DNA Methylation, Cancer Susceptibility, and Nutrient Interactions

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MINIREVIEW

DNA Methylation, Cancer Susceptibility, and Nutrient Interactions

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DNA methylation is an important epigenetic mechanism of transcriptional control. DNA methylation plays an essential role in maintaining cellular function, and changes in methylation patterns may contribute to the development of cancer. Aberrant methylation of DNA (global hypomethylation accompanied by region-specific hypermethylation) is frequently found in tumor cells. Global hypomethylation can result in chromosome instability, and hypermethylation has been associated with the inaction of tumor suppressor genes. Preclinical and clinical studies suggest that part of the cancer-protective effects associated with several bioactive food components may relate to DNA methylation patterns. Dietary factors that are involved in one-carbon metabolism provide the most compelling data for the interaction of nutrients and DNA methylation because they influence the supply of methyl groups, and therefore the biochemical pathways of methylation processes. These nutrients include folate, vitamin B_{12}, vitamin B_6, methionine, and choline. However, looking at individual nutrients may be too simplistic. Dietary methyl (folate, choline, and methionine) deficiency in combination causes decreased tissue S-adenosylmethionine, global DNA hypomethylation, hepatic steatosis, cirrhosis, and ultimately hepatic tumorigenesis in rodents in the absence of carcinogen treatment. Other dietary components such as vitamin B_{12}, alcohol, and selenium may modify the response to inadequate dietary folate. Exp Biol Med 229:988–995, 2004

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A wealth of evidence points to the diet as one of the most important modifiable determinants of cancer risk. A large number of bioactive components have been identified in food that are protective at different stages of cancer formation. Diet has been implicated in many pathways involved in carcinogenesis, including apoptosis, cell cycle control, differentiation, inflammation, angiogenesis, DNA repair, and carcinogen metabolism. These are also processes that are likely regulated by DNA methylation and other epigenetic events. Epigenetic events constitute an important mechanism by which dietary components can selectively activate or inactivate gene expression. This review will focus on how dietary components can affect DNA methylation and thus cancer susceptibility.

What Is DNA Methylation?

In humans, DNA methylation predominantly involves the covalent addition of a methyl group (CH_3) to the 5′ position of cytosine that precedes a guanosine in the DNA sequence (the CpG dinucleotide). This is referred to as an epigenetic modification because it does not change the coding sequence of the DNA. The distribution in the genome of the CpG dinucleotides at which DNA methylation occurs is asymmetric. In contrast to the relative paucity of CpGs in the genome as a whole, these dinucleotides can be clustered in small stretches of DNA termed "CpG islands" (1). These regions are often associated with the promoter regions of genes. Almost half of the genes in our genome have such CpG-rich promoter regions (2). In the bulk of the genome, about 80% of the CpG dinucleotides that are not associated with CpG islands are heavily methylated (2). In contrast, the dinucleotides in CpG islands, especially those associated with gene promoters, are usually unmethylated (3). The methylation of

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CpG islands in the promoter region silences gene expression and is a normal event that occurs in cells to regulate gene expression.

The mechanism for the CpG island–associated gene silencing seems to involve the link of specific methylated DNA binding proteins with a variety of proteins such as transcription factors, DNA methyltransferases (DNMT1, 2, 3L, 3A, and 3B), methyl-binding proteins (MeCP1 and 2, MBD1, 2, 3, and 4, and KAISO), histone-modifying enzymes (acetylases, deacetylases, methylases), chromatin-remodeling factors (SWI2/SNF2, ISW1, and Mi-2) and their multimolecular complexes (4). In general, these protein/DNA interactions alter the DNA conformation (5). The DNA structure changes to a compact, condensed chromatin configuration that results in a stable inhibition of messenger RNA and protein production (6).

Role of DNA Methylation in Carcinogenesis

Abnormal patterns of DNA methylation in cancer cells have been recognized for more than 20 years (4). The situation has been confusing because virtually all types of cancer examined have both global hypomethylation and gene-specific hypermethylation in the gene promoter regions (7). Hypermethylation of promoter regions, which is associated with transcriptional silencing, is at least as common as DNA mutation as a mechanism for inactivation of classical tumor suppressor genes in human cancers (8, 9). Furthermore, a number of candidate tumor suppressor genes that are not commonly inactivated by mutation are transcriptionally silenced by this mechanism (8). The aberrant methylation of genes that suppress tumorigenesis appears to occur early in tumor development and increases progressively, eventually leading to the malignant phenotype (10, 11). Genes involved in every step of tumorigenesis can be silenced by this epigenetic mechanism. In an excellent review on tumorigenesis, Hanahan and Weinberg (12) have pointed out the major hallmarks of cancer. The fundamental properties required to generate the characteristic malignant attributes associated with cancer cells are the ability to replicate without limitation, indifference to positive growth signals, disregard for growth inhibitory factors, evasion of programmed cell death, sustained angiogenesis, and the ability to invade and metastasize (12). Each of these traits is influenced by a gene or set of genes. Failure to express the gene correctly and produce functional regulatory proteins leads to the uncontrolled pattern of cell behavior observed in a typical neoplasm. Hypermethylation is associated with the inactivation of virtually all pathways involved with the cancer process, such as DNA repair (*hMLH1, BRCA1, MGMT*), cell cycle regulation (*p16, p14, p15*), apoptosis (*DAPK, APAF-1*), carcinogen metabolism (*GSTP1*), hormonal response (*RAR*β*2*), and cell adherence (*CDH1, CDH3*; Refs. 13–15). Furthermore, a tumor-type specific profile of CpG island hypermethylation exists in human cancer that allows the use of these aberrantly hypermethylated loci as biomarkers of the malignant disease (16).

