MicroRNAs as growth regulators, their function and biomarker status in colorectal cancer

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ABSTRACT

Gene expression is in part regulated by microRNAs (miRNAs). This review summarizes the current knowledge of miRNAs in colorectal cancer (CRC); their role as growth regulators, the mechanisms that regulate the miRNAs themselves and the potential of miRNAs as biomarkers. Although thousands of tissue samples and bodily fluids from CRC patients have been investigated for biomarker potential of miRNAs (>160 papers presented in a comprehensive tables), none single miRNA nor miRNA expression signatures are in clinical use for this disease. More than 500 miRNA-target pairs have been identified in CRC and we discuss how these regulatory nodes interconnect and affect signaling pathways in CRC progression.

INTRODUCTION

Western societies exhibit a high incidence and mortality of colorectal cancer (CRC), accounting for 10% of the total cancer burden with an individual lifetime risk of ~6% [1, 2]. CRC patients are stratified according to a clinical staging system (I - IV) at the time of diagnosis. Approximately 20–25% of patients present with metastatic disease, and another 25% will develop metastases in the follow-up period. There is a great need to identify precise biomarkers to facilitate the correct diagnosis, treatment and predict or monitor cancer recurrence.

MiRNAs are a non-protein coding class of small regulatory RNAs (22-nucleotides long) that play an essential role in post-transcriptional regulation of gene expression through binding to the 3’-untranslated region (3’-UTR) of protein-coding mRNAs. A single miRNA may regulate multiple target mRNAs and miRNAs are predicted to regulate approximately 60% of the human genes [3]. Identification and annotation of miRNAs ‘parallels’ technology development, and the introduction of e.g. deep sequencing technologies have provided tools for detection and discovery of the regulatory RNA species [4]. The recently released miRNA registry database (miRBASE v21, June 2014) reported a total of 1881 human miRNA genes, counting 2588 unique mature sequences. Independent of the challenges we still are facing regarding miRNA detection methods [5], there are no doubt that these molecules play essential roles in diverse cellular processes [6, 7] (Box 1).

The first evidence associating miRNAs with cancer was demonstrated by the Croce laboratory in 2002, showing that the common 13q14 deletion reduced expression of the miR-15a/16-1 cluster, located within the intron of the DLEU2 gene, ultimately leading to chronic lymphocytic leukemia [10]. The biological functions of miRNAs are highly dependent on the cellular context, which differ due to diverse compilation of the transcriptome in different tissues and cells. Consequently, and depending on their transcript targets, some miRNAs have increased expression and act as oncogenes in one cancer type, whereas they are downregulated and function as tumor suppressors in another cancer type. Such variability has for instance been seen for let-7, miR-15a/16-1, miR-17-92 cluster, miR-26, miR-29 and miR-125a/b [18, 19]. Therefore, care must be taken when generalizing interpretations of miRNA function across different contexts and tissues.

In this review, we summarize miRNAs that are relevant to CRC, describe research that has led to better understanding of the miRNA function and highlight miRNA involvement in the major signaling pathways.
OVERVIEW OF MAIN MIRNA RESEARCH AREAS IN CRC

MiRNA-induced deregulation in CRC has been well documented and continues to emerge as illustrated by the rapid increase of published studies (information retrieval and handling described in detail in Supplementary Methods) and growing numbers of analyzed clinical samples (Figure 1A). The aberrantly expressed miRNAs and their effects have been primarily addressed (Figure 1B). Approximately 70% of the reports that studied miRNAs in CRC analyzed clinical patient specimens and use of the patient samples increased in recent years. A similar increase in the size of the patient series is not seen (Figure 1C). The mechanisms that deregulate miRNAs, such as single nucleotide polymorphisms (SNPs), epigenetic alterations, mutations, amplifications and loss of genomic regions encoding miRNAs and transcriptional regulation have been addressed to a lower degree (Figure 1B). An overview of the main miRNA research activities in CRC have been generated by recording and ranking the keywords (Figure 2A). The following sections summarize the main research activities and compile the details on the miRNAs’ functional role with regards to CRC development and progression.

MIRNAS DRIVING INVASION, MIGRATION AND METASTASIS

Despite the fact that several miRNAs have been found deregulated early in CRC development, miRNAs have most frequently been described associated with invasion, migration and the progression of disease through epithelial mesenchymal transition (EMT) into metastases (Figure 2A). Cancer cells undergo several molecular changes to generate a mesenchymal cell phenotype necessary for cells to detach and leave the primary tumor. EMT is characterized by loss of cell adhesion, repression of CDH1, often caused by transcriptional repression by SNAI1 [20] and ZEB1/2 [21], and acquisition of mesenchymal markers, including CDH2, VIM, and FN1 [22]. A feed-forward loop consisting of SNAI1 and miR-34a has been suggested to control the activation of the EMT and mesenchymal-epithelial re-transition programs [23]. The downregulation of miR-34a occurred due to cancer-specific CpG methylation, repression by IL6R–mediated IL-6/STAT3 pathway and/or TP53 inactivation [23-28]. MiR-34a has also been involved in resistance to 5-FU in part through modulation of glucose metabolism [16]. Furthermore, miR-34a delivery represents a novel therapeutic approach. The first cancer-targeted miRNA drug, MRX34, reinforcing miR-34 expression, has already entered phase I clinical trials in patients with unresectable primary liver cancer and metastatic liver cancers [16]. The trial also includes a separate cohort of patients with hematological malignancies. The preliminary results of this trial have shown manageable safety and a dose-dependent repression of several key oncopgenes [29]. MiR-34 has recently been demonstrated to modulate tumor immune evasion pathways through direct downregulation of PDL1 in lung cancers. MRX34 therapy led to an increase in active tumor-infiltrating immune cells (CD8+) and a decrease in exhausted tumor-infiltrating immune cells (CD8+PD1+) that indicate an active immune response and reduced tumor tolerance [29]. This further supports the potential of miRNAs as therapeutics.

