

Review

The Diagnostic and Prognostic Role of microRNA in Colorectal Cancer – a Comprehensive review

Haggi Mazeh¹, Ido Mizrahi¹, Nadia Ilyayev¹, David Halle¹, Björn LDM Brücher^{1,2,3,4}, Anton Bilchik^{2,3,4,5}, Mladjan Protic^{2,3,4,6}, Martin Daumer^{2,3,7}, Alexander Stojadinovic^{2,3,4,8}, Itzhak Avital^{7,8}, Aviram Nissan^{2,3,4,9}✉

1. Department of Surgery, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
2. Theodor-Billroth-Academy®, Munich, Germany.
3. INCORE = International Consortium of Research Excellence of the Theodor-Billroth-Academy®.
4. United States Military Cancer Institute, Washington, D.C.
5. John Wayne Cancer Institute, Santa Monica, CA, USA.
6. Clinic of Abdominal, Endocrine, and Transplantation Surgery, Clinical Center of Vojvodina, Novi Sad, Serbia.
7. Sylvia Lawry Center for MS Research, Munich, Germany.
8. Department of Surgery, Walter Reed National Military Medical Center, Bethesda, MD, and the United States Military Cancer Institute, Washington, D.C. USA.
9. Bon Secours Cancer Institute, Richmond, VA, USA.

✉ Corresponding author: Aviram Nissan, M.D. Department of Surgery, Hadassah-Hebrew University Medical Center Ein Kerem, Kyriat Hadassah, P.O.B. 12000, Jerusalem, 91120 Israel. Tel: 011-972-3-7644587 Fax: 011-972-3-7645804 e-mail: anissan@cancer-surgery.co.il.

© Ivyspring International Publisher. This is an open-access article distributed under the terms of the Creative Commons License (<http://creativecommons.org/licenses/by-nc-nd/3.0/>). Reproduction is permitted for personal, noncommercial use, provided that the article is in whole, unmodified, and properly cited.

Received: 2013.01.20; Accepted: 2013.02.14; Published: 2013.03.20

Abstract

The discovery of microRNA, a group of regulatory short RNA fragments, has added a new dimension to the diagnosis and management of neoplastic diseases. Differential expression of microRNA in a unique pattern in a wide range of tumor types enables researches to develop a microRNA-based assay for source identification of metastatic disease of unknown origin. This is just one example of many microRNA-based cancer diagnostic and prognostic assays in various phases of clinical research.

Since colorectal cancer (CRC) is a phenotypic expression of multiple molecular pathways including chromosomal instability (CIN), micro-satellite instability (MIS) and CpG islands promoter hypermethylation (CIMP), there is no one-unique pattern of microRNA expression expected in this disease and indeed, there are multiple reports published, describing different patterns of microRNA expression in CRC.

The scope of this manuscript is to provide a comprehensive review of the scientific literature describing the dysregulation of and the potential role for microRNA in the management of CRC. A Pubmed search was conducted using the following MeSH terms, "microRNA" and "colorectal cancer". Of the 493 publications screened, there were 57 papers describing dysregulation of microRNA in CRC.

Key words: microRNA, colorectal cancer

Introduction

Colorectal cancer (CRC) is the second most common cancer in females and the third in males with 1.2 million annual new cases worldwide.¹ The incidence and mortality caused by CRC have been slowly

decreasing in the United States. Over 143,000 new cases of CRC are diagnosed annually and approximately 52,000 Americans die of the disease every year. These deaths account for approximately 9% of all

cancer mortality.¹

The lifetime incidence of CRC in the average-risk population living in North America and Western Europe is 5%. The vast majority of cases (90%) occur after the age of 50.¹ Due to this age-associated risk, current screening guidelines recommend routine testing after the age of 50. Patients with risk factors require age adjustment. The strongest risk factor is presence of hereditary CRC syndromes such as familial adenomatous polyposis (FAP) or its variants, MYH-associated polyposis, Lynch syndrome (hereditary non-polyposis colon cancer - HNPCC), BRCA2, juvenile polyposis, or any personal or family history of sporadic CRC or adenomatous polyps. Other risk factors that currently influence screening recommendations include presence of a long standing inflammatory bowel disease and history of abdominal radiation. Screening colonoscopy is recommended by the age of 45 in African Americans however, current screening recommendations do not stratify individuals by gender.^{2,4}

Despite the increased awareness with improved screening recommendations and techniques, CRC remains the second leading cause of cancer-related death of both men and women. Early detection was shown to have an important role in reducing CRC-related mortality rate. When detected at stage I or II, five year survival rates are 90% and 75%, respectively. Nevertheless, diagnosis is often delayed until patients become symptomatic with more advanced lesions and substantially increased associated mortality. Accordingly, the 5 year survival rates for stage III and IV are 65% and 5%, respectively.⁵

Pathogenesis of colorectal cancer

In most CRC patients, the progression of normal colonic mucosa to invasive cancer requires several molecular changes. The estimated time interval of the malignant transformation from normal mucosa through adenomatous polyp into an invasive carcinoma is 5-10 years in most cases.⁶ This long time-interval provides the ground for early detection and even prevention of CRC as was shown by the National Polyp Study. In this study, 1,418 patients were followed after all identifiable polyps were removed by colonoscopy. Within this follow-up period, only five subsequent malignancies were discovered and there were no deaths attributed to CRC. When compared to three other control groups, the study group had a decreased incidence of CRCs. The authors concluded that CRC arises mostly from adenomas, and colonoscopy-based screening may reduce both the incidence and the stage at diagnosis of CRC compared to non-screened populations.⁷

Screening for colorectal cancer

Fecal Occult Blood Testing (FOBT)

Guaiac-based fecal occult blood testing using rehydrated or nonrehydrated stool specimens in people aged 50 to 80 decreases mortality from colorectal cancer. In 1993 and 1996, 3 clinical trials provided evidence that FOBT screening reduces CRC-related mortality. A reduction of 33% in mortality was demonstrated among subjects who had annual rehydrated FOBT testing in a US clinical trial⁸, and approximately 15% mortality-rate reduction was demonstrated in two European studies using a bi-annual nonrehydrated FOBT testing protocol.^{9,10} The fact that occult gastrointestinal bleeding is more common in benign conditions and the fact that bleeding is not universal in adenomas and early cancers, results in relatively low sensitivity and specificity of FOBT for CRC detection. In recent years, there are multiple reports of improved sensitivity and specificity of immuno-based FOBT assays.¹¹ A large clinical trial is currently conducted in Spain with the main aim of comparing Ig-FOBT to colonoscopy-based screening.¹²

Endoscopic procedures

Flexible sigmoidoscopy

The role of flexible sigmoidoscopy for CRC screening is controversial. The 60 cm sigmoidoscope can reach the splenic flexure and potentially evaluate one-half of the colon. The benefit of flexible sigmoidoscopy is that it is cheaper and safer compared to colonoscopy and a full bowel preparation is not necessary. In a case-controlled study, CRC related mortality reduction was shown with screening by flexible sigmoidoscopy. However, this benefit was limited to the portion of colon examined.¹³ It is important to determine what percentage of malignancies is proximal to the splenic flexure and would, therefore, be missed by sigmoidoscopy alone. Imperiale et al. showed that, a strategy based on flexible sigmoidoscopy followed by full colonoscopy in cases where adenomatous polyp was found in the range of the sigmoidoscope, would detect additional invasive cancers and increase the detection rate to 70-80% of all cases of invasive colon cancer.¹⁴

Colonoscopy

Since colonoscopy has been shown to prevent CRC by the removal of large polyps prior to their conversion to invasive cancer, in recent years, colonoscopy has become the preferred choice for CRC screening. Additionally, colonoscopy can identify and biopsy most cancers during the initial procedure.

However, procedural competence varies among examiners and the cecum is reached in 80–98% of procedures, with the depth of penetration depending mainly on the experience of the endoscopist and the adequacy of bowel preparation. To date, there have been no studies examining the effectiveness of colonoscopy as a screening modality for CRC with reduction in CRC-related mortality as a study endpoint. Additionally, there are no controlled trials that address the question of how frequent colonoscopy should be performed. The National Polyp Study demonstrated that the removal of polyps reduces the incidence of CRC. Additionally, CRC mortality is lowered through the detection of early cancers and precancerous lesions.⁷ The limitations of colonoscopy remain its complication rate as well as compliance. The incidence of complications during colonoscopy is: 1 in 1,000 patients suffers perforation; 3 in 1,000 suffer major hemorrhage; and 1–3 in 10,000 die as a result of the procedure.¹⁵ The data on compliance with screening colonoscopy is even more distressing. When physicians, nurses, and spouses of the nurses were invited by letter to undergo a free screening colonoscopy, less than 15% complied.¹⁶ Colonoscopy has an important role in CRC screening, since it was an integral part of the FOBT screening trials that demonstrated a significant reduction in mortality in screened patients.

Imaging modalities for CRC screening

Barium Enema

Double contrast barium enema (DCBE), was the primary technique for the detection of polyps before colonoscopy became available. Despite the fact that it is the safest of the structural screening tests for CRC and the risk of serious complications is 1 in 10,000, it is hardly used anymore. The sensitivity of DCBE is variable and ranges between 70–90% for polyps >1 cm but only 50–80% for polyps <1 cm in diameter.¹⁷ The lack of sensitivity has been attributed to inadequate visualization of parts of the bowel as well as to errors in interpretation. DCBE has been considered an inaccurate examination for the rectum and sigmoid colon. No prospective randomized trials of DCBE screening have been completed, and most published studies of DCBE relate to symptomatic rather than asymptomatic patients. A randomized trial of DCBE plus flexible sigmoidoscopy versus colonoscopy in 383 symptomatic patients with gastrointestinal bleeding, suspected to be from the colon, found that colonoscopy detected more cases of polyps <9 mm than DCBE plus sigmoidoscopy.¹⁸ There was no difference between strategies in the number of patients detected

with cancer or polyps >9 mm. In summary, this method is currently used only in very selective cases and was replaced by colonoscopy or CT colonography.

