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Review

Epigenetic changes in colorectal cancer

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Abstract

Epigenetic changes frequently occur in human colorectal cancer. Genomic global hypomethylation, gene promoter region hypermethylation, histone modifications, and alteration of miRNA patterns are major epigenetic changes in colorectal cancer. Loss of imprinting (LOI) is associated with colorectal neoplasia. Folate deficiency may cause colorectal carcinogenesis by inducing gene-specific hypermethylation and genomic global hypomethylation. HDAC inhibitors and demethylating agents have been approved by the FDA for myelodysplastic syndrome and leukemia treatment. Non-coding RNA is regarded as another kind of epigenetic marker in colorectal cancer. This review is mainly focused on DNA methylation, histone modification, and microRNA changes in colorectal cancer.

Key words: DNA methylation, histone modification, microRNA (miRNA), epigenetics, colorectal cancer (CRC), field defect

Colorectal cancer (CRC) is the second most common malignant disease in developed countries, with 1 million new cases and 500 000 deaths worldwide every year[1], and it is the third leading cause of cancer-related death in both men and woman in industrialized countries[2]. The accumulation of gene mutations and epigenetic alterations may drive the initiation and progression of benign adenoma to malignant adenocarcinoma[3,4].

Epigenetics is defined as inheritable changes in gene expression without DNA sequence changes. The field of epigenetics includes DNA methylation, histone modification and non-coding RNAs. Increasing evidence shows that aberrant epigenetic changes play important roles in human cancer. Numerous DNA methylation and microRNA (miRNA) patterns have been regarded as tumor markers. Because of the reversible nature of epigenetic alterations, epigenetic associated agents are being developed[5,6]. In this review, we mainly focus on changes of DNA methylation, histone modification and miRNAs in colorectal cancer, especially on the potential clinical applications.

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DNA Methylation

DNA methylation is a normal procedure to maintain gene expression with normal patterns in mammalian cells. It is involved in the regulation of gene imprinting, X-chromosome inactivation and other biological activities[7,8]. Methylation of cytosine on DNA is well studied in epigenetics. CpG-rich regions constituted by CpG dinucleotides are known as CpG islands[9]. CpG islands in the gene promoter region are usually unmethylated, and the sporadic CpG sites in the gene body are normally methylated. However, during aging or carcinogenesis, the pattern will become global hypomethylation and promoter region hypermethylation[10,11].

The proteins involved in epigenetic regulation are DNA methyltransferase (DNMT), methyl-CpG–binding protein (MBP), histone deacetylase (HDAC), histone acetylase (HAT), and histone methyltransferase (HMT)[12-14]. DNMT3A and 3B are *de novo* methyltransferases that function mainly to establish methylation patterns, whereas DNMT1 is a methyltransferase that maintains methylation patterns. Hence, these enzymes cooperate to regulate cellular DNA methylation patterns[11,15]. The other DNMTs are DNMT3L and DNMT2. DNMT3L is reported to be required for the methylation of imprinted genes in germ cells and has been found to interact with DNMT3a and 3b in *de novo* methyltransferase activity. Although the biological function of DNMT2 remains unclear, its strong binding to DNA suggests that it may

target specific sequences in the genome[16,17]. Methyl-CpG binding proteins (MBPs), including methyl-CpG binding protein 2 (MeCP2) and methyl-CpG binding domains (MBD1, MBD2, MBD3, and MBD4), may block transcription factor binding to DNA by recruiting chromatin remodeling corepressor complexes[18,19]. HATs, HDACs and HMTs are mainly responsible for histone modification and chromatin remodeling[20,21].

DNA hypermethylation in CRC

Promoter region hypermethylation is found in a variety of cancers. Many of the affected genes are involved in cell cycle regulation, DNA repair, apoptosis, angiogenesis, invasion, and adhesion. The methylation profile varies in different types of cancer, but similar DNA methylation patterns were found in sporadic and inherited colon cancers[22].

