ANTIOXIDANTS & REDOX SIGNALING
Volume 17, Number 2, 2012

DOI: 10.1089/ars.2012.4554

FORUM REVIEW ARTICLE

Folate and DNA Methylation

Anna Ly,[1,2,*] Lesley Hoyt,[2,3,*] Julie Crowell,[1] and Young-In Kim[1–4]

Abstract

Significance: The progressive, dose-dependent, and potentially reversible epigenetic changes observed in cancer present new opportunities in cancer risk modification and prevention using dietary and lifestyle factors. Folate, a water-soluble B vitamin, has been of intense interest because of an inverse association between folate status and the risk of several malignancies (particularly colorectal cancer) and its potential to modulate DNA methylation. Aberrant patterns and dysregulation of DNA methylation are mechanistically related to carcinogenesis. ***Recent Advances:*** The effects of folate on DNA methylation patterns have recently been investigated in two important life stages: pre- and early postnatal life and aging. Recent studies have demonstrated that folate exposure in the intrauterine environment and early life and during the aging process may have profound effects on DNA methylation with significant functional ramifications, including the risk of cancer. ***Critical Issues:*** Evidence from animal, human, and *in vitro* studies suggest that the epigenetic effects of folate on DNA methylation are highly complex. The effects are gene and site specific and appear to depend on cell type, target organ, stage of transformation, the degree and duration of folate manipulations, interactions with other methyl group donors and dietary factors, and genetic variants in the folate metabolic pathways. ***Future Directions:*** The potential for folate to modulate DNA methylation and, thus, modify the risk of cancer in humans is worthy of further investigation. Due to the complex relationship between folate exposure and DNA methylation, more elaborate epidemiological, clinical, and mechanistic studies that determine the clinical, biological, and molecular effects of folate are warranted. *Antioxid. Redox Signal*. 17, 302–326.

Introduction

THE FIELD OF EPIGENETICS is the study of modifications of DNA and DNA-binding proteins 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 (38). DNA methylation is one epigenetic modification in particular, of which changes in DNA methylation patterns have been shown to occur with the development of cancer, specifically colorectal cancer (CRC). The provision of folate, a water-soluble B vitamin, and the synthetic form, folic acid (FA), are known to modulate DNA methylation patterns. This has led to extensive research on elucidating the role of folate and FA in epigenetic changes, particularly related to the progression of cancer. The aim of this review is to highlight the current literature investigating this relationship, as well as the growing research areas examining the effects of folate status on DNA methylation during the highly critical intrauterine developmental period and during aging.

DNA Methylation

The inheritance of information based on gene expression levels is known as epigenetics, as opposed to genetics, which refers to the information transmitted on the basis of gene sequence (40). Epigenetic mechanisms include DNA methylation, covalent modifications of histones, and RNA interference, all of which alter gene expression and function (38). In contrast to genetic changes in human diseases, epigenetic changes are gradual in onset and progressive, their effects are dose dependent, and are potentially reversible by dietary and pharmacologic manipulations (11, 86).

Of the various epigenetic mechanisms, DNA methylation of the cytosines located within the cytosine-guanine (CpG) sequences is the most widely studied and well characterized. DNA methylation is heritable, tissue- and, species specific, and an important epigenetic inverse determinant in gene expression (11, 72). Methylation also plays a pivotal role in the maintenance of DNA integrity and stability, and in chromatin modifications (41, 72). In normal cells, up to 80% of all CpG

[1]Department of Medicine, University of Toronto, Toronto, Ontario, Canada.
[2]Keenan Research Center of the Li Ka Shing Knowledge Institute at St. Michael's Hospital, Toronto, Ontario, Canada.
[3]Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada.
[4]Division of Gastroenterology, Department of Medicine, St. Michael's Hospital, Toronto, Ontario, Canada.
*These authors contributed equally to this work.

sites in human DNA are methylated (41, 72). However, this global 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 (Fig. 1) (61). By contrast, most CpG rich areas clustered in small stretches of DNA termed "CpG islands," which span the 5′ end of approximately half of all transcribed human genes including the promoter, untranslated region, and exon 1, are unmethylated in normal cells, thereby allowing transcription to occur (Fig. 1) (41, 72, 135). The methylation of promoter region CpG islands, termed gene-specific methylation, causes stable, heritable transcriptional silencing (41, 72). This is mediated by the transcriptional repressor, methyl-CpG binding protein-2 (MeCP2), which binds methylated CpG islands and recruits a complex containing a transcriptional co-repressor and a histone deacetylase (16, 73). The deacetylation of histones suppresses transcription by allowing tighter nucleosomal packaging, thus rendering an inactive chromatin conformation (15, 23).

DNA methylation is a dynamic process between active methylation, mediated by CpG DNA methyltransferases (DNMT1, 3a, 3b) using S-adenosylmethionine (SAM) as the methyl donor, and active and passive removal of methyl groups from 5-methylcytosine residues by a purported demethylase (MBD2) (99). During embryogenesis, active and passive demethylation of the paternal and maternal methylation patterns, respectively, occurs, which erases significant parts of the parental DNA methylation. This is followed by *de novo* methylation, which establishes a new DNA methylation pattern soon after implantation, with methylation limited to non-CpG island areas, except for the rare genes silenced in normal cells (Fig. 2) (99, 133). Themaintenance of CpG DNA methyltransferase (DNMT1) uses hemimethylated sites that ensure DNA methylation patterns, whereas *de novo* CpG DNA methyltransferases (DNMT3a, 3b) do not require preexisting methylation and, therefore, establish a new DNA methylation pattern (99).

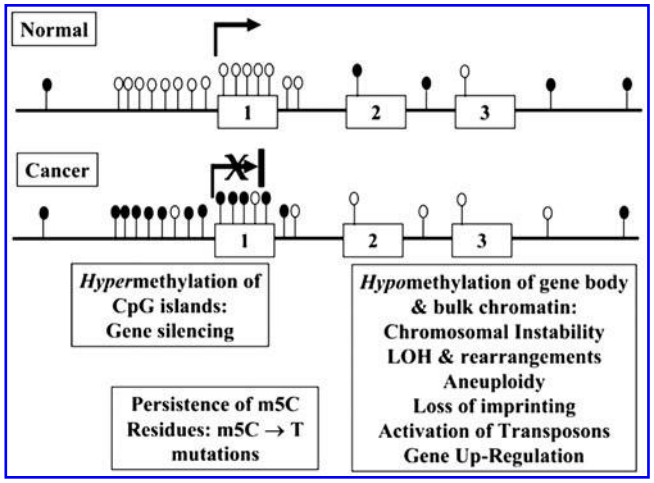

FIG. 1. Distribution of CpG dinucleotides in the human genome and CpG methylation patterns in normal and tumor cells. In contrast to methylated CpG sites in the CpG-poor bulk of the genome and unmethylated CpG islands in normal cells, cancer cells simultaneously harbor widespread loss of methylation in the CpG depleted regions, where most CpG dinucleotides should be methylated and gains in the methylation of CpG islands in gene promoter regions. *Open circles* represent unmethylated CpG sites, whereas *filled circles* are methylated CpG sites. *Boxes* 1, 2, and 3 represent exons, and *line* between exons are introns. *X* at the transcription start site represents transcriptional silencing.

DNA methylation is critically involved in regulating many developmental and cellular processes, including embryonic development, transcription, chromatin structure, X chromosome inactivation, genomic imprinting, and chromosome stability (134). Aberrant patterns and dysregulation of DNA methylation are mechanistically related to the development of several human diseases, including cancer (41, 72).

Folate

Folate is a water-soluble B vitamin that is naturally present in many foods, including green leafy vegetables, asparagus, broccoli, Brussels sprouts, citrus fruit, legumes, dry cereals, whole grain, yeast, lima beans, liver, and other organ meats. FA is the fully oxidized monoglutamyl synthetic form of folate that is commercially used in supplements and in fortified foods (Fig. 3). Naturally occurring folates are very unstable, rapidly lose their activity in foods, and are easily oxidized under low pH (114). Folate bioavailability varies widely depending on the food source and preparation method (114). Approximately 50%–75% of the original folate values are lost through food harvesting, storage, processing, and preparation (114). In contrast, FA is highly stable for months or even years and has a higher bioavailability compared with naturally occurring folates (114). FA is neither found in nature nor is it a normal metabolite. It should be reduced, first to dihydrofolate (DHF) and then to tetrahydrofolate (THF) by DHF reductase (DHFR) and methylated to 5-methylTHF (the predominant folate found in blood) in the liver and, to a lesser degree, in the intestine (179), before it can enter the folate cycle (Fig. 4). Since the capacity to reduce folate is limited, high intakes of FA result in its appearance of being unaltered in circulation (76).

Biochemical Functions of Folate

Folate participates in the transfer of one-carbon units involved in nucleotide biosynthesis, methionine cycle, and biological methylation reactions (Figs. 4 and 5) (141). As an essential cofactor for the *de novo* biosynthesis of nucleotides (106, 141, 169), folate is important in DNA synthesis, stability and integrity, and repair. In the methionine cycle, 5-methylTHF transfers single methyl groups to homocysteine, catalyzed by methionine synthase (20, 98), to synthesize methionine. This ensures the provision of SAM, the primary methyl group donor for most biological methylation reactions (84, 86). After donating the methyl group, 5-methylTHF is converted to THF and then to 5,10-methyleneTHF by serine hydroxymethyltransferase (58, 155). 5,10-methyleneTHF is a key substrate in folate metabolism, which can be directed toward nucleotide biosynthesis or toward methionine regeneration (59). Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methyleneTHF to 5-methylTHF (161).

Folate in Health and Disease

Folate plays an important role in human health and disease. Folate deficiency has been linked to the development of

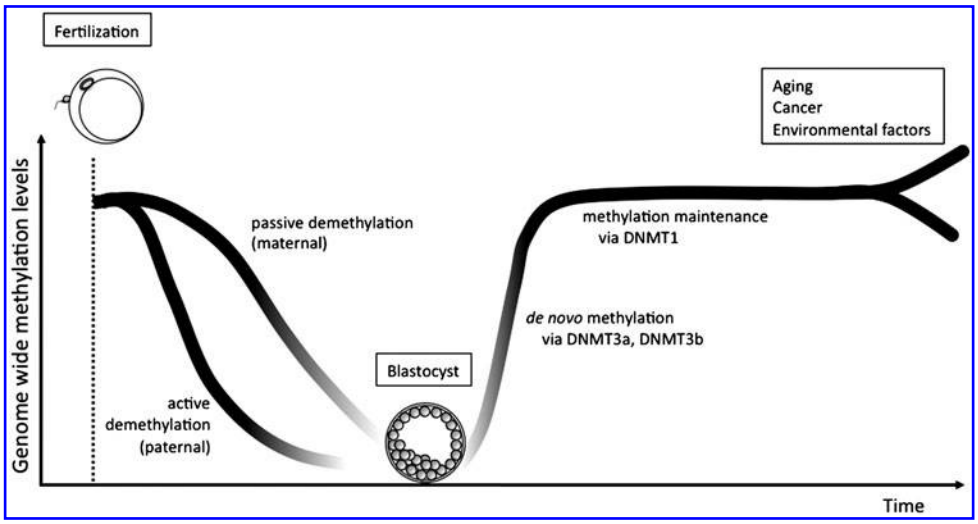

FIG. 2. DNA methylation throughout the life cycle. DNA methylation patterns are reprogrammed during embryogenesis by genome-wide demethylation after fertlization. Active demethylation of the paternal and passive demethylation of the maternal genome erases significant parts of the parental DNA methylation patterns, followed by *de novo* methylation, which establishes a new DNA methylation pattern soon after implantation when the blastocyst is formed. The maintenance of CpG DNA methyltransferase (DNMT1) uses hemimethylated sites to ensure DNA methylation patterns, whereas *de novo* CpG DNA methyltransferases (DNMT3a, 3b) do not require pre-existing methylation patterns. Later in life, factors such as aging, cancer, and environmental exposures can cause epigenetic divergence with increased or decreased methylation levels.

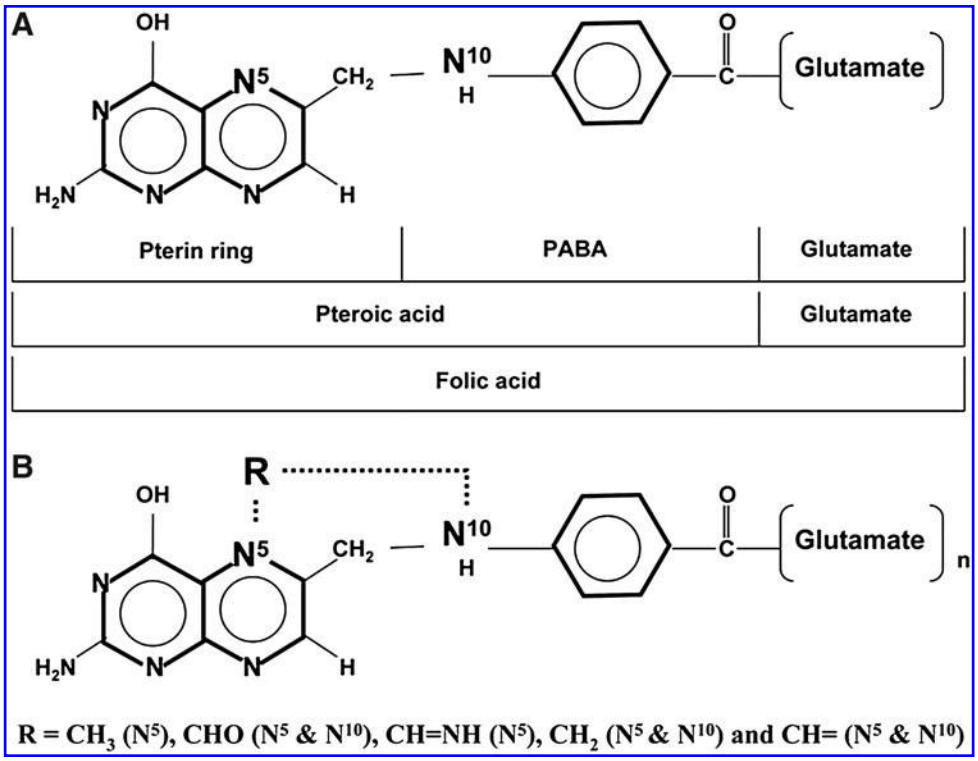

FIG. 3. Chemical structures of folic acid (A) and folate (B). Folic acid consists of three moieties: the pterin (or pteridine) ring, which is conjugated to para-aminobenzoic acid (PABA) by a methylene bridge, which is, in turn, joined to a glutamic residue *via* a peptide bond. Folic acid is the fully oxidized monoglutamyl form of this vitamin that is commercially used in supplements and in fortified foods. Folate is the generic term referring to compounds that have similar chemical structures and nutritional properties. All naturally occurring folates found in food differ from the oxidized folic acid in the oxidation state of the pteridine ring and are typically reduced. Furthermore, one-carbon units (R) can be linked to tetrahydrofolate (THF) at the N-5 and N-10 positions, which confers folate the role of mediating the transfer of one-carbon units. In addition, multiple glutamate residues of varying numbers (up to nine) can be added *via* a γ-peptide linkage.

