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Review

An overview of epigenetics and chemoprevention

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ABSTRACT

It is now appreciated that both genetic alteration, e.g. mutations, and aberrant epigenetic changes, e.g. DNA methylation, cause cancer. Epigenetic dysregulation is potentially reversible which makes it attractive as targets for cancer prevention. Synthetic drugs targeting enzymes, e.g. DNA methyltransferase and histone deacetylase, that regulate epigenetic patterns are active in clinical settings. In addition, dietary factors have been suggested to have potential to reverse aberrant epigenetic patterns. Uncovering the human epigenome can lead us to better understand the dynamics of DNA methylation in disease progression which can further assist in cancer prevention.

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1. Introduction

Epigenetics is defined as the study of heritable modifications on chromatin, without changing the nucleotide sequence of DNA, that regulates gene expression [1]. Gene silencing caused by epigenetic alternations has been associated with all stages of tumor development including initiation, progression, invasion, and metastasis. In comparison to genetic DNA coding which provides the blueprint for the manufacture of proteins to be made for a living cell, epigenetic information provides instructions on how, where, and when the genetic information should be used [1]. Although it is clear that genetic alternations, e.g. mutations, either germ line or somatic, cause cancer, aberrant epigenetic alternations are now appreciated as crucial processes in cancer development [2,3]. However, unlike genetic alternations which are almost impossible to reverse, the potential reversibility of epigenetic patterns suggests that it is a viable target for the prevention and/or treatment of cancers [2–4]. This review presents recent findings on the mechanisms causing epigenetic dysregulation and the clinical implications of epigenetic changes in cancer prevention and risk assessment.

2. Epigenetic alternations in carcinogenesis

2.1. Epigenetic patterns in normal and cancerous cells

Epigenetic mediated gene silencing can be generally divided into three related processes: DNA methylation, chromatin remodeling, and histone modification [5]. The best characterized and studied epigenetic modification is DNA methylation especially in the promoter regions of genes that regulate important cellular functions. A critical step in DNA methylation involves DNA methyltransferases (DNMTs). These enzymes transfer methyl group from *S*-adenosylmethionine (SAM) to the 5 position of the cytosine ring. As shown in Fig. 1, in general, CpG islands in promoter regions of genes in normal cells are protected against methylation. Only small portion of genes with promoter CpG islands are methylated in cancer cells. Importantly, these genes are involved in regulation of crucial cellular functions and encoding for cell cycle regulation (e.g. $p16^{INK4a}$, p15, $p14^{ARF}$), DNA repair (e.g. MLH1, GST3), tumor

Abbreviations: DNMT, DNA methyltransferase; SAM, *S*-adenosylmethionine; HAT, histone acetylase; HDAC, histone deacetylase; MBD, methyl-CpG-binding domain; IBD, inflammatory bowel disease; SFRP, secreted frizzled-related protein; APC, adenomatous polyposis coli; ACF, aberrant crypt foci; DES, diethylstilbestrol; S1P, sphingosine-1-phosphate; SphK2, sphingosine kinase 2; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin; BRBs, freeze-dried black raspberries; WIF, Wnt inhibitory factor; EGCG, (−)-epigallocatechin 3-gallate; RAR, retinoic acid receptor; NSCLC, non-small-cell lung cancer; CDH13, cadherin 13; RASSF1A, ras association domain family protein 1A; PITX2, pituitary homeobox 2; COMT, catechol-*o*-methyltransferase; CYP, cytochrome P450; NAT1, *N*-acetyltransferase type 1; SULT1A1, sulfotransferase 1A1; CIMP, CpG island methylator phenotype; BMP-4, bone morphogenetic protein-4; FGF4, fibroblast growth factor 4; CDO1, cysteine dioxygenase 1; MGMT, *O*-6-methylguanine-DNA methyltransferase; 5-FU, 5-fluorouracil

