

Epigenetics: A New Bridge between Nutrition and Health^{1,2}

Sang-Woon Choi^{3*} and Simonetta Friso⁴

³Vitamins and Carcinogenesis Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; and ⁴Department of Medicine, University of Verona School of Medicine, Verona 37134, Italy

ABSTRACT

Nutrients can reverse or change epigenetic phenomena such as DNA methylation and histone modifications, thereby modifying the expression of critical genes associated with physiologic and pathologic processes, including embryonic development, aging, and carcinogenesis. It appears that nutrients and bioactive food components can influence epigenetic phenomena either by directly inhibiting enzymes that catalyze DNA methylation or histone modifications, or by altering the availability of substrates necessary for those enzymatic reactions. In this regard, nutritional epigenetics has been viewed as an attractive tool to prevent pediatric developmental diseases and cancer as well as to delay aging-associated processes. In recent years, epigenetics has become an emerging issue in a broad range of diseases such as type 2 diabetes mellitus, obesity, inflammation, and neurocognitive disorders. Although the possibility of developing a treatment or discovering preventative measures of these diseases is exciting, current knowledge in nutritional epigenetics is limited, and further studies are needed to expand the available resources and better understand the use of nutrients or bioactive food components for maintaining our health and preventing diseases through modifiable epigenetic mechanisms. *Adv. Nutr.* 1: 8–16, 2010.

Introduction

Epigenetics can be defined as somatically heritable states of gene expression resulting from changes in chromatin structure without alterations in the DNA sequence, including DNA methylation, histone modifications, and chromatin remodeling. Over the past decades, epigenetic studies mainly have been focused on embryonic development, aging, and cancer. Presently, epigenetics is highlighted in many other fields, such as inflammation, obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and immune diseases. Because epigenetic modifications can be altered by external or internal environmental factors and have the ability to change gene expression, epigenetics is now considered an important mechanism in the unknown etiology of many diseases. Such induced epigenetic changes can be inherited during cell division, resulting in permanent maintenance of the acquired phenotype. Thus, epigenetics can provide a new framework for the search for etiological factors in environment-associated diseases as well as embryonic development and aging, which are also known to be affected by many environmental factors.

In the nutritional field, epigenetics is exceptionally important, because nutrients and bioactive food components can modify epigenetic

phenomena and alter the expression of genes at the transcriptional level. Folate, vitamin B-12, methionine, choline, and betaine can affect DNA methylation and histone methylation through altering 1-carbon metabolism. Two metabolites of 1-carbon metabolism can affect methylation of DNA and histones: S-adenosylmethionine (AdoMet)⁵, which is a methyl donor for methylation reactions, and S-adenosylhomocysteine (AdoHcy), which is a product inhibitor of methyltransferases. Thus, theoretically, any nutrient, bioactive component, or condition that can affect AdoMet or AdoHcy levels in the tissue can alter the methylation of DNA and histones. Other water-soluble B vitamins like biotin, niacin, and pantothenic acid also play important roles in histone modifications. Biotin is a substrate of histone biotinylation. Niacin is involved in histone ADP-ribosylation as a substrate of poly(ADP-ribose) polymerase as well as histone acetylation as a substrate of Sirt1, which functions as histone deacetylase (HDAC) (1). Pantothenic acid is a part of CoA to form acetyl-CoA, which is the source of acetyl group in histone acetylation. Bioactive food components directly affect enzymes involved in epigenetic mechanisms. For instance, genistein and tea catechin affects DNA methyltransferases (Dnmt). Resveratrol, butyrate, sulforaphane, and diallyl sulfide inhibit HDAC and curcumin inhibits histone acetyltransferases (HAT). Altered enzyme activity

¹ Supported by the USDA under agreement no. 581950-9-001. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the USDA. This project has been supported in part by NIH grants R21 AA016681 and R01 AG025834 (S-W.C.).

² Author disclosures: S-W. Choi and S. Friso, no conflicts of interest.

* To whom correspondence should be addressed. E-mail: sang.choi@tufts.edu.

⁵ Abbreviations used: 5hmC, 5-hydroxymethylcytosine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; DMR, differentially methylated region; H3K, H3 lysine; PRC, polycomb repressive complex; SWI/SNF, switch mating type/sucrose nonfermenting.

by these compounds may affect physiologic and pathologic processes during our lifetime by altering gene expression.

In this review, we update the most recent knowledge regarding nutritional epigenetics, which will be helpful for an understanding of how nutrients contribute to our health.

Current status of knowledge

DNA methylation

DNA methylation, which modifies a cytosine base at the CpG dinucleotide residues with methyl groups, is catalyzed by Dnmt and regulates gene expression patterns by altering chromatin structures. Currently, 5 different Dnmt are known: Dnmt1, Dnmt2, Dnmt 3a, Dnmt3b and DnmtL. Dnmt1 is a maintenance Dnmt and Dnmt 3a, 3b, and L are de novo Dnmt. The function of Dnmt2 is not yet clear. By affecting these Dnmt during our lifetime, nutrients and bioactive food components can change global DNA methylation, which is associated with chromosomal integrity, as well as gene-specific promoter DNA methylation, which is closely associated with gene expression. Furthermore, these DnmTs work together with enzymes that catalyze other epigenetic phenomena, and changes in the activity of these enzymes may be involved in the development of various diseases.

Compared with DNA methylation reactions, the DNA demethylation process has not been well delineated. However, the DNA demethylation mechanism is currently highlighted, because DNA demethylation is important in cellular processes during embryonic development and stem cell differentiation. Several candidate mechanism are suggested: 1) base excision repair initiated by 5-methylcytosine DNA glycosylase; 2) base excision repair initiated by coupled activities of 5-mC deaminase that converts 5-mC to T, and G/T mismatch DNA glycosylase that corrects the G/T mismatch; 3) nucleotide excision repair that removes methylated CpG dinucleotides; 4) oxidative removal of the methyl group; and 5) hydrolytic removal of the methyl group [reviewed in (2)]. Most recently, hydroxymethyl cytosine was found. The conversion of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) in mammalian DNA is mediated by methylcytosine oxygenase TET1 (3). In addition, 5hmC might be produced by the addition of formaldehyde to cytosines in DNA by Dnmt proteins (4). It appears that 5hmC might serve biologically important roles by itself, or it might serve as an intermediate in DNA demethylation. It was also suggested that a reversible enzymatic reaction catalyzed by Dnmt proteins can produce unmodified cytosine from 5hmC, supporting that 5hmC might be an intermediate in direct DNA demethylation. Because 5hmC is present in mammalian DNA at a significant level in a tissue-specific manner (5), further studies are needed to delineate the role of 5hmC, especially in aging and cancer, both of which demonstrate DNA hypomethylation.

