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REVIEW

MicroRNA dysregulation as a prognostic biomarker in colorectal cancer

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Abstract: Colorectal cancer (CRC) is one of the most potentially curable cancers, yet it remains the fourth most common overall cause of cancer death worldwide. The identification of robust molecular prognostic biomarkers can refine the conventional tumor–node–metastasis staging system, avoid understaging of tumor, and help pinpoint patients with early-stage CRC who may benefit from aggressive treatments. Recently, epigenetic studies have provided new molecular evidence to better categorize the CRC subtypes and predict clinical outcomes. In this review, we summarize recent findings concerning the prognostic potential of microRNAs (miRNAs) in CRC. We first discuss the prognostic value of three tissue miRNAs (miR-21-5p, miR-29-3p, miR-148-3p) that have been examined in multiple studies. We also summarize the dysregulation of miRNA processing machinery *DICER* in CRC and its association with risk for mortality. We also reviewe the potential application of miRNA-associated single-nucleotide polymorphisms as prognostic biomarkers for CRC, especially the miRNA-associated polymorphism in the *KRAS* gene. Last but not least, we discuss the microsatellite instability-related miRNA candidates. Among all these candidates, miR-21-5p is the most promising prognostic marker, yet further prospective validation studies are required before it can go into clinical usage.

Keywords: microRNA, colorectal cancer, prognostic biomarker, single-nucleotide polymorphism, microsatellite instability

Introduction

Colorectal cancer (CRC) is a malignant neoplasm affecting the lower gastrointestinal tract. CRC includes two major entities: colon cancer (CC), the malignance in the inner wall of the colon that constitutes two-thirds to three-quarters of all CRC cases; and rectal cancer (RC), defined as cancer located within 12 cm or less from the anal verge. CRC is a global public health problem: it is the third most common cancer and the fourth leading cause of cancer-related deaths in the world, with an estimated incidence of 1.2 million new cases and a mortality of >600,000 deaths annually (8% of all cancer deaths).[1] Both the incidence and death rates from CRC are increasing rapidly in Asian countries.[2]

Currently, the clinicopathologic tumor staging based on the tumor–node–metastasis (TNM) system is the basic prognostic marker for CRC clinical outcomes. The TNM system describes the degree to which the tumor has invaded the bowel wall and spread to the regional lymph nodes as well as to distant organs.[3] Although the TNM staging system is the mainstay of prognostication, this classification has weaknesses. Inadequate examination of lymph nodes may lead to understaging of the tumor and subsequent treatment failure.[3] Moreover, histologically identical CRC patients may have different

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genetic and epigenetic backgrounds that lead to distinctive disease progression and clinical outcomes. For example, TNM stage II patients with no lymph node metastasis have relatively better outcomes. However, approximately one-fourth of these patients can still have high risk for disease relapse after surgical resection. Unfortunately, no prognostic marker is currently available for identifying the patients who should benefit from more-aggressive treatments.[4] Recent epigenetic studies suggested that microRNAs (miRNAs) may help to better categorize the CRC subtypes and predict the outcomes.

miRNAs belong to a class of highly conserved ~22-nucleotide single-stranded RNAs that epigenetically regulate protein translation through binding to the 3′ untranslated region (UTR) of target messenger RNA (mRNA) and mediate either mRNA degradation or translational repression.[5] A single miRNA can manipulate multiple target gene expressions, initiate signaling pathways, and provoke signal crosstalk. It is estimated that miRNAs can fine-tune up to one-third of human gene translations.[6] By targeting multiple transcripts, miRNAs can epigenetically regulate fundamental cellular processes, such as cell proliferation, apoptosis, differentiation, and migration, which strongly indicates that they may function as potential oncogenes or tumor suppressors in cancer development. Indeed, a global impairment of miRNA has been described in various human cancers, including CRC.[7,8] A spectrum of dysregulated miRNAs was identified to be associated with CRC genesis, progression, and therapeutic response.

Herein, we summarize recent findings and discuss the potential value of miRNAs as prognostic biomarkers for CRC. For miRNA as a diagnostic marker and its therapeutic potential, readers can refer to recent reviews written by our group and others.[9–11] It is worth pointing out that in 2011 miRBase adopted a new "–5p/–3p" miRNA nomenclature to replace the conventional miR/miR* notation (http://www.mirbase.org). In this review, we will use the most updated miRNA identification nomenclature and list the original name used in the literature as reference.

miRNAs as prognostic biomarkers for CRC

miR-21-5p

miR-21-5p (accession number: MIMAT0000076), previously named miR-21, is one of the most abundantly expressed oncogenic miRNAs in CRC,[12,13] and has been extensively investigated for its prognostic potential in at least ten independent trials involving 2,039 patients since 2008 (Table 1).[14–23]

Slaby et al[24] first reported that elevated levels of miR-21-5p significantly correlated with lymph node positivity and the development of distance metastasis in a small cohort of 29 CRC patients, suggesting the potential prognostic value of miR-21-5p in CRC. This hypothesis was further tested by Schetter et al[14] in their multicenter study. Utilizing miRNA array profiling of 84 tumors and paired adjacent normal tissues, they identified 37 abnormal miRNAs, of which five promising miRNAs (miR-20a-5p [miR-20a], miR-21-5p, miR-106a-5p [miR-106a], miR-181b-5p [miR-181b], and miR-203a [miR-203]) were associated with unfavorable outcomes in the test cohort. Further quantitative real-time polymerase chain reaction (qRT-PCR)-based validation suggested that high miR-21-5p expression in tumor was significantly associated with a worse 5-year cancer-specific survival rate independent of demographic and clinicopathologic factors in a test cohort of 71 patients with sporadic colon adenocarcinomas. Moreover, the association of high miR-21-5p expression level in tumor and poor prognosis was confirmed by an external cohort of 103 colon adenocarcinoma patients from Hong Kong.[14]

The consistency of these associations has been proven by subsequent studies. Nielsen et al[15] performed a retrospective study based on a multicenter Danish and Scottish randomized clinical trial (RANX05) involving 130 stage II CC patients and 67 stage II RC patients. They evaluated the miR-21-5p expression using in situ hybridization on formalin-fixed paraffin-embedded tissue samples followed by image semi-quantitative analysis. Strong staining of miR-21-5p was significantly associated with shorter disease-free survival (DFS) and overall survival (OS) in stage II CC patients. By multivariate analysis, the intense signal of miR-21-5p was a prognostic factor for stage II CC group after adjustment for other clinical parameters, including tumor histology, *KRAS* mutational status, and microsatellite instability (MSI) status. Shibuya et al[16] further validated the prognostic role of miR-21-5p in a cohort of 156 CRC patients in Japan. They concluded that a high level of miR-21-5p was associated with venous invasion, liver metastasis, advanced Dukes' stage, and a marginal link with lymph-node metastasis using the mean expression as a cutoff value. The group with higher levels of miR-21-5p had significantly shorter 5-year DFS and worse OS after multivariate regression. However, the authors did not specify the percentage of rectal cancer cases in their study cohort.

