The Role of microRNAs in Colorectal Cancer

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Abstract

Over the last decade, it has become clear that aberrant microRNA expression has a functional role in the initiation and progression of colorectal cancer (CRC). Specific microRNAs can act as either tumor suppressors or oncogenes depending on the cellular environment in which they are expressed. The expression of microRNAs is reproducibly altered in CRC and their expression patterns are associated with diagnosis, prognosis and therapeutic outcome in CRC. Studies have begun to examine the association of microRNA related polymorphisms and their association with CRC incidence and prognosis as well as the possibility of using circulating microRNAs or fecal microRNA expression as non-invasive early detection biomarkers. These data suggest that microRNAs may be potential molecular classifiers, early detection biomarkers and therapeutic targets for CRC. Here, we will review the evidence demonstrating a role of microRNAs in CRC.

Introduction

Colorectal cancer (CRC) is responsible for 10% of the world-wide cancer incidence and mortality[1]; thus it represents a significant health burden. The more we understand about the etiological and biological nature of CRC, the better equipped we will be at designing effective preventive, diagnostic and therapeutic tools to help reduce the burden of this disease. Practical issues that are being addressed include identifying effective therapeutic strategies, identifying the precise patient populations that may benefit from adjuvant chemotherapy and identifying precise patient populations that would benefit from intensive screening for disease recurrence. For example, TNM staging is arguably the best prognostic classier tool that helps guide therapeutic decisions for CRC[2]. Adjuvant therapy after surgical resection is considered to be beneficial for TNM stage III patients, yet it is controversial if adjuvant therapy should be given to TNM stage II patients[3, 4]. Therefore there is a need to develop better classifiers to identify subpopulations of TNM stage II patients who would benefit from therapy.

Sporadic colon cancer progresses from a benign polyp to a malignant adenocarcinoma while cells accumulate a series of well documented genetic and epigenetic changes. Specific

genetic changes are starting to inform therapeutic approches and guide treatment decisions. For example, both vascular endothelial growth factor (VEGF) and epidermal growth factor (EGFR) pathways have been shown to be critical for colon cancer progression. Targeted therapies to both EGFR and VEGF have shown promise in late stage CRC.[5] KRAS is an important oncogene in CRC and mutations in KRAS predict a failure to respond to EGFR inhibitors. As we continue improve our understanding of the biology and both genetic and epigenetic changes in CRC, we may be able to develop additional biomarkers and therapies to help treat and even prevent this disease.

Historically, most research on CRC has focused on genetic and epigenetic changes in protein coding genes for their role in CRC initiation and progression. Recently, a class of small non-coding RNAs, called microRNAs, has come into focus. MicroRNAs are small, 18–24 nucleotide RNAs that regulate the translation and stability of specific target mRNAs. Ten years ago, microRNAs, were implicated in the initiation of chronic lymphocytic leukemia.[6] Since that discovery, microRNAs have been shown to be involved in almost every aspect of cancer biology, as tumor suppressor genes or oncogenes ostensibly depending on the cellular context in which they are expressed. Evidence supports a role for microRNAs at every stage of CRC initiation, progression and development. Extensive research is now aimed at determining if microRNAs can be used as diagnostic biomarkers and therapeutic targets for cancer (Figure 1).

In this review, we will focus on the role of microRNAs in CRC and discuss their potential as diagnostic and prognostic classifiers for CRC and as therapeutic targets.

MicroRNA expression is consistently altered in CRC

To date, more than twenty studies have examined microRNA expression patterns in CRC and confirmed that microRNAs are consistently and reproducibly altered in this disease.[7] These studies used a variety of techniques ranging from global miRNA expression profiling with deep sequencing[8] or microRNA microarrays[9–11] to examining the expression of selected microRNAs with quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). An underlying theme of all of these studies is that microRNA expression of CRC is distinctly different than nontumor tissues, which is consistent with the hypothesis that aberrant microRNA expression has a role in the CRC initiation and development.

In contrast to the original report that microRNA expression levels are globally reduced in cancer,[11] more microRNAs have been found to have elevated expression in CRC compared to those with reduced levels. A recent review of 23 microRNA expression studies found that of the 164 microRNAs that are significantly altered in CRC in at least one study, approximately 2/3 of them were elevated and 1/3 that were reduced in tumors.[7] This indicates that microRNAs may have more oncogenic than tumor suppressive functions in CRC. It also suggests that the microRNA processing machinery is not compromised in CRC. The number of microRNAs showing elevated expression in CRC corresponds with the findings that microRNAs are over-represented at regions of the genome that show copy-number gain while they are under-represented in regions that show copy number loss in CRC.[12, 13] Regardless of these findings, it is clear from functional studies that certain microRNAs have important oncogenic functions while others have important tumor suppressor functions and these functions need to be evaluated for each microRNA individually in the context of the specific tissue/tumor type.

