

Reciprocal regulation between microRNAs and epigenetic machinery in colorectal cancer (Review)

FENG WANG¹, YANLEI MA¹, HUAMIN WANG² and HUANLONG QIN¹

¹Department of Surgery, Shanghai Tenth People's Hospital Affiliated to Tongji University, Shanghai 200072, P.R. China;

²Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

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Abstract. Epigenetics encompasses changes in DNA methylation, histone and chromatin structure, and non-coding RNAs, specifically microRNA (miRNA) expression. Recent advances in the rapidly evolving field of colorectal cancer (CRC) epigenetics have revealed a complicated network of reciprocal interconnections between miRNAs and other epigenetic machinery. On the one hand, miRNA expression may be regulated by epigenetic mechanisms including DNA methylation and histone modifications. However, miRNAs may affect the epigenetic machinery by directly targeting its enzymatic components. In this study, we focus on the colorectal miRNA expression profile and further illustrate the reciprocal regulation in CRC, with the aim of offering new insights into the strategies of combatting the disease.

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1. Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide, ranking as the third most prevalent and fourth most frequent cause of cancer-related mortality (1). In the early era of modern molecular biology research, CRC was considered to be a disease caused by genetic alterations, which influenced the gene product by changing the amino acid sequence of a protein. With advances in cancer research, however, more studies have focused on the alterations in the regulation of gene expression that are independent from the DNA sequence of the cell and are referred to as 'epigenetic' changes. Epigenetic changes are likely to be a major feature of cancer, and the most prominent epigenetic modifications involve the aberrant methylation of CpG islands (CGIs) and deacetylation or methylation of histone proteins (2).

MicroRNAs (miRNAs or miRs) are non-coding, endogenous small RNAs that bind the 3' untranslated regions (UTRs) of their target messenger RNA (mRNA) transcripts to suppress protein translation or cause mRNA degradation (3). These newly identified small molecules have been classified as epigenetic since the regulation of mRNAs does not directly involve changing the DNA sequence. In the last decades, emerging evidence has indicated that miRNAs are deregulated in CRC and are directly connected with the epigenetic machinery, playing a significant role in the occurrence and progression of CRC.

In this review, we summarise recent findings on miRNAs in CRC, in particular the reciprocal regulation between miRNAs and other epigenetic machinery, to better understand the epigenetic mechanisms that contribute to CRC carcinogenesis and to identify new avenues for the diagnosis and treatment of this disease.

2. Deregulation of miRNAs in CRC

miRNAs are short 18-24 nucleotide single-stranded RNAs that mediate post-transcriptional gene repression (4). The miRNA family is comprised of gene miRNA clusters, which have high homogeneity. In animals, primary miRNA genes are transcribed by RNA polymerase II and are subsequently processed to precursor hairpin miRNAs (pre-miRNAs) that are ~70 nt long by Drosha in the nucleus. The pre-miRNAs are then transported to the cytoplasm, where they are recognised

Correspondence to: Dr Feng Wang or Professor Huanlong Qin, Department of Surgery, Shanghai Tenth People's Hospital Affiliated to Tongji University, 301 Middle Yanchang Road, Shanghai 200072, P.R. China

E-mail: wf03@live.com

E-mail: hlong_qin@live.com

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and processed by the RNase III enzyme Dicer into mature miRNAs of ~22 nt. The mature miRNAs induce the degradation of mRNAs and downregulate the target genes through the imperfect binding of the RNA-induced silencing complex to the 3' UTR of target mRNAs (5,6).

The first miRNA was reported in 1993, when the *Caenorhabditis elegans* heterochronic gene *lin-4* was identified as encoding small RNAs with antisense complementarity to *lin-14* (7). At the time of this review, 1,881 precursors and 2,588 mature human miRNAs have been annotated in the latest version of the miRBase (available at www.mirbase.org) (8). On the one hand, miRNAs are highly evolutionarily conserved across different species; on the other hand, the expression pattern of specific miRNAs may be correlated using a particular type of tissue. Compared with corresponding normal tissues, miRNA expression profiles in tumours have indicated widespread changes during tumorigenesis and appear to be related to the developmental stage of cancers as well as being associated with other clinical features (9-12).

