6

DNA Methylation: An Epigenetic Marker of Breast Cancer Influenced by Nutrients Acting as an Environmental Factor

Tarique N. Hasan, MSc, Gowhar Shafi, PhD, B. Leena Grace, PhD, Jesper Tegner, PhD, and Anjana Munshi, PhD

CONTENTS

ABSTRACT DNA methylation is an important regulator of gene expression and plays an essential role in maintaining cellular function. Its role in carcinogenesis has been a topic of considerable interest in recent years. Changes in DNA methylation patterns may contribute to the development of cancer in general and breast cancer in particular. Aberrant global methylation of DNA is frequently found in tumor cells. Global hypomethylation can result in chromosome instability, and hypermethylation has been associated with the inactivation of tumor suppressor genes. Several studies suggest that part of the cancer-protective effects associated with several bioactive components may involve modifications of the DNA methylation profile. Dietary factors that are involved in one-carbon metabolism provide the most compelling evidence supporting an interaction of nutrients and DNA methylation because these factors 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. This chapter examines alterations in DNA methylation in breast cancer, the effects on gene expression, and the role of nutrients in DNA methylation in the treatment of breast cancer.

6.1 Introduction

6.1.1 Epigenetics: Events Beyond Genetics

The concept of epigenetics was first introduced in 1942 by C. H. Waddington (Waddington, 1942), describing the influence of environmental factors on the development of specific traits through gene–environment interaction. Waddington's words, "the interaction of genes with their environment, which bring the phenotype into being," are fundamental to developmental biology, that is, the "idea that phenotype, or the morphologic and functional properties of an organism, arises sequentially under a program defined by the genome under the influence of the organism's environment" (Van Speybroeck, 2002). Modern aspects of epigenetics refer to the modification of DNA and/or related proteins without altering the nucleotide sequence, which passes the contained information to next generation. Only 6 years following Waddington's narration on epigenetics, DNA methylation was identified as an epigenetic marker (Hotchkiss, 1948). DNA methylation is currently the most studied and the best understood epigenetic modification. On the basis of many studies on a battery of housekeeping genes and growth regulator genes, DNA methylation is established as an additional mechanism for gene inactivation in different cell types including cancer cells (Lehmann et al., 2002).

6.2 DNA Methylation Stabilizes Chromatin Structure and Regulates Gene Expression Epigenetically

In normal cells, actively transcribing chromatin is hypomethylated, whereas nontranscribing chromatin is methylated in such a manner that it produces compact structures that sterically hinder RNA Pol II activities (Lorincz et al., 2004). The methylation of DNA is found to be involved in the stabilization of chromatin structure in an inactive conformation and inhibits gene transcription (Keshet et al., 1986). Therefore, it is believed that the

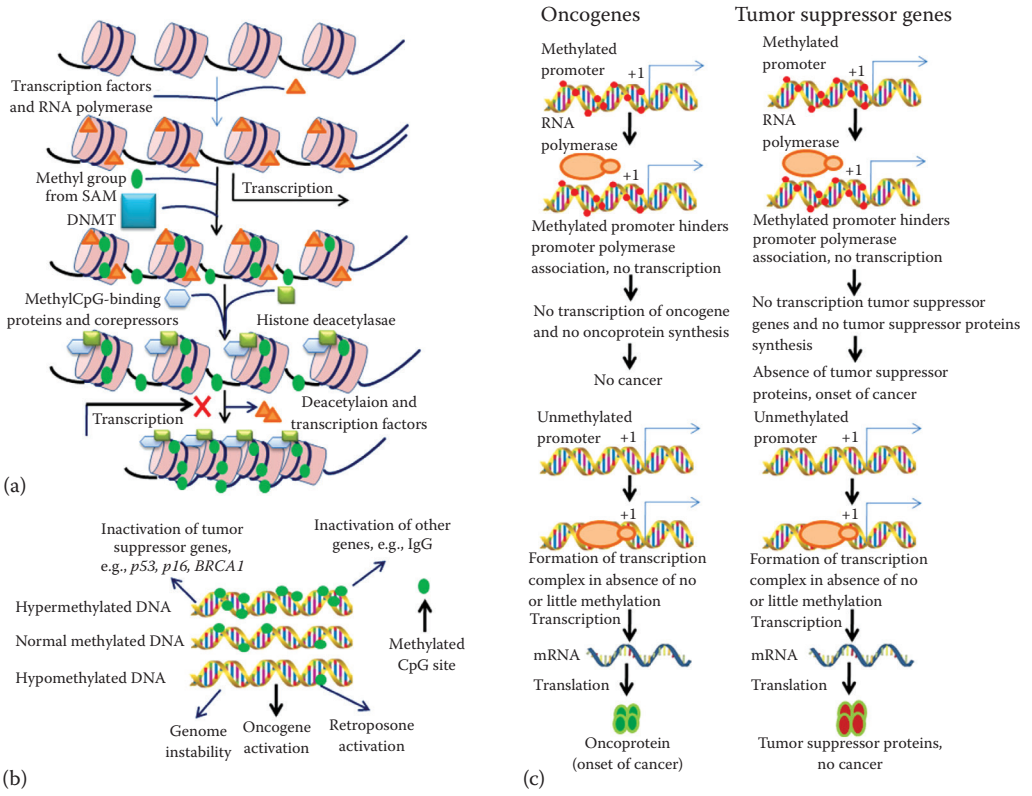

FIGURE 6.1
(a) Depiction of the epigenetic process in general methylation and, in particular, the subsequent stabilization of chromatin and inhibition of transcription. (b) Abnormal methylation of DNA leads either to inactivation of genes like tumor suppressor genes or activation of oncogenes, genome instability, and retroposon activation. (c) Methylation of oncogene promoters downregulates expression by blocking the formation of the transcriptional complex, while demethylation of oncogene promoters accelerates expression and leads toward cancer formation. Similarly, methylation of tumor suppressor genes downregulates expression by blocking the formation of the transcriptional complex, which in turn enhances the onset of cancer. Demethylation of tumor suppressor gene promoters facilitates transcriptional complex formation and increases tumor suppressor protein formation, thereby inhibiting tumor formation.

mechanism of gene regulation through DNA methylation is involved in essential genetic events including differentiation, genomic imprinting, and X-chromosome inactivation (Bernardino-Sgherri et al., 2002; Richardson and Yung, 2002) (Figure 6.1a).