Effective approaches are needed to screen premalignant adenomas and early stage cancers to reduce mortality of CRC. Epigenetic silencing of numerous tumor suppressor genes by promoter region hypermethylation has been found in a variety of cancers, including CRC[23,24]. Epigenetic changes were frequently found in precancerous lesions and adjacent tissues of CRC[25-28]. Growth-regulatory genes have been found to be epigenetically silenced in colonic mucosa in elder individuals, which may increase risk of cancer associated with aging[29]. Methylation of *ASC/TMS1* was reported to be a late-stage event in CRC[30], whereas hypermethylation of *SOX17* is an early event of CRC[31]. Methylation of *hMLH1*, *p16*, *DAP-kinase*, *APC*, *MGMT*, *RASSF2A*, and *Wif-1* were regarded as plasma or serum detection markers[32-36]. DNA methylation may serve as diagnostic, therapeutic, or prognostic markers for CRC (Table 1).

CpG island methylator phenotype in CRC

Cancer classification was mainly based on microscopic morphology and immunohistochemistry. Molecular classification was recognized as an important tool in clinic. Some molecular markers have been applied for cancer prevention, prognosis and chemosensitivity. Gefitinib-sensitizing mutation is one of the examples.

Fearon *et al.*[37] suggested in 1990 that most CRCs arise from adenoma and that multiple gene mutations were accumulated during carcinogenesis. One group of

Table 1. Clinical value of methylated genes in colorectal cancer

Gene	Authors	Year	Clinical value	Follow-up	Reference
CDH13/ FLBN3	Wang Z, *et al.*	2011	Poor prognosis	Median duration is 44 months (range, 3–60 months)	[114]
p16^{INK4a}	Mitomi H, *et al.*	2010	Larger size of tumor, frequent recurrence and poor prognosis	Median duration is 79 months (range, 60–123 months)	[115]
PPARG	Pancione M, *et al.*	2010	Poor prognosis	Average post-operative duration is 59.56 ± 26.5 months	[116]
EphA1	Herath NI, *et al.*	2009	Poor prognosis	Two years	[117]
DKK1	Rawson JB, *et al.*	2011	Related to MSI tumors; indicates favorable outcome	NA	[118]
SFRP1	Rawson JB, *et al.*	2011	Associated with MSI tumors inversely; indicates poor outcome	NA	[118]
GALR2	Kim JC, *et al.*	2011	Chemosensitive methylation candidates to bevacizumab	NA	[119]
ALX4	Kim JC, *et al.*	2011	Chemosensitive methylation candidates to cetuximab	NA	[119]
ER	Harder J, *et al.*	2009	High risk for local recurrence	NA	[120]
CHFR	Brandes JC, *et al.*	2005	Correlates with the microsatellite instability phenotype	NA	[121]
OSMR	Kim MS, *et al.*	2009	Highly specific diagnostic biomarker in fecal DNA	NA	[122]
SFRP2	Huang Z, *et al.*	2007	Detection of CRC and precancerous lesions in stool DNA	NA	[123]
SFRP2	Oberwalder M, *et al.*	2007	Detection of precancerous lesions of CRC in stool DNA	NA	[124]
DAPK	Mittag F, *et al.*	2006	Early event in CRC carcinogenesis	NA	[125]
TFPI2	Glockner SC, *et al.*	2009	Potential detection marker in stool DNA	NA	[126]
GATA4	Hellebrekers DM, *et al.*	2009	Potential detection marker in stool DNA	NA	[127]
GATA5	Hellebrekers DM, *et al.*	2009	Potential marker	NA	[127]
NGX6	Liu M, *et al.*	2010	Potential marker	NA	[128]
CDH4	Miotto E, *et al.*	2004	Potential marker	NA	[129]
Sox17	Zhang W, *et al.*	2008	Potential marker	NA	[31]
p15^{INK4b}	Ishiguro A, *et al.*	2006	Potential marker	NA	[130]

NA, not available.