With the application of high-throughput screening technology, including microarray-based miRNA profiling platforms and next-generation sequencing (NGS) approaches, more studies have focused on searching for biomarkers by identifying different miRNA expressions in different types of cancer. To date, a number of aberrantly expressed miRNAs and their gene targets have been identified in CRC. The first study to investigate a miRNA alteration in CRC was performed in 2003, in which miR-143 and miR-145 were expressed at reduced steady-state levels at the adenomatous and cancer stages of CRC (13). In another study, 37 miRNAs with various expression levels were identified in CRC using comprehensive array-based analyses in 84 CRC and matched normal colonic tissues (14). As a new emerging throughput screening platform, NGS technology easily enriches, detects and analyses miRNAs using a genome-wide scale. Moreover, the combination of miRNA and transcriptome sequencing enables the prediction of miRNA target genes, which aids in the identification of new and known miRNAs from a systematic and functional perspective. Using NGS technology in normal, tumour and metastasis tissue samples from the same patients with CRC, an earlier study investigated the complete set of miRNAs and their potential downstream regulated genes as well as the signalling network, and explored the power of miRNA-1 response prediction in individual patients (15).

Despite the use of various detection methods, including microarray, sequencing, real-time polymerase chain reaction-based approaches and *in situ* hybridisation, a high consistency of miRNA expression profiles exists among these studies, which indicates that these miRNAs are essential elements in cancer progression (16-19).

3. Function of miRNAs in CRC

As each miRNA has several different mRNA targets, miRNA genes are predicted to represent ~3% of the human genome, whereas ~30% of the genes are regulated by miRNAs (20). Evidence has demonstrated that miRNAs act either as tumour suppressors by suppressing the expression of target oncogenes, or as proto-oncogenes by inhibiting the expression of tumour suppressor genes (TSGs) (21-24). Both

effects correlate with cancer development and its progression in CRC (11,25,26).

miRNAs and Wnt/ β -catenin pathway. The deregulation of the Wnt/ β -catenin pathway is one of the earliest events during CRC development. In this pathogenic pathway, β -catenin acts as a transcriptional activator and upregulates the expression of Wnt target genes. Overexpression of constitutively active β -catenin may result in colorectal tumourigenesis (27). Through *in vitro* and *in vivo* experiments, Ma *et al* proved that miRNA-17-92 increases the expression of β -catenin indirectly by targeting P130, and subsequently promotes the tumourigenesis and progression of CRC (28). Strillacci *et al* revealed that miR-101 regulates Wnt/ β -catenin signalling in CRC through the strong impairment of β -catenin nuclear accumulation and β -catenin-driven transcriptional activity, following the control of downstream target gene expression and malignant phenotype in cancer cells (29).

miRNAs and cancer stem cells (CSC)s. CSCs are a group of heterogeneous cells that are vital for the initiation and progression of cancers, including CRC. The Wnt pathway plays an essential role in the induction of the symmetrical cell division (SCD) of CSCs, which disturbs the homeostasis of the stem cell pool and leads to carcinogenesis (30,31). miRNA-146a was identified by Hwang *et al* as an activator of the Wnt pathway in CRCSCs by stabilising β -catenin, which directs SCD to promote CRC progression. Notably, the study uncovered an upstream regulatory mechanism of miR-146a, in which Snail activates miR-146a transcription via a β -catenin-TCF4 complex. A feedback circuit of Snail-miR-146a- β -catenin loops exists in CRCSCs to maintain Wnt activity using a miRNA-dependent regulation method (32).

miRNAs and epithelial-to-mesenchymal transition (EMT). EMT is a cellular process of converting polarised epithelial cells into mesenchymal cells. During this process, cancer cells acquire malignant, migratory and invasive capabilities (33). The activation of EMT in CRC enables the cancer cells to detach, migrate and disseminate through the blood or lymphatic vessels, suggesting a mechanistic role of EMT in CRC metastasis.

