Nutritional “Omics” Technologies for Elucidating the Role(s) of Bioactive Food Components in Colon Cancer Prevention

Nutritional Epigenetics: Impact of Folate Deficiency on DNA Methylation and Colon Cancer Susceptibility1,2

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ABSTRACT The inheritance of information based on gene expression levels is known as epigenetics, as opposed to genetics, which refers to information transmitted on the basis of gene sequence. In contrast to genetic changes observed in cancer, epigenetic changes are gradual in onset and are progressive, their effects are dose-dependent and are potentially reversible. These observations present new opportunities in cancer-risk modification and prevention using dietary and lifestyle factors and potential chemopreventive drugs. In this regard, folate, a water-soluble B vitamin, has been a focus of intense interest because of an inverse association between folate status and the risk of several malignancies (in particular, colorectal cancer) and of its potential ability to modulate DNA methylation. DNA methylation is an important epigenetic determinant in gene expression, in the maintenance of DNA integrity and stability, in chromosomal modifications, and in the development of mutations. Aberrant patterns and dysregulation of DNA methylation are mechanistically related to colorectal carcinogenesis. Folate plays an essential role in one-carbon transfer involving remethylation of homocysteine to methionine, thereby ensuring the provision of S-adenosylmethionine, the primary methyl group donor for most biological methylation reactions. The portfolio of evidence from animal, human, and in vitro studies suggests that the effects of folate deficiency and supplementation on DNA methylation are gene and site specific, and appear to depend on cell type, target organ, stage of transformation, and the degree and duration of folate depletion. J. Nutr. 135: 2703–2709, 2005.

KEY WORDS: • folate • colorectal cancer • epigenetics • DNA methylation

Epigenetics

The inheritance of information based on gene expression levels is known as epigenetics, as opposed to genetics, which refers to information transmitted on the basis of gene sequence (1). The field of epigenetics therefore is the study of modifications of DNA and DNA-binding proteins and histones that alter the structure of chromatin without altering the nucleotide sequence of DNA; some of these modifications may be associated with heritable changes in gene function (2). Silencing is a subset of epigenetics, whereby gene expression and function are permanently lost. Although the involvement of RNA interference in epigenetic silencing has been clearly shown in yeasts and plants, it is not well understood in mammalian cells yet. Thus, in mammals, 2 systems, including DNA methylation and histone modifications (acetylation, methylation, ubiquitylation, and phosphorylation), are used to initiate and sustain epigenetic silencing (2).

DNA methylation

DNA methylation is an important epigenetic determinant in gene expression (an inverse relation), in the maintenance of DNA integrity and stability, in chromatin modifications, and in the development of mutations (3). The pattern of methylation at cytosine residues in the cytosine-guanine (CpG)4 sequences is a heritable, tissue- and species-specific, postsynthetic modification of mammalian DNA (3). Three to
4% of all cytosines in the human genome are methylated, and the resulting 5-methylcytosines make up 0.75–1% of all nucleotide bases in normal human DNA (1). CpG sites are unevenly distributed in the mammalian genome; vast stretches of sequence (~99% of the genome) are deficient for CpGs, and these are interspersed by CpG clusters called CpG islands (Fig. 1) (1). Seventy to 80% of all CpG sites in human DNA are normally methylated (1). However, this methylation occurs primarily in the bulk of the genome where CpG density is low, including exons, noncoding regions, and repeat DNA sites, and allows correct organization of chromatin in active and inactive states (4). Methylation of the CpG-depleted bulk of the genome facilitates transcriptional silencing of noncoding regions, which prevents the transcription of repeat DNA elements and parasitic DNA sequences (endogenous retroviruses and transposons that account for >35% of the human genome) (4). Parasitic DNA elements represent a significant threat to the structural integrity of the genome by promoting chromosome rearrangements or translocation or by directly disrupting genes or causing transcriptional interference (5).