Computed Tomography (CT) colonography (Virtual Colonoscopy)

In recent years, the utilization of virtual colonoscopy or CT colonography (CT-Co), is rapidly rising. Here the colon is prepared in the same manner as it is prepared for colonoscopy, distended with air inflated through the anus, and studied with the help of a computerized tomography (CT). In recent studies, CT-Co's sensitivity for the detection of large polyps ranged from 50 to 91%.¹⁹ The theoretical advantage to CT-Co is that it is rapid and completely visualizes the colon with a negligible risk. No sedation is required and the patient's acceptance is better than fiberoptic colonoscopy or barium enema. CT-Co can image the right colon and cecum in cases where fiberoptic colonoscopy was not completed, either due to technical difficulties or in a more common case scenario, because of a nearly obstructing lesion. Finally, it may also provide additional information by imaging the abdominal structures outside the colon. Its most significant limitation is that the colon should be thoroughly clean and that polypectomy is not possible with virtual colonoscopy. The size of polyp is also important, since the sensitivity of CT-Co to detect lesions smaller than 10mm is limited. There is also a concern regarding a flat adenoma, which cannot be detected by this modality.²⁰

Fecal DNA and RNA screening

Approximately one-sixth to one-third of normal adult colonic epithelial cells (colonocytes) are shed daily from the lower two-thirds of colon crypts, this corresponds to the daily exfoliation of approximately 10^{10} cells, each having a lifespan of 3–4 days.

Screening the feces for specific cancer-related DNA or RNA is appealing. However, technical complexity as well as the lack of specific CRC molecular markers prevented the development of a robust, commercially available, fecal DNA screening kit.

Since CRC is a common phenotype of several molecular diseases. Understanding the molecular genetics of colorectal cancer is essential for the analysis of fecal DNA or RNA.^{21,22} The vast majority of colorectal cancers result from chromosomal instability (CIN), with mutations progressively accumulating in the adenomatous polyposis coli (*APC*) gene, the *p53* tumor-suppressor gene, and the *K-ras* oncogene.²³ The second most common pathway (6%–25% of CRC) leads to a loss of genes involved in DNA-mismatch

repair, manifested by microsatellite instability (MSI).²⁴

Other molecular pathways leading to CRC are the CpG islands promoter hypermethylation (CIMP) including several different molecular pathways characterized by CIMP.²⁵⁻³⁰ Colorectal cancer may also be detectable through the use of DNA markers associated with disordered apoptosis.³¹ Several studies using fecal-based DNA testing have reported a sensitivity of 62-91% for cancer and 27-82% for advanced adenomas, with a specificity of 93-96% in persons with normal findings on colonoscopy.³²⁻³⁶ However, those studies were conducted in patients with advanced, symptomatic lesions. In a large-scale study comparing fecal DNA to FOBT, 5486 patients were screened.³⁶ The fecal DNA panel detected 16 of 31 invasive cancers, whereas FOBT identified 4 of 31 (51.6% vs. 12.9%, $P=0.003$). The DNA panel detected 29 of 71 invasive cancers, and adenomas with high-grade dysplasia, whereas FOBT identified 10 of 71 (40.8% vs. 14.1%, $P<0.001$). Specificity in subjects with negative findings on colonoscopy was 94.4% for the fecal DNA panel and 95.2% for the FOBT.³⁶

Another molecular screening strategy is to screen for fecal RNA and amplify cancer specific molecular markers using RT-PCR. Ahmed et al. described a method that permits extraction of intact non-degraded total RNA from human colonocytes in stool by utilizing commercially available kits.³⁷ They showed that RNA can be adequately reverse transcribed, making high-quality copy cDNA. This was followed by PCR using colon cancer-specific gene primers (guanylyl cyclase C and PYPAF5 genes).³⁷

Serum (blood) test for the detection of colon cancer

Currently, there is no commercially available FDA-approved blood test for CRC diagnosis. Literature search identified 93 studies evaluating an overall of 70 different blood markers for CRC, including CEA and carbohydrate antigens, as well as newly introduced ones, including mainly proteins identified through mass spectrometry analysis. The majority of studies evaluated protein markers, but in recent years, there is increasing interest in genetic and epigenetic (mRNA, DNA, microRNA) markers.³⁸⁻⁴³

Overall, a broad range of sensitivity and specificity was reported for the various markers. A direct comparison of results from different studies is complicated due to the diverse populations used and use of different cutoff points for the same marker. Furthermore, the majority of markers were evaluated in a single study. In addition, many studies had small sample size. Only in very few studies overall sample size was above 300 subjects. Another concern refers to

comparability of results across studies given potential differences in serum collection, processing and storage methods, and uncertainties in the stability of several biomarkers. Other concerns are the lack of patients with adenomatous polyps in most of these studies, and the fact that control patients in most studies did not undergo mandatory colonoscopy ruling out the presence of adenoma or carcinoma.

The current stage of evidence calls for prospectively planned and systematic evaluations of both the most promising blood tests and the most promising stool tests in a well-defined, large-scale screening population, in which special attention is drawn to standardized sample collection, processing, and storage.

microRNA

microRNAs (miRs) are a class of endogenous small, noncoding, RNA fragments (18-24 nucleotides in length) involved in multiple intracellular processes by regulating gene expression. Since the first miR discovery in the *Caenorhabditis Elegans* worm in 1993, numerous different miRs were identified in plants, animals, and humans.⁴⁴ At present, 2042 mature human miRs are listed in the updated database (<http://www.mirbase.org>). Approximately half of all human miR genes are contained within the introns of protein-coding genes, whereas others reside apart from known genes or in the exons of untranslated genes.^{45,46}

miRs biosynthesis begins with long double-stranded primary transcripts converted to a 70 nucleotide precursor. As many as 1600 human miR precursors are documented. miR precursors are cleaved in the cytoplasm in the short 22 nucleotide double stranded miR. The double strand is then unwound to produce single stranded active miRs. Mature miRs incorporate into the RNA-induced silencing complex (RISC) and are able to bind to the 3-untranslated region (UTR) of the target gene. This miR binding may cause either block of translation or mRNA degradation depending on the level of complementarity with the target DNA sequence.⁴⁷ It is thus that the effect of miRs on their target genes is based on the degree of homology between the sequences of the miR and the target gene. The homology that dictates the specificity of miRs is dependent on 6-7 nucleotides that bind to the 3-UTR of their target mRNAs. As a result a single gene can be targeted by many different miRs and a single miR can potentially affect hundreds of genes.⁴⁸

The homology diversity places the miRs in a perfect position to regulate multiple different intracellular processes. It is predicted that up to 30% of

protein-coding genes are controlled by miRs⁴⁹. It was also shown that miRs are involved in cellular development, differentiation, proliferation, and apoptosis.⁵⁰ As a result, miR expression level may affect tumor pathogenesis and behavior. Low expression of a specific miR may lead to upregulation of oncogenes and in contrast, high expression of other miRs may lead to downregulation of tumor-suppressor genes. In a similar manner, dysregulation of other miRs may have the opposite effect.^{47,51,52}

In the last decade, a rapidly increasing number of studies have identified miR-dysregulation in multiple malignancies. Since the first description of miR-15a and -16-1 in B-cell chronic lymphocytic leukemia other malignancies followed. To date, numerous miR dysregulations have been described in glioblastoma, breast cancer, acute myeloid leukemia, melanoma, pancreatic cancer, lung cancer, ovarian cancer, hepato-cellular carcinoma, thyroid cancer, bladder and kidney cancer, gastric cancer, colorectal cancer, and others.⁵³⁻⁶²

While the exact mechanism and target gene for every miR are yet to be elucidated, many oncologic aspects are currently being studied. The first step is to document the presence of miR-dysregulation in tumor versus control non-malignant tissues. Commercial kits are now available for RNA extraction and miR profile analysis. The mere documentation of miR-dysregulation in a specific malignancy does not shed light on the suspected target gene (or genes) nor on the specific mechanism of contribution to malignancy development. Lastly, targeting specific miRs may prove to have therapeutic value.

Due to their small size, miR levels are remarkably stable in tissue samples, serum, plasma, and even stool. Turchinovich et al. showed that extracellular miR remains stable for at least one month.⁶³ This stability may contribute to the value of specific miRs as biomarkers for malignancy. The focus of this review is the dysregulation of miR in CRC. The role of miRs as potential biomarkers for CRC will be investigated through studies that show miR dysregulation in the plasma and stool of patients with CRC. Furthermore, the prognostic role of miRs in CRC will be critically evaluated.

miR-dysregulation in tumor samples

The first study of miR expression in colorectal tumor tissue compared to normal colonic tissue was reported in 2003 by Michael et al.⁶⁴ In this study, miR-200c was first isolated in normal colonic tissue and miR-143 and -145 were found to be downregulated in tumor tissue compared to normal colon tissue. Since then, many studies have compared the

dysregulation of different miRs in CRC samples in comparison to normal colonic mucosa. We have evaluated 46 different studies.⁶⁴⁻¹⁰⁹ The studies differ dramatically in the number of miRs evaluated (Range 1-723 miRs), number of samples used (4-197 samples), and number of dysregulated-miRs found (1-71). The fold dysregulation for each specific miR also differed between the studies. Table 1 summarizes the studies that were evaluated and the miR-dysregulation that was found.

Overall, 170 different miRs were found to be upregulated in CRC. Upregulation of miR-21 was demonstrated in 15 studies followed by miR-31, miR-135b, and miR-183 (upregulated in 11, 9, and 8 studies, respectively). Other 110 different miRs were found to be upregulated in only one study each (Table 1). A total of 127 different miRs were found to be downregulated in CRC. Downregulation of miR-145 was demonstrated in 15 studies, followed by miR-143, downregulated in 9 studies, followed by miR-1, miR-195, and miR-378 downregulated in six studies each.

miR-dysregulation in serum or plasma samples

The first report of miRs detected in the serum of colorectal patients was by Chen et al in 2008.¹¹⁰ Although the miRs detected are not specified, there were 69 miRs detected in sera of CRC patients but not in sera of normal controls. Interestingly, the authors note that a large number of miRs were commonly detected in both sera obtained from colorectal and lung cancer patients. The only specific miRs mentioned are miR-23a, miR-134, miR-146a, miR-221, and miR-222. Since then, 13 reports were published of 33 miRs dysregulated in the plasma of CRC patients. In several studies, miR upregulation was found to be both in plasma and tumor tissue of CRC patients.