CRC is characterized by high level DNA microsatellite instability (MSI-H), CpG island methylator phenotype-high (CIMP-high), and *BRAF* mutation. This type of cancer, which predominantly affects females and usually occurs in the proximal colon, arises in serrated polyps and not in adenomas [38-40]. The existence of CIMP was initially suggested by Toyota *et al.*[24], who reported that CRC fell into two categories: one group that shows rare methylation (CIMP-negative) and another group that shows aberrant methylation of several genes simultaneously (CIMP-positive). The five "classic" CIMP markers, *CDKN2A*, *MINT1*, *MINT2*, *MINT3*, and *MLH1*, provide a simplified and representative approach in defining CIMP [24,41]. The mechanism underlying CIMP tumor development is unknown, however, it is known that sporadic CRC can be divided according to their degree of methylation of CIMP markers. It appears that cancers with high degrees of methylation (CIMP) represent a clinically and etiologically distinct group, one constituting "epigenetic instability," and seem to have distinct epidemiological, histological, and molecular features[40].

DNA hypomethylation, field defect, and LOI in CRC

Global DNA hypomethylation in human colonic cancer was first reported by Feinberg *et al.*[42] in 1983. Recently, hypomethylation of *CDH3* (P-cadherin) promoter was found in aberrant crypt foci (ACF) and CRC, with a potential field defect of *CDH3* hypomethylation in the adjacent epithelium of cancer. In another study, a significant association was found between aberrant demethylation of *CDH3* and tumor site or Dukes stage. Promoter region hypomethylation is associated with induction of *CDH3* expression in CRC[43].

The term *field defect* was used to describe the accumulation of genetic and epigenetic alterations in tissues with normal appearance [44]. Promoter region hypermethylation was considered an evaluation marker for field defect in lung and colonic cancer. The discovery of field defect markers could be of great use in mucosa that appears normal, both for early detection and risk assessment for colon cancer (such as *MGMT* promoter methylation in CRC)[25,45].

Genomic imprinting is an epigenetic modification of a specific parental chromosome in the gamete or zygote, leading to parental, origin-specific, differential expression of the two alleles of a gene in somatic cells of the offspring. In 1993, Rainier *et al.*[46] and Ogawa *et al.*[47] simultaneously reported loss of imprinting (LOI) of *IGF2* in Wilms tumor, and later, similar observations were made in many other malignancies, including CRC[48,49]. Cui *et al.*[48] analyzed LOI in 172 patients under colonoscopy and found a 4.7-fold increased likelihood of LOI among patients with CRC (past or present) and a 5.2-fold increased likelihood of LOI among patients with a positive family history of CRC among first-degree relatives. LOI is found in normal colonic mucosa of about 30% of CRC patients, but it is found in only 10% of healthy individuals. These results indicate that LOI, which can be assayed with a DNA-based blood test, may be a valuable predictive marker of an individual's risk for CRC[50].

The effect of diet on DNA methylation in CRC

Diet and lifestyle play important roles in cancer biology. Inappropriate diet may contribute to one third of cancer deaths [51]. Alcohol decreases folate absorption, alters its metabolism, increases its excretion, and therefore may interfere with both DNA methylation and thymidylate synthesis [52-54]. Several studies show an association between high alcohol intake and CRC, which suggests that the carcinogenic effect of alcohol in the colon is mediated through its adverse effect on folate status [55,56]. A few reports suggest that people who habitually consume high level of folate have a significantly reduced risk of developing colon polyps or cancer[57,58]. Folate maintains genomic stability by regulating DNA biosynthesis, repair and methylation (Figure 1). Folate deficiency may induce gene-specific DNA hypermethylation and global DNA hypomethylation [59]. The impact of certain micronutrients on DNA methylation adds to our current understanding of possible mechanisms linking diet to CRC.