Effects of nutrients on DNA methylation. Folate, a water-soluble B vitamin, has been extensively studied for its effect on DNA methylation, because folate carries a methyl group and ultimately delivers that methyl group for the synthesis of AdoMet, the unique methyl donor for DNA methylation reactions. However, folate is not the sole determinant of DNA methylation, because other methyl donor nutrients such as methionine, choline, betaine, and vitamin B-12 as well as other environmental factors can also change DNA methylation status. In a recent animal study, dietary folate levels were positively correlated with both genomic and *p16* promoter DNA methylation status, along with an altered *p16* gene expression level in aged mouse colon (6). This result is consistent with a human study that T lymphocytes showed DNA demethylation and overexpression of genes associated with autoimmunity after the age of 50 y when T lymphocytes from healthy adults

22–81 y old were cultured with a low-folate and -methionine medium. The effects were reproduced by *Dnmt1* knockdowns in T lymphocytes from young participants. Because it is known that *Dnmt1* expression is decreased by aging, we can speculate that age-dependent decreases in Dnmt levels and low dietary methyl donor nutrients synergistically alter the DNA methylation status and DNA methylation-mediated gene expression (7).

It appears that folate is essential for DNA methylation reprogramming during the early embryonic period. Because folate deficiency in early pregnancy is associated with an increased risk of neural tube defects, aberrant reprogramming of DNA methylation by low dietary folate has been suggested as a candidate mechanism. Steegers-Theunissen et al. (8) investigated whether periconceptual maternal folic acid supplementation affects methylation at the differentially methylated region (DMR) of the insulin-like growth factor 2 gene (*IGF2*) in 120 children aged 17 mo. Eight-six mothers of these children had used folic acid periconceptionally but 34 mothers had not. *IGF2* is an imprinting gene in which the methylated allele at DMR (imprinted allele) is repressed. Abnormal derepression of imprinted alleles (loss of imprinting) has been suggested to cause pediatric developmental diseases or cancer in later life. Children of mothers who used folic acid had a 4.5% higher methylation of the *IGF2* DMR than children who were not exposed to maternal folic acid supplementation ($P = 0.014$). This result indicates that periconceptual folic acid supplementation is associated with imprinting status of *IGF2* in the child, which may affect intrauterine programming of growth and development with consequences for health and disease throughout life. In an animal study using mature female sheep, restriction of folate, vitamin B-12, and methionine from the periconceptual diet induced obesity in adult offspring as well as altered immune responses to an antigenic challenge. In these adult offspring, methylation status of 4% of 1400 CpG islands was altered. This study indicates that dietary methyl nutrients during the periconceptual period can change DNA methylation patterns in offspring and it may modify adult health-related phenotypes (9).

Animal studies also suggest that dietary folate during the postweaning period also affects DNA methylation status in a way that may modify disease susceptibility in later life. Kotsopoulos et al. (10) reported that a low-folate diet provided from the postweaning period to puberty increased genomic DNA methylation by 34–48% ($P < 0.04$) in rat liver that persisted into adulthood. An animal study also indicated that a postweaning diet can affect imprinting status at the *IGF2* locus (11).

Vitamin B-12, a water-soluble B vitamin and essential cofactor of methionine synthase in 1-carbon metabolism, has been known to affect genomic DNA methylation. Most recently, Uekawa et al. (12) demonstrated that severe vitamin B-12 deficiency induces promoter hypomethylation of the cystathionine β -synthase gene and represses this gene transcription in rats, even though supplementation with methionine, the precursor of AdoMet and product of methionine synthase, could not reverse this effect. Choline is a methyl donor nutrient and maternal choline availability is essential for fetal neurogenesis such as hippocampal development as well as memory function throughout life. In a mouse study, choline deprivation during the embryonic period caused hypermethylation of a specific CpG site within the calbindin 1 (*Calb1*) gene, which is important in hippocampus development, along with increased expression of *Calb1* (13). This study indicates that choline deficiency during the embryonic period could change DNA methylation and thereby alter fetal brain development.

Effects of bioactive food components on DNA methylation. A growing body of evidence suggests that certain bioactive food

components, including tea polyphenols, genistein from soybean, or isothiocyanates from plant foods, might inhibit the development of cancer by reducing DNA hypermethylation status in critical genes associated with cancer, such as *p16* or retinoic acid receptor beta (*RARβ*) (14). The effects of dietary polyphenols appear to be either through their direct inhibition by interaction with the catalytic site of the Dnmt1 molecule or their influence on methylation status indirectly through metabolic effects associated with energy metabolism [reviewed in (15)]. In a human study, healthy premenopausal women demonstrated that a daily supplementation with isoflavones induced dose-specific changes in *RARβ2* and cyclin D2 (*CCND2*) gene methylation from the intraductal specimens, which are correlated with serum genistein levels (16). In a cultured cell study, genistein alone showed a significant antileukemic activity against murine cells, and this effect was enhanced when used in combination with 5-aza-2'-deoxycytidine, a potent inhibitor of Dnmt and an effective agent for the treatment of leukemia (17). These results suggest that genistein may have the potential to increase the clinical efficacy of 5-aza-2'-deoxycytidine for the treatment of cancer through its inhibitory effect on DNA methylation. Treatment with genistein could be more physiologic than that with potent cancer chemotherapeutic agents. On the other hand, transgenerational studies using CD-1 mice demonstrated that neonatal exposure to genistein can induce uterine adenocarcinoma, which is associated with abnormal hypomethylation of CpG islands in the nucleosomal binding protein 1 (*Nsbp1*) gene throughout life. The *Nsbp1* is purported to be involved in chromatin remodeling and transcriptional activation. This study indicates that the reprogramming of uterine *Nsbp1* expression by neonatal genistein exposure could be mediated by DNA methylation (18).

Effects of diet on DNA methylation. In rats moderate maternal dietary protein restriction is known to alter phenotypes in the offspring, which manifests as hypertension, dyslipidemia, and impaired glucose metabolism. However, these abnormalities can be reversed by folate supplementation. It has been shown that the induction of an altered phenotype by a maternal protein restriction diet during pregnancy involves changes in DNA methylation and histone modifications in specific genes, including the glucocorticoid receptor (*GR*) (33% lower; $P < 0.001$) and *PPARα* (26% lower; $P < 0.05$) in the liver of juvenile and adult offspring (19,20).

The honeybee model clearly demonstrated the epigenetic effects of diet on the phenotype, because honeybees grow to be either queens or workers depending on whether they are fed royal jelly or beebread. The different honeybee phenotype has been suggested to occur through epigenetic changes in DNA methylation patterns induced by the different types of honey (21). More recent studies found that ~35% of the annotated honeybee genes are expected to be methylated at the CpG dinucleotides by a highly conserved DNA methylation system, suggesting that honeybees use DNA methylation to control the levels of activity of the genes that might be needed for conserved core biological processes (22,23).