Out of 95 miRs analyzed, Ng et al identified five miRs (miR-17-3p, -92, -95, -135b, -222) that were upregulated in both plasma and tissue CRC samples.¹¹¹ Furthermore, miR-17-3p and miR-92 plasma levels significantly decreased following surgery for CRC, indicating these specific miRs as potential markers for response to therapy. In 2010, Huang et al. identified that out of 12 miRs studied, only miR-29a and -92a were upregulated in plasma obtained from CRC patients.⁹⁶ It was further demonstrated that these two miRs were also upregulated in patients with advanced adenomas, indicating a possible role in CRC diagnosis. Out of the three miRs reported as upregulated in CRC most often (miR-21, miR-221, and miR-222) only miR-221 levels were upregulated to a degree that could serve as biomarker.¹¹² In the same study, conducted by Pu et al, the high levels of

miR-221 also correlated with prognosis as well as with p53 expression. Wang et al confirmed the upregulation of miR-21 in CRC serum samples and further demonstrated its upregulation in breast, esophageal, gastric, and lung cancer.¹¹³ Cheng et al identified that plasma upregulation of miR-141 in the plasma of CRC patients, was associated with metastatic disease, and correlated with CEA levels and poor prognosis.¹¹⁴ In a study that analyzed 380 miRs, Kanaan et al identified only miR-21 as a possible serum marker accurately differentiating between CRC patients and healthy individuals, with 90% sensitivity and high specificity.⁷⁶ Most recently, Wang et al identified a panel of 22 miRs (miR-10a, miR-19a, miR-22*, miR-24, miR-92a, miR-125a-5p, miR-141, miR-150, miR-188-3p, miR-192, miR-210, miR-221, miR-224*, miR-376a, miR-425*, miR-495, miR-572, miR-601, miR-720, miR-760, miR-let-7a, and -let-7e) that were dysregulated in CRC plasma samples with a fold changes greater than five.¹¹⁵ After validation of this panel in a cohort of 191 CRC patients, it was noted that miR-601 and miR-760 were significantly downregulated in CRC plasma samples and could serve as markers accurately differentiating between plasma samples of CRC patients and of healthy controls, as well as between the plasma of patients with advanced adenomas and the plasma of normal controls (AOC of 0.792 and 0.683, respectively).¹¹⁵ All the data of miR-dysregulation in plasma or serum of CRC patients are summarized in Table 2.

miR-dysregulation in stool samples

The first study reporting miR-dysregulation in stool samples of patients with CRC was conducted by Ahmed et al in 2009.¹¹⁶ Fourteen miRs were dysregulated, of which, seven miRs were upregulated (miR-20a, miR-21, miR-92, miR-96, miR-106a, miR-203, and miR-326), and seven downregulated (miR-16, miR-125b, miR-126, miR-143, miR-145, miR-320, and miR-484-5p). Link et al also identified differentiated upregulation of miR-21 and miR-106b in stool samples obtained from CRC patients as compared to stool samples obtained from healthy controls.¹¹⁷ Koga et al using a large cohort of 206 CRC patients and 134 healthy controls, identified upregulation of miR-17-92 cluster, miR-21, and miR-135 in stool samples.¹¹⁸ The overall sensitivity and specificity of a stool assay based on upregulation of these three miRs were 74% and 79%, respectively.

Hypermethylation may occur not only in promoters of protein-coding genes but also in miRs. Hypermethylation of miR-34a/b and miR-148a was found by Kalimutho et al in stool samples of 98% and 75% of the patients with CRC, respectively.¹¹⁹ Another

report by the same group showed miR-144 to be upregulated in stool samples of CRC patients. Therefore it may be used for CRC detection with a sensitivity and specificity of 74% and 87%, respectively.¹²⁰ Wu et al. further validated the upregulation of miR-21 and miR-92a in both tissue and stool samples and following surgical removal of the tumor, stool levels of miR-21 and miR-92a were significantly reduced.⁷² Nevertheless, only miR-92a was significantly upregulated in stool of patients with adenomatous polyps as compared to healthy controls. Li et al. identified downregulation of miR-143 and -145 in stool samples of CRC patients as compared to healthy control subjects.¹²¹ However, there was no statistically significant difference in the expression levels of both miR-21 and miR-106a between stool samples of CRC patients and stool samples of the healthy human subjects.¹²¹ Data on miR dysregulation in feces samples are summarized in Table 2.

In summary, RNA can be extracted from stool samples, and dysregulated miRs in CRC may be amplified and provide a platform for a stool assay for early detection or follow up of CRC patients. The value of such as assay in detection of adenomatous polyps is yet to be defined.

miR-dysregulation and prognosis

Since the costs of modern therapy are rapidly increasing to a point that health authorities will not be able to fund therapy for cancer patients, a substantial research effort is carried out in order to identify patients that will benefit most from a given therapy. Despite this research effort, very few prognostic or predictive biomarkers exist in clinical practice. miRs may serve as prognostic or predictive markers in CRC patients.

In 2006 Xi et al reported that CRC patients in stage I & II (n=4 each) and 16 patients in stage III & IV (n=16 each) with higher expression of miR-200c in their tumor samples had a shorter median survival compared to patients with lower expression (26 vs. 38 months, respectively).⁹³ Schetter et al demonstrated that miR-21 was associated with advanced AJCC-TNM stage, poor survival independent of other clinical covariates, and poor therapeutic outcome.⁶⁷ Diaz et al showed that downregulation of miR-106a predicted shortened disease-free survival and overall survival regardless of tumor stage.¹²² Downregulation of miR-17-5p correlated with disease free survival in CRC patients diagnosed in early stages of disease, however this did not reach statistical significance.¹²² In a different study, Schepeler et al demonstrated that the expression levels of eight miRs (upregulation of miR-1, -let-7b, -let-7d, and miR-126, as well as down-

regulation of miR-320, miR-420, miR-498, and miR-526c) correlated with recurrence-free survival in AJCC-stage II CRC patients by a multivariate analysis.⁸⁸ Kaplan-Meier survival curves showed that patients diagnosed with AJCC-stage II CRC bearing tumors with high expression of miR-320 or miR-498 showed a significant difference in progression-free survival compared to patients bearing tumors with low expression.⁸⁸ Yantiss et al demonstrated that CRC patients younger than 40 years of age had more aggressive tumors and upregulation of miR-20a, miR-21, miR-145, miR-181b, and miR-203.¹²³ Wang et al identified positive correlation between miR-31 and advanced AJCC-TNM stage.⁸² Schetter et al identified that miR-21 expression was associated with inflammatory cytokines expression (IL-6, IL-8, IL-10, IL-12a, and NOS2a) as well as cancer-specific mortality.¹²⁴ Pu et al showed that circulating plasma levels of miR-221 are associated with poor overall survival in CRC patients.¹¹² Chiang et al identified that low expression of miR-203 was correlated with tumor size and pT stage.⁷¹ Nielsen et al using a multivariate analysis, showed that upregulation of stromal miR-21 significantly correlates with shorter disease-free survival in AJCC-stage II colon (but not rectal) cancer patients.¹²⁵ Feng et al confirmed that upregulation of miR-21 was associated with advanced tumor stage.¹²⁶ Wang et al showed that downregulation of miR-195 was an independent predictor of overall survival and it was upregulated more often in CRC patients with lymph node metastasis and advanced tumor stage, compared to patients with early, node-negative disease.⁹⁴ Upregulation of miR-125b was shown by Nishida et al to be an independent predictor of advanced tumor size, tumor invasion, and poor prognosis of CRC patients using a multivariate analysis.¹²⁷ In multiple studies, miR-21 was shown to be associated with advanced disease and worse outcome. Furthermore, Shibuya et al demonstrated that upregulation of miR-21 was associated with venous invasion, liver metastasis, and advanced tumor stage. Another predictor of tumor spread was reported by the same group, showing upregulation of miR-155 was correlated with lymph node metastases.¹²⁸ The downregulation of miR-124 correlated with tumor grade and poor overall and disease-free survival. Another downregulated miR associated with poor outcome is miR-144, shown by Iwaya et al to correlate with venous invasion, liver metastasis, liver recurrence, and poor survival.¹²⁹ Upregulation of miR-10b was shown by Nishida et al to be associated with high incidence of lymphatic invasion and poor prognosis, as well as resistance to fluorouracil-based therapy.¹³⁰ Once again, miR-21 expression levels in tumor tissue as well as miR-155

were independent prognostic factors for both overall survival and disease-free survival in CRC patients, regardless of clinical stage.¹³¹ Cheng et al, confirmed once again, that CRC patients with disease recurrence had higher levels of miR-141 in their tumor-tissues.¹¹⁴ Zhu et al identified miR-9 to be upregulated in CRC patients with distant metastasis in comparison to CRC patients diagnosed in earlier disease stages (without distant metastasis).¹³⁰ Akçakaya et al reported that upregulation of miR-185 and downregulation of miR-133b were correlated with distant metastasis and poor survival in CRC patients.¹³² Downregulation of miR-345 was associated with lymph node metastasis and worse histological type in CRC patients in a study conducted by Tang et al.¹⁰⁹ A panel of miRs containing upregulation of miR-31, miR-10b, and miR-139-5p and downregulation of miR-143 was typical for mucinous phenotype.⁷⁴ Furthermore, progressively increasing levels of miR-10b expression in tumor tissues were observed in correlation with AJCC T-stage, T1 to T4 lesions and with AJCC stage I to IV disease.⁷⁴ Karaayvaz et al reported that miR-215 upregulation in tumor tissue is closely associated with poor CRC patients' overall survival.¹³³ Ma et al identified that miR-150 downregulation was associated with decreased overall survival and a worse response to adjuvant chemotherapy.¹⁰³ Similarly, Nie et al demonstrated that downregulation of miR-365 is correlated with cancer progression and poor survival in CRC patients.¹⁰⁵ Upregulation of a small panel of three miRNA reported by Vickers et al, including miR-21, miR-135a, and miR-335, correlated with a stage-associated differential upregulation in CRC patients, while upregulation of miR-206 in tumor tissue was associated with better outcome. They also reported that upregulation of miR-let7a was associated with metastatic disease.¹³⁴ Both Liu et al and Horiuchi et al showed once more that miR-21 upregulation significantly correlates with advanced clinical stage and poor cell differentiation.^{135,136} Wiessmann-Brenner et al analyzed 903 miRs and their association to recurrence in patients with either AJCC stage-I or II CRC.¹³⁷ Only miR-29a was associated with increased recurrence rate. High expression of miR-29a was associated with a longer disease-free survival, on both univariate and multivariate analyses.¹³⁷ Yamashita et al used a cohort of 144 CRC patients and identified that upregulation of miR-372 was associated with synchronous liver metastasis and was an independent prognostic factor.¹³⁸ Pichler et al re-confirmed that downregulation of miR-143 was an independent prognostic factor with respect to cancer specific survival.¹³⁹