Michael and colleagues were the first to show that microRNA expression patterns were altered in CRC.[14] They reported that miR-143 and miR-145 were reduced in CRC and suggested that these microRNAs were tumor suppressors. Multiple studies have since validated these findings and demonstrated that miR-143 and miR-145 indeed have tumor

suppressive functions in CRC.[15] Another highly relevant microRNA in CRC is the oncogenic microRNA, miR-21. At least seven studies reported that miR-21 is elevated in CRC.[7] Furthermore, miR-21 has been found to be elevated in many other solid tumor types[10] and this microRNA has important roles in cancer initiation, progression and metastasis. Other microRNAs which have been found to be altered in CRC in multiple reports include the miR-17-92 cluster, miR-106a, miR-31, miR-181b, miR-183, miR-135a/b, the miR-200a/b/c family, miR-203 and miR-224.[7]

The transcriptional regulation of microRNAs is as complex and diverse as the transcriptional regulation of protein coding genes. Therefore, the causes of the altered expression of microRNAs in CRC are equally diverse and complex. Aberrant transcription of microRNAs in CRC can be the result of transcription factors that are activated through various oncogenic signaling cascades, the result of genomic amplification/loss, genotoxic stress or inflammatory stimuli. Epigenetic mechanisms also affect microRNA expression levels. Genome-wide profiling of chromatin signatures demonstrated that DNA methylation regulates miRNA expression of CRC cell lines in vitro.[16] Several microRNAs, including let-7,[17] miR-34,[18] miR-342,[19] miR-345,[19] miR-9,[20] miR-129,[20] and miR-137[20] are frequently hypermethylated in colon tumors and this is thought to lead to their reduced expression. MicroRNAs can also contribute to global epigenetic regulation in CRC. For example, miR-143 is a tumor suppressor microRNA that directly targets DNA methyltransferase 3A (DNMT3A) and loss of miR-143 expression leads to increased DNMT3A in CRC tissues.[21] Similarly, loss of miR-342 leads to increased DNA methyltransferase 1 (DNMT1) and this contributes to the hypermethylation of several tumor suppressor genes in CRC.[22]

MicroRNA expression patterns can also classify tissue types and tumor types and microRNA expression patterns perform at least as well as mRNA expression profiles for this purpose.[10, 11] Therefore, microRNA expression pattern may help classify different phenotypic subgroups of CRC. Important phenotypic subgroups of CRC include microsatellite instability (MSI), KRAS mutation status, and TP53 status. These subgroups differ in therapeutic response and, thus, projected survival. MicroRNA expression patterns are different between microsatellite stable (MSS) and MSI tumors[23–25] and these expression patterns can be used to correctly classify MSS versus MSI tumors. Oncogenic miR-155 targets the DNA mismatch repair proteins MLH1, MSH2, and MSH6 to contribute to the MSI phenotype in CRC.[26] MiR-21 also targets MSH2, which is thought to increase tumor resistance to 5 fluorouracil based therapies.[27] KRAS mutant tumors also show altered microRNA expression patterns when compared to wild type tumor.[28] These findings demonstrate the potential for microRNA expression patterns to classify CRC tumors into different phenotypic groups. Altered microRNA expression may also explain some of the biological differences between each subgroup.

MicroRNAs function at early stages in CRC development

Adenomas are benign growths that are frequently a precursor lesion of colon adenocarcinoma. If microRNAs are altered in adenomas, it suggests that microRNAs have a role in the initiation of cancer. This is indeed the case. MicroRNA expression patterns can distinguish normal colonic mucosa, colon adenomas, and colon carcinomas.[29] These expression patterns are consistent with the stepwise, multi-hit model for colon carcinogenesis and support a role for microRNAs in each step. MiR-21 is a good example of this in that it is elevated in both adenomas and colon carcinomas.[9] Higher expression levels of miR-21 correlate with more advanced stages of CRC indicating a potential a role for miR-21 in the initiation and progression of CRC.[9, 30] Consistent with this, the frequency and extent of miR-21 expression by *in situ* hybridization was found to be increased during the

transition from adenoma to advanced carcinoma.[31] Elevated expression of miR-21 in mice can induce malignancies that are addicted to high miR-21 expression, demonstrating potential for this microRNA to initiate malignancies.[32] MiR-135b may also have a role in the early stages of CRC. It is elevated in colon adenomas and one of its targets include the adenomatous polyposis coli (APC) gene, a component of the Wnt signaling pathway that is frequently mutated or repressed in adenomas.[33] The miR-17-92 cluster of oncogenic microRNAs has increased expression in adenomas and thought to have a role the adenoma to carcinoma transition.[34] Epigenetic silencing of miR-137[35] and miR-342[19] in colon adenomas also suggest a role for these microRNAs CRC.

