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MicroRNA and Colorectal Cancer

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Abstract MicroRNAs are small 19 to 22 nucleotide sequences of RNA that participate in the regulation of cell differentiation, cell cycle progression, and apoptosis. MicroRNAs act much like small interfering RNA, annealing with RISC, to cleave messenger RNA, and microRNAs exert translational inhibition that is incompletely understood. They are important factors in tumorigenesis and have been the subject of research in many types of cancers, including colon cancer. MicroRNAs may be abnormally down-regulated or up-regulated in colon-cancer tissue. Artificial dysregulation of certain microRNAs will trigger tumorigenesis or apoptosis depending on which microRNA is manipulated. Although the natural mechanisms for the dysregulation of microRNAs is still largely unknown, one theory tested in colon cancers proposes that DNA hypermethylation leads to down-regulation of certain microRNAs. Specific microRNA expression patterns help characterize specific cancers and may be used as a prognostication factor and in following patient response to 5-fluorouracil chemotherapy. This article reviews the existing literature pertaining to the study of microRNA in colorectal cancer.

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Introduction

MicroRNAs are small 19 to 22 nucleotide sequences of RNA found in both prokaryotes and eukaryotes that are intimately involved in cell differentiation, cell cycle progression, and apoptosis. MicroRNAs have been demonstrated recently to potentially play a significant role in tumorigenesis. Additionally, miRNAs may be useful tools for characterizing specific cancers and for determining patient prognosis and response to therapy. The study of microRNA has been extended into many types of cancer, including leukemias, lung, breast, and colon cancer.

The first description of microRNA appeared in 1993 by Lee et al., who proved that *lin-4* is involved in controlling the temporal progression of cell differentiation in *C. elegans* [1]. In 2000, another microRNA, *miR-7*, was found to share the control of developmental progression of nematodes [2]. Discoveries of other microRNAs that regulate apoptosis, proliferation, and differentiation in *Drosophila*, mice, and humans soon followed [3, 4]. Calin et al. [5] published the first study to link microRNAs to cancer in 2002. These authors demonstrated that *miR-15* and *miR-16* are located on chromosome 13 in a position where deletion of a putative tumor suppressor, known to be associated with greater than half of chronic lymphocytic leukemia cases, was identified [5]. Researchers have proposed that specific microRNA expression patterns could help identify human solid tumors, suggest patient prognosis, and even represent a novel molecular target for cancer treatment. In this review, we discuss the literature surrounding microRNAs and cancer, and with a specific focus on microRNAs in colorectal cancer. We also highlight the potential role of microRNAs in the regulation of GATA 6.

MicroRNA biochemistry

There are three steps in the maturation of microRNA: transcription of pri-miRNA, cleavage in the nucleus to form pre-miRNA, and a final cleavage in the cytoplasm to form mature microRNA [6–9] (reviewed in [8–11], Fig. 1). Pri-miRNA is synthesized from DNA by RNA polymerase II and may be up to 1 kb in length, forming hairpin loops. Pri-miRNAs are found as independent transcripts or within the introns of another gene. For example, *lin-4* sits within a host gene, is flanked by conventional splice donor and acceptor sites, and is transcribed as an intron [1]. Lagos-Quintana et al. described the genomic organization of *let-7* miRNAs as being gene clusters found on chromosomes 9 and 17 [10]. After the pri-miRNA is transcribed, it is cleaved by the RNase Drosha on the non-loop end to form 60–70 bp length precursor microRNA (pre-miRNA) [11–13]. Pre-microRNA then moves from the nucleus into the cytoplasm via a transporter on the nuclear membrane. The nature of this transporter was suspected but remained unknown until 2003, when two independent teams published their finding that the transporter is RanGTP-dependent Exportin 5 [14, 15].