Despite the well-documented presence of both global hypomethylation and regional DNA hypermethylation in cancer, the mechanisms of these events remain unclear, especially the paradox of why DNA remains hypomethylated in the presence of increased DNA methyltransferase activity and expression. It has been suggested that deregulation of DNA methyltransferases might lead to tumor-specific genome-wide hypomethylation in cancers (17). A significant correlation between overexpression of DNMT3b4, an inactive splice variant of DNMT3b, and degree of DNA hypomethylation on pericentromeric satellite regions of preneoplastic and neoplastic tissue provides support for this hypothesis (18). DNA methyltransferases have also been found to bind with higher affinity to DNA strand breaks, abasic sites, and uracil than they do to their cognate hemimethylated CpG sites, consistent with their ancestral function as DNA repair enzymes (19). These same DNA lesions are often present in human preneoplastic cells, which raises the possibility that DNA lesions may be a necessary prerequisite for the disruption of normal DNA methylation patterns in preneoplastic and neoplastic cells (19).

Nutrients That Affect DNA Methylation and Cancer Susceptibility

Several bioactive food components can modulate DNA methylation and cancer susceptibility (Table 1). Dietary factors that are involved in one-carbon metabolism provide the most compelling data for the interaction of nutrients and DNA methylation because they influence the supply of methyl groups and therefore the biochemical pathways of methylation processes. These nutrients include vitamin B_{12}, vitamin B_6, folate, methionine, and choline.

Folate has a central role in one-carbon metabolism (Figure 1). Normally in one-carbon metabolism, a carbon unit from serine or glycine is transferred to tetrahydrofolate to form 5,10-methylenetetrahydrofolate (20). Vitamin B_6 is a necessary co-factor for glycine hydroxymethyltransferase in the synthesis of 5,10-methylenetetrahydrofolate (21). This form of folate can be used for the synthesis of thymidine; oxidized to formyltetrahydrofolate for the synthesis of purines or reduced to 5-methyltetrahydrofolate and used to methylate homocysteine to form methionine (21). The vitamin B_{12}–dependent enzyme, methionine synthase (MS), catalyzes the synthesis of methionine from homocysteine. Methionine is subsequently converted to S-adenosylmethionine (SAM) by an ATP-dependent transfer of adenosine to methionine via methionine adenosyltransferase (21). S-adenosylmethionine then donates its labile methyl groups to more than 80 biological methylation reactions, including the methylation of DNA, RNA, and protein (22). When the supply of folate is limited, plasma and cellular levels of homocysteine increase. Although the

Table 1. Dietary Factors Known to Influence DNA Methylation and Cancer Susceptibility

Dietary component	References
Alcohol	36, 41–48, 74
Arsenic	51, 53, 57
Cadmium	64, 65
Choline	56, 58–61, 71–73
Coumestrol	55
Equol	55
Folate	22–34, 38, 46, 58–61, 63, 73, 75
Genistein	54, 55
Methionine	59–61, 73
Nickel	69, 70
Selenium	51, 63
Tea polyphenols	62
Vitamin A	50
Vitamin B_6	26, 59–61
Vitamin B_{12}	26, 59–61, 72, 73
Zinc	49, 66, 67, 73

alternative zinc-requiring enzyme for methionine synthesis, betaine-homocysteine methyltransferase, may partially compensate for the reduced MS activity, it is well known that dietary folate depletion alone is a sufficient perturbing force to diminish SAM pools (23). This leads to an increase in cellular levels of S-adenosylhomocysteine (SAH) because the equilibrium of the SAH-homocysteine interconversion actually favors SAH synthesis. Therefore, when homocysteine metabolism is inhibited (as in folate deficiency), cellular SAH will be increased. Increased SAH inhibits methyltransferase activity and, consequently, DNA methylation reactions (24). This inhibition of DNA methylation associated with inadequate dietary folate has also been associated with increased cancer susceptibility.