Single nucleotide polymorphisms (SNP) occur-

ring in various regions of the human genome were shown to have significant value in both risk for developing cancer in a given population and as predictive markers. Ryan et al analyzed a single nucleotide polymorphism (SNP) in miR-608 in a large cohort of 245 CRC samples and 446 control patients.¹⁴⁰ A specific genotype (GG) was associated with increased survival in African Americans and decreased survival in Caucasians.¹⁴⁰ Lin et al confirmed these findings in a very large study with over 1,000 patients, 26 miRs,

and 41 SNPs.¹⁴¹ The same SNP (rs4919510) in miR-608 was associated with increased risk for both recurrence and death. Also, miR-219-1:rs213210 showed consistent association with CRC related death.¹⁴¹ Zhou et al confirmed that upregulation of miR-92a correlated with advanced disease-stage, lymph node and distant metastases, and poor overall survival.¹⁴² Nishimura et al identified that upregulation of miR-181a was an independent significant prognostic factor for CRC.¹⁴³ These data are summarized in Table 3.¹⁴³

Table I. miR dysregulation in colorectal tissue samples

| Dysregulation | Number of studies | miRs and references | |
|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Upregulated | 15 | miR-21 ⁶⁵⁻⁷⁹ | |
| | 11 | miR-31 ^{65,68,70,74,76,77,79-83} | |
| | 9 | miR-135b ^{65,68,74,76,79,84-87} | |
| | 8 | miR-183 ^{65,68,70,74,76,80,81,84} ; miR-20a ^{66,67,70,74,80,88-90} | |
| | 7 | miR-19a ^{65,68,70,74,87,89,90} ; miR-203 ^{66,68,70,73,81,91} ; miR-96 ^{65,68,70,79,84,86} | |
| | 5 | miR-18a ^{70,80,87,89,90} ; miR-92 ^{68,80,81,83,88} ; miR-181b ^{67,69,70,92,93} | |
| | 4 | miR-15b ^{68,73,93,94} ; miR-177 ^{81,89,90} ; miR-17-5p ^{66,68,70,80} ; miR-19b ^{70,74,89,90} ; miR-20 ^{65,68,81,95} ; miR-25 ^{68,70,81,90} ; miR-93 ^{70,74,81,90} ; miR-106a ^{66-68,70} ; miR-182 ^{68,70,74,84} ; miR-200c ^{68,83,92,93} ; miR-224 ^{68,70,84,87} | |
| | 3 | miR-15a ^{68,74,94} ; miR-29a ^{68,70,73} ; miR-95 ^{68,70,96} ; miR-103 ^{68,83,95} ; miR-106b ^{70,87,90} ; miR-130b ^{68,70,74} ; miR-142-3p ^{68,74,83} ; miR-148a ^{68,73,74} ; miR-221 ^{66,74,97} ; miR-191 ^{66,68,93} | |
| | 2 | Let-7f ^{73,74} ; Let-7g ^{68,92} ; miR-10a ^{66,68} ; miR-17-3p ^{68,84} ; miR-27a ^{68,73} ; miR-29b ^{70,73} ; miR-32 ^{66,84} ; miR-34a ^{68,70} ; miR-92a ^{72,90} ; miR-98 ^{68,74} ; miR-105 ^{68,86} ; miR-107 ^{66,68} ; miR-133b ^{73,81} ; miR-135a ^{68,81} ; miR-182 ^{68,84} ; miR-188 ^{83,84} ; miR-200a ^{68,88} ; miR-210 ^{68,83} ; miR-213 ^{66,68} ; miR-223 ^{66,81} ; miR-301b ^{76,87} ; miR-320 ^{68,88} ; miR-324-5p ^{68,74} ; miR-424 ^{73,87} ; miR-493 ^{76,86} ; miR-513a-5p ^{73,75} ; miR-552 ^{84,86} ; miR-584 ^{84,86} | |
| | 1 | Let-7a ⁷³ ; Let-7c ⁷³ ; miR-1 ⁷³ ; miR-7 ⁸⁶ ; miR-16 ⁹⁴ ; miR-18b ⁸² ; miR-19b-1 ⁸⁹ ; miR-20a ⁸² ; miR-227 ³ ; miR-22a ⁷³ ; miR-23a ⁷³ ; miR-23b ⁷³ ; miR-24-1 ⁶⁶ ; miR-24-2 ⁶⁶ ; miR-26a ⁷³ ; miR-26b ⁷³ ; miR-27b ⁷³ ; miR-29b-2 ⁶⁶ ; miR-30b ⁷³ ; miR-30c ⁶⁶ ; miR-33a ⁸⁴ ; miR-91 ⁹⁵ ; miR-92a-1 ⁸⁹ ; miR-99b ⁶⁶ ; miR-122a ⁶⁸ ; miR-125b ⁷³ ; miR-126 ⁶⁶ ; miR-128a ⁶⁸ ; miR-128b ⁶⁶ ; miR-130a ⁷³ ; miR-134 ⁶⁸ ; miR-135b ⁸⁵ ; miR-137 ⁶⁶ ; miR-141 ⁶⁸ ; miR-142-5p ⁶⁸ ; miR-145 ⁷³ ; miR-146 ⁶⁸ ; miR-147 ⁶⁸ ; miR-150 ⁶⁶ ; miR-151 ⁶⁸ ; miR-154 ⁶⁸ ; miR-155 ⁶⁶ ; miR-181a ⁶⁸ ; miR-181c ⁶⁸ ; miR-183 ⁸⁵ ; miR-186 ⁶⁸ ; miR-191 ⁸⁸ ; miR-193b ⁷³ ; miR-194 ⁶⁸ ; miR-196b ⁸² ; miR-197 ⁶⁸ ; miR-199a-3p ⁷³ ; miR-200a ⁸³ ; miR-200b ⁶⁸ ; miR-214 ⁷⁴ ; miR-215 ⁶⁸ ; miR-216 ⁶⁸ ; miR-219 ⁶⁸ ; miR-222 ⁶⁸ ; miR-296-3p ⁸⁶ ; miR-301 ⁶⁸ ; miR-302a ⁸⁸ ; miR-330 ⁶⁸ ; miR-331-3p ⁷³ ; miR-335 ⁸² ; miR-338 ⁶⁸ ; miR-338-3p ⁷³ ; miR-339 ⁶⁸ ; miR-362 ⁷⁶ ; miR-370 ⁶⁸ ; miR-373 ⁶⁸ ; miR-374 ⁶⁸ ; miR-374a ⁸² ; miR-382 ⁷⁶ ; miR-432 ⁸⁸ ; miR-451 ⁷³ ; miR-483-3p ⁸⁶ ; miR-492 ⁸⁸ ; miR-494 ⁷⁵ ; miR-500 ⁷⁵ ; miR-503 ⁸⁴ ; miR-510 ⁸⁸ ; miR-512-5p ⁸⁸ ; miR-513 ⁸⁸ ; miR-513b ⁷⁵ ; miR-513c ⁷⁵ ; miR-526c ⁸⁸ ; miR-527 ⁸⁸ ; miR-542-5p ⁸⁴ ; miR-549 ⁸⁶ ; miR-582-5p ⁷⁴ ; miR-592 ⁸⁶ ; miR-622 ⁹⁸ ; miR-708 ⁷⁶ ; miR-766 ⁸⁵ ; miR-886 ⁷⁶ ; miR-892b ⁷⁵ ; miR-938 ⁹⁸ ; miR-1238 ⁹⁸ ; miR-1247 ⁸⁶ ; miR-1260 ⁷³ ; miR-1269 ⁸⁶ ; miR-1290 ⁸⁸ ; miR-1827 ⁸⁶ ; miR-3144-3p ⁸⁶ ; miR-3180-3p ⁸⁶ ; miR-4326 ⁸⁶ ; HS_287 ⁸⁴ ; HS_29 ⁸⁴ | |
| | Downregulated | 15 | miR-145 ^{64,65,68,70,75-77,80-83,85,86,88,99} |
| | | 9 | miR-143 ^{64,70,75,77,80,81,83,85,99} |
| | | 7 | miR-170 ^{74-76,84,86,98} ; miR-195 ^{68,70,75,85,86,94,100} ; miR-378 ^{70,75,84-87} |
| | | 5 | miR-133a ^{70,74-76,84} ; miR-133b ^{65,75,76,85,98} ; miR-139-5p ^{74-76,85,86} ; miR-192 ^{75,81,83,85,101} ; miR-215 ^{74,75,81,85,101} |
| | | 4 | miR-30a-3p ^{65,68,70,84} ; miR-375 ^{74,84,85,102} ; miR-422a ^{70,74,85,86} |
| 3 | | miR-10b ^{70,75,84} ; miR-26b ^{81,85,88} ; miR-30b ^{75,85,88} ; miR-30c ^{70,75,85} ; miR-138 ^{76,85,98} ; miR-139 ^{68,70,84} ; miR-194 ^{75,85,101} ; miR-363 ^{75,84,86} ; miR-378 ^{75,85,87} ; miR-490-3p ^{75,86,98} ; miR-497 ^{70,75,84} ; miR-551b ^{73,84,86} | |
| 2 | | miR-97 ^{6,84} ; miR-9 ^{75,84} ; miR-16 ^{81,85} ; miR-28-3p ^{73,74} ; miR-30a ^{75,85} ; miR-30a-5p ^{70,84} ; miR-30e ^{75,85} ; miR-101 ^{85,88} ; miR-125b ^{68,83} ; miR-137 ^{75,84} ; miR-149 ^{68,74} ; miR-150 ^{73,103} ; miR-192 ^{75,85} ; miR-204 ^{74,76} ; miR-320a ^{97,104} ; miR-328 ^{65,84} ; miR-365 ^{75,105} ; miR-486-5p ^{85,86} ; miR-598 ^{75,85} ; miR-642 ^{74,84} | |
| 1 | let-7a ⁸¹ ; miR-7-1 ⁷⁵ ; miR-9-3 ⁶⁶ ; miR-20b ⁸⁴ ; miR-22 ¹⁰⁶ ; miR-24-1 ⁷⁵ ; miR-26a ⁸⁵ ; miR-27b ⁷⁵ ; miR-28-5p ⁷⁵ ; miR-30a ⁷⁵ ; miR-30e ⁸⁵ ; miR-31 ⁷⁵ ; miR-31 ⁷⁵ ; miR-34 ¹⁰⁷ ; miR-34a ¹⁰⁷ ; miR-99a ⁸⁵ ; miR-100 ⁸⁵ ; miR-113 ⁷³ ; miR-122 ⁷⁵ ; miR-124a ⁶⁵ ; miR-125a ⁷⁰ ; miR-126 ¹⁰⁸ ; miR-127-3p ⁸⁵ ; miR-129 ⁶⁵ ; miR-133 ⁶⁹ | | |