Besides serving as a single marker, miR-21-5p has been combined with other potential indicators to improve prognostic accuracy. One year after their first report about

Table 1 Prognostic value of miR-21-5p, miR-29-3p, and miR-148a-3p in CRC

Location	Study type	Study period	Cohort description	Cohort size	Detection method	Endogenous control	Prognostic value	Validation cohort	Cutoff method	Ref
miR-21-5p (miR-21)										
Oslo region	P	1998–2000	TNM I, II, III	193 CRC	Taqman qRT-PCR	RNU44	No significant association with 5-year DFS	No	Mean, median and tertile	12
US, Hong Kong	R	US cohort: 1993–2002, Hong Kong cohort: 1991–2000	TNM I, II, III, IV	US cohort: 84 CC; Hong Kong cohort: 113 CC	Microarray and Taqman qRT-PCR	LOESS and RNU6B	High miR-21 associated with shorter OS of stage II CC: US cohort: HR 2.7, 95% CI =1.3–5.5, $P<0.008$; Hong Kong cohort: HR =2.4, 95% CI =1.4–4.1, $P<0.001$	Yes, independent cohort	Highest tertile	14
Denmark, Scotland	R	1991–1993	Dukes' B	149 CC, 85 RC	In situ hybridization		Strong stained miR-21 associated with shorter DFS and OS in stage II CC patients, independent prognosis factor; not associated with stage II RC	No	Tertile	15
Japan	NA	2000–2005	Dukes' A, B, C, D	156 CRC	Taqman qRT-PCR	RNU6B	High miR-21 associated with shorter OS (HR =0.513, 95% CI =0.280–0.956, $P=0.036$) and DFS (HR =0.396, 95% CI =0.186–0.897, $P=0.028$)	No	Mean value	16
US, Hong Kong	R	US cohort: 1993–2002, Hong Kong cohort: 1991–2000	TNM I, II, III, IV	US cohort: 83 CC; Hong Kong cohort: 113 CC	Taqman qRT-PCR	RNU6B	High miR-21 associated with shorter OS (HR =3.0, 95% CI =1.7–5.2, $P<0.0005$)	No	Highest tertile	17
People's Republic of China	R	2000–2008, 2012	TNM II	775 CC	miRNA array and SYBR Green qRT-PCR	RNU6B	The six-miRNA risk score was significantly associated with the DFS in the training, internal testing, and external validation cohorts (HR =3.79, 95% CI =2.82–5.09, $P<0.0001$)	Yes, independent cohort	Formula risk score	18
The Czech Republic	NA	2004–2005; 2002–2004	TNM I, II, III, IV	46 CRC, 30 CLM	Taqman qRT-PCR	Total RNA input; RNU6B; miR-191	High miR-21 associated with shorter DFI; no significant correlation with OS	No	Optimal cutoff value 8.1	19
Spain	P	2002–2003	TNM I, II, III, IV	28 CRC, 7 GC, 3 PC	SYBR Green qRT-PCR	5S rRNA and RNU6B	No significant association between PFS and OS	No	Mean value and the REST analysis	20
People's Republic of China	NA	2006–2008	TNM I, II, III, IV (serum sample)	200 CRC, 50 AA, 80 healthy control	Taqman qRT-PCR	miR-16	No significant association between serum miR-21 and OS	No	NA	22
Spain	P	2008–2010	TNM I, II, III, IV (serum sample)	102 CRC	Taqman qRT-PCR	miR-16	Low serum miR-21 has a borderline association with shorter OS; no significant association between serum miR-21 and DFS	No	Relative expression value 1	23

(*Continued*)

Table 1 (*Continued*)

Location	Study type	Study period	Cohort description	Cohort size	Detection method	Endogenous control	Prognostic value	Validation cohort	Cutoff method	Ref
The Czech Republic	NA	NA	TNM I, II, III, IV	29 CRC	Taqman qRT-PCR	let-7a-1	High miR-21 associated with lymph node positivity and the development of metastases in CRC patients	No	NA	24
miR-29a-3p (miR-29a)										
People's Republic of China	P	Not mentioned	TNM II, III, IV	58 CLM, 56 CRC	Taqman qRT-PCR	miR-16	No significant association between serum miR-29a and survival	No	Cutoff value 0.155	37
People's Republic of China	NA	2009	TNM I, II, III, IV	85 CRC	SYBR Green qRT-PCR	RNU6B	High miR-29a associated with CRC metastasis and shorter OS	No	Median level	38
The Czech Republic	NA	2009–2011	TNM I, II, III, IV	100 CRC, 30 healthy control	Taqman qRT-PCR	miR-16	Increased serum miR-29a associated with advanced stages	No	NA	40
Israel	R	1995–2005	TNM I, II	110 CC	miRNA array and Taqman qRT-PCR	miR-214, miR-221, miR-141, miR-185	Low miR-29a associated with shorter DFS (HR =0.194, 95% CI =0.063–0.597, *P*=0.0043)	No	Tertile	41
Taiwan	NA	NA	TNM I, II, III	78 CRC	Taqman qRT-PCR	RNU6B	Downregulated in the recurrence group	No	Median value	43
miR-148a-3p (miR-148a)										
People's Republic of China	NA	NA	TNM I, II, III, IV	101 CRC	SYBR Green qRT-PCR	RNU6B	Low miR-148a associated with increased tumor size and advanced primary tumor stage	No	Median value	44
Spain	NA	1996–2008	TNM II, III, IV	273 CRC, 20 healthy control	Taqman qRT-PCR	miR-16	Low miR-148a associated with shorter DFS (HR =1.83, 95% CI =1.12–2.99, *P*=0.017) in stage II/III, and worse therapeutic response in stage IV (HR =1.93, 95% CI =1.15–3.23, *P*=0.014)	No	ROC and median value	45
Taiwan	NA	NA	TNM II, III	195 CRC	Taqman qRT-PCR	RNU6B	Low miR-148a associated with shorter DFS and OS (HR =5.221, 95% CI =2.069–13.174, *P*<0.0001)	No	Mean value	46

Abbreviations: AA, advanced adenoma; CC, colon cancer; CI, confidence interval; CLM, colorectal liver metastases; CRC, colorectal cancer; DFI, disease-free interval; DFS, disease-free survival; HR, hazard ratio; LOESS, local regression; miRNA, microRNA; NA, not applicable; OS, overall survival; P, prospective study; PFS, progression-free survival; qRT-PCR, quantitative real-time polymerase chain reaction; R, retrospective study; RC, rectal cancer; REST, relative expression software tool; ROC, receiver operating characteristic curve; TNM, tumor–node–metastasis stage; ref, reference; rRNA, ribosomal RNA.

the prognostic values of miR-21-5p, Schetter et al[17] conducted a consecutive study to detect its prognostic value in combination with a panel of nine inflammatory-related genes (*PRG1*, *IL10*, *CD68*, *IL23a*, *IL12a*, *ANXA1*, *IL17a*, *FOXP3,* and *HLA-DRA*) utilizing the same cohorts as described earlier.[14] Consistent with their previous report,[14] miR-21-5p retained its strong association with stage II CC cancer-specific mortality with the updated 1-year follow-up. They observed that the combination of high miR-21-5p level and high inflammatory risk score (IRS) could predict the unfavorable outcomes of either all stages or the subset of stage II CC. Despite the fact that miR-21-5p expression was associated with two inflammatory-related genes (*IL10* and *IL12a*) in the IRS model, both miR-21-5p and IRS were found to be independent prognostic factors (adjusted for TNM stage) on multivariate analysis.

