MicroRNA dysregulation as a prognostic biomarker in colorectal cancer

Yujuan Dong1,2
Jun Yu2
Simon SM Ng1,2

1Division of Colorectal Surgery, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong; 2Institute of Digestive Disease, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong

Correspondence: Simon SM Ng
Division of Colorectal Surgery, Department of Surgery, Prince of Wales Hospital, Li Ka Shing Medical Sciences Building, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong
Tel +852 2632 1495
Fax +852 2637 7974
Email simonng@surgery.cuhk.edu.hk

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

<table>
<thead>
<tr>
<th>Location</th>
<th>Study type</th>
<th>Study period</th>
<th>Cohort description</th>
<th>Cohort size</th>
<th>Detection method</th>
<th>Endogenous control</th>
<th>Prognostic value</th>
<th>Validation cohort</th>
<th>Cutoff method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oslo region</td>
<td>P</td>
<td>1998–2000</td>
<td>TNM I, II, III</td>
<td>193 CRC</td>
<td>Taqman qRT-PCR</td>
<td>RNU44</td>
<td>No significant association with 5-year DFS</td>
<td>No</td>
<td>Mean, median and tertile</td>
<td>12</td>
</tr>
<tr>
<td>US, Hong Kong</td>
<td>R</td>
<td>1993–2002, Hong Kong cohort 1991–2000</td>
<td>US cohort: 84 CC; Hong Kong cohort: 113 CC</td>
<td>193 CRC</td>
<td>Microarray and Taqman qRT-PCR</td>
<td>RNU46 and LOESS</td>
<td>High miR-21 associated with shorter OS of stage II CC: US cohort: HR = 2.7, 95% CI = 1.3–5.5, P &lt; 0.008; Hong Kong cohort: HR = 2.4, 95% CI = 1.4–4.1, P &lt; 0.001</td>
<td>Yes, independent cohort</td>
<td>Highest tertile</td>
<td>14</td>
</tr>
<tr>
<td>Denmark, Scotland</td>
<td>R</td>
<td>1991–1993</td>
<td>Dukes’ B</td>
<td>149 CC, 85 RC</td>
<td>In situ hybridization</td>
<td>RNU6B</td>
<td>Strong stained miR-21 associated with shorter DFS and OS in stage II CC patients, independent prognosis factor; not associated with stage II RC</td>
<td>No</td>
<td>Tertile</td>
<td>15</td>
</tr>
<tr>
<td>Japan</td>
<td>NA</td>
<td>2000–2005</td>
<td>Dukes’ A, B, C, D</td>
<td>156 CRC</td>
<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>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)</td>
<td>No</td>
<td>Mean value</td>
<td>16</td>
</tr>
<tr>
<td>US, Hong Kong</td>
<td>R</td>
<td>1993–2002, Hong Kong cohort 1991–2000</td>
<td>US cohort: 83 CC; Hong Kong cohort: 113 CC</td>
<td>193 CRC</td>
<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>High miR-21 associated with shorter OS (HR = 3.0, 95% CI = 1.7–5.2, P &lt; 0.0005)</td>
<td>No</td>
<td>Highest tertile</td>
<td>17</td>
</tr>
<tr>
<td>People’s Republic of China</td>
<td>R</td>
<td>2000–2008, 2012</td>
<td>TNM II</td>
<td>775 CC</td>
<td>miRNA array and SYBR Green qRT-PCR</td>
<td>RNU6B</td>
<td>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 &lt; 0.0001)</td>
<td>Yes, independent cohort</td>
<td>Formula risk score</td>
<td>18</td>
</tr>
<tr>
<td>The Czech Republic</td>
<td>NA</td>
<td>2004–2005; 2002–2004</td>
<td>TNM I, II, III, IV</td>
<td>46 CRC, 30 CLM</td>
<td>Taqman qRT-PCR</td>
<td>Total RNA input; RNU6B; miR-191</td>
<td>No</td>
<td>High miR-21 associated with shorter DFI; no significant correlation with OS</td>
<td>Optimal cutoff value 8.1</td>
<td>19</td>
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<tr>
<td>Spain</td>
<td>P</td>
<td>2002–2003</td>
<td>TNM I, II, III, IV</td>
<td>28 CRC, 7 GC, 3 PC</td>
<td>SYBR Green qRT-PCR</td>
<td>RNU6B; miR-191</td>
<td>No significant association between PFS and OS</td>
<td>No</td>
<td>Mean value and the REST analysis</td>
<td>20</td>
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<tr>
<td>People’s Republic of Spain</td>
<td>NA</td>
<td>2006–2008</td>
<td>TNM I, II, III, IV (serum sample)</td>
<td>200 CRC, 50 AA, 80 healthy control</td>
<td>Taqman qRT-PCR</td>
<td>miR-16</td>
<td>No significant association between serum miR-21 and OS</td>
<td>No</td>
<td>NA</td>
<td>22</td>
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<tr>
<td>Spain</td>
<td>P</td>
<td>2008–2010</td>
<td>TNM I, II, III, IV (serum sample)</td>
<td>102 CRC</td>
<td>Taqman qRT-PCR</td>
<td>miR-16</td>
<td>Low serum miR-21 has a borderline association with shorter OS; no significant association between serum miR-21 and DFS</td>
<td>No</td>
<td>Relative expression value 1</td>
<td>23</td>
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</tbody>
</table>