An animal study using the obese Berlin fat mouse inbred line and the lean C57BL/6NCrl line of *Mus musculus* examined the methylation status and expression levels of the melanocortin-4 receptor (*Mc4r*) gene, which plays an important role in body weight regulation, in response to a standard and a high-fat diet. With the standard diet, the methylation status did not differ between the lines. With the high-fat diet, methylation of the CpGs near the transcription start site was decreased in both lines. The results suggest that a high-fat diet might affect the methylation status of the *Mc4r* gene. The *Mc4r* gene expression, however, was only marginally

increased in the obese mouse line, whereas there was no change in the lean mouse line (24).

Alcohol profoundly affects 1-carbon metabolism by limiting methyl transfer reactions. Recently, Kaminen-Ahola et al. (25) conducted an animal study using a model of gestational alcohol exposure. They observed changes in the expression of an epigenetically sensitive allele, *Agouti viable yellow* (*Avy*), in the offspring after maternal ad libitum ingestion of 10% (v:v) ethanol between gestational d 0.5 and 8.5. Maternal ethanol ingestion increased the probability of transcriptional silencing at this locus, resulting in more mice with an agouti-colored coat. This transcriptional silencing correlated with hypermethylation at *Avy*. In the ethanol-exposed group, 11% of the CpG dinucleotides were methylated compared with 2% in the control group. This demonstrates that ethanol can affect adult phenotype by altering the epigenotype of the early embryo.

In conclusion, individual nutrients and bioactive food components or total diet can change DNA methylation and subsequently alter gene expression. These epigenetic changes may affect physiologic and pathologic processes in our body.

Histone modification

Nucleosome and chromatin structure. A nucleosome, which consists of 146 bp DNA and an octamer of histone proteins (histone 2A, histone 2B, histone 3, and histone 4), is a building block of chromatin, which can regulate transcriptional processes through postsynthetic modifications of DNA and the histone (Fig. 1). In contrast to DNA that is modified only by methylation, histones can be modified by methylation, acetylation, phosphorylation, biotinylation, ubiquitination, sumoylation, and ADP-ribosylation. The location of the histone modifications is at the histone tails that consist of 15–38 amino acids. Lysine residues in the histone tails can be either methylated (mono-, di-, and tri-) or acetylated, and arginine residues can be mono- or di-methylated. Histone acetylation status is balanced by HAT and HDAC. Histone methylation is maintained by histone methyltransferases and histone demethylases.

Current epigenetic studies are determining the role of individual modifications as well as combinatorial effects of those modifications. One interesting question in histone modifications is how methylation patterns of DNA and histones are established, erased, recognized, and inherited. It appears that methyltransferases, demethylases, and accessory proteins interact and coordinate the status of chromatin. For example, mammalian DNA methylation is highly associated with the methylation status of histones, especially at histone H3 lysine (H3K) 4 and H3K9, which have reciprocal effects on gene expression; H3K4 methylation (active methyl mark) increases gene expression and H3K9 methylation (repressive methyl mark) decreases gene expression. Thus, the correlation of DNA methylation with unmethylated H3K4 and methylated H3K9 requires a mechanism to ensure that H3K4 and H3K9 are not simultaneously methylated or demethylated. Enzymatic and structural studies suggest that Jumonji demethylases, which demethylate H3K4 or H3K9, and histone methyltransferases, which contain domains for both synthesizing and recognizing, may play critical roles in the H3K4 and H3K9 reciprocal methylation (26).

Histone acetylation. Histone acetylation is one of the most extensively studied histone modifications. The reversible acetylation of N-terminal lysine residues at positions 9, 14, 18, and 23 of H3 and 5, 8, 12, and 16 of H4 mediates decondensation of the nucleosome structure, alters histone and DNA interactions, and facilitates access and binding of transcription factors. In general, increased histone acetylation at histone H4 lysine 5 or H4 lysine 8 is found in euchromatin regions where transcription is potentially

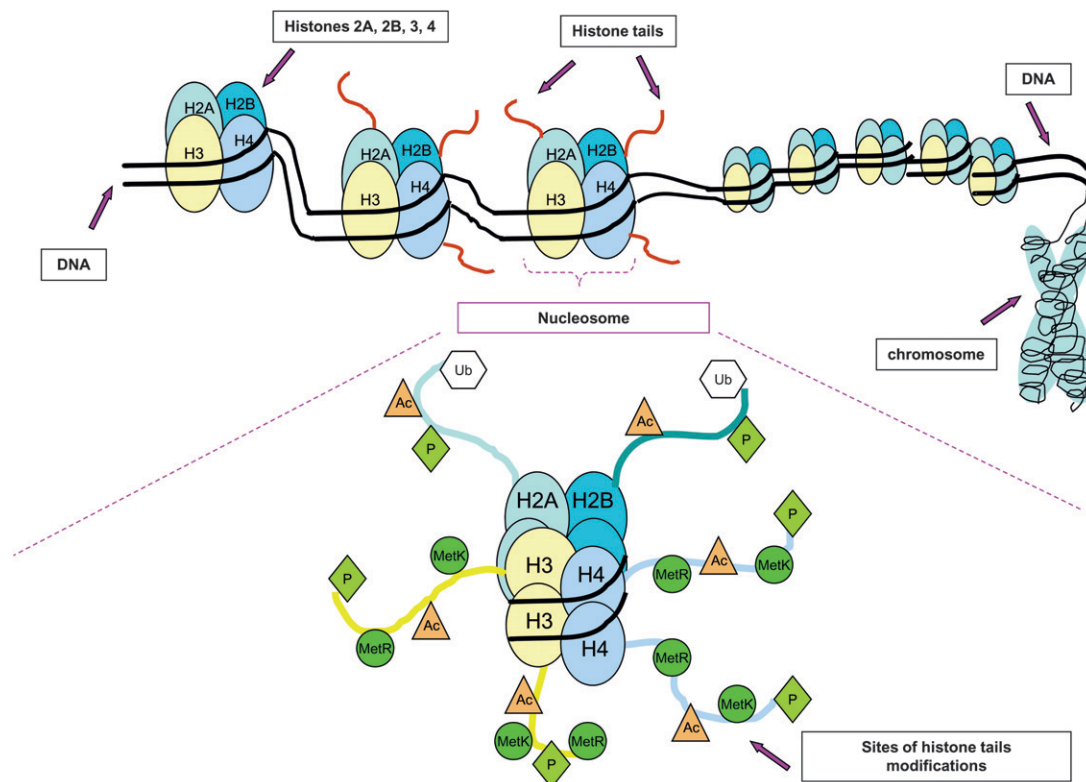


Figure 1 Nucleosome and histone modifications. Each nucleosome comprises an octamer of histone molecules and double-stranded DNA. The amino termini of histones, which are called histone tails, can be post-translationally modified and function as signal integration platforms. The main epigenetic modifications at histone tail sites are: lysine and arginine methylation (MetK and MetR, respectively), phosphorylation (P), acetylation (Ac), and ubiquitination (Ub).

active, whereas acetylation of H4 lysine 12 is increased in heterochromatin regions, where transcription is potentially inactive. HAT and HDAC regulate the steady-state balance of histone acetylation. Interestingly, HDAC inhibitors have been recognized as potential cancer therapeutic agents, because they induce cell cycle arrest and apoptosis by enhancing the expression of certain proapoptotic or cell cycle-mediating genes (27,28).