By contrast, about 1% of the genome consists of CpG-rich areas clustered in small stretches of DNA termed “CpG islands,” which are defined as a 500-base pair window with a G:C content of at least 55% and an observed overexpected CpG frequency of at least 0.65 (Fig. 1) (6). These motifs span the 5’ end of approximately half of the human genes, including promoter, untranslated region, and exon 1 (6). Most CpG islands are unmethylated in normal cells, thereby allowing transcription, with the exception of CpG islands on the inactive X chromosome in females and silenced alleles of imprinted genes (5). When methylated, CpG islands cause stable heritable transcriptional silencing.

DNA methylation is a dynamic process between active methylation, mediated by CpG methyltransferases (DNMT1, 3a, 3b) using S-adenosylmethionine (SAM) as the methyl donor, and removal of methyl groups from 5-methylcytosine residues by both passive and active mechanisms including demethylation by a purported demethylase (MBD2) (7).

**DNA methylation and cancer**

In contrast to methylated CpG sites in the CpG-poor bulk of the genome and unmethylated CpG islands in normal cells, cancer cells simultaneously harbor widespread loss of methylation in the CpG-depleted regions where most CpG dinucleotides should be methylated and gains in methylation of CpG islands in gene promoter regions (Fig. 1) (3,4).

Global hypomethylation is an early, and consistent, event in carcinogenesis (3,4). Global hypomethylation of the coding and the noncoding regions and demethylation of repetitive DNA sequences contribute to the development of cancer through the following mechanisms: chromosomal instability, increased mutations, reactivation of intragenomic parasitic sequences that could be transcribed and moved to other sites where they could disrupt normal cellular genes, mitotic recombination leading to loss of heterozygosity and promotion of rearrangements, aneuploidy, loss of imprinting, and upregulation of protooncogenes (1). However, animal studies have shown that genomic demethylation may protect against some cancers (e.g., intestinal tumors) (8,9) but may promote chromosomal instability and increase the risk of cancer in other tissues (e.g., lymphoma, sarcoma) (10,11).

Methylation at promoter CpG islands is an important mechanism of silencing transcription in carcinogenesis; the affected genes are silenced, and their function is stably lost in a clonally propagated fashion (1,4,12–14). Many genes inactivated by promoter CpG methylation in carcinogenesis have classic tumor-suppressor function or play critical roles in cell-cycle control, repair of DNA damage, apoptosis, differentiation, angiogenesis, metastasis, growth-factor response, drug resistance, and detoxification (4). Promoter CpG islands of over 60% of tumor suppressor and mismatch repair genes have been observed to be methylated in cancer (4).

Another means by which CpG methylation may contribute to carcinogenesis is the hypermutability of methylated cytosine. CpG dinucleotides within certain genes are not only the sites of DNA methylation but also mutational hot spots for human cancers (13). The majority of mutations observed in CpG sites are cytosine-to-thymine transitions mediated by the spontaneous deamination of 5-methylcytosine to thymine, by the enzymatic deamination of 5-methylcytosine to thymine by DNMT, and by the enzymatic deamination of unmethylated cytosine to uracil and subsequent methylation of uracil to thymine by DNMT (13). CpG sites have been shown to act as hot spots for germline mutations, contributing to 30% of all point mutations in the germ line, and for acquired somatic mutations that lead to cancer (5). For example, methylated CpG sites in the p53 tumor suppressor coding region contribute to as many as 50% of all inactivating mutations in colorectal cancer and to 25% of cancers in general (5).

Increased DNMT1, 3a, and 3b, and decreased MBD2 expression and activity have been observed in many human cancers (7). DNMT1 may promote tumorigenesis by its link to activation of the oncogenic ras signaling pathway, by increasing cellular proliferation by binding to proliferating cell nuclear antigen and by reducing cellular p21, a member of the cyclin-dependent kinase (CDK) inhibitor family that inhibits a wide range of cyclin-CDK complexes involved in G1 and S phase progression, by inhibition of p53-dependent apoptosis, and by promoter CpG island methylation of tumor suppressor and mismatch repair genes (7).
Folate and colorectal cancer risk