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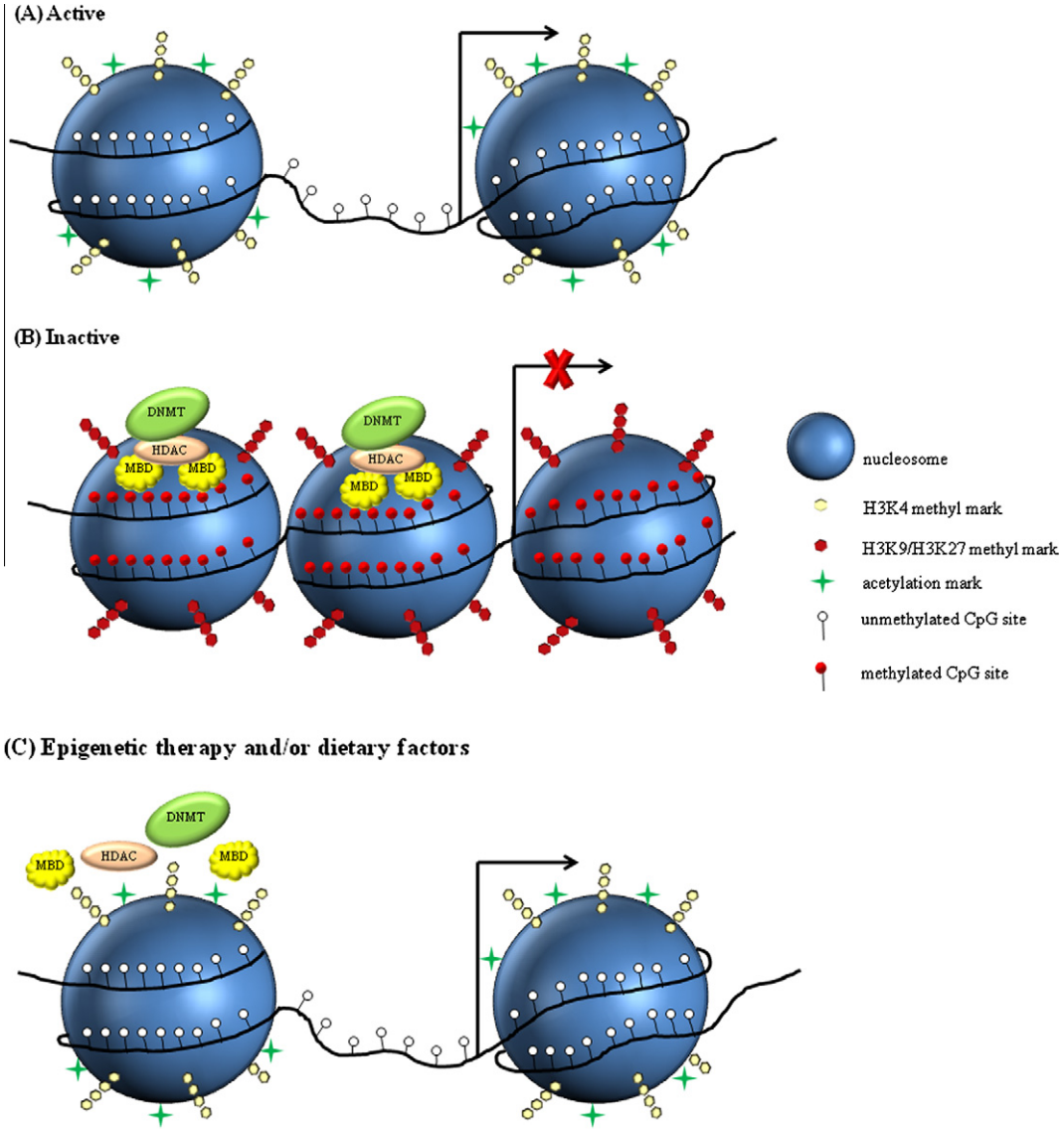

Fig. 1. Simplified diagram shows the epigenetic patterns in promoters of active and inactive genes, and epigenetic patterns of inactive genes affected by epigenetic therapy and/or dietary factors. (A) Promoters of active genes are often associated with unmethylated CpG sites (white circle), acetylation of histone (green cross), and methylation of lysine 4 on histone H3 (H3K4) (yellow hexagon), and are absence of a nucleosome (blue sphere). This configuration favors the access of proteins that activate transcription. (B) During carcinogenesis, CpG sites on the promoters of genes, frequently tumor suppressor genes, are methylated (red circle). MBD mediates transcriptional repression through binding to methylated CpG sites and interacting with HDAC and DNMT. In addition, promoters of inactive genes are associated with methylation of lysine 9 or 27 on histone H3 (H3K9 or H3K27) (red hexagon) and a nucleosome. This pattern renders the chromatin inaccessibility [3,11]. (C) Epigenetic therapy and/or dietary factors decrease DNMT, HDAC, and MBD, and increase acetylation of histones and methyl mark on H3K4 in the promoters of inactive genes. The chromatin might become more accessible to the transcription factors which then activate transcription.