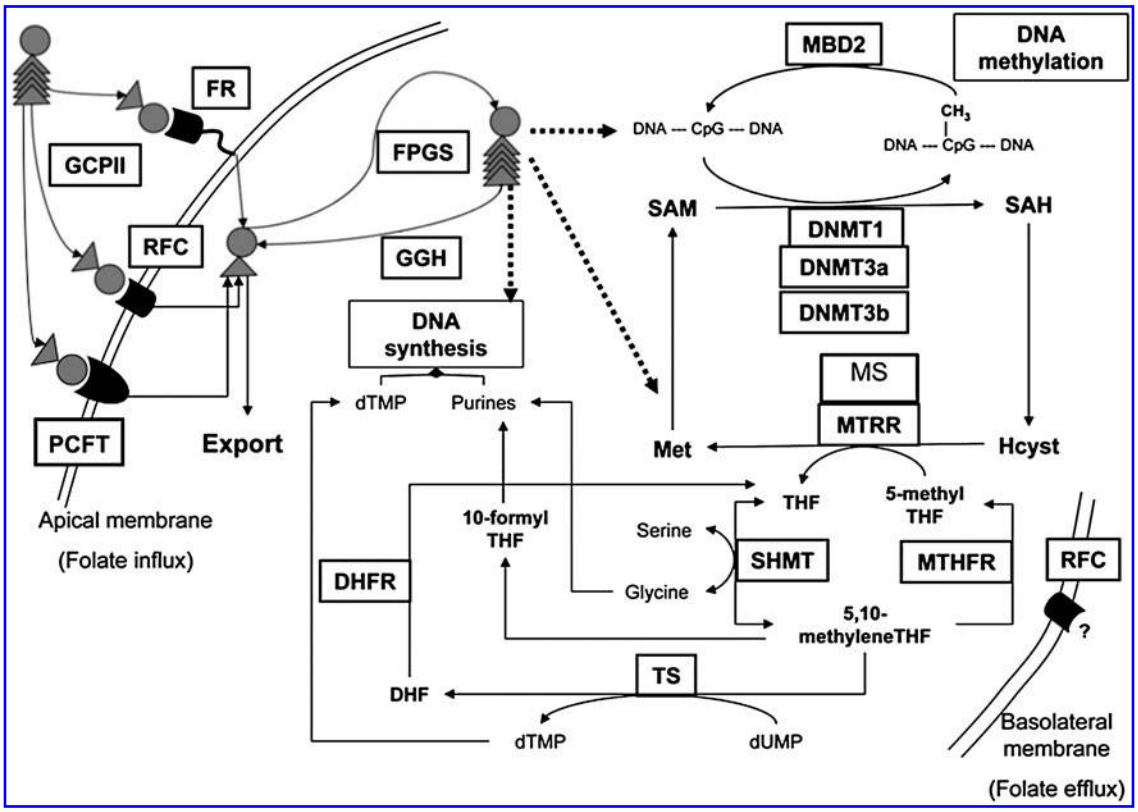

FIG. 4. Intracellular folate metabolism. Simplified scheme of intracellular folate metabolism and one-carbon transfer reactions in epithelial cells, highlighting the genes that are involved in intraluminal folate hydrolysis (GCPII, glutamate carboxypeptidase II), intracellular folate uptake (FR-α, folate receptor; RFC, reduced folate carrier; PCFT, proton coupled folate transporter), intracellular folate retention (FPGS, folylpolyglutamyl synthase) and hydrolysis and efflux (GGH, γ-glutamyl hydrolase), methionine cycle (MS, methionine synthase; MTRR, methionine synthase reductase; MTHFR, methylenetetrahydrofolate reductase), maintenance of intracellular folate pool (DHFR, dihydrofolate reductase; SHMT, serine hydroxylmethyltransferase), and nucleotide biosynthesis (TS, thymidylate synthase), DNA methylation (DNMT1, 3a, 3b, CpG DNA methyltransferases), and DNA demethylation (methyl-CpG binding domain protein 2 [MBD2], DNA demethylase). Intracellular folate exists primarily as polyglutamates. Intracellular folate is converted to polyglutamates by FPGS, while GGH removes the terminal glutamates. Polyglutamylated folates are better retained in cells and are better substrates than monoglutamates for intracellular folate-dependent enzymes involved in one-carbon transfer reactions. Folate mediates the transfer of one-carbon units necessary for DNA synthesis, methionine cycle, and biological methylation reactions. dTMP, deoxythymidine-5-monophosphate (thymidylate); dUMP, deoxyuridine-5-monophosphate; Hcyt, homocysteine; Met, methionine. *Filled circle* represents a pteridine ring conjugated to para-aminobenzoic acid. Each *filled triangle* represents a glutamate, which is linked *via* a peptide bond to form various chain lengths of polyglutamylated folate.

anemia, cardiovascular disease, neural tube defects (NTDs) and congenital disorders, adverse pregnancy outcomes, neuropsychiatric disorders, and cognitive impairments (114). In addition, a substantial amount of epidemiologic evidence suggests an inverse association between folate status (assessed by dietary folate intake or by the measurement of blood folate levels) and the risk of several malignancies, including cancer of the lungs, oropharynx, esophagus, stomach, colorectum, pancreas, cervix, ovary, prostate, and breast and the risk of neuroblastoma and leukemia (82, 83, 87, 88). More recently, an emerging body of evidence has warned of the potentially harmful effects of FA supplementation, including resistance or tolerance to antifolate drugs used against arthritis (34, 79) and cancer (19, 136, 182); decreased natural killer cell cytotoxicity (160); genetic selection of disease alleles (147); an increased risk of insulin resistance and obesity (181), and asthma (175) in children born to mothers supplemented with high levels of FA; and an increased risk of cognitive impairment in the elderly in combination with low vitamin B12 status (116, 137, 138, 140). Except for anemia and NTDs, the precise nature and magnitude of the relationship between folate status and the risk of these diseases have not been uniformly consistent and remain to be clearly elucidated (114, 147).

Folate and CRC

The highly controversial relationship between folate and carcinogenesis has been most widely studied for CRC. In general, the portfolio of epidemiologic and clinical evidence indicates ~20%–40% reduction in the risk of CRC or adenoma in subjects with the highest dietary intake or blood levels of folate compared with those with the lowest intake or blood levels (77, 82, 87, 88). The role of folate in colorectal carcinogenesis has been further strengthened by the observations that genetic polymorphisms in the folate metabolic pathway (*e.g.*, *MTHFR* C677T polymorphism) modify CRC risk (8, 89, 126).

Although there is no definitive evidence supporting the protective effect of folate supplementation on colorectal

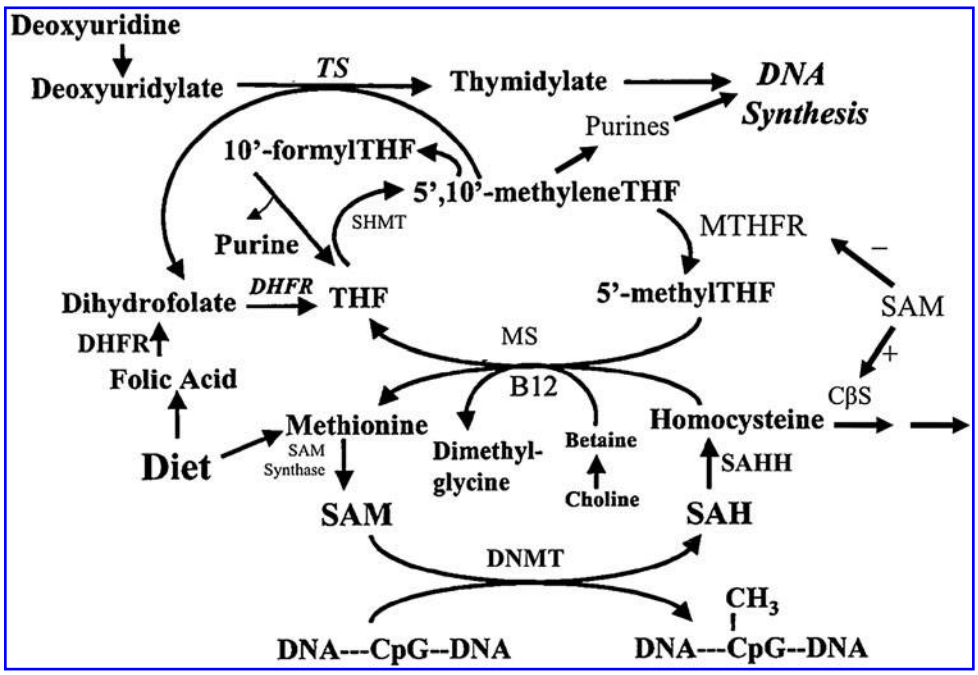

FIG. 5. Simplified scheme of folate metabolism and one-carbon transfer reactions involved in DNA synthesis and biological methylation reaction, including that of DNA. SAM is both an allosteric inhibitor of MTHFR and an activator of cystathionine *β*-synthase. B12, vitamin B12; C*β*S, cystathionine *β*-synthase; CH3, methyl group; CpG, cytosine-guanine dinucleotide sequence, DHF, dihydrofolate; DHFR, dihydrofolate reductase; DNMT, DNA methyltransferase; MS, methionine synthase; SAH, S-adenosylhomocysteine; SAHH, S-adenosylhomocysteine hydrolase; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; TS, thymidylate synthase.

carcinogenesis from human experiments at present, several small intervention studies have demonstrated that folate supplementation can improve or reverse surrogate endpoint biomarkers of CRC (14, 28, 30, 80, 90, 97, 121), and some epidemiologic studies have shown a beneficial effect of multivitamin supplements containing ≥0.4 mg FA on CRC risk and mortality (56, 57, 69). However, in the Aspirin/Folate Polyp Prevention Study, FA supplementation was associated with a 67% increased risk of advanced lesions with a high malignant potential in men and women with a history of colorectal adenomas (27). Furthermore, a positive association between FA supplementation and prostate cancer risk was reported as a secondary observation in this trial (43). In contrast, some human FA CRC chemoprevention trials have failed to demonstrate the tumor-promoting effect of FA supplementation (70, 104, 121, 180). In a study from Norway in which the primary endpoint was the cardiovascular outcome in response to FA and other B vitamin supplementation, treatment with FA (0.8 mg/day) and vitamin B12 (0.4 mg/day) for a median period of 36 months significantly increased overall cancer incidence and mortality by 21% and 38%, respectively (37). However, in a meta-analysis of 8 randomized trials of FA supplementation involving 37,485 individuals at increased risk of cardiovascular disease, FA supplementation of a median duration of 5 years had no significant effects on vascular outcomes (the primary endpoint), overall cancer incidence, cancer mortality, or all-cause mortality (25). Two recent ecologic studies that examined a temporal post-fortification trend of CRC incidence in the United States, Canada, and Chile reported increased CRC rates in these countries following fortification, suggesting that FA fortification may have been wholly or partly responsible for this disturbing trend (63, 111). However, two large prospective epidemiologic studies conducted after FA fortification in the United States have suggested a CRC protective of consuming adequate amounts of folate and have not demonstrated a CRC-promoting effect of FA supplementation (55, 153).

The data from animal studies generally support a causal relationship between folate depletion and CRC risk and an inhibitory effect of modest levels of folate supplementation on colorectal carcinogenesis (83). However, animals studies have also shown that folate supplementation may increase CRC risk and accelerate CRC progression if physiological levels are exceeded or if it is provided after neoplastic foci are established in the colorectum (83, 84).

Mechanisms of action. Several biologically plausible mechanisms exist that explain this seemingly dual role of folate in CRC cancer. As an essential cofactor for nucleotide biosynthesis, folate plays an important role in DNA synthesis, integrity, and repair, aberrancies of which are mechanistically related to cancer development (87). In normal tissues, FA supplementation provides nucleotide precursors for DNA synthesis and replication. This ensures DNA fidelity, maintenance of DNA integrity, and optimal DNA repair, therefore reducing the risk of neoplastic transformation (Fig. 6) (87). In contrast, FA supplementation promotes the progression of (pre)neoplastic lesions by providing nucleotide precursors to the rapidly replicating transformed cells, allowing accelerated proliferation (Fig. 6) (87). Folate also modulates DNA methylation of cytosine within the cytosine-guanine (CpG) sequences because of its role in the provision of SAM (86). Neoplastic cells simultaneously harbor widespread global DNA hypomethylation and gene-specific promoter CpG island hypermethylation (86). Global DNA hypomethylation is an early and consistent event in colorectal carcinogenesis and contributes to CRC development through several mechanisms, including chromosomal instability (86). DNA methylation at promoter CpG islands silences transcription and, hence, inactivates the function of a wide array of tumor suppressor and cancer-related genes (86). FA supplementation is able to reverse pre-existing global DNA hypomethylation and increase the extent of global DNA methylation above the pre-existing level (Fig. 6) (86), thereby reducing the risk of

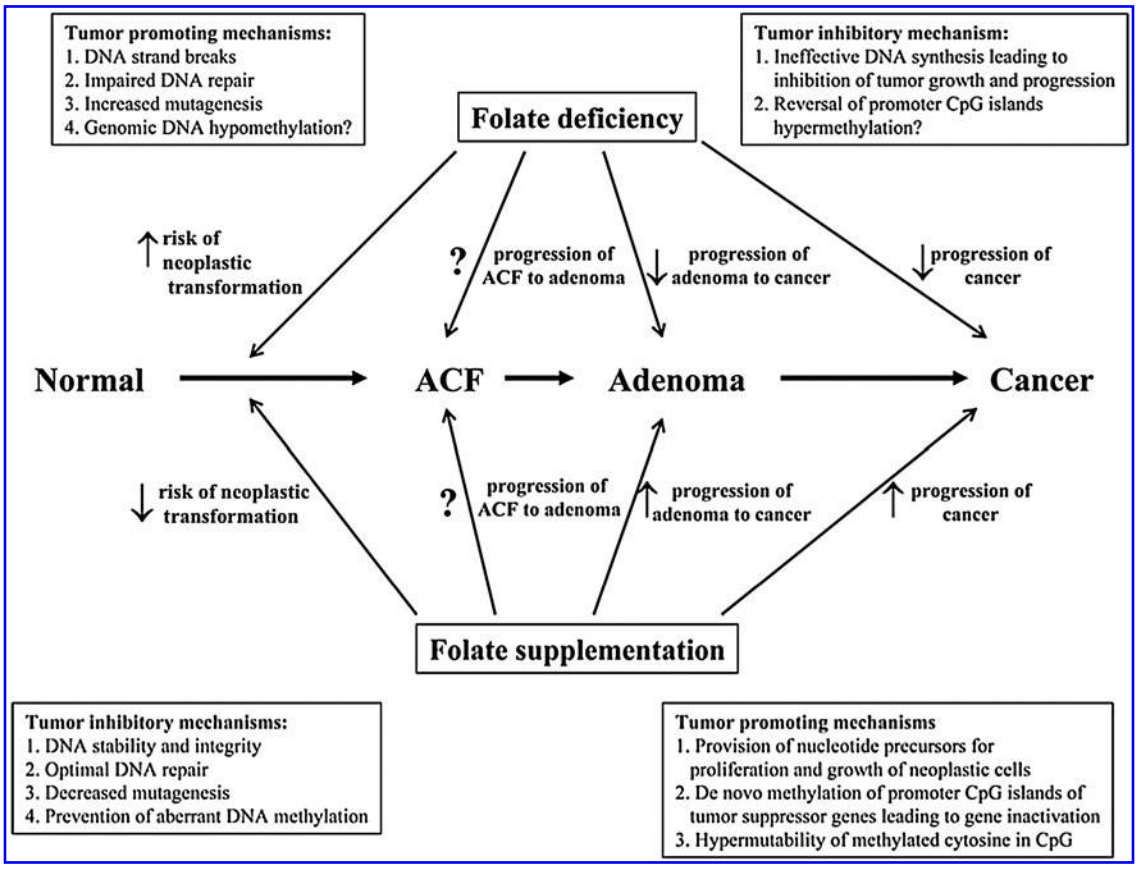

FIG. 6. Dual modulatory role of folate in carcinogenesis. Cancer develops over decades, if not lifetime, through different stages of premalignant lesions in the target organ. Folate deficiency in normal tissues predisposes them to neoplastic transformation, and modest supplemental levels suppress, whereas supraphysiologic doses of supplementation enhance, the development of tumors in normal tissues. In contrast, folate deficiency has an inhibitory effect, whereas folate supplementation has a promoting effect on the progression of established neoplasms. At present, the effect of folate deficiency and supplementation on the progression of early precursor or preneoplastic lesions of colorectal cancer is unknown (*e.g.*, aberrant crypt foci, ACF) to adenoma and to frank cancer. The mechanisms by which folate exerts dual modulatory effects on carcinogenesis depending on the timing and dose of folate intervention relate to its essential role in one-carbon transfer reactions involved in DNA synthesis and biological methylation reactions.

neoplastic transformation. In contrast, FA supplementation may cause *de novo* methylation of CpG islands of tumor suppressor genes, with consequent gene inactivation leading to tumor development and progression (Fig. 6) (87).

Folate and DNA methylation. Due to the folate's involvement in converting homocysteine to methionine, leading to the provision of SAM (Fig. 4) (141), we and others have been investigating whether folate deficiency or supplementation can modulate DNA methylation. In addition, there are certain periods of growth and development that are particularly vulnerable to environmental dietary exposures, and this may modify DNA methylation patterns with consequent permanent changes in phenotype and other functional ramifications.