In the cytoplasm, pre-miRNA is cleaved on the loop end by Dicer to form a miRNA-microRNA duplex that is unwound by a helicase to release two mature microRNAs,

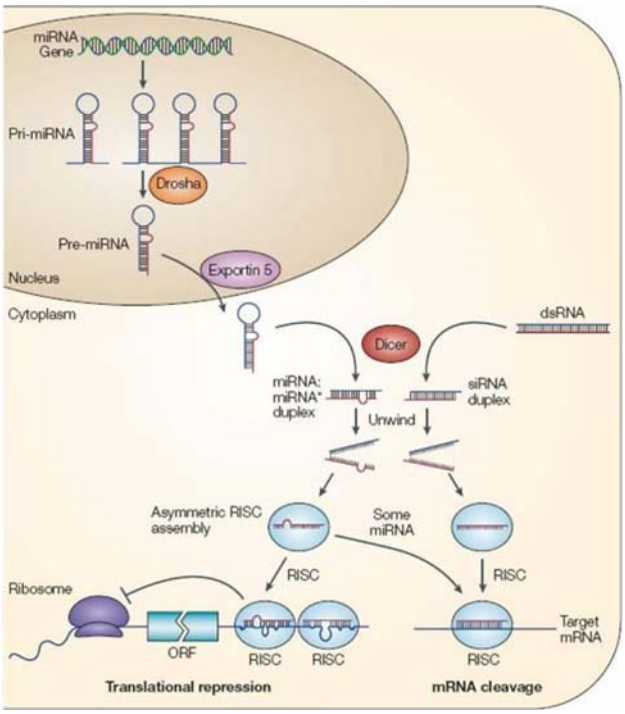

Fig. 1 microRNA biogenesis and mechanisms of action. Reprinted with permission from MacMillan (He L, Hannon GJ (2004) MicroRNAs: small RNSs with a big role in gene regulation. Nat Rev Genet 5:522–531)

of which one or both may be active [16]. The mature microRNAs inhibit protein expression in two ways. First, mature microRNA act through the RNA-induced silencing complex (RISC) to target [17] and cleave mRNA. RISC, already well known in association with siRNA, complexes with Argonaute 2 proteins Gemin 2 and Gemin 3 when it is charged by microRNA [18, 19]. Both microRNA and siRNA are small RNAs associated with RISC, but they differ in that siRNA matches exactly to its target mRNA and leads to cleavage of the mRNA. MicroRNAs can cleave their target mRNA without perfect complementarity; however, introduction of a synthesized microRNA with perfect complementarity can mediate cleavage of its target mRNA, in a nearly identical action to that of siRNA [20].

The second way that microRNAs act is through translational inhibition. Translational inhibition occurs when the microRNA does not perfectly align with its mRNA target. In fact, siRNAs altered to imperfectly complement targets also show translational inhibition, suggesting that the function of microRNAs and siRNAs depend greatly on the degree of sequence complementation [21]. The microRNA *lin-4* shows complementary pairing to the 3'UTR of mRNAs *lin-14 and lin-28* [1]. In 1999, Olsen and Ambros [22] demonstrated that microRNAs inhibit protein translation by isolating the *lin-4* microRNA from cytoplasmic ribosomal complexes, along with *lin-14* mRNA. This study also demonstrated that the *lin-4* mechanism of action was not mediated by cleavage or destabilization of the mRNA, because the level of *lin-14* remained steady and the poly-A tails were not shortened. Subsequently, Kim et al. [23] reported analogous results in mammalian neurons. These authors demonstrated that all microRNAs that were known to be temporally regulated localized to polyribosomes. A number of researchers also describe cytoplasmic processing bodies, or P bodies, where mRNAs localize in a microRNA dependent fashion [24]. In yeast, these P bodies contain decapping enzymes and mRNA degradation intermediates [25]. In humans, a decapping enzyme (hDcp1/2), an exonuclease (hXrnl1), and a mRNA degradation protein (LSm1-7) are found in similar cytoplasmic foci [26, 27]. In summary, it seems that microRNAs control the expression of mRNA by regulating the localization of mRNA to P bodies where the mRNAs are uncapped and degraded. Furthermore, microRNAs interfere with protein translation in polyribosomes via an unknown mechanism.