A large number of epidemiologic and clinical studies suggest that dietary folate intake and blood folate concentrations are inversely associated with colorectal cancer risk (25–29). Animal studies using chemical and genetically predisposed rodent models have provided considerable support for a causal relationship between folate depletion and colorectal carcinogenesis as well as a dose-dependent protective effect of folate supplementation (30–32). However, animal studies have also shown that the dose and timing of folate intervention are critical in providing safe and effective chemoprevention; exceptionally high supplemental folate concentrations and folate intervention after microscopic neoplastic foci are established in the colorectal mucosa promote rather than suppress carcinogenesis (25). Animal studies have shown that folate deficiency causes DNA hypomethylation prior to the development of tumors (33). DNA hypomethylation has also been found in lymphocytes of humans on low dietary folate and can be reversed by folate repletion (34). In contrast, folate deficiency with or without reductions in Dnmt1 did not affect overall genomic DNA methylation levels or the methylation levels of two candidate genes, *E-cadherin* or *p53*, in normal or neoplastic intestinal tissue (32). These studies suggest that the effects of folate deficiency on DNA

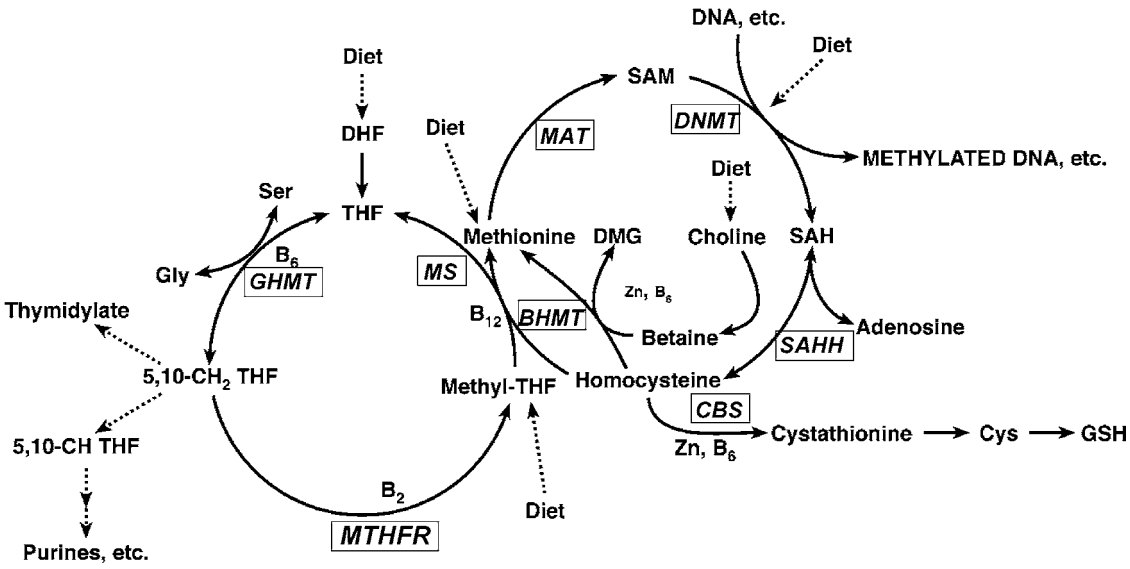

Figure 1. Dietary factors, enzymes, and substrates involved in methyl metabolism. Enzymes are shown in italics with a box around them. These include glycine hydroxymethyltransferase (GHMT; EC 2.1.2.1); methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20); 5-methyltetrahydrofolate:homocysteine S-methyltransferase (methionine synthase of MS; EC 2.1.1.13); betaine-homocysteine S-methyltransferase (BHMT; CD 2.1.1.5); methionine adenosyltransferase (MAT; EC 2.5.1.6); DNA methyltransferase (DNMT; EC 2.1.1.37); S-adenosyl-homocysteine hydrolase (SAHH; EC 3.3.1.1); and cystathionine-β-synthase (CBS; EC 4.2.1.22). Abbreviations: DHF, dihydrofolate; Ser, serine; Gly, glycine; Cys, cysteine; THF, tetrahydrofolate; B_6, vitamin B_6 or pyridoxine; B_{12}, vitamin B_{12} or cobalamin; B_2, vitamin B_2 or riboflavin; 5,10-CH_2 THF, 5,10-methyltetrahydrofolate; 5,10-THF, 5,10-methylenetetrahydrofolate; methyl-THF, 5-methyltetrahydrofolate; Zn, zinc; DMG, dimethylglycine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; GSH, glutathione.

methylation are highly complex; appear to depend on cell type, target organ, and stage of transformation; and are gene and site specific.