Epidemiologic studies over the past decade have suggested an inverse association of folate status (assessed by dietary folate intake or by the measurement of blood folate levels) with the risk of cancer of lungs, oropharynx, esophagus, stomach, colorectum, pancreas, cervix, ovary, prostate, and breast, and the risk of neuroblastoma and leukemia (15,16). Although the results from epidemiologic and clinical studies are not uniformly consistent, the portfolio of evidence indicates ~20–40% reduction in the risk of colorectal cancer in subjects with the highest dietary intake or blood levels of folate compared with those with the lowest intake or blood levels (16–18). Several small intervention studies have demonstrated that folate supplementation can improve or reverse surrogate end-point biomarkers of colorectal cancer (16,17), and some epidemiologic studies have shown a beneficial effect of multivitamin supplements containing ≥400 µg folic acid on colorectal cancer risk and mortality (19–21). The data from animal studies generally support a causal relation between folate depletion and colorectal cancer risk and an inhibitory effect of modest levels of folate supplementation on colorectal carcinogenesis (17). However, animals studies have also shown that folate supplementation may increase colorectal cancer risk and accelerate colorectal cancer progression if too much is given or if it is provided after neoplastic foci are established in the colorectum (17,22).

Folate and DNA methylation

Folate, in the form of 5-methyltetrahydrofolate, is involved in remethylation of homocysteine to methionine, which is a precursor of SAM, the primary methyl group donor for most biological methylation reactions, including that of DNA (Fig. 2) (23). After transfer of the methyl group, SAM is converted to S-adenosylhomocysteine (SAH), a potent inhibitor of most SAM-dependent methyltransferases (Fig. 2) (23). Cravo and Mason (24) first proposed that a mechanism by which folate deficiency enhances colorectal carcinogenesis might be through an induction of genomic DNA hypomethylation based on the biochemical function of folate in mediating one-carbon transfer and on evidence from animal experiments that demonstrated methyl group donor deficiency induced DNA hypomethylation.

Effect of methyl group deficiency and supplementation on DNA methylation in rodents

Diets deficient in methyl group donors (choline, folate, methionine, and vitamin B-12) are associated with spontaneous and chemically induced development of hepatocellular carcinoma in rats (15,16). Diets deficient in different combinations of methyl group donors have been consistently observed to induce genomic and protooncogene (c-myc, c-fos, c-Ha-ras) DNA hypomethylation and elevated steady-state levels of corresponding mRNAs and site-specific p53 hypomethylation in rat liver (15,16). Methyl group donor deficiency has also been shown to upregulate DNMT in rat liver (15,16). However, a recent study reported that a diet deficient in choline, methionine, and folate, which caused a 30% increase in DNA strand breaks, did not induce a significant degree of genomic DNA hypomethylation in rat colon, suggesting that the colorectum may be resistant to the hypomethylating effect of methyl group deficiency (25).

Recent animal studies using viable yellow agouti (A^vy) mice have unequivocally demonstrated that maternal dietary methyl group supplementation with a modest amount of folic acid, vitamin B-12, choline, and betaine permanently alters the phenotype of the offspring via increased methylation at the promoter CpG site of the agouti gene (26–28). Furthermore, Waterland and Jirtle (28) have shown that the methylation status of the promoter CpG region of the agouti gene was highly correlated with the methylation status of the adjacent transposon gene. This indicates that there is a localized epigenetic instability in methylation that arises from an interaction between the transposon and its nearby genetic region, and that genes that manifest a transposon region adjacent to a promoter region of DNA could be influenced by in utero exposure to a methyl supplemented diet.