Methods of screening microRNA

In 2006, Cummins et al. [28] developed a new method of identifying microRNA. In the process they realized that there are many more microRNAs than previously thought. This new approach, called microRNA serial analysis of

gene expression (miRAGE) involved the cloning of 18–22 nucleotide RNA molecules, reverse-transcription into cDNA, and sequence identification by the SAGE. The miRAGE technique was first performed on colorectal cancer, and it generated 273,966 cDNA tags. At that time, there were only 24 human microRNAs known, but miRAGE identified 100 novel microRNAs and confirmed the existence of 62 predicted microRNAs. At this point, we recognize that there may be as many as 1,000 human microRNAs and that each microRNA may have hundreds of potential mRNA targets.

Computerized screening modalities are essential tools for predicting microRNA targets. Current tools use four strategies and combine the modalities to increase the rate of true positives. First, computer analysis compares base-pair complementation of the 3' UTR recognition sites in potential target genes. This method results in a notoriously high false-positive rate, and because animal microRNAs act by imperfect base-pairing, probably predicts many false-negatives as well. Exacting rules have been developed to augment this prediction modality. Second, true-positive rates improve if programs stipulate that the putative target genes must be conserved in other animals. The TargetScanS prediction program uses the above-mentioned two methods in combination. Third, the DIANA-micro T program predicts the micro-RNA/mRNA complex and analyzes the number of bulges in the proposed construct. Finally, prediction models can use binding energy to predict microRNA targets. Programs, such as miRanda and PicTar, calculate the lowest energy alignment and free energy of binding, respectively, and combine this with base-pair complementation and conservation. Many of these screening modalities have been used throughout the colorectal microRNA literature to narrow in on mRNA target [29].

Recently, a method of screening microRNA expression using functional genomics was developed and tested in colon cancer cell lines (DLD-1 and HCT116) and breast cancer. Schlabach et al. created microarrays of barcoded short hairpin RNA (shRNA) that targeted the whole human genome, and they used the microarray to probe for microRNA expression down-regulation in tumor cells. This tool can screen the entire human genome rapidly. This technique will allow for the establishment of microRNA expression signatures for many cancers [30].

MicroRNA and cancer

Soon after the discovery that microRNA deletions were associated with CLL, microRNA abnormalities were reported in pediatric Burkitt lymphoma, lung carcinoma, and large-cell lymphoma [31]. For example, *miR-155,* located in the *BIC* gene, is highly expressed in many types of B cell lymphomas, including diffuse large B-cell lymphoma and pediatric Burkitt lymphoma [32, 33]. Takamizawa et al. [34] reported decreased expression of *let-7* microRNA in non-small–cell lung cancers, ranging from adenocarcinomas, to squamous carcinomas, to adenosquamous carcinomas, to large cell carcinomas of the lung. These authors also found that depressed *let-7* expression is correlated with poorer patient prognosis. In 2003, Michael et al. [35] published the first study of microRNA in colon cancer, identifying *miR-143* and *miR-145* as novel dysregulated microRNAs in colon cancer. Subsequent authors identified additional microRNAs by using new screening tools, and there are currently over 100 microRNAs implicated in colorectal cancer alone (Table 1). Recent studies have correlated specific microRNA expression patterns that are characteristic of certain cancers [36].

Specific candidates in colorectal cancer

Michael et al. [35] identified *miR-143* and *miR-145* as potential factors in colon tumorigenesis. These researchers isolated total RNA from matched normal and colon adenocarcinoma and cloned the 18–22 nucleotide RNA fragments. They identified the clones by comparing sequences against microRNA databases. They found that *miR-143* and *miR-145*, located close together on chromosome 5, are expressed at reduced levels in colon cancer epithelial cells. However, because tumor cells maintain normal levels of the microRNAs' precursor, it seemed that the microRNAs are unstable at the posttranscriptional level. Moreover, transfection of precursor *miR-143* and *miR-145* into cells expressing low levels of these microRNAs leads to inhibition of cell growth. This convincingly illustrated a tumor suppressor-like activity of the microRNAs.

