Mini-Review

MicroRNAs as Biomarkers in Colorectal Cancer

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Colorectal cancer is a leading cause of cancer-related morbidity and mortality in the Western world. While improved diagnostic surveillance and treatment strategies involving surgery, chemo-, and radiotherapy have all contributed to earlier detection and improved survival, treatment decisions are still made almost exclusively based on the cancer's clinicopathological stage at diagnosis. Therefore, the search for new biomarkers to facilitate early diagnosis and individualized treatment is particularly warranted. MicroRNAs (miRNAs) are short, noncoding RNAs that regulate gene expression through posttranscriptional interactions with mRNA, thereby potentially leading to a vast range of downstream effects that depend on the target proteins affected. The discovery that miRNAs may act as either oncogenes or tumor suppressors has initiated extensive research in the cancer field, leading to the identification of numerous miRNAs implicated in carcinogenesis and tumor progression. MiRNAs are chemically stable and can thus be detected in a broad range of clinical samples, making these molecules particularly attractive as potential biomarkers in cancer. While the knowledge of miRNA involvement in colorectal cancer biology is less extensive than for other cancer types and several targets with potential biological and clinical relevance have been identified, a significant amount of research is still needed. In this review, we explore the literature regarding the relevance of miRNAs in colorectal cancer, focusing in particular on miRNAs as potential diagnostic, prognostic, and predictive biomarkers. (*Am J Pathol* 2010, 177:1592–1599; DOI: 10.2353/ajpath.2010.100024)

Colorectal cancer (CRC) is a leading cause of cancer-related morbidity and mortality in the Western world, occurring almost equally in men and women. Intensified diagnostic surveillance along with improved treatment strategies involving surgery, chemo-, and radiotherapy

have contributed to earlier detection and improved survival from CRC. However, almost 50% of patients diagnosed with CRC will die of the disease, mainly because of metastasis development, most commonly in the liver and lungs. Although extensive research has attempted to identify useful prognostic and predictive tools in CRC, treatment decisions are still almost exclusively based on tumor stage as determined by radiological and/or pathological evaluation. This shortcoming results in failure to identify high- and low-risk patients, and patients may receive either insufficient or unnecessary treatment, respectively. Thus, with the aim of achieving earlier detection and better tailoring of treatment in CRC outcome, the search for new biomarkers is highly warranted.

MicroRNAs (miRNA) are short (20–22nt) noncoding RNAs which regulate gene expression posttranscriptionally. The first miRNA was discovered in 1993 in the nematode *Caenorhabditis elegans*,¹ where it was found to regulate postembryonic development. After their discovery miRNAs have been identified in plants, viruses, animals, and humans, and many have been evolutionarily conserved between species, underscoring their pivotal biological role. More than 700 human miRNAs have been discovered to date, and the number is increasing rapidly (<http://microrna.sanger.ac.uk/sequences/index.shtml>, last accessed January 15, 2010).² Studies have shown that miRNA expression levels differ between normal and tumor tissues and vary among tumor types.³ The implication that they play an important role in oncogenesis has led to the notion of miRNA as potential biomarkers in early diagnosis of cancer, predicting prognosis or treatment response or even as targets in cancer treatment strategies.⁴ This review will briefly introduce miRNA biogenesis and function, suggested importance in cancer, and further explore the potential utility of miRNAs as biomarkers and therapeutic tools in CRC.

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MiRNA Biogenesis and Function

MiRNAs are transcribed by polymerase II and then cleaved by the RNaseIII enzyme Drosha into precursor miRNA (pre-miRNA) which are 60–70 nucleotide stem-loop structures.⁵ Pre-miRNAs are transported out of the nucleus and further processed by another RNaseIII enzyme, Dicer, to generate the mature double-stranded product, from which one strand is incorporated into the RNA-induced silencing complex (RISC) to form miRISC. Negative regulation by miRNA can either lead to translational inhibition or transcript degradation depending on the complementarities between the miRNA and the 3'-untranslated region of the target mRNA.⁶ Imperfect pairing results in weak binding between miRNA and mRNA, followed by translation inhibition, while high degree of complementarity results in strong binding, giving the miRISC access to mRNA for degradation by proteolytic cleavage. Interestingly, although translation inhibition is the best described outcome of miRNA activity, opposite effects have been shown. Thus it was demonstrated that miRNAs could activate translation in response to experimentally induced quiescence via miRNA target sites in AU-rich elements in the 3'-untranslated region of the tumor necrosis factor α mRNA,⁷ further illustrating the biological diversity of miRNAs.