suppression (e.g. BRCA1, VHL), tissue remodeling (e.g. TIMP3, E-cadherin), and hormone receptor (e.g. ESR1, ESR2) [6]. Summarizing results from studies on the correlation between the position of the methylation within a given gene and gene expression indicates that methylation degree within and/or around the promoter region is negatively associated with gene expression level [7,8]. Dose hypermethylation alone cause gene silencing? Studies have shown that DNA methylation may not initiate gene silencing and itself alone does not directly repress transcription. The constitution of chromatin surrounding a hypermethylated gene promoter contributes to the functional state of a gene [8,9]. Chromatins are composed of nucleosomes which contain histone proteins and are winded by DNA. Nucleosomes associated with active and non-methylated gene promoters are normally widely and irregularly spaced with acetylated core histone which favors the access of proteins that activate transcription. In contrast, nucleosomes are tightly and regularly spaced around heavily methylated gene promoters and contain de-acetylated histones [8,9]. This indicates that histone acetylases (HATs) and histone deacetylases (HDACs) are associated with active and silent state of genes, respectively [8,9]. In addition to acetylation, active genes are often associated with methylation of lysine 4 on the core histone H3. The promoters of silenced genes are often marked by methylation of lysine 9 or 27 on histone H3 [8,9]. Although only discovered relatively recently, methyl-CpG-binding domain (MBD) family has been shown to possess a significant role in controlling gene expression. The study on the profile of MBD occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancers indicates

that most hypermethylated promoters are occupied by MBD proteins, whereas unmethylated promoters are generally devoid of MBDs; the profile of MBD occupancy is gene and tumor type specific [10]. Further, the MBD proteins 'read' and 'interpret' the methylation moieties on DNA, and thus are critical mediators of many epigenetic processes, e.g. MBD1 binds to methylated CpGs and mediates repression through interactions with HDAC and DNMT proteins [11].

3. Dysregulation of epigenetic controls in cancer development

3.1. Epigenetic and genetic alternations in carcinogenesis

A complete loss of function of a tumor suppressor gene requires the disruption of both copies of a given gene – the two-hit hypothesis proposed by Knudson [12]. It is now accepted that both genetic changes, e.g. mutations, and epigenetic changes, e.g. promoter hypermethylation, cause silencing of genes. In familial cancers, the first hit is the germ-line mutations, and both genetic and epigenetic changes can cause the second hit in which the cells lose both copies of a given gene [8]. In somatic cancers, the loss of one or both alleles of a tumor suppressor gene by promoter hypermethylation has been observed in non-familial cancers [8,12].

The number of tumor suppressor genes affected by epigenetic inactivation equals or exceeds the number that is inactivated by mutations [8,13]; the number of mutations in a cancer cell has been estimated to be approximately 80 whereas aberrant methylation of promoter CpG islands can reach several hundreds to thousands [4]. Although the frequency of mutations of a specific gene in non-cancerous tissues is very low (0.0001–0.1% of cells), aberrant methylation can be up-to 10% of cells [4]. Mutations are generally induced by mutagenic chemicals, radiations, and oxygen radicals whereas chronic inflammation and aging might be associated with the induction of aberrant methylation [4,14]. For example, high levels of aberrant promoter methylation of tumor suppressor genes have been detected in *Helicobacter pylori*-infected gastric mucosa from healthy volunteers; these high degrees of methylation are similar with mucosa from *H. pylori*-positive gastric cancer patients [14]. Recently, aberrant promoter methylation of genes in Wnt signaling pathway has been observed in inflammatory bowel disease (IBD) associated neoplasia [15]. Furthermore, aging has been suggested as a factor causes methylation and age-related methylation seems to be gene and tissue specific [16]. Age-related methylation has been suggested as a risk factor for human colorectal [16] and prostate [17] cancers. In an attempt to study if age-related methylation is restricted to humans because of the relatively long lifespan or is common physiologic aging event, a recent study compares gene methylation of gastrointestinal tract (esophagus, stomach, small intestine, cecum, and large intestine), lung, kidney, liver, and spleen tissues from mice at 3-mo-old and 35-mo-old [18]. Age-related methylation is observed with a strong tissue-specificity. The largest differences of age-related increase and decrease in methylation are seen in small intestine and cecum; variation of age-related methylation is less frequent and to a lesser degree in lung, liver, and spleen than the changes observed in small intestine. Therefore, epigenetic deregulation is a common feature of aging in mammals [18]. Because genes with increased methylation in aging tissue are frequently hypermethylated in cancer, especially in colorectal cancer [18], it is reasonable to speculate that age-related alternations in methylation may result from accumulation of errors in cell proliferation and/or environmental exposures; gene expression diversity caused by mixture of graded methylation may trigger selective growth such as tumor growth [9,18]. However, the methylation of some genes, e.g. secreted frizzled-related protein 2 (SFRP2) and genes associated with DNA repair, are not affected by aging; rather, their methylation levels are increased with the progression of cancer. This is especially true of colorectal cancer in human [16,19]. Factors other than aging must also be important in the regulation of methylation. Genetic changes and their interaction with epigenetic and environmental factors may contribute to part of the originality of tumor growth.

Is there a connection between epigenetic and genetic deregulation? A recent study investigating the relationships between gene copy number alterations and DNA methylation profiles in a case series of pleural mesotheliomas has shown that the overall extent of copy number alteration is significantly associated with DNA methylation profile; this association has been suggested to be partially attributable to prevalent allele loss at the DNA methyltransferase gene DNMT1 [20].