Gene polymorphisms may modulate the effect of dietary folate on DNA methylation and cancer susceptibility. A single nucleotide substitution at position 677 of the methylenetetrahydrofolate reductase (*MTHFR*) gene (C677T) has been associated with a reduced risk of colon cancer (35–38) but an increased risk of breast cancer (39, 40). Slattery et al. (37) reported that colon cancer risk was reduced 30%–40% in individuals homozygous for the variant TT genotype of the *MTHFR* gene who consumed adequate dietary intakes of folate, vitamin B_6, and vitamin B_{12} relative to those with the CC genotype who consumed low nutrient intakes. When folate status is low, the presence of the TT genotype is associated with an increase in homocysteine concentrations and DNA hypomethylation. These studies emphasize the importance of taking into consideration interactions between folate status and critical genes on the folate and one-carbon metabolic pathways when investigating the effect of folate on DNA methylation.

Alcohol consumption has been shown to alter folate metabolism and increase cancer susceptibility. Epidemiologic studies demonstrate that alcohol consumption increases cancer of the oral cavity, pharynx, esophagus, and liver, and that it causes a small increase in risk for breast cancer (40–45). Excessive alcohol consumption has been shown to alter DNA methylation in the colon of both humans and rats (46, 47). Chronic alcohol exposure has also been found to affect the mRNA levels of DNMT in sperm and thus disturb paternal genomic imprinting, which is suggested as a potential mechanism for the paternal effects of alcohol on abnormal fetal development and growth (48). Thus, many of the adverse effects of alcohol consumption may be related to alterations in DNA methylation.

Other bioactive food components have also been shown to affect DNA methylation (Table 1). Many of these nutrients, including zinc, selenium, genistein and vitamin A, have also been associated with cancer susceptibility. Whereas deficiencies of some food components induce global DNA hypomethylation, deficiencies of other food components induce global DNA hypermethylation. For example, zinc deficiency (49) and retinoic acid excess (50) have been shown to reduce the utilization of methyl groups from SAM in rat liver and to result in global DNA hypomethylation. Selenium deficiency decreased DNA methylation in Caco-2 cells and in rat liver and colon (51). In contrast, vitamin C deficiency has been associated with DNA hypermethylation in lung cancer cells (52). However, there may be optimum amounts for certain dietary components based on DNA methylation. For example, either an absence or an excess of dietary arsenic has been shown to cause global hypomethylation in rat liver (53).

Soy phytoestrogens, such as genistein, are thought to be involved in preventing the development of certain prostate and mammary cancers by maintaining a protective DNA methylation pattern (39). Consumption of genistein was positively correlated with changes in prostate DNA methylation at CpG islands of specific mouse genes as assessed using mouse differential hybridization arrays (54). This assay allows one to screen the entire genome for aberrant methylation patterns and locate candidate sequences for further study (54). Other phytoestrogens have also been shown to modify DNA methylation. Neonatal exposure to the phytoestrogens coumestrol and equol have been found to lead to specific hypermethylation of the c-*Ha-ras* proto-oncogene in pancreatic cancer (55).

The influence of bioactive food components on gene-specific DNA methylation is less clearly understood than the effect of bioactive food components on global DNA methylation. In a rat model of hepatocellular carcinoma, a choline-deficient diet induced hypomethylation of CpG sites of the *c-myc* gene as well as overexpression of this gene (56). Methylation of the promoter region of *p53* in Caco-2 cells decreased when cells were cultured in the absence of selenium (51). Arsenic has been shown to induce hypomethylation of the 5′ regulatory region of Ha-*ras* in animals (57). Interestingly dietary factors that alter global DNA methylation can simultaneously cause opposite effects on gene-specific methylation. For example, folate deficiency causes global DNA hypomethylation but hypermethylation of the 5′ regulatory sequence of the *H-cadherin* gene (58). Caco-2 cells treated with 1 μmol arsenite/L had significantly higher global methylation but significantly less methylation of the *p53* tumor suppressor gene than Caco-2 cells treated with 2 μmol arsenite/L (51). Furthermore, retinoic acid leads to global hypomethylation but region-specific hypermethylation (5). Thus, the pattern of gene-specific methylation may not be in concert with the direction of changes in genomic DNA methylation.

Nutrients have also been shown to affect gene transcription by modifying exon-specific DNA methylation. For example, in animals fed methyl-deficient diets, increased levels of mRNA for c-fos, c-Ha-ras, and c-myc were correlated with hypomethylation at specific sites within the exons of these genes (59, 60). In a hepatocarcinogenesis study with chronic dietary methyl deficiency, the methylation status of the *p53* gene coding region decreased and then increased, which corresponded to a high *p53* mRNA level in preneoplastic liver tissue and then a lower *p53* mRNA level after tumor formation (61). This suggests that methylation changes in the coding region of genes can affect gene transcription and that gene-specific methylation can vary during the carcinogenic process.