Because miRNAs can bind with different degrees of complementarity, these molecules can recognize and bind a variety of mRNAs to potentially regulate translation, and ultimately the expression of corresponding proteins. This again implies that changes in the expression level of a specific miRNA could in principle influence several biological pathways, either positively or negatively, depending on the activity of the proteins affected. Also, as imperfect strand pairing may inhibit translation without affecting mRNA integrity, miRNA action may explain observed discrepancies between mRNA and protein expression levels, and knowledge of miRNA expression and function may increase the understanding of regulation of protein expression. Notably, the small size and stem-loop structure of miRNA render these molecules less prone to degradation by RNases than mRNAs, and are thus likely to be conserved and amenable for detection in body fluids such as blood, pleural and peritoneal effusions, and urine.⁸ Because the number of identified miRNAs is relatively low (~700) compared to the several thousand known mRNAs, the task of gaining an overview of miRNA expression is less complicated, while downstream regulatory action is potentially highly complex. By identifying conserved complementarity of mRNA with that of miRNA, it has been suggested that up to 30% of the human genome may be regulated by miRNAs,⁹ indicating that miRNAs are indeed potentially important regulators of biological processes and clarification of their role in various diseases such as cancer is warranted.

MiRNA and Colorectal Cancer

MiRNAs are involved in multiple biological pathways that can influence tumor formation and progression^{10–13} and can act either as oncogenes or tumor suppressors, de-

pending on the downstream effects elicited by affected mRNA. Even if the complexity of miRNA function is still incompletely understood, miRNAs have been associated with several important molecular players and pathways relevant in carcinogenesis and tumor progression through work involving experimental models and clinical studies in different cancer types, including CRC. Results from expression analyses indicate that specific miRNAs are associated with disease stage and survival in several tumor types, such as chronic lymphocytic leukemia, prostate cancer, breast cancer, nonsmall cell lung cancer, and CRC.^{14–17} Although research into the relevance of miRNA expression in CRC has been less extensive than in other cancer types, several miRNAs have been identified with potential biological and clinical relevance in CRC (Table 1). The main points of interest have been divided between studying miRNA expression levels, exploring functions of differentially expressed targets and determining how this information can be used for diagnostic purposes, and for predicting prognosis and treatment response. In the following, we attempt to give an overview of current knowledge regarding the significance of miRNAs in CRC with particular attention to the potential utility of miRNA as biomarkers exemplified in Figure 1.

Differential Expression of miRNAs in CRC

Differential expression of a molecule in tumor and normal tissues often represents an important basis for cancer biomarker exploration and development. For diagnostic biomarkers, one would commonly focus on overexpressed targets, while for prognostic and predictive biomarkers, as well as for defining potential therapeutic targets, both over- and underexpression could be biologically interesting. In general, miRNA expression levels have been shown to be decreased in cancerous compared to corresponding normal tissue.³ Moreover experimental global repression of miRNA biogenesis in cancer cell lines promoted proliferation and invasion *in vitro* and increased tumor growth and metastasis development in animal models.²⁶ However, contrasting these findings are numerous studies on clinical samples showing that specific miRNAs can be differentially up- and down-regulated in different cancer types.^{4,27–29}

Among the first miRNAs to be studied in CRC were miR-143 and miR-145, which were both reported to be down-regulated in cancers compared to corresponding normal tissue.^{23,30} One of the suggested targets of miR-143 is the MAP kinase ERK5, which is involved in cancer relevant processes such as proliferation and differentiation.^{27,31,32} When CRC cell lines (DLD-1 and SW480) were transfected with pre-miR-143, ERK5 was down-regulated and cell growth was significantly inhibited in a dose-dependent manner.³¹ Thus, reduced expression of miR-143 could be associated with tumorigenesis through elevated levels of ERK5. MiR-145 has been shown to target insulin receptor substrate 1 (IRS-1) and type I insulin-like growth factor receptor (IGF-IR), which sends antiapoptotic signals and promotes cell growth, and its low expression in CRC could be hypothesized to promote