Using Wnt signaling pathway as an example, Baylin and Ohm have provided a new mechanism from which abnormalities arise in colorectal tumorigenesis [2,21]. Wnt pathway deregulation can lead to the expansion of stem and progenitor cell populations. Mutations of adenomatous polyposis coli (APC), a tumor suppressor gene in Wnt pathway, are often found in colorectal cancer and are thought to be responsible for the initial progression of this cancer [22]. Promoter methylation, however, has been detected in negative Wnt regulators, e.g. SFRPs, in early stage lesion of colorectal cancer, aberrant crypt foci (ACF), in which most ACF cells do not contain APC mutations and methylation of SFRP-gene promoter persists in colorectal adenomas [21]. Interestingly, in the same study, the authors have shown that restoration of SFRP function in colorectal cancer cells attenuates Wnt signaling even in the presence of downstream APC mutations. Therefore, the epigenetic loss of SFRP function occurring early in colorectal cancer progression may lead to constitutive activation of Wnt signaling thereby complement downstream mutations in the evolution of colorectal cancer. Gene silencing can lead to oncogenic pathway activation and addiction of precancerous and cancerous cells to oncogenic pathways [2].

3.2. Environmental factors that dysregulate DNA methylation

Environmental agents including hormone, cigarette smoke, metals, etc., affect tumor development and have been reported to alter DNA methylation pattern [23]. We use environmental estrogens in the modulation of DNA methylation as an example.

Studies have shown that abnormal epigenetic silencing of genes occurs most frequently during the early stage, precancerous stage, of the neoplastic process, although it can occur any stage of tumor development [2,24]. The exposure to a wide variety of xenobiotics, e.g. diethylstilbesterol (DES), during critical period of mammalian development can persistently alter the methylation pattern leading to aberrant gene expression [25]. Perinatal exposure of DES, one of the environmental hormones possessing estrogenic activity, has been found to induce epithelial tumors of the uterus in mice [26]. Women who exposed to DES for the purpose of preventing miscarriage during the first three months of pregnancy have changes in the tissues and/or structure of their uterus, cervix or vagina which put them at high-risk of developing cancers from these organs later in life [25,27]. Recently, it has been demonstrated that xenoestrogen induces epigenetic repression of microRNA-9-3, playing a role in the p53-related apoptotic pathway, in cultured progenitor-containing mammospheres [28]. In addition, estrogen-mediated epigenetic repression has been shown to be associated with large chromosomal regions through DNA looping; together with the acquisition of DNA methylation and repressive chromatin modifications at the 16p11.2 loci [29]. An inflexible DNA scaffold may be a novel determinant used by breast cancer cells to reinforce estrogen-mediated repression [29]. Nutritional factors and stress may also alter DNA methylation and modulate cancer and other chronic diseases [25].

3.3. Regulators of enzymes involved in epigenetic regulation

3.3.1. DNMTs

Using cultured human lung cancer cells, Lin et al. have suggested that dysregulation of p53/Sp1 leads to DNMT1 overexpression in lung cancer. In the same study, in lung cancer patients, overexpression of DNMT1 is associated with p53 mutation and high expression of Sp1 protein; patients with overexpression of both DNMT1 and Sp1 proteins have poor prognosis [30]. These results identify a core mechanism of transcriptional deregulation on DNA methyltransferase that promotes lung tumorigenesis and progression.

Mice carrying a truncated DNMT3B isoform, DNMT3B7, exhibit altered embryonic development, including lymphopenia, craniofacial abnormalities, and cardiac defects, similar to Dnmt3b-deficient animals but rarely develop cancer [31]. However when these transgenic mice are bred with Eμ-Myc transgenic mice, the frequency of mediastinal lymphomas increases in Eμ-Myc/DNMT3B7 animals. Mediastinal lymphomas from these mice have more chromosomal rearrangements, increased global DNA methylation levels, and more locus-specific perturbations in DNA methylation pattern in comparison with Eμ-Myc lymphomas. This is the first in vivo model of cancer-associated DNA methylation changes suggesting that truncated DNMT3B isoforms, commonly found in cancer cells, may contribute to the re-distribution of DNA methylation characterizing most if not all human tumors.

3.3.2. HDACs

The function of HDACs is the removing of acetyl groups from histones which can induce chromatin condensation and transcriptional repression [32]. Although their function has been elucidated, the environmental stimuli that change nuclear HDAC function remain largely unknown. New evidence from a recent study has demonstrated that lipid sphingosine-1-phosphate (S1P) inhibits the activity of HDAC1 and HDAC2 [32]. Both S1P and sphingosine kinase 2 (SphK2), the enzyme that synthesizes S1P, are assembled in co-repressor complexes containing HDAC1 and HDAC2. S1P is among the few endogenous HDAC inhibitors that is synthesized in the nucleus in response to extracellular stimulation and is the first nuclear lipid associated with an epigenetic modification [32].

