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miRNAs as biomarkers for management of patients with colorectal cancer

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Abstract

miRNAs serve as micromanagers, negatively regulating gene expression. Since altered miRNA expression is implicated in the pathobiology of various cancers, including colorectal cancers (CRCs), these molecules serve as potential therapeutic targets. Manipulation of miRNAs may offer an alternative therapy for chemo- and radio-resistant CRCs. For CRC patients, miRNA expression patterns can be used for diagnosis, and to predict prognosis and efficacy of therapy. This article describes the methodological approaches for miRNA measurement, their function in the pathobiology of CRCs and their potential clinical utility.

Keywords

biomarker; colorectal cancer; diagnosis; miRNA; prognosis; therapeutic target

miRNAs are endogenously produced, noncoding RNAs that are 18–25 nucleotides in length. They are involved in the temporal and tissue-specific negative regulation of gene expression at the post-transcriptional and post-translational levels, and function by binding to target mRNAs involved in cellular differentiation, growth, proliferation and apoptosis [1]. To date, there are more than 700 miRNA sequences characterized in humans [101]. The first miRNA to be discovered, *lin-4*, is involved in the development of the nematode *Caenorhabditis elegans*, which produces this 22-nucleotide RNA that binds to the 3'-untranslated region (UTR) of the *lin-14* mRNA and represses its expression [2].

miRNAs are genetically linked to cancers [3]. Their altered expression patterns are implicated in the development of various cancers [4], including colorectal cancers (CRCs) [5]. The probable mechanisms of miRNA deregulation are amplification, deletion, mutation and epigenetic silencing [6]. In CRCs, characterization of plasma miRNAs can be used as a

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minimally invasive, early diagnostic tool [7–10], and for assessing patient prognosis [11–16] and the efficacy of therapeutic drugs [13,17–22]. This article describes the function of miRNAs in CRCs and highlights their potential clinical utility.

Biosynthesis & mechanism of action of miRNA

As most miRNA genes are located in intergenic regions (>1 kb away from the gene annotations or the predicted regions of genes), they are transcribed as autonomous transcription units [1]. The miRNA genes are transcribed from their own promoters and products are generated by RNA polymerase II as primary transcripts. These primary miRNA transcripts are converted to 70-nucleotide, stem-loop structures, known as pre-miRNAs, by a multiprotein, microprocessor complex consisting of a ribonuclease, Drosha and Pasha, or DGCR8 (a dsRNA-binding protein). The pre-miRNAs are translocated to the cytoplasm by RanGTP-dependent exportin 5 (a transporter protein). In the cytoplasm, these pre-miRNAs are cleaved to form miRNA-miRNA duplexes by an endonuclease enzyme, Dicer. Subsequently, helicase unwinds the miRNA-miRNA duplex to release two mature miRNAs, only one of which is functional [23].

Mature miRNAs bind to the 3'UTR of their mRNA targets, without perfect complementation, and integrate into the RNA-induced silencing complex, with the central part formed by proteins of the Argonaute family; identical to those in the RNAi pathway [24]. Therefore, miRNAs repress target-gene expression post-transcriptionally, resulting in reduced protein levels of their target genes, without affecting mRNA levels. Furthermore, either by endonuclease 'Slicer'-mediated mRNA cleavage or by induction of deadenylation, miRNAs induce mRNA degradation and affect protein translation in the polyribosomes (reviewed in [25]). Translation can be affected by the 3'UTR-binding miRNA-induced silencing complex through physical or biochemical blocking of the translation initiation complex, eIF4F [25].