Effects of folate status on DNA methylation in animal studies

Folate deficiency. The effect of folate deficiency and supplementation on DNA methylation in rodents has been previously reviewed (31, 85, 86). Briefly, it appears that prolonged and severe folate deficiency induces global DNA hypomethylation in the rodent liver (10, 22), while moderate deficiency does not have a consistent effect. Despite altering hepatic levels of one-carbon metabolism intermediates and reducing SAM to S-adenosylhomocysteine (SAH) concentration ratios, moderate folate deficiency did not induce hepatic global hypomethylation (36, 91, 162). In contrast, there are some reports of folate deficiency increasing genomic methylation. Moderate folate deficiency for 5 weeks in mice was shown to induce a significant 56% increase in genomic DNA methylation in the liver followed by the return of genomic DNA methylation value to that of the baseline by 8 weeks (149). In a more recent study, dietary folate deficiency provided during the postweaning period through childhood to puberty significantly increased genomic DNA methylation by 34%–48% in rat liver that persisted into adulthood after a return to the control diet at puberty (93).

In the colorectum, the majority of animal studies demonstrate a resistance to altered SAM and SAH levels and do not support an association between folate deficiency and genomic DNA methylation (31, 85, 86). However, a recent study reported that chronic, severe folate deficiency in older adult

mice induced significant genomic DNA hypomethylation in the colon, and a nonsignificant degree of genomic DNA hypomethylation in the small intestine and spleen (103). Contrary to this observation, a study by Sohn *et al.* (148) demonstrated that folate deficiency of a short duration and severe degree may induce genomic DNA hypermethylation in the colon (148). The paradoxical effect of folate deficiency on increasing genomic methylation in hepatic and colonic tissue is likely due to the compensatory up-regulation of Dnmt and of the choline and betaine-dependent transmethylation pathway. If adequate levels of dietary folate and other methyl group donors are provided in adolescence and continued into adulthood, then this pattern of genomic DNA hypermethylation is maintained. On the other hand, if continual dietary folate deficiency is imposed, then this compensatory hypermethylation pattern will not be maintained.

Folate supplementation. The effect of folate supplementation alone on DNA methylation in rodent liver has not yet been clearly elucidated. A recent study has found that dietary folate supplementation at four times the basal dietary requirement of the rat (8 mg/kg) did not affect genomic DNA methylation in adult rat liver, regardless of the timing or duration of supplementation (93). Another study found that dietary folate supplementation at 20 times the basal dietary requirement of the rat (40 mg/kg) provided for 4 weeks in weanling rats did not alter SAM and SAH, SAM to SAH concentration ratios, and genomic DNA methylation in the liver (1). Interestingly, in older rats fed a folate deficient (0 μmol/kg), replete (4.5 μmol/kg), or supplemented (18 μmol/kg) diet for 8 and 20 weeks, hepatic SAH levels decreased, while genomic DNA methylation in liver increased incrementally with increasing levels of dietary folate, and folate-supplemented rats demonstrated a significantly greater degree of genomic DNA methylation compared with folate-deplete rats at both 8 and 20 weeks (22). In the colon, one study found that dimethylhydrazine administration in conjunction with folate supplementation of 8 and 40 mg/kg for 20 weeks in weanling rats did not alter concentrations of SAM, SAH, SAM to SAH concentration ratios, and DNA methylation (92). At the same time, weanling rats fed a folate-supplemented diet (8 mg/kg) for 5 weeks showed no change in colonic concentrations of SAM, SAH, SAM to SAH concentration ratios, and colonic DNA methylation, and, in addition, *p53*-specific DNA methylation was not altered (148). Interestingly, dietary folate supplementation (18 μmol/kg) in both young and older rats for 8 and 20 weeks resulted in a decrease in colonic SAH concentrations, although genomic DNA methylation in the colon was not altered (21).

The results from these studies suggest that in rodents, the liver and colon are generally resistant to changes in the SAM and SAH levels from folate supplementation, and, consequently, are resistant to changes in DNA methylation. Taken together, the results from animal studies suggest that DNA methylation patterns are gene and site specific and depend on cell type, target organ, and stage of transformation as well as on the timing, degree, and duration of folate intervention.

Effects of folate status on global DNA methylation in human studies

Folate deficiency. There are several observations in humans suggesting that an altered folate status can affect genomic DNA methylation (see Table 1). In a metabolic unit setting, folate depletion in older female volunteers (49–63 years of age) for 7–9 weeks has been demonstrated as reducing genomic DNA methylation in leukocytes (67, 132). Furthermore, FA supplementation, even at modest levels, was shown to reverse the extent of genomic hypomethylation in one study (67). However, in an earlier study by the same group, changes 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 (25 μg folate/day) and methyl group restriction for 30 days was not observed in healthy male volunteers (33–46 years of age) (68).

Folate supplementation. In some human intervention studies, folate supplementation at 12.5–25 times the daily requirement for 3–12 months significantly increased the extent of colonic genomic DNA methylation in subjects with resected colorectal adenoma or cancer (28, 30, 90), 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 months (29) or in participants from the Aspirin/Folate Polyp Prevention Study, as determined by the methylation of long interspersed nucleotide elements (LINE-1) (44). Folate supplementation at three and five times the daily requirement, which was sufficient to improve and correct a marker of DNA damage, also failed to modulate genomic DNA methylation in the lymphocytes of healthy volunteers (12, 42). In another study, daily folate supplementation with 15 mg 5-methyltetrahydrofolate for 8 weeks has been observed as restoring genomic DNA methylation in lymphocytes to normal levels in 32 men with uremia, hyperhomocysteinemia, and pre-existing genomic DNA hypomethylation (65). In patients with colorectal adenomas, a physiological dose of FA (0.4 mg/day) for 10 weeks has been demonstrated as significantly increasing genomic DNA methylation in both lymphocytes (by 31%) and colonic mucosa (by 25%) compared with placebo (127).

Observational studies. Several human observational studies have investigated correlations between DNA methylation and folate status (see Table 2). In subjects with adequate folate, no significant correlations between genomic lymphocyte DNA methylation and red blood cell (RBC) folate and plasma homocysteine concentrations have been reported (42, 118), whereas a positive relationship has been found in adults chronically exposed to arsenic (123). Studies evaluating the relationship between serum folate status and methylation-related intermediates have also generally failed to demonstrate an association in healthy adults (62) and the elderly (13). On the other hand, colonic global DNA methylation is positively correlated with serum and RBC folate concentrations and negatively correlated with plasma homocysteine concentrations in individuals with colonic adenomas and adenocarcinomas (3, 128) and in those without these lesions (129). Folate levels and SAM to SAH concentration ratios have also been reported as being lower (by 28%) in malignant tissue compared with normal-appearing adjacent colon mucosa in subjects with CRC (4). Additional evidence lends support to a positive relationship between folate status and global DNA methylation. In a combined analysis of CRCs from

Table 1. Summary of Dietary Folate Deficiency and Supplementation on DNA Methylation in Human Studies

Study (references)	*Subjects*	*Dose*	*Duration*	*DNA methylation*	*Effect*
Rampersaud *et al.*, 2000 (132)	Women 60–85 years	118 μg/day	7 weeks	Leukocytes, genomic	10% decrease ($p = 0.0012$)
Jacob *et al.*, 1998 (67)	Women 49–63 years	56–111 μg/day	9 weeks	Lymphocytes, genomic	120% decrease ($p < 0.05$)
Jacob *et al.*, 1995 (68)	Men	25 μg/day	30 days	Methylation capacity (not DNA)	No change
Cravo *et al.*, 1994 (28)	Patients with colon cancer and adenoma	10 mg/day	6 months	Rectal mucosa, genomic	93% increase ($p < 0.002$)
Cravo *et al.*, 1998 (30)	Patients with colonic adenoma	5 mg/day	3 months	Rectal mucosa, genomic	37% increase in patients with 1 adenoma ($p = 0.05$) and no change in those with >1 adenomas
Kim *et al.*, 2001 (90)	Patients with colonic adenoma	5 mg/day	6 months 1 year	Rectal mucosa, genomic	57% increase ($p = 0.001$) No change
Cravo *et al.*, 1998 (29)	Patients with inflammatory bowel disease	5 mg/day	6 months	Rectal mucosa, genomic	No change
Figueiredo *et al.*, 2009 (44)	Patients with colonic adenoma	1 mg/day	6–8 years	Colonic mucosa, genomic	No change
Fenech *et al.*, 1998 (42)	Normal subjects	2 mg/day	12 weeks	Lymphocytes, genomic	No change
Basten *et al.*, 2006 (12)	Normal subjects	1.2 mg/day	12 weeks	Lymphocytes, genomic	No change
Ingrosso *et al.*, 2003 (65)	Uremic patients with hyper-homocyteinemia and pre-existing DNA hypomethylation	15 mg/day 5-methyltetrahydrofolate	8 weeks	Lymphocytes, genomic	Restored to normal levels
Pufulete *et al.*, 2005 (127)	Patients with colonic adenoma	400 μg/day	10 weeks	Lymphocytes, genomic Rectal mucosa, genomic	31% increase ($p = 0.05$) 25% increase ($p = 0.09$)

participants from the Nurse's Health Study and the Health Professionals Follow-up Study, the risk of genomic hypomethylation (determined by <55% LINE-1 methylation) was 43% lower in subjects with a high (≥0.4 mg) total daily folate intake compared with those with a low (<0.2 mg) total daily folate intake (139). In a study that stratified folate intake according to pre- and postfortification levels, the observed inverse association between leukocyte genomic DNA methylation and adenoma was stronger among subjects with a low (<0.317 mg pre- and <0.413 mg postfortification) total folate intake as compared with those with a high (≥0.317 mg pre- and ≥0.413 mg postfortification) total folate intake in either of the fortification periods (102). Furthermore, in women positive for the human papillomavirus, supraphysiological concentrations of plasma folate (>19 ng/ml) in combination with sufficient levels of vitamin B12 (≥200.6 ng/ml) were significantly associated with greater genomic methylation of peripheral blood mononuclear cells compared with women with lower folate and lower vitamin B12 (124). One human study reported that serum and cervical tissue folate concentrations correlated inversely, albeit weakly, with cervical genomic DNA methylation (47); however, such a relationship was not observed for RBC (46) or circulating plasma folate (124) in exfoliated cervical cells.

In summary, the data from human clinical studies suggest that controlled folate depletion in a metabolic unit appears to reduce genomic DNA methylation in leukocytes of older individuals. However, there are no conclusive data suggesting that folate deficiency of a physiologically and clinical relevant degree induces significant genomic DNA hypomethylation in the colorectum. In contrast, folate supplementation, even at modest supplemental levels, appears to be able to increase genomic DNA methylation in the colorectum in certain situations. The majority of observational studies have described a direct relationship between dietary and blood levels of folate and genomic DNA methylation in both lymphocytes and colonic tissues such that a low folate status is associated with genomic hypomethylation. This positive association is more consistent in individuals with colorectal adenomas, adenocarcinomas, or previously resected neoplastic tumors as well as in those at a greater risk of health complications compared with normal subjects.

Table 2. Summary of Associations Between Dietary and Blood Folate and Genomic DNA Methylation in Human Epidemiological Studies

Study (references)	*Design*	*Subjects*	*Folate source and levels*	*DNA methylation*	*Effect*
Fenech *et al.*, 1998 (42)	Cross-sectional	Normal subjects	RBC (n*M*) 363.8–440.0	Lymphocytes, genomic	No associations
Narayanan *et al.*, 2004 (118)	Cross-sectional	Normal subjects	Plasma (n*M*) 15.2–20.6 RBC (n*M*) 191.9–254.2	Lymphocytes, genomic	No associations
Pilsner *et al.*, 2007 (123)	Cross-sectional	Bangladeshi adults chronically exposed to arsenic	Plasma (n*M*) <9 *versus* ≥ 9 Plasma (n*M*) 8.6	Leukocytes, genomic	3% decrease ($p=0.03$) $r=0.12$ ($p\leq 0.05$)
Hirsch *et al.*, 2008 (62)	Cross-sectional	Men	Serum (n*M*) 62.9 (high) *versus* 20.5 (normal)	Blood, genomic	No associations
Becker *et al.*, 2003 (13)	Cross-sectional	Normal subjects	RBC (n*M*) 551	Blood, genomic	No associations
Pufulete *et al.*, 2003 (128)	Case–control	Patients with colon cancer or adenoma	Serum (n*M*) 12.2 *versus* 18.1	Colon mucosa, genomic Leukocytes, genomic	26% decrease ($p=0.009$) 14% decrease ($p<0.001$)
Al-Ghnaniem *et al.*, 2007 (3)	Case–control	Patients with colon cancer or adenoma	Serum (n*M*) 12.3 *versus* 17.9	Colon mucosa, genomic	38% decrease ($p<0.001$)
Pufulete *et al.*, 2005 (129)	Cross-sectional	Patients without colon cancer or adenoma	Serum (n*M*) 18.6 RBC (n*M*) 648.1 Hcy (μM) 9.9	Colon mucosa, genomic	$r=-0.311$ ($p=0.01$) $r=-0.356$ ($p=0.03$) $r=0.256$ ($p=0.04$)
Alonso-Aperte *et al.*, 2008 (4)	Cross-sectional	Patients with colon cancer	Colon (nmol/g) 0.49 *versus* 0.95	Neoplastic *versus* normal colon, genomic	28% decrease ($p=0.08$)
Schernhammer *et al.*, 2009 (139)	Prospective	Patients with colon cancer	Dietary intake (μg/day) ≥ 400 *versus* <200	Colon cancer, genomic	43% decreased risk of hypomethylation ($p=0.05$)
Lim *et al.*, 2008 (102)	Case–control	Women 50–79 years with colonic adenoma	Prefortification dietary intake (μg/1000 kcal/day) <317 *versus* ≥ 317 Postfortification dietary intake (DFE): <413 *versus* ≥ 413	Leukocytes, genomic	Methylation inversely associated with colonic adenoma risk, especially in subjects with low folate intake in pre- and postfortification
Fowler *et al.*, 1998 (47)	Prospective	Patients with cervical dysplasia	Serum (n*M*) 5.2 Cervical tissue (ng/mg protein) 1.2	Cervical tissue, genomic	$r=-0.239$ ($p=0.06$) $r=-0.279$ ($p=0.02$)
Flatley *et al.*, 2009 (46)	Cross-sectional	Patients with cervical cancer	RBC (n*M*) 685	Cervical cells, genomic	No associations
Piyathilake *et al.*, 2011 (124)	Prospective	Women positive for high-risk human papillomavirus	Plasma folate (ng/ml) and B12 (pg/ml) >19.8 and >200 *versus* ≤ 19.8 and <200	Peripheral blood mononuclear cells, genomic Cervical cells, genomic	$\sim$4-fold increase ($p=0.04$) No associations

RBC, red blood cell; DFE, dietary folate equivalent.