The main target for DNA methylation is cytosine. The majority of 5′-methylcytosine in mammalian DNA is present in cytosine–guanine (CpG) dinucleotides, which are present in the promoter regions of several genes. Among all human promoters, 72% contain high CpG content, and 28% of promoters are those with CpG content characteristic of the overall genome, that is, low CpG content (Saxonov et al., 2006). As far as the whole human genome is concerned, CpGs are present approximately once per 80 dinucleotides in 98% of the genome; however, in the CpG islands, which comprise 1%–2% of the human genome, the frequency is five times higher (Bird, 1986).

Methylation and/or demethylation of DNA are essential features of a normal genome. But whenever DNA becomes abnormally methylated, several crucial functions become severely impeded. Abnormal DNA methylation accounts for (1) the excess of methylation

(hypermethylation) of genes that must be transcriptionally active in normal conditions and (2) the excess of genome-wide demethylation (global hypomethylation) (Figure 6.1b). A major aberration associated with hypermethylation of the promoter regions of tumor suppressor genes and DNA repair genes (*p15, p16, p57, p53, SLC5A8*, and *BRCA1*) is the onset of different kinds of cancers including breast cancer (Dobrovic and Simpfendorfer, 1997; Chanda et al., 2006; Hayslip and Montero, 2006; Whitman et al., 2008). Hypomethylation of DNA causes instability of the genome and is also related with breast and other cancers. For example, a case–control study from Spain reports that leukocyte genomic DNA hypomethylation is associated with increased risk of developing bladder cancer (Moore et al., 2008) (Figure 6.1c). Hypermethylation takes place mostly at CpG islands of promoter region of a gene, but hypomethylation usually is associated with repeated DNA sequences, such as long interspersed nuclear elements (Ehrlich, 2002). Global hypomethylation also activates transposable elements (TEs) to transcribe in both sense and antisense directions (Roman-Gomez et al., 2005) (Figure 6.1b).

6.3 Essentials of DNA Methylation

The methylation status of DNA (hypo or hyper) is mainly determined by (1) availability of primary methyl group sources in diet, (2) availability of *S*-adenosyl-L-methionine (SAM), and (3) availability of functional DNA methyltransferases (DNMTs) to transfer methyl groups from SAM to DNA, specifically to 5-C of cytosine. This is explained in more detail in the following.

6.3.1 Methyl Group Source

The major methyl group sources in the human diet are methionine, choline, and de novo one-carbon metabolism via methylfolate, which provides the methyl groups for methionine synthesis (Institute of Medicine and National Academy of Sciences, USA, 1998). Homocysteine is also converted to methionine through two alternative pathways: (1) choline derivative betaine donates a methyl group to homocysteine in the presence of betaine homocysteine *S*-methyltransferase (Varela-Moreiras et al., 1992; Lu et al., 2001), and (2) homocysteine receives a methyl group from the de novo one-carbon pool by the action of 5-methyltetrahydrofolate (MTHF)–homocysteine *S*-methyltransferase (Barak and Kemmy 1982; Horne et al., 1989) to regenerate methionine (Figure 6.2).

6.3.2 *S*-Adenosyl-L-Methionine

Available methionine for the formation of SAM directly comes from food and via the conversion of homocysteine. Methionine adenosyltransferase converts methionine to SAM, which acts as a methyl group donor for DNA methylation (Lu et al., 2001).

6.3.3 Mammalian DNA Methyltransferase Families

The methylation of DNA is a product of the activities of a family of enzymes known as DNMTs, which take up the methyl group from cofactor SAM (Jeltsch et al., 2007) and mediate the transfer to a nucleotide (Bujnicki and Radlinska, 1999), particularly cytosine. Three families of mammalian DNMTs have been found, called DNMT1, DNMT2, and DNMT3 (Hermann et al., 2004). Among these, DNMT2 has little involvement in methyl group