Table 1. MiRNAs That Are Differentially Expressed in Colorectal Cancer (CRC) Samples Compared to Normal Tissues and Suggested Clinical Relevance

miRNA	Expression in CRC versus normal	Patients (n)	miRNA detection method	Clinical relevance	Comment	Reference
Let-7	Up	46	RT-PCR	CT response	Low expression associated with improved response to folate-based CT	18
miR-17-5p	Up	110	RT-PCR	DFS	Low expression associated with increased DFS in low-stage disease (TNM 1 and 2)	19
miR-20a	Up	197	μ A and RT-PCR	No*	High expression associated with poor OS in test cohort	17
miR-29a	Up	157	RT-PCR	Plasma marker	Upregulation as a diagnostic marker	20
miR-21	Up	22	RT-PCR	NA		21
		197	μ A and RT-PCR	OS	High expression associated with poor OS	17
		29	RT-PCR	Tumor stage	High expression associated with advanced tumor stage	22
miR-31	Up	98	RT-PCR	Tumor stage	High expression associated with advanced tumor stage	23
		12	RT-PCR	Tumor stage	High expression associated with advanced tumor stage	24
		29	RT-PCR	Tumor differentiation	Low expression associated with poorly differentiated tumors	22
miR-92a	Up	90	RT-PCR	Plasma marker	Upregulation as a diagnostic marker	25
		157	RT-PCR	Plasma marker	Upregulation as a diagnostic marker	20
miR-106a	Down	110	RT-PCR	DFS and OS	Downregulation associated with shorter DFS	19
	Up	197	μ A and RT-PCR	No*	High expression associated with poor OS in test cohort	17
miR-126	Down	110	RT-PCR	No		19
miR-143	Down	98	RT-PCR	NA		23
		29	RT-PCR	Tumor size	Low expression associated with large tumor size	22
miR-145	Down	98	RT-PCR	NA		23
		29	RT-PCR	Tumor size	Low expression associated with large tumor size	22
miR-181b	Up	197	μ A and RT-PCR	No*	High expression associated with poor OS in test cohort	17
		46	RT-PCR	CT response	Low expression associated with improved response to folate-based CT	18
miR-203	Up	197	μ A and RT-PCR	No*	High expression associated with poor OS in test cohort	17

CT indicates chemotherapy; DFS, disease-free survival; μ A, microarray analysis; OS, overall survival; NA, not assessed.

*These miRNAs were selected for association with OS in the test set, but the correlation was not confirmed in the validation set.

proliferation through this mechanism.³³ In a study of 29 primary CRC samples, low expression of miR-143 and miR-145 was associated with large primary tumor size, supporting the suggested role of these molecules in CRC biology.²² MiR-126 was shown to be down-regulated in CRC compared to normal tissue through unknown mechanisms.¹⁹ *In vitro* studies have suggested that one target for miR-126 may be the p85 β subunit, a component in the phosphatidylinositol 3-kinase signaling network, and loss of negative regulation by miR-126 could lead to a selective growth advantage during colon carcinogenesis.³⁴

One explanation offered to explain reduced miRNA expression levels in human cancer is that many miRNA encoding genes are located in clusters at fragile sites in the genome, which are commonly methylated or deleted in cancer.³⁵ In CRC, p53 targets miR-34b and miR-34c

were shown to be epigenetically silenced through methylation of neighboring CpG islands.³⁶ This particular methylation pattern was present in 90% of examined CRC specimens (101 of 111), but not in corresponding normal tissue, and miR-34b/c were shown to be down-regulated in CRC cell lines and tumors. This suggests that miR-34b/c could be important mediators of p53-dependent tumor suppression in colorectal epithelium, and that their epigenetic silencing may be a potential mechanism in CRC carcinogenesis.

Three independent groups have reported up-regulation of miR-31 in CRC, but the clinical significance is unclear, as are the biological functions of this molecule, because no mRNA targets have so far been verified for miR-31. Over-expression was associated with advanced tumor stage in two of the reports,^{23,24} while in the third

Figure 1. Potential uses of miRNAs as biomarkers of diagnosis, prognosis, and therapy response and as possible therapeutic target.

study, no correlation was observed between miR-31 expression and tumor stage.²² The discrepancy could be explained by the low number of cases included in two of the three studies ($n = 29$ and $n = 12$), underscoring the importance of study design when aiming to determine the clinical relevance of molecular targets, particularly with regard to sample size.

The most comprehensive analysis of miRNA expression in CRC performed to date used microarrays containing 389 miRNA probes, and 37 miRNAs exhibited differential expression in the training set of 84 colon cancer samples.¹⁷ In particular, miR-20a, miR-21, miR-106a, miR-181b, and miR-203 were found to be overexpressed in tumor tissues with high tumor to normal ratios, as well as being associated with poor overall survival in the testing set. Prognostic relevance could be confirmed in the validation set for only one of the candidate miRNA (miR-21, see discussion below), and the clinical and biological implications of differential expression of the remaining miRNAs are unclear.