Methods of miRNA estimation

Until recently, the most common method for quantifying miRNAs was northern blotting. Over the past few years, other approaches, including cDNA arrays, a modified Invader[®] assay, a bead-based flow cytometric assay and real-time PCR (RT-PCR), have been used [26]. Oligonucleotide-based microarrays are most commonly used for high-throughput analysis of expression levels of miRNAs [27]. These allow the simultaneous measurement of differential gene expression of hundreds of miRNAs in large numbers of samples. Analysis of miRNAs presents several challenges. First, their short sequences make it difficult to achieve sufficient specificity with standard DNA oligonucleotide technologies. Furthermore, some miRNAs differ from each other by as little as a single nucleotide, emphasizing the importance of mismatch discrimination. Locked nucleic acids are useful in overcoming these obstacles, particularly for miRNA profiling and *in situ* hybridization. Locked nucleic acid probes are superior to DNA probes with respect to specific and selective detection of miRNAs [28,29]. The main advantage of RT-PCR is that it is more quantitative and more sensitive than other high-throughput assays. However, the accuracy, reproducibility and biological relevance of the miRNA, assessed by RT-PCR, are influenced by protocol-dependent RT-PCR inconsistencies. Use of reliable reference genes for normalization in RT-PCR would avoid these problems, but use of a common single reference gene for normalization in all studies is inappropriate and may lead to erroneous results [30,31]. In this regard, from a set of six miRNAs (*let-7A*, and *miR-16*, *-26a*, *-345*, *-425* and *-454*), *miR-16* and *-345* were the most stably expressed reference genes. Furthermore, two small nucleolar RNA genes (*RNU48* and *Z30*) were used to establish the oncogenic effect of *miR-21* and *-31* and the tumor suppressor role of two target miRNAs

(*miR-143* and *-145*) in CRCs. The results of this investigation suggested that there is a need to identify a suitable set of reference genes for determining dysregulation of specific target miRNAs [32].

The expressions of miRNAs can be used to classify cancer types with a greater reliability than the profiles of protein-coding genes [33]. For routine diagnoses, measurements of miRNA expression have advantages over profiling by conventional mRNA biomarkers. First, a single miRNA can simultaneously regulate the expression of more than 100 mRNAs, owing to its imperfect, complementarily based binding to target mRNAs. Therefore, it is easier to discover reliable biomarkers from the hundreds of miRNAs than from more than 40,000 genes. Second, owing to their small size and stem-loop structure, miRNAs are relatively more stable and less subject to degradation during fixation and sample processing. A recent study [34], and our unpublished results [Bovell L, Katkoori VR, Shanmugam C *et al.*, Unpublished data], demonstrate that there are no substantial differences in the miRNA levels in fresh-frozen versus formalin-fixed, paraffin-embedded CRC tissues. Therefore, miRNAs are apparently more stable than mRNAs. Furthermore, for 40 CRC specimens, differing times of formalin fixation do not alter the expression of miRNAs [34,35]. Third, cellular and subcellular localization of miRNAs can be established by conventional hybridization and by fluorescence *in situ* hybridization [36].

miRNA & cancer

Dysregulation of miRNAs influences tumorigenesis if their target mRNAs are encoded by tumor-suppressor genes or oncogenes [37]. Mature miRNAs may be overexpressed owing to transcriptional activation or amplification of the miRNA-encoding gene, whereas reduced expression or silencing results from deletion of a particular chromosomal region, epigenetic silencing or biogenetic defects [38]. Characterization of expression profiles of human cancer miRNAs reveals frequent miRNA deregulation in various cancers, including those of the lung [39], breast [40], pancreas [41], colorectum [14,42], brain [43], ovary [44], prostate [45] and blood [46]. In both solid tumors and hematological malignancies, upregulation of *miR-21* is the most common alteration of miRNAs that is implicated in cancers [47]. Other miRNAs, including *miR-155*, *-181b*, *-221* and *-22*, are frequently overexpressed in cancers of the blood, brain, thyroid and the GI tract. These miRNAs are also upregulated, to a lesser extent, in liver, lung and breast cancers [48]. By contrast, the *let-7/miR-98* cluster, *miR-143* and *miR-145* are frequently downregulated in malignancies of the breast, lung, thyroid, GI tract, genitourinary tract and blood [48]. The similarly altered miRNA expression patterns among different tumor types indicate their involvement in the cellular pathways altered in cancer pathogenesis. Although these aberrant patterns of miRNA expression that are associated with various cancers are well characterized, there is little information on the functional significance of such miRNA deregulation and on their intracellular targets. Further efforts are needed to clarify the function of miRNAs in cellular differentiation, proliferation and apoptosis.