The targets of *miR-143* and *miR-145* remain speculative, but there are several candidates. Transfection of precursor *miR-143* into deficient cells leads to decreased ERK 5 protein and normal *ERK5* mRNA levels, suggesting that *miR-143* inhibits *ERK 5* posttranscriptionally in a way that does not involve mRNA degradation [37]. Using four microRNA target prediction methods—miRanda, TargetScan, miRBase, miRNAMap method—Shi et al. [38] predicted that miR-145 targets insulin receptor substrate-1 (IRS-1), a known mitogen, at a sequence specific binding site in the 3' UTR of the *IRS-1* mRNA. They verified the prediction by three methods: they showed that IRS-1 is down-regulated by *miR-145*; they created a luciferase reporter hybrid to the putative 3' UTR target sequence of *miR-145* and showed that *miR-145* transfection decreases

Table 1 Expression and proposed targets of microRNA in colorectal cancer

Up-regulated microRNA	Proposed targets	Down-regulated microRNA	Proposed targets
let-7b		let-7a	
let-7 g	TGFR2	miR-10a	
miR-9	TCF4, MSH2	miR-15b	
miR-21		miR-23a	
miR-26a		miR-25	
miR-30a-3p		miR-27a	
miR-30a-5p		miR-27b	
miR-31		miR-30c	
miR-96	K-Ras	miR-107	
miR-124b	MLH1	miR-124a	CDK6, Rb
miR-132		miR0125a	
miR-135a	MSH2	miR-125b	VEGF, IGFR1, VEGFR
miR-135b		miR-127	
miR-141	APC, MSH2	miR-130a	TGFR2
miR-142-3p	APC	miR-133a	BAX, K-Ras
miR-142-5p		miR-133b	K-Ras
miR-181a		miR-134	
miR-181b		miR-137	TGF2I
miR-182	IGFR1	miR-143	
miR-183		miR-145	TGFR2, APC
miR-194		miR-147	
miR-200a	MSH2	miR-154	MLH1
miR-200b	MLH1	miR-191	
miR-200c	MLH1, SMAD2	miR-199a	
miR-203		miR-199b	
miR-205	K-Ras, SMAD4, MSH2, PTEN	miR-214	TP53, B-catenin, TGFR2, BAX, CDKN2b, EGFR
miR-215		miR-296	
miR-219	TGFR2	miR-299	B-catenin, CDKN1a
miR-320		miR-337	CDKN2a
miR-338		miR-339	
miR-372	TGFR2, SMAD2, MLH1, AKT1	miR-342	
		miR-368	
		miR-370	BAX, AKT1
		miR-582	

reporter activity; and they showed that *miR-145* leads to cell growth inhibition. Also, like *miR-143,* the target of *miR-145* seems to be at the translational or posttranscriptional level, because *IRS-1* mRNA levels are normal. These studies suggest that *miR-143 and miR-145* exert a tumor suppressor-like effect by targeting tumorigenic elements.

The *let-7* microRNA family is comprised of 14 isomers, many of which are dysregulated in colorectal cancer (Table 1). Akao et al. [39] reported down-regulation of *let-7a-1* in colon cancer tumors and in DLD-1 (colon cancer) cell lines. An independent study in China corroborated this finding in a small number of patients with colorectal cancer [40]. In DLD-1 cells that express low levels of *let-7a-1*, transfection with *let-7a-1* precursor causes reduced cell growth and decreased RAS and c-myc protein expression. This inhibitory effect resembles *miR-143* and *miR-145* inhibition in that the mRNA levels of both proteins remain at normal levels. *Let-7a* inhibition of RAS, and c-myc protein expression also has been reported in lung cancer and Burkitt lymphoma [41, 42]. The specific function and targets of many of the other *let-7* isomers remains unknown.

miR-34a is another microRNA that inhibits cell proliferation. Tazawa et al. [43] demonstrated its cell-cycle

activity by introducing *miR-34a* into HCT116 and RKO colon cancer cells and finding that the cells entered into a senescence-like state. The results of gene expression array and immunoblot analysis show that *miR-34a* down-regulates transcription factor E2F and up-regulates p53.