Histone Modifications

Histone modifications, such as phosphorylation, acetylation, or methylation, in localized promoter regions are histone codes for chromatin packing and transcription [60]. In general, methylation of H3K4, H3K36, and H3K79 are linked to gene expression activation, whereas H3K9me2, H3K9me3, H3K27me3 and H4K20 are associated with gene repression [61-64]. The global pattern of histone modifications has been considered a predictor for the risk of recurrence of human cancers [65,66]. Histone acetyltransferases (HATs) and deacetylases (HDACs) are responsible for the addition and removal of acetyl groups from lysine residues. In cancer cells, disruption of the balance between HATs and HDACs contributes to transcriptional inactivation of tumor suppressor genes (TSGs). Cyclin-dependent kinase inhibitor $p21^{WAF1}$ is repressed by promoter hypoacetylation in the absence of CpG island hypermethylation, and expression can be reactivated by inhibition of HDAC activity[67]. Interestingly, some TSGs with CpG island hypermethylation can also be re-expressed through inhibition of SIRT1, a class III HDAC that increases H4K16 and H3K9 acetylation at promoters, without affecting the hypermethylation status[68].

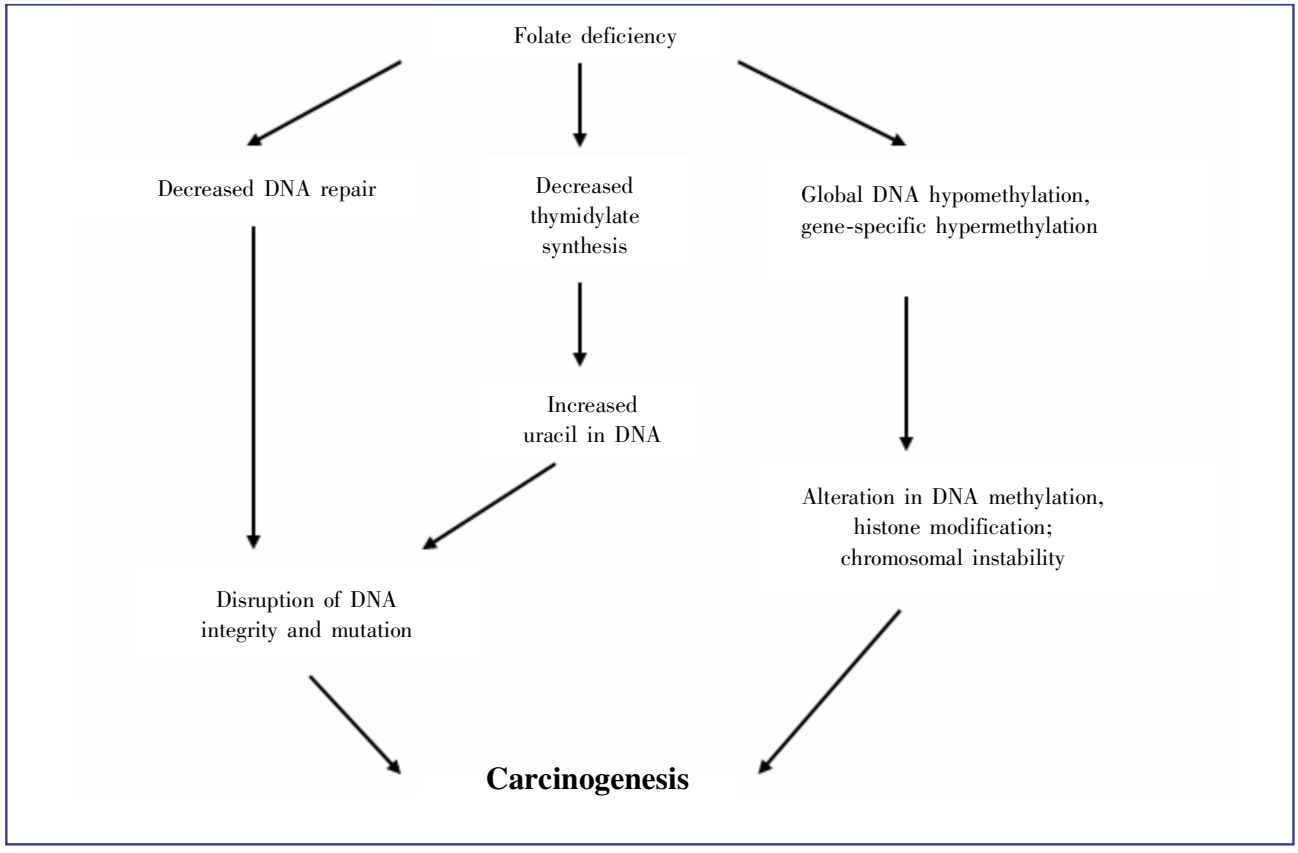

Figure 1. **Folate and regulation of DNA synthesis, repair, and methylation.** Folate deficiency may decrease thymidylate syntheses, inhibit DNA repair, induce imbalance of DNA methylation, histone modification, and finally cause carcinogenesis.

Similar to histone acetylation, histone methylation is dynamically regulated by both histone methyltransferases (HMTs) and histone lysine demethylases (HDMTs). Methylation takes place on both lysine and arginine residues and has different degrees, including mono-, di-, and