A number of studies investigated the effects of bioactive food components, especially their effects on HDAC and HAT [reviewed in (29)]. Because inhibition of HDAC could derepress epigenetically silenced genes in cancer cells, it has been investigated whether certain bioactive food components can act as HDAC inhibitors; e.g. sulforafane, an isothiocyanate from broccoli and broccoli sprouts, diallyl sulfide, an organosulfur compound from garlic, and butyrate, a SCFA from fiber. In vitro cell culture studies performed using B16 and S91 melanoma cells showed that sulforaphane inhibited growth and proliferation of cancer cells by downregulating deacetylation enzymes (30). Another study demonstrated that diallyl sulfide increases histone H3 and H4 acetylation in colonocytes isolated from rats along with alterations in the expression of a subset of genes (31). In another rat study, treatment of tributyrin, a butyrate prodrug that increases hepatic butyrate levels, increased hepatic nuclear H3K9 acetylation in preneoplastic lesions (4-fold; $P < 0.05$) and increased p21 protein expression (1.5-fold; $P < 0.05$), which could be associated with HDAC inhibitory effects (32). Each of these studies shows a promising link between bioactive food components and histone acetylation.

Histone acetylation is highly associated with inflammation. HDAC regulate proinflammatory genes such as interleukin (IL)-1, 5, 8, 12, and antiinflammatory genes such as IL-10 (33). The

expression of *COX-2* is also regulated by histone acetylation of the promoter region (34) and suppressed by sirtuin 1 (Sirt1), which functions as HDAC (35). The expression of inducible nitric oxide synthase (*iNOS*) is also known to be regulated by p300 HAT.

Calorie restriction reduces the expression of inflammatory genes such as *NF- κ B*, *API*, *COX-2*, and *iNOS*. *NF- κ B* is known to be activated by histone acetylation. p300 HAT acetylates the p50 subunit of *NF- κ B*, thereby increasing *NF- κ B* binding and *NF- κ B* mediated transactivation. Further, caloric restriction reduces the expression of *Sirt1*, a major mediator of calorie restriction (36), which functions as a HDAC (37) and regulates p300 HAT (38). *Sirt1* is also a regulator of histone methyltransferases (39).

Resveratrol, a bioactive component in grape skins and novel potent activator of *Sirt1*, has an antiinflammatory effect against colitis and colitis-associated colon cancer (40,41). The antiinflammatory effect of resveratrol is conveyed through the inhibitory effects of *iNOS*, *COX-2*, and *NF- κ B* (42–44). It has been suggested that histone acetylation by activated *NF- κ B* can be repressed by resveratrol. Butyrate, a SCFA that enters into the active site of the HDAC enzymes and inhibits the activity, enhances derivation of induced pluripotent stem cells by increasing histone H3 acetylation and DNA demethylation. Butyrate stimulation may provide an effective method for reprogramming various human adult somatic cells (45).

Because HAT, especially p300/CREB-binding protein (CBP), have been associated with cancer cell growth and survival, HAT could be novel molecular targets for the development of cancer chemotherapeutic agents. Interestingly, curcumin, whose medicinal properties have long been recognized in India and Southeast Asia, is an inhibitor of p300/CBP HAT. In cells, curcumin promotes proteasome-dependent degradation of p300 and the closely related

CBP protein. In addition to inducing p300 degradation, curcumin inhibits the acetyltransferase activity of p300. These data indicate that curcumin can be a novel compound for the development of therapeutic p300/CBP-specific inhibitors (46). Additionally, curcumin prevents diabetes-associated abnormalities in the kidneys by inhibiting p300 and NF- κ B (47).

Histone methylation. Compared with histone acetylation, the effects of nutrients or bioactive components on histone methylation have not yet been extensively studied, even though cancer and aging demonstrated substantial changes in histone methylation. Because AdoMet is the methyl donor to histone methylation and AdoHcy is an inhibitor of histone methyltransferases, the effects of dietary donor nutrients on histone methylation have been studied. Ara et al. (48) investigated whether the treatment with AdoMet in RAW cells, a mouse leukemic monocyte macrophage cell line, blocks the lipopolysaccharide-induced binding of trimethylated H3K4 to the tumor necrosis factor- α promoter. Exogenous AdoMet was unstable and converted spontaneously to methylthioadenosine and AdoHcy, both of which are known to inhibit histone methylation. In the same study, similar effects were also observed with lipopolysaccharide-mediated induction of *i*NOS. Because AdoHcy inhibits histone methylation reactions, AdoHcy hydrolase, which reversibly hydrolyzes AdoHcy to homocysteine and adenosine, can be a promising target for the treatment of epigenetic diseases. It also indicates that nutritional conditions that can increase intracellular AdoHcy may have a similar effect (49).

After being converted to betaine, choline, a dietary methyl donor, remethylates homocysteine. Mehedint et al. (50) reported that C57BL/6 mice with choline deficiency during gestational d 12–17 had altered methylation of the histone H3 in E17 fetal hippocampi. In the ventricular and subventricular zones, H3K9 monomethylation was decreased by 25% ($P < 0.01$) and in the pyramidal layer, H3K9 dimethylation was decreased by 37% ($P < 0.05$), indicating that choline deficiency can reduce histone methylation in the brain. In a liver cancer animal model of a methyl-deficient diet, which is low in methionine, choline and folic acid, Pogribny et al. (51) demonstrated that the liver tumors decreased in histone H4 lysine 20 trimethylation (44%) and increased in H3K9 trimethylation (40%), as well as gradually decreased in the expression of Suv4-20h2 histone methyltransferase that catalyzes H4 lysine 20 methylation and increased in the expression of Suv39h1 that catalyzes H3K9 methylation. This study indicates that the development of liver tumors by a methyl-deficient diet may be through the effect on histone methylation.

One of the most interesting observations is that histone methylation is associated with obesity. The H3K9-specific demethylase Jhd2a (also known as Jmjd1a and Kdm3a) has an important role in nuclear hormone receptor-mediated gene activation and male germ cell development. The loss of Jhd2a function in mice results in obesity and hyperlipidemia, indicating that H3K9 methylation status is important in regulating the expression of metabolic genes (52). Further studies are needed to understand the role of histone methylation in obesity.