The other CDH1 repressors, ZEB1/2 were the most prominent targets of the miR-200 family [30-35]. The family includes five members encoded in two clusters: miR-200a/b, miR-429 and miR-200c, miR-141, on chromosomes 1 and 12, respectively. The miR-200b/200a/429 and miR-200c/141 polycistronic transcripts have been shown to be repressed due to CpG
Figure 1: An overview of miRNA studies in colorectal cancer (CRC). A. A rapid growth of the published research reports illustrated by the number of studies (with and without clinical samples) per year; the box plots represent numbers of the analyzed patient samples per year; B. The proportion of studies reporting aberrant miRNA (expression) and miRNA regulation by copy number variation (CNV), miRNAs regulated by epigenetic modifications, miRNA regulation by transcription factors (TF); single nucleotide polymorphism (SNP) in miRNA coding genes or binding sites of their target genes. C. The quantity of patient cohorts segregated by the type of analysis performed.
Figure 2: Main miRNA research themes in colorectal cancer (CRC) studies. A. The trends of miRNA research themes exemplified by article keywords. B. An illustration and a heatmap of miRNAs validated to regulate CDH1, its regulators and other mesenchymal markers in CRC. The miRNA/mRNA was obtained from CRC patients registered in TCGA. values represent RPM normalized, median-centered values (for further information see Supplementary Material).
methylation [33, 35]. The reduced miR-200 expression was observed at the invasive front of primary CRC that had disrupted basement membrane [34]. In contrast, adenomas and adenocarcinomas with intact basement membranes exhibited uniform miR-200 expression from the tumor core to the tumor-host interface. Interestingly, the metastatic CRCs displayed strong expression of miR-200 and were involved in the recapitulation of the primary tumor phenotype at the metastatic sites [34]. Furthermore, miR-200c increased the sensitivity to chemotherapeutic drugs through repression of MAPK9 and blocking multidrug resistance [36]. Several other miRNAs, among them, miR-19b, miR-194 and let-7b, had also altered expression when front-specific CRC liver metastases were compared with the tumor center [37].

So far only one study addressed regulation of miR-9 and its direct repression of CDH1 was confirmed at the protein level in CRC [38]. We previously showed that miR-9 was downregulated at early CRC stages and that its re-expression reduced cell proliferation [39]. However, our and others attempts to establish CDH1 as miR-9 target in CRC at the mRNA level have failed. This is most likely explained by the fact that miR-9 inhibit CDH1 translation. Nonetheless, an inverse expression pattern is observed between multiple miRNAs reported to be in control of metastatic progression and selected EMT regulators in TCGA CRC patients [40] (Figure 2B).

The effect of miRNA deregulation on migration and invasion has been frequently measured after perturbations by single synthetic miRNAs in cell line models using wound healing or transfer assays. For example, overexpression of miR-361-5p markedly suppressed proliferation, migration and inhibited tumor growth and lung metastasis in vivo [41]. By using a large functional screen, over 20% of miRNAs have been shown to affect migration across diverse cell types, indicating a general involvement of miRNAs in this process [42]. Loss of miR-23b expression in colon cancer resulted in increased activity of prometastatic genes, including FZD7 and MAP3K1, and facilitated tumor growth, invasion and angiogenesis in vivo [42]. By employing “dropout” screens of a miRNA library in a mouse model of liver metastasis, Okamoto and colleagues have shown that upregulation of miR-493 during carcinogenesis prevented liver metastasis via the induction of cell death of metastasized cells [43]. To colonize the liver, CRC metastatic lesions must overcome hypoxia and energy starvation. Tumor cells have been demonstrated to release a metabolic enzyme CKB, upregulated through loss of miR-551a and miR-483-5p, into the extracellular matrix and catalyzing liver metabolites into ATP-rich products, which further were taken up by the cell to fuel metastatic survival and subsequent liver colonization [41].

The crucial miRNA function in metastasis is also supported by a meta-analysis employing TCGA data across multiple cancer types, including CRC, that reported metastasis as the most prominent process under miRNA regulation [44].

**MIRNAS WITH BIOMARKER POTENTIAL**

Due to high tissue specificity, stability and altered expression in neoplastic development, miRNAs have been suggested as diagnostic, prognostic and predictive biomarkers. The Cancer Origin Test, based on the expression of 64 miRNAs, is the first promising assay available for determining the tumor origin with 90% sensitivity and 99% specificity for metastatic cancer of unknown or uncertain primary site [15]. MiRNA-based tests for improved subclassification of lung, kidney cancers and mesothelioma subtypes are also on the market [45]. However, despite the long list of miRNAs suggested as biomarkers in CRC, none have made it to the clinic yet. The individual studies that have reported miRNAs as circulating biomarkers in blood serum or plasma and feces as well as prognostic and/or predictive biomarkers in tumor tissues in CRC are summarized in Tables 1 and 2. Approximately 40% of the reported CRC miRNA biomarkers were suggested by two or more studies, among them, miR-21, miR-29a, miR-92a, miR-200c, miR-215 and miR-375.

**miRNA tissue biomarkers**

Aberrantly expressed miRNAs have been found in adenomas and carcinomas of the large bowel as compared to normal colonic mucosa, and several of these miRNAs have been suggested as biomarkers. A high miR-31 expression was observed in serrated lesions and suggested as diagnostic biomarker and therapeutic target for BRAF mutated CRCs [46]. A signature of miR-92a, miR-375 and miR-424 has been shown to discriminate invasive carcinoma from adenoma with high-grade neoplasm in biopsies from colonoscopy, representing promising clinical utility in early diagnosis [47]. A predictive value of miR-29a, where high levels of this were associated with longer disease-free survival, has been shown in patients with stage II CRC [48]. With few exceptions, stage II CRC patients are treated with surgery alone. If considered to have a high risk of relapse based on clinicopathological parameters they may be offered adjuvant chemotherapy [49]. A 6 miRNA classifier, consisting of miR-21-5p, miR-20a-5p, miR-103a-3p, miR-106b-5p, miR-143-5p, and miR-215 has been shown to distinguish between stage II colon cancer patients with high and low risk of disease progression, with 5-year disease-free survival of 60% and 89%, respectively [50]. The miRNA panel outperformed clinicopathological risk factors and DNA mismatch repair status when it came to predicting the prognosis, and patients in the high-risk group were found to have a favorable response to adjuvant chemotherapy [50].
Table 1: Tissue miRNAs as potential biomarkers for Colorectal Cancer (CRC).

