REVIEW ARTICLE

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# MicroRNA Promoter Methylation in Colorectal Cancer

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## ABSTRACT

Colorectal cancer (CRC) is one of the most common cancers worldwide. The beginning and progression of the disease are thought to be determined by combinations of epigenetic and genetic changes that trigger multistep programs of carcinogenesis. In colorectal cancer, epigenetic alterations, in particular promoter CpG island methylation, occur more commonly than genetic mutations. Hyper-methylation contributes to carcinogenesis via inducing transcriptional silencing or down-regulation of tumor suppressor genes. DNA methylation alteration has a high potential for minimally invasive biomarker identification. Genome analysis has confirmed that microRNA expression is deregulated in most cancer types through several mechanisms, including failings in the microRNA biogenesis machinery.

Moreover, microRNAs can be dysregulated by abnormal CpG methylation in cancer. Since it is believed that epigenetic changes occur in the early stages of the disease, these changes can be used for the early detection of cancer. In this review, we intend to study the role of microRNA gene promoter methylation in colorectal cancer.

**Keywords: Colorectal Neoplasm, microRNA, epigenetics, DNA hypermethylation**



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## INTRODUCTION:

Epigenetic abnormalities, like genetic alteration, are involved in cancer. It has been proposed that epigenetic changes are early events in tumor development and may lead to genetic variation, therefore providing a rationale for the development of molecular biomarkers[1].

CRC is one of the most common cancers in the world and the fourth leading cause of cancer death worldwide [1,2]. Genetic changes, DNA methylation, and histone modification are genetic and epigenetic events that are involved in the initiation and progress of colorectal carcinoma[1,2]. The CpG dinucleotide methylation in the gene promoter is the principal mechanism of gene regulation. Furthermore, abnormal DNA methylation patterns play an essential role in various human tumors, including CRC[1,2].

MicroRNA (miRNA) is one of the subdivisions of small noncoding RNAs, which controls the expression of genes through mRNA degradation or translational inhibition[3,2]. In the regulation of biological processes, miRNAs can act as both tumor suppressors or oncogenes, with roles in cell differentiation, proliferation, and apoptosis[3]. It has been shown that unusual miRNA expressions are involved in human cancers[5,6]. Though the biological importance of miRNA is becoming progressively apparent, the molecular mechanisms underlying the regulation of miRNA expression in cancer are not entirely understood. Variation in DNA methylation of promoter-associated CpG dinucleotides is a significant mechanism leading to the dysregulation of miRNAs in disease, including CRC[4].

In this review, we focus on the importance and impact of miRNA promoter methylation in CRC and summarize the association between miRNA expression level and promoter methylation as a diagnostic or prognostic biomarker in CRC.

### The dual role of miRNA in CRC:

Various studies have shown that miRNA are involved in colorectal carcinogenesis based on CRC tissues or cell line investigation. It has been confirmed that the participation of miRNA in CRC seems to be context-specific, with evidence indicating that miRNAs have a dual character in tumor-suppressing and tumor-promoting activities[5]. Oncogenic miRNAs mostly constrain the expression of tumor-suppressor genes and hasten carcinogenesis. OncomiRs has an essential effect on the development of CRC. Some current miRNAs seem to interfere with the regular activity of important gene functions as oncogenes associated with CRC[5-7]. Tumor-suppressive miRNAs play an essential role in hindering tumor progression through down-regulating oncogenes related to proliferation, apoptosis, invasion, and migration (**Figure1**).

### Epigenetic Mechanisms in CRC:

Epigenetic and genetic changes of the cellular genome in CRC lead to the transformation of normal epithelium into adenocarcinoma[8]. Abnormal epigenetic change in CRC was first identified in the1980s[9]. Other epigenetic mechanisms such as chromatin looping, noncoding RNAs, and nucleosomal remodeling, also play essential roles in CRC development[10]. The key epigenetic tools which are supposed to play a critical part in cancer development consist of DNA methylation of cytosine bases in CpG dinucleotides and post-translational modifications of histone proteins that facilitate the packaging of DNA into chromatin and thus regulate gene expression through controlling chromatin conformation[11].

Hypermethylation of numerous gene promoters in CRC has been reported (**Table1**). The abnormal methylation of CpG islands within gene promoters is a known epigenetic event that leads to transcriptional silencing. Besides biological values of methylation-induced silencing of tumor suppressor genes, this epigenetic modification constitutes a molecular alteration that can