Pathobiology of miRNAs in colorectal neoplasias

Premalignant colorectal neoplasias

Adenomatous polyposis coli (*APC*) gene inactivation, leading to stimulation of the Wnt pathway via free β -catenin, is a central initiating event in colorectal carcinogenesis, and is observed in more than 60% of colorectal adenomas and carcinomas [49]. There is upregulation of *miR-135a* and *-135b* in colorectal adenomas and CRCs, and their levels correlate with low levels of *APC* mRNA. This genetic interaction is also evident in cancer cells expressing *miR-135a* and *-135b*, regardless of the mutational status of *APC* [50]. Increased expression of the *miR-17–92* cluster during progression of colorectal adenomas to

adenocarcinomas is associated with gain in DNA copy numbers of the *miR-17-92* locus on chromosome 13q31 and with *c-myc* expression [51]. The downregulation of *miR-122a*, mediated by aberrant APC/ β -catenin signaling, is a factor in the pathogenesis of gastrointestinal cancers [52].

Plasma levels of *miR-29a* and *-92a* can be used to discriminate advanced adenomas from normal tissues. Combined receiver-operating characteristic analyses using these two miRNAs revealed an elevated area under the curve of 0.883, with 83% sensitivity and 85% specificity in discriminating CRC, and an area under the curve of 0.773, with 73% sensitivity and 80% specificity in discriminating advanced adenomas [8]. Therefore, there is a potential use for plasma miRNAs, which can be obtained by a minimally invasive procedure, for the early detection of CRCs. Since dysregulation of the *Pdcd4* gene by *miR-21* is implicated in invasion, intravasation and metastasis of CRCs, this could provide a diagnostic tool for discriminating normal colonic tissues from benign adenomas and colorectal carcinomas [53,54]. Alterations in *miR-21* and in the *miR-135* family, or *miR17-92* or *-122a* could be early events in CRC pathogenesis.

Colorectal cancer

The pathogenesis of CRCs involves chromosomal instability or microsatellite instability pathways. As described later in this article, these pathways are influenced by miRNA regulation. Commonly dysregulated miRNAs in CRCs are summarized in Table 1

Chromosomal instability pathways

In CRCs, KRAS and PI3K, downstream molecules in the EGF receptor pathway, promote tumor growth, survival, angiogenesis and metastasis [55]. KRAS is a direct target of the *let-7* miRNA family [56], and the PI3K signal is stabilized and propagated by the p85 β regulatory subunit, which is a *miR-126* target [57]. Expression of KRAS is regulated by *miR-143* [58] and *-18a* [59], and the suppression of PTEN by *miR-21* is associated with augmented PI3K signaling and tumor progression [37].

The tumor-suppressor gene, *p53*, which is mutated in more than half of all CRCs [49], transcriptionally regulates various other genes [60]. The basis for this phenomenon is a link between *p53* and the regulatory miRNA network [61]. Several miRNAs are induced in wild-type but not in *p53*-null HCT-116 colon cancer cells, suggesting that *p53* mediates miRNA expression in CRCs [62]. Specifically, an increased expression of *miR-34a* with wild-type *p53* modulates the expression of genes that are involved in cell-cycle arrest and apoptosis [62]. The *SIRT1* gene, which encodes NAD⁺-dependent deacetylases and is a negative regulator of apoptosis in response to cellular stress, is a target for *miR-34a*. Suppression of *SIRT1* by *miR-34a* results in apoptosis in *p53* wild-type colon cancer cells but not in *p53*-null cells [63]. Indeed, there is reduced expression of the *miR-34* family in nine out of 25 CRCs [64]. The reduced expression of this family is either caused by loss of 1p36 [62] (harbors *miR-34a*) or CpG island hypermethylation [65]. These findings establish a direct link between *p53* and *miR-34*, and implicate the *miR-34* family in the pathogenesis of CRCs.

In addition, the *miR-17-92* cluster, located on chromosome 13q31.3, negatively regulates the antiangiogenic factors, thrombospondin-1 and CTGF, which are induced by a proto-oncogene, *c-myc* [66]. The growth of colon cancer cells is inhibited by *miR-145* binding to the 3'UTR of insulin-receptor substrate-1 [67]. Moreover, *miR-145* targets the IGF-I receptor [18]. In CRC cell lines, there is an inverse correlation between *miR-101* and *COX-2* expression [68]. The upregulation of *miR-21* in CRC cells is associated with increased migration and invasion [69], and its inhibition by anti-*miR-21* results in increased levels of

PDCD4, an independent prognostic factor for CRCs and a negative regulator of intravasation and invasion [70].