| Application       | miRNA biomarker                        | Type of analysis | Total nr of specimens | Ref.  |
|-------------------|----------------------------------------|------------------|-----------------------|-------|
| **MIRNA BIOMARKERS IN TISSUE BIOPSIES** |                                        |                  |                       |       |
| Anti-angiogenetic therapy | miR-126 | ↑ | 109 | [172] |
|                   | let-7a | ↑ | 59 | [173] |
|                   | let-7c, miR-99a, miR-125b | signature | 183 | [174] |
|                   | miR-7 | ↓ | 105 | [58] |
|                   | miR-31-5p | ↑ | 102 | [175] |
|                   | miR-125b, miR-137 | ↑ | 66 | [176] |
| Anti-EGFR therapy  | let-7g, miR-132, miR-224, miR-320a | signature | 128 | [177] |
|                   | miR-16, miR-590-5p, miR-153, miR-519c-3p, miR-561 | signature | 32 | [178] |
|                   | miR-17-5p | ↑ | 390 | [179] |
|                   | miR-21 | ↑ | 84 | [180] |
|                   | miR-21 | ↑ | 301 | [181] |
|                   | miR-21, miR-143, miR-145 | expression, diverse | 120 | [116] |
|                   | miR-107, miR-99a-3a | ↑ | 78 | [182] |
| Chemotherapy       | miR-1183, miR-483-5p, miR-622, miR-125a-3p, miR-1224-5p, miR-188-5p, miR-1471, miR-671-5p, miR-1909*, miR-630, miR-765, miR-622, miR-630 | signature | 37 | [183] |
|                   | miR-126 | expression, not significant | 158 | [184] |
|                   | miR-143 | ↓ | 175 | [185] |
|                   | miR-148a | ↓, methylation | 293 | [186] |
|                   | miR-148b | ↓ | 192 | [187] |
|                   | miR-150 | ↓ | 625 | [54] |
|                   | miR-181d, miR-139-3p, miR-892b, miR-338-5p, miR-150, miR-154, miR-454 | signature | 70 | [188] |
|                   | miR-200a, miR-200c, miR-141, miR-429 | ↓ | 127 | [189] |
|                   | miR-200c | ↓ | 103 | [190] |
|                   | miR-214 | ↓ | 242 | [191] |
|                   | miR-215 | ↑ | 48 | [192] |
|                   | miR-215, miR-190b, miR-29b-2*, miR-196b, let-7c, miR-450a, miR-450b-5p, miR-99a* | signature | 20 | [193] |
|                   | miR-320 | ↓ | 100 | [194] |
|                   | miR-451 | ↓ | 35 | [195] |
|                   | miR-622 | ↑ | 17 | [55] |
|                   | miR-625-3p, miR-181b, miR-27b | ↑ | 283 | [196] |
| **Diagnosis**      | miR-21 | ↑ | 360 | [197] |
|                   | miR-21 | ↑ | 73 | [198] |
|                   | miR-31 | ↑ | 1382 | [46] |
|                   | miR-34b | ↓, methylation | 113 | [199] |
|                   | miR-155 | ↑ | 80 | [200] |
|                   | miR-194 | ↓ | 446 | [201] |
|                   | miR-375, miR-424, miR-92a | signature | 282 | [64] |
|                   | miR-378*, miR-145 | ↓ | 64 | [202] |
| miR-21       | ↑ | 554 | [203] |
| miR-21       | ↑ | 412 | [204] |
| miR-21       | ↑ | 392 | [205] |
| miR-21       | ↑ | 7 studies [206] |
| miR-21       | ↑ | 14 studies [207] |
| miR-21, miR-155 | ↑ | 312 | [208] |
| miR-21-5p, miR-20a-5p, miR-103a-3p, miR-106b-5p, miR-143-5p, miR-215 | ↑ | signature 775 | [209] |
| let-7a | ↓ | 40 | [210] |
| let-7c | ↓ | 254 | [211] |
| miR-1, miR-129, miR-215, miR-497, miR-135b, miR-493 | ↑ | signature 49 | [212] |
| miR-101 | ↓ | 36 | [213] |
| miR-106a, miR-143, miR-125b | ↑ | expression, diverse 48 | [214] |
| miR-126 | ↓ | 560 | [215] |
| miR-130b | ↑ | 160 | [216] |
| miR-139-3p | ↓ | 126 | [217] |
| miR-139-5p, miR-31, miR-17-92, miR-143, miR-10b | ↑ | signature 142 | [218] |
| miR-143 | ↓ | 154 | [219] |
| miR-148a, miR-152 | ↓ | 202 | [220] |
| miR-15, miR-16 | ↓ | 126 | [221] |
| miR-15b, miR-135b | ↓ | 189 | [222] |
| miR-17-92, miR-17 | ↑ | 48 | [223] |
| miR-182 | ↑ | 296 | [52] |
| miR-182 | ↑ | 176 | [75] |
| miR-183 | ↑ | 188 | [224] |
| miR-195 | ↓ | 170 | [225] |
| miR-195, miR-1280, miR-140-3p, miR-1246 | ↑ | signature 19 | [226] |
| miR-196a, miR-190b | ↑ | 252 | [227] |
| miR-200a, miR-17, miR-106a, miR-375, miR-29a, miR-18a, miR-200b | ↑ | signature 267 | [228] |
| miR-215 | ↑ | 116 | [229] |
| miR-224 | ↑ | 230 | [230] |
| miR-338-3p | ↓ | 80 | [231] |
| miR-340 | ↓ | 155 | [232] |
| miR-34-5p | ↓ | 278 | [28] |
| miR-375 | ↓ | 190 | [233] |
| miR-429 | ↑ | 214 | [234] |
| miR-638 | ↓ | 312 | [70] |
| miR-21 | ↑ | 20 | [235] |
| miR-21, miR-103, miR-93, miR-566 | ↑ | signature 187 | [236] |
| miR-25-3p, miR-339-5p | ↓ | 93 | [237] |
| miR-27a | ↓ | 82 | [238] |
| miR-122 | ↑ | 12 | [239] |
| miR-133a | ↓ | 338 | [240] |
| miR-133b | ↓ | 62 | [241] |
| miR-133b | ↓ | 100 | [242] |
| miR-185 | ↓ | 40 | [243] |
| miR-185, miR133b | ↑ | expression, diverse 50 | [244] |
| miR-193a-5p | ↓ | 360 | [245] |
| miR-199a-5p | ↑ | 20 | [246] |
| miR-200b, miR-200c | ↓ | 13 | [247] |
| miR-210 | ↑ | 193 | [248] |
| miR-210, miR-133b | ↑ | expression, diverse 54 | [249] |
| miR-224 | ↓ | 158 | [250] |
| miR-224, miR-221* | ↓ | 60 | [251] |
| miR-625 | ↓ | 192 | [252] |
| miR-21 | ↑ | 277 | [253] |
| miR-29a | ↓ | 110 | [254] |
| miR-215 | ↓ | 125 | [255] |
miRNAs appear to be protected from degradation by DNA-based test. In addition, in circulation, tumor-derived principle be detected earlier with a miRNA- compared to a feces. Considering that a single cell may express hundreds evaluated in blood derivatives (plasma or serum) and circulating miRNAs have in recent years also been Circulating miRNA biomarkers independent validation studies are needed. biomarkers’ clinical significance is not conclusive and miRNA tissue biomarkers offer great promise, the miRNA targeted anti-EGFR therapy [58]. Despite the fact that in patients with CRC whose tumors were resistant to prognostic biomarker and a candidate for targeted therapy miR-7, a direct regulator of with EGFR inhibitor [57]. Whereas, reduced levels of KRAS wild-type metastatic CRC that responded to anti-EGFR therapy [56]. Reduced expression of miR-181a was associated with poor clinical outcome in patients treated with EGFR inhibitor [57]. Whereas, reduced levels of miR-7, a direct regulator of EGFR, was suggested as a prognostic biomarker and a candidate for targeted therapy in patients with CRC whose tumors were resistant to targeted anti-EGFR therapy [58]. Despite the fact that miRNA tissue biomarkers offer great promise, the miRNA biomarkers’ clinical significance is not conclusive and independent validation studies are needed.