A range of miRNAs were identified as regulators in this CRC process. For example, miR-29c expression in primary CRC and distant liver metastases tissues was observed to be negatively related to the mRNA levels of E-cadherin and vimentin, which are known to be specifically upregulated and established as markers of EMT (34). Moreover, ectopic miR-29c expression suppresses the expression of these EMT markers and induces morphological transformations to reverse EMT in CRC cells (34). Additional mechanistic research revealed that GNA13 and PTP4A are direct targets of miR-29c that act through the ERK/GSK3 β / β -catenin and AKT/GSK3 β / β -catenin pathways, respectively, to regulate EMT (34).

Using an azoxymethane and dextran sodium sulfate-induced colitis-associated tumour mouse model, Rokavec *et al* demonstrated that exposure of CRC cells to the cytokine IL-6 represses the miR-34a gene by activating the STAT3 transcription factor. Furthermore, the IL-6 receptor, which mediates IL-6-dependent STAT3 activation, was identified as a direct miR-34a target.

Such miRNA-related self-stabilising circuits consisting of IL-6R/STAT3/miR-34a are crucial for the induction of EMT and capacitation of metastasis in CRC cells (35).

miRNAs and epidermal growth factor receptor (EGFR) pathway. Human EGFR comprises an extracellular ligand-binding domain and an intracellular domain with tyrosine kinase activity. The selective induction of EGFR activates two major downstream signalling pathways, namely KRAS/RAF/ERK and PI3K/AKT, and is responsible for the progression of a broad spectrum of solid tumours, including CRC.

KRAS is described as a major driver of CRC progression and may be regulated directly by a number of miRNAs with tumour suppressor functions, including miR-143 (36,37), miR-145 (37), miR-18a* (38), miR-30b (39) and let-7 (40). In contrast, KRAS upregulates the expression of oncogenic miR-200c, miR-221/222, miR-181a and miR-210 in CRC cells (41). In an inflammatory bowel disease-associated CRC study, researchers identified a correlation between miR-133a, miR-143, miR-145 and miR-223 dysregulation and alteration of PI3K/Akt signalling. Furthermore, the authors observed that miR-223 directly targeted IGF1R to regulate this pathway, representing molecular links between inflammation and cancer (37).

Additionally, the EGFR pathway serves as an essential anticancer agent target (42), and anti-EGFR chemotherapy has been routinely used in advanced CRC. However, a response to this therapy was observed in less than 40% of patients with KRAS wild-type tumours (43). Additional factors are required to facilitate patient selection for this therapy to avoid the prescription of ineffective and potentially harmful side effects. In related studies, miRNAs including miR-31-3p (44), let-7 (45), miR-181a (46) and miR-143 (47) have been identified as significant markers for predicting an effective response in patients treated with anti-EGFR therapy.

4. Epigenetic regulation of miRNAs in CRC

Since aberrant miRNAs are well recognised as representing the hallmark of and playing a key role in several biological processes of CRC, increasing numbers of studies have focused on exploring the mechanisms responsible for disturbed miRNA expression. Dysregulated miRNAs are known as epigenetic alterations. Notably, a previous study has suggested that gene encoding for miRNAs may be affected by the same epigenetic regulatory mechanisms that modulate any other protein-coding genes (48). We summarise the altered miRNAs regulated by epigenetic factors in Table I, including promoter methylation, histone acetylation and chromatin changes in CRC. The data are derived from various array platforms and supported by validation experiments and function assays (49-72).

DNA methylation-mediated regulation of miRNAs in CRC. The chemical modification of DNA methylation is mediated by DNA methyltransferases (DNMTs), which catalyse the covalent attachment of a methyl group to the 5' carbon of cytosine in CpG dinucleotides. The majority of CpGs are maintained in a methylated state, whereas the CpGs located in CGIs, local enrichments of CpG sequences, are normally

unmethylated. The methylation of CGIs in promoters and start sites inhibits interactions with transcription factors and induces a reduction in gene expression (73). A comprehensive analysis of miRNA gene sequences has revealed that ~50% are associated with CGIs, suggesting that they could be subjected to a DNA methylation mechanism of regulation (74).