Microsatellite instability pathway

In approximately 15% of sporadic cases, CRCs develop via the microsatellite instability (MSI) pathway. The CRCs with MSI exhibit a stable karyotype, low frequencies of allelic losses and diploid nuclear DNA content [71]. In an evaluation of combined miRNA and mRNA gene expression, a molecular signature of 27 differentially expressed genes, including eight miRNAs, distinguished CRCs with and without MSI. Members of the oncogenic *miR-17-92* cluster were upregulated in CRCs without MSI [72]. In addition, there was a significant difference in expression of *miR-92*, *-223*, *-155*, *-196a*, *-31* and *-26b* in MSI subgroups (MSI high and low). *miR-31* and *-223* are upregulated in CRCs with hereditary nonpolyposis CRC syndrome (Lynch syndrome) [73]. These findings indicate that miRNA expression profiles are not only distinct for MSI versus microsatellite stability, but also vary within MSI subgroups. These findings may aid in selection of patients for treatment based on their CRC molecular classification.

miRNAs & epigenetics

miRNAs, which exhibit reciprocal interconnections with epigenetic machinery, may be directly regulated by epigenetics either by aberrant methylation in the promoter region or by histone modifications [74,75]. Bandres *et al.* identified five miRNAs located around/on the CpG islands that were downregulated in CRCs and restored the expression of three of these miRNAs by exposing the cells to methyltransferase inhibitors and a histone deacetylase inhibitor [76]. As determined in the same investigation, the *miR-9-1*, *miR-129-2* and *miR-137* CpG islands were frequently methylated in CRCs but not in the normal colonic mucosa. Furthermore, DNMT3A, a key enzyme in DNA methylation, is targeted by *miR-143* in CRCs [77]. The *miR-34b/c* CpG island, which bidirectionally regulates *miR-34b/c* and B-cell translocation gene 4, may be a target of epigenetic silencing in CRCs [78]. These findings point to a link between the miRNA network and epigenetic machinery. Such a connection warrants further investigation.

miRNAs as metastatic signatures

miRNAs have been considered as metastatic signatures in various cancers, including CRCs. Gene expression analysis of metastatic versus nonmetastatic isogenic CRC cell lines indicate that *miR-145* targets involved in cell-cycle and neuregulin pathways are downregulated in metastatic cell lines [79]. In CRCs, however, *miR-21* [70] and *-17-92* [66] are believed to be prometastatic and potentially useful for the assessment of metastatic risk.

Utility of miRNAs in risk assessment, early detection & diagnosis of CRCs

CRC risk assessment

Single-nucleotide polymorphisms (SNPs) can occur either in the miRNA binding sites of mRNAs, at any step in the miRNA biogenetic process or in the miRNA target genes, and thereby affect the expression of the gene products [80]. Identification of such SNPs was accomplished and their biological relevance was evaluated for a Czech population with CRCs. The evaluated SNPs were positively associated with CRC risk [81]. Furthermore, computer simulations predicted eight candidate SNPs, of which two genes for CD86, a lymphocytic ligand and the gene for the insulin receptor carried an SNP that was associated with CRC risk [81]. However, the biological significance of these SNPs has not been determined.

CRC early detection

miRNAs are present in the plasma and sera of humans. Their levels, as compared with other circulating nucleic acids, are more stable and consistent among individuals [7,9]. The potential diagnostic utility of serum miRNAs has been assessed for patients with prostate cancers, ovarian cancers and CRCs [7,82,83]. There are differential serum miRNA profiles between CRC patients and healthy controls [7]. All examined CRC patients had 69 miRNAs that were not present in healthy controls; many of these were also detected in the sera of lung cancer patients. This observation is consistent with the existence of common, tumor-related miRNAs in the plasma/serum. Indeed, investigations that assessed whether plasma miRNAs could discriminate between patients with and without CRCs have demonstrated that *miR-17-3p* and *-92* are elevated in patients with CRCs and that their levels are reduced after surgery [82]. As further validation, analysis of an independent set of plasma samples indicated that *miR-92* not only differentiates CRCs from normal controls, but also from patients with gastric cancer or inflammatory bowel disease [82]. The method compares favorably with the fecal occult blood test; thus, *miR-92*, which can be obtained by a minimally invasive procedure, is a potential molecular marker for early detection of CRCs [82].