Circulating miRNA biomarkers

The diagnostic and prognostic potential of circulating miRNAs have in recent years also been evaluated in blood derivatives (plasma or serum) and feces. Considering that a single cell may express hundreds of copies of a single miRNA, cancers may at least in principle be detected earlier with a miRNA- compared to a DNA-based test. In addition, in circulation, tumor-derived miRNAs appear to be protected from degradation by endogenous ribonucleases. Thus miRNAs are promising candidates for minimally invasive biomarkers.

Furthermore, stromal expression of miR-21 alone was found as an independent predictor of early tumor relapse in a large colon cancer series, indicating that these patients could be considered for more intensive treatment [45]. As mentioned above, miR-34a and miR-200 family members have a functional role in CRC metastases. Supporting this notion, miR-34a has also been suggested as an independent predictor of recurrence among stage II/III patients [28], while expression levels of miR-200 family members identified those CRC patients, including stage II, who are most likely to benefit from adjuvant chemotherapy [51]. High expression of miR-182, identified as anti-apoptotic miRNA [39], has been associated with lymph node metastases and poor survival, and suggested to have clinical potential not only as a promising predictor of aggressive phenotype but also as an independent prognostic predictor to identify patients with particularly low rate of survival [52]. Interestingly, anti-miR-182 therapy has been demonstrated as a promising therapeutic strategy for metastatic melanoma [53]. Drug resistance is the major obstacle of effective cancer therapy, and a number of miRNAs have been shown to induce chemoresistance and to be associated with poor prognosis. Patients whose tumors had low miR-150 expression exhibited shorter survival and a worse response to adjuvant chemotherapy [54]. Overexpression of miR-622 was induced by radiotherapy in rectal cancers, causing poor response to therapy [55]. A signature of miR-99a, let-7c and miR-125b distinguished patients with KRAS wild-type metastatic CRC that responded to anti-EGFR therapy [56]. Reduced expression of miR-181a was associated with poor clinical outcome in patients treated with EGFR inhibitor [57]. Whereas, reduced levels of miR-7, a direct regulator of EGFR, was suggested as a prognostic biomarker and a candidate for targeted therapy in patients with CRC whose tumors were resistant to targeted anti-EGFR therapy [58]. Despite the fact that miRNA tissue biomarkers offer great promise, the miRNA biomarkers’ clinical significance is not conclusive and independent validation studies are needed.

*↑, upregulated expression in CRC vs. normal control; ↓, downregulated expression in CRC vs. normal control; methylation, cancer specific DNA methylation; signature, multiple miRNAs considered collectively; expression, diverse, multiple miRNAs considered separately, “total number of samples included in the study, CRCs, adenomas and normal control samples collectively; specimens” – tissue samples from normal colonic mucosa, adenomas and cancer biopsies.
Table 2: Circulatory miRNAs as potential biomarkers for Colorectal Cancer (CRC).

| Application                  | miRNA biomarker | Type of analysis* | Total nr of samples# | Ref.   |
|------------------------------|-----------------|-------------------|----------------------|--------|
| Anti-angiogenetic therapy    | miR-126         | ↑, SNP            | 178                  | [256]  |
| Anti-EGFR therapy            | miR-345         | ↑                 | 138                  | [257]  |
| Chemotherapy                 | miR-19a         | ↑                 | 88                   | [258]  |
|                              | miR-20a, miR-150, miR-145, miR-216, miR-372 | signature | 253                  | [118]  |
|                              | miR-27b, miR-148a, miR-320, miR-106a, miR-484, miR-130b | signature | 328                  | [259]  |
|                              | miR-126         | ↑                 | 178                  | [260]  |
|                              | miR-143         | ↑                 | 51(175)              | [185]  |
|                              | miR-155, miR-200c, miR-210 | ↑           | 35                   | [261]  |
| Diagnosis                    | miR-17-3p, miR-29a, miR-92a, miR-135b | expression, not significant | 130 | [262]  |
|                              | miR-18a, miR-19a, miR-15b, miR-92a, miR-335 | signature | 196                  | [263]  |
|                              | miR-19a-3p, miR-223-3p, miR-92a-3p, miR-422a | signature | 697                  | [264]  |
|                              | miR-21           | ↑                 | 5 studies            | [62]   |
|                              | miR-21           | ↑                 | 366(532)             | [265]  |
|                              | miR-21           | ↑                 | 100(192)             | [59]   |
|                              | miR-21           | ↑                 | 75(189)              | [60]   |
|                              | miR-21           | ↑                 | 80                   | [266]  |
|                              | miR-21, let-7g, miR-31, miR-92a, miR-181b, miR-203 | signature | 202                  | [268]  |
|                              | miR-21, miR-378  | ↑                 | 135                  | [269]  |
|                              | miR-21, miR-92a  | ↑                 | 98                   | [270]  |
|                              | miR-29a, miR-92  | ↑                 | 216                  | [271]  |
|                              | miR-34a          | ↑                 | 108                  | [272]  |
|                              | miR-92           | ↑                 | 255(265)             | [273]  |
|                              | miR-145          | ↑                 | 59(170)              | [274]  |
|                              | miR-145          | ↑                 | 60                   | [275]  |
|                              | miR-199a-3p, miR-23a, miR-338-5p | signature | 162(222)             | [276]  |
|                              | miR-375, miR-206, miR-150, miR-125b, miR-126* | ↑ | 140(228) | [277]  |
|                              | miR-409-3p, miR-7, miR-93 | ↑ | 241 | [278]  |
|                              | miR-423-5p, miR-210, miR-720, miR-320a, miR-378, miR-106a, miR-143, miR-103, miR-199a-3p, miR-382, miR-151-5p | signature | 90 | [279]  |
|                              | miR-423-5p, miR-484 | ↓ | 103 | [280]  |
|                              | miR-532-5p, miR-331, miR-195, miR-142-3p, miR-15b, miR-532, miR-452, miR-453, miR-13b, miR-139-3p | signature | 128 | [281]  |
|                              | miR-574-5p      | ↑                 | 14(146)              | [281]  |
|                              | miR-601, miR-760 | ↑                 | 191                  | [282]  |
| Prognosis/Survival/Metastasis | miR-7, miR-17-3p, miR-20a, miR-21, miR-92a, miR-96, miR-183, miR-196a and miR-214, miR-124, miR-127-5p, miR-138, miR-143, miR-146a, miR-222 | expression, diverse | 20(40) | [283]  |
|                              | miR-21           | ↑                 | 102                  | [284]  |
|                              | miR-124-5p, miR-26a | ↑ | 71(142) | [285]  |
|                              | miR-141          | ↑                 | 278                  | [286]  |
|                              | miR-155          | ↑                 | 206                  | [287]  |
|                              | miR-182          | ↑                 | 51(161)              | [288]  |
|                              | miR-200a         | ↑                 | 230(446)             | [288]  |
|                              | miR-218          | ↑                 | 90(331)              | [289]  |
|                              | miR-221          | ↑                 | 140                  | [289]  |
|                              | miR-23a          | ↑                 | 102(162)             | [290]  |
|                              | miR-29a, miR-92a | ↑, no associations for miR-92a | 74(114) | [291]  |
|                              | miR-92a          | ↑                 | 36(94)               | [292]  |
|                              | let-7i, miR-18b, miR-221, miR-320a, miR-185-5p | signature | 169(478) | [293]  |
|                              | miR-126, Let-7a, miR-141, miR-21 | ↑ | 224 | [294]  |
|                              | miR-15a, miR-103, miR-148a, miR-320a, miR-451, miR-596 | signature | 69 | [295]  |
|                              | miR-18a, miR-29a | ↑                 | 56(680)              | [296]  |
|                              | miR-92c          | ↑                 | 84(91)               | [297]  |
|                              | miR-148a         | ↑                 | 85(195)              | [298]  |
|                              | miR-183          | ↑                 | 179(195)             | [65]   |
| Mirna BIOMARKERS IN STOOL    |                 |                   |                      |        |
## MIRNAS REGULATING PROLIFERATION AND APOPTOSIS