Colon adenomas have high levels of cellular senescence that serve as a barrier to a malignant transformation[36]. MicroRNA expressions patterns may help maintain this barrier to prevent tumor progression. The p53 isoforms Δ133p53 and p53β are endogenous regulators of cellular senescence and their expression patterns are consistent with preventing the progression from colon adenoma to carcinoma.[37] MiR-34a is a p53 regulated microRNA that influences cellular senescence.[37] The indirect repression of miR-34a by Δ133p53 through the inhibition of p53 function may be one of the mechanisms that p53 isoforms utilize to bypass this senescence barrier.

MicroRNA expression affects cell growth, proliferation, invasion and metastasis in CRC

There is overwhelming evidence that supports a mechanistic role for specific microRNAs in CRC. Several microRNAs have been shown to have roles in CRC and their targets have been experimentally verified. While we cannot discuss all of those findings in this review, we will present some of the important findings on specific microRNAs, their targets, and their role colon cancer (Table 1).

The first study examining microRNA expression in colon tumors identified miR-143 and miR-145 as potential tumor suppressors.[14] Since that time evidence has continued to support a tumor suppressive role for these microRNAs. Both miR-143 and miR-145 can regulate cell growth and proliferation in vitro,[38] each by targeting different oncogenic protein coding genes. MiR-143 functions to suppress cell growth and proliferation by directly repressing the translation of KRAS,[39] DNMT3A[21] and extracellular signal-regulated kinase-5 (ERK5).[38] Furthermore, replacement of miR-143 in xenograft mouse models of colon cancer reduced tumor growth[15] supporting a functional role for miR-143 expression in colon cancer progression. The tumor suppressor activity of miR-145 functions in part through its inhibition of the oncogenic insulin receptor substrate-1 (IRS-1),[40] c-Myc,[41] Yamaguchi sarcoma viral oncogene homolog 1 (YES1),[42] signal transducer and activator of transcription 1 (STAT1)[42] and Friend leukemia integration 1 (FLI1).[43] MiR-145 may also have a role in stem cell maintenance by targeting octamer-4 (OCT4), sex determining region Y-box 2 (SOX2) and Krüppel-like factor-4 (KLF4).[44]

MiR-21 is an important oncogenic microRNA with roles in tumor initiation, progression and metastasis. This microRNA is over expressed in at least 18 malignancies, indicating that miR-21 biology is relevant to most cancer types.[10, 45] Elevated miR-21 expression leads to increased cell proliferation, decreased apoptosis, increased cell migration, intravasation and metastasis by targeting several tumor suppressor genes. Tumor suppressor genes experimentally-verified to be targeted by miR-21 include programmed cell death 4 (PDCD4),[46, 47] phosphatase and tensin homolog (PTEN),[48] Cell division cycle 25 homolog A (Cdc25a),[49] reversion-inducing-cysteine-rich protein with kazal motifs (RECK),[50] TIMP3,[50] maspin,[51] nuclear factor 1 B-type (NFIB),[52] tropomyosin 1 (TPM1),[53] sprouty 2 (SPRY2),[54] T-lymphoma invasion and metastasis-inducing protein 1 (TIAM1),[55] and Ras

homolog gene family, member B (RHOB).[56] The targeting of PDCD4 by miR-21 is of particular interest because it has been thoroughly validated in vitro, in mouse models, and the reverse association between miR-21 and PDCD4 protein expression has been confirmed in human tissues and appears to be relevant in multiple tumor types.

Several other microRNAs have been implicated in CRC. MiR-30a-5p is a tumor suppressor microRNA that targets denticleless homolog (DTL) to suppress tumor growth in CRC[57]. MiR-192 and miR-215 are both effectors and regulators of p53 function to suppress colon carcinogenesis.[58] Another p53 related microRNA, miR-34a, has been shown inhibit cell invasion in colon cancer cell lines by targeting FRA1.[59] Cyclooxygenase 2 (COX-2) can be negatively regulated by miR-101[60] and this may contribute to the initiation and progression of colon tumors. MiR-451 overexpression in colon cancer cells leads to reduced cell proliferation through targeting of the oncogene macrophage migration factor (MIF).[61] Over expression of miR-499-5p in CRC cell lines targets FOXO4 and PDCD4 to promote cell migration and invasion in vitro and lung and liver metastases in mouse models.[62] MiR-675 can target the retinoblastoma (RB) tumor suppressor gene to increase tumor growth.[63] MiR-365 acts as a tumor suppressor to inhibit cell cycle progression and promotes apoptosis of colon cancer cells by targeting Cyclin D1 (CCND1) and Bcl-2.[64] Loss of miR-29 leads to increased expression of MMP2 to promote metastases in mouse models of colon cancer.[65] The oncogenic miR-95 promotes tumorgenicity by targeting sorting nexin 1 (SNX1).[66] Because altered microRNA expression can influence the initiation and progression of colon cancer, it suggests that microRNAs have potential as therapeutic targets for CRC.