Most recently, Zhang et al[18] carried out the largest multicenter retrospective trial to date to dissect the association between miRNA and stage II CC outcomes in a Chinese population. In their study, miRNA array identified 35 miRNAs as highly dysregulated in stage II CC. They further selected six potential indicator miRNAs (four upregulated miRNAs in cancer: miR-21-5p, miR-20a-5p, miR-103a-3p, and miR-106b-5p, and two downregulated miRNAs in cancer: miR-143-5p and miR-215) using the least absolute shrinkage and selection operator Cox regression model.[18,25] They then developed a formula to calculate the disease recurrence risk score based on the expression levels of the six miRNAs and dichotomized patients into high-risk and low-risk groups. The high-risk panel score was significantly associated with poor prognosis: among an internal testing group of 137 stage II patients, 43% of the high-risk patients developed recurrence after a 5-year follow-up, whereas recurrence only occurred in 15% of the low-risk patients. Similarly, among an external validation set of 460 patients, 46% of the high-risk patients experienced relapse, whereas only 15% of the low-risk group had progressive disease. The six-miRNA panel as a predictor for 5-year DFS was independent of conventional clinicopathologic risk factors. They suggested that the combination of miR-21-5p with other indicators significantly enhanced the prognostic accuracy for CC.

However, among the top aberrant miRNAs identified by the two large-scale miRNA screening studies mentioned above,[14,18] only nine (miR-17-5p, miR-20a-5p, miR-21-5p, miR-92a-3p, miR-106b-5p, miR-181b-5p, miR-203a, miR-215, and miR-221-3p) in Zhang et al's China cohort overlapped with Schetter et al's US cohort.[14,18] Specifically, the two miRNAs (miR-103a-3p and miR-143-5p) in Zhang et al's risk score panel were not considered to be dramatically altered in the US cohort. Although technical variations, such as different miRNA array platforms used and bioinformatics methods applied for data mining, may partially explain the inconsistency, it is possible that miRNA prognostic signature may differ across populations. Previous studies also suggested that miRNA transcriptome varied according to tumor sites and molecular alterations, such as CpG island methylator phenotype, MSI, *KRAS*, and *TP53* status.[26,27] Considering that their findings were restricted only to the Chinese population and specific CC subtypes, the generalizability of the multimarker signature on other ethnicities and subgroups still needs further validation.

miR-21-5p was significantly overexpressed in colon adenomas and adenocarcinoma.[14] Initially it was identified to be upregulated in colonic epithelial cells.[14] Further studies indicated that miR-21-5p was predominantly overexpressed in cancer-associated fibroblasts in CRC.[15,28] Laboratory evidence of its role in CRC progression through fibroblast-to-myofibroblast transdifferentiation provided coherence to the abovementioned epidemiological findings.[29,30] Although multiple studies supported miR-21-5p as a promising CC prognostic marker, it is uncertain whether miR-21-5p is of relevance in certain clinical stages. Studies of its role in other less-common histologic subtypes, such as signet ring cell carcinoma, are also scant. Moreover, the association between miR-21-5p and RC is conflicting. Despite comparable expression levels and patterns in CC and RC, miR-21-5p failed to predict the outcomes of patients with stage II RC in Nielson et al's study.[15] No or reverse correlation of miR-21-5p with disease progression and mortality were also observed in several studies with heterogeneous population covering both CC and RC.[12,13,20–23] The contradictory findings might be due to inadequate sample size, insufficient follow-up time, and different medical intervention for the CC/RC patients, but may also be rooted in the different molecular pathways for CC/RC metastasis.

miR-29a-3p

The prognostic value of miR-29a-3p (previous name: miR-29a; accession number: MIMAT0000086) in CRC is not straightforward. Several studies suggested that miR-29a-3p was significantly elevated in primary CRC compared with the matched adjacent normal tissue.[31–34] Higher levels of plasma miR-29a-3p were also detected in CRC and advanced adenoma patients compared with normal healthy donors.[35,36] Liver is one of the most common sites for CRC distant metastatic spread. Further study indicated that both

serum and tissue miR-29a-3p were significantly higher in colorectal liver metastatic patients than in nonmetastatic patients, suggesting that miR-29a-3p might be associated with disease progression.[37] In line with the prior study,[37] another China-based single-centered study observed a higher miR-29a-3p level in the primary tumor tissues of M1-stage patients compared to those of M0-stage patients.[38] They found that miR-29a-3p high expression correlated with CRC metastasis and poor OS. They also reported experimental evidence that overexpression of miR-29a-3p regulated Kruppel-like factor 4/matrix metalloproteinase-2/cadherin 1 cascade, and promoted cell invasion and dissemination in vitro and in vivo.[38] Although it has not been published in a peer reviewed journal, a United States patent (US8338106 B2) claimed that the tumor:normal ratio of miR-29a-3p was shown to be an independent predictive marker of CC prognosis.[39] A higher tumor:normal ratio of miR-29a-3p was associated with significantly worse DFS in a cohort of 77 CC patients.[39]

Meanwhile, several independent studies suggested a completely opposite prognostic value of miR-29a-3p. Although gradual increase of serum miR-29a-3p expression was associated with advanced stages of CRC, Faltejskova et al[40] documented a comparable expression of miR-29a-3p in primary CRC serum and healthy subject serum in a Caucasian population. Weissmann-Brenner et al[41] performed a retrospective study in a cohort of 110 early-stage CC patients who had not received adjuvant systemic therapy. They classified those patients who developed locoregional or distant recurrence within 36 months after initial complete resection into the poor-prognosis groups. On the basis of miRNA screening and a 10-year follow-up, they identified a significantly lower level of miR-29a-3p in stage II patients with poor prognosis. Decreased miR-29a in tumor was strongly associated with shorter DFS for stage II patients, which was independent of tumor grade and location.[41] Despite the high specificity and sensitivity for miR-29a-3p in discrimination of good and poor prognosis for stage II CC, miR-29a-3p was incapable of predicting the clinical outcome of stage I CC patients.

Lee et al[42] developed a reverse engineering approach (IMRE) to predict the altered expression of microRNAs using the currently available genome-wide gene expression datasets. This IMRE algorithm is based on the in-silicon miRNA target prediction databases, and the assumption that all miRNAs generally induce target cleavage and therefore inversely correlate with target mRNA level. Using four published human CRC gene expression array datasets (GSE12032, GSE17538, GSE4526, and GSE17181), Kuo et al[43] performed a pooled IMRE computational analysis to infer putative recurrence-related miRNAs. IMRE identified miR-29a-3p and miR-29c-3p (miR-29c) as potential recurrence candidate markers for both stage II/III CRC patients. To verify their in-silicon prediction, they experimentally tested the miR-29a-3p/29c-3p expression level in 43 CRC patients who experienced early recurrence within 1 year after curative surgery and 35 patients who remained free of disease progression. Kaplan–Meier analysis suggested that lower level of miR-29a-3p was significantly associated with early recurrence.[43] However, no multivariate analysis was performed in this study. Whether miR-29a-3p is an independent prognosis factor needs further investigation.