4. Epigenetics in cancer prevention

Although demehtylation of tumor suppressor genes may have a beneficial effect, the decrease of methylation of oncogenes which reactivate these genes may have an adverse effect. However, it has been shown that hypomethylation agents exert therapeutic activities related to the fact that tumor cells are much more dependent on gene silencing to maintain their phenotype than normal adult cells. Thus, the overall effect of decreasing methylation appears to be positive [33]. We present the recent findings on epigenetic prevention and/or therapy using synthetic drugs and dietary factors. The clinical implications of global DNA methylation and epigenetic changes in cancer prevention are discussed as well.

4.1. Synthetic drugs

4.1.1. DNA methylation inhibitors

There are two kinds of DNA methylation inhibitors: nucleoside and non-nucleoside analogues (Table 1). Nucleoside analogues have a modified cytosine ring, e.g. carbon at the 5 position of the ring is replaced by nitrogen in drug 5-azacytidine (Fig. 2). DNMTs transfer methyl group from SAM to the 5 position of the cytosine ring. These drugs inhibit methylation when they are integrated into DNA and block the release of DNMTs by forming a covalent complex with these enzymes [34]. They have been found to have clinical activities especially on hematopoietic malignancies [35–38]. Although aberrant promoter methylation is corrected by DNA methylation inhibitors, once the drug being stopped, the aberrant promoter methylation and gene silencing return [8]. Therefore, the prolonged use of the drug is necessary for the purpose of cancer therapy and even more important for the purpose of prevention. The toxic effect probably caused by their presence in the DNA has been reported. Because these drugs need to be incorporated into DNA to have effects, the quiescent cells, e.g. stem cells, can be less responsive [2]. Non-nucleoside analogues are

Table 1
Effects of DNA methylation and histone-deacetylase inhibitors on patients.

Drug	Clinical trial and targeted disease	Findings	Ref.
DNA methylation inhibitors – nucleoside analogues			
5-Azacytidine (azacitidine)	Phase I, II, III Myelodysplastic syndrome subtypes	The first demethylating agent approved for treatment of myelodysplastic syndrome.	[35]
5-Aza-2′-deoxycytidine (decitabine)	Phase I, II, III Hematopoietic malignancies	Better demethylation response was observed in low dose regimen than high does group.	[36–38]
DNA methylation inhibitors – non-nucleoside analogues			
Hydralazine	Phase I Cervical cancer	Demethylated and reactivated tumor suppressor genes without affecting global methylation. There is no dose-related effect.	[39]
MG98	Phase I Solid tumors	– Antisense oligonucleotide of human DNMT1 – No evidence of antitumor activity was observed.	[40]
Histone-deacetylase inhibitor – short-chain fatty acids			
Combination of valproic acid and all-trans retinoic acid	Phase I Acute myeloid leukemia in elder patients	Complete marrow response was observed in 3 patients, including 1 complete remission. Two additional patients had hematologic improvement.	[41]
Histone-deacetylase inhibitor – hydroxamic acids			
Suberoylanilide hydroxamic acid (SAHA)	Phase I Hematological and solid tumors	– Increase of acetylated histones – Tumor regression in four (2 lymphoma and 2 bladder) patients	[42]
LBH589	Phase I Hematological tumor	Increase of H2B and H3 acetylation	[43]
Histone-deacetylase inhibitor – cyclic tetrapeptides			
Depsipeptide (FK228)	Phase I chronic lymphocytic leukemia and acute myeloid leukemia	Effectively inhibits HDAC but there was no partial or complete response.	[44]

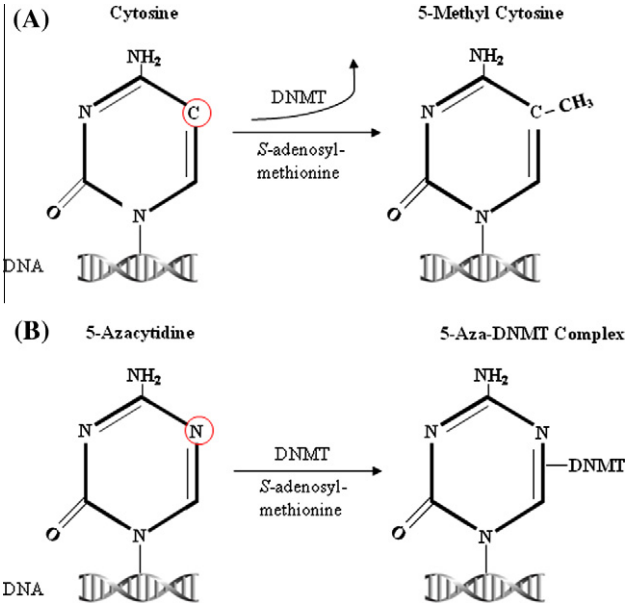

Fig. 2. Methylation of cytosine by DNMTs and inhibiting methylation with 5-azacytidine. (A) Using *S*-adenosylmethionine as the methyl group (CH_3) donor, DNMTs catalyze the methylation of the 5 position of the cytosine ring. (B) 5-Azacytidine, a cytosine analogue, is a hypomethylation drug which can block this reaction by replacing cytosine and acts as a direct and irreversible inhibitor of DNMTs [8].