MicroRNA related polymorphisms and the risk and prognosis of CRC

Recent studies suggest that 35% of all CRC can be attributed to inherited genetic factors.[67] These inherited risks are likely due to single-nucleotide polymorphisms (SNPs) and other genetic abnormalities in both coding and noncoding genes. Because microRNAs have an important role in CRC initiation and development, it is possible that SNPs that disrupt microRNAs, microRNA binding sites, or microRNA processing may alter an individual's susceptibility to CRC.[68] SNPs in the 3' untranslated region (UTR) of protein coding genes can also introduce microRNA binding sites to hasten translational repression of that gene. Therefore, microRNA related SNPs can deregulate biological pathways in a variety of ways and it is important to evaluate the association between these SNPs and CRC risk.

Studies evaluating the association between SNPs in microRNAs and microRNA binding sites with risk of CRC are starting to emerge. Zhan and colleagues reported that a SNP in *miR-196a* led to altered *miR-196a* expression and increased CRC incidence in a Chinese population consisting of 252 CRC patients and 543 healthy controls.[69] A global analysis of the 3'UTRs of candidate genes led to the identification of two SNPs; one which disrupts the binding of *miR-337*, *miR582*, *miR-200a**, *miR-184* and *miR-212* for *CD86* and another which disrupts the binding of *miR-612* to *INSR*. In a study population of 697 cases and 624 controls, each of these SNPs were associated with cancer risk.[70] Zanetti and colleagues have recently reported that a SNP disrupts the binding of *miR-27a* to the 3' UTR *MBL2* and this SNP was associated with colon cancer risk in an African American population of 100 cases and 186 controls while a similar association was not found in Caucasians.[71] While the findings of each of these studies require validation in additional, independent populations and the biological mechanism that are responsible for these associations need to be further explored, these studies support the hypothesis that SNPs in microRNAs and microRNA binding sites modify colon cancer risk.

Similar studies are evaluating microRNA related SNPs and their association with prognosis and response to therapy. For example, a study of 426 CRC patients found that a SNP in

miR-492 was modestly associated with improved survival[72]. KRAS-LCS6 is a particular SNP of interest as it disrupts a let-7 binding site in the 3'UTR of KRAS. CRC patients with activating KRAS mutations do not respond to anti-EGFR therapies. Because KRAS-LCS6 may lead to altered levels of KRAS protein, it leads to the hypothesis that KRAS-LCS6 may have some association with therapeutic response. In a study of 121 metastatic CRC patients without BRAF mutations who received anti-EGFR therapy, patients with KRAS-LCS6 showed significantly worse overall survival.[73] Although a similar study evaluated 130 metastatic, KRAS wild type CRC patients and found that KRAS-LCS6 was associated with improved response to anti-EGFR therapy.[74] In a third study of 409 early stage, 182 stage III, and 69 stage IV CRC cases, KRAS-LCS6 was associated with improved survival only in early stage cases and no survival benefit was observed in late stage patients.[75] These seemingly contradictory results highlight the need for independent validation in multiple cohorts with special attention to KRAS and BRAF mutation status and their interaction with specific chemotherapeutic regimens.

micoRNAs as diagnostic biomarkers for CRC

Undoubtedly, early detection of CRC provides the best chance for successful treatment. While current chemotherapeutic regimens have significantly improved overall survival of CRC patients, surgery before metastatic spreading of the disease is considered the only curative form of treatment. The screening methods of colonoscopies and fecal occult blood tests (FOBT) have improved survival rates for CRC by detecting patients and earlier stages of cancer, but there is still much room for improvement and neither test is ideal. While colonoscopies are considered the best screening tool because it can also remove precancerous polyps during the procedure, they are both invasive and expensive, which leads to lower compliance rates. FOBT is less invasive, but also less sensitive and specific. New non-invasive, accurate biomarkers are needed to improve both the accuracy and screening rates for CRC. MicroRNAs are being evaluated for their potential in this area.