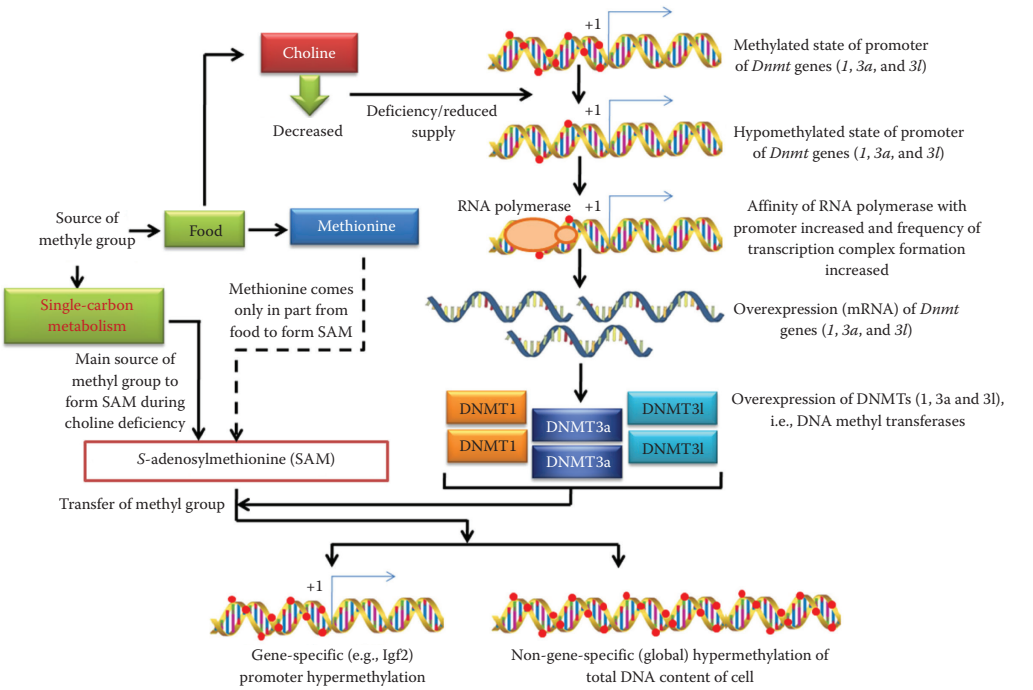

FIGURE 6.2
Methyl groups come from various sources in the human diet via methionine to be used in methylation process. This transfer of methyl groups is mediated by DNMT, while SAM is the final donor of the methyl group for the reaction.

transfer to DNA; recently, it has been found to be involved in the methylation of a cytosine residue in the anticodon loop of tRNAAsp (Goll et al., 2006).

DNMT1 is the first mammalian DNMT (Bestor et al., 1988). This is the sole mammalian DNMT identified biochemically and is responsible for copying the pattern of DNA methylation after each round of DNA replication. DNMT1 has a higher (5–30-fold) affinity to hemimethylated CpG sites (Yoder et al., 1997). This 1620-amino-acid-long protein has a C-terminal domain that more closely resembles many of the bacterial methyltransferases than those of either DNMT2 or DNMT3 (Bestor et al., 1988). The long N-terminal domain is involved in cellular targeting, protein–protein interaction, and catalytic domain regulation (Fatemi et al., 2001).

Three proteins have been recognized as member of DNMT3 family, namely, DNMT3A, DNMT3B, and DNMT3L. DNMT3L is not directly involved in methylation; rather, it is required for the activation of DNMT3A and DNMT3B. A long N-terminal is also found in DNMT3A and DNMT3B similar to that of DNMT1 and serves the same purpose. However, the isolated catalytic domain of DNMT3A and DNMT3B is enzymatically active, whereas that of DNMT1 is not (Gowher and Jeltsch, 2001; Reither et al., 2003). DNMT3A and DNMT3B are not capable of distinguishing between unmethylated or hemimethylated CpG sites. They do not show large sequence specificity except for CpG and a few flanking sequences (Gowher and Jeltsch, 2001; Handa and Jeltsch, 2005).

Although the roles of the availability of methyl group sources (choline, methionine, and 1-C metabolism mediated by MTHF), SAM, and functional DNMTs are primarily accounted for in DNA methylation, it is the availability of DNMTs specifically that governs

the DNA methylation, given that SAM is a donor of methyl groups for more than 80 biological methylation reactions, including the methylation of DNA (Choi and Mason, 2002). *Hence, as far as the regulation of genes through methylation of DNA is concerned, the prime factor might be a process that regulates the presence of active DNMTs.*

In a nutshell, DNA methylation, along with histone tail modification and noncoding RNA expression, constitutes the cellular memory that stores the transcriptional profile of the cell, which is passed to the next generation. Hence, DNA methylation is involved in the regulation of gene expression during development and in response to environmental signals. In addition, DNA methylation is implicated in genetic imprinting, inactivation of one X-chromosome, and contributing to the stability of the genome.

6.4 Altered DNA Methylation May Cause Breast Cancer While Bioactive Components of Common Food and Nutrients Act as Epigenetic Factors

There is a battery of nutrients (Table 6.1) whose presence or absence to a large extent contributes to the determination of the methylation status of DNA globally and to certain genes in particular. For example, dietary zinc deficiency leads to the global hypomethylation of DNA in rat liver (Wallwork and Duerre, 1985), while hypomethylation of *c-Ha-ras* results from deficiency of methionine and choline (Zapisek et al., 1992). Here, we discuss the bioactive compounds present in common food and nutrients and their roles in DNA methylation and breast cancer (Table 6.1).

6.4.1 Alcohol

Alcohol is known for folate antagonist activities and is found to affect one-carbon metabolism adversely (Seitz and Stickel, 2007; Jung et al., 2008). Alcohol consumption was associated with DNA methylation in postmenopausal breast tumors, suggesting that the association of alcohol and breast cancer may be related, at least in part, to altered methylation and may differ by drinking pattern (Tao et al., 2011). It has been almost a half century since alcohol was recognized as risk factor for oral cancer (Wynder and Bross, 1957). Since then, several epidemiological studies have been carried out and have now established that alcohol is a major risk factor for head and neck cancers (Yokoyama et al., 2006; Freedman et al., 2007; Brooks et al., 2009) and laryngeal cancer (Bosetti et al., 2002; Altieri et al., 2005). Chronic alcohol consumption is found to induce genomic hypomethylation in rat colon (Choi et al., 1999). An interesting study by Bonsch et al. (2006) revealed that in patients with chronic alcoholism, DNMT (*Dnmt-3B*) mRNA expression is lowered and associated with genomic DNA hypermethylation. Furthermore, the chronic alcohol exposure was found to affect the genetic imprinting of sperm by altering the mRNA level of *Dnmt* (Bielawski et al., 2002). This may constitute one example of paternal sharing in abnormal fetal growth and development. Thus, chronic exposure of alcohol is associated with cancers, both somatically and autosomally.