CRC diagnosis

There are reduced levels of mature *miR-143* and *-145* in the adenomatous and carcinoma stages of colorectal neoplasia [42]. In CRCs and colon cancer cell lines, the most extensively altered miRNAs are *miR-31*, *-96*, *-135b* and *-183* (upregulated), and *miR-133b* and *-145* (downregulated) [26]. Evaluation of the expression of miRNAs and mRNAs in CRC samples, characterized by microsatellite stability or by high levels of microsatellite instability, demonstrates that the combined miRNA/mRNA fingerprint is the best discriminator for micro-satellite stability versus high levels of micro-satellite instability CRCs [72]. There is a similar pattern of expression of the *miR-17-92* cluster and its target, *E2F1*, in development of the human colon and in colonic carcinogenesis, indicating that they function in the regulation of cell proliferation [84].

Metastasis from an unknown primary site usually presents as an aggressive disease with a poor prognosis. Identifying CRCs as the primary site, among adenocarcinomas of unknown primary sites, may improve prognosis of these patients by allowing them to be selected for appropriate therapy. From a diagnostic standpoint, miRNA expression patterns are also evaluated for the identification of the tissue origin of cancers of unknown primary sites. By using an miRNA array classifier, 12 out of 17 poorly differentiated tumors were accurately classified by miRNA profiling [33]. Another analysis, involving a set of 48 miRNAs, demonstrated a 90% overall accuracy in classifying more than 400 malignant tumor samples of 22 tissue origins [85]. Three miRNAs (*miR-148a*, *-34b/c* and *-9*), which are downregulated by CpG island hypermethylation, could identify lymph node metastases of several malignant tumors, including CRCs [86]. These results demonstrated the potential usefulness of miRNA expression, not only for the early diagnosis, but also for the assessment of risk of CRCs.

Prognostic utility of miRNAs in CRCs

There are associations between altered expression of specific miRNAs and poor prognostic parameters in cancers, but little information is available on the impact of miRNA levels on patient survival [11]. In one study of the prognostic value of a panel of ten miRNAs for CRC samples, only higher *miR-200c* expression was associated with shorter patient survival [16].

Overexpression of *miR-21* was reported in various cancers and in CRCs; its overexpression was correlated with established prognostic factors, such as stage, nodal status and metastasis [15]. For CRC patients, this association of high *miR-21* expression in tumors with poor survival was validated for two separate study cohorts (in the USA and China). In each cohort, the association was independent of other clinical covariates, indicating that *miR-21* expression is a useful independent prognostic indicator [14]. Furthermore, overexpression of *miR-18a* in CRCs was correlated with poor clinical outcome [12]. A predictive molecular signature composed of only four miRNAs (*miR-142-3p*, *-212*, *-151* and *-144*) was associated with tumor microsatellite status in stage II colon cancer [13]. Furthermore, the miRNA expression profiles predicted recurrence of disease with an overall performance accuracy of 81%, indicating the potential value of miRNAs in determining tumor aggressiveness. Although these findings are of considerable importance, they need further validation to determine their prognostic value before being introduced into routine oncologic practice.

Predicting therapeutic response by miRNA profiles in CRCs

A major concern in the management of CRC patients is prediction of therapeutic efficacy. Of five miRNAs evaluated, *let-7g* and *miR-181b* proved to be indicators for response to S-1-based chemotherapy [19]. In cultures of HT-29 and HCT-116 human colon cancer cells, 5-fluorouracil up- or down-regulated the expression of 19 and three miRNAs, respectively, by a factor at least twofold. *miR-21* was one of the upregulated miRNAs overexpressed in tumor tissue [20]. By contrast, *miR-200b*, which is upregulated in CRCs, is downregulated in tumors treated with 5-fluorouracil. *miR-200b* targets mRNA that codes for a protein tyrosine phosphatase, PTPN12, which inactivates products of oncogenes, such as *ABL*, *SRC* or *KRAS* [13]. In humans rectal tumors, many miRNAs (*miR-10a*, *-21*, *-145*, *-212*, *-339* and *-361*) responded to capecitabine and radiotherapy. However, in most samples only two miRNAs, *miR-125b* and *-137*, demonstrated significant increases in expression levels after 2 weeks of therapy [22].