Circulating microRNAs can be detected in blood serum or plasma. Ng and colleagues were the first to report that circulating levels of microRNAs were different in blood plasma in CRC cases and controls.[76] In a population of 90 cases and 50 controls, they found that miR-92 was expressed at higher levels in the plasma cases and could distinguish cases from healthy control patients with 70% specificity and 89% sensitivity. The expression of miR-92 was reduced following surgical removal of the tumor suggesting that circulating levels of microRNAs may be useful marker of disease recurrence. A second study of 120 cases and 29 controls validated these findings showing that plasma levels of miR-92 can discriminate CRC cases and controls with 65% sensitivity and 82% specificity.[77] While this classifier may not be accurate enough on its own to serve as a screening marker, it may be developed along with additional markers to improve screening accuracy. A similar study found that circulating levels of miR-141 has been shown to be elevated in metastatic CRC and its expression is associated with poor prognosis, suggesting that this microRNA may be used in conjunction with carcinoembryonic antigen (CEA) to detect CRC with distant metastases[78].

Measuring microRNAs in stool offers another non-invasive approach to detect CRC. One small study of 29 CRC cases and 8 healthy controls found that stool from CRC cases expressed higher levels of miR-21 and miR-106a[79]. A larger study of 197 CRC cases and 134 healthy controls investigated microRNA expression patterns of colonocytes isolated from feces and was able to demonstrate that microRNA expression patterns could distinguish cases from controls with 74% sensitivity and 79% specificity.[80] A similar strategy found that microRNA methylation patterns from DNA isolated from stool may have promise as a screening tool for CRC.[81] Hypermethylation pattern of miR-34b/c in stool samples could distinguish CRC cases from controls with 75% sensitivity and 84%

specificity. Further tests are warranted to determine if microRNA expression or methylation patterns in stool can be utilized, alone or in combination with FOBT, as an effective screening strategy for CRC.

microRNA expression as prognostic and predictive biomarkers

Molecular classifiers can serve as prognostic and predictive tools to help stratify cancer patients into appropriate risk groups to aid physicians in making therapeutic decisions. These decisions can include whether or not to provide adjuvant chemotherapy or what types of therapy are appropriate. Expression patterns of microRNAs are associated with both prognosis and therapeutic outcomes in CRC; therefore they have potential as prognostic and predictive biomarkers.

Elevated expression of miR-21 has a robust and reproducible association with colon cancer prognosis. We first reported this association based on both microRNA microarray expression data on an American cohort of 84 colon cancer patients and by qRT-PCR in an additional cohort of 113 Chinese patients.[9] In both cohorts, elevated miR-21 expression in tumors was associated with worse survival prognosis and therapeutic outcomes. These associations were independent of staging and other clinical characteristics demonstrating the potential of miR-21 as an independent prognostic and predictive biomarker. This association was also significant in TNM stage II patients, demonstrating that miR-21 expression may be a useful, early stage biomarker to identify subjects at high risk of cancer progression that have no evidence for metastasis or advanced disease. The association of elevated miR-21 expression and worse survival outcomes in CRC has now been validated in at least three additional studies (Figure 2A). These include studies of 156 Japanese CRC patients,[82] 46 CRC patients from the Czech Republic[83] and 130 TNM stage II colon cancer patients from Denmark.[84] The fact that these associations are found in diverse ethnic populations using a variety of methods to measure miR-21 argues that miR-21 expression is a robust prognostic classifier for CRC that is valid for most populations regardless of ethnicity. In fact, the association of elevated miR-21 and worse prognosis has now been reported in at least 9 additional cancer types including lung cancer,[85, 86] breast cancer,[87] pancreatic cancer,[88] tongue,[89] gastric cancer[90], head and neck cancer,[91] chronic lymphocytic leukemia,[92] melanoma[93] and astrocytomas[94]. These findings are consistent with the hypothesis that miR-21 expression is a prognostic classifier for many types of malignancies.

Additional studies have identified microRNA expression patterns that are associated with either prognosis or therapeutic outcome. The expression levels of miR-106b,[95] miR-320,[25] miR-498,[25] miR-125b,[96] miR-145,[97] miR-185,[98] miR-133b,[98] miR-215,[99] and miR-17[100] have each been reported to be associated with prognosis or therapeutic outcome in CRC in at least one study. Elevated expression of Dicer, an important gene encoding and RNA nuclease that is involved in microRNA processing, is associated with poor prognosis in CRC.[101] Further validation of these associations is warranted and may reveal additional prognostic classifiers for CRC.

The combination of multiple, independent validated biomarkers may provide improved risk stratification of patients (Figure 2B). As an example of this, we have reported that the combination miR-21 expression data with an inflammatory gene classifier and demonstrated significantly improved risk stratification with cancer-specific mortality.[102] The combination was also significant in TNM stage II colon cancer patients indicating that these may also be useful in identifying patients that are more likely to have undetectable micro-metastases.