Whereas most of the microRNAs discussed above are down-regulated, microRNAs also may be up-regulated in colorectal tumors. Bandres et al. [44] demonstrate, by using RT PCR and microarray analysis, elevated levels of *miR-31, miR-96, miR-31, miR-135b*, and *miR-183* in colorectal tumor cells. The authors found that the cells exhibiting microRNA up-regulation also harbored oncogenic mutations of either KRAS or BRAF. Xi et al. [45] studied colon cancer cell lines that were knocked out for p53, and found both overexpressed and underexpressed microRNAs. Some of the microRNA that were overexpressed in p53-null cells are: *miR-181b* (+11.87-fold change), *miR-7 g* (+4.93-fold change), *miR-21*(+1.89-fold change), and *miR-181a* (+1.34-fold change). Many of these microRNAs are potential targets for the p53 tumor suppressor and have p53 binding sites in their promoter sequences.

MicroRNA genomics in colorectal cancer

From the studies reviewed above, microRNA up-regulation or down-regulation may play a role in colorectal cancer development, but the question of how the microRNA becomes up- or down-regulated in cancer cells is still unclear. MicroRNA over-expression is not related to the number of copies in the genome as is found in mRNA expression. Lamy et al. [46] studied the overexpressed micro-RNA in colon cancer cells and compared these microRNA to areas of the genome known to harbor duplicate expansions of genes or gain of function expression. They found no significant association between overexpressed micro-RNA and these unusual regions of the genome.

Many microRNA abnormalities are associated with p53 dysregulation, an important tumor suppressor whose activity is lost during colorectal tumorigenesis. Xi et al. [45] identified numerous potential p53 binding sites in microRNA promoters. They also demonstrated a difference in the level of actively translated mRNAs in p53 knocked out cells (HCT116) compared with p53 wild-type cells. These authors explored the effect of p53 on microRNAs by knocking out p53 and demonstrating increased levels of 11 microRNAs and decreased levels of 43 microRNAs. This data suggest that loss of normal p53 regulation may be an important mechanism of microRNA dysregulation.

Decreased expression of microRNA in colorectal cancer may be due to epigenetic mechanisms, such as hypermethylation. Hypermethylation of target microRNA can occur when the microRNA gene is located near a CpG island. Published studies demonstrate methylation control of four microRNAs: *miR-124a, let-7a-3, miR-10a,* and *miR-342* [41, 47–49]. Lujambio et al. [47] showed that the *miR-124a* gene is located in a CpG island and is methylated in colon cancer tissues but unmethylated in matched normal colon tissue. This CpG island is hypermethylated in breast, lung, and leukemic cells. Double knockout of DNA methyltransferases in HCT116 cells causes the cells to overexpress *miR-124a*. *miR-124a* was predicted to target CDK6 and Rb translation. Thus, in tumor cells, hypermethylation signals down-regulation of *miR-124a*, releasing the inhibition on CDK6 translation, and leading to tumor formation. Similarly, *let-7-3*, which often is down-regulated in colon cancer cells, also is regulated by methylation at a CpG island. *let-7-3* targets RAS, a known human oncogene. Hypermethylation of the *miR-10a* gene leads to down-regulation of the microRNA, which is predicted to target HOXA3 and HOXD10 [48]. The *miR-342* gene is somewhat unusual because it is located in an intron of the EVL gene. The EVL gene is regulated by methylation at an upstream CpG island. By a similar mechanism, this island is methylated in colorectal adenocarcinoma, leading to down-regulation of *miR-342*. Interestingly, the CpG island is hypermethylated in the normal tissue of patients with history of colorectal cancer, but not in patients without a history of colorectal cancer. The authors suggest that there is a "field defect" in the total mucosa of patients with a history of colon cancer that is present in both tumor and apparently normal epithelium, but they do not specifically propose the possibility of an inherited mechanism. Transfection of *miR-342* into a colon cancer cell line leads to apoptosis. Thus, *miR-342* has a tumor-suppressor-like effect that is inhibited by methylation [49].