Since overexpression of *miR-21* is associated with low sensitivity and a poor response to chemotherapy, its inhibition may improve the response [17]. On the other hand, restoration of *miR-145* expression is associated with inhibition of tumor cell growth via downregulation of insulin receptor substrate-1. Expression levels of *miR-145*, downregulated in tumor tissues of CRC patients, are increased *in vitro* and cause reduced cell proliferation and increased sensitivity to radiotherapy [18]. In osteosarcoma and colon cancer cells, *miR-215*, the suppression of denticleless protein homolog, a cell-cycle-regulated nuclear and centrosome protein, causes decreased cell proliferation through G2 arrest, thereby leading to an increase in chemo-resistance to the chemotherapeutic agents methotrexate and raltitrexed [21]. These are examples of miRNAs validated as oncogenes or tumor suppressors in CRCs and are candidates for miRNA-based CRC therapy. Despite these results, additional, prospectively validated studies are needed to examine the effects of chemotherapeutic agents on the miRNA expression profiles and to evaluate their possible usage for predicting therapy response in CRC patients.

miRNAs as therapeutic targets

Inhibition of the mRNAs of genes involved in the pathogenesis of cancers, specifically in CRCs, is being evaluated in various preclinical and clinical settings using antisense oligonucleotides, ribozymes, DNazymes, siRNAs, shRNAs, locked nucleic acids and, more recently, antagomirs (reviewed in [87]). The miRNAs are considered as drugs, owing to their secondary structures (stem-loops in pre-miRNAs and bulges in mature miRNAs).

Although these structures could be targeted by small inhibitory molecules [88], the targeted delivery of such molecules is a major challenge.

Conclusion & future perspective

Since the complex miRNA network is involved in the regulation of various genes, miRNAs are potential therapeutic targets. Manipulation of miRNAs using synthetic miRNA mimics or chemically modified oligonucleotides can alter biogenesis, maturation, activity and binding sites on the target mRNAs. However, a major obstacle to the use of these miRNAs for therapeutic purposes is the occurrence of nonspecific side effects, either caused by cross-sensitivity to endogenous miRNAs and/or nonspecific binding to noncancerous cells. The use of a directed, site-specific delivery system and synthesis of less toxic anti-miRNA oligonucleotides may minimize such side effects [89,90]. Targeting miRNAs that are overexpressed in CRCs may not only prevent the recurrence of high-risk tumors, but also control the growth of advanced metastatic tumors. Such miRNA manipulation could serve as alternative therapy in chemo- and radio-resistant cancer patients. Although results from experimental models are available, the clinical utility of miRNAs, both in preclinical and clinical settings, should be validated in order to develop a miRNA-based therapy for CRCs.

The understanding of gene regulation, its complexity and its relation to cancers, especially CRCs, increased after the discovery of miRNAs. Dysregulation of miRNAs is closely associated with cancer pathobiology. miRNAs have potential clinical utility in early diagnosis, in assessing patient prognosis and in predicting efficacy of therapy, specifically in CRCs. The CRC-specific miRNAs await further validation in larger prospective cohorts prior to application in clinical oncologic practice.

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Table 1

Altered miRNA expression in colorectal cancers.