small molecular inhibitors. They directly bind to the catalytic region of DNMTs or are antisense oligonucleotide of DNMTs which suppress the translation. Both of which lead to DNA demethylation without integration into DNA [3]. These groups of drugs however are less and/or not active in solid tumors [39,40].

4.1.2. HDAC inhibitors

HDAC inhibitors include short-chain fatty acid, hydroxamic, and cyclic tetrapeptides (Table 1). They contain different functional groups but all inhibit HDAC leading to the accumulation of acetylation in histones [3]. In combination with all-trans retinoic acid, valproic acid produces complete marrow response in elder patients with acute myeloid leukemia [41]. Suberoylanilide hydroxamic acid (SAHA) is probably the most successful HDAC inhibitor. SAHA can bind to a zinc ion in the catalytic domain of HDAC resulting in inhibition of the enzyme [42]. SAHA treatment produces good results and improves symptoms in patients with hematological and solid tumors [42]. LBH, a cinnamic hydroxamic acid analogue histone deacetylase inhibitor, increases H2B and H3 acetylation in patients with refractory hematologic malignancies [43]. Further, depsipeptide (FK228) effectively inhibits HDAC but does not produce partial or complete response in chronic lymphocytic leukemia and acute myeloid leukemia [44].

4.1.3. Combination of DNA methylation and histone deacetylase inhibitors

DNA methylation inhibitors and HDAC inhibitors have been shown to have synergistic effects and they work together in mediating the function of genes in cultured cells and rodents [3]. Trichostatin A (TSA), a HDAC inhibitor, can not transcriptionally reactivate hypermethylated genes in tumor cells whereas TSA alone can up-regulate the expression of non-methylated genes [45]. TSA treatment results in robust re-expression of methylated gene only after the presence of low dose 5-aza-2′-deoxycytidine which causes minimal demethylation and slight gene reactivation. These results suggest that methylation seems to be the dominant component that locks genes in the silence state. The combination of 5-aza-2′-deoxycytidine and TSA is being tested in clinical setting [24].

4.2. The modulation of dietary factors on epigenetics

As mentioned above, using SAM as the methyl group donor, DNMTs catalyze the methylation of the 5 position of the cytosine ring [8]. Dietary factors, e.g. folate, vitamin B12, vitamin B6, vitamin B2, methionine, choline, and alcohol, etc., may influence the supply of methyl groups available for the formation of SAM [46]. Dietary factors may also affect the activity of DNMTs which in turn modify the utilization of methyl groups. The effects of dietary factors on promoter methylation status and on enzymes involved in regulation of epigenetics, e.g. DNMTs, HATs, etc., are depicted in Table 2. Results from our recent study demonstrate the DNA demethylation capabilities of freeze-dried black raspberries (BRBs) in human colorectal cancer patients; average 4 weeks of dietary intervention of BRBs inhibits DNMT1 protein expression and demethylates promoters of tumor suppressor genes, e.g. $p16^{INK4a}$, negative Wnt pathway regulators, SFRP2 and Wnt inhibitory factor 1 (WIF1), leading to a reduction of cell proliferation and protective modulation of Wnt pathway downstream genes, e.g. β-catenin, E-cadherin [47]. Micronutrients, e.g. isoflavones, flavonols, and catechins, etc., have received much attention due to their ability to influence activities of chromatin-modifying enzymes [48]. (−)-Epigallocatechin 3-gallate (EGCG) from green tea has been demonstrated to inhibit DNMTs and reactivate tumor suppressor genes in cultured human cancer cell lines including esophageal [49], lung [50], and breast [51]. Similarly, genistein from soybean

Table 2
Effects of dietary factors on promoter methylation status and on enzymes associated with the regulation of epigenetics.