The effect of miRNAs on cellular proliferation could be subdivided into miRNAs targeting 1) mitogen receptor tyrosine kinases, or G-protein signal transducers such as KRAS, and the intermediary signal transducing molecules that convey mitogenic information to its intracellular targets, 2) cell cycle regulators, such as late-G1 checkpoint regulators RB and CDKs, and 3) reciprocal miRNA interactions with MYC and TP53. The comparisons of the proliferative potential of two cell populations, frequently addressed in many studies, may reflect not only efficiency of cell cycle, but also cell differentiation and senescence, survival or apoptosis. In addition, cell proliferation and metabolism are tightly linked cellular processes, since cells need to increase their biomass and replicate their genome prior to proliferation. The effect of miRNAs on apoptosis is mediated by their regulation of pro-apoptotic and anti-apoptotic mRNAs or their positive regulators. Augmented cell proliferation together with suppressed apoptosis constitutes the minimal common platform upon which all neoplastic development occurs. MiRNAs with pro-proliferative and/or anti-apoptotic activities would likely promote oncogenesis and thus may be overexpressed in cancer cells. Likewise, miRNAs with anti-proliferative and/or pro-apoptotic activities are likely to function as tumor suppressor genes and thus may be downregulated in cancer cells.

The effect of miRNA deregulation on cell proliferation and apoptosis has frequently been measured after perturbations of single miRNAs in cell line models. The ectopic expression of miR-638 inhibited CRC cell proliferation by targeting TSP4N1 and arresting the cell cycle in G1 phase [70]. Overexpression of miR-29b induced apoptosis and arrested the cell cycle in the G1/S transition by repressing MCL1 and CDK6 [71]. High throughput approaches analyzing the effect of multiple miRNAs simultaneously, have also been performed [39, 43, 72]. The perturbations of entire population of miRNAs by employing a comprehensive miRNA library screen, have shown that approximately 15% and 30% of miRNAs had an effect on proliferation and apoptosis, respectively [39]. However, only some of these are relevant to CRC development and progression. By integrating miRNA expression from CRC patients and applying in-depth functional validation, the strongest candidates have been identified, including miR-9, miR-31, miR-182, miR-375 and miR-491 [39, 72, 73]. MiR-491 induced apoptosis via silencing of BCL2L1 that is commonly overexpressed in CRC, and treatment with miR-491 suppressed in vivo tumor growth [72]. Interestingly, the genomic micro-deletions at chromosome 9 (cytoband 9p21.3) and chromosome 20 (cytoband 20q13.33) that include the mir-491/KIAA1797 and AK309218/mir-646 genes, respectively, have been associated with familial and early-onset CRC [74]. Enhanced expression of miR-182 in CRC has been reported in multiple studies [39, 52, 75, 76] and associated with lymph node metastasis and poor prognosis [52, 75]. MiR-182 exhibited anti-apoptotic effect by targeting ENTPD5 [77], FBXW7, SATB2 [78] and THBS1 [79], and stably blocking its expression in animal models has been shown to inhibit tumor formation [80].
MIRNA ABERRATIONS IN THE MOLECULAR PHENOTYPES OF CRC

CRCs are primarily subdivided into three molecular phenotypes. The chromosomal instability phenotype (CIN) accounts for about 85% of all sporadic CRCs and is characterized by large-scale genomic rearrangements [81]. The microsatellite instability ( MSI) phenotype accounts for about 15% of all CRCs and is associated with a defect DNA mismatch-repair system (MMR), and an accordingly hypermutated genome [82, 83]. Cancers with the CpG island methylator phenotype (CIMP) display promoter hypermethylation and subsequent inactivation of multiple genes. CIMP cancers overlap with both the CIN and MSI types, but are mainly associated with MSI [84]. Recently, transcriptome instability (TIN) was introduced as a fourth molecular phenotype, characterized by excess aberrant pre-mRNA splicing [85].

The impact of abnormalities of the miRNA-ome is still limited for these individual molecular phenotypes. Most studies have focused on comparison of the most common phenotypes CIN to MSI where differential expression of miRNAs has been reported. Further, in MSI cancers, expression levels of certain miRNAs have been associated with resistance to chemotherapy [86-93]. Furthermore, miR-155 and miR-21, which are frequently overexpressed in CRC, have been demonstrated to down-regulate the core MMR proteins MSH2, MSH6, and MLH1, inducing the MSI mutator phenotype [88, 92, 94]. MiR-155 has been found overexpressed in MSI tumors with unknown cause of MMR inactivation and therefore suggested as a potential mechanism of MSI induced cancer pathogenesis [88]. The MSI phenotype is characterized by a dramatic increase of insertions and deletions at repetitive sequences. Even though mutated DNA repeats in miRNA hairpin sequences are relatively rare, cancers with MSI phenotype also have an elevated rate of single nucleotide mutations. Thus, miRNA genes are as well potential mutation targets MSI CRCs. A single miRNA, miR-1303, displayed frequent mutations due to MSI and was expressed in colonic tissues [95]. Functional studies are required to conclude whether this miRNA might have a role in MSI tumorigenesis.