Histone biotinylation. Biotin, an essential water-soluble B vitamin, has been known to modify tails of histone H2A, H3, and H4 through a covalent attachment of biotin to specific lysine residues catalyzed by the enzymes biotinidase and holocarboxylase synthase. Biotinylations at histone H4 lysine 8 and lysine 12 have been associated with heterochromatin structures, gene silencing, mitotic condensation of chromatin, and DNA repair (53,54). Histone biotinylation is a reversible process, even though debiotinylases have

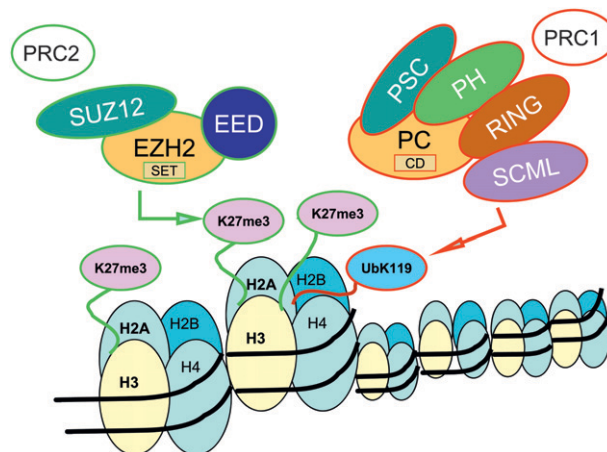


Figure 2 Scheme for the PRC signaling. Polycomb group proteins are structurally and functionally diverse and form large multimeric complexes of 2 general types: PRC1 and PRC2. The PRC2 (green contours) possesses H3K27 methyltransferase activity through the function of the EZH2 subunit, together with embryonic ectoderm development (EED) and suppressor of Zeste 12 homolog (SUZ12). The PRC1 complex (red contours) is recruited by the affinity of chromodomains in chromobox proteins to the H3K27 trimethylation mark. PRC1 exists in multiple forms that contain polycomb proteins (PC), RING proteins, PH proteins, and Sex combs on midleg proteins (SCML) and catalyzes the ubiquitination of histone H2A on lysine 119 following the H3K27 methylation.

not been characterized. Dietary supplementation of biotin is required for biotinylation and a deficiency in biotin may have profound effects on chromatin structure (55), although a recent cultured cell study strongly argues that biotin is absent in native histones (56). Because there are many unanswered questions in histone biotinylation, further studies are needed to delineate the significance of histone biotinylation, a modification of the histone directly by a nutrient.

Chromatin remodeling

ATP-dependent chromatin remodeling. ATP-dependent chromatin remodeling complexes, which are broadly divided into 4 main families [switch mating type/sucrose nonfermenting (SWI/SNF), imitation switch, chromodomain helicase DNA binding, and INO80 complexes], are crucial for the assembly of chromatin structures. About 30 genes encode the ATPase subunits of these complexes in mammals (57). The SWI/SNF complex uses the free energy derived from ATP hydrolysis to actively alter nucleosomal structure to make DNA accessible to transcription factors and repair enzymes. The SWI/SNF complex plays essential roles in a variety of cellular processes, including differentiation, proliferation, and DNA repair and replication (58). The SWI/SNF complex is also involved in immune responses (59) and carcinogenesis (60). A more recent study demonstrated that whole body disruption of a novel double bromodomain protein Brd2, a transcriptional coactivator/corepressor with SWI/SNF-like functions that regulates chromatin, causes lifelong severe obesity in mice with pancreatic islet expansion, hyperinsulinemia, hepatosteatosis, and elevated proinflammatory cytokines (61). This study indicates that the SWI/SNF chromatin remodeling mechanism may play an important role in the prevention of obesity.

Polycomb repressive complex. Polycomb repressive complex (PRC), a chromatin remodeling complex, is an epigenetic gene silencer and crucial regulator of genomic programming and differentiation (62) (Fig. 2). This complex cooperates in transcriptional repression of target genes by altering chromatin structure (63). Polycomb group proteins form large multimeric complexes of 2 general types, PRC1 and PRC2. The PRC2 possesses histone H3K27 methyltransferase activity and the PRC1 components ubiquitinate H2A following the H3K27 trimethylation. Enhancer of Zeste homolog (EZH2) histone methyltransferase is an enzymatic subunit of PRC2 and methylates H3K27 to mediate gene silencing. The Bmi-1 (RING finger protein 51) is a PRC1 protein that binds to the H3K27 trimethylation and catalyzes the ubiquitination of histone H2A.

Histone modifications via PRC are important during embryonic stem cell differentiation. During differentiation, PRC2 binding that mediates trimethylation of H3K27 is lost in transcriptionally induced developmental genes. Retinoic acid is involved in differentiation of embryonic stem cells as well as differentiation of various cancer cells in culture. Interestingly, a global decrease in H3K27 trimethylation was observed 3 d after differentiation of mouse embryonic stem cells induced by retinoic acid treatment. The global level of EZH2 also decreased with retinoic acid treatment. A loss of EZH2 binding and H3K27 trimethylation was observed locally on PRC2 target genes induced after 3 d of retinoic acid treatment. In contrast, direct retinoic acid-responsive genes that are rapidly induced, such as Homeobox protein Hox-A1 (Hoxa1), showed a loss of EZH2 binding and H3K27 trimethylation after a few hours of retinoic acid treatment and increased histone acetylation to override the H3K27 trimethylation repressive mark to induce gene transcription (64).

Bmi-1, a PRC1 protein, is overexpressed in some human cancers, including colorectal cancer, and human non-small cell lung cancer and epidermal squamous cell carcinoma cells. Balasubramanian et al. (65) reported that (-)-epigallocatechin-3-gallate treatment of SCC-13 cells, a human epidermal squamous carcinoma cell line, reduced Bmi-1 and EZH2 level and was associated with

reduced cell survival. The reduced survival was also associated with a global reduction in H3K27 trimethylation. This change in PRC protein expression was associated with reduced expression of key proteins that enhance progression through the cell cycle. These findings suggest that green tea polyphenols reduce skin cancer cell survival through PRC-mediated epigenetic regulatory mechanisms.

MicroRNA

MicroRNA is a new class of noncoding, endogenous, small RNA that regulates gene expression by translational repression, representing a new important class of regulatory molecules. MicroRNA can play important roles in controlling DNA methylation and histone modifications, creating a highly controlled feedback mechanism. Interestingly, epigenetic mechanisms such as promoter methylation or histone acetylation, can also modulate microRNA expression. A connection between epigenetic phenomena and microRNA has been described in several physiological processes and an altered balance between them represents one of the mechanisms leading to pathological conditions such as cancer. An aberrant expression of microRNA has been associated with the development or progression of human cancers by altering cell proliferation and apoptosis processes (66).