Effects of folate status on gene-specific methylation in human studies

Observational studies investigating CRC. For gene-specific DNA methylation, the majority of human observational studies have investigated the role of folate status in CRC (see Table 3). Aberrant CpG island methylation is characteristic of tumor development, and specific promoter CpG islands are frequently and simultaneously methylated in sporadic CRC, leading to transcriptional silencing (32, 75, 110, 158). In the

Table 3. Summary of Associations Between Dietary and Blood Folate and Gene-Specific DNA Methylation in Human Epidemiological Studies

Study (references)	*Design*	*Subjects*	*Folate source and levels*	*DNA methylation*	*Effect*
van Engeland *et al.*, 2003 (165)	Prospective	Patients with colon cancer	Dietary intake (μg/day)/alcohol <215/high *versus* ≥215/low	Colon cancer: *APC-1A*, $p14^{ARF}$, $p16^{INK4A}$, *hMLH1*, O^6-*MGMT RASSFIA*	Increased prevalence for all genes ($p>0.05$)
de Vogel *et al.*, 2008 (33)	Prospective	Patients with colon cancer	Dietary intake (μg/day) 142.4–163.2 *versus* 247–279.9	Colon cancer: *MLH1*	No associations
Al-Ghnaniem *et al.*, 2007 (3)	Case–control	Patients with colon cancer or adenoma	Serum (n*M*) 12.3 *versus* 17.9	Colon mucosa: *ERα*, *MLH1*	No associations
van Guelpen *et al.*, 2009 (166)	Case–control	Patients with colon cancer	Plasma (n*M*) ≥6.8 *versus* <6.8	Colon cancer: *CDKN2A*, *MLH1*, *CACNA1G*, *NEUROG1*, *RUNX3*, *SOCS1*, *IGF2*, *CRABP1*	~3-fold increase ($p<0.05$)
Kawakami *et al.*, 2003 (75)	Cross-sectional	Patients with colon cancer	5,10-methylene-tetrahdrofolate (pmol/g tissue) 2.95 *versus* 1.53 Tetrahydrofolate (pmol/g tissue) 3.71 *versus* 1.99	Colon cancer: *hMLH1*, *p16*, *TIMP3*, *ARF*, *MINT2*, *DAPK*, *APC*	Tumors with methylated promoters had higher tissue concentration of folate intermediates
Other sites					
Flatley *et al.*, 2009 (46)	Cross-sectional	Patients with cervical cancer	RBC (n*M*) 685	Cervical cells: *hMLH1*, *DAPK*, *CDH1*, *HIC*, *MGMT*, *RARβ*, *GSTP1*	No associations
Tao *et al.*, 2009 (157)	Case–control	Patients with breast cancer	Dietary intake (mg/day) 267 *versus* 275–286	Breast cancer: *E-cadherin*, *p16*, *RAR-B_2*	No associations
Stidley *et al.*, 2010 (154)	Prospective	Subjects with a history of smoking (≥15 years)	Dietary intake per 750 μg/day	Sputum: *p16*, *MGMT*, *DAPK*, *RASSF1A*, *PAXα5*, *PAX5β*, *GATA-4,5*	16% decrease in methylation status (≥2 genes methylation) ($p=0.04$)
Wang *et al.*, 2008 (171)	Prospective	Patients with ESCC	Dietary intake (μg/day) 0 *versus* 311.1	ESCC: *p16* *hMLH1* *MGMT*	No associations No associations 34% decrease prevalence ($p=0.03$)
Jin *et al.*, 2009 (71)	Cross-sectional	Patients with NSCLC	5,10-methylene-THF & THF (pmol/g protein) 3.23	NSCLC: *LINE-1* *CDH13* *RUNX3*	$r=0.243$ ($p=0.041$) $r=0.007$ ($p=0.034$) $r=0.247$ ($p=0.038$)

ESCC, esophageal squamous cell carcinoma; NSCLC, nonsmall cell lung cancer.

Netherlands Cohort Study on Diet and Cancer, the prevalence of CpG island promoter hypermethylation was higher, albeit nonsignificantly, in CRCs derived from patients with low folate/high alcohol intake compared with CRCs from patients with high folate/low alcohol intake for each of the six tested genes (*APC*, *p14*, *p16*, *hMLH1*, O^6-*MGMT*, and *RASSF1A*) (165). The number of CRCs with at least one gene methylated was higher (84%) in the low folate intake/high alcohol intake group compared with the high folate intake/low alcohol intake group (165). A later follow-up analysis in a subcohort of this population did not report any effect of isolated dietary folate intake on the risk of CRCs specifically presenting with *MLH1* hypermethylation (33). Al-Ghnaniem *et al.* also examined gene-specific methylation in biopsies of normal-appearing colorectal mucosa from subjects with and without colorectal neoplasia (3). In general, patients with neoplasia were reported to have lower serum folate and promoter CpG hypermethylation of the *ERα* and *MLH1* genes compared with disease-free patients. Estrogen receptor alpha (*ERα*) methylation was also positively correlated with plasma

homocysteine in all subjects, but significant inverse correlations between promoter CpG methylation and folate status were not observed (3). In contrast, a modest degree of colorectal CpG hypermethylation of the *ERα* and *SFRP1* genes was significantly associated with higher RBC folate levels in participants from the Aspirin/Folate Polyp Prevention Study (170). The odds of promoter methylation of *CDKN2A, MLH1, CACNA1G, NEUROG1, RUNX3, SOCS1, IGF2,* and *CRABP1* in colorectal tumors are also greater in patients with high circulating levels of plasma folate (166). Colorectal carcinomas with frequent promoter methylation have been shown as having higher tumor concentrations of different folate metabolites, including 5,10-methyleneTHF and THF (75).

Observational studies investigating other cancers. The effect of folate status on gene-specific methylation patterns at other tissue sites has also been examined. In women with cervical dysplasia, although lower RBC folate and cervical promoter hypermethylation of several tumor suppressor genes were both independently associated with increasing severity of cervical cancer, inverse correlations between folate and methylation were not observed (46). Similarly, no associations were found between dietary folate intake and promoter methylation of *E-cadherin, p16, and RAR-*β_2 in breast tumors (157). For the *ERα* gene, however, lower dietary folate intake was associated with twofold greater odds, albeit nonsignificantly, of breast cancer with *ERα* promoter methylation. In addition, high folate intake has been shown to offer protection against methylation of several genes in subjects with a long history of smoking (154). The dietary folate intake derived from fruits was positively associated with a 34% increase in methylation frequency of the *MGMT* gene in esophageal squamous cell carcinoma (171) and tissue folate levels, measured as the sum of 5,10-methyleneTHF and THF, whereas it positively correlated with *LINE-1, CDH13*, and *RUNX3* methylation in nonsmall cell lung cancer (71).

In summary, the direction and magnitude of effect due to dietary and blood folate concentrations on gene-specific methylation remain unclear. Some studies demonstrate a greater prevalence or risk of aberrant hypermethylation of certain genes involved in colorectal carcinogenesis in subjects with low folate, while others have reported this in subjects with high folate. The discrepancies in identifying a clear association between folate status and gene-specific methylation may be explained, in part, by the different methods of stratifying folate levels for comparison and the use of different markers to evaluate folate status. Dietary intake and serum levels of folate may not necessarily be reflective of folate concentrations in the target organ. Moreover, blood as a surrogate marker of methylation is not always representative of tissue-specific methylation (113). These studies are also complicated by the lack of consistency in the specific genes investigated and the sampling of different CpG sites in different tissues.

Recently, the possibility of an inverse relationship between folate supplementation and DNA methylation status has been raised. Among reproductive-age women not previously exposed to FA in China, FA supplementation (0.1, 0.4, and 4 mg/day) for 1 month significantly decreased genomic methylation by 13% and continued to remain significantly lower with further intervention (130). An interesting observation in this study is that after a 3 month washout period after FA treatment, genomic methylation decreased even further (by 23%) relative to baseline values. This seemingly paradoxical effect of FA supplementation on global DNA methylation may be partly explained by the preferential shuttling of the flux of one-carbon units to the nucleotide synthesis pathway over the methionine cycle necessary for biological methylation reactions in response to FA supplementation. Although FA is an inhibitor of DHFR (9), and an enzyme important in the maintenance of the intracellular folate pool, in certain situations, it may upregulate DHFR (74), and this upregulation may increase thymidylate synthase activity, because the transcription of these genes is co-regulated by several transcription factors (Fig. 4) (145, 150). A mathematical modeling framework has indicated that this would increase thymidylate production, thereby increasing cellular proliferation, at the expense of biological methylation reactions (119).

MTHFR Polymorphisms and DNA Methylation

There is evidence that folate status influences DNA methylation through an interaction with the *MTHFR* C677T polymorphism (see Table 4). MTHFR is a critical enzyme in folate metabolism that catalyzes the irreversible conversion of 5,10-methyleneTHF to 5-methyltetrahydrofolate, thereby playing an important role in DNA synthesis, maintenance of nucleotide pool balance, and DNA methylation (Fig. 4) (49, 82). The *MTHFR* C677T polymorphism causes thermolability and reduced MTHFR activity, leading to lower levels of 5-methyltetrahydrofolate, an accumulation of 5,10-methyleneTHF, increased plasma homocysteine levels (a sensitive inverse indicator of folate status), and changes in the cellular composition of one-carbon folate derivatives (Fig. 4) (49, 82). Studies have also indicated that another polymorphism in the *MTHFR* gene (A1298C) may modulate the genomic DNA methylation in human lymphocytes, although the degree and direction of change have not been clearly established (17, 50). More recent investigations of folate status and DNA methylation in humans include analysis of common *MTHFR* polymorphisms.

In human intervention trials, daily folate restriction with 135 μg of dietary folate equivalents (DFEs) for 7 weeks followed by a repletion with 400 or 800 μg DFE for 7 weeks did not influence leukocyte genomic DNA methylation in a group of young and healthy women wild type for *MTHFR* C677T (5). However, another study by the same group observed significantly lower leukocyte genomic DNA methylation in women homozygous for the polymorphism relative to women with the CC or CT genotype at the end of the 14-week period of folate treatment (6). Similarly, Shelnutt *et al.* reported a nonsignificant decrease in leukocyte genomic DNA methylation in women depleted of folate (115 μg DFE/day for 7 weeks) (142). This was corrected by the folate repletion of 400 μg DFE/day for 7 weeks but only in women with the *MTHFR* 677TT genotype. However, an *in vivo* analysis of genomic monocyte DNA methylation in a subgroup of women from this same population, as determined by methyldeoxycytidine enrichment after radiolabeled infusions of [$^{13}C_5$]methionine, indicated that folate-dependent intracellular one-carbon metabolism was suppressed after 7 weeks of folate restriction (115 $\pm$ 20 μg DFE/day), but this effect was independent of the *MTHFR* genotype (131). Among a small

Table 4. Summary of Studies That Examined the Interaction Between the *MTHFR* C677T Genotype and Dietary Folate Intake in Association with DNA Methylation

Study (references)	*Design*	*Subjects*	*Folate dose, source and levels*	*Duration*	*DNA methylation*	*Effect*
Axume *et al.*, 2007 (6)	Clinical	Normal female subjects with *MTHFR* 677TT, CT or CC	135 μg/day, then 400 or 800 μg/day	7 weeks 7 weeks	Leukocytes, genomic	~4% decrease ($p<0.05$) in women with *MTHFR* 677TT
Shelnutt *et al.*, 2004 (142)	Clinical	Normal female subjects 20–30 years with *MTHFR* 677TT or CC	115 μg/day, then 400 μg/day	7 weeks 7 weeks	Leukocytes, genomic	5% decrease ($p=0.08$) after folate depletion then ~8% increase ($p=0.04$) in women with *MTHFR* 677TT
Quinlivan *et al.*, 2005 (131)	Clinical	Normal female subjects with *MTHFR* 677TT or CC	[$^{13}C_5$]methionine infusion and folate depletion (115 ± 20 μg DFE/day)	7 weeks	Monocytes, methyldeoxycytidine 1-carbon enrichment	MdC enrichment of monocyte DNA increased ($p=0.012$) with folate depletion but no effect by genotype
Pizzolo *et al.*, 2011 (125)	Clinical	Hyperhomocysteinemic men with *MTHFR* 677TT	5 mg/day	8 weeks	Peripheral blood mononuclear cells, genomic	No change
Friso *et al.*, 2002 (49)	Cross-sectional	Patients with and without coronary artery disease with *MTHFR* 677TT or CC	Plasma (n*M*) <12 *versus* ≥12 RBC (nmol/g Hb) <1.1 *versus* ≥1.1	NA	Peripheral blood mononuclear cells, genomic	61% decrease ($p<0.0001$) in subjects with *MTHFR* 677TT 65% decrease ($p<0.0001$) in subjects with *MTHFR* 677TT
Stern *et al.*, 2000 (152)	Cross-sectional	Healthy volunteers with *MTHFR* 677 TT or CC	RBC (nmol/g Hb) 2.3	NA	Leukocytes, genomic	$r=-0.738$ ($p=0.02$) in subjects with *MTHFR* 677TT
La Merrill *et al.*, 2011 (96)	Prospective	Pregnant women	Dietary intake (mg/day) <0.6 *versus* ≥0.6	NA	Blood, genomic	No associations with genotype
Van den Donk *et al.*, 2007 (164)	Case–control	Patients with colorectal adenoma and *MTHFR* TT genotype	Dietary intake (μg/day) >212 *versus* <183	NA	CRC adenomas: *APC-1A* *$p14^{ARF}$* *$p16^{INK4A}$* *hMLH1* *O^6-MGMT* *RASSFIA*	70% decrease (P-int >0.05) 51% decrease (P-int >0.05) 28% decrease (P-int >0.05) 74% decrease (P-int >0.05) 63% decrease (P-int = 0.02) 22% decrease (P-int = 0.06)
Curtin *et al.*, 2007 (32)	Case–control	Patients with colon cancer	Dietary intake (μg/1000 kcal/day) <135–152 (low), 135–201 (medium), >180–201 (high)	NA	Colon cancer: *p16, MLH1, MINT-1, −2, −3*	Increased in subjects with low folate/low methionine/high alcohol and *MTHFR* 1228AC/CC
Kraunz *et al.*, 2006 (94)	Case–control	Patients with HNSCC	Dietary intake low *versus* high	NA	HNSCC: *$p16^{INK4A}$*	>2.3 increase ($p\leq0.05$) and the *MTHFR* 677T polymorphism augmented the risk of methylation associated with low folate
Mokarram *et al.*, 2008 (115)	Prospective	Patients with sporadic CRC	Serum (ng/ml) >5.5 *versus* <5.5	NA	CRC tumors: *p16, hMLH1, hMSH2*	Frequency of methylated tumors was significantly higher in high *versus* low folate group in those with *MTHFR* 677CT ($p=0.01$) and CT/TT ($p=0.002$) genotypes

MTHFR, methylenetetrahydrofolate reductase; CRC, colorectal cancer; NA, not applicable; HNSCC, head and neck squamous cell carcinoma.

group of hyperhomocysteinemic men with normal renal function and homozygous for the *MTHFR* C677T polymorphism, supplementation with 5 mg FA/day for 8 weeks also did not induce any genomic methylation changes in peripheral blood mononuclear cells (125).

In observational studies, genomic DNA hypomethylation in peripheral blood monouclear cells has been observed in subjects homozygous for the *MTHFR* 677TT genotype when compared with CC wild-type individuals (49). When analyzed according to folate status, however, only TT subjects with low levels of folate had hypomethylated DNA (49). In healthy human volunteers, leukocyte genomic DNA methylation was positively and significantly related to RBC folate concentrations in subjects with the *MTHFR* 677TT genotype, but not in those with wild-type *MTHFR* (152). Furthermore, a strong trend toward diminished DNA methylation was also observed in subjects with the TT variant with lower plasma folate levels (152). However, in a population of pregnant women, where the majority were deficient in folate (< 0.6 mg/day according to World Health Organization and Institute of Medicine guidelines), dietary folate consumption did not influence global methylation in maternal blood, while women carrying the *MTHFR* 677 T allele had a greater risk of global DNA hypomethylation if they were vitamin B6 deficient (96).

In a study that investigated the combined effects of FA (12.5 times the daily requirement) and vitamin B12 (1.25 mg/day) supplementation for 6 months on the promoter methylation of six tumor suppressor and DNA repair genes frequently reported to be aberrantly methylated in CRC, a trend toward a 67% increase in promoter hypermethylation was reported in the rectal mucosa of patients with resected colorectal adenomas, although this did not reach statistical significance (163). However, further investigation of the six genes revealed that folate intake interacted with the *MTHFR* C677T polymorphism to influence CpG promoter methylation in colorectal adenomas such that among individuals homozygous for this variant, the risk of promoter methylation was inversely related to dietary folate intake, but statistical significance was only observed for the O^6-*MGMT* DNA-methyltransferase gene (164). The results from this research group suggest that higher folate intakes may increase methyltransferase expression and subsequent methylation activity, particularly in individuals with adenomas and reduced MTHFR enzyme activity (164). Furthermore, Slattery *et al.* initially failed to identify a significant association between dietary folate and colon tumor CpG island methylation of *p16, MLH1 and MINT-1, −2, and −3 loci* (146), but in their follow-up analysis, subjects heterozygous or homozygous for the *MTHFR* A1298C genotype with low folate/low methionine/high alcohol intake had more than twofold greater odds of developing tumors presenting CpG island hypermethylation compared with subjects with the wild-type genotype and high folate/high methionine/low alcohol intake (32). A greater risk of *p16* hypermethylation in head and neck squamous cell carcinomas was also observed in subjects with low dietary folate compared with those with high dietary folate, which was further exacerbated in subjects with the *MTHFR* 677TT genotype (94). In contrast, one study reported that the prevalence of promoter methylation of *p16*, but not *hMLH1* or *hMSH2*, was significantly higher in CRCs from patients with high serum folate concentrations, that the odds of tumor promoter methylation were significantly higher in patients with high circulating folate levels, and that this positive association was further modified by the *MTHFR* C677T polymorphism, reaching significance only in subjects heterozygous or homozygous for the *MTHFR* C677T polymorphism (115).