trimethylation. H3K27-specific HMT (enhancer of zeste homolog 2, EZH2), catalytic subunit of polycomb-repressive complex 2 (PRC2), is overexpressed in human cancers, including colon cancer[69]. H3K27me3 is also regulated by RAS signaling pathway and further affects cyclin D1 and E-cadherin expression. Overexpression of oncogenic RAS influences global and gene-specific histone modification during the epithelial-mesenchymal transition (EMT) in Caco-2 CRC cells[70].

DNA methylation-mediated gene silencing is closely linked to histone deacetylation[71,72]. Histone methylation at key lysine residues has been shown to work in concert with acetylation and other modifications to provide a histone code that may determine heritable transcriptional states[73]. In lower eukaryotes, methylated H3K9 determines DNA methylation and correlates with transcription repression[74,75] (Figure 2). DNA methylation maintains key repressive elements of the histone code at a hypermethylated gene promoter in RKO colon cancer cells. *hMLH1*, a mismatch repair gene, is often silenced by aberrant CpG island hypermethylation in colorectal cancers[76]. Deacetylated histone H3 (deacetylated histone H3K9 and H3K14) plus methyl-H3-K9 surround the hypermethylated and inactive *hMLH1* promoter, whereas unmethylated and active *hMLH1* promoter is embedded in methyl-H3-K4 and acetylated H3 (acetylated histone H3K9 and H3K14). Promoter demethylation, gene reexpression, and finally complete histone code reversal were induced only by inhibiting DNA methyltransferases, not HDAC[77].

HDACs, HDAC Inhibitors, and Demethylating Agents

Alterations in HDACs are found in many human cancers including CRC[14,78]. The expression of HDAC1, HDAC2, HDAC3, and HDAC8 are reported to be increased in colon cancer[78-80]. Retinoblastoma (Rb) is a