Although clinical relevance has not been verified for the differentially expressed miRNAs discussed in this section, the targets of many of these molecules are associated with pathways and processes relevant for CRC carcinogenesis and progression. Several of the mentioned studies were hampered by suboptimal design, and lack of significant correlations should not discourage inclusion of some of these candidate molecules in future studies in the search for potential biomarkers in CRC.

MiRNA as Diagnostic Biomarkers in CRC

The only current CRC biomarker in serum and plasma is carcinoembryonal antigen, but low specificity and sensitivity limits its clinical use, particularly for early detection of CRC. MiRNAs have been shown to be stably and differentially present in serum and plasma of cancer patients, and additionally, studies are ongoing to clarify the potential use as biomarkers in extracellular biological materials such as stool and urine.^{8,37–39}

Based on high expression levels in tumors and plasma from CRC patients and after a selection process starting with 95 miRNAs, miR-17-3p and miR-92a were chosen for further study in plasma from CRC patients ($n = 90$), healthy donors ($n = 50$), and patients with inflammatory bowel disease ($n = 20$) and gastric cancer ($n = 20$).²⁵ High expression of both miRNAs was associated with the presence of CRC, but miR-92a was by far the best performer in statistical analyses. MiR-92a expression levels discriminated CRC patients from healthy controls with a quite promising sensitivity of 89% and specificity of 70%. However no correlation was observed with tumor stage in the CRC patients. Interestingly, miR-92a expression was lower in inflammatory bowel disease than in controls, and levels in gastric cancer patients were equal to controls. The conclusions from this report, suggesting miR-92a as a possible biomarker for early detection of CRC were corroborated by a recent study of plasma samples from 100 CRC patients in which miR-92a was also found to be

elevated relative to controls along with a second candidate miRNA, miR-29a.²⁰ MiR-92a has been shown to be overexpressed in many solid tumors, and as part of the mir-17-92 gene cluster, which is a known oncomir, it may contribute to promote cell proliferation, inhibit apoptosis, and induce tumor angiogenesis. The biological explanation for the presence of miRNA in serum and plasma is incompletely understood, but recent work suggests that miRNA is secreted from cells and transported in the blood in exosomes, and may affect the recipient cells by inhibiting mRNA translation.^{39,41} A stage independent, sensitive, and specific marker for CRC in plasma would be clinically important, and clearly these promising results support further assessment of miRNAs as potential biomarkers in extracellular fluids.

The most widely applied screening method for CRC is the fecal occult blood test, but interpretation of results is hampered by low test specificity. However, the principle of detecting products shed from a tumor located in the colorectum into the stool offers the possibility of increased diagnostic sensitivity and specificity compared to blood tests, and attempts have been made to identify targets for such purposes. Among candidate molecules are miRNA because of their unique stability, and in a recent study, the levels of 15 miRNAs were measured in stool and colorectal tissue samples from 15 patients with CRC and 5 healthy individuals.⁴² Although the variability was more pronounced among the stool samples than the tissue samples, the authors concluded that specific miRNA expression profiles could be defined, suggesting that stool is yet another biological material in which miRNAs are preserved and amenable for detection.

MiRNA as Prognostic Biomarkers in CRC

The search for prognostic biomarkers in CRC has in recent years been extended to study the potential of analyzing expression of single or multiple miRNAs in tumor tissue, and in the following individual miRNAs which have been shown to have prognostic relevance in CRC will be discussed.

From the 37 miRNAs identified as differentially expressed in the previously mentioned microarray profiling study, overexpression of miR-21 was identified as an independent predictor of overall survival (OS) in the validation set containing tumor samples from 113 colon cancer patients.¹⁷ Similar conclusions were drawn from a study of 29 tumor samples, in which miR-21 expression was associated with poor survival and therapeutic outcome in stage II and III CRC.³⁴ Interestingly, miR-21 has been shown to down-regulate Pdc4, a 64-kDa protein that inhibits tumor progression by interacting with translation initiation factors eIF4A and eIF4G,²¹ and this has been suggested as a possible mechanism by which miR-21 might promote tumor invasion and metastasis and potentially influence clinical outcome.