MicroRNA posttranscriptional inhibition is dependent on sequence complementation to the 3'UTR of mRNA. Mutations in the 3'UTR of mRNA may interfere with microRNA inhibition, and if these mutations occur in oncogenes, they may nullify an important mechanism of inhibition in tumor cells. Landi et al. [50] studied eight single nucleotide polymorphisms (SNP) in the 3'UTR of *CD86* and *INSR* and found that polymorphisms in these genes are correlated with increased risk for colorectal cancer. CD86 is a proinflammatory protein and is the putative target of five microRNAs: *miR-337, miR-582, miR-200a, miR-184*, and *miR-122*. Certainly, it is suggestive that polymorphisms in the *CD86* mRNA 3'UTR releases it from microRNA mediated regulation, resulting in overexpression of a proinflammatory protein, and leading to tumorigenesis. Interestingly, *miR-582* is down-regulated in colon cancer tissues, but it is uncertain whether there is a link between the SNP in the target mRNA and down-regulation of the microRNA.

MicroRNA expression and microenvironmental conditions

There is a paucity of data concerning microenvironment factors that trigger dysregulation of microRNAs. There is great potential for studies in local inflammation, hypoxia, dietary elements, obesity, *et cetera,* and the effect of such on microRNA expression. Currently, there is one study on the effect of hypoxia on microRNA expression in colon cancer tissues [51]. Hypoxia is a characteristic feature of tumors and is associated with greater invasion and poorer response to treatment [52]. The hypoxic cells express anti-apoptotic characteristics and cell-cycle arrest. A low oxygen environment causes the release of hypoxia induced factors (HIF), which induces expression of certain microRNAs via HIF-binding sites on the microRNA genes. These hypoxia-regulated microRNAs (HRM) seem to inhibit cell death or cell-cycle progression. The anti-apoptosis HRM inhibit caspases and other components of apoptosis: *miR-26* inhibits BAK1, *miR-181* inhibits BIM, *miR-23* inhibits caspase 7. HRM that inhibit cell-cycle progression affect cyclins, such as *miR-26* and cyclin D2/cyclin E1/cdk6, *miR-23* and cyclin H, and *miR-21* and cdc25A.

MicroRNA and colorectal cancer prognosis

Accumulating evidence shows that microRNA expression patterns are unique to certain cancers and may be used clinically as prognostic factors. There are many advantages to using microRNAs as biomarkers: (1) microRNAs are involved in the tumorigenesis process, (2) certain microRNAs show specific up- or down-regulation response to chemotherapy, and (3) certain microRNA patterns may be related to patients' survival. The role of microRNAs as prognostic biomarkers in CLL, lung cancer, pancreatic cancer, neuroblastoma, and colon cancer has been explored [53, 54]. Xi et al. [55] performed Kaplan-Meier analysis for patients with UICC stages 1 through 4 adenocarcinoma of the colon and rectum and found that tumors expressing high levels of *miR-200c* are correlated with poorer prognosis, regardless of tumor stage: approximately 12 months decreased survival compared with patients whose tumor expresses low levels of *miR-200c*. These investigators also found that p53 mutation, commonly found in colorectal cancers, is strongly associated with greater than twofold *miR-200c* overexpression. *miR-21* is overexpressed in many solid tumors, including colon adenocarcinoma and adenomas. Targets for *miR-21* include human tumor suppressor phosphatase and tensin homolog (*PTEN*) and Pdcd4, a tumor suppressor that inhibits transformation and tumor invasion [56]. Schetter et al. [57] showed that overexpression of *miR-21* is associated with worse prognosis and poorer response to chemotherapeutics in colorectal cancer. Asangani et al. [56] studied the inhibition of Pdcd4 by *miR-21* and found that overexpression of *miR-21* causes tumor cells to invade, intravasate, and metastasize more aggressively when implanted into mouse models. Slaby et al. [58] showed that patients overexpressing *miR-21* are more likely to have lymph node and distant metastasis. Low expression levels of certain microRNA also are associated with certain cancer characteristics. Revisiting *miR-143* and *miR-145*, Slaby et al. reported that low expression of these two miRNAs is associated with tumors larger than 55 mm [58].