(Continued)
### Table 1 (Continued)

<table>
<thead>
<tr>
<th>Location</th>
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<th>Study period</th>
<th>Cohort description</th>
<th>Cohort size</th>
<th>Detection method</th>
<th>Endogenous control</th>
<th>Prognostic value</th>
<th>Validation cohort</th>
<th>Cutoff method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Czech Republic</td>
<td>NA</td>
<td>NA</td>
<td>TNM I, II, III, IV</td>
<td>29 CRC</td>
<td>Taqman qRT-PCR</td>
<td>let-7a-1</td>
<td>High miR-21 associated with lymph node positivity and the development of metastases in CRC patients</td>
<td>No</td>
<td>NA</td>
<td>24</td>
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<tr>
<td><strong>miR-29a-3p (miR-29a)</strong></td>
<td>People’s Republic of China</td>
<td>P</td>
<td>Not mentioned</td>
<td>TNM II, III, IV</td>
<td>58 CLM, 56 CRC</td>
<td>Taqman qRT-PCR</td>
<td>miR-16</td>
<td>No significant association between serum miR-29a and survival</td>
<td>No</td>
<td>Cutoff value 0.155</td>
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<tr>
<td>People’s Republic of China</td>
<td>NA</td>
<td>2009</td>
<td>TNM I, II, III, IV</td>
<td>85 CRC</td>
<td>SYBR Green qRT-PCR</td>
<td>RNU6B</td>
<td>High miR-29a associated with CRC metastasis and shorter OS</td>
<td>No</td>
<td>Median level</td>
<td>38</td>
</tr>
<tr>
<td>The Czech Republic</td>
<td>NA</td>
<td>2009–2011</td>
<td>TNM I, II, III, IV</td>
<td>100 CRC, 30 healthy control</td>
<td>Taqman qRT-PCR</td>
<td>miR-16</td>
<td>Increased serum miR-29a associated with advanced stages</td>
<td>No</td>
<td>NA</td>
<td>40</td>
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<tr>
<td>Israel</td>
<td>R</td>
<td>1995–2005</td>
<td>TNM I, II</td>
<td>110 CC</td>
<td>miRNA array and Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-29a associated with shorter DFS (HR = 0.194, 95% CI = 0.063–0.597, P = 0.0043)</td>
<td>No</td>
<td>Tertile</td>
<td>41</td>
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<tr>
<td>Taiwan</td>
<td>NA</td>
<td>NA</td>
<td>TNM I, II, III</td>
<td>78 CRC</td>
<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>Downregulated in the recurrence group</td>
<td>No</td>
<td>Median value</td>
<td>43</td>
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<tr>
<td><strong>miR-148a-3p (miR-148a)</strong></td>
<td>People’s Republic of China</td>
<td>NA</td>
<td>NA</td>
<td>TNM I, II, III, IV</td>
<td>101 CRC</td>
<td>SYBR Green qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-148a associated with increased tumor size and advanced primary tumor stage</td>
<td>No</td>
<td>Median value</td>
</tr>
<tr>
<td>Spain</td>
<td>NA</td>
<td>1996–2008</td>
<td>TNM II, III, IV</td>
<td>273 CRC, 20 healthy control</td>
<td>Taqman qRT-PCR</td>
<td>miR-16</td>
<td>Low miR-148a associated with shorter DFS (HR = 1.83, 95% CI = 1.12–2.99, P = 0.017) in stage III, and worse therapeutic response in stage IV (HR = 1.93, 95% CI = 1.15–3.23, P = 0.014)</td>
<td>No</td>
<td>ROC and median value</td>
<td>45</td>
</tr>
<tr>
<td>Taiwan</td>
<td>NA</td>
<td>NA</td>
<td>TNM I, II, III</td>
<td>195 CRC</td>
<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-148a associated with shorter DFS and OS (HR = 5.211, 95% CI = 2.069–13.174, P &lt; 0.0001)</td>
<td>No</td>
<td>Mean value</td>
<td>46</td>
</tr>
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</table>