MiR-106a and miR-17-5p are both believed to target the multifunctional E2F1 transcription factor and may as such take part in p53-E2F cross talk in regulating cell proliferation and death.⁴³ Low expression of miR-106a was in a panel of

110 CRC samples found to correlate with advanced disease stage and OS, and expression levels were inversely correlated with E2F1 expression levels.¹⁹ The finding would support miR-106a as a tumor suppressor as loss of negative regulation of E2F1 could result in a proliferative tumor response. However, for miR-17-5p, which also varied inversely with E2F1 expression levels, overexpression was associated with advanced disease stage (although not with OS). This would suggest the opposite biological interpretation although the resulting effect on the effector molecule E2F1 would be expected to be the same. To complicate the picture, Schetter and co-workers found that high expression levels of miR-106a was associated with shorter survival in the test cohort, although the correlation was not confirmed in the validation population.¹⁷

These results illustrate several important points regarding the interpretation of miRNA expression data, particularly when trying to use the data to explain clinical tumor progression. The lack of standardized test methods for miRNA analysis may in part explain the contradictory results of miR-106a being up-regulated relative to normal tissues in one study and down-regulated in another. The ability of miRNAs to variably influence expression levels of multiple target molecules depending on strand complementarity may make outcome analyses complicated, because several pathways with variable downstream responses may be influenced by one, or in E2F1s case, several miRNAs. When correlating these data to complex, multifactor outcomes, such as survival from cancer, it is not surprising that conclusions may differ.

MiRNAs as Predictive Biomarkers for Chemotherapy Response in CRC

With the implementation of new drugs and the addition of biological agents, chemotherapy is becoming an increasingly important treatment modality in CRC, particularly in metastatic disease, but also in the neoadjuvant and adjuvant settings. These drugs have significant side effects and treatment is costly, rendering the search for predictive biomarkers important with the aim to possibly individualize treatment. miRNAs have been explored for their association with tumor sensitivity and cellular responses, in particular to fluoropyrimidine based chemotherapy which is one of the mainstays in CRC treatment.

Most of the work studying the relevance of miRNAs in predicting chemotherapy effects and tumor cell sensitivity has been performed using preclinical models, in particular CRC cell lines grown *in vitro*. In a study of two subclones from the much-used human CRC cell lines HT29 and HCT116, the expression of multiple miRNAs was shown to change on exposure to 5-fluorouracil (5FU) compared with untreated cells (19 miRNAs were up-regulated and 3 down-regulated).⁴⁴ While some changes in miRNA expression were consistent with the antitumor effects of the drug, others were not, such as up-regulation of the polycistronic mir-17-92 cluster (which include miR-19a and miR-20) and miR-21, thought to have anti-apoptotic and oncogenic effects and in theory to be involved in drug resistance in these clones.

Several individual miRNAs have been suggested to be of importance for drug sensitivity in CRC. MiR-192 was shown to down-regulate the anticancer target dihydrofolate reductase which participates in a cycle feedback loop together with thymidylate synthase and is involved in DNA and RNA synthesis.⁴⁵ Dihydrofolate reductase is an important target for antifolate-based anticancer chemotherapy, and its down-regulation could influence 5FU efficacy in CRC, as 5FU blocks thymidylate synthase and therefore also growth and proliferation by inhibiting DNA/RNA synthesis. While miR-192 could indirectly sensitize cells for 5FU, elevated levels of miR-196a, which targets the *HoxA7*, *HoxB8*, *HoxC8*, and *HoxD8* genes, increased apoptotic cell death in response to oxaliplatin and *cis*-platin, but not to 5FU, exposure in the CRC cell line SW480.⁴⁶ However, up-regulation of miR-196a also promoted cell migration and invasion *in vitro*, as well as increased frequency of lung colony formation on tail vein injection in nude mice, rendering the effects ambiguous. As previously mentioned, miR-143 is expressed at low levels in CRC compared with normal tissues. Experimental up-regulation of miR-143 in HCT116 cells resulted in reduced cell viability and increased apoptotic cell death on 5FU exposure, but its implications in the clinical setting has not been examined.⁴⁷ From a recent study of osteosarcoma xenografts, miR-140 was identified as associated with multiple drug resistance, and subsequent experimental up-regulation by transfection of miR-140 in a CRC cell line (HCT116), resulted in p53-dependent cell cycle arrest accompanied by resistance to 5FU and methotrexate.⁴⁸ MiR-140 was highly expressed in stem-like subpopulations of HCT116 which were also shown to be resistant to 5FU treatment, and this phenotype could be partially reverted by transfection of anti-miR-140, leading the authors to suggest that miR-140 might be a candidate target for developing novel therapeutic strategy to overcome drug resistance.