Due to the insufficient evidence from both sides, current studies have not yet yielded a clear-cut picture of the miR-29a-3p dysregulation and its prognostic value in CRC. There are several possible explanations for this observation. First, these contradictory findings could be explained by the less-informative clinical data, especially lack of the definition of "healthy" control subjects. Some studies[37,38] did not specify whether the patients enrolled accepted any radiotherapy or chemotherapy prior to specimen collection, which will very likely affect the miRNA expression. The clinical endpoints among studies[41,43] varied as well. Second, the miR-29a-3p expression pattern in colorectal tissue is largely unknown. Considering the different percentages of stromal tissues in normal tissue and its cancerous counterpart, a simple qRT-PCR based on RNA isolated from whole surgical specimens may distort the result. Therefore, an in situ hybridization or a qRT-PCR analysis with laser-captured stromal or epithelial compartment is necessary for carefully determining the source and expression pattern of miR-29a-3p. Third, several studies also suffered from flaws like heterogeneous populations and failure to stratify the CC and RC, the two distinct clinical entities. An miRNA array study based on 57 RC cases suggested that miR-29a-3p showed no significant difference between RC tissues and adjacent normal mucosa.[26] Lack of stratification may have led to those contradictory findings. Fourth, most studies mentioned above[37,38,43] were based on very small sample sizes, as shown in Table 1. None of the studies gave any justification for the sample size used, which may bring type I and II errors in analysis. Last but not least, population ethnicity may be one of the potential confounding variables. Future strictly designed studies are certainly warranted.

miR-148a-3p

miR-148a-3p (previous name: miR-148a; accession number: MIMAT0000243) showed reduced expression in gastrointestinal

cancer.[44] Further study suggested that miR-148a-3p presented a comparable level between normal colonic mucosa and CRC tissues in stage II disease, whereas significant downregulation of miR-148a-3p was observed in more-advanced stages of CRC.[45] This suggests that dysregulation of miR-148a-3p is one of the later events in CRC progression. Although tissue miR-148a-3p levels were not associated with 5-year DFS or OS in the stage II group, lower miR-148a-3p expression was significantly associated with worse 5-year DFS in stage III CRC. The group with low miR-148a-3p expression showed a trend toward a worse progression-free survival (PFS) and significantly worse OS in stage IV patients. After a statistical correction for multivariate testing, miR-148a-3p expression status was still independently associated with unfavorable outcomes for stage III/IV patients.[45] Tsai et al[46] tested the miR-148a-3p expression level in a Chinese population and observed a 2.5-fold decrease in the expression in the early-relapse patients than in the late-relapse patients. Similar to the prior study,[45] they observed strong associations between a lower miR-148a-3p level and worse DFS and OS in a cohort of 110 stage II/III patients. They also reported experimental evidence that overexpression of miR-148a-3p inhibited cell migration but not invasion. These available findings suggest that miR-148a-3p expression status has potential as a prognostic biomarker for advanced-stage CRC. Further replication studies are needed for validation.

Great efforts have been taken to identify new prognostic miRNA biomarkers for CRC. For example, high levels of miR-10b-5p, miR-17-5p, miR-18a-5p, miR-19b-3p, miR-92a-3p, miR-125b-5p, miR-155-5p, miR-181a-5p, miR-185-5p, miR-194-5p, miR-200c-3p, miR-215, and miR-372 in tumor tissues were found to be associated with unfavorable clinical outcomes; similarly, low levels of miR-16-5p, miR-22-3p, miR-93-5p, miR-106a-5p, miR-124-3p, miR-126-3p, miR-128, miR-133b, miR-135b-5p miR-195-5p, miR-212-3p, and miR-362-3p were associated with worse survival (Table 2). In plasma, high levels of miR-140-5p, miR-141-3p, and miR-221-3p were associated with shorter OS, whereas low levels of miR-143-3p and miR-1224-5p predicted worse survival (Table 2). A major problem with the aforementioned miRNA marker studies (Table 2) is that many of the analyses were based on limited number of specimens and there was a lack of replication of the initial findings. Each study analyzed only a small number of cases, ranging from 24–273, with a median sample size of 89. So far only four studies,[12,20,26,87] which included 28, 48, 57, and 193 cases, respectively, were prospective studies. The rest of the studies (Table 2) were either retrospective in nature or of uncertain study type. Retrospective study has disadvantages, such as selection bias and information bias. It is therefore impossible to rule out the likelihood of chance findings due to the nature of the study itself. Further prospective studies are warranted for validation of the prognostic power of the candidate miRNAs in CRC.

miRNA processing machinery: DICER1

RNase III endonuclease DICER1 performs a fundamental role in miRNA biogenesis by excising the stem-loop pre-miRNAs into functional miRNAs. Human DICER1 is an L-shaped 219-kilodalton multidomain protein including a DEAD-like helicase domain for double-stranded RNA translocation, a Piwi/Argonaute/Zwille domain for RNA-binding, a ruler domain, and a RNase III domain for double-stranded RNA cleavage.[47] Unlike other organisms that have multiple Dicer proteins, DICER1 is the only form of Class 3 RNase III enzyme that is involved in both small interfering RNA (siRNA) and miRNA maturation in human cells.[48] DICER1 is a haploinsufficient tumor suppressor, and deletion of DICER1 has been evidenced in various human cancers.[49] Experimental evidence suggested that impaired DICER1 causes a global reduction in mature miRNA levels and promotes tumor growth and metastasis.[49–51] Giving the central role of DICER1 in miRNA production, several studies have tried to evaluate the correlation between DICER1 level and its prognostic significance in CRC.

DICER1 is located on chromosome 14q32.13. A frequent loss of heterozygosity of this region was linked with metastatic recurrence of early-stage CRC.[52] Akahane[53] evaluated the association between the expression levels of DICER1 mRNA and the clinical outcomes in 260 CRC patients from Japan who did not receive any chemoradiotherapy prior to surgery. Based on laser microdissection and qRT-PCR, mRNA of DICER1 was significantly reduced in tumor compared to that in the adjacent normal tissue. Lower mRNA level of DICER1 was significantly associated with larger tumor size, greater invasion depth, more lymph node metastasis and lymphatic invasion, and more-advanced Dukes' stages. The OS and DFS of patients in the lower DICER1 group showed worse survival rates compared with the high DICER1 group. On the protein level, Faggad et al[54] examined the expression of DICER1 in 331 CRC patients by immunohistochemistry, of which 65 patients (19.6%) showed a negative stain for DICER1. The mean OS time for the DICER1 negative group was 64.1 months, which was significantly shorter than in the positive group

