scirilas A. Enhanced microRNA-182 transcription is a predictor of poor overall survival in colorectal adenocarcinoma patients. *Clin Chem Lab Med* 2014; 52: 1217-1227 [PMID: 24615484 DOI: 10.1515/ccm-2013-0950]

Wu W, Yang P, Feng X, Wang H, Qiu Y, Tian T, He Y, Yu C, Yang J, Ye S, Zhou Y. The relationship between and clinical significance of MicroRNA-32a and phosphatase and tensin homologue expression in colorectal cancer. *Genes Chromosomes Cancer* 2013; 52: 1133-1140 [PMID: 24123284 DOI: 10.1002/gcc.22108]

Chen DL, Wang ZQ, Zeng ZL, Wu WJ, Zhang DS, Luo HY, Wang F, Qiu MZ, Wang DS, Ren C, Wang FH, Chiao LJ, Peliciano R, Huang P, Li YH, Xu RH. Identification of microRNA-214 as a negative regulator of colorectal cancer liver metastasis by way of regulation of fibroblast growth factor receptor 1 expression. *Hepatology* 2014; 60: 598-609 [PMID: 24616020 DOI: 10.1002/hep.27118]

Liu H, Du L, Wen Z, Yang Y, Li J, Wang L, Zhang X, Liu Y, Dong Z, Li W, Zheng G, Wang C. Up-regulation of miR-182 expression in colorectal cancer tissues and its prognostic value. *Int J Colorectal Dis* 2013; 28: 697-703 [PMID: 23474644 DOI: 10.1007/s00384-013-1674-0]

Zhou T, Zhang G, Liu Z, Xia S, Tian H. Overexpression of microRNA-92a correlates with tumor metastasis and poor prognosis in patients with colorectal cancer. *Int J Colorectal Dis* 2013; 28: 19-24 [PMID: 22772712 DOI: 10.1007/s00384-012-1528-1]

Wang MJ, Li Y, Wang R, Wang C, Yu YY, Yang L, Zhang Y, Zhou B, Zhou ZG, Sun XF. Downregulation of microRNA-124 is an independent prognostic factor in patients with colorectal cancer. *Int J Colorectal Dis* 2013; 28: 183-189 [PMID: 22885837 DOI: 10.1007/s00384-013-1550-3]

Liao WT, Ye YP, Zhang NZ, Li TT, Wang SY, Cui YM, Qi L, Wu P, Jiao HL, Xie YJ, Zhang C, Wang JX, Ding YQ. MicroRNA-308 functions as a tumour suppressor in human colorectal cancer by targeting KRAS, PIK3CD and BCL2L1. *J Pathol* 2014; 232: 415-427 [PMID: 24293274 DOI: 10.1002/path.4309]

Lou X, Qi X, Zhang Y, Long H, Yang J. Decreased expression of microRNA-625 is associated with tumor metastasis and poor prognosis in patients with colorectal cancer. *J Surg Oncol* 2013; 108: 230-235 [PMID: 23861214 DOI: 10.1002/jso.23380]

Li S, Gao J, Gu J, Yuan J, Hua D, Shen L. MicroRNA-215 inhibits relapse of colorectal cancer patients following radical surgery. *Med Oncol* 2013; 30: 549 [PMID: 23532818 DOI: 10.1007/s12032-013-0459-0]
He J, Xie G, Tong J, Peng Y, Huang H, Li J, Wang N, Liang H. Overexpression of microRNA-122 re-sensitizes 5-FU-resistant colon cancer cells to 5-FU through the inhibition of PKM2 in vitro and in vivo. Cell Biochem Biophys 2014; 70: 1343-1350 [PMID: 24898807 DOI: 10.1007/s12013-014-0062-x]