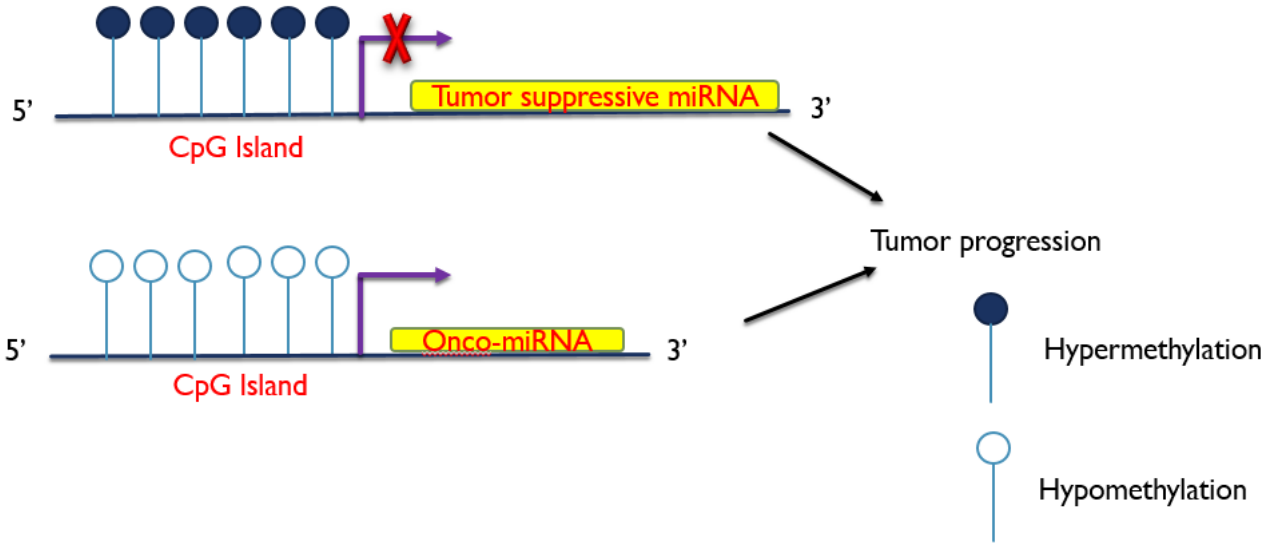


**Figure 1.** Schematic representation of miRNA promoter methylation

hopefully serve as a biomarker in early diagnosis[12,13]. Several epigenetic mechanisms are involved in CRC pathogenesis, and crosstalk between different epigenetic mechanisms, such as histone modifications and DNA methylation in the gene promoter region has been studied. Moreover, evidence suggests the existence of primary intermediaries of CRC epigenetic inheritance in cancer cells[14].

**Table 1. DNA methylation of several of genes and their significance in CRC**

| Gene | Function in CRC | Reference |
|---|---|---|
| **APC** | Wnt signaling pathway | **16** |
| **MLH1** | MSI | **17** |
| **MGMT** | MSI-H pathway of CRC neoplasia | **18** |
| **SFRP1** | Wnt signaling pathway | 19 |
| **CDKN2A** | uncontrolled cell proliferation | 20 |
| **TIMP3** | Invasion and metastasis | 21 |
| **VIM** | cellular structure and integrity | 22, 23 |
| **SEPT** | Genomic instability | 24 |
| **CDH1** | Intercellular adhesion | 25 |
| **HLTF** | genomic instability | 26 |

DNA methylation refers to the enzymatic addition of a methyl group to the 5' position of cytosine by DNA methyltransferases (DNMTs) using S-adenosyl-methionine as a methyl-donor to produce 5-methyl cytosine. The DNMTs act on exact CG dinucleotide sequences, recognized as CpGs. A high percentage of cytosine bases (over 70%) involved in the context of CpG dinucleotides are exposed to methylation. It is a usual procedure used by cells to preserve the physiological expression of genes and to maintain the mono-allelic expression of imprinted genes[18]. The non-cancerous cell genome in mammalian cells in the non-promoter region contains around 70–80% methylated CpGs, but CpG islands located in the areas surrounding the promoter are typically unmethylated[15].

## Epigenetic regulation of miRNAs in CRC:

MiRNAs, as epigenetic regulators, disturb the protein levels of target genes without altering the sequences of the gene. Furthermore, miRNAs can be controlled by epigenetic changes, including DNA methylation, RNA modification, and histone modifications. Disruption of the regulatory cycle in the miRNA-epigenetic feedback loop affects all processes which contribute to disease diversity[27]. MiRNAs play a crucial role in numerous cellular processes, including proliferation, cell cycle control, programmed cell death, differentiation, and invasiveness. All these processes are concerned with the evolution and development of cancer[28-31]. Genome-wide analysis has verified that miRNA expression is deregulated in most cancer types through numerous mechanisms, including faults in the miRNA biogenesis machinery, amplification/deletion of the region surrounding the miRNA, or abnormal transcriptional control[28]. Different evidence suggests that miRNAs could furthermore be deregulated in cancer by inappropriate CpG methylation or histone modification[19, 35].