Dietary factor	System	Gene demethylated	Enzymes examined	Ref.
Freeze-dried black raspberries	Phase I clinical trial: 20 colorectal cancer patients	SFRP2, SFRP5, WIF1, PAX6a	↓ DNMT1	[47]
EGCG	Human esophageal squamous cell carcinoma cell line KYSE510	RARβ, MGMT, hMLH1, p16	↓ DNMTs	[49]
EGCG	Human lung cancer cell lines H460 and A549	WIF1	–	[50]
EGCG	Human breast cancer cell line MCF7	RARβ	↓ DNMT1	[51]
Genistein	Human esophageal squamous cell carcinoma cell line KYSE510	RARβ, MGMT, p16	↓ DNMT1	[52]
Genistein	Human prostate cancer cell lines LNCaP and PC3	–	↑ Acetylated histones 3, 4, and H3/K4 at the p21 and p16 transcription start sites	[53]
Genistein	Human renal cell carcinoma (RCC) cell lines A498, ACHN, and HEK-293	BTG3	↓ DNMTs ↓ MBD2 ↑ HAT	[54]
Caffeic acid, chlorogenic acid (coffee polyphenols)	Human breast cancer cell lines MCF7 and MDA-MB-231	RARβ	↓ DNMT1	[55]

modulates enzymes that regulate DNA methylation and histone acetylation, and reactivates tumor suppressor genes in esophageal [52], prostate [53], and renal [54] cancer cells. Catechol-containing coffee polyphenols, caffeic acid, and chlorogenic acid demethylate retinoic acid receptor β (RARβ) possibly through the inhibition of DNMT1 in human breast cancer cells [55]. Therefore it is possible that bioactive food components possess DNA demethylation activity and influence DNA methylation pattern which in turn regulate gene expression and delay cancer development [48,56].

4.3. Global DNA methylation

Although global DNA hypomethylation has been closely linked to chromatin restructuring and nuclear disorganization in cancer cells leading to chromosomal instability [3,33], the effects of global DNA hypomethylation on tumor development in animals have been controversial. Mice carrying a hypomorphic Dnmt1 allele, which reduces Dnmt1 expression to 10% of wild-type level, exert substantial genome-wide hypomethylation in all tissues. These mutant mice are runty at birth and at 4–8 months of age they develop aggressive T cell lymphomas that display a high frequency of chromosome 15 trisomy [57]. Apc(Min/+) mice carrying Dnmt1 hypomorphic alleles have reduced genomic methylation and elevated incidence of microadenomas; however, the incidence and growth of macroscopic intestinal tumors in the same animals are strongly suppressed [58]. In contrast to the overall inhibition of intestinal tumorigenesis in these animals, hypomethylation causes development of multifocal liver tumors [58]. These results clearly demonstrate the opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. In humans, a study examining global methylation in cancer cell lines, including breast, central nervous system, colon, leukemia, liver, lung, ovary and prostate, has shown that 85% of tested cell lines (51 out of 60) is globally hypomethylated [59]. Interestingly, the same study has demonstrated that global methylation in colorectal tumors is highly variable and increased, no change or decreased global methylation is observed in comparison with their adjacent normal tissues. The global hypomethylation is partially reversed in tumor with microsatellite instability which may reflect alternative progression pathways in tumors [59]. Therefore, the concept of global DNA hypomethylation in cancers might be too simplified and needs to be further investigated.

The DNA global methylation changes caused by DNA methylation inhibitors or dietary factors have been studied in humans. For example, patients, with chronic myelogenous leukemia who are resistant to imatinib mesylate, are treated with low dose decitabine, a DNA methylation inhibitor. The decreases of DNA global methylation, measured by LINE1 methylation, in responders and non-responders are 14.5 ± 3.0% and 26.8 ± 2.7%, respectively; the resistant cells can withstand more hypomethylation [37]. Further, a study on the prevention of colorectal adenomas in 388 patients after 3 years of folic acid supplementation (1 mg/day) reports that overall the global hypomethylation is not influenced by folic acid but difference between left and right colon is observed in which right side of the normal colon has significantly lower mean LINE-1 methylation levels than those on the left [60].

4.4. Clinical implications of epigenetic changes in cancer prevention

Chemoprevention is the administration of one or more chemical entities, either as individual drugs or as dietary supplements, to prevent the initiation of premalignant lesions or their progression to cancer or cancer recurrence [61]. Therefore, cancer prevention can be to prevent cancer in high-risk populations, e.g. frequent exposure to carcinogens or family history of cancer. It also can be to prevent cancer from recurrence and metastasis after surgery and chemotherapy, or to prevent cancer from developing resistance to chemotherapeutic drugs. Using epigenetic changes as markers for cancer prevention has being greatly advanced in the past few years because numbers of human studies have been conducted. Few examples are given below.