Hypermethylation-silenced miRNAs in CRC. Evidence has confirmed that DNA methylation may be responsible for the altered expression of miRNAs in CRC by silencing potential tumour suppressor miRNAs. The DNA demethylating agent 5-aza-2'-deoxycytidine (5-AZA) reduces the methylation levels of genes by covalently binding to DNMTs and inhibiting their function (75), and is widely applied in the search for methylation-regulated miRNAs. By combining the treatment of CRC cells using 5-AZA and high-throughput analysis, eight miRNAs were identified as novel miRNAs regulated by DNA methylation (59). The authors subsequently investigated the functions of these hypermethylation-silenced miRNAs by ectopically expressing select candidates, which resulted in the repression of proliferation and the migration of cancer cells (59). After treating the CRC cells with 5-AZA and the histone deacetylase (HDAC) inhibitor trichostatin A, Grady *et al* demonstrated that the expression of miR-342 is commonly suppressed by hypermethylation in CRC and that the reconstitution of miR-342 in the HT-29 cells induced apoptosis, implying its function as a pro-apoptotic tumour suppressor. More significantly, the methylated CGI located in the promoter of EVL, which has been proven to be the host gene of miR-342, has a higher frequency of methylation in cancer and adenoma samples compared with normal samples. This study addressed a novel mechanism for silencing intronic miRNAs by epigenetic alterations of cognate host genes in CRC (57).

A similar approach aimed at identifying epigenetically regulated miRNAs was represented by the miRNA profiling of CRC cells genetically deficient for the DNA methyltransferase enzymes. DNA hypomethylation induces a release of miRNA silencing in cancer cells. One of the primary targets is miRNA-124a, which is embedded in a large CGI and is capable of targeting cyclin D kinase 6 (CDK6); CDK6 has an impact on the phosphorylation status of downstream effector retinoblastoma TSG (51). To identify methylation-silenced miRNAs and to clarify their role in CRC, our group performed a microarray analysis to screen for miRNAs in CRC cells using 5-AZA treatment or a genetic knockdown of DNA methyltransferases. We observed that miR-149 was epigenetically silenced in CRC and that the downregulation of miR-149 was associated with the hypermethylation of the neighbouring CGI. The transfection of miR-149 inhibited the cell growth and invasion of CRC cells *in vitro* by targeting specificity protein 1 (67).

Methylation-regulated miRNAs have been observed to be closely linked with the progression of CRC. Balaguer *et al* reported that methylation of the miR-137 CGI was a cancer-specific event that was observed in virtually all CRC cell lines, in 82% of adenomas, in 82% of CRCs but in only 14% of normal mucosae, suggesting that the results constituted an early event in colorectal carcinogenesis (58). Other studies in CRC samples identified five miRNAs that were downregulated and located around or on a CGI, and 5-AZA-restored

Table I. Epigenetically regulated miRNAs in CRC.

miRNA	Chromosomal location	Regulation	Target gene	Expression in CRC	References
miR-34b/c	11q23.1	Hypermethylation	CDK6, c-MYC; E2F3	Downregulated	(49)
miR-124a	8p23.1 (miR-124a-1) 8q12.3 (miR-124a-2) 20q13.33 (miR-124a-3)	Hypermethylation	CDK6	Downregulated	(50)
miR-9-1	1q22	Hypermethylation		Downregulated	(51)
miR-1/133a cluster	20q13.33	Hypermethylation		Downregulated	(52)
miR-124a	8p23.1	Hypermethylation		Downregulated	(53)
miR-342	14q32.2	Hypermethylation		Downregulated	(54)
miR-137	1p21.3	Hypermethylation	LSD1	Downregulated	(55)
miR-129-2	11p11.2	Hypermethylation		Downregulated	(51)
miR-941	20q13.33	Hypermethylation		Downregulated	(56)
miR-1237	11	Hypermethylation		Downregulated	(56)
miR-1247	14	Hypermethylation		Downregulated	(56)
miR-17-92 cluster	13q31.3	Histone deacetylation	PTEN, BCL2L1, CDKN1A	Upregulated	(57)
miR-34a	1p36.22	Hypermethylation	c-Met, Snail, β -catenin	Downregulated	(58)
miR-128	2q21.3	Hypermethylation	NEK2	Downregulated	(59)
miR-148a	7p15.2	Hypermethylation		Downregulated	(60)
miR-126	9q34.3	Hypermethylation	VEGF	Downregulated	(61)
miR-27b	9q22.32	Hypermethylation	VEGFC	Downregulated	(62)
miR-149	2q37.3	Hypermethylation	SP1	Downregulated	(63)
miR-497/195	17p13.1	Hypermethylation		Downregulated	(64)
miR-212	17p13.3	Hypermethylation	MnSOD	Downregulated	(65)
miR-200c/141	12p13.31	Hypomethylation	ZEB1, ETS1, FLT1	Upregulated	(66)
miR-345	14q32.2	Hypermethylation	BAG3	Downregulated	(67)
miR-373	19q13.42	Hypermethylation	RAB22A	Downregulated	(68)