A methyl-deficient diet, which induces liver tumors in rats, also induced prominent early changes in expression of microRNA genes, including miR-34a, miR-127, miR-200b, and miR-16a involved in the regulation of apoptosis, cell proliferation, cell-to-cell connection, and epithelial-mesenchymal transition in the rat liver (67). Mice fed a methyl-deficient diet contracted nonalcoholic steatohepatitis, which was accompanied by changes in the expression of microRNA, including miR-29c, miR-34a, miR-155, and miR-200b. Interestingly, changes in the expression of these microRNA are in parallel with changes in protein levels of their targets. These studies suggest that alterations in the expression of microRNA are a prominent event during the development of liver cancer and nonalcoholic steatohepatitis caused by dietary methyl deficiency (68).

Similar to the methyl-deficient diet, folate deficiency induced a marked global increase in microRNA expression in human lymphoblastoid cells. miR-222 was significantly overexpressed under folate-deficient conditions in vitro. This finding was confirmed in vivo in human peripheral blood from individuals with low folate intake, suggesting that microRNA expression might be a potential biomarker of nutritional status in humans (69). The Göttingen minipig, a high-fat diet animal model of obesity, was fed either a high-cholesterol or standard diet. Body weight, total cholesterol, and HDL were higher and miR-122 was lower (1.4-fold; $P < 0.0015$) in pigs fed the high-cholesterol diet compared with those fed the standard diet, indicating the implication of microRNA in obesity (70).

Table 1. Epigenetic roles of nutrition in physiologic and pathologic processes

	Nutrient or diet	Epigenetic mechanism	References
Embryonic development	Folate	DNA methylation, imprinting	(8,9)
	Choline	DNA methylation	(13)
	Protein restriction	DNA methylation, histone modifications	(19,20)
Stem cell	Alcohol	DNA methylation	(25)
	Butyrate	Histone acetylation, DNA methylation	(45)
Aging	Retinoic acid	PRC	(64)
	Folate	DNA methylation	(6,7)
Immune function	Calorie restriction	Histone acetylation	(36,37)
	Folate	DNA methylation	(7)
Cancer	Methyl-deficient diet	Histone modification, microRNA	(51,67)
	Genistein	DNA methylation, microRNA	(16,18,72)
	(-)-Epigallocatechin-3-gallate	DNA methylation, PRC	(14,65)
	Curcumin	microRNA	(73,74)
Obesity, insulin resistance	High-fat diet	DNA methylation, microRNA	(24,70)
	Methyl-deficient diet	DNA methylation	(9)
Inflammation	Curcumin	Histone acetylation	(47)
	Resveratrol	Histone acetylation	(40,41)
	AdoMet	Histone methylation	(48)
	Methyl-deficient diet	microRNA	(68)
Neurocognition	Choline	DNA methylation, histone methylation	(13,50)

A few reports have suggested that bioactive food components may reduce carcinogenesis through microRNA [reviewed in (71)]. Genistein represses human uveal melanoma cells and murine chronic lymphocytic leukemia cells by altering miR-16 (72). Curcumin represses human pancreatic cancer cells by upregulating miR-22 and downregulating miR-199a (73). Curcumin also upregulates miR-15a and miR-16 expression, both of which could inhibit the expression of B-cell lymphoma 2 (*Bcl-2*), thereby inducing apoptosis in MCF-7 breast cancer cells (74). miR-10a, a key mediator of metastatic behavior in pancreatic cancer, is a retinoic acid target. Retinoic acid receptor antagonists effectively repress miR-10a expression and block metastasis (75). miR-34a functions as a potential tumor suppressor in neuroblastoma cells, and retinoic acid-induced differentiation of the neuroblastoma cell line enhanced miR-34a expression and decreased expression of its target, E2F transcriptional factor 3 (75).

Conclusion

Epigenetics is an inheritable phenomenon that affects gene expression without base pair changes. Epigenetic phenomena include DNA methylation, histone modifications, and chromatin remodeling. Chromatin is quite dynamic and is much more than a neutral system for packaging and condensing genomic DNA. It is a critical player in controlling the accessibility of DNA for transcription. Modifications of chromatin structure can give rise to a variety of epigenetic effects. Due to its reversible character, epigenetics is now considered an attractive field of nutritional intervention.

During our lifetime, nutrients can modify physiologic and pathologic processes through epigenetic mechanisms that are critical for gene expression (summarized in Table 1). Modulation of these processes through diet or specific nutrients may prevent diseases and maintain health. However, it is very hard to delineate the precise effect of nutrients or bioactive food components on each epigenetic modulation and their associations with physiologic and pathologic processes in our body, because the nutrients also interact with genes, other nutrients, and other lifestyle factors. Furthermore, each epigenetic phenomenon also interacts with the others, adding to the complexity of the system.

Our knowledge regarding nutritional epigenetics is still limited. In particular, the effects of nutrients or bioactive food components on histone methylation or chromatin remodeling complexes are largely unknown. In the future, we need to investigate more nutrients or bioactive food compounds to find better ones for our health. Understanding the role of nutrients or bioactive food components in altering epigenetic patterns will aid our ability to find a better way to maintain our health through nutritional modulation that could be more physiologic than any other pharmacotherapies.

Acknowledgments

Both authors participated in the conception, design, and writing of this manuscript. Both authors read and approved the final manuscript.