6.4.2 Arsenic

Arsenic exposure is associated with increased risk of cancers of the breast, skin, liver, lung, and bladder (WHO, 2001). A study of the effects of long-term arsenic exposure on human HaCaT keratinocytes revealed that arsenic causes the depletion of

TABLE 6.1

Role of Dietary Factors in DNA Methylation

Dietary Factors	Effect	References
Alcohol	Float antagonist; adversely affects C1 metabolism.	Jung et al. (2008), Seitz and Stickel (2007)
Arsenic	DNMT inhibitor.	Benbrahim-Tallaa et al. (2005), Benbrahim-Tallaa and Waalkes, (2008), Reichard et al. (2007), Pilsner et al. (2007)
Cadmium	Noncompetitive DNMT inhibitor; increases the expression of *Dnmt1*, *Dnmt3a*, and *Dnmt3b* mRNAs.	Takiguchi et al. (2003), Zhang et al. (2009), Jiang et al. (2008)
Choline	Deficiency causes hypomethylation of CpGs of *Dnmt1*; *c-Ha-ras* increases the gene-specific and global DNA methylation.	Kovacheva et al. (2007), Xu et al, (2008), Zapisek et al. (1992)
Genistein	Modulates CpG methylation; inhibits cancer development in *BRCA1*-mutated cells.	Zheng et al. (2008), King-Batoon et al. (2008), Privat et al. (2009)
Lycopene	Modulates CpG methylation.	King-Batoon et al. (2008)
Folate	Aberrant genomic and site-specific DNA methylation, associated with adenomas; decreases SAM:SAH; affects one-carbon metabolism.	Kim (2004), Van den Donk et al. (2007), Balaghi and Wagner (1993), Kim et al. (2009)
Methionine	Inhibited proliferation of MCF-7 breast cancer cells; inhibits cellular growth dependent on the p53 status of cells; absence induces apoptosis of prostate cancer and hypomethylation of *c-Ha-ras*.	Larsson et al. (2007), Kim and Park (2003), Benavides et al. (2007), Lu et al. (2002), Zapisek et al. (1992)
Nickel	Induces carcinogenesis by manipulating DNA methylation, de novo DNA hypermethylation of tumor suppressor genes and senescence genes.	Lee et al. (1995, 1998), Costa (2002), Zhou et al. (2009)
Selenium	Deficiency led to liver and colon DNA hypomethylation.	Davis et al. (2000), Zeng and Combs (2008)
Vitamin A	Induces DNA hypomethylation in rat liver; hypomethylation of genes related to retinoid signaling; may play role in gastric carcinogenesis.	Vainio and Rautalahti (1999), Rowling et al. (2002), Shutoh et al. (2005)
Vitamin B_6	Restriction decreases the supply of single-unit carbon for thymidylate and methionine; higher intakes reduce the risk of colorectal cancer; may impair de novo methionine synthesis.	Perry et al. (2007), Zhang et al. (2006), Cravo et al. (1997)
Vitamin B_{12}	Elevated level associated with prostate cancer; plasma/blood level inversely associated with breast cancer among premenopausal women; one of the determinants of DNA hypomethylation.	Johansson et al. (2008), Zhang et al. (2003, 2008), Brunaud et al. (2003)
Zinc	Global hypomethylation of DNA in liver.	Wallwork and Duerre (1985)

S-adenosylmethionine, the main cellular methyl donor, and represses the expression of the DNMT genes *Dnmt1* and *Dnmt3A* (Reichard et al., 2007). Low-dose (5 μM) long exposure was found to transform the nonmalignant prostate cancer epithelial cell line (RWPE-1) to the tumorigenic (CAsE-PE) cell line with DNA hypomethylation and *K-Ras* expression as coevents (Benbrahim-Tallaa et al., 2005). Although the mechanism of the

involvement of arsenic in DNA methylation has not been deciphered exactly, a population study indicates that the presence of adequate folate is essential for increased genomic DNA methylation (Pilsner et al., 2007).

6.4.3 Cadmium

Cadmium is a noncompetitive inhibitor of DNMTs. In vitro studies have shown that short-term cadmium exposure leads to genomic DNA hypomethylation, whereas long-term exposure results in hypermethylation of DNA (Takiguchi et al., 2003). Recent in vivo and in vitro studies demonstrate that chronic and subchronic exposures of cadmium cause the overexpression of *Dnmt1*, *Dnmt3A*, and *Dnmt3B* mRNAs (Jiang et al., 2008; Zhang et al., 2009). These findings suggest a possible underlying mechanism of cadmium carcinogenesis.

6.4.4 Choline

Choline is an essential micronutrient required for methyl group metabolism; it donates a methyl group to SAM via betaine (Varela-Moreiras et al., 1992; Lu et al., 2001). The nutritional role of choline is very well studied, and it also has been convincingly demonstrated in several studies that a deficiency of choline is associated with higher risk of breast cancer (Xu et al., 2008). However, the role in carcinogenesis and tumor progression is not well understood.

Choline deficiency results from the hypomethylation of *Dnmt1* CpG islands, consecutively increasing the expression of associated mRNAs including those mRNAs of *Dnmt3a* and *Dnmt3l*. Choline deficiency modulates fetal DNA methylation machinery in a complex fashion that includes hypomethylation of the regulatory CpGs within the *Dnmt1* gene, leading to overexpression, and the resultant increased global and gene-specific (e.g., *Igf2*) DNA methylation in fetal rat liver and brain (Kovacheva et al., 2007).