Effect of isolated folate deficiency on genomic DNA methylation in rodent liver and colon

Although isolated folate deficiency has been shown to reduce SAM levels and SAM to SAH ratios and increase SAH concentrations in rat liver (29–33), conflicting data exist for the effect of isolated folate deficiency on DNA methylation in rodent liver (Table 1). Severe folate deficiency of a short duration (4–6 wk) has been shown to either decrease (34) or increase (33) the extent of genomic DNA methylation in rat liver. A prolonged (15–24 wk), moderate degree of dietary folate deficiency failed to induce significant genomic DNA hypomethylation in rat liver (32). Recently, the same moderate folate deficient diet was shown to induce a significant 56% increase in genomic DNA methylation in the liver of mice after 5 wk; however, genomic DNA methylation returned to baseline values after 8 wk (35). Taken together, the results from these studies suggest that folate deficiency of a moderate degree or short duration appears to induce genomic DNA hypermethylation in rodent liver, likely due to compensatory upregulation of DNMT, and that the effect of sustained or
severe folate deficiency on genomic DNA methylation in rodent liver is not consistent.

One intriguing observation from one of these animal studies was that severe folate deficiency produced significant hypomethylation (by 40%) within a mutation hot spot (exons 6–7), but not in exon 8, of the p53 tumor suppressor gene, despite a 56% increase in genomic DNA methylation in rat liver (33). This observation raises the possibility that the effect of folate deficiency on DNA methylation may be site and gene-specific, and suggests that the changes in genomic and site-specific DNA methylation in response to folate deficiency may not be in the same direction.

The effect of isolated folate deficiency on DNA methylation in the colorectum, a tissue that is particularly susceptible to increased carcinogenesis with folate deficiency, has not yet been clearly elucidated (Table 1). A moderate degree of folate deficiency alone or in conjunction with a colorectal carcinogen (dimethylhydrazine [DMH] or azoxymethane [AOM]) for 10–26 wk failed to induce significant genomic and genespecific DNA hypomethylation in rat colon (25,32,36–39). In contrast, significant p53 hypomethylation in exon 8 but not in exons 6–7 was observed in the DMH-treated rat colon in conjunction with folate deficiency. It remains unclear whether this was due to the DMH, the folate deficiency, or the combination of the two. This was effectively overcome in a dose-dependent manner by increasing levels of dietary folate (40).

A recent study showed that, despite a marked reduction in plasma and colonic folate concentrations, a large increase in plasma homocysteine concentrations, and a progressive decrease in colonic SAM to SAH ratios, isolated folate deficiency did not induce significant genomic DNA hypomethylation in the colon (41). Paradoxically, isolated folate deficiency significantly increased (by 30%) the extent of genomic DNA methylation in the colon at an intermediate time point (41), which is consistent with prior observations made in rodent liver (33,35). Folate supplementation did not modulate colonic SAM, SAH, SAM to SAH ratios, or genomic DNA methylation at any time point (41). The extent of p53 methylation in the promoter and exons 6–7 was variable over time at each of the CpG sites examined, and no associations with time or dietary folate were observed at any CpG site (41).

Effect of folate deficiency on DNA methylation in in vitro systems

In one study by Duthie et al. (43), normal human colonic epithelial cells were immortalized by SV40 T antigen and cultured in folate-deficient (<2.3 nmol/L) and control (9.1

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Folate deficiency</th>
<th>Duration, wk</th>
<th>Species</th>
<th>Organ</th>
<th>DNA methylation</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balaghi and Wagner (34)</td>
<td>Severe</td>
<td>4</td>
<td>Rat</td>
<td>Liver</td>
<td>Genomic</td>
<td>20% decrease ($P = 0.032$)</td>
</tr>
<tr>
<td>Kim et al. (33)</td>
<td>Severe</td>
<td>6</td>
<td>Rat</td>
<td>Liver</td>
<td>Genomic</td>
<td>60% increase ($P = 0.1$)</td>
</tr>
<tr>
<td>Song et al. (35)</td>
<td>Mild</td>
<td>5</td>
<td>Mouse</td>
<td>Liver</td>
<td>Genomic</td>
<td>56% increase ($P &lt; 0.005$)</td>
</tr>
<tr>
<td>Kim et al. (36)</td>
<td>Mild + DMH</td>
<td>20</td>
<td>Rat</td>
<td>Liver</td>
<td>Genomic</td>
<td>No effect</td>
</tr>
<tr>
<td>Le Leu et al. (37)</td>
<td>Mild + AOM</td>
<td>13</td>
<td>Rat</td>
<td>Liver</td>
<td>Genomic</td>
<td>No effect</td>
</tr>
<tr>
<td>Davis and Uthus (39)</td>
<td>Mild + DMH</td>
<td>13</td>
<td>Rat</td>
<td>Colon</td>
<td>Genomic</td>
<td>No effect</td>
</tr>
<tr>
<td>Duthie et al. (25)</td>
<td>Mild</td>
<td>10</td>
<td>Rat</td>
<td>Colon</td>
<td>Genomic</td>
<td>30% increase at wk 3 ($P = 0.022$) and no effect at other time points</td>
</tr>
<tr>
<td>Sohn et al. (41)</td>
<td>Severe</td>
<td>5</td>
<td>Rat</td>
<td>Colon</td>
<td>Genomic</td>
<td>No effect</td>
</tr>
<tr>
<td>Choi et al. (42)</td>
<td>Mild</td>
<td>8 and 20</td>
<td>Rat (young and old)</td>
<td>Colon</td>
<td>Genomic</td>
<td>No effect</td>
</tr>
</tbody>
</table>