miR-139-3p⁷³; miR-140-5p⁸⁵; miR-143⁷⁵; miR-144⁷⁵;
 miR-144^{*85}; miR-147⁸⁴; miR-186⁸⁵; miR-190⁸⁵; miR-191⁸¹;
 miR-193b⁸³; miR-196a⁸¹; miR-200b⁸⁵; miR-203⁷¹; miR-212⁸³;
 miR-214⁸³; miR-218⁷⁵; miR-299-5p⁷⁴; miR-342-3p⁸⁵;
 miR-345¹⁰⁹; miR-362-3p⁷⁵; miR-376c⁸⁵; miR-378c⁸⁶; miR-383⁸⁶;
 miR-411⁸⁵; miR-422b⁷⁰; miR-424⁹⁴; miR-451⁸⁵; miR-455⁸⁸;
 miR-484⁸⁸; miR-485-3p⁷⁴; miR-486⁸⁴; miR-490-5p⁹⁸;
 miR-500⁷³; miR-501-5p⁷³; miR-511⁸⁴; miR-582-5p⁷⁵;
 miR-590-5p⁷⁵; miR-627⁷³; miR-628-3p⁸⁶; miR-628-5p⁸⁶;
 miR-629⁷³; miR-636⁸⁵; miR-650⁸⁴; miR-760⁷³; miR-885-5p⁷⁶;
 miR-886-3p⁷⁴; miR-892b⁷³; miR-1288⁷³; miR-1290⁷³; miR-1295⁷³; miR-1297⁸⁶; miR-1299⁷³; miR-1305⁷³;
 miR-3151⁸⁶; miR-3163⁸⁶; miR-3622a-5p⁸⁶; miR-3656⁸⁶

Table 2. miR dysregulation in serum or fecal samples

| Source | Dysregulation | Number of studies | miRs and references |
|--------|---------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Serum | Upregulated | 3 | miR-221 ^{110,112,115} |
| | | 2 | miR-21 ^{76,113} ; miR-92a ^{96,115} ; miR-222 ^{110,111} |
| | | 1 | miR-Let-7e ¹¹⁵ ; miR-17-3p ¹¹¹ ; miR-19a ¹¹⁵ ; miR-22 ^{*115} ; miR-23a ¹¹⁰ ; miR-24 ¹¹⁵ ; miR-29a ⁹⁶ ; miR-92 ¹¹¹ ; miR-95 ¹¹¹ ; miR-125-5p ¹¹⁵ ; miR-134 ¹¹⁰ ; miR-135b ¹¹¹ ; miR-141 ¹¹⁴ ; miR-146 ¹¹⁰ ; miR-210 ¹¹⁵ ; miR-376a ¹¹⁵ |
| | Downregulated | 1 | miR-10a ¹¹⁵ ; miR-141 ¹¹⁵ ; miR-150 ¹¹⁵ ; miR-188-3p ¹¹⁵ ; miR-192 ¹¹⁵ ; miR-224 ^{*115} ; miR-425 ^{*115} ; miR-495 ¹¹⁵ ; miR-572 ¹¹⁵ ; miR-601 ¹¹⁵ ; miR-720 ¹¹⁵ ; miR-760 ¹¹⁵ ; Let-7a ¹¹⁵ |
| Feces | Upregulated | 4 | miR-21 ^{72,116-118} |
| | | 1 | miR-17 ¹¹⁸ ; miR-18a ¹¹⁸ ; miR-19a ¹¹⁸ ; miR-19b-1 ¹¹⁸ ; miR-20a ¹¹⁶ ; miR-92 ¹¹⁶ ; miR-92a ⁷² ; miR-96 ¹¹⁶ ; miR-106a ¹¹⁶ ; miR-106b ¹¹⁷ ; miR-135 ¹¹⁸ ; miR-144 ¹²⁰ ; miR-203 ¹¹⁶ ; miR-326 ¹¹⁶ |
| | Downregulated | 2 | miR-143 ^{116,121} ; miR-145 ^{116,121} |
| | | 1 | miR-16 ¹¹⁶ ; miR-125b ¹¹⁶ ; miR-126 ¹¹⁶ ; miR-320 ¹¹⁶ ; miR-484-5p ¹¹⁶ |

Table 3. Studies on the association between miR dysregulation and staging or prognosis

| Author | Year | miR | N | Endpoint |
|-------------------------|------|--------------------|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Schetter ¹²⁴ | 2009 | miR-21 | 115 | High miR-21 expression was associated with higher rates of cancer-specific mortality (P <0.0001) |
| Horiuchi ¹³⁶ | 2011 | miR-21 | 326 | PDCD4 mRNA levels were negatively regulated by miR-21 in each tumor stage of CRC. OS and DFS rates of low PDCD4 patients were significantly worse than those of patients with high expression. |
| Schetter ⁶⁷ | 2008 | miR-21 | 197 | 5-year cancer-specific survival rate: 57.5% - Maryland cohort and 49.5% - Hong Kong cohort High miR-21 expression associated with advanced TNM staging, poor survival, and poor therapeutic outcome. |
| Feng ¹²⁶ | 2011 | miR-21 | 54 | Highly expressed miR-21 was more common in stage IV cancer than in stage II and stage III cancer |
| Liu ¹³⁵ | 2011 | miR-21 | 42 | High expression of miR-21 was significantly correlated with advanced clinical stage and poor cell differentiation. miR-21 may predict pathological tumor response to chemotherapy. |
| Shibuya ¹²⁸ | 2010 | miR-21, miR-155 | 156 | High miR-21 expression was significantly associated with venous invasion, liver metastasis and tumor stage. High miR-155 expression was significantly correlated with lymph node metastases. The OS and DFS rates of patients with high miR-21, and high miR-155 expression were significantly worse than those of patients with low expression. |

| | | | | |
|--------------------------|------|-----------------------------------------------|------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | | | | miR-21 and miR-155 expression levels in CRC tissue were independent prognostic factors for OS and DFS. |
| Nielsen ¹²⁵ | 2011 | miR-21 | 130 - stage II colon cancer 67 - stage II rectal cancer | Higher miR-21 expression correlated significantly with shorter DFS (p = 0.004) in the stage II colon cancer patient group, but not in the stage II rectal cancer group. |
| Vickers ¹³⁴ | 2012 | miR-335, miR-206, miR-135a, miR-21, miR let7a | 34 | Increased expression of miR-21, miR-135a and miR-335 was associated with clinical progression of CRC. miR-206 demonstrated an opposite trend. miR-let7a, showed elevated expression in metastatic disease compared to normal mucosa or non-metastatic disease, and only in KRAS mutation positive tumors. Prognostic signature of miR 21,135a, 335, 206 and let-7a for detecting the presence of metastases had a specificity of 87% and sensitivity of 76% for the presence of metastases. |
| Xi ⁹³ | 2006 | hsa-miR-200c | 24 | Shorter median survival- (26 vs. 38 months) for patients with hsa-miR-200c over expression. |
| Diaz ¹²² | 2008 | miR-17-5p miR-106a | 110 | Lower levels of miR-17-5p and miR-106a were associated with pathological tumor features of poor prognosis. Downregulation of miR-106a predicted shortened DFS (P = 0.03) and OS (P = 0.04), independent of tumor stage. |
| Schepeler ⁸⁸ | 2008 | miR-320 miR-498 | 37 | Stage II colon tumors with high expression of miR-320 or miR-498 showed a significant difference in progression-free survival compared with tumors with low expression. |
| Wang ⁸² | 2009 | miR-31 | 98 | Higher miR-31 expression was positively related to advanced TNM stage (p = 0.026) and deeper invasion of tumors (p = 0.024). |
| Pu ¹¹² | 2010 | miR-221 | 103 | Patients with higher plasma miR-221 levels have a dramatically lower survival rate than that in the low expression group (P < 0.05) |
| Chiang ⁷¹ | 2011 | miR-203 | 107 | Significant low expression of miR-203 associated with increased tumor size (p=0.015) and an advanced pT stage (p=0.005) |
| Wang ⁹⁴ | 2012 | miR-195 | 85 | Reduced expression of miR-195 occurred more often in patients with lymph node metastasis and advanced tumor stage (all P < 0.01). Patients with reduced miR-195 had a poor OS (P < 0.01). Reduced expression of miR-195 was an independent predictor of OS. |
| Nishida ¹²⁷ | 2011 | miR-125b | 89 | The high miR-125b expression group showed a greater incidence of advanced tumor size and tumor invasion, as well as a significantly poorer prognosis compared to the low miR-125b expression group (P<0.05). Multivariate analysis indicated that high miR-125b expression was an independent prognostic factor for survival. |
| Cheng ¹¹⁴ | 2011 | miR-141 | 102- First cohort 156- Second cohort | Circulating plasma miR-141 was significantly associated with stage IV colon cancer. High levels of plasma miR-141 predicted poor survival. |
| Zhu ¹³⁰ | 2012 | miR-9 | 25 | Significant up regulation of miR-9 expression was observed in patients with distant metastasis (P < 0.001). |
| Akçakaya ¹³² | 2011 | miR-185, miR-133b | 50 | High expression of miR-185 and low expression of miR-133b were correlated with poor survival (p=0.001 and 0.028, respectively) and metastasis (p=0.007 and 0.036, respectively). |
| Tang ¹⁰⁹ | 2011 | miR-345 | 31 | Low expression of miR-345 was associated with lymph node metastasis and worse histological type. |
| Chang ⁷⁴ | 2011 | miR-31, miR-139-5p, miR-143, miR-10b | 102 | Elevated expression of miR-31, miR10b (p=0.004) and miR-139-5p (p<0.001) and reduced expression of miR-143 (p=0.016) were associated with aggressive mucinous phenotype. Progressively increasing levels of miR-10b expression were observed from T1 to T4 lesions and from stage I to IV disease. |
| Karaayvaz ¹³³ | 2011 | miR-215 | 34 | High levels of miR-215 expression (P=.025) are closely associated with poor patient's OS. |
| Ma ¹⁰³ | 2012 | miR-150 | 239 | Patients with low miR-150 expression had shorter survival and a worse response to adjuvant chemotherapy than patients with high expression. |
| Nie ¹⁰⁵ | 2011 | miR-365 | 97 | Decreased levels of miR-365 were related to colon cancer progression. Low miR-365 levels significantly correlated with reduced DFS. |