Bioactive food components can also modulate DNA methylation by interfering with DNMT activity. Green tea has been shown to inhibit carcinogenesis in many different animal models (62). Recently, epigallocatechin-3-gallate, the major polyphenol from green tea, was found to inhibit DNMT activity by binding to the enzyme, which resulted in the reactivation of methylation-silenced genes in cancer cells (62). Rats fed selenium- or folate-deficient diets had

significantly reduced liver and colon DNMT activity; however, the mechanism for their inhibitory effect is not known (63). Cadmium is another active inhibitor of DNMT as shown in studies on cultured rat liver cells and in animals (64). Furthermore, both cadmium and zinc inhibited DNMT activity in the nuclear extracts from rats fed either a control or a methyl-deficient diet (65). The inhibitory effects of cadmium and zinc may be caused by binding of these metals to the cysteine residue of the active center of DNMT (65).

Modifications of histone acetylation are another epigenetic mechanism whereby bioactive food components can modulate gene expression. Histone deacetylase (HDAC) inhibitors have been shown to bind through zinc at zinc-binding sites (66). This has led to the development of a novel class of HDAC inhibitors in which short-chain fatty acids are tethered to a zinc-chelating moiety through hydrophobic linkages (67). The short-chain fatty acid, butyrate, has been shown to promote hyperacetylation of the *RET* proto-oncogene and increase its transcription (68). Nickel is a potent environmental carcinogen, and exposure to nickel can induce *de novo* methylation of tumor suppressor genes due to formation of heterochromatic structure at these gene loci (69). Furthermore, a suppressive effect of nickel on histone H4 acetylation *in vitro* has been reported for both yeast and mammalian cells (70). Thus, alterations in histone acetylation may be another mechanism whereby dietary components modulate gene expression and cancer susceptibility.

There Is a Need to Look at Nutrient Interactions

Although many bioactive food components have been shown to modify epigenetic events and cancer susceptibility, looking at individual nutrients may be too simplistic. Dietary methyl (folate, choline, and methionine) deficiency in combination causes decreased tissue SAM, global DNA hypomethylation, hepatic steatosis, cirrhosis, and ultimately hepatic tumorigenesis in rodents in the absence of carcinogen treatment (71). The percentage of CpG sites that lose methyl groups on both stands progressively increases in the liver after 9, 18, and 36 weeks of folate/methyl deficiency despite the presence of elevated DNA methyltransferase activity (17). Thus, it appears that DNA methyltransferase is incapable of methylating double-stranded unmethylated DNA present in preneoplastic liver and this may result in the establishment of a cancer-specific DNA methylation profile. Changes in the DNA methylation profile probably explain why these animals develop cancer in the absence of carcinogen treatment. However, it is also possible that the increased mitogenesis (as a result of folate deficiency) leads to mutations and it is a combination of mutagenesis and altered DNA methylation that leads to cancer. Furthermore, other tissues, such as pancreas, spleen, kidney, and thymus, displayed no changes in DNA methylation level or DNA methyltransferase activity after 36 weeks of folate/methyl deficiency (17). These findings suggest that DNA hypomethylation is specific to the liver, the target tissue for carcinogenesis, following a folate/methyl-deficient diet. These findings also further support the hypothesis that changes in the DNA methylation profile can cause cancer and that it is important to look at nutrient interactions.

Diets that are deficient in choline or in choline and methionine have also been shown to cause hepatocellular carcinoma in 20%–50% of animals after 12–24 months of consumption (72). However, the carcinogenicity of a methyl-deficient diet is much higher in the presence of adequate vitamin B_{12}, compared with a methyl-deficient diet without vitamin B_{12} (72). Dietary arsenic has also been shown to interact with a methyl-deficient diet. Administration of arsenic with a methyl-deficient diet to mice resulted in genome-wide hypomethylation and reduced methylation of the promoter region of the oncogene Ha-*ras* (57). This process would be expected to switch on expression of the oncogene and contribute to tumor development.

Prenatal feeding of a methyl-supplemented diet can increase the level of DNA methylation and phenotypic expression of genes in the offspring (73). The coat color in mice is determined by agouti gene expression (73). This is determined by the DNA methylation status of the long terminal repeat of the agouti gene in the hair follicle. If this region is hypermethylated, the mouse is agouti in color, whereas if the region is hypomethylated, the mouse is yellow. When pregnant female mice were fed a methyl-supplemented diet enriched in zinc, methionine, betaine, choline, folate, and vitamin B_{12}, there was an alteration in the methylation status of the agouti long terminal repeat and none of the pups had a yellow coat (73). Interestingly, the expression of the yellow coat has been linked to an increased risk of obesity, adult diabetes, cancer, and mortality (74). It should be noted that the dietary supplements were added to a control diet that was considered adequate for meeting nutritional needs. Thus, *in utero* exposure to nutrients can lead to epigenetic modifications of the genome in the offspring and potentially modify cancer risk.