CRC, colorectal cancer; miR/miRNA, microRNA.

expression of three miRNAs (namely miR-9, miR-129 and miR-137) in three CRC cell lines. Methylation of the miR-9-1, miR-129-2 and miR-137 CGIs was observed in CRC cell lines and in primary CRC tumours, but not in normal colonic mucosae. Furthermore, the methylation of miR-9-1 occurred more frequently in the advanced stages of CRC and was associated with nodal invasion ($P=0.008$), vascular invasion ($P=0.004$) and distant metastasis ($P=0.016$) (52). Using a case-control study containing 94 primary colon cancer samples with and without liver metastases, Siemens *et al* determined CGI methylation frequencies of miR-34 promoters and prognostic values. Their results revealed that miR-34a methylation was strongly associated with metastasis to the liver ($P=0.003$) and lymph nodes ($P=0.006$). In a confounder-adjusted multivariate regression model, miR-34a methylation provided the most significant prognostic information concerning metastasis to the liver ($P=0.014$) (61).

Inactivation of miR-200 members by DNA methylation has been described in various cancers (76-78). In a previous

study, the authors demonstrated that the 5'-CGIs of both miR-200 loci are unmethylated and are associated with the expression of the corresponding miRNAs with epithelial features, whereas CGI hypermethylation-associated silencing is observed in transformed cells with mesenchymal characteristics (78). Additional studies revealed that miR-200ba429 and miR-200c141 transcripts undergo a dynamic epigenetic regulation linked to EMT or mesenchymal-to-epithelial transition (MET) phenotypes in tumour progression. These findings confirmed that the epigenetic silencing plasticity of the miR-200 family contributes to EMT and MET, and the development of CRC tumourigenesis (79).

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease, is one of the most key high-risk conditions for CRC (80). Although it is known that epithelial dysplasia occurring in long-standing IBD is a typical precancerous lesion, the pathogenesis of colon cancer in IBD is poorly understood. Several studies have demonstrated the crucial role of altered miRNAs in the carcinogenesis of

IBD-associated CRC (56,81-83), which includes the detection of epigenetic regulation of miRNAs. By comparing the expression and promoter methylation status of miR-124a in tissue samples from UC patients with or without CRC, patients with sporadic CRC and healthy volunteers, Ueda *et al* observed significantly decreased expression and higher methylation levels of miR-124a in UC-associated CRC (56). Downregulated miR-124 by hypermethylation was also identified in another study on UC, which promoted inflammation and the pathogenesis of UC by targeting STAT3 in paediatric patients (84).

Hypomethylation-reactivated miRNAs in CRC. Compared with silencing TSGs through DNA hypermethylation, hypomethylation activates oncogenes and promotes cancer progression. As a member of the let-7 miRNA family, let-7a has been reported as a tumour suppressor in CRC (85,86). However, its expression was reactivated by DNA hypomethylation in lung cancer and was associated with enhanced tumour phenotypes and oncogenic changes (87). In a previous study of metastasis-related miRNAs in CRC, the authors observed a higher expression of miR-200c in liver metastases tissues than in primary CRCs (70). A functional analysis indicated that the overexpression of miR-200c induces MET in liver metastasis by targeting ZEB1, ETS1 and FLT1. More significantly, the increased expression of miR-200c was significantly associated with hypomethylation of the promoter region and was proven to be reactivated by the demethylation in CRC (70).