Findings from Kovacheva and coworkers (2007) suggest a tentative mechanism for DNA methylation that is modulated by choline, with the conclusion that (1) deficiency of choline does not cause global or gene-specific DNA hypomethylation, but rather hypomethylates *Dmnt1*; (2) choline deficiency is associated with overexpression of *Dnmt1*, *Dmnt3a*, and *Dmnt3l*; (3) choline is not the sole source of methyl groups for the formation of SAM, but rather the derivatives of one-carbon metabolism and homocysteine might be the sources; and (4) one-carbon metabolism is a housekeeping metabolism, and homocysteine comes only partially from diet. Thus, it seems that choline deficiency modulates global and gene-specific DNA methylation only by increasing the bioavailability of different DNMTs, while the major source of methyl groups is single-carbon metabolism derivatives (Figure 6.3).

This model of DNA methylation regulation by choline from Kovacheva and coworkers needs to be confirmed by using other animal models and in different organs. Additionally, a lot of work will be needed to ensure the fidelity of the hypothesized mechanism.

6.4.5 Genistein

Genistein is a phytoestrogen and is the main protein found in soy. It has been recognized for its antitumor properties. Genistein at very low, dietary-relevant concentrations can potentially mitigate tumorigenic processes via promoter methylation modulation of gene expression (King-Batoon et al., 2008). In a previous study, 0.5 mg of genistein was sufficient to hypermethylate the CpG islands of certain genes (e.g., glucose transporter 4, *GLUT4*)

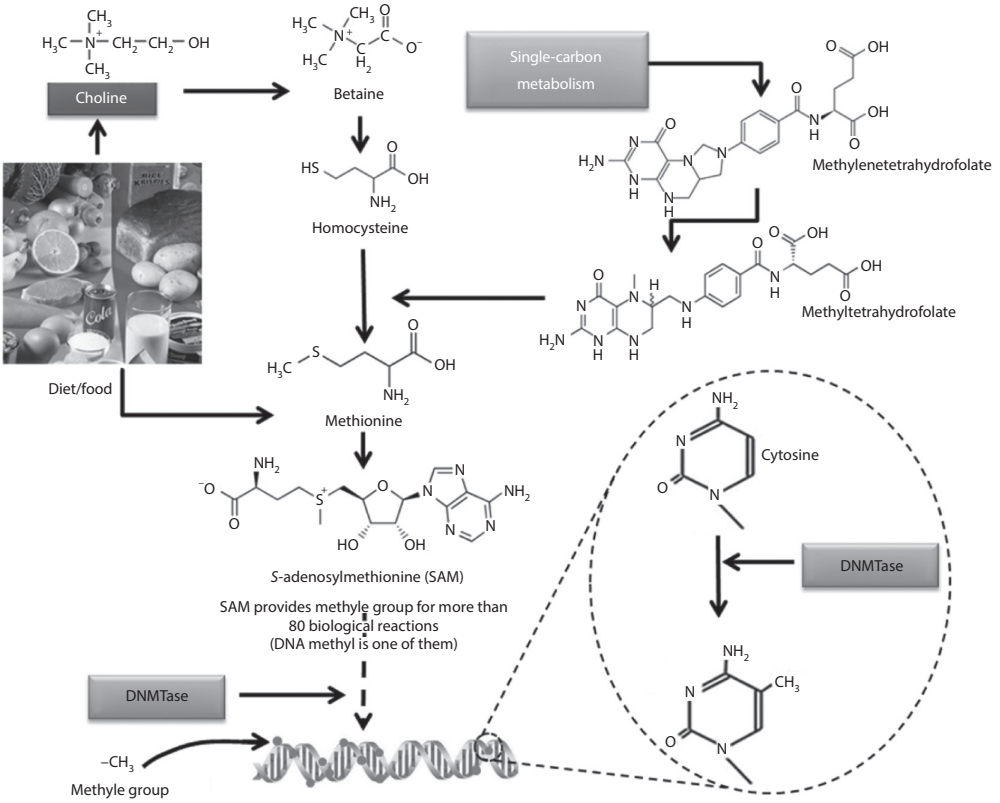

FIGURE 6.3
Deficiency of choline hypomethylates *Dnmt*s, which in turn could increase the expression of DNMTs. This could lead to an increase in gene-specific and non-gene-specific global methylation. Because choline is deficient with only a little amount of methionine from food, the sole source of methyl groups could be *single-carbon metabolism*—a housekeeping metabolism.

and lower mRNA expression (Zheng et al., 2008). Genistein may, therefore, be an efficient inhibitor of cancer development in *BRCA1* mutant breast cancer cells (Privat et al., 2009). However, additional studies are required to support the role of genistein as an antitumorigenic and anticarcinogenic agent. Also, relevant dietary concentration must be established to determine the required physiologically available concentration sufficient to exert its anticancer actions.

6.4.6 Folate

Folate is one of the main components of single-carbon metabolism and the pathway for synthesis of SAM and SAM:SAH (*S*-adenosylhomocysteine) (Balaghi and Wagner, 1993), as folate in the form of MTHF provides methyl groups to homocysteine (Figure 6.1). Folate excess and deficiency both affect the formation of SAM and the status of more than 80 different biological methylation reactions, including DNA methylation. Folate intake in adenoma patients has been shown to be inversely associated with promoter methylation in case–case comparison, whereas it is positively associated with promoter methylation in case–control comparison (van den Donk et al., 2007). A recent study on maternal folate status in hyperhomocysteinemic rats concluded that folate status influences the homeostasis

of folate-mediated one-carbon metabolism and the methyl pool, which would in turn affect placental DNA methylation by altering the methylation potential of the liver (Kim et al., 2009). Although large body of research has been performed addressing whether DNA hypomethylation is the mechanism through which folate deficiency leads to certain types of cancer (e.g., breast cancer, colorectal cancers), the details remain elusive. The evidence from animal, human, and in vitro studies suggests that the effects of folate deficiency on DNA methylation are highly complex and context dependent, since they appear to depend on cell type, target organ, and stage of transformation and are gene and site specific (Kim, 2004).