**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\textsuperscript{12} 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.\textsuperscript{14} Consistent with their previous report,\textsuperscript{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\textsuperscript{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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{14} Initially it was identified to be upregulated in colonic epithelial cells.\textsuperscript{14} Further studies indicated that miR-21-5p was predominantly overexpressed in cancer-associated fibroblasts in CRC.\textsuperscript{15,28} Laboratory evidence of its role in CRC progression through fibroblast-to-myofibroblast transdifferentiation provided coherence to the abovementioned epidemiological findings.\textsuperscript{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.\textsuperscript{13} 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,\textsuperscript{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.\textsuperscript{31–34} Higher levels of plasma miR-29a-3p were also detected in CRC and advanced adenoma patients compared with normal healthy donors.\textsuperscript{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.\(^\text{37}\) In line with the prior study,\(^\text{37}\) 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.\(^\text{38}\) 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.\(^\text{38}\) 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.\(^\text{39}\) A higher tumor:normal ratio of miR-29a-3p was associated with significantly worse DFS in a cohort of 77 CC patients.\(^\text{39}\)

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, Faltejsekova et al\(^\text{40}\) documented a comparable expression of miR-29a-3p in primary CRC serum and healthy subject serum in a Caucasian population. Weissmann-Brenner et al\(^\text{41}\) 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.\(^\text{41}\) 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\(^\text{42}\) 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\(^\text{43}\) 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.\(^\text{44}\) 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\(^\text{37,38}\) 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\(^\text{41,43}\) 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.\(^\text{26}\) Lack of stratification may have led to those contradictory findings. Fourth, most studies mentioned above\(^\text{37,38,41}\) 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
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. 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. Tsai et al 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, 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-29a-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, 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 exciting the stem-loop premiRNAs 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. 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. DICER1 is a haploinsufficient tumor suppressor, and deletion of DICER1 has been evidenced in various human cancers. Experimental evidence suggested that impaired DICER1 causes a global reduction in mature miRNA levels and promotes tumor growth and metastasis. 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. Akahane 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 Duke’s 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 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