The task of carrying out prospective studies for identification of predictive markers in clinical cancer therapy is challenging, and often retrospective strategies are implemented. In a study of 46 patients with residual or recurrent colon cancer treated with the 5FU analog S-1, RNA was isolated from formalin-fixed paraffin-embedded samples, followed by RT-PCR analysis of miRNA expression levels. MiRNAs let-7g and miR-181b were shown to be overexpressed in tumor samples relative to normal colon tissues, and the expression was inversely correlated with response to S-1 treatment but had no impact on patient survival.¹⁸ The above-mentioned reports exploring the potential relevance of miRNAs for chemotherapy response represent important first steps toward identifying possible predictive biomarkers, but verification of targets in adequately designed clinical panels is necessary to assess the potential value of suggested candidates.

MiRNAs as Therapeutic Targets in CRC

MiRNAs are important biological regulators and are therefore potentially interesting therapeutic targets in cancer. By influencing one miRNA, a cascade of pathways could be affected, and herein exist the potential for

sequence specific gene regulation but also the challenge of carefully determining the range of possible desired and undesired effects. Although there are at present no clinical reports describing therapy targeting miRNAs, most authors publishing miRNA results seem to have optimistic views on the future utility of miRNAs as therapeutic targets.

By using antagomirs, modified RNA with the ability to inhibit miRNA by stably and rapidly hybridizing to single stranded RNA, it is possible to inhibit sequence-specific miRNA and study their function *in vitro* and *in vivo*. The feasibility of *in vivo* miRNA inhibition was demonstrated in a proof of principle report in 2005 showing that in mice injected with antagomirs against several miRNAs normal tissue expression of the targets was reduced up to 23 days after a single injection.⁴⁹ However, although these results demonstrate the effectiveness of miRNA inhibition *in vivo*, the obvious challenge of obtaining tumor cell specificity must be solved to allow clinical implementation. In search of a specific, effective, and stable delivery system, one could look toward progress made with closely related silencing systems such as small interfering RNA (siRNA). Advances have been made using siRNA as a delivery system for cancer therapy through local or targeted administration *in vivo*,⁵⁰ and the similarity between the miRNA and siRNA systems in silencing mRNA makes the findings applicable to both.

There are several promising potential therapeutic miRNA candidates in CRC, many of which have been mentioned in the previous sections, and if the levels of miRNAs could be manipulated *in vivo*, effects relevant for cancer progression and treatment efficacy could be obtained. For instance, if levels of let-7g and miR-181b could be lowered, improved response to S-1 treatment might be achieved, while augmenting miR-143 levels could inhibit tumor proliferation. However, it would be important to ascertain comprehensively the biological implications of such manipulations, as well as the downstream effects induced by miRNAs over time. Thus, using miRNA in treatment is an intriguing idea, but effective delivery systems must be available, and for each potential target, the challenge of foreseeing down-stream regulatory effects of manipulating levels of a single miRNA will require extensive research.

Conclusions

Since their discovery, the field of miRNA research has grown tremendously, and we presently know that these molecules are important regulators of gene expression and may influence cancer development and progression as oncogenes or tumor suppressors depending on effects brought about by targeted mRNAs. The relatively low number of miRNA, the possibility of using standardized, rapid, and relatively inexpensive detection methods suitable for high-throughput analysis, together with their unique chemical stability make miRNAs promising candidates for *ex vivo* detection as biomarkers of cancer. A number of miRNAs are dysregulated in CRC and have been linked to biological processes relevant for tumor

growth and progression and for chemotherapy response, as discussed in this review. However, with respect to identifying clinically useful biomarkers we are currently only scratching the surface; a significant amount of research is still required to determine whether any of the mentioned candidates will fulfill their promise.

In this regard, one important task is the clinical validation of already suggested miRNA biomarkers in independent clinical panels, an undertaking that feasible, and most likely broadly ongoing in research groups with access to patient samples. As the number of identified human miRNAs steadily increases, novel candidates will be added to screening tools, and particular attention should be focused toward applying these tools in adequately designed clinical panels. However, the most daunting challenge will be to identify miRNA targets and understand the potentially complex downstream cellular signaling. Although such knowledge is not absolutely necessary for early detection incomplete characterization would hamper the use of biomarkers for prognostic and predictive purposes or as therapeutic targets. Generating a more complete picture of miRNA expression and clinical relevance in CRC and gaining knowledge of targets and cellular effects is a major task, but acknowledging the potential utility of mRNA as biomarkers it is likely to be worth the effort.

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