6.4.7 Methionine

Although little is known regarding the effect of dietary methionine supplementation on mammalian DNA methylation, the available data suggest that methionine supplementation can induce hypermethylation of DNA in specific genomic regions (Waterland, 2006). In vitro as well as in vivo studies have suggested that a higher dose of methionine interferes with expression of some crucial oncogenes like *p53* in MCF-7 breast cancer cells. Methionine suppresses the expression of *p53* up to 70%, hence interfering with *p53*-dependent cell growth (Kim and Park, 2003; Benavides et al., 2007). Methionine restriction induces apoptosis in prostate cancer cells via the c-Jun N-terminal kinase-mediated signaling pathway (Lu et al., 2002). Such data advocate the hypothesis that methionine restricts the growth of cancer cells through hypermethylation of oncogenes. This hypothesis has received support from a population study conducted by Larsson et al. (2007), which concludes with "higher methionine intake may reduce the risk of pancreatic cancer."

6.4.8 Nickel

Nickel is a heavy metal that has been reported for its carcinogenicity. Although nickel is not mutagenic, as confirmed by most bacterial and mammalian cell assays, it does produce a little genotoxicity because of the ability to induce small DNA damage. Lee et al. (1995) reported in the case of nickel carcinogenesis that it is DNA methylation that leads to the condensation of chromatin and does not allow the expression of certain genes. The finding from Lee et al. (1995) also explains that nickel carcinogenesis does not account for genetic alterations and DNA damage. It has been well studied and documented that nickel is involved in carcinogenesis through DNA hypermethylation of tumor suppressor genes and senescence genes (Lee et al., 1998), but the exact mechanism of involvement of nickel is still nebulous. Probably the nickel ion (Ni^{2+}) directly affects chromatin and leads to de novo DNA methylation (Costa, 2002; Zhou et al., 2009).

6.4.9 Selenium

Selenium is a micronutrient. A large amount of data suggest its possible role as an effective, naturally occurring, anticarcinogenic agent. It is found to be involved in hypomethylation of tumor suppressor genes like *p53* (Davis et al., 2000), which might be a possible mechanism through which selenium shows its anticancer property.

6.4.10 Vitamin A

Initially, it was Fujimaki (1926) who pulled the attention toward the possibility of the anticancer properties of vitamin A (Sporn and Roberts, 1983). In the past two decades, vitamin A has been extensively studied as a cancer chemopreventive agent. Retinol can activate

nuclear retinoid receptors via some of its metabolites and may prevent or delay carcinogenesis at both the initiation and promotion steps (Vainio and Rautalahti, 1999). The mechanism involved in vitamin A acting as anticancer agent has not yet been detailed, but the work of Rowling et al. (2002) provides important clues. Glycine *N*-methyltransferase (GNMT) is the cytosolic enzyme that maintains the ratio of SAM and the SAM:SAH. Hence, inappropriate activation of GNMT may lead to the loss of methyl groups vital for many SAM-dependent transmethylation reactions, for example, DNA methylation. Vitamin A and its retinoic acid derivatives are able to induce GNMT. This observation suggests that hypomethylation or no further methylation of DNA because of inappropriate activation of GNMT in the presence of adequate vitamin A, and vice versa, may be the possible mechanism of methylation of DNA by vitamin A. This possibility got support by the finding that gastric carcinogenesis involves transcriptional inactivation by aberrant DNA methylation of genes related to retinoid signaling (Shutoh et al., 2005).

6.4.11 Vitamin B_6

Cravo and coworkers (1997) predicted that restriction of vitamin B_6 may interfere with DNA methylation status by impairing de novo methionine synthesis. Actually vitamin B_6 restriction decreases the activity and stability of serine hydroxymethyltransferase and may impair the supply of single-unit carbon for homocysteine remethylation for methionine formation (Perry et al., 1997) and probably affect the DNA methylation through restricting methionine availability for the formation of SAM, the ultimate donor of methyl groups to DNA via DNMT enzymes.

6.4.12 Vitamin B_{12}

Vitamin B_{12} is one of the determinants of one-carbon metabolism. Elevated circulating levels of vitamin B_{12} may be associated with risk for advanced stage prostate cancer (Johansson et al., 2008). Vitamin B_{12} and low methionine synthase activity also have been reported as the two determinants of DNA hypomethylation (Brunaud et al., 2003). Zhang et al. (2003) reported that plasma vitamin B_{12} levels were inversely associated with breast cancer risk among premenopausal women but not among postmenopausal women. Recently, it was found that treatment with vitamin B_{12} does not have any significant effect on the risk of breast cancer (Zhang et al., 2008).

6.5 Methylation of Genes Associated with Breast Cancer

Several recent studies have shown that DNA methylation is the most well-researched epigenetic mark that differs between normal cells and cancer cells in humans. In normal cells, CpG islands preceding gene promoters are generally unmethylated, while other individual CpG dinucleotides throughout the genome tend to be methylated. However, in cancer cells, CpG islands preceding tumor suppressor gene promoters are often hypermethylated, while CpG methylation of oncogene promoter regions and parasitic repeat sequences is often decreased. Hypermethylation of gene promoters can result in silencing of those genes. This type of epigenetic mutation is dangerous when genes that regulate the cell cycle are silenced, allowing cells to grow and reproduce uncontrollably, leading

to tumorigenesis. Hundreds of hypermethylated genes have been described in breast cancer. Several genes involved in cell cycle regulation and apoptosis (*CCND2, CDKN2A/p16, RASSF1A*), DNA damage response (*BRCA1*), cell adhesion (*CDH1*), and cell signaling (*ER, RAR*β 2) have been reported to undergo promoter hypermethylation in breast carcinoma. DNA methylation has been reported to play diverse functions like genome imprinting, normal development, and repression of gene transcription.