## Hyper methylation of miRNA promotor in CRC:

Chen et al. proposed that miR-125a/b expression plays a key role in chemoresistance through the upregulation of ALDH1A3 and Mcl1[32]. Several studies have shown that methylation status of miRNA promoters in CRC miR-125a, and miR-125b significantly downregulate in CRC tissues. MiR-125a and miR-125b expression levels are inversely correlated with CpG island methylation in CRC. Increased DNA methylation could be one of the most important causes of the inactivation of miR-125a and miR-125b in CRC, and hypermethylation in miR-125 gene is a potential biomarker for predicting clinical outcomes[33]. Studies have shown that miR-132 was meaningfully down-regulated in CRC patients with distant metastasis. Furthermore, miR-132 could predict disease-free survival and distant metastasis in CRC patients. Most research has shown that the ZEB2 as an EMT regulator is a direct target of miR-132[34]. Down-regulation of miR-132 by DNA hyper-methylation is related to cell invasion in CRC. MiR-132 is significantly reduced in CRC in both tissue and cell lines. Reduced expression of miR-132 in CRC tissues is owing to the hyper-methylation of CpG islands[35]. MiR-133b reduced the expression of HOXA9, and then decreased the expression of ZEB1 and upregulated E-cadherin expression[36]. Lvlv and colleagues identified that the expression of miR-133b significantly decreases in CRC cells with miR-133b promoter hyper-methylation, indicating that miR-133b is silenced by epigenetic mechanisms. They observed a high occurrence of hypermethylation in miR-133b in tissue samples and CRC cells, indicating that methylation of the miR-133b gene may be a useful diagnostic marker for patients with CRC[37]. MiR-148a promotes apoptosis by targeting Bcl-2 in CRC[38]. Takahashi et al. showed that in advanced CRC tissues, miR-148a expression was meaningfully down-regulated. Also,

miR-148a methylation status is correlated inversely with its expression and is associated with worse survival in stage IV of CRC. In multivariate analysis, miR-148a expression is an independent prognostic/predictive biomarker for advanced CRC patients[39]. MiR-32 expression is related to CRC lymphatic attack, metastasis, and is associated with poor survival. Practical training verified that overexpression of miR-32 in LoVo cells promotes cell proliferation and migration, whereas inhibition of miR-32 in HCT 116 cells had the opposite effect[40]. Another study showed that methylation does not affect miR-32 expression in CRC cell lines and tissues[41]. DNA methylation-persuaded silencing of miR-126 donates to tumor invasion and angiogenesis in CRC[42]. It has been indicated that miR 486 5p-like compounds suppressed the progression of CRC by constraining the initiation of AKT signaling pathway through targeting PIK3R1. Therefore, miR 486 5p may be a potential target for CRC treatment[43]. Liu and colleagues discovered that miR-486-5p is unusually down-regulated in CRC, which is caused by higher DNA methylation in the promoter region[44]. MiR-34a upsurges the affinity of CRC cells to 5-fluorouracil in vivo and in vitro[45]. MiR-34a/b/c-deficient adenomas displayed high proliferation and decreased apoptosis[46]. MiR-34a and miR-34b/c promoter hyper-methylation have been shown in CRC tissue compared to healthy tissue. P53 could exert its tumor inhibitory role via the induction of miR-34 expression[47]. Association of miR-181a/135a/302c promoter methylation with microsatellite instability and chemoresistance to 5-fu treatment in CRC has confirmed the tumor-suppressive action of these miRNAs[48] (**Table 2**).

**Table 2. Hyper-methylated miRNAs and their effects on CRC**

| MiR-name | Target gene | Role in CRC | Reference |
|---|---|---|---|
| **miR-125a/b** | ALDH-1,MCL1 | Reduces resistance to chemotherapy | **31-32** |
| **miR-132** | ZEB2 | Distant metastasis Reduces | **33-34** |
| **miR-133b** | HOXA9 | Reduces metastasis | **35-36** |
| **miR-148a** | BCL2 | Improved survival in stage IV of CRC | 37-38 |
| **miR-32** | PTEN - | Reduces lymphatic attack | 39-40 |
| **miR-126** | VEGF | Metastasis,angiogenesis | 41 |
| **miR-486-5P** | AKT,PIK3R1 | Increased cell growth control | 42-43 |
| **miR-34a/b/c** | TGFBR1,MDM4,B-CATENIN | Reduces resistance to 5fu | 44-46 |
| **miR-181a miR-135a miR-302a** | PLAG1/IGF2 | Reduces resistance to 5fu | 47 |

## CONCLUSION

CRC remains a global problem, affecting both health and the economy. This is in spite of the fact that patients have a 90% chance of 5- year survival when detected and cured early[1, 2]. It is therefore essential for the development of techniques which not only aid current diagnostic processes but also permit non-invasive and more precise screening, diagnosis, follow-up, and response to therapy in cancer patients. A large number of miRNAs that could function as tumor-suppressors are epigenetically silenced because of hypermethylation in CRC. MiRNA expression abnormalities often occur in CRC and appear to play an essential role in tumor development and progression. Because of the reduction in the expression of a large number of miRNAs in CRC, evaluation of the methylation status of the gene promoter region can be helpful in early detection and treatment.

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