Another important dietary interaction occurs between alcohol and folate to affect DNA methylation and cancer susceptibility. Alcohol has been shown to cleave folate, impair folate absorption, increase folate excretion, and interfere with methionine synthase activity (75). A high alcohol intake may lead to a localized folate deficiency and DNA hypomethylation in the colon, which can occur when dietary folate intake and blood folate concentrations are normal (75). Furthermore, in the Netherlands Cohort Study, the prevalence of promoter hypermethylation (six selected genes previously found to be involved in colorectal cancer) was higher in colorectal cancers derived from patients with low-folate/high-alcohol intake when compared with colorectal cancers from patients with high-folate/low-alcohol intake (46).

Dietary selenium has been shown to modulate many of the adverse effects of folate deficiency, including alterations in one-carbon metabolism and aberrant crypt formation, a preneoplastic lesion for colon cancer (63). For example, when rats were fed a folate-deficient diet, there were large increases in colonic aberrant crypts, plasma homocysteine, and liver SAH concentrations and decreased liver SAM concentrations and methionine synthase activities that were somewhat ameliorated by a selenium-deficient diet (63). In contrast, colonic SAM, SAH, and genomic methylation were not affected by a dietary interaction between selenium and folate. These results suggest that selenium modifies some of the effects of folate deficiency, probably by shunting the buildup of homocysteine (as a result of folate deficiency) through the transsulfuration pathway.

Summary

Numerous epidemiologic, animal, and human studies suggest that several dietary components can modify cancer risk. Imbalances of nutrients and other bioactive components have been shown to lead to global DNA hypomethylation and gene-specific hypomethylation and/or hypermethylation. Although most methylation research has been done with dietary folate, a number of these studies indicate that the dose and timing of folate intervention, as well as interactive effects with other dietary components, are important in determining its chemopreventive effects.

1. Robertson KD, Wolffe AP. DNA methylation in health and disease. Nat Rev Genet 1:11–19, 2000.
2. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Eng J Med 349:2042–2054, 2003.
3. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21, 2002.
4. Brown R, Strathdee G. Epigenomics and epigenetic therapy of cancer. Trends Mol Med 8:S43–S48, 2002.
5. Singh SM, Murphy B, O'Reilly RL. Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. Clin Genet 64:451–460, 2003.
6. Santini V, Kantarjian HM, Issa JP. Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications. Ann Intern Med 134:573–586, 2001.
7. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa J-P. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 72:141–196, 1998.
8. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 3:415–428. 2002.
9. Tsou JA, Hagen JA, Carpenter CL, Laird-Offringa IA. DNA methylation analysis: a powerful new tool for lung cancer diagnosis. Oncogene 21:5450–5461, 2002.
10. Fearon EA, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 61:759–767, 1990.
11. Kim Y, Mason J. Folate, epithelial dysplasia and colon cancer. Proc Assoc Am Physicians 107:218–227, 1995.
12. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 100:57–70, 2000.
13. Costello JF, Plass C. Methylation matters. J Med Genet 38:285–303, 2001.
14. Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. Oncogene 21:5427–5440, 2002.
15. Momparler RL. Cancer epigenetics. Oncogene 22:6479–6483, 2003.
16. Esteller M. Cancer epigenetics: DNA methylation and chromatin alterations in human cancer. In: Llombart-Boschm F, Ed. New Trends in Cancer for the 21st Century. New York: Academic/Plenum Publishers, pp39–49, 2003.
17. Pogribny IP, James SJ, Jernigan S, Pogribna M. Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues. Mutat Res 548:53–59, 2004.
18. Saito Y, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S. Overexpression of a splice variant of DNA methyltransferase 3b, DNMT3b4, associated with DNA hypomethylation on pericentromeric satellite regions during human hepatocarcinogenesis. Proc Natl Acad Sci U S A 99:10060–10065, 2002.
19. James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, Melnyk S. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr 133:3740S–3747S, 2003.
20. Scott JM, Weir DG. Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. J Cardiovas Risk 5:223–227, 1998.
21. Ross SA. Diet and DNA methylation interactions in cancer prevention. Ann NY Acad Sci 983:197–207, 2003.
22. Choi S.-W., Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr 132:2413S–2418S, 2002.
23. Miller JW, Nadeau MR, Smith J, Smith D, Selhub J. Folate-deficiency-induced homocysteinaemia in rats: disruption of S-adenosylmethionine=s co-ordinate regulation of homocysteine metabolism. Biochem J 298:415–419, 1994.
24. De Cabo SF, Santos J, Fernandez-Piqueras J. Molecular and cytological evidence of S-adenosyl-L-homocysteine as an innocuous undermethylating agent in vivo. Cytogenet Cell Genet 71:187–192, 1995.
25. Kim Y.-I. Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? Cancer Epidemiol Biomarkers Prev 13:511–519, 2004.
26. Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. Nutr Cancer 43:152–158, 2002.
27. La Vecchia C, Negri E, Pelucchi C, Franceschi S. Dietary folate and colorectal cancer. Int J Cancer 102:545–547, 2002.
28. Konings EJ, Goldbohm RA, Brants HA, Saris WH, van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. Cancer 95:1421–1433, 2002.
29. Fuchs CS, Willett WC, Colditz GA, Hunter DJ, Stampfer MJ, Speizer FE, Giovannucci EL. The influence of folate and multivitamin use on the familial risk of colon cancer in women. Cancer Epidemiol Biomarkers Prev 11:227–234, 2002.
30. Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Res 60:5434–5440, 2000.
31. Kim YI, Salomon RN, Graeme-Cook F, Choi SW, Smith DE, Dallal GE, Mason JB. Dietary folate protects against the development of macroscopic colonic neoplasia in a dose responsive manner in rats. Gut 39:732–740, 1996.
32. Fang JY, Xiao SD. Folic acid, polymorphism of methyl-group metabolism genes, and DNA methylation in relation to GI carcinogenesis. Gastroenterol 38:821–829, 2003.
33. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, Miller BJ, Henning SM, Swendseid ME. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J Nutr 128:1204–1212, 1998.
34. Trasler J, Deng L, MeInyk S, Pogribny I, Hiou-Tim F, Sibani S, Oakes C, Li E, James SJ, Rozen R. Impact of Dnmt1 deficiency, with and