MiRNAs are frequently located at fragile sites of the genome, which are usually either amplified or deleted in human cancers [96]. The aberrant miRNA expression in CRC could be due to these genomic rearrangements associated with CIN phenotype. DNA copy number gain on chromosome band 13q31 has been shown to increase the expression of the oncogenic miR-17-92 cluster [97]. Reduced expression of miR-497 and miR-195 in CRC was associated with DNA copy number loss of a segment of chromosome band 17p13.1 [98]. However, to which extent these miRNAs are oncogenic drivers for cancers with the recurring rearrangements is not proven.

One of the most common causes of the loss of tumor suppressor miRNAs in CRC is the silencing of their primary transcripts by cancer-specific DNA methylation in associated CpG islands [99-102]. However, there is no evidence whether more miRNA genes are affected in CIMP positive tumors displaying higher frequency and extent of DNA methylation as compared to other subtypes. So far, only two miRNAs, miR-31 [103] and miR-146a [104], have been associated with the CIMP positive CRCs.

MIRNA REGULATION OF TARGET GENES

Approximately 450 unique miRNAs have been associated with CRC, of which twenty account for one third of all miRNA quotations. MiRNAs miR-21, miR-143 and miR-145 were the most frequently reported, followed by miR-31, miR-34a, miR-200c, miR-20a and miR-92a. Recent experimental analyses have validated a total of 530 miRNA-mRNA pairs in CRC, 200 unique miRNAs and 347 unique targets (Figure 3A-3B).

Modeling cross-talk in miRNA and their target networks based on validated interactions

Most of the studies reporting miRNAs and their targets in CRC validated only a single miRNA-mRNA pair, despite that 1) miRNAs may have target sites in multiple transcripts and 2) several miRNAs may play together in controlling a single mRNA transcript. To explore the underlying regulatory circuits of miRNAs and their targets, we extracted all experimentally validated interactions and built a miRNA-target network by combining all miRNA-mRNA pairs reported in the reviewed studies. The four nodes of miR-21-PTEN, PDCD4, miR-143/miR-145-KRAS-let-7a, miR-34a-SIRT1/LEF1 and miR-200c-ZEB1 were prominent in the miRNA-target interaction network. In addition, a small number of miRNA-mRNA nodes included two to four partners, and the bulk of miRNAs that so far have reported with only one validated target. The miRNA-target interaction network is available in Supplementary Figure S1, and selected enlarged nodes are highlighted in Figure 3C. The node of miR-143/miR-145-KRAS should be interpreted with precaution in light of the recent results by Chivukala et. al. [105] (further discussed on page 13).

Although the network analysis illustrates the complexity of miRNA-induced regulation, it is strongly biased towards well-studied protein coding genes and miRNAs. Since the miRNA functions are dependent on available cellular transcripts, the cell composition of a tumor may further influence this interaction map. Moreover, some of the miRNAs targets may be repressed more strongly than others. Ideally, one should therefore predict not only miRNA-target pairs, but also the expected degree of translational suppression, taking into account...
Figure 3: MiRNA regulation of target genes. A. and B. The most frequently validated miRNA and target genes. C. The enlarged leading miRNA-targets nodes generated using the validated miRNA-target pairs. The network edges are coded by degree of expressional miRNA-mRNA associations obtained in TCGA CRC patients. MiRNA nodes are indicated by rectangles, target nodes as ovals. Size of nodes reflects the number of connections, with bigger nodes representing more densely connected regions. The node of miR-143/miR-145-KRAS should be taken with precaution in light of the recent results by Chivukala et al.[105].
seed complementarity and mRNA secondary structure [106]. Recently, the distribution of the recurrent miRNA-target interactions have been analyzed employing TCGA data across multiple cancer types, including CRC [44, 107]. We have integrated miRNA-mRNA associations obtained in the TCGA CRC patients [44] with the network of validated miRNA-target pairs. More than 60% of validated miRNA-target interactions collected across all reviewed studies had, as expected, negative expression associations in the TCGA CRC patients (Figure 3C). This also suggests that a part of these experimentally validated interactions in CRCs and/or CRC cell lines had no association or even displayed positive associations in the TCGA CRC patients. This might be explained by some targets that reciprocally control the level and function of miRNAs. Furthermore, we cannot exclude the patient cohort specificity, or that some miRNA-mRNA interactions validated in CRC cell lines only, are not relevant to CRC patients.

MIRNAS REGULATE KEY COLORECTAL CANCER SIGNALING PATHWAYS

CRC tumorigenesis is driven by molecular alterations resulting in activation of pro-survival signaling pathways such as the canonical WNT signaling pathway, the EGFR pathway, the TP53 network, and the transforming growth factor beta (TGF-β) (Figure 4).

Activation of canonical WNT signaling

WNT signals are pivotal for the regulation of stem cell activity to the crypt bottom in the intestines and ultimately for the renewal of the epithelial cells [108]. Inappropriate activation of WNT signaling promotes cell survival, inhibits cell death and differentiation and triggers the development of upper- and lower- gastrointestinal polyps and carcinoma [109]. The APC tumor suppressor is a negative regulator of free CTNNB1, a central player of WNT signaling, and up to 80% of CRCs have mutations in the APC gene resulting in a truncated protein [110]. MiRNAs may also contribute to reduced APC levels. The elevated expression of miR-135a/b, negative regulator of APC, has been observed in adenocarcinomas as well as premalignant colorectal adenomas and correlated with concomitant reduced levels of APC leading to WNT pathway activation [111]. Interestingly, miR-135a/b suppressed APC expression, even when APC mutations were present, suggesting that miR-135a/b may function as complementary players in WNT pathway addiction. Furthermore, overexpression of miR-135b, activated by the WNT pathway, has been observed in sporadic as well as inflammatory bowel disease-associated CRCs and

Figure 4: An overview of key signaling pathways in CRC and the regulation of their components by miRNAs. MiRNAs that are involved in multiple pathways and contribute to the cross-talk among the pathways are marked in bold.
correlated with tumor stage and poor clinical outcome [112].