Histone modification-mediated regulation of miRNAs in CRC. Methylation is not the only epigenetic mechanism that affects miRNA expression. Histone modifications may also lead to either activation or repression of miRNAs depending on which residues are modified and which type of modification is present. The N-terminal tails of histones undergo several different covalent modifications, including acetylation, methylation, ubiquitylation, sumoylation and phosphorylation on specific residues (88).

Several studies have confirmed that HDAC inhibitor alters miRNA expression in CRC cells (60,88-90). Acetylated lysine in histone tails was correlated with a more relaxed chromatin state and gene-transcription activation, whereas histone deacetylation led to a more compact chromatin structure and suppressed gene transcription (91). By combining the treatment of CRC cells with an HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) and a microarray assay, Shin *et al* revealed that 32 of 275 human miRNAs were upregulated in HCT-116 cells, with the potential target genes related to apoptosis, cell cycle and differentiation of the cancer cells (90). Using another HDAC inhibitor, butyrate, which may decrease gene transcription by reducing histone H3 and H4 acetylation near the transcription start sites (92), Hu *et al* downregulated the expression of miR-106b in HCT-116 cells, restored p21 protein expression and decreased cell proliferation (89). Similarly, the expression of the oncogenic miR-17-92 cluster was inhibited by butyrate in HT29 and HCT116 CRC cells, with a corresponding increase in the target TSGs, including PTEN, BCL2L1 and CDKN1A (60).

In addition to performing their individual roles, DNA methylation and histone modifications interact with each other at manifold levels to influence chromatin organisation and the

expression status of genes, including miRNAs. By comparing miRNA expression and histone modifications (H3K4me3, H3K27me3 and H3K79me2) before and after DNA demethylation, 47 miRNAs, including miR-1-1, were identified to be potential targets of epigenetic silencing and may act as a tumour suppressors in early and advanced CRC. DNA demethylation induced the upregulation of H3K4me3 and H3K27me3, but not H3K79me2, at the promoters of these miRNAs. The conclusion provided additional insight into the association between hypermethylation, chromatin modifications and miRNA dysregulation in cancer (54).

5. miRNA regulatory effects on epigenetic machinery in CRC

The significance of inhibitory signals that contribute to epigenetic gene silencing, including DNA methylation, histone modification and miRNAs, has been increasingly recognised in recent years. However, the crosstalk between these epigenetic regulators is not fully understood. An increasing amount of data exists to support miRNA regulation of the expression of components of the epigenetic machinery, creating a highly controlled feedback mechanism.

miRNAs regulate DNA methylation in CRC. Methylation changes to the genome are controlled by DNMTs. To date, three catalytically active DNMTs, namely DNMT1, DNMT3A and DNMT3B, have been identified (93). miR-143 has been confirmed to be a tumour suppressor miRNA and to decrease in CRC cells and tissues according to several studies (47,94-97). Moreover, DNMT3A, which represents an essential controller of methylation changes to the genome and is frequently increased in various malignancies including CRC, was defined as a direct target of miR-143 (98). Specifically, miR-143 was inversely correlated with mRNA and protein expression of DNMT3A in CRC. *In silico* prediction illustrated the binding of miR-143 with the DNMT3A 3' UTR, which was confirmed by a luciferase reporter assay. Furthermore, the restoration of miR-143 expression in colon cell lines downregulated DNMT3A expression at the mRNA and protein level.