MicroRNAs also can help distinguish microsatellite unstable colon cancer from microsatellite stable colon cancers. The identification of cancer type is a powerful tool because microsatellite unstable tumors have better prognosis, are more often mucinous or medullary colon cancers, and tend to be respond more poorly to chemotherapy drugs [59]. The use of 14 microRNAs correctly predicted 36 of 39 tumors compared with the use of 451 mRNA that correctly predicted 39 of 39 tumors. It may be possible to use a combination of microRNA/mRNA expression signatures to identify microsatellite instability in colon cancers and thus guide clinical decisions.

MicroRNA and chemotherapy

Chemotherapeutic drugs are a major form of treatment of colorectal cancer. Thus far, the literature in colorectal cancer is limited to a handful of studies on 5-fluorouracil (5-FU) and a phytochemical, alpha-Mangostin. 5-FU is an antimetabolite that is commonly used, alone or in combination regimens, against colorectal cancers. A study published by Rossi et al. [60] reported a suggestive pattern of microRNA rearrangement in HT-29 and HCT-116 human colon cancer cell lines after exposure to 5-FU. 5-FU leads to down-regulation of *miR-200*, which is a microRNA known to inhibit a tumor-suppressor gene, protein tyrosine phosphatase, non-receptor type 12 (PTPN12) [61]. PTPN12 is a phosphatase that inactivates well-established oncogenic agents, such as Ras, Src, and c-Abl. 5-FU treatment induces up-regulation of *miR-133a,* which is thought to inhibit the proto-oncogene *K-Ras.* Strangely, treatment with 5-FU also causes up-regulation of microRNA known to be mitogenic. To this, Rossi suggests that the cytotoxic effect of 5-FU induces cells to express anti-apoptotic factors, of which are these abnormally up-regulated—and tumorigenic—microRNAs. Besides these, 5-FU treatment leads to significant (twofold or greater) elevated expression of many other microRNAs, and it remains to be seen what genes these microRNAs target.

5-FU induces p53 protein expression at a posttranscriptional level without correspondingly elevated mRNA level in a pattern that has become a hallmark for microRNA involvement. What is so interesting about the p53 protein is that it may have three mechanisms of action: (1) its familiar transcription factor role that probably includes microRNA transcription, (2) as a pro-apoptotic signal in the mitochondria, and (3) as an RNA binding factor regulating translation, very likely in association with microRNAs. When wild-type HCT-116 cells are treated with 5-FU, they express high levels of certain microRNAs, and a great majority of these affected microRNA have a binding site for p53 in the gene. When HCT-116 cells knocked out for p53 are treated with 5-FU, these microRNAs are not up-regulated. These results suggest that 5-FU acts as a switch to turn on p53 and, through p53, a cascade of microRNAs that may act with or independently of p53.