Table 2 miRNAs as potential prognostic biomarkers for CRC

Mature miRNA ID	Previous miRNA ID	Location	Study type	Study period	Cohort description	Cohort size	Detection method	Endogenous control	Prognostic value	Validation cohort	Cutoff method	Ref
Tissue miRNA												
miR-10b-5p	miR-10b	Japan	NA	1993–2006	Dukes' A, B, C, D	88 CRC	Taqman qRT-PCR	RNU6B	High miR-10b associated with shorter 10-year OS (HR =1.56, 95% CI =1.06–2.38, *P*=0.025)	No	Median value	85
miR-16-5p	miR-16	People's Republic of China	NA	2002–2006	TNM I, II, III, IV	143 CRC	Taqman qRT-PCR	RNU6B	Low miR-16 associated with shorter 5-year OS (HR =1.67, 95% CI =1.22–2.54, *P*=0.018)	No	ROC curve	86
miR-17-5p	miR-17	People's Republic of China	P	2006	TNM I, II, III, IV	48 CC	Taqman qRT-PCR	RNU6B	High miR-17 associated with shorter 5-year OS (HR 2.67, 95% CI, 1.31–6.82, *P*=0.007)	No	Highest tertile	87
miR-17-5p	miR-17	Spain	P	2002–2003	TNM I, II, III, IV	28 CRC, 7 GC, 3 PC	SYBR Green qRT-PCR	5S rRNA, RNU6B	High miR-17 associated with shorter PFS (HR =2.11, 95% CI =1.29–3.54, *P*=0.003) and OS (HR =2.62, 95% CI =1.55–4.49, *P*<0.001)	No	Mean value and the REST analysis	20
miR-18a-5p	miR-18a	People's Republic of China	R	1999–2003	TNM I, II, III	45 RC	Taqman qRT-PCR	miR-16	High miR-18a associated with shorter 6-year PFS (*P*=0.005), no multivariate analysis	No	Highest tertile	88
miR-19b-3p	miR-19b	Germany	NA	NA	TNM II, III, IV	30 CRC	SYBR Green qRT-PCR	18S rRNA	High miR-19b associated with shorter RFS and OS, no multivariate analysis	No	Median value	89
miR-20a-5p	miR-20a	Spain	P	2002–2003	TNM I, II, III, IV	28 CRC, 7 GC, 3 PC	SYBR Green qRT-PCR	5S rRNA, RNU6B	No significant association with PFS and OS	No	Mean value and the REST analysis	20
miR-22-3p	miR-22	People's Republic of China	NA	2005–2008	T1–T4	86 CRC	SYBR Green qRT-PCR	RNU6B	Low miR-22 associated with shorter 5-year OS (HR =2.217, 95% CI =1.028–4.780, *P*=0.042)	No	Median value	90
miR-31-5p	miR-31	Oslo region	P	1998–2000	TNM I, II, III	193 CRC	Taqman qRT-PCR	RNU44	No significant association with 5-year DFS	No	Mean, median and tertile	12
miR-92a-3p	miR-92a	Oslo region	P	1998–2000	TNM I, II, III	193 CRC	Taqman qRT-PCR	RNU44	No significant association with 5-year DFS	No	Mean, median and tertile	12
miR-92a-3p	miR-92a	People's Republic of China	NA	2005–2008	TNM I, II, III, IV	82 CRC	SYBR Green qRT-PCR	RNU6B	High miR-92a associated with shorter 5-year OS (HR =2.342, 95% CI =1.072–5.115, *P*=0.033)	No	Median value	91
miR-93-5p	miR-93	People's Republic of China	NA	2001–2006	TNM I, II, III, IV	138 CC	Taqman qRT-PCR	RNU6B	Low miR-93 associated with shorter OS (HR =4.3, 95% CI =0.8–17.2, *P*=0.02)	No	Median value	92
miR-101-3p	miR-101	Oslo region	P	1998–2000	TNM I, II, III	193 CRC	Taqman qRT-PCR	RNU44	No significant association with 5-year DFS	No	Mean, median and tertile	12
miR-106a-5p	miR-106a	Oslo region	P	1998–2000	TNM I, II, III	193 CRC	Taqman qRT-PCR	RNU44	No significant association with 5-year DFS	No	Mean, median and tertile	12

miR-106a-5p	miR-106a	Spain	NA	1998–2000	TNM I, II, III, IV	110 CC	SYBR Green qRT-PCR	5S rRNA	Low miR-106a associated with shorter 5-year DFS (HR =2.8, 95% CI =1.3–6.0, *P*=0.009) and OS (HR =1.9, 95% CI =0.9–3.8, *P*=0.07)	No	Tertile	93
miR-124-3p	miR-124	People's Republic of China	NA	2006–2007	TNM I, II, III, IV	96 CRC	Taqman qRT-PCR	5S rRNA	Low miR-124 associated with shorter DFS (HR =4.533, 95% CI =1.733–11.856, *P*=0.002) and OS (HR =4.634, 95% CI =1.731–12.404, *P*=0.002)	No	Tumor/ normal ratio	94
miR-125b-5p	miR-125b	Japan	NA	1993–2000	Not mentioned	89 CRC	Taqman qRT-PCR	RNU6B	High miR-125b associated with shorter 8-year OS (HR =1.84, 95% CI =1.14–3.15, *P*=0.011)	No	Median value	95
miR-126-3p	miR-126	Denmark	R	2004–2009	TNM IV	89 CRC	In situ hybridization	NA	Low miR-126 associated with shorter PFS and OS, no multivariate analysis	No	Median value	96
–	miR-128	Japan	NA	1992–2002	TNM 0, I, II, III	108 CRC	Taqman qRT-PCR	RNU6B	Low miR-128 associated with shorter DFS, no multivariate analysis	No	Median value	97
miR-133b	miR-133b	Sweden	NA	1993–1998	TNM I, II, III, IV	50 CRC	Taqman qRT-PCR	miR-16	Low miR-133b associated with shorter OS (*P*=0.028), no multivariate analysis	No	Median value	21
miR-135b-5p	miR-135b	Germany	P	2001–2010	TNM II, III, IV	173 RC	Taqman qRT-PCR	RNU66, RNU44, RNU48	Low miR-135b associated with shorter DFS and CSS, no multivariate analysis	Yes, independent cohort	Median value	26
miR-140-5p	miR-140-5p	Finland	NA	NA	TNM IV	33 CRC, wild-type KRAS and BRAF	miRNA array and SYBR Green qRT-PCR	RNU6B	High miR-140-5p associated with shorter OS	No	NA	98
miR-143-3p	miR-143	Austria	R	2005–2011	TNM II, III, IV	77 CRC, KRAS wild-type	SYBR Green qRT-PCR	RNU6B, miR-16, miR-345	Low miR-143 associated with shorter CSS (HR =1.86, 95% CI =1.06–3.25, *P*=0.031)	No	Optimal cutpoints	99
miR-143-3p	miR-143	Germany	NA	1999–2007	uT3/T4 Nx	40 RC	SYBR Green qRT-PCR	RNU6B	No significant association with OS	No	ROC curve	100
miR-145-5p	miR-145	Oslo region	P	1998–2000	TNM I, II, III	193 CRC	Taqman qRT-PCR	RNU44	No significant association with 5-year DFS	No	Mean, median, and tertile	12
miR-145-5p	miR-145	Germany	NA	1999–2007	uT3/T4 Nx	40 RC	SYBR Green qRT-PCR	RNU6B	No significant association with OS	No	ROC curve	100
miR-155-5p	miR-155	Japan	NA	2000–2005	Dukes' A, B, C, D	156 CRC	Taqman qRT-PCR	RNU6B	High miR-155 associated with shorter OS (HR =0.427, 95% CI =0.223–0.838, *P*=0.014) and DFS (HR =0.387, 95% CI =0.179–0.872, *P*=0.023)	No	Mean value	16