A critical point of WNT signaling is nuclear translocation of CTNNB1 that binds to the LEF/TCF transcription complex and activates transcription of multiple target genes, including CCND1 and MYC [113]. MiR-26b and miR-34a/b/c directly targeted LEF1 3′UTR and inhibited its expression [114], while miR-145 directly targeted CTNNG1, contributing to the aberrant translocation of CTNNB1 through impaired nuclear shuttling with PAK4 [115]. A reduced miR-143/145 expression in CRC [11] was reported in the first study associating miRNA and CRC, and roughly 40 other studies have reproduced these results. Nevertheless, the tumor suppressor role of miR-143/145 in CRC has been challenged by the recent results of Chivukula and colleagues, who reported that miR-143/145 were expressed exclusively within the mesenchymal compartment of the normal intestine and that the repeatedly observed repression of miR-143/145 in CRC was rather the result of depletion of mesenchymal cells in tumors relative to normal mucosal biopsies [105]. Nonetheless, loss of miR-143/145 expression has been shown to predict poor prognosis and serve as CRC biomarkers [116-118]. In light of the new evidence regarding the lack of miR-143/145 functionality in intestinal epithelial cells, one may hypothesize that miR-143/145 may function as a surrogate marker for tumor infiltration with mesenchymal cells rather than repression of target genes, such as KRAS. Thus it would be interesting to see an expression analysis of miR-143/145 using in situ hybridization of CRC tissue microarrays, and examine co-localization with their validated target genes, such KRAS, NKRAS, CTNNG1, ERK and KLF5.

The LEF/TCF complex activates numerous pro-survival signaling cascades and some miRNAs may be under direct LEF/TCF control and/or being regulated by downstream effectors [119, 120]. By using either a bioinformatics approach to discover miRNA transcription start sites within close proximity to TCF4 chromatin occupancy sites [120] or profiling miRNA expression in CRC cells with disrupted CTNNB1/TCF4 activity followed by integration of TCF4 chromatin data [119], roughly 30 TCF4-responsive miRNAs have been identified. However, only miR-23b was found in both studies.

The TCF/LEF–dependent transcriptional activity is most likely regulated by miR-34 family members, since the UTRs of multiple WNT pathway genes contain binding sites for this family of miRNAs [27, 121-123]. MiR-34, activated by TP53, inhibited activity of the TCF/LEF complex and linked the canonical WNT pathway with TP53 activity [122] (Figure 4). Loss of TP53 function resulted in increased activity of WNT signaling cascade and promoted the Snail-dependent EMT program [122]. Interestingly, UTRs of oncogenes, including CTNNB1, are often shortened in cancer cells as a consequence of alternative cleavage, leading to loss of miR-34 mediated regulation [121, 122]. Furthermore MYC, induced by WNT, upregulated the oncogenic miR-17-92 miRNA cluster that encode miRNAs, directly targeting the key effector of TGFR signaling pathway, SMAD4 [124].

**Activation of EGFR signaling**

The epidermal growth factor receptor (EGFR) signaling pathway coordinates a variety of cellular activities [125]. The cancers with mutated EGFR have been suggested to have an “oncogene addiction”, serving as the basis for EGFR targeted therapies. However, the clinical benefit from EGFR-targeted therapies has so far been rather limited in CRC. Activating KRAS mutations, functioning downstream of EGFR, have been reported in 30–60% of CRCs [126]. These patients do not benefit from anti-EGFR treatment. Furthermore, among patients with wild type KRAS, a clinical benefit is only seen in 10–20% [127, 128]. Activating mutations of BRAF, as well as of PIK3CA and loss of PTEN are also associated with lack of response to anti-EGFR therapy. Nevertheless, they cannot alone explain the low success rate of the treatment. In search of additive deregulation mechanisms, multiple studies have focused on miRNA association with KRAS and PTEN, while other genes of the EGF pathway have received less attention.

The KRAS gene is directly targeted by the let-7 miRNA family [129]. A SNP, present in the KRAS 3′UTR, disturbs the let-7 binding site, resulting in increased KRAS mRNA levels. KRAS-let-7 SNP variant carriers were shown to have an increased presentation of advanced colon cancer [130], however, conflicting results are published regarding the response to chemotherapy and CRC outcome [130-133]. Recently, the KRAS-let-7 SNP variant was reported to correctly predict a clinical response to anti-EGFR therapy in a large cohort of patients with metastatic CRC [134]. Other miRNAs have been shown to repress KRAS, in particular, miR-143 and miR-145 have received a lot of attention [135]. Restoring miR-143/145 in colon cancer cells decreased proliferation, migration and chemoresistance, however, in light of the recent results by Chivukala and colleagues [105], the biological relevance of miR-143/145 in epithelial malignancies needs re-examination. Other studies revealed that the star strand of mir-18a and miR-4689 function as potential tumor suppressors by targeting KRAS [136, 137]. In addition, miR-31 activated the RAS signaling pathway by repressing RASA1 [138].

Multiple miRNAs have been shown to target the PIK3/akt hub downstream in the EGFR signaling pathway [139]. Mutations in the PIK3CA coding sequence (exons 9 and 20) and a novel 3′UTR mutation, reducing binding affinity for miR-520a and miR-525a, were associated to increased sensitivity to saracatinib, a Src kinase inhibitor [140]. This mutation could be used
as a predictive marker for saracatinib therapy and may improve effectiveness of this drug. Importantly, a phase II study of saracatinib in unselected metastatic CRC patients showed no improvement in progression-free survival [141]. MiR-126, which expression is frequently lost in CRC, modulated the activity of PI3K at the level of signal initiation by limiting PIK3R2 levels in normal colon epithelium [142], while miR-30a, which expression is also reduced in metastatic CRC, has been reported to target PIK3CD [143].

**PTEN**, a central negative regulator of the PIK3/AKT, is inactivated by mutations and/or deletions in many primary and metastatic human cancers [144, 145]. In CRC the *PTEN* transcript has been shown to be targeted by multiple miRNAs (Figure 4C), including miR-19 [146], miR-21 [147], miR-32 [148] and miR-92-1-5p [149]. Despite the fact that *PTEN* was most frequently reported to be repressed by miR-21, miR-21 and *PTEN* expression showed no association in TCGA CRC patient cohort [44]. Upon enforced miR-26a in *APC*min/+ mice, a model known to be sensitive to PTEN dosage [19], potent reduction of tumor number and size was observed, suggesting a therapeutic potential of miR-26a.