6.5.1 *BRCA1* Gene Methylation and BRCA1

BRCA1 is located on chromosome 17q21 (Wakefield and Alsop, 2006). Its protein product is 1863 amino acid (NCBI amino acid sequence) and known for its association with tumor suppressor activity in breast and ovarian cancers. There is abundant evidence that BRCA1 plays a number of roles in maintenance of genome integrity. It is an important participant in the response to DNA damage, operating in both the repair of double-strand breaks (DSB) and in enaction of certain cell cycle checkpoint responses. There is considerable circumstantial evidence suggesting that at least part of its repair and/or checkpoint response function is linked to its tumor-suppressing activity (Boulton, 2006). Inherited mutations in *BRCA1* gene account for one-half of inherited breast carcinomas.

6.5.1.1 Breast Cancer and BRCA1 Hypermethylation

Southern analysis of the *BRCA1* promoter revealed methylation in 11% sporadic breast cancer cases (Catteau et al., 1999). Another study with 19% of breast cancer carcinoma demonstrated that *BRCA1* promoter is methylated in 13% of unselected primary breast tumors (Esteller et al., 2000). Loss of heterozygosity (LOH) for *BRCA1*, in familial breast cancer. Surprising fact is even in the presence of heterozygosity, and homozygosity of *BRCA1* occurrences of breast cancer had been reported, in the case of sporadic breast cancer cases. As expected, the normal tissues do not have the *BRCA1* gene with hypermethylated status. A study from Chinese population revealed that the frequency of *BRCA1* promoter hypermethylation is 32% (Jing et al., 2008). Hypermethylation of *BRCA1* is very rare to found outside breast cancer except ovarian cancers (Dobrovic and Simpfendorfer, 1997). Hence, it was strongly advocated that *BRCA1* promoter hypermethylation plays a significant role in breast cancer pathogenesis (Bianco et al., 2000).

6.5.1.2 Other Gene Hypermethylation in Breast Cancer

Lehmann et al. (2002) studied methylation status of some growth regulatory genes (e.g., *p16, RASSF1A, cyclinD2, 14-3-3*σ) in breast cancer patients in a German population. It was reported that promoter methylation is an early and frequent event in breast cancer development but displays great quantitative and gene-specific differences and changes in a gene-specific manner during tumor progression.

RAS association domain family protein 1A (*RASSF1A*), *adenomatous polyposis coli* (*APC*), and *death-associated protein* (*DAP*) *kinase* are three normally unmethylated and biologically significant genes. These three genes were studied by sensitive methylation-specific PCR in 34 breast tumors and paired preoperative serum DNAs. Collectively hypermethylation in one or more genes was found in 94% samples. *APC*, *RASSF1A*, and *DAP kinase* found individually as 47%, 65%, and 50% of samples, respectively (Dulaimi et al., 2004).

To evaluate whether hypermethylation identifies breast cancer with distinctive clinical and pathological features, Li et al. (2006) studied *RAR*β*2*, *CDH1*, *ER*, *BRCA1*, *CCND2*,

p16, and *TWIST* genes in 193 breast carcinomas. It was found that methylation frequencies ranged from 11% for *CCND2* to 84% for *ER*. They concluded that gene methylation may be linked to various pathological features of breast cancer. Seniski et al. (2009) narrated that *ADAM33* gene promoter methylation may be a useful molecular marker for differentiating invasive lobular carcinoma and invasive ductal carcinoma.

E-cadherin gene located on chromosome 16 encodes a cell surface adhesion protein. It has an important role to play in maintaining hemophilic cell–cell adhesion in epithelial tissues (IIyas and Tomlinson, 1997). Epigenetic silencing of E-cadherin gene by 5 CpG methylation has been reported to occur in some human breast cancer cell lines. Nass et al. (2000) demonstrated that hypermethylation of the E-cadherin CpG islands was evident in 30% ductal carcinomas in situ and increased significantly to nearly 60% of metastatic lesions suggestive of a role of methylation in tumor progression.

6.6 Sources of DNA for the Methylation Studies in Cancer Patients

Almost one decade earlier, the presence of abnormally high DNA concentration in the serum of patients of various malignant diseases was reported. Numerous studies showed tumor-specific alteration in cell-free DNA recovered from blood, plasma, or serum of patients. The nucleic acid markers described in plasma and serum include oncogene mutation, microsatellite alteration, gene rearrangements, and epigenetic alteration, such as aberrant promoter hypermethylation (Chen et al., 1999). Methylation status of certain genes (*RASSF1A* and *APC*) found in cell-free DNA from sera of breast cancer patients had been reported as more powerful prognostic marker than other prognostic parameters (Müller et al., 2003). Several other studies showed that the cell-free DNA isolated from sera of cancer patients can be used to study the methylation status of cancer-related genes (Widschwendter et al., 2004; Hu et al., 2006; Ikoma et al., 2006).