without low folate diets, on tumor numbers and DNA methylation in Min mice. Carcinogenesis 24:39–45, 2003.
35. Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 13:285–292, 2004.
36. Giovannucci E, Chen J, Smith-Warner SA, Rimm EB, Fuchs CS, Palomeque C, Willett WC, Hunter DJ. Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. Cancer Epidemiol Biomarkers Prev 12:970–979, 2003.
37. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 8:513–518, 1999.
38. Rampersaud GC, Kauwell GP, Hutson AD, Cerda JJ, Bailey LB. Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. Am J Clin Nutr 72:998–1003, 2000.
39. Beilby J, Ingram D, Hahnel R, Rossi E. Reduced breast cancer risk with increasing serum folate in a case-control study of the C677T genotype of the methylenetetrahydrofolate reductase gene. Eur J Cancer 40:1250–1254, 2004.
40. Ergul E, Sazci A, Utkan Z, Canturk NZ. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biol 24:286–290, 2003.
41. Freudenheim JL, Bonner M, Krishnan S, Ambrosone CB, Graham S, McCann SE, Moysich KB, Bowman E, Nemoto T, Shields PG. Diet and alcohol consumption in relation to p53 mutations in breast tumors. Carcinogenesis 25:931–939, 2004.
42. Menvielle G, Luce D, Goldberg P, Bugel I, Leclerc A. Smoking, alcohol drinking and cancer risk for various sites of the larynx and hypopharynx. A case-control study in France. Eur J Cancer Prev 13:165–172, 2004.
43. Visapaa JP, Gotte K, Benesova M, Li J, Homann N, Conradt C, Inoue H, Tisch M, Horrmann K, Vakevainen S, Salaspuro M, Seitz HK. Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. Gut 53:871–876, 2004.
44. Dal Maso L, La Vecchia C, Polesel J, Talamini R, Levi F, Conti E, Zambon P, Negri E, Franceschi S. Alcohol drinking outside meals and cancers of the upper aero-digestive tract. Int J Cancer 102:435–437, 2002.
45. Munaka M, Kohshi K, Kawamoto T, Takasawa S, Nagata N, Itoh H, Oda S, Katoh T. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. J Cancer Res Clin Oncol 129:355–360, 2003.
46. van Engeland M, Weijenberg MP, Roemen GM, Brink M, de Bruine AP, Goldbohm RA, van den Brandt PA, Baylin SB, de Goeij AF, 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.
47. Choi SW, Stickel F, Baik HW, Kim YI, Seitz HK, Mason JB. Chronic alcohol consumption induces genomic but not p53-specific DNA hypomethylation in rat colon. J Nutr 129:1945–1950, 1999.
48. Bielawski DM, Zaher FM, Svinarich DM, Abel EL. Paternal alcohol exposure affects sperm cytosine methyltransferase messenger RNA levels. Alcohol Clin Exp Res 26:347–351, 2002.
49. Wallwork JC, Duerre JA. Effect of zinc deficiency on methionine metabolism, methylation reactions and protein synthesis in isolated perfused rat liver. J Nutr 115:252–262, 1985.
50. Rowling MJ, McMullen MH, Schalinske KL. Vitamin A and its derivatives induce hepatic glycine N-methyltransferase and hypomethylation of DNA in rats. J Nutr 132:365–369, 2002.
51. Davis CD, Uthus EO, Finley JW. Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon. J Nutr 130 2903–2909, 2000.
52. Haliwell B. Vitamin C and genomic stability. Mutat Res 475:29–35, 2001.
53. Uthus EO. Estimation of safe and adequate daily intake for arsenic. In: Mertz W, Abernathy CO, Olin SS, Eds. Risk Assessment of Essential Elements. Washington, DC: ILSI Press, pp273–282, 1994.
54. Day JK, Bauer AM, desBordes C, Zhuang Y, Kim B-E, Newton LG, Nehra V, Forsee KM, MacDonald RS, Besch-Williford C, Huang THM, Lubahn DB. Genistein alters methylation patterns in mice. J Nutr 132:2419S–2423S, 2002.