Altered microRNA expression may contribute to chemoresistance

We have shown that miR-21 expression is associated with therapeutic outcome to 5FU-based therapies in both American and Chinese cohorts.[9] This association, in combination with the known oncogenic role for miR-21, suggests that increased miR-21 expression is in part responsible for the resistance to 5FU. Elevated miR-21 induces resistance to 5FU in colon cancer cell lines by down regulating DNA repair protein MutS homolog 2 (MSH2).[27] Exposure of colon cancer cells to 5FU leads to the increased miR-21 expression and this may be a response to genotoxic stress to help cells overcome the effects of 5FU.[103] Additional in vitro data support roles for altered expression of miR-140,[104] miR-215,[105] miR-224[106] and miR-20a[107] in developing chemoresistance. Further studies are warranted to determine if the expression of these microRNAs can predict response to chemotherapy and if those microRNAs can be used as therapeutic targets themselves.

Potential for microRNAs a therapeutic target

There is an emphasis to determine if microRNA-based therapies can be used to treat cancer. The two general strategies for microRNA-based therapies are to inhibit oncogenic microRNAs or restoring tumor suppressor microRNAs. Preclinical models have shown that both strategies can be effective.

Direct inhibition of microRNAs can be achieved by using antisense oligonucleotides or microRNA sponges to bind and sequester the target microRNA. Antagomirs are modified, antisense oligonucleotides which have been used to inhibit microRNAs in vivo[108] in mouse studies. Similar strategies have been used to effectively inhibit miR-122 function in primates[109] and anti-miR-122 treatments in chimpanzees chronically infected with HCV can improve HCV-related liver pathology[110]. This anti-miR-122 drug has progressed to Phase II clinical trials to treat HCV in humans suggesting that anti-sense based microRNA therapeutics may soon be available. Indirect inhibition of microRNAs may be achieved through treatment with various chemical compounds. In fact, screens have found small molecule inhibitors that can inhibit the function of at least miR-21[111] and miR-122[112] in vitro.

MicroRNA replacement involves reintroducing synthetic microRNA mimics or expression vectors that will produce the microRNA of interest. This has shown promise in preclinical mouse models where reintroduction of miR-145 and miR-33a had an antitumor effect in a model of colon cancer.[113]

The critical issues to overcome with microRNA-based therapies is to have a delivery mechanism that allows prolonged stability of the therapy and provide sufficient uptake in target cells while minimizing off-target side effects. These strategies include optimizing the chemistry and size of the synthetic oligonucleotides to achieve better binding efficiency and increased stability. Delivery mechanisms range from the use adenoviral vector-based delivery, to cationic liposomes to polymer-based nanoparticles. There are great expectations that these strategies will result in effective therapies for CRC.

Future Prospective and Conclusion

MicroRNAs have a clear role in the initiation and progression of CRC. Future research will have to specifically address the potential role for microRNA-based classifiers and therapeutics in medicine.

Many studies have reported that microRNA expression patterns or microRNA related polymorphisms are associated with diagnosis or prognosis in CRC indicating that they have

potential as early detection markers or predictive/prognostic classifiers. Like all population-based studies, these too require validation in additional populations to determine if these results are representative of the majority of CRC. Most of these studies are retrospective analyses on historical cohorts; therefore, there is a need to conduct prospective studies asking how relevant the reported associations are to current CRC patients. We need to determine if microRNA classifiers can provide actionable information, such as how or even if to treat a patient. Assays measuring microRNAs have to be standardized in ways that will allow an assay to give clinically relevant information on a single patient.

The successful development of microRNA-based therapeutics for CRC has many challenging hurdles to overcome. First, the best microRNAs to be targeted in CRC are yet to be defined. In order to do this, a better understanding the biology of how these microRNAs affect CRC is required. Anti-miR-21 based therapies or microRNA replacement therapies for miR-143 and miR-145 may provide useful strategies based on what is already known about their biology. Another major obstacle is determining how to achieve effective delivery without causing unnecessary side effects. Finally, the appropriate patient populations have to be selected in order to determine if there strategies can have therapeutic benefit.

While there is still much to be done, we remain optimistic that microRNA related diagnostics and therapeutics have substantial potential for the prevention and treatment of CRC.