<table>
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<th>Study type</th>
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<th>Cohort description</th>
<th>Cohort size</th>
<th>Detection method</th>
<th>Endogenous control</th>
<th>Prognostic value</th>
<th>Validation cohort</th>
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<td>High miR-10b associated with shorter 10-year OS (HR = 1.56, 95% CI = 1.06–2.38, P = 0.025)</td>
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<td>2002–2006</td>
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<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-16 associated with shorter 5-year OS (HR = 1.67, 95% CI = 1.22–2.54, P = 0.018)</td>
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<td>ROC curve</td>
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<td>High miR-17 associated with shorter 5-year OS (HR = 2.67, 95% CI = 1.31–6.82, P = 0.007)</td>
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<td>2002–2003</td>
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<td>28 CRC, 7 GC, 3 PC</td>
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<td>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 &lt; 0.001)</td>
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<td>SS rRNA, RNU6B</td>
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<td>Taqman qRT-PCR</td>
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<td>Oslo region</td>
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<td>RNU6B</td>
<td>High miR-92a associated with shorter 5-year OS (HR = 2.342, 95% CI = 1.072–5.115, P = 0.033)</td>
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<td>Low miR-93 associated with shorter OS (HR = 4.3, 95% CI = 0.8–17.2, P = 0.02)</td>
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<td>TNM i, ii, iii, iv</td>
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<td>Taqman qRT-PCR</td>
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<td>Taqman qRT-PCR</td>
<td>RNU44</td>
<td>No significant association with 5-year DFS</td>
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<td>Year Range</td>
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<td>Site</td>
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<td>P Value</td>
<td>OS (HR) ± CI</td>
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<td>110 CC</td>
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<td>SS rRNA</td>
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<td>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)</td>
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<td>SS rRNA</td>
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<td>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)</td>
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<td>RNU6B</td>
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<td>Taqman qRT-PCR</td>
<td>RNU6B, RNU4, RNU4B</td>
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<td>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)</td>
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(Continued)
Table 2 (Continued)

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<td>TNM II, III, IV</td>
<td>30 CRC</td>
<td>SYBR Green qRT-PCR</td>
<td>185 rRNA</td>
<td>High miR-194 associated with shorter RFS and OS, no multivariate analysis</td>
<td>No</td>
<td>Median value</td>
<td>89</td>
</tr>
<tr>
<td>miR-195-5p</td>
<td>miR-195</td>
<td>People's Republic of China</td>
<td>NA</td>
<td>2005–2010</td>
<td>TNM I, II, III, IV</td>
<td>85 CRC</td>
<td>SYBR Green qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-195 associated with shorter OS (HR =2.44, 95% CI =1.12–5.30, P&lt;0.05)</td>
<td>No</td>
<td>Median value</td>
<td>102</td>
</tr>
<tr>
<td>miR-200c-3p</td>
<td>miR-200c</td>
<td>Germany</td>
<td>NA</td>
<td>NA</td>
<td>TNM I, II, III, IV</td>
<td>24 CRC</td>
<td>SYBR Green qRT-PCR</td>
<td>18S rRNA</td>
<td>No</td>
<td>dCt value</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>miR-212-3p</td>
<td>miR-212</td>
<td>People's Republic of China</td>
<td>NA</td>
<td>2004–2010</td>
<td>TNM I, II, III, IV</td>
<td>180 CRC</td>