Literature Cited

- Kirkland JB. Niacin status impacts chromatin structure. *J Nutr.* 2009; 139:2397–401.
- Zhu JK. Active DNA demethylation mediated by DNA glycosylases. *Annu Rev Genet.* 2009;43:143–66.
- Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science.* 2009;324:930–5.
- Liutkeviciute Z, Lukinavicius G, Masevicius V, Daujotyte D, Klimasauskas S. Cytosine-5-methyltransferases add aldehydes to DNA. *Nat Chem Biol.* 2009;5:400–2.
- Jin SG, Kadam S, Pfeifer GP. Examination of the specificity of DNA methylation profiling techniques towards 5-methylcytosine and 5-hydroxymethylcytosine. *Nucleic Acids Res.* 2010;38:e125.
- Keyes MK, Jang H, Mason JB, Liu Z, Crott JW, Smith DE, Friso S, Choi SW. Older age and dietary folate are determinants of genomic and p16-specific DNA methylation in mouse colon. *J Nutr.* 2007;137:1713–7.
- Li Y, Liu Y, Strickland FM, 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.* 2010;45:312–22.
- Stegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, Slagboom PE, 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.* 2009;4:e7845.
- Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, et al. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci USA.* 2007;104:19351–6.
- Kotsopoulos J, Sohn KJ, Kim YI. Postweaning dietary folate deficiency provided through childhood to puberty permanently increases genomic DNA methylation in adult rat liver. *J Nutr.* 2008;138:703–9.
- Waterland RA, Lin JR, Smith CA, Jirtle RL. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (*Igf2*) locus. *Hum Mol Genet.* 2006;15:705–16.
- Uekawa A, Katsushima K, Ogata A, Kawata T, Maeda N, Kobayashi K, Maekawa A, Tadokoro T, Yamamoto Y. Change of epigenetic control of cystathionine beta-synthase gene expression through dietary vitamin B12 is not recovered by methionine supplementation. *J Nutrigenet Nutrigenomics.* 2009;2:29–36.
- Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. *FASEB J.* 2006;20:43–9.
- Fang M, Chen D, Yang CS. Dietary polyphenols may affect DNA methylation. *J Nutr.* 2007;137:S223–8.
- Li Y, Tollefsbol TO. Impact on DNA methylation in cancer prevention and therapy by bioactive dietary components. *Curr Med Chem.* 2010; 17:2141–51.
- Qin W, Zhu W, Shi H, Hewett JE, Ruhlen RL, MacDonald RS, Rottinghaus GE, Chen YC, Sauter ER. Soy isoflavones have an antiestrogenic effect and alter mammary promoter hypermethylation in healthy premenopausal women. *Nutr Cancer.* 2009;61:238–44.
- Raynal NJ, Charbonneau M, Momparler LF, Momparler RL. Synergistic effect of 5-Aza-2'-deoxycytidine and genistein in combination against leukemia. *Oncol Res.* 2008;17:223–30.
- Tang WY, Newbold R, Mardilovich K, Jefferson W, Cheng RY, Medvedovic M, Ho SM. Persistent hypomethylation in the promoter of nucleosomal binding protein 1 (*Nsnp1*) correlates with overexpression of *Nsnp1* in mouse uteri neonatally exposed to diethylstilbestrol or genistein. *Endocrinology.* 2008;149:5922–31.
- Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring. *Br J Nutr.* 2008;100:278–82.
- Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr.* 2007;97:1064–73.
- Kucharski R, Maleszka J, Foret S, Maleszka R. Nutritional control of reproductive status in honeybees via DNA methylation. *Science.* 2008; 319:1827–30.
- Foret S, Kucharski R, Pittelkow Y, Lockett GA, Maleszka R. Epigenetic regulation of the honey bee transcriptome: unraveling the nature of methylated genes. *BMC Genomics.* 2009;10:472.
- Elango N, Hunt BG, Goodisman MA, Yi SV. DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. *Proc Natl Acad Sci USA.* 2009;106:11206–11.