Taken together, these studies suggest that common *MTHFR* polymorphisms associated with impaired enzyme activity interact with folate in a manner that modulates both genomic and gene-specific DNA methylation. Human observational studies provide evidence that the *MTHFR* C677T polymorphism is associated with genomic hypomethylation in leukocytes, which may be mediated, in part, by a low status in folate or other methyl donors. For the colorectum and other tissue sites, whether or not the *MTHFR* C677T and A1298C polymorphism in conjunction with marginal folate status affects DNA methylation needs to be further characterized. These studies emphasize the importance of taking into consideration the interactions between folate status and critical genes in the folate and one-carbon metabolic pathway when investigating the effect of folate nutrition on DNA methylation.

Periods of Increased Susceptibility to Folate and DNA Methylation Changes

Pregnancy and early postnatal life

The importance of the immediately surrounding environment during critical periods of prenatal and early postnatal development has been known to affect lifelong health and the risk of disease. This field of study, known as the developmental origins of health and disease (DOHaD), has been in existence for nearly 50 years, but recent developments have generated tremendous interest in this area of research. Since epigenetic patterns are established *in utero*, aberrations in this dynamic process have been proposed to be an underpinning mechanism in the DOHaD hypothesis. One possibility is such that maternal exposures, including nutrition, can alter the intrauterine one-carbon precursor milieu and, as a result, disrupt one-carbon metabolism in the developing offspring. At present, the study of maternal nutrition and its effects on DNA methylation patterns as a predictor of health and risk of disease in the offspring has become a rapidly emerging field in the research community. Several preliminary studies have shown that maternal FA supplementation during pregnancy can modify the offspring's methylome with subsequent changes in phenotype.

Effects of maternal environment on DNA methylation in the offspring. Studies using viable yellow *agouti* mice have unequivocally demonstrated that maternal dietary methyl group supplementation containing FA can permanently alter the phenotypic coat color of the offspring *via* increased CpG methylation in the promoter region of the *agouti* gene (174, 178). Similarly, a methyl group-rich diet has been shown as significantly reducing the proportion of progeny with a kinked tail phenotype, and this was paralleled to a higher degree of CpG methylation in the promoter region of the *AxinFused* gene (173). In the *agouti* mouse model, folate has also been shown as interacting with other environmental exposures during the intrauterine period to modulate methylation patterns in the developing offspring. Bisphenol A is an estrogenic xenobiotic chemical used in the manufacturing of

polycarbonate plastics and is associated with higher body weight, increased risk of cancer, and other chronic health conditions (108). Exposure to this chemical has been shown to shift the coat color of *agouti* mice by decreasing CpG methylation of the *agouti* gene when provided *in utero*. Moreover, maternal methyl donor supplementation including FA successfully reversed the epigenetic and phenotypic effects of bisphenol A (35). In rats, promoter methylation of the *Pparγ* and *Gr* genes has been observed as being significantly lower, by 20% and 22.8%, respectively, in offspring from dams fed a protein-restricted diet compared with those fed a control diet during pregnancy (101). Accompanying increases in protein expression of the *Pparγ* and *Gr* genes were also reported, and maternal supplementation with FA prevented these changes (101).

Effects of maternal folate status on DNA methylation in the animal offspring. The effect of FA supplementation alone *in utero* on epigenetic modulation in the offspring has been displayed in other animal studies (see Table 5). In mice heterozygous for the folate binding protein gene *(Folbp1+/−)*, daily administration of folinic acid by gavage during the periconceptional period until day 15.5 of gestation significantly decreased genomic DNA methylation in both the liver and brain tissues of the offspring (45). In other rodent studies, maternal folate deficiency and supplementation with other nutritional factors were not observed as affecting offspring liver genomic methylation (39, 109). However, in hyperhomocysteinemic rats, maternal dietary folate deficiency was significantly associated with placenta genomic DNA hypomethylation (81). In addition, positive significant correlations between placental genomic DNA methylation and folate levels in the placenta, plasma, and liver were reported (81). Kulkarni *et al.* also investigated the effects of altered maternal FA in the presence or absence of vitamin B12 on placental global DNA methylation in rats and reported that FA supplementation (8 mg/kg) in the absence of vitamin B12 results in genomic DNA hypomethylation, suggesting that the ratio of FA and vitamin B12 that may have an important role in determining genomic methylation patterns (95). More recently, a study examining both maternal and postweaning folate supply on global methylation in the small intestine of adult mice offspring observed that maternal folate depletion (0.4 mg/kg) during pregnancy and lactation is associated with DNA hypomethylation compared with control (2 mg/kg) and supplemented (8 mg/kg) groups, regardless of postweaning folate diet (112). In an animal model involving sheep, maternal periconceptional folate and vitamin B12 restriction led to aberrant methylation patterns in 4% of the 1400 CpG islands examined (144). Furthermore, the adult male offspring displayed increased adiposity, insulin resistance, altered immune function, and high blood pressure (144).

Experimental animal tumorigenesis models have shown that maternal dietary folate manipulation can modify both DNA methylation patterns and the risk of cancer in the developing offspring. Maternal FA supplementation, equivalent to ∼1 mg FA/day in humans, provided *in utero* and during lactation significantly decreased mammary genomic methylation by 7%, and increased the risk of mammary tumors in the offspring (107). In contrast, maternal FA supplementation at the same level and duration significantly increased colorectal genomic methylation by 3%, and reduced the odds of developing colorectal tumors in the offspring (143). Similarly, in the Apc^{1638N} spontaneous mouse model of CRC, the offspring of B-vitamin (including FA) supplemented mothers displayed a mild degree of genomic hypomethylation in the small intestine mucosa and decreased tumor occurrence (24). This suggests that the effect of maternal FA supplementation on cancer risk in the offspring may be organ specific, and the outcome may be mediated by changes in global DNA methylation.

Effects of maternal folate status on DNA methylation in the human offspring. Recent human studies provide further support of the effect of folate supplementation *in utero* on epigenetic consequences in the offspring (see Table 5). A preliminary prospective study in the United Kingdom found an inverse correlation between cord plasma homocysteine concentrations and global DNA methylation using methylation of *LINE-1* sequences as a surrogate in cord lymphocytes in the offspring of 24 pregnant women (52). Although the results of this study are consistent with the biological functions of folate, significant associations between maternal FA use and cord blood folate and genomic lymphocyte methylation were not reported. Interestingly, an association between genomic lymphocyte methylation and fetal birth weight was identified (52). Further interrogation of CpG loci associated with specific genes in a subset of the same cohort by Fryer *et al.* found that CpG dinucleotide methylation patterns were also significantly correlated with plasma homocysteine, *LINE-1* methylation, and birth weight centile (51).

A limited number of studies have investigated the effect of maternal folate exposure on CpG methylation of the imprinted insulin-like growth factor-2 gene (*IGF2*), which is important for growth and development. A cross-sectional examination of a population unexposed to FA supplements before and during pregnancy revealed no significant associations between cord blood promoter methylation of the *IGF2* gene and serum folate levels in either mother's or umbilical cord blood (7). However, in an observational study conducted in the Netherlands, periconceptional maternal FA use of 400 μg/day significantly increased, by 4.5%, the methylation of the *IGF2* differentially methylated region (*IGF2* DMR) in whole blood derived from children at 17 months of age (151). An independent inverse association was also observed between *IGF2* DMR methylation and birth weight. More recently, the North American Newborn Epigenetic Study also evaluated the effect of the exposure of maternal FA supplementation before and during pregnancy on the methylation profile of two DMRs known to regulate *IGF2* expression (64). Using cord blood leukocytes, no evidence for methylation differences at the *IGF2* DMR was found while methylation levels at the second *H19* DMR decreased significantly among FA users compared with nonusers (64). Both *IGF2* DMR hypomethylation and *H19* DMR hypermethylation have been independently associated with increased *IGF2* transcriptional activity and loss of imprinting, and have been observed in several malignancies (64). While both studies report favorable shifts in methylation changes of the *IGF2* and *H19* DMRs with FA supplementation, the functional outcomes of these methylation changes require further investigation. More recently, the question of whether mothers themselves may be susceptible to changes in DNA methylation as a result of modified folate nutrition during pregnancy has been raised. In mice fed folate-adequate (2 mg/kg) and folate-deficient (0.4 mg/kg)

Table 5. Summary of *In Utero* and Perinatal Folate Supplementation on DNA Methylation in the Offspring

Study (references)	*Species*	*Folate source and levels*	*Duration*	*Tissue*	*DNA methylation*	*Effect*
Finnell *et al.*, 2002 (45)	Mice *Folbp1*$^{+/-}$	25 mg/kg/day folinic acid by gavage	2 weeks before mating to gestational day 15.5 Periconceptional	Liver Brain	Genomic	~4-fold decrease ($p<0.05$) ~2-fold decrease ($p<0.05$)
Maloney *et al.*, 2007 (109)	Rats	1. Control 2. Folate – / – 3. Folate – / – and low methionine 4. Folate – / – and low choline 5. Folate – / – and low methionine and low choline	2 weeks before mating to D21 of gestation Periconceptional	Liver	Genomic	No change
Kim *et al.*, 2009 (81)	Rats	1. 8 mg/kg FA 2. 8 mg/kg FA and 0.3% Hcy 3. 0.3% Hcy	4 weeks before day 20 of pregnancy Periconceptional	Placenta	Genomic correlation with Plasma folate Liver folate Placenta folate	$r=0.752$ ($p=0.0003$) $r=0.700$ ($p=0.0012$) $r=0.819$ ($p<0.0001$)
Kulkarni *et al.*, 2011 (95)	Rats	8 mg/kg FA and B12 – / – *versus* 2 mg/kg FA and B12 – / –	Pregnancy	Placenta	Genomic	Decreased ($p<0.05$)
McKay *et al.*, 2011 (112)	Mice	0.4 mg/kg FA *versus* 2 mg/kg FA or 8 mg/kg FA	Mating, pregnancy, and lactation	Small intestine	Genomic	Decreased ($p=0.009$) Decreased ($p=0.006$)
Sinclair *et al.*, 2007 (144)	Sheep	Vitamin B12 and folate deficient *versus* control	8 weeks before 6 days after conception Periconceptional	Liver	1400 CpG sites	4% of CpG sites had altered status ($p<0.001$)
Ly *et al.*, 2011 (107)	Rats	5 mg/kg FA *versus* 2 mg/kg FA	3 weeks before mating through pregnancy and lactation	Mammary	Genomic	7% decrease ($p=0.03$)
Sie *et al.*, 2011 (143)	Rats	5 mg/kg FA *versus* 2 mg/kg FA	3 weeks before mating through pregnancy and lactation	Colorectum	Genomic	3% increase ($p=0.007$)
Ciappio *et al.*, 2011 (24)	*Apc*^{1638N} Mice	Vitamin B supplemented (8 mg/kg FA) *versus* control (2 mg/kg FA)	4 weeks before mating through pregnancy and lactation	Small intestine mucosa	Genomic	~3% decrease ($p=0.07$)
Fryer *et al.*, 2009 (52)	Humans	FA Supplement 400 mg/day Cord serum 15.8 μM Cord Hcy 10.8 μM	Pregnancy	Cord blood	Genomic	$r=0.364$ ($p=0.08$) $r=0.209$ ($p>0.05$) $r=-0.688$ ($p=0.001$)
Ba *et al.*, 2011 (7)	Humans	Cord serum 7.29 ng/ml Maternal serum 2.29 ng/ml	NA (observational design)	Cord blood	*IGF2* promoter2 *IGF2* promoter3	NS
Steegers-Theunissen *et al.*, 2009 (151)	Humans	FA Supplement 400 μg/day	Periconceptional	Whole blood at 17 months old	*IGF2*	4.5% higher ($p=0.014$)
Hoyo *et al.*, 2011 (64)	Humans	FA users *versus* nonusers before or during pregnancy	NA (observational design)	Cord blood	*IGF2* DMR *H19* DMR	No associations 2.8% decrease ($p=0.03$) before pregnancy, 4.9% decrease ($p=0.04$) during pregnancy

FA, folic acid; IGF2, insulin-like growth factor-2; DMR, differentially methylated region; NS, not significant; NA, not applicable.

diets before mating and during pregnancy and lactation, postpartum methylation of *Igf*2 DMR1 and *Slc39a4* at specific CpG sites was significantly lower in folate-deficient rats (DNA methylation measured as an average across blood, liver, and kidney) (112). Interestingly, for *Igf*2 DMR2, a significant interaction between dietary folate and tissue type for overall methylation was observed where CpG methylation was lower in blood and higher in liver in low-folate dams (112).

Given the association between folate deficiency and the risk of NTDs, a limited number of human studies have investigated whether aberrant DNA methylation patterns can be an underlying mechanism of congenital malformations. Two case-control studies have examined the relationship between maternal folate status and genomic methylation of neural tissue from NTD-affected human fetuses (18, 172). While both studies reported lower maternal circulating folate concentrations and genomic brain hypomethylation in NTD fetuses compared with controls, only the study by Chang *et al.* observed a significant correlation between the methylation changes and folate status (18). Both studies suggest that folate inadequacy may interfere with one-carbon metabolism and affect normal fetal development.

Although it is difficult to directly compare the outcomes of the studies just reviewed due to inherent differences in study design and research models utilized, several lines of evidence support the theory that DNA methylation patterns of the developing offspring can be significantly modulated by environmental intrauterine exposures, including varying levels of FA alone or in combination with other methyl donors. Furthermore, the changes in methylation status may be associated with developmental changes and permanent alterations in phenotype with potential health consequences in later adult life. Whether or not folate-mediated epigenetic changes *in utero* can affect the risk of chronic disease development in adulthood remains unclear. Several studies indicate that maternal nutrition during pregnancy accurately predicts early life nutrition of the developing offspring (117, 120). Maternal biomarkers of B vitamins, including folate, have been shown as significantly correlating with cord blood folate (120) and infant folate at 6 months (60). Furthermore, folate levels in umbilical cord blood are thrice the level found in the mother (156). Thus, in this specific population of pregnant women, the possibility exists that an increased exposure to dietary and circulating folate translates to a heightened intrauterine folate environment, and this may have significant implications in the growth, development, and subsequent risk for disease in the offspring.

Aging

The study of age-related epigenetic changes has recently become a growing topic in research. As we age, a variety of internal and external factors play a role in disease susceptibility. Exposure to numerous environmental factors throughout life alters epigenetic patterns previously established *in utero*. Studies involving monozygotic twins clearly illustrate how environmental factors, including diet, can lead to the divergence of epigenetic modifications with age (48). Therefore, research on modifying or limiting exposures, which lead to epigenetic changes, is of significant interest.

Mechanism of action. Aging has been shown as modifying patterns of DNA methylation in a variety of species and tissues and is generally associated with genomic DNA hypomethylation and gene-specific promoter hypermethylation in a tissue-specific manner (53, 66, 167, 176). Genomic DNA hypomethylation is thought to occur with decreased activity of DNMT1, the methyltransferase responsible for maintaining and translating methylation patterns to subsequent DNA strands (168). Although not yet clear, gene-specific hypermethylation is thought to be due to upregulation of methyltransferases DNMT3a and 3b, which are responsible for *de novo* methylation (105).

Age-related methylation is a common event in human tissues and an important contributor to promoter CpG island hypermethylation of several genes with consequent gene inactivation of tumor suppressor genes observed in colorectal and other cancers (159). Furthermore, aging is an independent determinant of CRC risk (177). It is, therefore, possible that age-related DNA methylation of certain genes serves as a functional link between aging and CRC by providing a selective advantage for normal colon cells through deregulating growth and differentiation (159). These cells may then be at a higher susceptibility for acquiring genetic lesions such as mutations.

Effects of folate status and aging on DNA methylation in animal studies. It has been proposed that environmental exposures or modifier genes may play a role in age-related methylation changes (2). FA exposure plays a significant role in this phenomenon, as folate participates in methylation reactions. A number of studies have shown that folate and aging may act synergistically to alter DNA methylation patterns (see Table 6). A recent animal study reported diminished genomic and increased *p16* promoter DNA methylation in the colon of aged mice compared with those of young mice (78). Interestingly, both genomic and *p16* promoter DNA methylation increased in a manner that was directly related to dietary folate in old mice, whereas this pattern was not evident in young mice (78). In older rat liver, dietary folate over a wide range of intakes was also shown as modulating genomic DNA methylation, as genomic DNA methylation increased with increasing levels of dietary folate (22). Another rodent study has shown the elder rat colon to be highly susceptible to folate depletion, with consequent changes in SAM and SAH, compared with the young colon (21). Therefore, folate deficiency in the aging colon may predispose it to changes in SAM and SAH and consequent DNA methylation changes more readily than that in the young colon. Therefore, folate status and DNA methylation changes may serve as a functional link between aging and CRC.