<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-212 associated with shorter DFS and OS (HR =0.403, 95% CI =0.195–0.829, P=0.014)</td>
<td>No</td>
<td>Median value</td>
<td>104</td>
</tr>
<tr>
<td>miR-215</td>
<td>miR-215</td>
<td>US</td>
<td>NA</td>
<td>1998–2003</td>
<td>TNM II, III</td>
<td>34 CC</td>
<td>Taqman qRT-PCR</td>
<td>RNU6B</td>
<td>High miR-215 associated with shorter OS (HR =3.516, 95% CI =1.007–12.280, P=0.025)</td>
<td>No</td>
<td>NA</td>
<td>105</td>
</tr>
<tr>
<td>miR-320a</td>
<td>miR-320</td>
<td>Denmark</td>
<td>NA</td>
<td>NA</td>
<td>TNM II</td>
<td>49 CC, 10 healthy control</td>
<td>miRNA array normalized with TiGR MIDAS 2.19 software</td>
<td>RNU6B</td>
<td>Low miR-320 associated with shorter PFS (HR =6.6, 95%CI =1.5–28.1, P=0.011)</td>
<td>No</td>
<td>Median value</td>
<td>81</td>
</tr>
<tr>
<td>miR-362-3p</td>
<td>miR-362-3p</td>
<td>Denmark, Poland, Australia</td>
<td>NA</td>
<td>1999–2006, 2005–2008</td>
<td>TNM II, III</td>
<td>89 MSS CRC and 14 healthy control</td>
<td>Taqman qRT-PCR</td>
<td>miR-3-40, miR-151-3p, RNU6B</td>
<td>Low miR-362-3p associated with shorter DFS and OS (HR =3.23, 95% CI =1.6–5.6, P=0.015)</td>
<td>Yes, independent cohort</td>
<td>ROC curve</td>
<td>106</td>
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<tr>
<td>miR-498</td>
<td>miR-498</td>
<td>Denmark</td>
<td>NA</td>
<td>NA</td>
<td>TNM II</td>
<td>49 CC, 10 healthy control</td>
<td>miRNA array normalized with TiGR MIDAS 2.19 software</td>
<td>RNU6B</td>
<td>No</td>
<td>Median value</td>
<td>81</td>
<td></td>
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<tr>
<td>miR-1224-5p</td>
<td>miR-1224-5p</td>
<td>Finland</td>
<td>NA</td>
<td>NA</td>
<td>TNM IV</td>
<td>33 CRC, wild-type KRAS and BRAF</td>
<td>miRNA array and SYBR Green qRT-PCR</td>
<td>RNU6B</td>
<td>Low miR-1224-5p associated with shorter OS</td>
<td>No</td>
<td>NA</td>
<td>98</td>
</tr>
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</table>
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 may occasionally perturb the protein expression level and yield unanticipated protein products, whereas SNPs residing in the 3′UTR do not alter the function of the protein, but they may have pathogenic consequences. In 2006, a team of researchers first corroborated that a G to A substitution in the 3′UTR of the GDF8 gene created an illegitimate miRNA octamer motif that could be transcriptionally downregulated by two miRNAs: miR-1-3p (miR-1) and miR-221-3p. This discovery motivated intensive research on the potential application of miRNA-associated polymorphisms as biomarkers for the clinical outcomes of cancer, especially the presence of miRNA target SNPs. Compared to the wild-type T genotype, of the most intensively studied polymorphism-associated complementary site in the let-7a, LCS6, located in the let-7a miRNA complementary site, were associated with worse clinical outcomes compared to those with a lower DICER1 expression. The intense staining of DICER1 in CRC showed a strong correlation between DICER1 expression and tumor stage. Faber et al. examined 27 patients with negative patients who did not experience any relapse in a 10-year follow-up, although the authors did not specify any association with poor cancer-specific survival and reduced association with poor cancer-specific survival and reduced RFS. Fifteen out of the 27 stage III CRCs were DICER1-negative patients who did not experience any relapse in a 10-year follow-up, although the authors did not specify any association with poor cancer-specific survival and reduced association with poor cancer-specific survival and reduced RFS.
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. In 2010, Graziano et al first reported that the homzygous 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. 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 observed that the G allele correlated with a lower mortality risk in stage I/II patients. Most recently, Sha et al 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). There is no clear explanation for the conflicting observations among studies. It is speculated that the chemotherapy backbone would be one confounding factor.