24. Widiker S, Karst S, Wagener A, Brockmann GA. High-fat diet leads to a decreased methylation of the Mc4r gene in the obese Bfmi and the lean B6 mouse lines. *J Appl Genet*. 2010;51:193–7.
25. Kaminen-Ahola N, Ahola A, Maga M, Mallitt KA, Fahey P, Cox TC, Whitelaw E, Chong S. Maternal ethanol consumption alters the epigenotype and the phenotype of offspring in a mouse model. *PLoS Genet*. 2010;6:e1000811.
26. Cheng X, Blumenthal RM. Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. *Biochemistry*. 2010;49:2999–3008.
27. Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. *Proc Natl Acad Sci USA*. 2000;97:10014–9.
28. Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov*. 2002;1:287–99.
29. Nian H, Delage B, Ho E, Dashwood RH. Modulation of histone deacetylase activity by dietary isothiocyanates and allyl sulfides: studies with sulforaphane and garlic organosulfur compounds. *Environ Mol Mutagen*. 2009;50:213–21.
30. Do DP, Pai SB, Rizvi SA, D'Souza MJ. Development of sulforaphane-encapsulated microspheres for cancer epigenetic therapy. *Int J Pharm*. 2010;386:114–21.
31. Druesse-Pecollo N, Chaumontet C, Pagniez A, Vaugelade P, Bruneau A, Thomas M, Cherbuy C, Duee PH, Martel P. In vivo treatment by diallyl disulfide increases histone acetylation in rat colonocytes. *Biochem Biophys Res Commun*. 2007;354:140–7.
32. Kuroiwa-Tzmielina J, de Conti A, Scolastici C, Pereira D, Horst MA, Purgatto E, Ong TP, Moreno FS. Chemoprevention of rat hepatocarcinogenesis with histone deacetylase inhibitors: efficacy of tributyrin, a butyric acid prodrug. *Int J Cancer*. 2009;124:2520–7.
33. Villagra A, Sotomayor EM, Seto E. Histone deacetylases and the immunological network: implications in cancer and inflammation. *Oncogene*. 2010;29:157–73.
34. Coward WR, Watts K, Feghali-Bostwick CA, Knox A, Pang L. Defective histone acetylation is responsible for the diminished expression of cyclooxygenase 2 in idiopathic pulmonary fibrosis. *Mol Cell Biol*. 2009;29:4325–39.
35. Zhang R, Chen HZ, Liu JJ, Jia YY, Zhang ZQ, Yang RF, Zhang Y, Xu J, Wei YS, et al. SIRT1 suppresses activator protein-1 transcriptional activity and cyclooxygenase-2 expression in macrophages. *J Biol Chem*. 2010;285:7097–110.
36. Qiu X, Brown KV, Moran Y, Chen D. Sirtuin regulation in calorie restriction. *Biochim Biophys Acta*. 2010;1804:1576–83.
37. Vaquero A, Sternglanz R, Reinberg D. NAD⁺-dependent deacetylation of H4 lysine 16 by class III HDACs. *Oncogene*. 2007;26:5505–20.
38. Yang T, Fu M, Pestell R, Sauve AA. SIRT1 and endocrine signaling. *Trends Endocrinol Metab*. 2006;17:186–91.
39. Vaquero A, Scher M, Erdjument-Bromage H, Tempst P, Serrano L, Reinberg D. SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. *Nature*. 2007;450:440–4.
40. Cui X, Jin Y, Hofseth AB, Pena E, Habiger J, Chumanevich A, Poudyal D, Nagarkatti M, Nagarkatti PS, et al. Resveratrol suppresses colitis and colon cancer associated with colitis. *Cancer Prev Res (Phila Pa)*. 2010;3:549–59.
41. Sanchez-Fidalgo S, Cardeno A, Villegas I, Talero E, de la Lastra CA. Dietary supplementation of resveratrol attenuates chronic colonic inflammation in mice. *Eur J Pharmacol*. 2010;633:78–84.
42. Donnelly LE, Newton R, Kennedy GE, Fenwick PS, Leung RH, Ito K, Russell RE, Barnes PJ. Anti-inflammatory effects of resveratrol in lung epithelial cells: molecular mechanisms. *Am J Physiol Lung Cell Mol Physiol*. 2004;287:L774–83.
43. Zykova TA, Zhu F, Zhai X, Ma WY, Ermakova SP, Lee KW, Bode AM, Dong Z. Resveratrol directly targets COX-2 to inhibit carcinogenesis. *Mol Carcinog*. 2008;47:797–805.
44. Youn J, Lee JS, Na HK, Kundu JK, Surh YJ. Resveratrol and piceatannol inhibit iNOS expression and NF- κ B activation in dextran sulfate sodium-induced mouse colitis. *Nutr Cancer*. 2009;61:847–54.
45. Mali P, Chou BK, Yen J, Ye Z, Zou J, Dowey S, Brodsky RA, Ohm JE, Yu W, et al. Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. *Stem Cells*. 2010;28:713–20.
46. Marcu MG, Jung YJ, Lee S, Chung EJ, Lee MJ, Trepel J, Neckers L. Curcumin is an inhibitor of p300 histone acetyltransferase. *Med Chem*. 2006;2:169–74.
47. Chiu J, Khan ZA, Farhangkhoe H, Chakrabarti S. Curcumin prevents diabetes-associated abnormalities in the kidneys by inhibiting p300 and nuclear factor- κ B. *Nutrition*. 2009;25:964–72.
48. Ara AI, Xia M, Ramani K, Mato JM, Lu SC. S-adenosylmethionine inhibits lipopolysaccharide-induced gene expression via modulation of histone methylation. *Hepatology*. 2008;47:1655–66.
49. Kim BG, Chun TG, Lee HY, Snapper ML. A new structural class of S-adenosylhomocysteine hydrolase inhibitors. *Bioorg Med Chem*. 2009;17:6707–14.
50. Mehedint MG, Niculescu MD, Craciunescu CN, Zeisel SH. Choline deficiency alters global histone methylation and epigenetic marking at the Re1 site of the calbindin 1 gene. *FASEB J*. 2010;24:184–95.
51. Pogribny IP, Ross SA, Tryndyak VP, Pogribna M, Poirier LA, Karpinets TV. Histone H3 lysine 9 and H4 lysine 20 trimethylation and the expression of Suv4-20h2 and Suv-39h1 histone methyltransferases in hepatocarcinogenesis induced by methyl deficiency in rats. *Carcinogenesis*. 2006;27:1180–6.
52. Tateishi K, Okada Y, Kallin EM, Zhang Y. Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. *Nature*. 2009;458:757–61.
53. Zempleni J, Chew YC, Bao B, Pestinger V, Wijeratne SS. Repression of transposable elements by histone biotinylation. *J Nutr*. 2009;139:2389–92.
54. Hassan YI, Zempleni J. A novel, enigmatic histone modification: biotinylation of histones by holocarboxylase synthetase. *Nutr Rev*. 2008;66:721–5.
55. Camporeale G, Giordano E, Rendina R, Zempleni J, Eissenberg JC. Drosophila melanogaster holocarboxylase synthetase is a chromosomal protein required for normal histone biotinylation, gene transcription patterns, lifespan, and heat tolerance. *J Nutr*. 2006;136:2735–42.
56. Healy S, Perez-Cadahia B, Jia D, McDonald MK, Davie JR, Gravel RA. Biotin is not a natural histone modification. *Biochim Biophys Acta*. 1789;2009:719–33.
57. Ho L, Crabtree GR. Chromatin remodelling during development. *Nature*. 2010;463:474–84.
58. Simone C. SWI/SNF: the crossroads where extracellular signaling pathways meet chromatin. *J Cell Physiol*. 2006;207:309–14.
59. Jeong SM, Lee C, Lee SK, Kim J, Seong RH. The SWI/SNF chromatin-remodeling complex modulates peripheral T cell activation and proliferation by controlling AP-1 expression. *J Biol Chem*. 2010;285:2340–50.
60. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. *Oncogene*. 2009;28:1653–68.
61. Wang F, Liu H, Blanton WP, Belkina A, Lebrasseur NK, Denis GV. Brd2 disruption in mice causes severe obesity without Type 2 diabetes. *Biochem J*. 2009;425:71–83.
62. Schwartz YB, Pirrotta V. Polycomb complexes and epigenetic states. *Curr Opin Cell Biol*. 2008;20:266–73.
63. Kanno R, Janakiraman H, Kanno M. Epigenetic regulator polycomb group protein complexes control cell fate and cancer. *Cancer Sci*. 2008;99:1077–84.
64. Lee ER, Murdoch FE, Fritsch MK. High histone acetylation and decreased polycomb repressive complex 2 member levels regulate gene specific transcriptional changes during early embryonic stem cell differentiation induced by retinoic acid. *Stem Cells*. 2007;25:2191–9.
65. Balasubramanian S, Adhikary G, Eckert RL. The Bmi-1 polycomb protein antagonizes the (–)-epigallocatechin-3-gallate-dependent suppression of skin cancer cell survival. *Carcinogenesis*. 2010;31:496–503.
66. Iorio MV, Piovano C, Croce CM. Interplay between microRNAs and the epigenetic machinery: an intricate network. *Biochim Biophys Acta*. Epub May 20.
67. Tryndyak VP, Ross SA, Beland FA, Pogribny IP. Down-regulation of the microRNAs miR-34a, miR-127, and miR-200b in rat liver during

- hepatocarcinogenesis induced by a methyl-deficient diet. *Mol Carcinog.* 2009;48:479–87.
68. Pogribny IP, Starlard-Davenport A, Tryndyak VP, Han T, Ross SA, Rusyn I, Beland FA. Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab Invest.* Epub 2010 Jun 14.
69. Marsit CJ, Eddy K, Kelsey KT. MicroRNA responses to cellular stress. *Cancer Res.* 2006;66:10843–8.
70. Cirera S, Birck M, Busk PK, Fredholm M. Expression profiles of miRNA-122 and its target CAT1 in minipigs (*Sus scrofa*) fed a high-cholesterol diet. *Comp Med.* 2010;60:136–41.
71. Saini S, Majid S, Dahiya R. Diet, microRNAs and prostate cancer. *Pharm Res.* 2010;27:1014–26.
72. Salerno E, Scaglione BJ, Coffman FD, Brown BD, Baccarini A, Fernandes H, Marti G, Raveche ES. Correcting miR-15a/16 genetic defect in New Zealand Black mouse model of CLL enhances drug sensitivity. *Mol Cancer Ther.* 2009;8:2684–92.
73. Sun M, Estrov Z, Ji Y, Coombes KR, Harris DH, Kurzrock R. Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. *Mol Cancer Ther.* 2008;7:464–73.
74. Yang J, Cao Y, Sun J, Zhang Y. Curcumin reduces the expression of Bcl-2 by upregulating miR-15a and miR-16 in MCF-7 cells. *Med Oncol.* Epub 2009 Nov 12.
75. Weiss FU, Marques IJ, Woltering JM, Vlecken DH, Aghdassi A, Partecke LI, Heidecke CD, Lerch MM, Bagowski CP. Retinoic acid receptor antagonists inhibit miR-10a expression and block metastatic behavior of pancreatic cancer. *Gastroenterology.* 2009;137:2136–45.e1–7.