Similarly, Wang *et al* demonstrated that DNMT1 is regulated by miR-342 in CRC (99). Low expression of miR-342 and high expression of DNMT1 were observed in CRC tissues and cell lines. Restoring miR-342 resulted in reduced DNMT1 expression and reduced cell proliferation as well as invasiveness in CRC cells and inhibition of tumour growth and lung metastasis formation in nude mice. More significantly, the ectopic expression of miR-342 decreased DNMT1 expression and reactivated TSGs, including ADAM23, Hint1, RASSF1A and RECKS, through promoter hypomethylation.

miRNAs regulate histone deacetylation in CRC. In addition to DNA methyltransferases, miRNA has been proven to regulate histone deacetylases in CRC. As a nicotinamide adenine dinucleotide-dependent deacetylase, silent information regulator 1 (SIRT1) may function as an oncogene and play a role in cancers including CRC (100-102). Yamakuchi *et al* demonstrated that miR-34a inhibits SIRT1 expression through a special binding site within the 3' UTR of SIRT1. miR-34a inhibition of SIRT1 leads to an increase in acetylated p53

Table II. Regulation of epigenetic factors by miRNAs in CRC.

miRNA	Chromosomal location	Target gene	Expression in CRC	References
miR-342	14q32.2	DNMT1	Downregulation	(99)
miR-140		HDAC4	Downregulation	(105)
miR-627	16q22.1	JMJD1A	Downregulation	(106)
miR-34a	1p36.22	MTA2, HDAC1	Downregulation	(104)
miR-34a	1p36.22	SIRT1	Downregulation	(103)
miR-137	1p21.3	LSD1	Downregulation	(58)
miR-143	5q32	DNMT3A	Downregulation	(98)

CRC, colorectal cancer; miR/miRNA, microRNA.

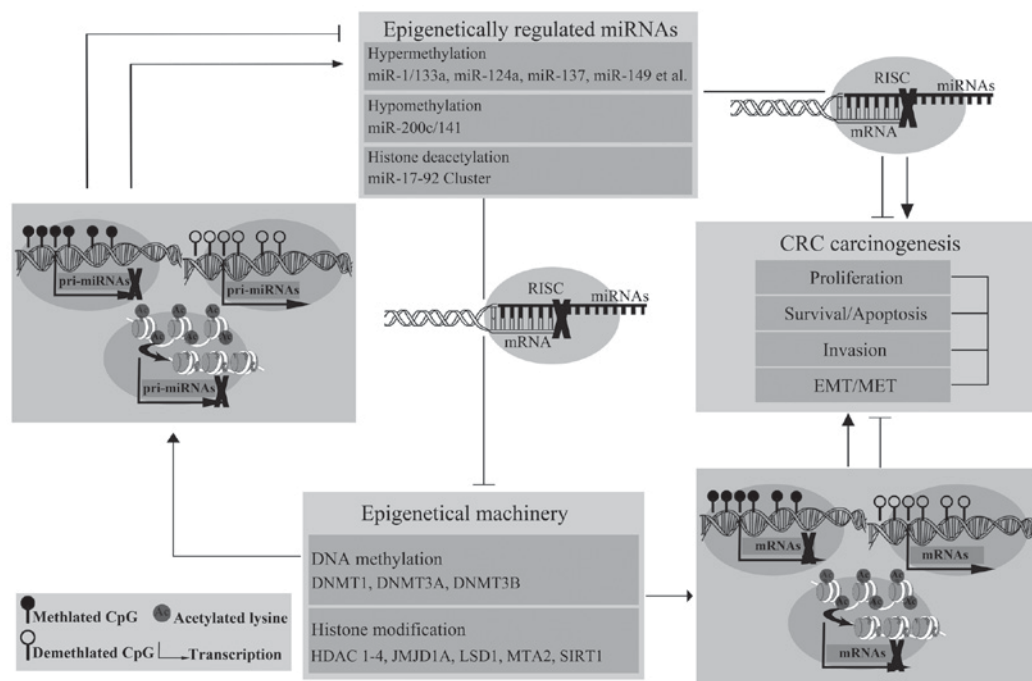


Figure 1. Schematic diagram of reciprocal crosstalk between microRNAs (miRNAs) and epigenetic regulators in colorectal cancer (CRC). Special pathological conditions result in the formation of an intricate regulatory circuit between miRNAs and various epigenetic mechanisms, involving DNA methylation and histone modifications. The epigenetic-miRNA interplay accounts for the development of CRC by post-transcriptional regulating oncogenes and tumour suppressor genes involved in CRC carcinogenesis. Regular arrow, positive interaction; T-shaped arrow, negative interaction.