AOM, Azoxymethane; DMH, dimethylhydrazine.
µmol/L folic acid) medium for 14 d (43). Folate deficiency led to genomic DNA hypomethylation, increased uracil misincorporation, and inhibition of DNA excision repair in colonic epithelial cells. In contrast, a recent study, using 2 human colon adenocarcinoma cell lines, Caco2 and HCT116, has shown that the extent and the direction of the changes of SAM and SAH in response to folate deficiency (0.6 nmol/L folic acid in the medium vs. 2.3 µmol/L in control medium) are cell specific, and that genomic-, site- and gene-specific DNA methylation are not affected by the changes of SAM and SAH induced by folate depletion (44). In a similar experiment using nontransformed cell lines, folate deficiency (0.6 nmol/L in the medium) was shown to induce significant genomic DNA hypomethylation in both a mouse fibroblast cell line, NIH/3T3, and a Chinese hamster ovarian cell line, CHO-K1, by cell-specific mechanisms as indicated by cell-specific differential effects of folate deficiency on intracellular SAM, SAH, and DNMT (44). In another study, human nasopharyngeal carcinoma KB cells grown in folate-deplete (2–10 nmol/L folic acid) medium was associated with paradoxical hypermethylation in a 5' CpG island (by 40%) and consequent downregulation of the H-cadherin gene compared with cells grown in folate-replete (2.0 µmol/L folic acid) medium (45).

The results from these studies collectively suggest that the effects of folate deficiency on DNA methylation are site and gene specific. Also, the direction of methylation changes may be cell, target organ, and stage of transformation specific, and may not be the same between genomic and gene or site-specific DNA methylation. The major limitation of the in vitro system to study the effect of folate on DNA methylation is that the degree of folate deficiency and supplementation used in this system is not physiologically and clinically relevant and applicable to in vivo situations.

### Effect of folate status on DNA methylation in humans

There are some observations in humans suggesting that altered folate status can affect genomic DNA methylation. Folate depletion in healthy human volunteers in a metabolic unit setting has been observed to diminish genomic DNA methylation in leukocytes (Table 2) (46,47). Rampersaud et al. (47) showed that lymphocyte genomic DNA methylation significantly decreased by 10% in response to moderate (118 µg folate/d) folate depletion for a period of 7 wk in elderly women (60–85 y of age). However, no significant changes in leukocyte genomic DNA methylation were detected during the 7-wk period of folate repletion with either 200 or 415 µg of folate/d (47). Another study by Jacob et al. (46) housed healthy, postmenopausal women (49–63 y of age) in a metabolic unit and fed them folate-deplete diets (56–111 µg folate/d) for 9 wk. This resulted in a significant (by 120%) degree of lymphocyte genomic DNA hypomethylation, which was reversed during the 3-wk period of folate supplementation (285–516 µg folate/d). However, an earlier study by the same group using healthy, younger males (33–46 y of age) failed to show a change in in vivo methylation capacity (as measured by the ability to methylate orally administered nicotinamide as detected in the urine as methylated metabolites) in response to dietary folate and methyl group restriction (25 µg folate/d for 30 d) (Table 2) (48).