Effects of folate status and aging on DNA methylation in human studies. In human studies, moderate folate depletion has been shown as being associated with diminished genomic DNA methylation in lymphocytes (67) and leukocytes (132) in healthy, postmenopausal women. This direct relationship between folate status and genomic DNA methylation is similar to the trends demonstrated in previous animal studies. Interestingly, however, genomic hypomethylation in lymphocytes was reversed after 3 weeks of folate repletion (286–516 μg/day) (67), but no such reversal was observed in leukocyte genomic methylation after folate

Table 6. Summary of Effects of Folate Status and Aging on DNA Methylation

Study (references)	*Species*	*Folate source and levels*	*Duration*	*Tissue*	*DNA methylation*	*Effect*
Keyes *et al.*, 2007 (78)	C57BL/6 mice, old (18 months) *versus* young (4 months)	0 μmol FA/kg diet	20 weeks	Colonic mucosa	Genomic *p16*	4.5% in old *versus* 6.0% in young ($p<0.001$) 61.0 *versus* 10.8% ($p<0.05$)
		4.5 μmol FA/kg diet				4.8% in old *versus* 5.3% in young (NS) 69.7% *versus* 8.4% ($p<0.05$)
		18 μmol FA/kg diet				4.9% in old *versus* 5.9% in young ($p<0.001$) 87.1% *versus* 4.9% ($p<0.05$)
Choi *et al.*, 2003 (21)	Rats, Weanling *versus* 1 year old	0 *versus* 4.5 *versus* 18 μmol FA/kg diet	8 weeks 20 weeks	Colonic mucosa	Genomic	No associations
Choi *et al.*, 2005 (22)	Rats	0 *versus* 4.5 *versus* 18 μmol FA/kg diet	8 weeks 20 weeks	Liver	Genomic	Stepwise increase in DNA methylation with increasing folate at 8 (NS) and 20 weeks ($p=0.025$)
Rampersaud *et al.*, 2000 (132)	Humans Female 60–85 years	118 μg/day	7 weeks	Leukocytes	Genomic	10% decrease ($p=0.0012$)
Jacob *et al.*, 1998 (67)	Humans Female 49–63 years	56–111 μg/day	9 weeks	Lymphocytes	Genomic	120% decrease ($p<0.05$)
Wallace *et al.*, 2010 (170)	Humans	Placebo *versus* 1 mg FA/day	3 years	Colorectal mucosa	*ERα* *SFRP1*	1.7% increase ($p<0.001$) 2.9% increase ($p<0.001$) for each 10 years of age No associations with FA
Li *et al.*, 2010 (100)	Human mononuclear cells	40 or 10 n*M* folate	NA	CD4+ T cells	*KIR2DL2*	Decreased in older cells in 40 n*M* *versus* younger cells in either folate concentration ($p<0.05$)

NS, not significant.

repletion (415 μg/day) for 7 weeks (132). It has been suggested that older age and lower levels of folate may increase the response time of returning methylation levels to normal levels (132).

In terms of gene-specific methylation, a recent multi-center chemoprevention trial found a 1.7% increase ($p<0.001$) in methylation levels in normal colorectal mucosa of the *ERα* and a 2.9% methylation increase ($p<0.001$) in secreted frizzled related protein-1 (*SFRP1*) for each 10 years of age. *ERα* and *SFRP1* are thought to regulate cell growth and differentiation and are, therefore, two genes important for cancer development. There was a positive, nonsignificant association between year 3 RBC folate levels and methylation levels in *ERα* and *SFRP1*. However, there were no associations indicating percentage *ERα* or *SFRP1* methylation levels leading to an increased risk of adenoma or hyperplastic polyps (170), indicating that the epigenetic changes may not lead to detrimental phenotypic outcomes.

Li *et al.* found that human cells from older subjects (>50 years old) cultured in low folate (10 n*M*) resulted in decreased killer cell immunoglobulin-like receptor 2DL2 (*KIR2DL2*) promoter region DNA methylation levels in T cells than in cells from younger subjects (100). Interestingly, the cells from older subjects exposed to higher folate levels (40 n*M*) had less methylation in the *KIR2DL2* promoter region than those in younger subjects exposed to either folate concentration, indicating that adequate folate levels did not return methylation levels to those found in younger subjects. The partial demethylation in *KIR2DL2* resulted in increased gene expression of *KIR2DL2* but only in the older subjects, and homocysteine exacerbated this relationship (100). However, these data are based on a small subset (three older, three younger) of study subjects. The resulting promoter region hypomethylation of *KIR2DL2* is the opposite of what is usually associated with aging. Nonetheless, the study indicates low folate and age lead to changes in methylation patterns that result in the differential expression of a gene involved in autoimmunity and acute coronary syndromes.

Since folate and increased age have been shown as having a synergistic effect on altered DNA methylation patterns, it is important to consider folate exposure in this population. Recent data indicate folate exposure in the elderly and are

adequate across all age groups with <1% of the Canadian population being folate deficient (26). Furthermore, median RBC folate levels were the highest among 60–79 year olds at 1409 n*M*. This is above the high RBC folate concentration cutoff of 1360 n*M*, which reflects the 97th percentile from the National Health and Nutrition Examination Survey (1999–2004) (122). The effects of supraphysiological FA levels in a population who is at a higher risk for cancer development are currently unknown; however, the potential for increased aberrant DNA methylation patterns and subsequent altered gene expression cannot be ignored. Concerns for increased FA levels in this vulnerable population have recently been raised (54).

The results from these studies suggest that the aging process can alter DNA methylation, and folate may further modify DNA methylation in the elderly in a tissue-specific manner. At present, the precise mechanism of how folate and aging interact to modulate DNA methylation has not been clearly elucidated. Folate intakes in the elderly seem to be adequate, and there is some concern that this population is reaching supraphysiological levels. However, data on epigenetic changes in the elderly suggest that it is important to consider environmental exposures over a lifetime and short-term folate exposure, in this case, may not be enough to return methylation levels to those found in younger individuals. However, therapeutic measures targeted at epigenetic modifications are desirable and worth researching while considering that epigenetic pathways are potentially reversible. Further studies are required to investigate the interaction between folate and aging on genomic and gene-specific DNA methylation and subsequent disease susceptibility.

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. In contrast, epigenetic changes are gradual in onset and 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 has been a focus of intense interest because of an inverse association between folate status and the risk of several malignancies and due to its potential ability to modulate DNA methylation. The portfolio of evidence from animal, *in vitro*, and human studies collectively suggests that the effects of folate deficiency and supplementation 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 depend on the magnitude and duration of folate manipulations, on interactions with other methyl group donors and dietary factors, and on genetic variants in the folate metabolic and one-carbon transfer pathways. Although some similarities exist, animal models differ from humans in several important physiological aspects, including bioavailability, metabolism, and excretion of folate. Therefore, any extrapolation of the observations from these models to human situations should be very cautiously made. Furthermore, animal models may produce variable results owing to species differences, different diet compositions, and variable dose, time, and duration of folate manipulations. Recent studies have demonstrated the exposure to folate deficiency, and supplementation in the intrauterine environment and early life and during the aging process may have profound effects on DNA methylation with significant functional ramifications. Although the jury is still out, the potential for folate to modulate DNA methylation and, thus, modify the risk of chronic diseases including cancer in humans remains provocative and is worthy of further studies.

References

1. Achon M, Alonso-Aperte E, Ubeda N, and Varela-Moreiras G. Supranormal dietary folic acid supplementation: effects on methionine metabolism in weanling rats. *Br J Nutr* 98: 490–496, 2007.
2. Ahuja N, Li Q, Mohan AL, Baylin SB, and Issa JP. Aging and DNA methylation in colorectal mucosa and cancer. *Cancer Res* 58: 5489–5494, 1998.
3. Al-Ghnaniem R, Peters J, Foresti R, Heaton N, and Pufulete M. Methylation of estrogen receptor alpha and mutL homolog 1 in normal colonic mucosa: association with folate and vitamin B-12 status in subjects with and without colorectal neoplasia. *Am J Clin Nutr* 86: 1064–1072, 2007.
4. Alonso-Aperte E, Gonzalez MP, Poo-Prieto R, and Varela-Moreiras G. Folate status and S-adenosylmethionine/S-adenosylhomocysteine ratio in colorectal adenocarcinoma in humans. *Eur J Clin Nutr* 62: 295–298, 2008.
5. Axume J, Smith SS, Pogribny IP, Moriarty DJ, and Caudill MA. Global leukocyte DNA methylation is similar in African American and Caucasian women under conditions of controlled folate intake. *Epigenetics* 2: 66–68, 2007.
6. Axume J, Smith SS, Pogribny IP, Moriarty DJ, and Caudill MA. The MTHFR 677TT genotype and folate intake interact to lower global leukocyte DNA methylation in young Mexican American women. *Nutr Res* 27: 1365–1317, 2007.
7. Ba Y, Yu H, Liu F, Geng X, Zhu C, Zhu Q, Zheng T, Ma S, Wang G, Li Z, and Zhang Y. Relationship of folate, vitamin B12 and methylation of insulin-like growth factor-II in maternal and cord blood. *Eur J Clin Nutr* 65: 480–485, 2011.
8. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C—>T polymorphism affect cancer risk: intake recommendations. *J Nutr* 133: 3748S–3753S, 2003.
9. Bailey SW and Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. *Proc Natl Acad Sci U S A* 106: 15424–15429, 2009.
10. Balaghi M, Horne DW, and Wagner C. Hepatic one-carbon metabolism in early folate deficiency in rats. *Biochem J* 291 (Pt 1): 145–149, 1993.
11. Ballestar E and Esteller M. The impact of chromatin in human cancer: linking DNA methylation to gene silencing. *Carcinogenesis* 23: 1103–1109, 2002.
12. Basten GP, Duthie SJ, Pirie L, Vaughan N, Hill MH, and Powers HJ. Sensitivity of markers of DNA stability and DNA repair activity to folate supplementation in healthy volunteers. *Br J Cancer* 94: 1942–1947, 2006.
13. Becker A, Smulders YM, Teerlink T, Struys EA, de Meer K, Kostense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ, Bouter LM, and Stehouwer CD. S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine are not related to folate, cobalamin and vitamin B6 concentrations. *Eur J Clin Invest* 33: 17–25, 2003.

14. Biasco G, Zannoni U, Paganelli GM, Santucci R, Gionchetti P, Rivolta G, Miniero R, Pironi L, Calabrese C, Di Febo G, and Miglioli M. Folic acid supplementation and cell kinetics of rectal mucosa in patients with ulcerative colitis. *Cancer Epidemiol Biomarkers Prev* 6: 469–471, 1997.
15. Bird AP and Wolffe AP. Methylation-induced repression—belts, braces, and chromatin. *Cell* 99: 451–454, 1999.
16. Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, Appella E, Kastan MB, and Siliciano JD. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* 281: 1677–1679, 1998.
17. Castro R, Rivera I, Ravasco P, Camilo ME, Jakobs C, Blom HJ, and de Almeida IT. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C—>T and 1298A—>C mutations are associated with DNA hypomethylation. *J Med Genet* 41: 454–458, 2004.
18. Chang H, Zhang T, Zhang Z, Bao R, Fu C, Wang Z, Bao Y, Li Y, Wu L, Zheng X, and Wu J. Tissue-specific distribution of aberrant DNA methylation associated with maternal low-folate status in human neural tube defects. *J Nutr Biochem* 22:1172–1177, 2011.
19. Chattopadhyay S, Tamari R, Min SH, Zhao R, Tsai E, and Goldman ID. Commentary: a case for minimizing folate supplementation in clinical regimens with pemetrexed based on the marked sensitivity of the drug to folate availability. *Oncologist* 12: 808–815, 2007.
20. Chen LH, Liu ML, Hwang HY, Chen LS, Korenberg J, and Shane B. Human methionine synthase. cDNA cloning, gene localization, and expression. *J Biol Chem* 272: 3628–3634, 1997.
21. Choi SW, Friso S, Dolnikowski GG, Bagley PJ, Edmondson AN, Smith DE, and Mason JB. Biochemical and molecular aberrations in the rat colon due to folate depletion are age-specific. *J Nutr* 133: 1206–1212, 2003.
22. Choi SW, Friso S, Keyes MK, and Mason JB. Folate supplementation increases genomic DNA methylation in the liver of elder rats. *Br J Nutr* 93: 31–35, 2005.
23. Choi SW, Kim YI, Weitzel JN, and Mason JB. Folate depletion impairs DNA excision repair in the colon of the rat. *Gut* 43: 93–99, 1998.
24. Ciappio ED, Liu Z, Brooks RS, Mason JB, Bronson RT, and Crott JW. Maternal B vitamin supplementation from preconception through weaning suppresses intestinal tumorigenesis in Apc1638N mouse offspring. *Gut* 60: 1695–1702, 2011.
25. Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, Bonaa KH, Spence JD, Nygard O, Jamison R, Gaziano JM, Guarino P, Bennett D, Mir F, Peto R, and Collins R. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med* 170: 1622–1631, 2010.
26. Colapinto CK, O'Connor DL, and Tremblay MS. Folate status of the population in the Canadian Health Measures Survey. *CMAJ* 183: E100–E106, 2011.
27. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, McKeown-Eyssen G, Summers RW, Rothstein RI, Burke CA, Snover DC, Church TR, Allen JI, Robertson DJ, Beck GJ, Bond JH, Byers T, Mandel JS, Mott LA, Pearson LH, Barry EL, Rees JR, Marcon N, Saibil F, Ueland PM, and Greenberg ER. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA* 297: 2351–2359, 2007.
28. Cravo M, Fidalgo P, Pereira AD, Gouveia-Oliveira A, Chaves P, Selhub J, Mason JB, Mira FC, and Leitao CN. DNA methylation as an intermediate biomarker in colorectal cancer: modulation by folic acid supplementation. *Eur J Cancer Prev* 3: 473–479, 1994.
29. Cravo ML, Albuquerque CM, Salazar de Sousa L, Gloria LM, Chaves P, Dias Pereira A, Nobre Leitao C, Quina MG, and Costa Mira F. Microsatellite instability in non-neoplastic mucosa of patients with ulcerative colitis: effect of folate supplementation. *Am J Gastroenterol* 93: 2060–2064, 1998.
30. Cravo ML, Pinto AG, Chaves P, Cruz JA, Lage P, Nobre Leitao C, and Costa Mira F. Effect of folate supplementation on DNA methylation of rectal mucosa in patients with colonic adenomas: correlation with nutrient intake. *Clin Nutr* 17: 45–49, 1998.
31. Crowell J, Ly A, and Kim YI. Folate and DNA methylation. In: *Nutrition, Epigenetic Mechanisms, and Human Disease*. edited by Maulik N and Maulik G. Boca Raton, FL: CRC Press, 2011, pp. 31–75.
32. Curtin K, Slattery ML, Ulrich CM, Bigler J, Levin TR, Wolff RK, Albertsen H, Potter JD, and Samowitz WS. Genetic polymorphisms in one-carbon metabolism: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. *Carcinogenesis* 28: 1672–1679, 2007.
33. de Vogel S, Bongaerts BW, Wouters KA, Kester AD, Schouten LJ, de Goeij AF, de Bruine AP, Goldbohm RA, van den Brandt PA, van Engeland M, and Weijenberg MP. Associations of dietary methyl donor intake with MLH1 promoter hypermethylation and related molecular phenotypes in sporadic colorectal cancer. *Carcinogenesis* 29: 1765–1773, 2008.
34. Dervieux T, Furst D, Lein DO, Capps R, Smith K, Caldwell J, and Kremer J. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study. *Ann Rheum Dis* 64: 1180–1185, 2005.
35. Dolinoy DC, Huang D, and Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc Natl Acad Sci U S A* 104: 13056–13061, 2007.
36. Duthie SJ, Grant G, Pirie LP, Watson AJ, and Margison GP. Folate deficiency alters hepatic and colon MGMT and OGG-1 DNA repair protein expression in rats but has no effect on genome-wide DNA methylation. *Cancer Prev Res (Phila)* 3: 92–100, 2010.
37. Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, Rasmussen K, Njolstad I, Refsum H, Nilsen DW, Tverdal A, Meyer K, and Vollset SE. Cancer incidence and mortality after treatment with folic acid and vitamin B12. *JAMA* 302: 2119–2126, 2009.
38. Egger G, Liang G, Aparicio A, and Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429: 457–463, 2004.
39. Engeham SF, Haase A, and Langley-Evans SC. Supplementation of a maternal low-protein diet in rat pregnancy with folic acid ameliorates programming effects upon feeding behaviour in the absence of disturbances to the methionine-homocysteine cycle. *Br J Nutr* 103: 996–1007, 2009.
40. Esteller M. Relevance of DNA methylation in the management of cancer. *Lancet Oncol* 4: 351–358, 2003.

41. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 8: 286–298, 2007.
42. Fenech M, Aitken C, and Rinaldi J. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis* 19: 1163–1171, 1998.
43. Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS, Burke CA, McKeown-Eyssen GE, and Baron JA. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst* 101: 432–435, 2009.
44. Figueiredo JC, Grau MV, Wallace K, Levine AJ, Shen L, Hamdan R, Chen X, Bresalier RS, McKeown-Eyssen G, Haile RW, Baron JA, and Issa JP. Global DNA hypomethylation (LINE-1) in the normal colon and lifestyle characteristics and dietary and genetic factors. *Cancer Epidemiol Biomarkers Prev* 18: 1041–1049, 2009.
45. Finnell RH, Spiegelstein O, Wlodarczyk B, Triplett A, Pogribny IP, Melnyk S, and James JS. DNA methylation in Folbp1 knockout mice supplemented with folic acid during gestation. *J Nutr* 132: 2457S–2461S, 2002.
46. Flatley JE, McNeir K, Balasubramani L, Tidy J, Stuart EL, Young TA, and Powers HJ. Folate status and aberrat DNA methylation are associated with HPV infection and cervical pathogenesis. *Cancer Epidemiol Biomarkers Prev* 18: 2782–2789, 2009.
47. Fowler BM, Giuliano AR, Piyathilake C, Nour M, and Hatch K. Hypomethylation in cervical tissue: is there a correlation with folate status? *Cancer Epidemiol Biomarkers Prev* 7: 901–906, 1998.
48. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, and Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Nat Acad Scis U S A* 102: 10604–10609, 2005.
49. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, and Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci U S A* 99: 5606–5611, 2002.
50. Friso S, Girelli D, Trabetti E, Olivieri O, Guarini P, Pignatti PF, Corrocher R, and Choi SW. The MTHFR 1298A>C polymorphism and genomic DNA methylation in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* 14: 938–943, 2005.
51. Fryer AA, Emes RD, Ismail KM, Haworth KE, Mein C, Carroll WD, and Farrell WE. Quantitative, high-resolution epigenetic profiling of CpG loci identifies associations with cord blood plasma homocysteine and birth weight in humans. *Epigenetics* 6:86–94, 2011.
52. Fryer AA, Nafee TM, Ismail KM, Carroll WD, Emes RD, and Farrell WE. LINE-1 DNA methylation is inversely correlated with cord plasma homocysteine in man: a preliminary study. *Epigenetics* 4: 394–398, 2009.
53. Fuke C, Shimabukuro M, Petronis A, Sugimoto J, Oda T, Miura K, Miyazaki T, Ogura C, Okazaki Y, and Jinno Y. Age related changes in 5-methylcytosine content in human peripheral leukocytes and placentas: an HPLC-based study. *Ann Hum Genet* 68: 196–204, 2004.
54. Garcia A. Folic acid in older adults. *CMAJ* 183: 827, 2011.
55. Gibson TM, Weinstein SJ, Pfeiffer RM, Hollenbeck AR, Subar AF, Schatzkin A, Mayne ST, and Stolzenberg-Solomon R. Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. *Am J Clin Nutr* 94: 1053–1062, 2011.
56. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, and Willett WC. Alcohol, low-methionine—low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* 87: 265–273, 1995.
57. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, and Willett WC. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med* 129: 517–524, 1998.
58. Girgis S, Nasrallah IM, Suh JR, Oppenheim E, Zanetti KA, Mastri MG, and Stover PJ. Molecular cloning, characterization and alternative splicing of the human cytoplasmic serine hydroxymethyltransferase gene. *Gene* 210: 315–324, 1998.
59. Green JM, MacKenzie RE, and Matthews RG. Substrate flux through methylenetetrahydrofolate dehydrogenase: predicted effects of the concentration of methylenetetrahydrofolate on its partitioning into pathways leading to nucleotide biosynthesis or methionine regeneration. *Biochemistry* 27: 8014–8022, 1988.
60. Hay G, Clausen T, Whitelaw A, Trygg K, Johnston C, Henriksen T, and Refsum H. Maternal folate and cobalamin status predicts vitamin status in newborns and 6-month-old infants. *J Nutr* 140: 557–564, 2010.
61. Herman JG and Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 349: 2042–2054, 2003.
62. Hirsch S, Ronco AM, Guerrero-Bosagna C, de la Maza MP, Leiva L, Barrera G, Llanos M, Alliende MA, Silva F, and Bunout D. Methylation status in healthy subjects with normal and high serum folate concentration. *Nutrition* 24: 1103–1109, 2008.
63. Hirsch S, Sanchez H, Albala C, de la Maza MP, Barrera G, Leiva L, and Bunout D. Colon cancer in Chile before and after the start of the flour fortification program with folic acid. *Eur J Gastroenterol Hepatol* 21: 436–439, 2009.
64. Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR, Iversen ES, Kurtzberg J, Overcash F, Huang Z, and Murphy SK. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics* 6: 928–936, 2011.
65. Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML, Vacca M, D'Esposito M, D'Urso M, Galletti P, and Zappia V. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet* 361: 1693–1699, 2003.
66. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, and Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* 7: 536–540, 1994.
67. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, Miller BJ, Henning SM, and Swendseid ME. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr* 128: 1204–1212, 1998.
68. Jacob RA, Pianalto FS, Henning SM, Zhang JZ, and Swendseid ME. *In vivo* methylation capacity is not impaired in healthy men during short-term dietary folate and methyl group restriction. *J Nutr* 125: 1495–1502, 1995.

69. Jacobs EJ, Connell CJ, Patel AV, Chao A, Rodriguez C, Seymour J, McCullough ML, Calle EE, and Thun MJ. Multivitamin use and colon cancer mortality in the Cancer Prevention Study II cohort (United States). *Cancer Causes Control* 12: 927–934, 2001.
70. Jaszewski R, Misra S, Tobi M, Ullah N, Naumoff JA, Kucuk O, Levi E, Axelrod BN, Patel BB, and Majumdar AP. Folic acid supplementation inhibits recurrence of colorectal adenomas: a randomized chemoprevention trial. *World J Gastroenterol* 14: 4492–4498, 2008.
71. Jin M, Kawakami K, Fukui Y, Tsukioka S, Oda M, Watanabe G, Takechi T, Oka T, and Minamoto T. Different histological types of non-small cell lung cancer have distinct folate and DNA methylation levels. *Cancer Sci* 100:2325–2330, 2009.
72. Jones PA and Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 3: 415–428, 2002.
73. Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, and Wolffe AP. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* 19: 187–191, 1998.
74. Kamen BA, Nylen PA, Whitehead VM, Abelson HT, Dolnick BJ, and Peterson DW. Lack of dihydrofolate reductase in human tumor and leukemia cells *in vivo*. *Cancer Drug Deliv* 2: 133–138, 1985.
75. Kawakami K, Ruszkiewicz A, Bennett G, Moore J, Watanabe G, and Iacopetta B. The folate pool in colorectal cancers is associated with DNA hypermethylation and with a polymorphism in methylenetetrahydrofolate reductase. *Clin Cancer Res* 9: 5860–5865, 2003.
76. Kelly P, McPartlin J, Goggins M, Weir DG, and Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. *Am J Clin Nutr* 65: 1790–1795, 1997.
77. Kennedy DA, Stern SJ, Moretti M, Matok I, Sarkar M, Nickel C, and Koren G. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. *Cancer Epidemiol* 35: 2–10, 2011.
78. Keyes MK, Jang H, Mason JB, Liu Z, Crott JW, Smith DE, Friso S, and Choi SW. Older age and dietary folate are determinants of genomic and p16-specific DNA methylation in mouse colon. *J Nutr Biochem* 137: 1713–1717, 2007.
79. Khanna D, Park GS, Paulus HE, Simpson KM, Elashoff D, Cohen SB, Emery P, Dorrier C, and Furst DE. Reduction of the efficacy of methotrexate by the use of folic acid: *post hoc* analysis from two randomized controlled studies. *Arthritis Rheum* 52: 3030–3038, 2005.
80. Khosraviani K, Weir HP, Hamilton P, Moorehead J, and Williamson K. Effect of folate supplementation on mucosal cell proliferation in high risk patients for colon cancer. *Gut* 51: 195–199, 2002.
81. Kim JM, Hong K, Lee JH, Lee S, and Chang N. Effect of folate deficiency on placental DNA methylation in hyperhomocysteinemic rats. *J Nutr Biochem* 20: 172–176, 2009.
82. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. *J Nutr Biochem* 10: 66–88, 1999.
83. Kim YI. Role of folate in colon cancer development and progression. *J Nutr* 133: 3731S–3739S, 2003.
84. Kim YI. Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? *Cancer Epidemiol Biomarkers Prev* 13: 511–519, 2004.
85. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr* 80: 1123–1128, 2004.
86. Kim YI. Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. *J Nutr* 135: 2703–2709, 2005.
87. Kim YI. Folate and colorectal cancer: an evidence-based critical review. *Mol Nutr Food Res* 51: 267–292, 2007.
88. Kim YI. Folic acid supplementation and cancer risk: point. *Cancer Epidemiol Biomarkers Prev* 17: 2220–2225, 2008.
89. Kim YI. Role of the MTHFR polymorphisms in cancer risk modification and treatment. *Future Oncol* 5: 523–542, 2009.
90. Kim YI, Baik HW, Fawaz K, Knox T, Lee YM, Norton R, Libby E, and Mason JB. Effects of folate supplementation on two provisional molecular markers of colon cancer: a prospective, randomized trial. *Am J Gastroenterol* 96: 184–195, 2001.
91. Kim YI, Christman JK, Fleet JC, Cravo ML, Salomon RN, Smith D, Ordovas J, Selhub J, and Mason JB. Moderate folate deficiency does not cause global hypomethylation of hepatic and colonic DNA or c-myc-specific hypomethylation of colonic DNA in rats. *Am J Clin Nutr* 61: 1083–1090, 1995.
92. Kim YI, Salomon RN, Graeme-Cook F, Choi SW, Smith DE, Dallal GE, and Mason JB. Dietary folate protects against the development of macroscopic colonic neoplasia in a dose responsive manner in rats. *Gut* 39: 732–740, 1996.
93. Kotsopoulos J, Sohn KJ, and Kim YI. Postweaning dietary folate deficiency provided through childhood to puberty permanently increases genomic DNA methylation in adult rat liver. *J Nutr* 138: 703–709, 2008.
94. Kraunz KS, Hsiung D, McClean MD, Liu M, Osanyingbemi J, Nelson HH, and Kelsey KT. Dietary folate is associated with p16(INK4A) methylation in head and neck squamous cell carcinoma. *Int J Cancer* 119: 1553–1557, 2006.
95. Kulkarni A, Dangat K, Kale A, Sable P, Chavan-Gautam P, and Joshi S. Effects of altered maternal folic acid, vitamin B12 and docosahexaenoic acid on placental global DNA methylation patterns in Wistar rats. *PLoS One* 6: e17706, 2011.
96. La Merrill M, Torres-Sanchez L, Ruiz-Ramos R, Lopez-Carrillo L, Cebrian ME, and Chen J. The association between first trimester micronutrient intake, MTHFR genotypes, and global DNA methylation in pregnant women. *J Matern Fetal Neonatal Med* 25: 133–137, 2012.
97. Lashner BA, Shapiro BD, Husain A, and Goldblum JR. Evaluation of the usefulness of testing for p53 mutations in colorectal cancer surveillance for ulcerative colitis. *Am J Gastroenterol* 94: 456–462, 1999.
98. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, Watkins D, Heng HH, Rommens JM, Scherer SW, Rosenblatt DS, and Gravel RA. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. *Proc Natl Acad Sci U S A* 95: 3059–3064, 1998.
99. Li E and Jaenisch R. DNA methylation and methyltransferases. In: *DNA Alterations in Cancer: Genetic and Epigenetic Changes*, edited by Ehrlich M. Natick, MA: Eaton Publishing, 2000, pp. 351–365.
100. Li Y, Liu Y, Strickland FM, and Richardson B. Age-dependent decreases in DNA methyltransferase levels and low transmethylation micronutrient levels synergize to promote overexpression of genes implicated in autoimmunity and acute coronary syndromes. *Exp Gerontol* 45: 312–322, 2010.
101. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, and Burdge GC. Dietary protein restriction of pregnant rats

induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 135: 1382–1386, 2005.
102. Lim U, Flood A, Choi SW, Albanes D, Cross AJ, Schatzkin A, Sinha R, Katki HA, Cash B, Schoenfeld P, and Stolzenberg-Solomon R. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. *Gastroenterology* 134: 47–55, 2008.
103. Linhart HG, Troen AM, Bell GW, Cantu E, Chao WH, Moran E, Steine E, He T, and Jaenisch R. Folate deficiency induces genomic uracil misincorporation and hypomethylation but does not increase DNA point mutations. *Gastroenterology* 136: 227–235, 2009.
104. Logan RF, Grainge MJ, Shepherd VC, Armitage NC, and Muir KR. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology* 134: 29–38, 2008.
105. Lopatina N, Haskell JF, Andrews LG, Poole JC, Saldanha S, and Tollefsbol T. Differential maintenance and *de novo* methylating activity by three DNA methyltransferases in aging and immortalized fibroblasts. *J Cell Biochem* 84: 324–334, 2002.
106. Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol Genet Metab* 71: 121–138, 2000.
107. Ly A, Lee H, Chen J, Sie KK, Renlund R, Medline A, Sohn KJ, Croxford R, Thompson LU, and Kim YI. Effect of maternal and postweaning folic acid supplementation on mammary tumor risk in the offspring. *Cancer Res* 71: 988–997, 2011.
108. Maffini MV, Rubin BS, Sonnenschein C, and Soto AM. Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol* 254–255: 179–186, 2006.
109. Maloney CA, Hay SM, and Rees WD. Folate deficiency during pregnancy impacts on methyl metabolism without affecting global DNA methylation in the rat fetus. *Br J Nutr* 97: 1090–1098, 2007.
110. Markowitz SD and Bertagnolli MM. Molecular origins of cancer: molecular basis of colorectal cancer. *N Engl J Med* 361: 2449–2460, 2009.
111. Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, Dallal G, and Rosenberg IH. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev* 16: 1325–1329, 2007.
112. McKay JA, Waltham KJ, Williams EA, and Mathers JC. Folate depletion during pregnancy and lactation reduces genomic DNA methylation in murine adult offspring. *Genes Nutr* 6: 189–196, 2011.
113. McKay JA, Xie L, Harris S, Wong YK, Ford D, and Mathers JC. Blood as a surrogate marker for tissue-specific DNA methylation and changes due to folate depletion in postpartum female mice. *Mol Nutr Food Res* 55: 1026–1035, 2011.
114. A report of the standing committee on the scientific evaluation of dietary reference intakes and its panel on folate, other B vitamins, and choline and subcommittee on upper reference levels of nutrients. Food and Nutrition Board. Institute of Medicine. *Dietary Reference Intakes for Thiamin, Roboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press, 1998, pp. 196–305.
115. Mokarram P, Naghibalhossaini F, Saberi Firoozi M, Hosseini SV, Izadpanah A, Salahi H, Malek-Hosseini SA, Talei A, and Mojallal M. Methylenetetrahydrofolate reductase C677T genotype affects promoter methylation of tumor-specific genes in sporadic colorectal cancer through an interaction with folate/vitamin B12 status. *World J Gastroenterol* 14: 3662–3671, 2008.
116. Morris MS, Jacques PF, Rosenberg IH, and Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 85: 193–200, 2007.
117. Murphy MM, Scott JM, Arija V, Molloy AM, and Fernandez-Ballart JD. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. *Clin Chem* 50: 1406–1412, 2004.
118. Narayanan S, McConnell J, Little J, Sharp L, Piyathilake CJ, Powers H, Basten G, and Duthie SJ. Associations between two common variants C677T and A1298C in the methylenetetrahydrofolate reductase gene and measures of folate metabolism and DNA stability (strand breaks, misincorporated uracil, and DNA methylation status) in human lymphocytes *in vivo*. *Cancer Epidemiol Biomarkers Prev* 13: 1436–1443, 2004.
119. Nijhout HF, Reed MC, Budu P, and Ulrich CM. A mathematical model of the folate cycle: new insights into folate homeostasis. *J Biol Chem* 279: 55008–55016, 2004.
120. Obeid R, Munz W, Jager M, Schmidt W, and Herrmann W. Biochemical indexes of the B vitamins in cord serum are predicted by maternal B vitamin status. *Am J Clin Nutr* 82: 133–139, 2005.
121. Paspatis GA and Karamanolis DG. Folate supplementation and adenomatous colonic polyps. *Dis Colon Rectum* 37: 1340–1341, 1994.
122. Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, Fisher KD, Mulinare J, and Osterloh JD. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988 2004. *Am J Clin Nutr* 86: 718–727, 2007.
123. Pilsner JR, Liu X, Ahsan H, Ilievski V, Slavkovich V, Levy D, Factor-Litvak P, Graziano JH, and Gamble MV. Genomic methylation of peripheral blood leukocyte DNA: influences of arsenic and folate in Bangladeshi adults. *Am J Clin Nutr* 86: 1179–1186, 2007.
124. Piyathilake CJ, Macaluso M, Alvarez RD, Chen M, Badiga S, Siddiqui NR, Edberg JC, Partridge EE, and Johanning GL. A higher degree of LINE-1 methylation in peripheral blood mononuclear cells, a one-carbon nutrient related epigenetic alteration, is associated with a lower risk of developing cervical intraepithelial neoplasia. *Nutrition* 27: 513–519, 2011.
125. Pizzolo F, Blom HJ, Choi SW, Girelli D, Guarini P, Martinelli N, Stanzial AM, Corrocher R, Olivieri O, and Friso S. Folic acid effects on S-adenosylmethionine, S-adenosylhomocysteine, and DNA methylation in patients with intermediate hyperhomocysteinemia. *J Am Coll Nutr* 30: 11–18, 2011.
126. Potter JD. Methyl supply, methyl metabolizing enzymes and colorectal neoplasia. *J Nutr* 132: 2410S–2412S, 2002.
127. Pufulete M, Al-Ghnaniem R, Khushal A, Appleby P, Harris N, Gout S, Emery PW, and Sanders TA. Effect of folic acid supplementation on genomic DNA methylation in patients with colorectal adenoma. *Gut* 54: 648–653, 2005.
128. Pufulete M, Al-Ghnaniem R, Leather AJ, Appleby P, Gout S, Terry C, Emery PW, and Sanders TA. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. *Gastroenterology* 124: 1240–1248, 2003.
129. Pufulete M, Al-Ghnaniem R, Rennie JA, Appleby P, Harris N, Gout S, Emery PW, and Sanders TA. Influence of folate