(*Continued*)

Table 2 (*Continued*)

Mature miRNA ID	Previous miRNA ID	Location	Study type	Study period	Cohort description	Cohort size	Detection method	Endogenous control	Prognostic value	Validation cohort	Cutoff method	Ref
miR-181a-5p	miR-181a	Japan	NA	1992–2000	TNM 0, I, II, III, IV	162 CRC	Taqman qRT-PCR	RNU6B	High miR-181a associated with shorter OS (HR =1.83, 95% CI =1.26–2.76, *P*=0.0013)	No	Median value	101
miR-185-5p	miR-185	Sweden	NA	1993–1998	TNM I, II, III, IV	50 CRC	Taqman qRT-PCR	miR-16	High miR-185 associated with shorter OS (*P*=0.001), no multivariate analysis	No	Median value	21
miR-194-5p	miR-194	Germany	NA	NA	TNM II, III, IV	30 CRC	SYBR Green qRT-PCR	18S rRNA	High miR-194 associated with shorter RFS and OS, no multivariate analysis	No	Median value	89
miR-195-5p	miR-195	People's Republic of China	NA	2005–2010	TNM I, II, III, IV	85 CRC	SYBR Green qRT-PCR	RNU6B	Low miR-195 associated with shorter OS (HR =2.44, 95% CI =1.12–5.30, *P*<0.05)	No	Highest tertile	102
miR-200c-3p	miR-200c	Germany	NA	NA	TNM I, II, III, IV	24 CRC	SYBR Green qRT-PCR	5S rRNA	High miR-200a associated with shorter OS (*P*=0.0122), no multivariate analysis	No	dCt value	103
miR-212-3p	miR-212	People's Republic of China	NA	2004–2010	TNM I, II, III, IV	180 CRC	Taqman qRT-PCR	RNU6B	Low miR-212 associated with shorter DFS and OS (HR =0.403, 95% CI =0.195–0.829, *P*=0.014)	No	Median value	104
–	miR-215	US	NA	1998–2003	TNM II, III	34 CC	Taqman qRT-PCR	RNU6B	High miR-215 associated with shorter OS (HR =3.516, 95% CI =1.007–12.280, *P*=0.025)	No	NA	105
miR-320a	miR-320	Denmark	NA	NA	TNM II	49 CC, 10 healthy control	miRNA array	LOWESS normalized with TIGR MIDAS 2.19 software	Low miR-320 associated with shorter PFS (HR =6.6, 95%CI =1.5–28.1, *P*=0.011)	No	Median value	81
miR-362-3p	miR-362-3p	Denmark, Poland, Australia	NA	1999–2006, 2005–2008	TNM II, III	89 MSS CRC and 14 healthy control	Taqman qRT-PCR	miR-340, miR-151-3p, RNU44	Low miR-362-3p associated with RFS (HR =3.23, 95% CI =1.26–8.32, *P*=0.015)	Yes, independent cohort	ROC curve	106
–	miR-372	Japan	NA	1992–2000	TNM I, II, III, IV	144 CRC	Taqman qRT-PCR	RNU6B	High miR-372 associated with shorter 5-year OS (HR =2.76, 95% CI =1.32–6.11, *P*=0.006)	No	Median value	107
miR-498	miR-498	Denmark	NA	NA	TNM II	49 CC, 10 healthy control	miRNA array	LOWESS normalized with TIGR MIDAS 2.19 software	Low miR-498 associated with shorter PFS (HR =11.5, 95% CI =2.3–59.0, *P*<0.003)	No	Median value	81
miR-1224-5p	miR-1224-5p	Finland	NA	NA	TNM IV	33 CRC, wild-type KRAS and BRAF	miRNA array and SYBR Green qRT-PCR	RNU6B	Low miR-1224-5p associated with shorter OS	No	NA	98

Plasma/serum miRNA												
miR-29c-3p	miR-29c	Taiwan	NA	NA	TNM II, III	107 CRC, 23 healthy control	Taqman qRT-PCR	RNU6B	High serum miR-29c associated with early relapse; low tissue miR-29c associated with early relapse (HR =2.722, 95% CI =1.301–6.172, *P*=0.007)	No	NA	108
miR-141-3p	miR-141	US and People's Republic of China	NA	US cohort: 2002–2008; People's Republic of China cohort: 2007–2009	TNM I, II, III, IV	US cohort: 74 CRC, 28 healthy control; People's Republic of China cohort: 111 CRC, 48 healthy control	Taqman qRT-PCR	Equal sample input, cel-miR-39	High miR-141 associated with shorter OS (HR =2.40, 95% CI =1.18–4.86, *P*=0.016)	Yes, independent cohort	Median value	109
miR-221-3p	miR-221	People's Republic of China	NA	2002–2009	TNM I, II, III, IV	103 CRC, 37 healthy control	SYBR Green qRT-PCR	Equal sample input; standard curve	High miR-221 associated with shorter OS (HR =3.478, 95% CI =1.038–11.654, *P*=0.043)	No	Youden index	110

Abbreviations: CC, colon cancer; CSS, cancer specific survival; CI, confidence interval; CRC, colorectal cancer; dCt, delta cycle threshold; DFS, disease free survival; HR, hazard ratio; ID, identification; LOWESS, locally weighted scatterplot smoothing; miRNA, microRNA; MSS, microsatellite stable; NA, not applicable; OS, overall survival; P, prospective study; PFS, progression free survival; qRT-PCR, quantitative real-time polymerase chain reaction; R, retrospective study; RC, rectal cancer; REST, relative expression software tool; RFS, relapse-free survival; ROC, receiver operating characteristic curve; TNM, tumor–node–metastasis stage; rRNA, ribosomal ribonucleic acid; ref, reference.

(88.6 months).[53,54] Both of the studies supported DICER1 as an independent prognostic factor for OS.

These associations were challenged by other studies. Comparable expression of DICER1 was observed in primary CRC tissues and the corresponding normal mucosa in several independent studies.[55–57] Faber et al[58] examined 237 patients with moderately differentiated CRC by immunohistochemistry. The intense staining of DICER1 in CRC showed a strong association with poor cancer-specific survival and reduced PFS. Fifteen out of the 237 stage I/II CRCs were DICER1-negative patients who did not experience any relapse in a 10-year follow-up, although the authors did not specify any correlation between DICER1 expression and tumor stage.[58] Stratmann et al[55] claimed that patients with high DICER1 mRNA expression in normal mucosa, but not in cancerous sites, were associated with worse clinical outcomes compared to those with a lower DICER1 expression.

miRNA-associated single-nucleotide polymorphism

The whole genome is constantly evolving and generates many germ-line single nucleotide alterations among individuals in a population. These alterations are known as single-nucleotide polymorphisms (SNPs). SNPs that locate in the protein-coding sequence may have an obvious biological effect by changing the amino acid sequence or yielding truncated protein product, whereas SNPs residing in the UTR do not alter the function of the protein, but they may occasionally perturb the protein expression level and may have pathogenic consequences.[59] In 2006, a team of researchers first corroborated that a G to A substitution in the 3′UTR of *GDF8* created an illegitimate miRNA octamer motif that could be transcriptionally downregulated by two miRNAs: miR-1-3p (miR-1) and miR-206.[60] This discovery had promoted intensive research on the potential application of miRNA-associated polymorphisms as biomarkers for the clinical outcomes of cancer, especially the miRNA-related SNP on the 3′UTR of the *KRAS* gene.