Several human studies have investigated correlations between DNA methylation and folate status. In human subjects with normal folate status, no significant correlations between genomic lymphocyte DNA methylation and red blood cell folate and plasma homocysteine concentrations were observed (49). Two recent studies, however, have shown that colonic DNA methylation was positively correlated with serum and

### TABLE 2

**Summary of dietary folate deficiency and supplementation on DNA methylation in human studies**

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Subjects</th>
<th>Dose</th>
<th>Duration</th>
<th>DNA methylation</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Folate deficiency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rampersaud et al. (47)</td>
<td>Women 60–85 y</td>
<td>118 µg/d</td>
<td>7 wk</td>
<td>Leukocytes, genomic</td>
<td>10% decrease (P = 0.0012)</td>
</tr>
<tr>
<td>Jacob et al. (46)</td>
<td>Women 49–63 y</td>
<td>56–111 µg/d</td>
<td>9 wk</td>
<td>Lymphocytes, genomic</td>
<td>120% decrease (P &lt; 0.05)</td>
</tr>
<tr>
<td>Jacob et al. (48)</td>
<td>Men</td>
<td>25 µg/d</td>
<td>30 d</td>
<td>Methylation capacity (not DNA)</td>
<td>No change</td>
</tr>
<tr>
<td><strong>B. Folate supplementation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cravo et al. (53)</td>
<td>Patients with colon cancer and adenoma</td>
<td>10 mg/d</td>
<td>6 mo</td>
<td>Rectal mucosa, genomic</td>
<td>93% increase (P &lt; 0.002)</td>
</tr>
<tr>
<td>Cravo et al. (54)</td>
<td>Patients with colonic adenoma</td>
<td>5 mg/d</td>
<td>3 mo</td>
<td>Rectal mucosa, genomic</td>
<td>37% increase in patients with 1 adenoma (P = 0.05) and no change in those with &gt;1 adenomas (P = 0.001)</td>
</tr>
<tr>
<td>Kim et al. (55)</td>
<td>Patients with colonic adenoma</td>
<td>5 mg/d</td>
<td>6 mo</td>
<td>Rectal mucosa, genomic</td>
<td>57% increase, (P = 0.001)</td>
</tr>
<tr>
<td>Cravo et al. (56)</td>
<td>Patients with inflammatory bowel disease</td>
<td>5 mg/d</td>
<td>1 y</td>
<td>Rectal mucosa, genomic</td>
<td>No change</td>
</tr>
<tr>
<td>Fenech et al. (49)</td>
<td>Normal subjects</td>
<td>2 mg/d</td>
<td>12 wk</td>
<td>Lymphocytes, genomic</td>
<td>No change</td>
</tr>
<tr>
<td>Ingrosso et al. (57)</td>
<td>Uremic patients with hyperhomocysteinemia and preexisting DNA hypomethylation</td>
<td>15 mg/d</td>
<td>8 wk</td>
<td>Lymphocytes Genomic</td>
<td>Restored to normal levels</td>
</tr>
<tr>
<td>Pufulete et al. (58)</td>
<td>Patients with colonic adenoma</td>
<td>400 µg/d</td>
<td>10 wk</td>
<td>Lymphocytes, genomic</td>
<td>31% increase (P = 0.05)</td>
</tr>
</tbody>
</table>

THF, Tetrahydrofolate.
red blood cell folate concentrations and negatively correlated with plasma homocysteine concentrations in individuals with (50) and without (51) colonic adenomas and adenocarcinomas. In the Netherlands Cohort Study on Diet and Cancer (52), the prevalence of CpG island promoter hypermethylation was higher, albeit nonsignificantly, in colorectal cancer derived from patients with low folate/high alcohol intake compared with colorectal cancer from patients with high folate/low alcohol intake for each of the 6 tested genes (APC, p14, p16, hMLH1, O6-MGMT, and RASSF1A). The number of colorectal cancers with at least one gene methylated was higher (84%) in the low folate/high alcohol intake group compared with the high folate/low alcohol intake group (70%; P = 0.085) (52).