55. Lyn-Cook BD, Blann E, Payne PW, Bo J, Sheehan D, Medlock K. Methylation profile and amplification of proto-oncogenes in rat pancreas induced with phytoestrogens. Proc Soc Exp Biol Med 208:116–119, 1995.
56. Tsujiuchi T, Tsutsumi M, Sasaki Y, Takahama M, Konishi Y. Hypomethylation of CpG sites and *c-myc* gene overexpression in hepatocellular carcinomas, but not hyperplastic nodules, induced by a choline-deficient L-amino acid-defined diet in rats. Jpn J Cancer Res 90:909–913, 1999.
57. Okoji RS, Yu RC, Maronpot RR, Froines JR. Sodium arsenite administration via drinking water increases genome-wide and Ha-ras DNA hypomethylation in methyl-deficient C57BL/6J mice. Carcinogenesis 23:777–785, 2002.
58. Jhaveri MS, Wagner C, Trepel JB. Impact of extracellular folate levels on global gene expression. Mol Pharmacol 60:1288–1295, 2001.
59. Bhave MR, Wilson MJ, Poirier LA. c-H-ras and c-K-ras gene hypomethylation in the livers and hepatomas of rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis 9:343–348, 1988.
60. Zapisek WF, Cronin GM, Lyn-Cook BD, Poirier LA. The onset of oncogene hypomethylation in the livers of rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis 13:1869–1872, 1992.
61. Sohn KJ, Stempak JM, Reid S, Shirwadkar S, Mason JB, Kim YI. The effect of dietary folate on genomic and p53-specific DNA methylation in rat colon. Carcinogenesis 24:81–90, 2003.
62. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang CS. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 63:7563–7570, 2003.
63. Davis CD, Uthus EO. Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats. Nutr 133:2907–2914, 2003.
64. Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation Exp Cell Res 286:355–365, 2003.
65. Poirier LA, Vlasova TI. The prospective role of abnormal methyl metabolism in cadmium toxicity. Environ Health Perspect 110:793–795, 2002.
66. Furumai R, Matsuyama A, Kobashi N, Lee KH, Nishiyama M, Nakajima H, Tanaka A, Komatsu Y, Nishino N, Yoshida M, Horinouchi S. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. Cancer Res 62:4916–4921, 2002.
67. Lu Z, Yang Y-T, Chen C-S, Davis M, Byrd JC, Eterton MR, Umar A, Chen C-S. Zn^{2+}-chelating motif-tethered short-chain fatty acids as a novel class of histone deacetylase inhibitors. J Med Chem 47:467–474, 2004.
68. Griseri P, Patrone G, Puppo F, Romeo G, Ravazzolo R, Ceccherini I. Rescue of human RET gene expression by sodium butyrate: a novel powerful tool for molecular studies in Hirschsprung disease. Gut 52:1154–1158, 2003.
69. Lee YW, Klein CB, Kargacin B, Salnikow K, Kitahara J, Dowjat K, Zhitkovich A, Christie NT, Costa M. Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. Mol Cell Biol 15:2547–2557, 1995.
70. Broday L, Peng W, Kuo MH, Salnikow K, Zoroddu M, Costa M. Nickel compounds are novel inhibitors of histone H4 acetylation. Cancer Res 60:238–241, 2000.
71. Steinmetz KL, Pogribny IP, James SJ, Pitot HC. Hypomethylation of the rat glutathione S-transferase pi (GSTP) promoter region isolated from methyl-deficient livers and GSTP-positive liver neoplasm. Carcinogenesis 19:1487–1494, 1998.

72. Brunaud L, Alberto JM, Ayav A, Gerard P, Namour F, Antunes L, Braun M, Bronowicki JP, Bresler L, Gueant JL. Effects of vitamin B_{12} and folate deficiencies on DNA methylation and carcinogenesis in rat liver. Clin Chem Lab Med 41:1012–1019, 2003.
73. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 132:2393S–2400S, 2002.
74. Waterland RA, Jirtle RL. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition 20:63–68, 2004.
75. Pufulete M, Al-Ghnaniem R, Leather AJ, Appleby P, Gout S, Terry C, Emery PW, Sanders TA. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology 124:1240–1248, 2003.