and apoptosis in CRC cells. Furthermore, miR-34a is a transcriptional target of p53, suggesting a positive feedback loop between p53 and miR-34a through SIRT1-dependent regulation (103). Notably, another study reported that HDAC1 also exhibits a robust decrease in protein levels following the induction of miR-34a in CRC cells (104). In a previous study evaluating the role of miRNAs in the antitumour action of calcitriol, miRNA expression profiles were examined in colon cancer cells treated with calcitriol (105). The results revealed that calcitriol selectively induces the expression of miR-627. Moreover, histone demethylase JMJD1A was identified as the direct target of miR-627, suggesting that miR-627-mediated JMJD1A downregulation is an essential intracellular target for calcitriol and mediates epigenetic activities of calcitriol. The series of studies showing miRNAs regulating DNMTs or HDACs in CRC are listed in Table II.

6. miRNAs mediate crosstalk between epigenetic regulators in CRC

The same miRNA that provides epigenetic regulation may in turn affect the epigenetic machinery. miR-143, targeting DNMT3A in CRC cells, was hypermethylated and decreased in acute lymphoblastic leukaemia (ALL) cells. Demethylation restored functional endogenous miR-143 expression and significantly inhibited proliferation and induced apoptosis in ALL (106). On the one hand, CGI methylation of miR-148a and miR-152 genes regulated by DNMT1 silenced the TSG-like miRNAs in CRC (107-109). On the other hand, the levels of DNMT1, a direct target of miR-148a and miR-152, were inhibited by miR-148a and miR-152 overexpression. A negative feedback loop between DNMT1 and miR-148a/152 promoted carcinogenesis in cancer cells (110,111), implying

that miRNAs mediate the crosstalk between epigenetic regulators.

miR-137 has been identified as another significant mediator of crosstalk between epigenetic regulators in CRC. Balaguer *et al* revealed that miR-137 is constitutively expressed in the normal colonic epithelium and is silenced by CGI methylation of the promoter in neoplastic tissues (58). Restoring miR-137 expression in CRC cells resulted in a significant decrease in proliferation, suggesting its potential as a tumour suppressor miRNA in CRC. An additional analysis of candidate targets was performed using a combination of bioinformatic and transcriptomic approaches and identified that LSD1, an HDAC, is a target of miR-137. Notably, as a new class of histone demethylating enzymes, LSD1 was essential for the maintenance of global DNA methylation through the demethylation of a non-histone substrate, DNMT1, by increasing its stability. The results implied that miR-137 mediates the interplay between epigenetic regulators in CRC (58). All of these observations suggested a reciprocal crosstalk between miRNAs and epigenetic regulators. In this scenario, miRNAs functioned as a crucial factor in the valid transmission of various patterns of epigenetic modulation and thereby interfered with CRC carcinogenesis (Fig. 1).

7. Clinical significance and future prospects

The evidence discussed here indicates a strong interplay between miRNAs and epigenetic regulators in CRC. Epigenetically regulated miRNAs by DNA methylation or histone modifications are capable of silencing specific target molecules at the post-transcriptional level, including members of the epigenetic machinery, thus contributing to the conversation between other epigenetic events. These results have extended our comprehension of the pathogenesis and pathophysiology of CRC.

Additional exploration is warranted to annotate the regulatory loop involving miRNAs and the epigenetic machinery, and to explore how to translate these findings into clinical applications. First, it is crucial to identify new avenues for anti-cancer therapy based on the epigenetic regulation of miRNAs. To date, therapy using several epigenetic-based synthetic agents has been based on conventional protein-coding TSGs. With the identification of a greater number of epigenetically silenced tumour-suppressor miRNA genes in CRC, restoring these miRNAs using epigenetic agents is likely to become a potentially powerful approach to the remedy. Second, endeavours utilising miRNAs to modulate the expression levels of oncogenes and suppressor genes or to control the effector enzymes using epigenetic machinery and thus affecting the expression of a broad range of modulated molecules would permit the development of novel preventative therapies or adjunctive therapeutic approaches in CRC.

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