In some human intervention studies, folate supplementation at 12.5–25 times the daily requirement for 3–12 mo significantly increased the extent of colonic genomic DNA methylation in subjects with resected colorectal adenoma or cancer (53–55), whereas no such effect was observed in patients with chronic ulcerative colitis who were given folate supplementation at 12.5 times the daily requirement for 6 mo (56) (Table 2). Folate supplementation at 5 times the daily requirement, which was sufficient to correct a marker of DNA damage, failed to modulate genomic DNA methylation in lymphocytes in healthy volunteers (49) (Table 2). In another study, folate supplementation with 15 mg methyltetrahydrofolate a day for 8 wk restored genomic DNA methylation in lymphocytes to normal levels in 32 men with uremia, hyperhomocysteinemia, and preexisting genomic DNA hypomethylation (Table 2) (57). A recent study has demonstrated that a physiological dose of folic acid (400 μg/d) for 10 wk increases genomic DNA methylation in lymphocytes (by 31%; P = 0.05) and in colonic mucosa (by 25%; P = 0.09) compared with placebo in patients with colorectal adenomas (Table 2) (58).

The data from these human studies collectively raise a possibility that the effect of folate status on genomic DNA methylation may be site and tissue specific, and may depend on the degree of folate depletion and supplementation. However, there is no conclusive data suggesting that folate deficiency, of a physiologically and clinically relevant degree, induces significant genomic DNA hypomethylation and/or site and gene-specific aberrant DNA methylation in the colorectum. In contrast, folate supplementation, even at modest levels, appears to be able to increase genomic DNA methylation in the colorectum in certain situations.

Conclusions

Genetic changes in cancer are abrupt in onset, their effects are often all-or-nothing, the loss of function occurs at a fixed level, and they are not reversible in most cases (14). In contrast, epigenetic changes are gradual in onset and progressive, their effects are dose-dependent, and are potentially reversible (14). These observations present new opportunities in cancer-risk modification and prevention using dietary and lifestyle factors and potential chemopreventive drugs. In this regard, folate has been a focus of intense interest because of an inverse association between folate status and the risk of several malignancies and of its potential ability to modulate DNA methylation (15). The portfolio of evidence from animal and in vitro studies collectively suggests that the effects of folate deficiency on DNA methylation are highly complex and appear to depend on cell type, target organ, and stage of transformation, and are gene and site specific. These studies also suggest that changes in DNA methylation induced by folate deficiency may be mediated via both SAM and SAH dependent and independent pathways. Also, there is evidence suggesting that the pattern of site- and gene-specific DNA methylation may not be in concert with the direction of changes in genomic DNA methylation. Currently available data pertaining to the effects of folate deficiency on DNA methylation in the liver and colon in animal studies are inconsistent and do not support the hypothesis that folate deficiency induces DNA hypomethylation in the colon. Although some similarities do exist, these animal models differ in several important physiological aspects from humans, including bioavailability, metabolism, and excretion of folate (17). Therefore, any extrapolation of the observations from these models to human situations should be made very cautiously. Furthermore, these animal models may produce variable results owing to species differences, different diet compositions, and variable dose, time, and duration of folate manipulations.

In contrast, human feeding studies in metabolic unit settings have shown a consistent, significant hypomethylating effect of folate deficiency in genomic lymphocytes DNA (46,47). However, the effect of physiologically and clinically relevant folate deficiency on DNA methylation in the colorectum has not been studied in humans. Folate supplementation appears to be capable of increasing the degree of DNA methylation in the colorectum in some human studies (53–55,58). Although the jury is still out, the potential for folate to modulate DNA methylation and thus modify colorectal cancer risk remains biologically plausible and is worthy of further studies.

LITERATURE CITED

FOLATE, COLORECTAL CANCER, AND DNA METHYLATION

2709