The germline variation rs61764370 (also called let-7 miRNA complementary site, LCS6), located in the let-7 complementary site in the *KRAS* 3′UTR mRNA, is one of the most intensively studied polymorphism-associated miRNA target SNPs. Compared to the wild-type T genotype, the less-frequent variant G transcript of *KRAS* exerts a high stability through escaping the let-7 translational repression and causes a high level of KRAS in the cell.[61,62] Generally, Caucasians have a higher frequency of the G allele (17.2%) compared to the other races.[63] While the G allele frequency

is comparable between healthy control, adenoma, and CRC, an increasing frequency is observed when the tumor stage increases, with 14% in the early stages and 21.4%–25.0% in the terminal stage.[64–66] In 2010, Graziano et al[66] first reported that the homozygous and heterozygous G allele carriers exhibited a significantly worse PFS and OS than the wild-type TT genotype metastatic patients who carried a *BRAF* V600-wildtype and received salvage cetuximab-irinotecan therapy. They also reported that, in a subgroup of 55 unresponsive patients carrying *KRAS* mutation, G type carriers showed a median OS of 5.9 months and PFS of 2.5 months, which was significantly shorter than the TT genotype patients, who had a median OS of 9.7 months and PFS of 3.4 months. On the contrary, conflicting results were reported by Ryan et al.[67] Based on a cohort of 237 cases of African-American and European American patients who were primarily treated with 5-fluorouracil, they found that the stage III/IV G allele carriers had a significantly reduced risk for death compared to the TT genotype, whereas no benefit was observed in the stage I and II subset. Smits et al[65] observed that the G allele correlated with a lower mortality risk in stage I/II patients. Most recently, Sha et al[63] carried out the largest cohort study to date and genotyped 2,834 stage III CC patients who received FOLFOX alone or combined with cetuximab. The variant-containing genotype showed no statistically significant association with DFS or time to recurrence in the whole cohort or in any treatment arm. Further, no correlations were observed between rs61764370 and molecular/clinical status, such as *KRAS*, *BRAF,* and mismatch repair, tumor grade, lymph-node status, and body mass index. In agreement with their findings, previous studies also suggested no association between rs61764370 and clinical outcomes of CRC, or stage IV CRC patients who were treated with Nordic FLOX, cetuximab, or both (Table 3).[64,68,69] There is no clear explanation for the conflicting observations among studies. It is speculated that the chemotherapy backbone would be one confounding factor.[64]

A SNP presented in pri-, pre-, or mature miRNA itself or in the miRNA processing machinery will potentially affect the miRNA expression and function.[70] Lin et al[71] performed a very informative study. On the basis of data mining of several SNP datasets and an miRNA prediction algorithm, they selected 41 SNPs located in eleven genes related to miRNA biogenesis, and 15 in pri-, pre-, or mature miRNA sequences. In the training phase, after stratifying by stage, they found that *RAN*/rs14035 and miR-373/rs12983273 showed a highly significant association with recurrence-free survival in stage II patients, whereas miR-608/rs4919510, *GEMIN3*/rs197412, *XPO5*/rs11077, *AGO2*/rs4961280, *GEMIN4*/rs2740348, *GEMIN3*/rs197388, and *GEMIN4*/rs7813 did so in stage III patients. Among the 218 cases with stage IV disease, four SNPs (let-7f-2/rs17276588, miR-30c-1/rs16827546, *DROSHA*/rs6877842, and *DICER1*/rs13078) were linked with the risk for recurrence. For the OS, *AGO2*/rs4961280, miR-608/rs4919510, miR-219a-1 (miR-219-1)/rs213210, miR-604/rs2368392, *DICER1*/rs13078, and *TRBP*/rs784567 were associated with the risk of death. The authors further verified the prognostic power of the 16 SNPs, and two of them retained the strong association with stage III patients. In the independent validation cohort, training cohort, or the combined cohorts, the C>G substitution in rs4919510 was associated with a higher risk for both recurrence and death, and a C>T substitution in rs213210 showed a significantly more adverse OS than the wild-type CC genotype.[71] The SNP rs4919510 is located in the mature miR-608 sequence, whereas the functional consequence of miR-219a-1/rs213210 is still unknown. It is speculated that rs213210 might affect the miR-219a-1 maturation. Lee et al[72] validated all 16 SNPs identified in Lin's study,[71] including miR-608/rs4919510 and miR-219a-1/rs213210. Unfortunately, none of the above-mentioned SNPs retained the prognostic power in a Korean cohort.[72] Intriguingly, a completely opposite clinical outcome and result for miR-608/rs4919510 was observed in a Chinese Han population.[73] Xing et al[73] found that the G allele carriers had a significantly favorable recurrence-free survival than the CC wild-type. Moreover, the association between rs4919510 and clinical outcome was more prominent in a subset of patients who received chemotherapy.

SNP rs2910164 resides in the stem region opposite to the mature miR-146a-5p (miR-146a). Experimental evidence demonstrated that the presence of the rare C allele caused a less-efficient processing reaction in vitro and ultimately led to a decreased level of mature miR-146a-5p.[74] Although the biological function of miR-146a-5p in CRC progression is still unknown, previous studies suggested that miR-146a-5p could negatively regulate the immune response.[75] Chae et al[76] observed that the GG or GC genotypes of rs2910164 were associated with better relapse-free and disease-specific survival compared with the homozygote CC genotype. However, in another Korea-based study, rs2910164 was shown to have no association with OS or relapse-free survival.[77]

miRNA and microsatellite instability

CRC mainly arises through two distinct mutational pathways. The first pathway is chromosomal instability characterized

Table 3 The prognostic value of miRNA-associated single-nucleotide polymorphisms in CRC