Author (year)	Sample size and type	miRNA	Tumor levels	Quantification technique	Clinical outcome	Ref.
Michael <i>et al.</i> (2003)	16 CRCs and matched normals (FFPE)	miR-143, -145	Down	Northern blot analysis	ND	[42]
Volinia <i>et al.</i> (2006)	46 CRCs and eight normals (FFPE)	miR-9-3	Down	miRNA microarray	ND	[47]
Bandres <i>et al.</i> (2006)	12 CRCs and matched normals (FFPE)	miR-133b, -145, -129, -124a, -30-3p, -328	Down	Real-time qRT-PCR	ND	[26]
Slaby <i>et al.</i> (2007)	29 CRCs and six normals (FFPE)	miR-143, -145	Down	Real-time qRT-PCR	miR-143, miR-145 tumors >50 mm	[15]
Schepeler <i>et al.</i> (2008)	49 CRCs (stage II) and ten normals (FFPE)	miR-145, -320, -498	Down	Real-time qRT-PCR and miRNA <i>in situ</i> hybridization	High miR-320, miR-498 associated with recurrence-free survival	[13]
Diaz <i>et al.</i> (2008)	110 CRCs and matched normals (frozen)	miR-17-5p, -106a,	Down	Real-time qRT-PCR	miR-106a associated with short disease-free survival	[11]
Motoyama <i>et al.</i> (2009)	69 CRCs and matched normals (frozen)	miR-143, -145	Down	miRNA microarray	ND	[12]
Volinia <i>et al.</i> (2006)	46 CRCs and eight normals (FFPE)	miR-21, -17-5p, -191, -29b-2, -223, -128b, -24-1, -24-2, -155, -20a, -107, -32, -30c, -221, -106a	Up	miRNA microarray	ND	[47]
Bandres <i>et al.</i> (2006)	12 CRCs and matched normals (FFPE)	miR-19a, -20, -21, -183, -96, -31, -135b	Up	Real-time qRT-PCR	miR-31 stage IV > stage II	[26]
Xi <i>et al.</i> (2006)	24 CRCs and matched normals (frozen)	miR-15b, -181b, -191, -200c	Up	Real-time qRT-PCR	miR-200c short median survival	[16]
Lanza <i>et al.</i> (2007)	39 CRCs (23 MSS and 16 MSI-H) (FFPE)	miR-17-92 family	Up	miRNA microarray and northern blot analysis	miR-17-92 MSS > MSI-H	[72]
Slaby <i>et al.</i> (2007)	29 CRCs and six normals (FFPE)	miR-21, -31	Up	Real-time qRT-PCR	miR-21 node positivity and distant metastases	[15]
Nakajima <i>et al.</i> (2006)	46 CRCs treated with 5-FU-based antimetabolite S-1 (FFPE)	let-7g, miR-181b, -200c	Up	Real-time qRT-PCR	let-7g, miR-181b associated with therapy response but not with survival	[19]
Monzo <i>et al.</i> (2008)	22 CRCs (six stage I and 16 stage II) and matched normals (FFPE)	miR-17-92 family	Up	Real-time qRT-PCR and miRNA <i>in situ</i> hybridization	miR-17-92 family associated with CRC carcinogenesis	[84]
Schetter <i>et al.</i> (2008)	197 CRCs (84 test and 113 validation) and matched normals (FFPE)	miR-20a, -21, -106a, -181b, -203	Up	miRNA microarray and miRNA <i>in situ</i> hybridization	miR-21 associated with poor survival in both cohorts	[14]
Svoboda <i>et al.</i> (2008)	35 rectal tumors before and after treatment with capecitabine (frozen)	miR10a, -21, -31, -125b, -137, -145, -212, -339, -361	Up	Real-time qRT-PCR	miR-125b and -137 associated with worse response to the therapy	[22]
Motoyama <i>et al.</i> (2009)	69 CRCs and matched normals (frozen)	miR-31, -183, -17-5p, -18a, -20a, -92	Up	miRNA microarray	miR-18a associated with poor clinical prognosis	[12]
Ng <i>et al.</i> (2009)	95 plasma (five test and 90 validation) and 50 healthy controls	miR-17-3p, -92	Up	miRNA microarray	miR-92 differentiates CRCs from healthy subjects	[10]

Author (year)	Sample size and type	miRNA	Tumor levels	Quantification technique	Clinical outcome	Ref.
Yamamichi <i>et al.</i> (2009)	73 tumors (includes 34 polyps and 39 CRCs; FFPE)	miR-21	Up	Locked nucleic acid <i>in situ</i> hybridization	Precancerous adenomas and CRCs but not in nontumorigenic polyps	[54]
Huang <i>et al.</i> (2010)	157 plasma (120 CRCs and 37 advanced adenomas) and 59 healthy controls	miR-29a, -92a	Up	Real-time qRT-PCR	miR-29a and -92a differentiates adenomas from CRCs and healthy controls	[8]

5-FU: 5-Fluorouracil; CRC: Colorectal cancer; FFPE: Formalin-fixed paraffin embedded; MSI-H: Microsatellite instable-high; MSS: Microsatellite stable; ND: Not determined; qRT-PCR: Quantitative reverse transcription-PCR.