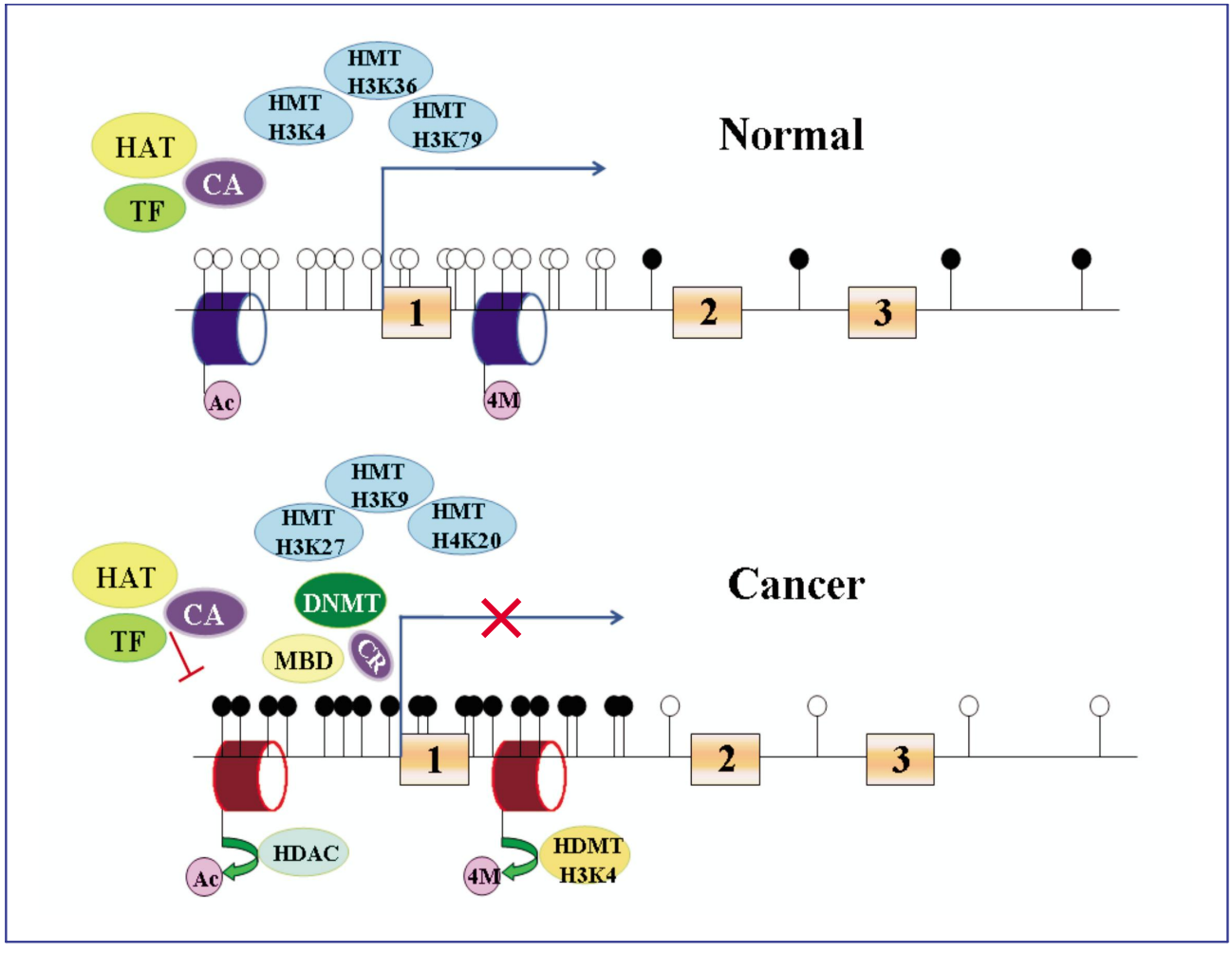

Figure 2. **Epigenetic regulation of gene expression.** Promoter region of tumor suppressor gene is unmethylated in normal cells and methylated in cancer cells. Filled cycles represent methylated DNA; unfilled cycles represent unmethylated DNA. Blue cylinder represents active histone modification; red cylinder represents repressive histone modification. 1, 2 and 3 represent exons 1, 2 and 3. HMT, histone methyltransferase; HAT, histone acetylase; DNMT, DNA methyltransferase; MBP, methyl-CpG binding protein; HDAC, histone deacetylase; TF, transcription factors; CA, co-activator; CR, co-repressor; Ac, acetylation; 4M, H3K4 methylation.

tumor suppressor that represses gene expression by modulating the architecture of chromatin. Rb recruits HDAC to E2F and cooperates with HDAC1 to repress E2F-regulated promoter of genes encoding cell cycle protein cyclin E [81,82]. HDAC1 facilitates the removal of highly charged acetyl groups from core histones, causing a tighter association between DNA and nucleosomes and preventing transcription factor from accessing to DNA. This repression is released when, on exposure to proliferative signals, G1 cyclin dependent kinases phosphorylate Rb [83]. Histone deacetylase inhibitors (HDACi), such as short chain fatty acid and butyrate, have been recognized and utilized to induce growth arrest, differentiation, and apoptosis for several decades [84,85]. Trichostatin A (TSA) was the first natural hydroxamate discovered to inhibit HDACs. Vorinostat (SAHA) is structurally similar to TSA and the first HDACi to be approved for clinical application [86,87]. A number of dietary factors with HDAC inhibitory activity and antitumor effects in the colon have been described [85,88]. To date, several clinical trials of HDACi have shown a preferential clinical efficacy. Elucidation the mechanism of HDACi may help to develop more effective therapeutic drugs [89]. HDACi are also potent sensitizers of radiation therapy in multiple cell types, including colon cancer cells.