miRNA/SNP	Variation (M/m)	Ethnicity	Stages	Cohort size (case/control)	Method	Prognosis value	Validation	Ref
let-7 rs61764370	T/G	European population	TNM IV	138 CRC	Pyrosequencing	The G allele associated with shorter PFS (HR =1.59, 95% CI =1.04–2.75, *P*=0.03) and OS (HR =1.68, 95% CI =1.14–2.7, *P*=0.002) compared to the wild-type TT genotype	No	66
let-7 rs61764370	T/G	European population	TNM I, II, III, IV	734 CRC	Taqman PCR	The G allele associated with better survival in stage I/II	No	65
let-7 rs61764370	T/G	Norwegian	TNM IV	535 mCRC in the NORDIC-VII cohort; 197 CRC, 1,060 adenoma, 358 healthy control in the KAM cohort	Taqman PCR	No significant difference of OS and PFS between TT genotype and G allele	No	64
let-7 rs61764370	T/G	African-American, European American	TNM I, II, III, IV	237 CRC, 441 healthy control	Not mentioned	The G allele associated with better OS in stage III and IV compared to the TT genotype (HR =0.38, 95% CI =0.17–0.92, *P*=0.025)	No	67
let-7 rs61764370	T/G	Caucasian, African-American, Asian	TNM III	2,834 CC	Taqman PCR	The G allele showed no significant association with either DFS or TTR, in the whole cohort or any treatment arms	No	63
let-7 rs61764370	T/G	Caucasian, African-American, Asian, others	TNM IV	130 mCRC	PCR-RFLP	The G allele showed no significant association with OS and PFS	No	69
let-7 rs61764370	T/G	Caucasian, African-American, others	TNM I, II, III, IV	1,103 CRC	Taqman PCR	The G allele showed no significant association with OS, RFS, and PFS	Yes	68
miR-146a rs2910164	G/C	Korean	TNM I, II, III, IV	399 CRC, 568 healthy control	PCR-RFLP	The CC genotype associated with shorter RFS (HR =2.120, 95% CI =1.257–3.574, *P*=0.005) and DSS (HR =2.349, 95% CI =1.257–4.390, *P*=0.007) compared to the G allele	No	76
miR-146a rs2910164	G/C	Korean	TNM I, II, III, IV	446 CRC	PCR-RFLP	No significant association with OS and RFS	No	77
miR-149 rs2292832	C/T	Korean	TNM I, II, III, IV	446 CRC	PCR-RFLP	No significant association with OS and RFS	No	77
miR-196a2 rs11614913	C/T	Korean	TNM I, II, III, IV	446 CRC	PCR-RFLP	C allele associated with unfavorable OS in rectal cancer	No	77
miR-219-1 rs213210	C/T	Caucasian, African-American, others	TNM I, II, III, IV	1,097 CRC	SNPlex	The T allele associated with shorter OS	Yes	71
miR-423 rs6505162	A/C	Han Chinese	TNM I, II, III, IV	408 CRC	iPLEX	The C allele associated with worse OS and RFS	No	73
miR-492 rs2289030	C/G	Korean	TNM I, II, III, IV	426 CRC	Real-time PCR genotyping assay	The G allele associated with worse PFS	No	72

(*Continued*)

Table 3 (*Continued*)

miRNA/SNP	Variation (M/m)	Ethnicity	Stages	Cohort size (case/control)	Method	Prognosis value	Validation	Ref
miR-499 rs3746444	G/A	Korean	TNM I, II, III, IV	446 CRC	PCR-RFLP	No significant association with OS and RFS	No	77
miR-608 rs4919510	C/G	Caucasian, African-American, others	TNM I, II, III, IV	1,097 CRC	SNPlex	The G allele associated with a higher risk for both recurrence and death in stage III CRC	Yes	71
miR-608 rs4919510	C/G	Han Chinese	TNM I, II, III, IV	408 CRC	iPLEX	The G allele associated with better OS and RFS	No	73

Abbreviations: CC, colon cancer; CI, confidence interval; CRC, colorectal cancer; DFS, disease-free survival; DSS, disease-specific survival; HR, hazard ratio; mCRC, metastatic colorectal cancer; M/m, majority/minority; OS, overall survival; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; PFS, progression-free survival; RFS, recurrence-free survival; SNP, single-nucleotide polymorphism; TNM, tumor–node–metastasis stage; TTR, time to recurrence ; miRNA, microRNA; ref, reference.

by an imbalance in chromosome number, subchromosomal genomic amplifications, and a high frequency of loss of heterozygosity.[78] The other pathway is the MSI pathway, featured by increased short tandem repeats (microsatellites) due to a malfunctioning DNA mismatch repair system, and it accounts for 15% of all cases of CRC.[79] Although MSI and microsatellite stable (MSS) are two histologically similar CRC subtypes, they have different clinical and pathologic features. In general, MSI patients have better survival and are less likely to develop metastasis.[79] It is therefore assumed that MSI-related miRNAs have prognostic potential as well.

Indeed, it has been proven that CRC tumors have different miRNA expression signatures according to their MSI status.[80–83] Lanza et al firstly reported a list of 27 predictors of mRNA/miRNA that can discriminate MSI-high (MSI-H) from MSS tumors.[80] Schepeler et al[81] focused on MSI-related miRNA profiles in a study of 49 patients with stage II CC. They identified a four-miRNA-signature (miR-142-3p, miR-212-3p [miR-212], miR-151a-3p [miR-151], and miR-144-3p [miR-144]) that can specifically discriminate stage II CC according to microsatellite status. Sarver et al[82] dichotomized 80 subjects into sporadic MSI-H group and MSS/MSI-low (MSI-L) group. They revealed that four miRNAs (miR-552, miR-592, miR-181c-5p [miR-181c], and miR-196b-5p [miR196b]) were decreased in MSS/MSI-L patients compared with the MSI-H group, whereas miR-625 and miR-31 exhibited increased expression in MSI-H group. In addition to the sporadic MSI cases, Balaguer et al[83] included hereditary nonpolyposis CC cases in their study. They demonstrated that a signature of 59 miRNAs was able to distinguish MSI from MSS tumors. Moreover, they reported that an miRNA signature (miR-622, miR-362-5p, and miR-486-5p) was able to accurately discriminate hereditary nonpolyposis colorectal cancer cases from sporadic MSI patients. Earle et al[84] selected 23 miRNAs' based on previous work and evaluated these miRNAs' expression in a cohort of 55 CRC cases. They characterized the study cohort as MSI-H, MSI-L, and MSS as determined by microsatellite marker polymerase chain reaction or immunohistochemistry. Elevated relative expression of miR-155-5p (miR-155), miR-31-5p (miR-31), miR-223-3p (miR-223), and miR-26b-5p (miR-26b) was significantly associated with MSI-H status, whereas increased relative expression of miR-92a-3p (miR-92), let-7a-5p (let-7a), and miR-145-5p (miR-145) was associated with MSI-L. Increased relative expression of miR-196a was associated with MSS status. Five independent studies[80–84] described MSI-associated candidate miRNAs, but only part of the candidates overlapped with each other (eg, miR-155-5p and miR-223-3p).

Furthermore, they have seldom been validated at the MSI/MSS background. Finally, caution has to be taken when interpreting these MSI-related miRNA markers. For example, increased miR-155-5p was identified as a MSI-H marker, which theoretically should be regarded as a favorable prognostic factor.[80,84] On the other hand, high tissue miR-155-5p was observed to be associated with lymph-node metastasis and independently predicted higher risk for mortality.[16]

Conclusion and future perspectives

In this article, we have introduced the recent findings concerning the prognostic potential of miRNAs in CRC. Although the literature of identification of novel miRNA markers has increased rapidly in the last 7 years, we are still in the very initial stage of the clinical-application realm. So far, three tissue miRNAs (miR-21-5p, miR-29-3p, miR-148-3p) have been examined in multiple studies, of which miR-21-5p is the most promising prognostic marker, yet further prospective validation studies are required before it can go into clinical use. Most of the current research comprises initial exploratory studies that suffered from methodologic flaws, including small sample size, nontransparent patient information, lack of replication, and poor statistical analysis. We are expecting a multimarker signature in the future that can accurately predict clinical outcomes, although the cost-efficiency issue should be also considered. Moreover, for each potential prognostic biomarker, it is necessary to understand its molecular function and the associated mechanisms behind its dysregulation, which may help support its clinical use and provide novel therapeutic targets.

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Disclosure

The authors report no conflicts of interest in this work.

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