Acknowledgments

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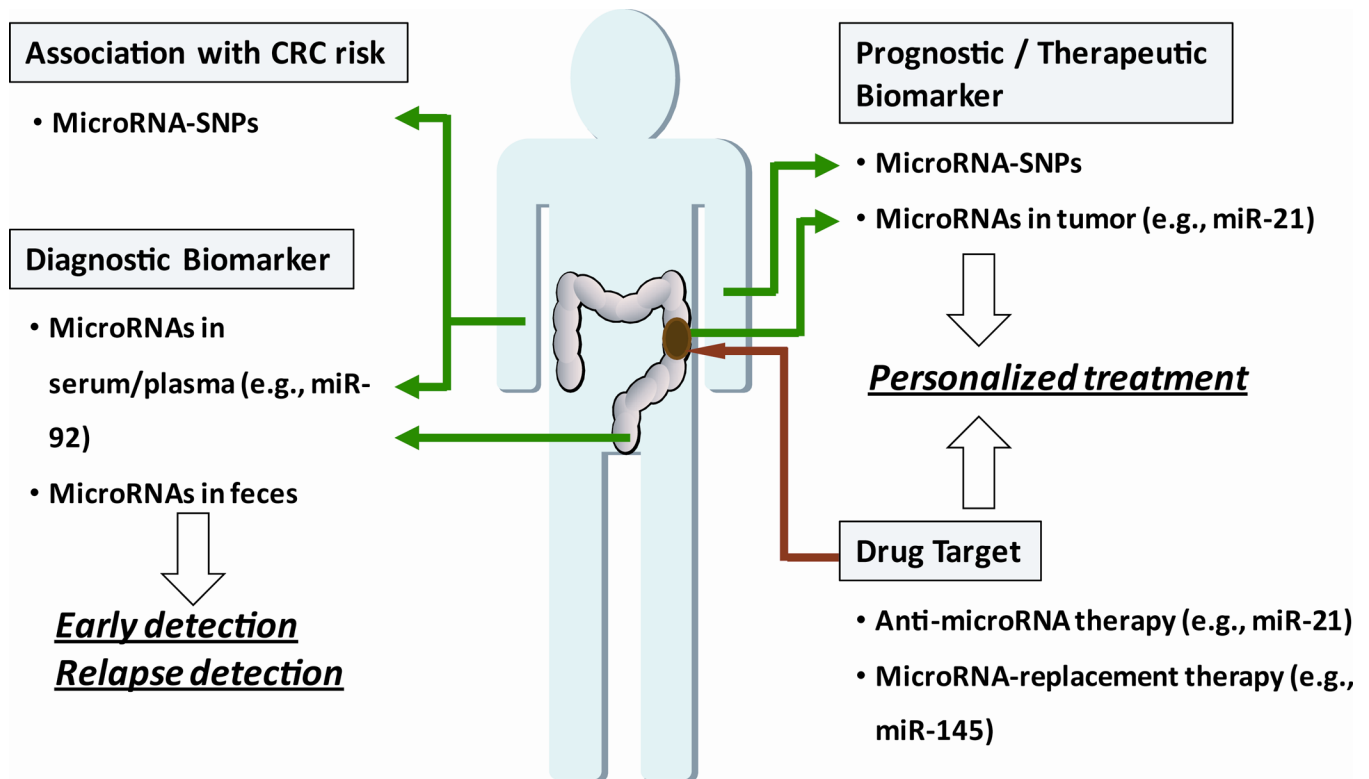
Figure 1.
Potential strategies for microRNA-based diagnostics and therapeutics for the detection and management of colon cancer. MicroRNA-related DNA polymorphisms may modify cancer risk. MicroRNAs detected in blood or feces may aid in the early detection of colon cancer. MicroRNA expression levels in tumors may help guide therapeutic decisions and have utility as a therapeutic target through microRNA inhibition or replacement strategies.
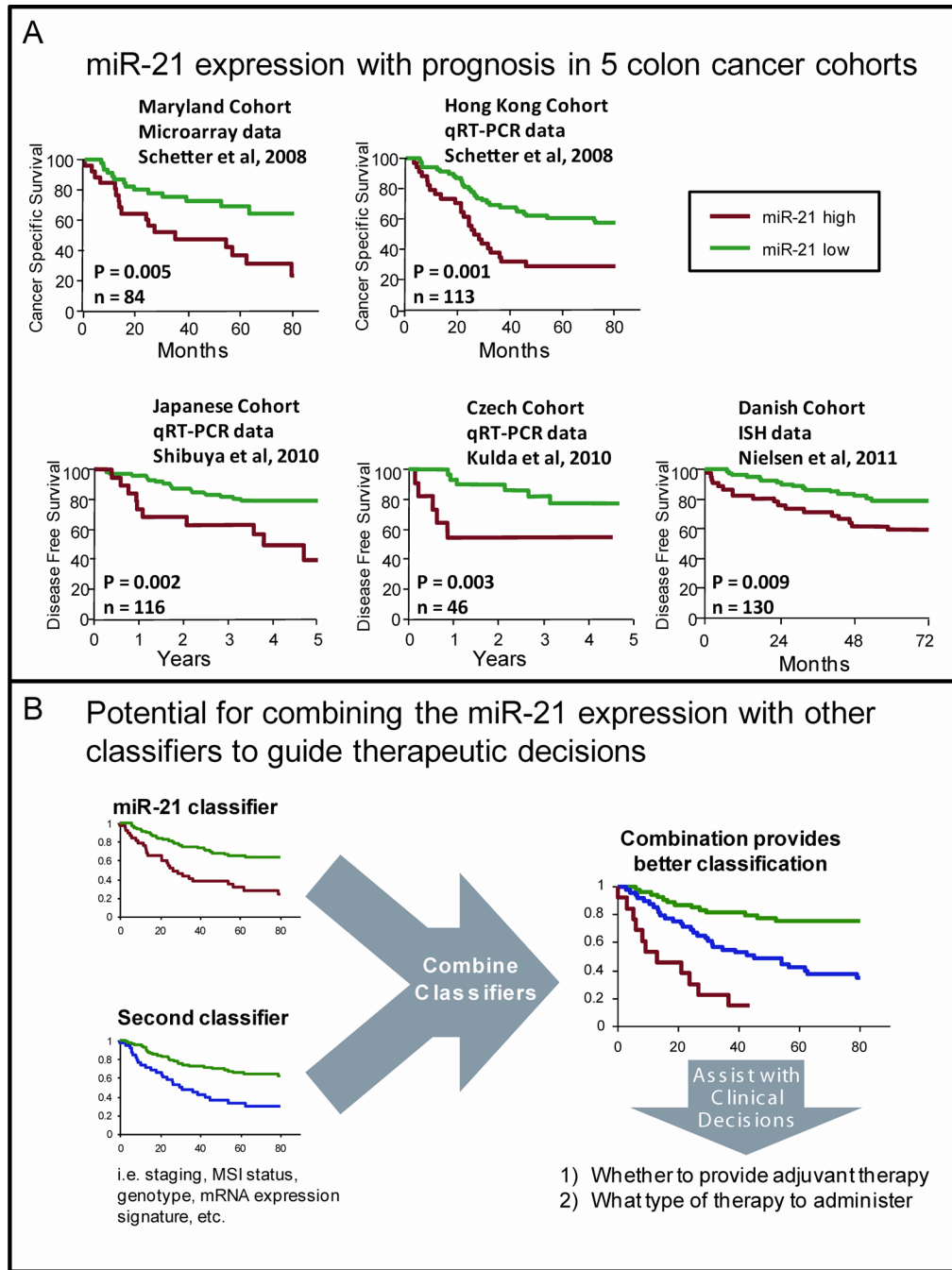
Figure 2.
MicroRNA-based prognostic and predictive classifiers for colon cancer. (A) High expression of *miR-21* is consistently associated with worse prognosis and therapeutic outcome in five colon cancer cohorts from four studies, suggesting miR-21 may have use as a prognostic or predictive classifier. (B) The combination of multiple, validated prognostic classifiers may provide improved risk stratification to help guide therapeutic decisions. ISH = in situ hybridization; qRT-PCR = quantitative reverse transcription polymerase chain reaction.