| | | | | |
|----------------------------------|------|-------------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Nishida ¹³¹ | 2012 | miR-10b | 88 | miR-10b over expression was associated with high incidence of lymphatic invasion (P = 0.0257) and poor prognosis (P = 0.0057). High miR-10b expression is an independent prognostic factor for survival. Over expression of miR-10b confers chemoresistance in colorectal cancer cells to 5-FU. |
| Wiessmann-Brenner ¹³⁷ | 2012 | miR-29a | 110 | High expression of miR-29a was associated with a longer DFS in stage II CRC patients. |
| Yamashita ¹³⁸ | 2012 | miR-372 | 144 | High miR-372 expression was an independent prognostic factor (p = 0.006). High miR-372 expression was associated with synchronous liver metastasis (p = 0.035). |
| Pichler ¹³⁹ | 2012 | miR-143 | 77 | Low levels of miR-143 were an independent prognostic factor with respect to cancer specific survival (p=0.024). miR-143 expression levels serve as an independent prognostic biomarker for CRC in KRAS wild-type patients. |
| Ryan ¹⁴⁰ | 2012 | miR-608 | 245 | A specific genotype (GG) was associated with increased survival in African Americans and decreased survival in Caucasians. |
| Lin ¹⁴¹ | 2012 | miR-608, mir219-1 | 1097 | mir608:rs4919510 was associated with increased risk for both recurrence and death in patients with stage III disease. mir219-1:rs213210 showed consistent association with death. Patients carrying the variant genotypes at both sites exhibited a 5.6-fold increased risk of death. |
| Zhuo ¹⁴² | 2012 | miR-92a | 82 | High expression of miR-92a correlated with advanced clinical stage (p = 0.025), lymph node metastases (p = 0.015), distant metastases (p = 0.046), and poor OS (p = 0.001). Increased expression of miR-92a was an independent predictor of OS. |
| Wang ¹⁴⁴ | 2012 | miR-124 | 96 | Decreased miR-124 expression correlated significantly with the grade of differentiation (P = 0.021). Downregulated miR-124 was significantly correlated with worse prognosis, both in terms of OS (P = 0.017) and DFS (P = 0.014). Downregulated miR-124 was demonstrated as an independent prognostic factor for OS (P = 0.002) and DFS (P = 0.002). |
| Iwaya ¹²⁹ | 2012 | miR-144 | 137 | Low expression levels of miR-144 were associated with enhanced malignant potential such as venous invasion (P = 0.0013), liver metastasis (P = 0.08), liver recurrence (P = 0.0058) and poor prognosis (P = 0.0041). Low miR-144 expression was an independent prognostic factor for survival. |
| Nishimura ¹⁴³ | 2012 | miR-181a | 162 | Patients with high expression of miR-181a had a significantly poorer prognosis than those with low expression (P=0.011). High miR-181a expression was an independent significant prognostic factor for CRC. However, no correlation was observed between miR-181a expression and clinicopathological parameters. |

Summary

Large body of evidence has accumulated in recent years regarding the dysregulation of miRs in CRC. Multiple different miRs were shown to be up-regulated or downregulated in tumor tissue (Table 1). The variability in results is caused by the fact that most studies were small sampled in size and used heterogenic groups of patients. Different sources of tissue used for the studies, paraffin-embedded tissue versus frozen tissue, and the timing from colon resection to fixation either in formaldehyde or in liquid nitrogen may all have a significant impact on the quality, quantity, and composition of RNA extracted. The most frequently upregulated miRs are miR-21 in fifteen studies, miR-31 in eleven studies, and miR-135b

in nine studies while the most downregulated are: miR-145 in fifteen studies and miR 143 in nine (see Table 1 for more details).

In stool samples, the content of microRNA may change following bowel preparation. Therefore, stool samples used from patients undergoing colonoscopy may vary in their miR content compared to stool samples obtained from patients that did not undergo bowel preparation. Again, the time interval between stool sampling and freezing may have a major impact on RNA degradation. The most frequently upregulated miR in stool samples is miR-21 in four studies, while the most downregulated are miR-145 and miR 143 in two studies each (see Table 2 for more details).

The variability in the results obtained from blood sampling may be attributed to many factors: some studies report plasma dysregulation of miRs while

others used serum, the timing between blood sampling and separation of the serum/plasma is critical, and so is the methods in which the samples were stored. On top of that, different microRNA-enriched RNA extraction kits were used with variability in performance as well as heterogenic patient populations. The most frequently upregulated miRs are miR-221 in three studies, miR-21, miR-92a, and miR-222 in two studies each, and there were many miRs downregulated in one study each (see Table 2 for more details). There is new and interesting data showing that miRs are carried in the plasma by small micelles, therefore may resist the hostile environment of peripheral blood.

The current AJCC staging system is based on histopathologic parameters. Additional molecular prognostic markers are essential for better stratification of CRC patients. The accumulated data of dysregulated miRs associated with better or worse outcome may be incorporated into staging CRC patients and used for better treatment selection. There is also important data regarding the potential role of miRs in the regulation of the carcinogenesis process with possible implications for therapy; however, this is beyond scope of the current review. Overall, the vast amount of data generated by multiple studies should be verified in a uniform and controlled manner in order to establish miRs with a potential to become future predictive or prognostic markers.

Competing Interests

The authors have declared that no competing interest exists.

References

- Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012;62:10-29.
- Rex DK, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *Am J Gastroenterol* 2009;104:739-50.
- Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008;134:1570-95.
- Cash BD, Banerjee S, Anderson MA, et al. Ethnic issues in endoscopy. *Gastrointest Endosc* 2010;71:1108-12.
- O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J Natl Cancer Inst* 2004;96:1420-5.
- Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;319:525-32.
- Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N Engl J Med* 1993;329:1977-81.
- Mandel JS, Bond JH, Church TR, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med* 1993;328:1365-71.
- Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996;348:1467-71.
- Hardcastle JD, Chamberlain JO, Robinson MH, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996;348:1472-7.
- Fraser CG, Allison JE, Halloran SP, Young GP. A proposal to standardize reporting units for fecal immunochemical tests for hemoglobin. *J Natl Cancer Inst* 2012;104:810-4.
- Quintero E, Castells A, Bujanda L, et al. Colonoscopy versus fecal immunochemical testing in colorectal-cancer screening. *N Engl J Med* 2012;366:697-706.
- Selby JV, Friedman GD, Quesenberry CP, Jr., Weiss NS. A case-control study of screening sigmoidoscopy and mortality from colorectal cancer. *N Engl J Med* 1992;326:653-7.
- Imperiale TF, Wagner DR, Lin CY, Larkin GN, Rogge JD, Ransohoff DF. Risk of advanced proximal neoplasms in asymptomatic adults according to the distal colorectal findings. *N Engl J Med* 2000;343:169-74.
- Jentschura D, Raute M, Winter J, Henkel T, Kraus M, Manegold BC. Complications in endoscopy of the lower gastrointestinal tract. Therapy and prognosis. *Surg Endosc* 1994;8:672-6.
- Rex DK, Lehman GA, Ulbright TM, et al. Colonic neoplasia in asymptomatic persons with negative fecal occult blood tests: influence of age, gender, and family history. *Am J Gastroenterol* 1993;88:825-31.
- Fork FT. Double contrast enema and colonoscopy in polyp detection. *Gut* 1981;22:971-7.
- Rex DK, Weddle RA, Lehman GA, et al. Flexible sigmoidoscopy plus air contrast barium enema versus colonoscopy for suspected lower gastrointestinal bleeding. *Gastroenterology* 1990;98:855-61.
- Hawes RH. Does virtual colonoscopy have a major role in population-based screening? *Gastrointestinal endoscopy clinics of North America* 2002;12:85-91.
- Galdino GM, Yee J. Carpet lesion on CT colonography: a potential pitfall. *AJR Am J Roentgenol* 2003;180:1332-4.
- Sidransky D, Tokino T, Hamilton SR, et al. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992;256:102-5.
- Ahlquist DA, Shuber AP. Stool screening for colorectal cancer: evolution from occult blood to molecular markers. *Clin Chim Acta* 2002;315:157-68.
- Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. *Nature* 1997;386:623-7.
- Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;260:816-9.
- Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* 1994;7:536-40.
- Schubel KE, Chen W, Cope L, et al. Comparing the DNA hypermethylome with gene mutations in human colorectal cancer. *PLoS genetics* 2007;3:1709-23.
- Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. *Science* 2007;318:1108-13.
- Samowitz WS. Genetic and epigenetic changes in colon cancer. *Experimental and molecular pathology* 2008;85:64-7.
- Nosho K, Irahara N, Shima K, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. *PLoS One* 2008;3:e3698.
- Toyota M, Ohe-Toyota M, Ahuja N, Issa JP. Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype. *Proc Natl Acad Sci U S A* 2000;97:710-5.
- Koornstra JJ, de Jong S, Hollema H, de Vries EG, Kleibeuker JH. Changes in apoptosis during the development of colorectal cancer: a systematic review of the literature. *Crit Rev Oncol Hematol* 2003;45:37-53.
- Ahlquist DA, Skoletsky JE, Boynton KA, et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000;119:1219-27.
- Dong SM, Traverso G, Johnson C, et al. Detecting colorectal cancer in stool with the use of multiple genetic targets. *J Natl Cancer Inst* 2001;93:858-65.
- Syngal S, Stoffel E, Chung D, et al. Detection of stool DNA mutations before and after treatment of colorectal neoplasia. *Cancer* 2006;106:277-83.
- Tagore KS, Lawson MJ, Yucaitis JA, et al. Sensitivity and specificity of a stool DNA multitarget assay panel for the detection of advanced colorectal neoplasia. *Clinical colorectal cancer* 2003;3:47-53.
- Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704-14.
- Ahmed FE, James SI, Lysle DT, et al. Improved methods for extracting RNA from exfoliated human colonocytes in stool and RT-PCR analysis. *Dig Dis Sci* 2004;49:1889-98.