status on genomic DNA methylation in colonic mucosa of subjects without colorectal adenoma or cancer. *Br J Cancer* 92: 838–842, 2005.
130. Quinlivan EP, Crider K, Berry RJ, Hao L, Li Z, Zhu JH, Maneval D, Young TP, and Bailey LB. Global DNA methylation changes in response to chronic consumption and withdrawal of low, moderate, and high folic acid doses. *FASEB J* 22: 689.7, 2008.
131. Quinlivan EP, Davis SR, Shelnutt KP, Henderson GN, Ghandour H, Shane B, Selhub J, Bailey LB, Stacpoole PW, and Gregory JF 3rd. Methylenetetrahydrofolate reductase 677C->T polymorphism and folate status affect one-carbon incorporation into human DNA deoxynucleosides. *J Nutr* 135: 389–396, 2005.
132. Rampersaud GC, Kauwell GP, Hutson AD, Cerda JJ, and Bailey LB. Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. *Am J Clin Nutr* 72: 998–1003, 2000.
133. Reik W, Dean W, and Walter J. Epigenetic reprogramming in mammalian development. *Science* 293: 1089–1093, 2001.
134. Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 6: 597–610, 2005.
135. Robertson KD and Wolffe AP. DNA methylation in health and disease. *Nat Rev Genet* 1: 11–19, 2000.
136. Robien K. Folate during antifolate chemotherapy: what we know…and do not know. *Nutr Clin Pract* 20: 411–422, 2005.
137. Savage D, Gangaidzo I, Lindenbaum J, Kiire C, Mukiibi JM, Moyo A, Gwanzura C, Mudenge B, Bennie A, Sitima J, *et al.* Vitamin B12 deficiency is the primary cause of megaloblastic anaemia in Zimbabwe. *Br J Haematol* 86: 844–850, 1994.
138. Savage DG and Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects. *Baillieres Clin Haematol* 8: 657–678, 1995.
139. Schernhammer ES, Giovannucci E, Kawasaki T, Rosner B, Fuchs C, and Ogino S. Dietary folate, alcohol, and B vitamins in relation to LINE-1 hypomethylation in colon cancer. *Gut* 2009 [Epub ahead of print]; DOI: 10.1136/gut.2009.183707.
140. Selhub J, Morris MS, Jacques PF, and Rosenberg IH. Folate-vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency. *Am J Clin Nutr* 89: 702S–706S, 2009.
141. Shane B. Folate chemistry and metabolism. In: *Folate in Health and Disease*, edited by Bailey LB. New York: Marcel Dekker, 1995, pp. 1–22.
142. Shelnutt KP, Kauwell GP, Gregory JF 3rd, Maneval DR, Quinlivan EP, Theriaque DW, Henderson GN, and Bailey LB. Methylenetetrahydrofolate reductase 677C—>T polymorphism affects DNA methylation in response to controlled folate intake in young women. *J Nutr Biochem* 15: 554–560, 2004.
143. Sie KK, Medline A, van Weel J, Sohn KJ, Choi SW, Croxford R, and Kim YI. Effect of maternal and postweaning folic acid supplementation on colorectal cancer risk in the offspring. *Gut* 60: 1687–1694, 2011.
144. Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG, and Young LE. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci U S A* 104: 19351–19356, 2007.
145. Slansky JE, Li Y, Kaelin WG, and Farnham PJ. A protein synthesis-dependent increase in E2F1 mRNA correlates with growth regulation of the dihydrofolate reductase promoter. *Mol Cell Biol* 13: 1610–1618, 1993.
146. Slattery ML, Curtin K, Sweeny C, Levin TR, Potter JD, Wolff RK, Albertsen H, and Samowitz WS. Diet and lifestype factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. *Int J Cancer* 120: 656–663, 2006.
147. Smith AD, Kim YI, and Refsum H. Is folic acid good for everyone? *Am J Clin Nutr* 87: 517–533, 2008.
148. Sohn KJ, Stempak JM, Reid S, Shirwadkar S, Mason JB, and Kim YI. The effect of dietary folate on genomic and p53-specific DNA methylation in rat colon. *Carcinogenesis* 24: 81–90, 2003.
149. Song J, Sohn KJ, Medline A, Ash C, Gallinger S, and Kim YI. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/-Msh2−/− mice. *Cancer Res* 60: 3191–3199, 2000.
150. Sowers R, Toguchida J, Qin J, Meyers PA, Healey JH, Huvos A, Banerjee D, Bertino JR, and Gorlick R. mRNA expression levels of E2F transcription factors correlate with dihydrofolate reductase, reduced folate carrier, and thymidylate synthase mRNA expression in osteosarcoma. *Mol Cancer Ther* 2: 535–541, 2003.
151. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, Slagboom PE, and Heijmans BT. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. *PLoS One* 4: e7845, 2009.
152. Stern LL, Mason JB, Selhub J, and Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. *Cancer Epidemiol Biomarkers Prev* 9: 849–853, 2000.
153. Stevens VL, McCullough ML, Sun J, Jacobs EJ, Campbell PT, and Gapstur SM. High levels of folate from supplements and fortification are not associated with increased risk of colorectal cancer. *Gastroenterology* 141: 98–105.e1, 2011.
154. Stidley CA, Picchi MA, Leng S, Willink R, Crowell RE, Flores KG, Kang H, Byers T, Gilliland FD, and Belinsky SA. Multivitamins, folate, and green vegetables protect against gene promoter methylation in the aerodigestive tract of smokers. *Cancer Res* 70: 568–574, 2010.
155. Stover PJ, Chen LH, Suh JR, Stover DM, Keyomarsi K, and Shane B. Molecular cloning, characterization, and regulation of the human mitochondrial serine hydroxymethyltransferase gene. *J Biol Chem* 272: 1842–1848, 1997.
156. Tamura T and Picciano MF. Folate and human reproduction. *Am J Clin Nutr* 83: 993–1016, 2006.
157. Tao MH, Shields PG, Nie J, Marian C, Ambrosone CB, McCann SE, Platek M, Krishnan SS, Xie B, Edge SB, Winston J, Vito D, Trevisan M, and Freudenheim JL. DNA promoter methylation in breast tumors: no association with genetic polymorphisms in MTHFR and MTR. *Cancer Epidemiol Biomarkers Prev* 18: 998–1002, 2009.
158. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, and Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A* 96: 8681–8686, 1999.
159. Toyota M and Issa JP. CpG island methylator phenotypes in aging and cancer. *Sem Cancer Biol* 9: 349–357, 1999.
160. Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, Selhub J, McTiernan A, Yasui Y, Oral E, Potter JD,

and Ulrich CM. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr* 136: 189–194, 2006.
161. Ueland PM, Hustad S, Schneede J, Refsum H, and Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol Sci* 22: 195–201, 2001.
162. Uthus EO, Ross SA, and Davis CD. Differential effects of dietary selenium (se) and folate on methyl metabolism in liver and colon of rats. *Biol Trace Elem Res* 109: 201–214, 2006.
163. van den Donk M, Pellis L, Crott JW, van Engeland M, Friederich P, Nagengast FM, van Bergeijk JD, de Boer SY, Mason JB, Kok FJ, Keijer J, and Kampman E. Folic acid and vitamin B-12 supplementation does not favorably influence uracil incorporation and promoter methylation in rectal mucosa DNA of subjects with previous colorectal adenomas. *J Nutr* 137: 2114–2120, 2007.
164. van den Donk M, van Engeland M, Pellis L, Witteman BJ, Kok FJ, Keijer J, and Kampman E. Dietary folate intake in combination with MTHFR C677T genotype and promoter methylation of tumor suppressor and DNA repair genes in sporadic colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 16: 327–333, 2007.
165. van Engeland M, Weijenberg MP, Roemen GM, Brink M, de Bruine AP, Goldbohm RA, van den Brandt PA, Baylin SB, de Goeij AF, and Herman JG. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. *Cancer Res* 63: 3133–3137, 2003.
166. Van Guelpen B, Dahlin AM, Hultdin J, Eklof V, Johansson I, Henriksson ML, Cullman I, Hallmans G, and Palmqvist R. One-carbon metabolism and CpG island methylator phenotype status in incident colorectal cancer: a nested case-referent study. *Cancer Causes Control* 21: 557–566, 2010.
167. Vanyushin BF, Mazin AL, Vasilyev VK, and Belozersky AN. The content of 5-methylcytosine in animal DNA: the species and tissue specificity. *Biochim Biophys Acta* 299: 397–403, 1973.
168. Vertino PM, Issa JP, Pereira-Smith OM, and Baylin SB. Stabilization of DNA methyltransferase levels and CpG island hypermethylation precede SV40-induced immortalization of human fibroblasts. *Cell Growth Diff* 5: 1395–1402, 1994.
169. Wagner C. Biochemical role of folate in cellular metabolism. In: *Folate in Health and Disease*, edited by Bailey LB. New York: Marcel Dekker Inc., 1995, pp. 23–42.
170. Wallace K, Grau MV, Levine AJ, Shen L, Hamdan R, Chen X, Gui J, Haile RW, Barry EL, Ahnen D, McKeown-Eyssen G, Baron JA, and Issa JP. Association between folate levels and CpG island hypermethylation in normal colorectal mucosa. *Cancer Prev Res (Phila)* 3: 1552–1564, 2010.
171. Wang J, Sasco AJ, Fu C, Xue H, Guo G, Hua Z, Zhou Q, Jiang Q, and Xu B. Aberrant DNA methylation of P16, MGMT, and hMLH1 genes in combination with MTHFR C677T genetic polymorphism in esophageal squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 17: 118–125, 2008.
172. Wang L, Wang F, Guan J, Le J, Wu L, Zou J, Zhao H, Pei L, Zheng X, and Zhang T. Relation between hypomethylation of long interspersed nucleotide elements and risk of neural tube defects. *Am J Clin Nutr* 91: 1359–1367, 2010.
173. Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, and Tahiliani KG. Maternal methyl supplements increase offspring DNA methylation at axin fused. *Genesis* 44: 401–406, 2006.
174. Waterland RA and Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23: 5293–5300, 2003.
175. Whitrow MJ, Moore VM, Rumbold AR, and Davies MJ. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. *Am J Epidemiol* 170: 1486–1493, 2009.
176. Wilson VL, Smith RA, Ma S, and Cutler RG. Genomic 5-methyldeoxycytidine decreases with age. 262: 9948–9951, 1987.
177. Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, Mulrow CD, Woolf SH, Glick SN, Ganiats TG, Bond JH, Rosen L, Zapka JG, Olsen SJ, Giardiello FM, Sisk JE, Van Antwerp R, Brown-Davis C, Marciniak DA, and Mayer RJ. Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology* 112: 594–642, 1997.
178. Wolff GL, Kodell RL, Moore SR, and Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J* 12: 949–957, 1998.
179. Wright AJ, Dainty JR, and Finglas PM. Folic acid metabolism in human subjects revisited: potential implications for proposed mandatory folic acid fortification in the UK. *Br J Nutr* 98: 667–675, 2007.
180. Wu K, Platz EA, Willett WC, Fuchs CS, Selhub J, Rosner BA, Hunter DJ, and Giovannucci E. A randomized trial on folic acid supplementation and risk of recurrent colorectal adenoma. *Am J Clin Nutr* 90: 1623–1631, 2009.
181. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, Bhat DS, Naik SS, Coyaji KJ, Joglekar CV, Joshi N, Lubree HG, Deshpande VU, Rege SS, and Fall CH. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 51: 29–38, 2008.
182. Zhao R and Goldman ID. Resistance to antifolates. *Oncogene* 22: 7431–7457, 2003.

Address correspondence to:
Dr. Young-In Kim
Division of Gastroenterology
Department of Medicine
St. Michael's Hospital
16CC-038, 30 Bond St.
Toronto
Ontario M5B 1W8
Canada

E-mail: youngin.kim@utoronto.ca

Date of first submission to ARS Central, February 5, 2012; date of acceptance, February 10, 2012.

Abbreviations Used

5-methylTHF = 5-methyltetrahydrofolate
5,10-methyleneTHF = 5,10-methylenetetrahydrofolate
ACF = aberrant crypt foci
CH3 = methyl group
CpG = cytosine-guanine dinucleotide sequence
CRC = colorectal cancer
CβS = cystathionine β-synthase
DFE = dietary folate equivalent
DHF = dihydrofolate
DHFR = dihydrofolate reductase
DMR = differentially methylated region
DNMT = DNA methyltransferase
ERα = estrogen receptor alpha
FA = folic acid

Abbreviations Used (Cont.)

FPGS = folylpolyglutamyl synthase
FR-α = folate receptor
GCPII = glutamate carboxypeptidase II
GGH = γ-glutamyl hydrolase
IGF2 = insulin-like growth factor 2
KIR2DL2 = killer cell immunoglobulin-like receptor 2DL2
LINE-1 = long interspersed nucleotide element 1
MBD2 = methyl-CpG binding domain protein 2
MeCP2 = methyl-CpG binding protein 2
MS = methionine synthase
MTHFR = methylenetetrahydrofolate reductase
MTRR = methionine synthase reductase
NA = not applicable
NS = not significant
NTD = neural tube defect
PABA = para-aminobenzoic acid
PCFT = proton coupled folate transporter
RBC = red blood cell
RFC = reduced folate carrier
SAH = S-adenosylhomocysteine
SAHH = S-adenosylhomocysteine hydrolase
SAM = S-adenosylmethionine
SFRP1 = secreted frizzled related protein-1
SHMT = serine hydroxymethyltransferase
THF = tetrahydrofolate
TS = thymidylate synthase

This article has been cited by:

1. Tom C. Karagiannis , Nilanjana Maulik . 2012. Factors Influencing Epigenetic Mechanisms and Related Diseases. *Antioxidants & Redox Signaling* **17**:2, 192-194. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]