A SNP presented in pri-, pre-, or mature miRNA itself or in the miRNA processing machinery will potentially affect the miRNA expression and function. Lin et al 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/rs1687546, 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. 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 validated all 16 SNPs identified in Lin’s study, including miR-608/rs4919510 and miR-219a-1/rs213210. Unfortunately, none of the above-mentioned SNPs retained the prognostic power in a Korean cohort. Intriguingly, a completely opposite clinical outcome and result for miR-608/rs4919510 was observed in a Chinese Han population. Xing et al 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. 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. Chae et al 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.

**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

<table>
<thead>
<tr>
<th>miRNA/SNP</th>
<th>Variation (M/m)</th>
<th>Ethnicity</th>
<th>Stages</th>
<th>Cohort size (case/control)</th>
<th>Method</th>
<th>Prognosis value</th>
<th>Validation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>European population</td>
<td>TNM IV</td>
<td>138 CRC</td>
<td>Pyrosequencing</td>
<td>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</td>
<td>No</td>
<td>66</td>
</tr>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>European population</td>
<td>TNM I, II, III, IV</td>
<td>734 CRC</td>
<td>Taqman PCR</td>
<td>The G allele associated with better survival in stage VII</td>
<td>No</td>
<td>65</td>
</tr>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>Norwegian</td>
<td>TNM IV</td>
<td>535 mCRC; 197 CRC, 1,060 adenoma, 358 healthy control in the KAM cohort</td>
<td>Taqman PCR</td>
<td>No significant difference of OS and PFS between TT genotype and G allele</td>
<td>No</td>
<td>64</td>
</tr>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>African-American, European American</td>
<td>TNM I, II, III, IV</td>
<td>237 CRC, 441 healthy control</td>
<td>Not mentioned</td>
<td>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)</td>
<td>No</td>
<td>67</td>
</tr>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>Caucasian, African-American, Asian</td>
<td>TNM III</td>
<td>2,834 CC</td>
<td>Taqman PCR</td>
<td>The G allele showed no significant association with either DFS or TTR, in the whole cohort or any treatment arms</td>
<td>No</td>
<td>63</td>
</tr>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>Caucasian, African-American, Asian, others</td>
<td>TNM IV</td>
<td>130 mCRC</td>
<td>PCR-RFLP</td>
<td>The G allele showed no significant association with OS and PFS</td>
<td>No</td>
<td>69</td>
</tr>
<tr>
<td>let-7</td>
<td>T/G</td>
<td>Caucasian, African-American, others</td>
<td>TNM I, II, III, IV</td>
<td>1,103 CRC</td>
<td>Taqman PCR</td>
<td>The G allele showed no significant association with OS, RFS, and PFS</td>
<td>Yes</td>
<td>68</td>
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<tr>
<td>miR-146a</td>
<td>G/C</td>
<td>Korean</td>
<td>TNM I, II, III, IV</td>
<td>399 CRC, 568 healthy control</td>
<td>PCR-RFLP</td>
<td>The G allele showed no significant association with OS, RFS, and PFS</td>
<td>No</td>
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</tr>
<tr>
<td>rs2910164</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>77</td>
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<tr>
<td>miR-146a</td>
<td>G/C</td>
<td>Korean</td>
<td>TNM I, II, III, IV</td>
<td>446 CRC</td>
<td>PCR-RFLP</td>
<td>No significant association with OS and RFS</td>
<td>No</td>
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<td>rs2910164</td>
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<td>No</td>
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<td>miR-149</td>
<td>C/T</td>
<td>Korean</td>
<td>TNM I, II, III, IV</td>
<td>446 CRC</td>
<td>PCR-RFLP</td>
<td>No significant association with OS and RFS</td>
<td>No</td>
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<td>rs2292832</td>
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<tr>
<td>miR-196a2</td>
<td>C/T</td>
<td>Korean</td>
<td>TNM I, II, III, IV</td>
<td>446 CRC</td>
<td>PCR-RFLP</td>
<td>C allele associated with unfavorable OS in rectal cancer</td>
<td>No</td>
<td>77</td>
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<td>rs11614913</td>
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<tr>
<td>miR-219-1</td>
<td>C/T</td>
<td>Caucasian, African-American, others</td>
<td>TNM I, II, III, IV</td>
<td>1,097 CRC</td>
<td>SNPlex</td>
<td>The T allele associated with shorter OS</td>
<td>Yes</td>
<td>73</td>
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<td>rs213210</td>
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<tr>
<td>miR-423</td>
<td>A/C</td>
<td>Han Chinese</td>
<td>TNM I, II, III, IV</td>
<td>408 CRC</td>
<td>iPLEX</td>
<td>The G allele associated with worse OS and RFS</td>
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<td>rs6505162</td>
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<td></td>
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<tr>
<td>miR-492</td>
<td>C/G</td>
<td>Korean</td>
<td>TNM I, II, III, IV</td>
<td>426 CRC</td>
<td>Real-time PCR genotyping assay</td>
<td>The G allele associated with worse PFS</td>
<td>No</td>
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<tr>
<td>rs2289030</td>
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</table>

(Continued)
by an imbalance in chromosome number, subchromosomal genomic amplifications, and a high frequency of loss of heterozygosity.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{80} Schepeler et al\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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. 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.

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 multimer 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.

References