**Impairment of TP53 function**

The tumor suppressor *TP53* responds to diverse stress signals by coordinating specific cellular responses, including cell cycle arrest, senescence, apoptosis, invasion and metastasis, as well as cell–cell communication within the tumor microenvironment [150]. The importance of *TP53* in tumor suppression is unequivocal, as shown by its inactivation in more than half of all sporadic human cancers, including CRC [151]. Wild-type *TP53* encodes a sequence-specific activator which exerts its function through transcriptional regulation of protein-coding genes to initiate cellular responses. Many genes have been identified to contain *TP53*-responsive elements and be induced upon *TP53* activation. Despite that global bioinformatic sequence analysis suggested that up to 46% of the miRNA putative promoters contain a potential *TP53*-binding site [152], only approximately 50 miRNAs have been experimentally validated to be under transcriptional control of *TP53*. Among them, miRNAs, let-7i, miR-20a, miR-21, miR-25, miR-34a/b/c, miR-145, miR-181b, miR-183, miR-195, miR-215, and miR-451 have been reported by two or more studies. MiR-34a and other members of the miR-34 family were identified as *TP53*-inducible miRNAs in several cancer types [153, 154]. The miR-34 family comprises three miRNAs; miR-34a is encoded by its own transcript, whereas miR-34b and miR-34c share a common primary transcript. MiR-34a is expressed at high levels in CRC patients, while miR-34b/c expression is not detectable. An epigenetic silencing of miR-34b/c has been reported in CRC cell lines [155]. Silencing of miR-34 by aberrant CpG methylation was dominant over its transactivation by TP53 after DNA damage [102], impairing the tumor suppressive role of TP53, particularly for cancer cells not exhibiting TP53 mutation. Finally, miR-34a was found to activate TP53 by inhibiting SIRT1, the major regulator of TP53 [156, 157] suggesting a positive feedback loop between TP53 and miR-34a.

Interestingly, miR-192 and miR-215 may act as effectors as well as regulators of *TP53* by suppressing tumorigenesis through *CDKN1A* [158]. The fact that miR-192 and miR-215 were reduced in CRC while being highly expressed in normal colon further supports the idea that these miRNAs carry out a tumor suppressive function. Among other *TP53* responsible miRNAs, miR-200 cluster family members play a critical role in metastasis, as previously discussed.

*TP53* itself is subjected to miRNA induced regulation and miR-125b has been validated as such a negative regulator in CRC [159], which upon high expression is associated with tumor initiation, progression, invasiveness, and poor prognosis. Intriguingly, in relation to miR-125 expression levels, no significant difference was observed between patients with wild type *TP53* or *TP53* mutation [159]. Other miRNAs have been reported to control *TP53* indirectly, by regulating *MDM2* (miR-339-5p) [160] and *RFFL* (miR-133a) [138].

Although, several of the examples described above illustrate importance of miRNA induced regulation, we have only begun to understand the role of the complex regulatory loops in the *TP53*-miRNA network. It also should be noted that besides the expression regulation of miRNA-coding genes, *TP53* may directly affect the processing of miRNAs [161].

**Inactivation of TGF-β signaling**

A wide spectrum of cellular functions such as proliferation, apoptosis, differentiation, migration and interactions with the microenvironment are regulated by TGF-β family members. The TGF-β pathway is activated when the ligand binds to TGFBR2 at the cell surface and TGFBR1 is recruited and phosphorylated. TGFBR2 is pivotal to trigger the signaling pathway, and signals through SMADs or non-SMAD pathways [162]. Tumor cells often escape from the anti-proliferative effects of TGF-β by mutational inactivation of its components. Mutations in *TGFBR2* are estimated to occur in approximately 90% of MSI and in 15% of microsatellite stable (MSS) CRCs [163]. Germline mutations in *SMAD4* and *BMPRI A* are found in patients with familial juvenile polyposis, an autosomal dominant condition, and associated with increased risk of CRC [164]. Multiple miRNAs have been confirmed to regulate *TGFBR2*, such as miR-17-5p, miR-20a, miR-21, miR-23b, miR-106a and miR-301a (Figure 3C). The miR-21-†TGFBR2† pair had also significant negative associations in TCGA CRC
patients, confirming this regulatory node in large patient series. Notably, miR-21, activated by the WNT signaling pathway, regulated stemness by modulating TGFBR2 signaling, which induced chemoresistance in animal models [165].

The oncogenic miR-17-92 cluster, regulated by MYC, has executed TGF-β responses by targeting TGFBR2 and SMAD4 [124], interconnecting the TGF-β and WNT signaling pathways. On the other hand, overexpression of MYC and DNA copy number gain of the miR-17-92 locus on 13q31 were frequently seen in non-MSI tumors, indicating that miR-17-92 induced repression could be an alternative mechanism for inactivation of the TGF-β pathway. Interestingly, the miR-106a/363 and miR-106b/25 clusters encode paralogous miRNAs to those in the miR-17-92 cluster and, consequently, target some of the same genes and pathways including TGFBR2 and SMAD2/SMAD4 [124, 166]. Furthermore, miR-130a, miR-301a, and miR-454, frequently upregulated in CRC tissues, have been shown to target SMAD4 resulting in enhanced cell proliferation and migration [167]. The activation of SMAD7, a negative regulator of the TGF-β signaling pathway, promoted EMT and metastasis as a result of reduced miR-25 expression [168]. The non-SMAD TGF-β pathways include various branches of MAP/PI3K/AKT and RHO-like GTPase signaling. Alterations in Rho GTPase gene expression levels, rather than constitutive mutations, are often associated with tumorigenesis and cancer progression [169]. The reduced expression may be caused, at least in part, by the mismatch 3’UTRs repression by miRNAs. For example, miR-185 inhibited the expression of RHOA and CDC42 resulting in reduced proliferation and induced G1 cell cycle arrest and apoptosis [170]. CDC42 has also been validated as a target for miR-137 [171], while RHOB and RHOB/BR1 have been repressed by miR-21 and miR-31, respectively. This links regulation of RHO GTPase gene expression with WNT and EGFR signaling pathways.

CONCLUDING REMARKS

The field of miRNA research has grown tremendously due to methodological developments and discoveries of new cellular roles of miRNAs. They seem to play a role at every stage of the tumor development, however, their necessity in driving of tumor growth still needs to be proven. So far, metastasis stands out as the most tightly regulated by miRNAs. Despite the fact that miRNAs are known to have multiple targets, pair-wise miRNA-target validation has dominated target investigations in CRC as summarized and discussed in this review. As a result, our understanding of the regulatory miRNA networks and how family miRNAs could compensate for each others’ functions remains limited.

The identified altered miRNA expression in CRC has also raised exciting opportunities for clinical applications and we have here reviewed several miRNAs with potential as biomarkers for diagnosis and prognosis. However, the challenge remains to validate miRNA biomarkers in large, independent patient cohorts. Evidence that miRNAs contribute to several aspects of tumorigenesis suggests that inhibition of highly expressed miRNAs, or replacement of miRNAs with reduced expression, could become treatment strategies. On the other hand, the ability of miRNAs to regulate multiple targets might increase the efficacy of miRNA-based drugs as well as lead to undesirable side effects. Understanding the molecular and cellular pathways that are controlled by miRNAs, as illustrated for the main pathways of CRC development, may facilitate development of miRNA-therapeutics.

Taken together, these directions are innovative and promising. Nonetheless, the clinical impact for CRC of miRNAs identified in the proof-of-concept studies in cell lines, animal models and small patient cohorts has to be confirmed in carefully designed clinical studies.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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