38. Kuusela P, Jalanko H, Roberts P, et al. Comparison of CA 19-9 and carcinoembryonic antigen (CEA) levels in the serum of patients with colorectal diseases. *Br J Cancer* 1984;49:135-9.
39. Wang FM, Tsai LC, Chang ZN, Han SH, Tsao D. The significance of CA19-9 tumor antigen in the serum of patients with carcinomas. *Proceedings of the National Science Council, Republic of China Part B, Life sciences* 1985;9:119-25.
40. Kornek G, Depisch D, Temsch EM, Scheithauer W. Comparative analysis of cancer-associated antigen CA-195, CA 19-9 and carcinoembryonic antigen in diagnosis, follow-up and monitoring of response to chemotherapy in patients with gastrointestinal cancer. *Journal of cancer research and clinical oncology* 1991;117:493-6.
41. Kuusela P, Haglund C, Roberts PJ. Comparison of a new tumour marker CA 242 with CA 19-9, CA 50 and carcinoembryonic antigen (CEA) in digestive tract diseases. *Br J Cancer* 1991;63:636-40.
42. Meltzer PS. Cancer genomics: small RNAs with big impacts. *Nature* 2005;435:745-6.
43. Gusev Y, Schmittgen TD, Lerner M, Brackett D. Computational analysis of biological functions and pathways collectively targeted by co-expressed microRNAs in cancer. *BMC bioinformatics* 2007;8 Suppl 7:S16.
44. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993;75:843-54.
45. Kim VN. Small RNAs: classification, biogenesis, and function. *Mol Cells* 2005;19:1-15.
46. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004;14:1902-10.
47. van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. *Nat Rev Cancer* 2011;11:644-56.
48. Santarpia L, Nicoloso M, Calin GA. MicroRNAs: a complex regulatory network drives the acquisition of malignant cell phenotype. *Endocr Relat Cancer* 2010;17:F51-75.
49. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008;9:102-14.
50. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281-97.
51. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. *Cell* 2005;122:6-7.
52. Lovat F, Valeri N, Croce CM. MicroRNAs in the Pathogenesis of Cancer. *Semin Oncol* 2011;38:724-33.
53. Sandhu S, Garzon R. Potential Applications of MicroRNAs in Cancer Diagnosis, Prognosis, and Treatment. *Semin Oncol* 2011;38:781-7.
54. Marcucci G, Radmacher MD, Mrozek K, Bloomfield CD. MicroRNA expression in acute myeloid leukemia. *Curr Hematol Malig Rep* 2009;4:83-8.
55. Wang J, Sen S. MicroRNA functional network in pancreatic cancer: from biology to biomarkers of disease. *J Biosci* 2011;36:481-91.
56. Fanini F, Vannini I, Amadori D, Fabbri M. Clinical Implications of MicroRNAs in Lung Cancer. *Semin Oncol* 2011;38:776-80.
57. Li SD, Zhang JR, Wang YQ, Wan XP. The role of microRNAs in ovarian cancer initiation and progression. *J Cell Mol Med* 2010;14:2240-9.
58. Wu WK, Lee CW, Cho CH, et al. MicroRNA dysregulation in gastric cancer: a new player enters the game. *Oncogene* 2010;29:5761-71.
59. Catto JW, Alcaraz A, Bjartell AS, et al. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. *Eur Urol* 2011;59:671-81.
60. Negrini M, Gramantieri L, Sabbioni S, Croce CM. microRNA involvement in hepatocellular carcinoma. *Anticancer Agents Med Chem* 2011;11:500-21.
61. Menon MP, Khan A. Micro-RNAs in thyroid neoplasms: molecular, diagnostic and therapeutic implications. *J Clin Pathol* 2009;62:978-85.
62. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. *J Clin Endocrinol Metab* 2008;93:1600-8.
63. Turchinovich A, Weiz L, Langheinza A, Burwinkel B. Characterization of extracellular circulating microRNA. *Nucleic acids research* 2011;39:7223-33.
64. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Molecular cancer research : MCR* 2003;1:882-91.
65. Bandres E, Cubedo E, Agirre X, et al. Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. *Molecular cancer* 2006;5:29.
66. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 2006;103:2257-61.
67. Schetter AJ, Leung SY, Sohn JJ, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* 2008;299:425-36.
68. Monzo M, Navarro A, Bandres E, et al. Overlapping expression of microRNAs in human embryonic colon and colorectal cancer. *Cell research* 2008;18:823-33.
69. Schmitz KJ, Hey S, Schinwald A, et al. Differential expression of microRNA 181b and microRNA 21 in hyperplastic polyps and sessile serrated adenomas of the colon. *Virchows Arch* 2009;455:49-54.
70. Arndt GM, Dossey L, Cullen LM, et al. Characterization of global microRNA expression reveals oncogenic potential of miR-145 in metastatic colorectal cancer. *BMC Cancer* 2009;9:374.
71. Chiang Y, Song Y, Wang Z, et al. Aberrant expression of miR-203 and its clinical significance in gastric and colorectal cancers. *J Gastrointest Surg* 2011;15:63-70.
72. Wu CW, Ng SS, Dong YJ, et al. Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. *Gut* 2012;61:739-45.
73. Slattery ML, Wolff E, Hoffman MD, Pellatt DF, Milash B, Wolff RK. MicroRNAs and colon and rectal cancer: differential expression by tumor location and subtype. *Genes Chromosomes Cancer* 2011;50:196-206.
74. Chang KH, Miller N, Kheirleisid EA, et al. MicroRNA signature analysis in colorectal cancer: identification of expression profiles in stage II tumors associated with aggressive disease. *Int J Colorectal Dis* 2011;26:1415-22.
75. Mosakhani N, Sarhadi VK, Borze I, et al. MicroRNA profiling differentiates colorectal cancer according to KRAS status. *Genes Chromosomes Cancer* 2012;51:1-9.
76. Kanaan Z, Rai SN, Eichenberger MR, et al. Plasma miR-21: a potential diagnostic marker of colorectal cancer. *Ann Surg* 2012;256:544-51.
77. Slaby O, Svoboda M, Fabian P, et al. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. *Oncology* 2007;72:397-402.
78. Chang KH, Mestdagh P, Vandesompele J, Kerin MJ, Miller N. MicroRNA expression profiling to identify and validate reference genes for relative quantification in colorectal cancer. *BMC Cancer* 2010;10:173.
79. Xu XM, Qian JC, Deng ZL, et al. Expression of miR-21, miR-31, miR-96 and miR-135b is correlated with the clinical parameters of colorectal cancer. *Oncology letters* 2012;4:339-45.
80. Motoyama K, Inoue H, Takatsuno Y, et al. Over- and under-expressed microRNAs in human colorectal cancer. *Int J Oncol* 2009;34:1069-75.
81. Earle JS, Luthra R, Romans A, et al. Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. *The Journal of molecular diagnostics : JMD* 2010;12:433-40.
82. Wang CJ, Zhou ZG, Wang L, et al. Clinicopathological significance of microRNA-31, -143 and -145 expression in colorectal cancer. *Dis Markers* 2009;26:27-34.
83. Chen X, Guo X, Zhang H, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene* 2009;28:1385-92.
84. Sarver AL, French AJ, Borrallho PM, et al. Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states. *BMC Cancer* 2009;9:401.
85. Faltejskova P, Svoboda M, Srutova K, et al. Identification and functional screening of microRNAs highly deregulated in colorectal cancer. *J Cell Mol Med* 2012.
86. Hamford J, Stangeland AM, Hughes T, et al. Differential expression of miRNAs in colorectal cancer: comparison of paired tumor tissue and adjacent normal mucosa using high-throughput sequencing. *PLoS One* 2012;7:e34150.
87. Wang YX, Zhang XY, Zhang BF, Yang CQ, Chen XM, Gao HJ. Initial study of microRNA expression profiles of colonic cancer without lymph node metastasis. *Journal of digestive diseases* 2010;11:50-4.
88. Scheepeler T, Reinert JT, Ostenfeld MS, et al. Diagnostic and prognostic microRNAs in stage II colon cancer. *Cancer Res* 2008;68:6416-24.
89. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, et al. MiR-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. *Br J Cancer* 2009;101:707-14.
90. Nishida N, Nagahara M, Sato T, et al. Microarray analysis of colorectal cancer stromal tissue reveals upregulation of two oncogenic miRNA clusters. *Clin Cancer Res* 2012;18:3054-70.