Table 1

MicroRNAs with roles in colon cancer and their targets

microRNA	Tumor Suppressor or Oncogene	Examples of experimentally validated microRNA Targets
let-7	Tumor Suppressor	*KRAS*
miR-17-92 cluster	Oncogene	*E2F1*
miR-21	Oncogene	*PDCD4, PTEN, RECK, NFIB, TPM1, SPRY2, RHOB, TIMP3, maspin, CDC25a, TIAM1, MSH2*
miR-29	Tumor Suppressor	*MMP2, DNMT3A/B*
miR-30a	Tumor Suppressor	*DTL*
miR-34a	Tumor Suppressor	*FRA1, SIRT1, MYC, BCL2*
miR-95	Oncogene	*SNX1*
miR-101	Tumor Suppressor	*COX2*
miR-135a/b	Oncogene	*APC*
miR-137	Tumor Suppressor	*CDC42*
miR-143	Tumor Suppressor	*KRAS, DNMT3A, ERK5*
miR-145	Tumor Suppressor	*IRS-1, c-Myc, YES1, STAT1, OCT4, SOX2, KLF4,FLI1*
miR-155	Oncogene	*MLH1, MSH2, MSH6*
miR-200c		*ZEB1, ZEB2*
miR-342	Tumor Suppressor	*DNMT1*
miR-365	Tumor Suppressor	*CCND1, BCL-2*
miR-451	Tumor Suppressor	*MIF*
miR-499	Oncogene	*FOXO4, PDCD4*
miR-675	Tumor Suppressor	*RB*