DNA methylation is reversible under certain circumstances. It is possible to induce reexpression of silenced genes by demethylating agents in cancer cell lines. Two such agents, 5-azacytidine (Vidaza) and 5-aza-2'deoxycytidine (decitabine), have been approved for both myelodysplastic syndrome and leukemia [90,91].

MicroRNAs in Colorectal Carcinogenesis

MicroRNA (miRNA) is a kind of non-coding RNA and the length is about 18–22 nucleotide. The major role of miRNA is regulation of gene expression[92-94]. miRNAs have been found to play an important role in cancer initiation and progression[95]. Furthermore, the patterns of miRNAs expression were considered diagnostic, prognostic, and chemosensitivity markers in various types of cancer.

Loss of miR-133a and gain of miR-224 are associated with CRC tumorigenesis. Reduced expression of miR-143 and miR-145 were found in CRC and adenomatous polyps[96]. Chemically modified miR-143 (miR-143BP) has improved nuclease-resistance and may serve as RNA medicine for the treatment of CRC[97]. The level of miR-92 and miR-17-3p has been reported to be significantly higher in the plasma of colon cancer patients compared with healthy controls, and is suggested as potential markers for CRC. Stool miR-17-92 clusters and miR-135 are also significantly increased in CRC patients[98,99]. MiR-31 is up-regulated in CRC, and suppression of miR-31 may increase sensitivity to 5-FU and inhibit cell migration and invasion[100-103]. Plasma miR-141 is reported to be a novel biomarker in detecting metastatic colon cancer, and high level in plasma is associated with poor prognosis of CRC[104]. In stage II colon cancer, high level of miR-320 and/or miR-498 is correlated with progression-free survival[105]. MiR-21, miR-20a, and miR155 are also highly expressed in CRC. High level of miR-21 is associated with poor benefit from 5-FU adjuvant chemotherapy in CRC[106-108], and miR-21 expression level was considered an independent predictor of colon cancer prognosis[109].

Epigenetic regulation of miRNAs expression, including DNA methylation and histone deacetylation, was found in CRC. Frequent methylation of miR-9-1, miR-129-2, and miR-137 was observed in CRC but not in normal mucosa. Methylation of miR-9-1 was more frequent in advanced cancer and was significantly associated with regional nodal invasion, vascular invasion, and metastasis. Expression of these miRNAs was restored after treatment with 5-aza-2'-deoxycytidine (AZA, a DNA methyltransferase inhibitor) and 4-phenylbutyric acid (PBA, a HDAC inhibitor) in CRC cell lines[110,111]. Loss of miR-127 expression was found in HCT116 cells, though expression could be restored by AZA and PBA in a dose-dependent manner[112]. MiR-124a expression was down-regulated by DNA methylation in HCT116 cells compared with DKO cells (double knockout of *DNMT1* and *DNMT3b* in HCT116 cells)[113].

Conclusions

Changes of DNA methylation may serve as diagnostic, prognostic, and chemosensitive markers in CRC. The reversibility of epigenetic changes makes it possible to treat CRC with DNA methyltransferase inhibitors and histone deacetylase inhibitors. However, the available demethylating agents are globally effective. It is great beneficial for cancer treatment to develop gene-specific demethylating approaches. MiRNAs expression patterns may associate with CRC, but further study is necessary to develop diagnostic and prognostic markers. The era is coming to apply epigenetic methods to CRC therapy.

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