91. Kulda V, Pesta M, Topolcan O, et al. Relevance of miR-21 and miR-143 expression in tissue samples of colorectal carcinoma and its liver metastases. *Cancer genetics and cytogenetics* 2010;200:154-60.
92. Nakajima G, Hayashi K, Xi Y, et al. Non-coding MicroRNAs hsa-let-7g and hsa-miR-181b are Associated with Chemoresponse to S-1 in Colon Cancer. *Cancer genomics & proteomics* 2006;3:317-24.
93. Xi Y, Formentini A, Chien M, et al. Prognostic Values of microRNAs in Colorectal Cancer. *Biomarker insights* 2006;2:113-21.
94. Wang X, Wang J, Ma H, Zhang J, Zhou X. Downregulation of miR-195 correlates with lymph node metastasis and poor prognosis in colorectal cancer. *Med Oncol* 2012;29:919-27.
95. Luo H, Zou J, Dong Z, Zeng Q, Wu D, Liu L. Up-regulated miR-17 promotes cell proliferation, tumour growth and cell cycle progression by targeting the RND3 tumour suppressor gene in colorectal carcinoma. *The Biochemical journal* 2012;442:311-21.
96. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer* 2010;127:118-26.
97. Sun K, Wang W, Zeng JJ, Wu CT, Lei ST, Li GX. MicroRNA-221 inhibits CDKN1C/p57 expression in human colorectal carcinoma. *Acta pharmacologica Sinica* 2011;32:375-84.
98. Balaguer F, Moreira L, Lozano JJ, et al. Colorectal cancers with microsatellite instability display unique miRNA profiles. *Clin Cancer Res* 2011;17:6239-49.
99. Akao Y, Nakagawa Y, Naoe T. MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol Rep* 2006;16:845-50.
100. Liu L, Chen L, Xu Y, Li R, Du X. microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. *Biochem Biophys Res Commun* 2010;400:236-40.
101. Chiang Y, Song Y, Wang Z, et al. microRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. *Experimental and therapeutic medicine* 2012;3:560-6.
102. Dai X, Chiang Y, Wang Z, et al. Expression levels of microRNA-375 in colorectal carcinoma. *Molecular medicine reports* 2012;5:1299-304.
103. Ma Y, Zhang P, Wang F, et al. miR-150 as a potential biomarker associated with prognosis and therapeutic outcome in colorectal cancer. *Gut* 2012;61:1447-53.
104. Zhang Y, He X, Liu Y, et al. microRNA-320a inhibits tumor invasion by targeting neuropilin 1 and is associated with liver metastasis in colorectal cancer. *Oncol Rep* 2012;27:685-94.
105. Nie J, Liu L, Zheng W, et al. microRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting Cyclin D1 and Bcl-2. *Carcinogenesis* 2012;33:220-5.
106. Yamakuchi M, Yagi S, Ito T, Lowenstein CJ. MicroRNA-22 regulates hypoxia signaling in colon cancer cells. *PLoS One* 2011;6:e20291.
107. Roy S, Levi E, Majumdar AP, Sarkar FH. Expression of miR-34 is lost in colon cancer which can be re-expressed by a novel agent CDF. *Journal of hematology & oncology* 2012;5:58.
108. Li XM, Wang AM, Zhang J, Yi H. Down-regulation of miR-126 expression in colorectal cancer and its clinical significance. *Med Oncol* 2011;28:1054-7.
109. Tang JT, Wang JL, Du W, et al. MicroRNA 345, a methylation-sensitive microRNA is involved in cell proliferation and invasion in human colorectal cancer. *Carcinogenesis* 2011;32:1207-15.
110. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell research* 2008;18:997-1006.
111. Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut* 2009;58:1375-81.
112. Pu XX, Huang GL, Guo HQ, et al. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. *J Gastroenterol Hepatol* 2010;25:1674-80.
113. Wang B, Zhang Q. The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *Journal of cancer research and clinical oncology* 2012;138:1659-66.
114. Cheng H, Zhang L, Cogdell DE, et al. Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. *PLoS One* 2011;6:e17745.
115. Wang Q, Huang Z, Ni S, et al. Plasma miR-601 and miR-760 Are Novel Biomarkers for the Early Detection of Colorectal Cancer. *PLoS One* 2012;7:e44398.
116. Ahmed FE, Jeffries CD, Vos PW, et al. Diagnostic microRNA markers for screening sporadic human colon cancer and active ulcerative colitis in stool and tissue. *Cancer genomics & proteomics* 2009;6:281-95.
117. Link A, Balaguer F, Shen Y, et al. Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol Biomarkers Prev* 2010;19:1766-74.
118. Koga Y, Yasunaga M, Takahashi A, et al. MicroRNA expression profiling of exfoliated colonocytes isolated from feces for colorectal cancer screening. *Cancer Prev Res (Phila)* 2010;3:1435-42.
119. Kalimutho M, Di Cecilia S, Del Vecchio Blanco G, et al. Epigenetically silenced miR-34b/c as a novel faecal-based screening marker for colorectal cancer. *Br J Cancer* 2011;104:1770-8.
120. Kalimutho M, Del Vecchio Blanco G, Di Cecilia S, et al. Differential expression of miR-144* as a novel fecal-based diagnostic marker for colorectal cancer. *Journal of gastroenterology* 2011;46:1391-402.
121. Li JM, Zhao RH, Li ST, et al. Down-regulation of fecal miR-143 and miR-145 as potential markers for colorectal cancer. *Saudi medical journal* 2012;33:24-9.
122. Diaz R, Silva J, Garcia JM, et al. Deregulated expression of miR-106a predicts survival in human colon cancer patients. *Genes Chromosomes Cancer* 2008;47:794-802.
123. Yantiss RK, Goodarzi M, Zhou XK, et al. Clinical, pathologic, and molecular features of early-onset colorectal carcinoma. *Am J Surg Pathol* 2009;33:572-82.
124. Schetter AJ, Nguyen GH, Bowman ED, et al. Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma. *Clin Cancer Res* 2009;15:5878-87.
125. Nielsen BS, Jorgensen S, Fog JU, et al. High levels of microRNA-21 in the stroma of colorectal cancers predict short disease-free survival in stage II colon cancer patients. *Clinical & experimental metastasis* 2011;28:27-38.
126. Feng YH, Wu CL, Tsao CJ, et al. Deregulated expression of sprouty2 and microRNA-21 in human colon cancer: Correlation with the clinical stage of the disease. *Cancer Biol Ther* 2011;11:111-21.
127. Nishida N, Yokobori T, Mimori K, et al. MicroRNA miR-125b is a prognostic marker in human colorectal cancer. *Int J Oncol* 2011;38:1437-43.
128. Shibuya H, Iinuma H, Shimada R, Horiuchi A, Watanabe T. Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. *Oncology* 2010;79:313-20.
129. Iwaya T, Yokobori T, Nishida N, et al. Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. *Carcinogenesis* 2012.
130. Zhu L, Chen H, Zhou D, et al. MicroRNA-9 up-regulation is involved in colorectal cancer metastasis via promoting cell motility. *Med Oncol* 2012;29:1037-43.
131. Nishida N, Yamashita S, Mimori K, et al. MicroRNA-10b is a Prognostic Indicator in Colorectal Cancer and Confers Resistance to the Chemotherapeutic Agent 5-Fluorouracil in Colorectal Cancer Cells. *Ann Surg Oncol* 2012;19:3065-71.
132. Akcakaya P, Ekelund S, Kolosenko I, et al. miR-185 and miR-133b deregulation is associated with overall survival and metastasis in colorectal cancer. *Int J Oncol* 2011;39:311-8.
133. Karaayvaz M, Pal T, Song B, et al. Prognostic significance of miR-215 in colon cancer. *Clinical colorectal cancer* 2011;10:340-7.
134. Vickers MM, Bar J, Gorn-Hondermann I, et al. Stage-dependent differential expression of microRNAs in colorectal cancer: potential role as markers of metastatic disease. *Clinical & experimental metastasis* 2012;29:123-32.
135. Liu K, Li G, Fan C, Zhou X, Wu B, Li J. Increased expression of microRNA-21 and its association with chemotherapeutic response in human colorectal cancer. *The Journal of international medical research* 2011;39:2288-95.
136. Horiuchi A, Iinuma H, Akahane T, Shimada R, Watanabe T. Prognostic significance of PDCD4 expression and association with microRNA-21 in each Dukes' stage of colorectal cancer patients. *Oncol Rep* 2012;27:1384-92.
137. Weissmann-Brenner A, Kushnir M, Lithwick Yanai G, et al. Tumor microRNA-29a expression and the risk of recurrence in stage II colon cancer. *Int J Oncol* 2012;40:2097-103.
138. Yamashita S, Yamamoto H, Mimori K, et al. MicroRNA-372 is associated with poor prognosis in colorectal cancer. *Oncology* 2012;82:205-12.
139. Pichler M, Winter E, Stotz M, et al. Down-regulation of KRAS-interacting miRNA-143 predicts poor prognosis but not response to EGFR-targeted agents in colorectal cancer. *Br J Cancer* 2012;106:1826-32.
140. Ryan BM, McClary AC, Valeri N, et al. rs4919510 in hsa-mir-608 is associated with outcome but not risk of colorectal cancer. *PLoS One* 2012;7:e36306.

141. Lin M, Gu J, Eng C, et al. Genetic polymorphisms in MicroRNA-related genes as predictors of clinical outcomes in colorectal adenocarcinoma patients. *Clin Cancer Res* 2012;18:3982-91.
142. Zhou T, Zhang G, Liu Z, Xia S, Tian H. Overexpression of miR-92a correlates with tumor metastasis and poor prognosis in patients with colorectal cancer. *Int J Colorectal Dis* 2012.
143. Nishimura J, Handa R, Yamamoto H, et al. microRNA-181a is associated with poor prognosis of colorectal cancer. *Oncol Rep* 2012;28:2221-6.
144. Wang MJ, Li Y, Wang R, et al. Downregulation of microRNA-124 is an independent prognostic factor in patients with colorectal cancer. *Int J Colorectal Dis* 2012.