Alpha-mangostin is a chemotherapeutic agent from the mangosteen plant with cytotoxic effect. It is not yet used for colon cancer, but it has been applied in treatment of skin infections and wounds in Southeast Asian traditional medicine. Early investigations into α-mangostin activity in leukemic cells showed that the drug causes apoptosis through caspase-3 pathway, but the same researchers found that α-mangostin does not act through the caspase in colon cancer cells [62]. Rather, apoptosis occurs in colon cancer cells through Akt and Erk1/2 signaling. Exposure to α-mangostin also leads to significantly increased expression of *miR-143* and decreased expression of Erk5, a cell growth signaling protein. As reviewed, *miR-143* inhibits the translation of *ERK5* mRNA, and, in a characteristic pattern of microRNA action, exposure to α-mangostin induces low protein expression but normal *ERK5* mRNA level. When applied with 5-FU to DLD-1 colon cancer cell line, α-mangostin sensitizes cells so that a lower level of the antimetabolite is required to induce apoptosis. This may be due to overlapping and additive alteration of microRNA expression or an unclear mechanism; however, it is evident that microRNAs play a key role in action of the chemotherapeutic drugs used in clinical treatment of colorectal cancers. More studies into the effect of other chemotherapy are needed.

GATA 6 is a potential target of microRNA

The GATA 6 protein is a zinc finger transcription factor that is expressed during the development of the cardiovascular and gastrointestinal systems. In cardiogenesis, GATA 6 is involved in the development of the septum and the valves through downstream targets, such as BMP4 and periostin. Niu et al. [63] showed that *miR-1-2* inhibits the expression of GATA 6 in developing myocytes and that *miR-1-2* knockout results in fatal ventricular septal defects, arrhythmias, myocyte hyperplasia. In gut epithelium, GATA 6 expression is localized at the base of intestinal crypts where cell proliferation occurs and decreases at the tips of the villi [64]. Interestingly, there is increased

Table 2 MicroRNAs that target GATA 6

	Proposed microRNA	
Colon	let-7a	miR-10a
	let-7g	miR-181b
		miR-203
Other tissues	let-7b	miR-339
	let-7c	miR-340
	let-7d	miR-345
	let-7f	miR-361
	let-7i	miR-363
	miR-10b	miR-367
	miR-19a	miR-372
	miR019b	miR-374a
	miR-23a	miR-377
	miR-25	miR-429
	miR-27a	miR-431
	miR-27b	miR-450b
	miR-28	miR-452
	miR-32	miR-488
	miR-92a	miR-491
	miR-92b	miR-496
	miR-93	miR-499
	miR-98	miR-502
	miR-105	miR-507
	miR-124	miR-508
	miR-125a	miR-512
	miR-128a	miR-513
	miR-128b	miR-519c
	miR-129	miR-520a
	miR-141	miR-520b
	miR-146b	miR-520c
	miR-155	miR-520d
	miR-181d	miR-525
	miR-185	miR-526b
	miR-188	miR-539
	miR-196a	miR-548
	miR-196b	miR-548a
	miR-200a	miR-548b
	miR-200b	miR-548c
	miR-297	miR-548d
	miR-302a	miR-568
	miR-302b	miR-590
	miR-302d	miR-708
	miR-338	miR-871

expression of GATA 6 in premalignant intestinal adenomas, ranging from low-grade to high-grade dysplasia [65]. Additionally, GATA 6 expression is increased in colorectal cancers and at metastatic sites. We have found a number of microRNAs that potentially target GATA 6 (Table 2). Of these, *let-7a, miR-10a*, *miR-181b,* and *miR-203* have been found in colon cancers, and some of these microRNAs have been cited in numerous colorectal studies. It will be interesting to see whether there is a stronger connection between these microRNAs and GATA 6.

Conclusions and future perspective

The identification of mirocRNAs and their role adds another layer to the complexity of control of protein expression. Recent data suggests that dysregulation of microRNAs to be an important step in the development of many cancers, including colorectal cancer. We believe that microRNA dysregulation may be an important mechanism for increased expression of a host of genes not affected during tumorigenesis directly by genetic mutation or transcriptional regulation. Continued investigation into the role of microRNAs in colorectal cancer is likely to yield important information on new potential targets for therapy, diagnosis, and prognosis.

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