Epigenetic changes have been detected in a high-risk population. For example, $p16^{INK4a}$ promoter methylation has already been found in smokers with bronchial epithelial atypia, classified as pre-neoplastic pathologically; increased $p16^{INK4a}$ methylation is detected in sputum from 3 of 7 patients with cancer and 5 of 26 cancer-free individuals at high risk [62]. Can epigenetic changes be used to predict recurrence or metastasis? A study in the US investigating the methylation of genes in tumor and lymph nodes from patients with recurrent and non-recurrent non-small-cell lung cancer (NSCLC) has reported that $p16^{INK4a}$, cadherin 13 (CDH13), ras association domain family protein 1A (RASSF1A), and APC in tumors and in histologically tumor-negative lymph nodes are associated with tumor recurrence; the methylation of these genes in histologically normal regional lymph nodes probably indicates the presence of microscopically undetectable micrometastases which can be detected by gene methylation [63]. Similar findings from a study in Japan targeting patients with NSCLC have demonstrated that $p16^{INK4a}$ and CDH13 methylation are associated with poor prognosis [64,65]. Further, methylation of pituitary homeobox 2 (PITX2) has been suggested to predict risk of metastasis and distant recurrence in steroid hormone receptor-positive and node-negative breast cancer patients who received tamoxifen as their only systemic adjuvant therapy [66].

In addition, epigenetic changes have been associated with drug resistance and chemotherapy outcome. For instance, the application of MGMT methylation as a predictor of response to alkylating agents has been confirmed in two human clinical trials [67,68]. These agents cause cell death by cross-linking of double-stranded DNA which can be inhibited by *O*-6-methylguanine-DNA methyltransferase (MGMT) [67]. Inactivation of MGMT gene by promoter methylation which comprises DNA repair has been associated with longer survival in glioblastoma patients treated with carmustine [67] or temozolomide [68]. Further, methylation patterns of genes coding for drug-metabolizing enzymes, e.g. catechol-*o*-methyltransferase (COMT), cytochrome P450 1A1 (CYP1A1), CYP2D6, *N*-acetyltransferase type 1 (NAT1), and sulfotransferase 1A1 (SULT1A1), have been examined in tissues from tamoxifen-resistant breast cancers. Methylation of NAT1 is significantly higher in tamoxifen-resistant tissues accompanying with lower NAT1 mRNA expression, and higher Ki67 and cyclin D1 protein expression [69]. In patients with metastatic and microsatellite stable colorectal carcinomas, CpG island methylation and CpG island methylator phenotype (CIMP)-positive are associated with worse survival [70]. Another trial has reported that DNA methylation markers, PITX2, bone morphogenetic protein-4 (BMP4), fibroblast growth factor 4 (FGF4), and C20orf55, can be used to predict outcome in lymph node-positive and HER-2-negative breast cancer patients treated with anthracycline-based chemotherapy [71]. In a similar patient population, anthracycline treated, estrogen receptor-positive, and lymph node-positive breast cancer patients, cysteine dioxygenase 1 (CDO1) promoter methylation has been suggested as a biomarker for outcome prediction in these patients [72]. Interestingly, however, $p16^{INK4a}$ methylation is associated with longer survival and a longer recurrence-free period in gastric cancer patients who received 5-fluorouracil (5-FU)-based adjuvant chemotherapy [73]. Many more investigations are necessary to understand the large scale methylation pattern changes caused by different chemotherapeutic drugs and heterogeneous nature of tumors needs to be taken into consideration.

Chemoprevention and chemotherapeutic drugs, and dietary factors can lead to methylation pattern changes in a group of genes

rather than a single or few genes. Our recent review article summarizes the technologies of quantitative methylation detection providing high-throughput screening with increased sensitivity. It is now possible to globally map the DNA cytosine methylation at single base resolution, e.g. genome-scale sequencing [74]. New genome-wide technologies might be applied to central questions such as how cells from the same person differ in epigenetics over time and environmental exposure. For example, a recent study uses genome-wide methylation analysis to study age-related methylation changes in mice and discovers the possible epigenetic component that changes with aging [18]. These technologies can be applied to study the involvement of epigenetic components in common aging events, e.g. insulin resistance, neuron degeneration, etc., which might be caused by the environmental exposures and/or their interaction with genetic and epigenetic factors [18]. Finally, they can be used to discover new genes whose methylation statuses are associated with risk assessment, chemotherapeutic outcome, recurrence, and metastasis.

5. Conclusions

Cancer prevention might be achieved by understanding the initiation and maintenance of epigenetic gene silencing which ultimately reverses the process of gene silencing. Epigenetic therapy and dietary factors regulate enzymes controlling the functional state of genes which might turn chromatin to be more accessible to transcriptional factors and activate transcription (Fig. 1C). The best understood epigenetic gene silencing is gene promoter hypermethylation. The profiling of DNA methylomes might provide new insights into the regulation and dynamics of DNA methylation in genomes for cancer prevention. Hypomethylation drugs are helping patients live longer with fewer side effects than conventional cytotoxic therapy [33]. Because it is a relatively easy and a non-invasive method to obtain DNA from small amount of blood, stool, or sputum for the detection of CpG methylation, DNA methylation markers might be clinically valuable for cancer risk assessment, early detection, and prognosis.

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