A good quality of cell-free DNA can be isolated from saliva. DNA from saliva had been used for the study of methylation of cancer-related genes for head and neck cancers as well as oral cancer (Viet and Schmidt, 2008). Saliva and urine were used as the source for studying methylation of cancer-related genes in breast cancer patients. But it was only the urine, not the saliva, that showed the significant alteration in the methylation of *BRCA1* gene (Hansmann et al., 2012). However, Bryan et al. (2013) had reported a differential methylation pattern in breast cancer genes assayed from saliva.

6.7 DNA Methylation–Based Biomarkers in Serum and Other Body Fluids of Breast Cancer Patients

Although DNA methylation analysis is a rapidly developing field, a reproducible epigenetic blood (serum) and other body fluids–based assay for diagnosis and follow-up of breast cancer has yet to be successfully developed into a routine clinical test. There is a tug nowadays to find and establish DNA methylation–based biomarkers in urine and saliva in addition to blood and serum (Viet and Schmidt, 2008; Hansmann et al., 2012; Bryan et al., 2013). With a little success with saliva and urine, blood and serum have been found more promising

in terms of developing such markers. The use of such body fluids–based methylated DNA biomarkers is based on the fact that these fluids are readily available for molecular diagnosis. As far as the DNA methylation–based biomarkers in serum are concerned, the list of hypermethylated genes in breast cancer is heterogeneous, and no single gene is methylated in all breast cancer types. There is increasing evidence that a panel of epigenetic markers is essential to achieve a higher sensitivity and specificity in breast cancer detection.

6.7.1 Effect of Nutrients and Lifestyle on DNA Methylation–Based Biomarkers

The reported percentages of methylation in biomarkers are highly variable. This can be partly explained by the different sensitivities and the different intra-/interassay coefficients of variability of the analysis methods (De Voorde et al., 2012). Simultaneously, we know nutrients play a great role in maintaining and alteration of the DNA methylation pattern. Hence, they also can affect the DNA methylation–based biomarkers (Kim et al., 2010; Dominguez-Salas et al., 2013). These two are the main hurdles in developing efficient DNA methylation–based biomarkers in body fluids. So, while developing such biomarkers, one have to keep these issues in mind. Hence, developing such marker is still challenging while that is interesting as well as promising.

6.8 Conclusions and Future Perspective

Numerous scholarly investigations have demonstrated that DNA methylation is very dynamic and it is the most well-characterized epigenetic mark. DNA methylation, along with histone tail modifications, gives stability to the genome and is involved in cell differentiations and X-chromosome inactivation. DNA methylation (hypo- and hyper-) is a normal cellular event that determines the expression of genes according to the environment, at the transcriptional level. Hypo- and/or hypermethylation of DNA can initiate a cascade of abnormal events, which may be transcriptional activation and inactivation of oncogenes and tumor suppressor genes, respectively. As an outcome, cancer may occur. The source of the methyl groups (choline, homocysteine, and single-carbon metabolism), SAM, and families of DNMTs are the three main determinants of the DNA methylation status. The main target of DNMTs is the CpG island of promoters of different genes. Although SAM is the ultimate donor of methyl groups to cytosine residues in CpG islands, the reaction is mediated by DNMTs. However, SAM donates methyl groups to more than 80 biological reactions. Thus, the limiting factor for DNA methylation reactions is the presence of active DNMTs. Hence, the key factor for regulation of genes through methylation of DNA is something that regulates the presence of active DNMTs in appropriate concentrations. Further studies are needed to identify such factors.

There are large numbers of nutrients and food components that alter intracellular molecular environments, thus affecting DNA methylation machinery and mechanisms and, in turn, the methylation status of DNA. Several nutrients that have been studied with respect to the status of DNA methylation in cancers include alcohol, arsenic, cadmium, choline, folate, methionine, nickel, selenium, and vitamins like A, B_6, and B_{12}. Alcohol, arsenic, and cadmium interfere with DNA methylation processes and cause global hypomethylation. Choline is one of the methyl group sources that come from food. Choline deficiency causes gene-specific hypomethylation, for example, CpGs of *Dnmt1* and *c-Ha-ras*. A study in fetal rat liver and brain suggests that the modulation of DNA methylation machinery is performed

by choline, and as a consequence of hypomethylation of *Dnmt1*, mRNA expression of *Dnmt1*, *Dnmt3a*, and *Dnmt3l* increases. There are global and gene-specific hypermethylation. Although this study does not give a complete picture of the complex mechanisms of modulation of DNA methylation, it supports some putative interpretations such as (1) choline deficiency does not cause hypomethylation; (2) choline is not the sole source of methyl groups; rather, it is a single-carbon metabolism because homocysteine is contributed in part from food; and (3) choline somehow modulates DNA methylation by stabilizing DNMTs in the intracellular environment. All of these assertions need to be carefully experimentally evaluated in order to decipher the exact mechanisms of DNA methylation. Both methionine and nickel have been demonstrated to induce gene-specific hypermethylation. It is assumed that methionine hypermethylates oncogenes, whereas nickel is involved in hypermethylation of tumor suppressor genes, both preventing and causing cancer, respectively. Selenium is anticarcinogenic via hypomethylation of tumor suppressor genes, thus altering expression of these genes. It is yet unclear how vitamins are involved in DNA methylation and, consequently, breast cancer. However, vitamins A, B_6, and B_{12} have been found to be involved in transcriptional inactivation by aberrant DNA methylation of genes related to retinoid signaling and impairing de novo methionine synthesis.

Although extensive research has been performed in the field of epigenetics and breast cancers where nutrients have been considered as environmental factors, there remain several challenges and questions to be answered. Among many crucial questions is determining the key factor(s) for regulation of genes through methylation of DNA through the regulation of the presence of active DNMT in appropriate concentrations.

References

Altieri A, Garavello W, Bosetti C et al. (2005) Alcohol consumption and risk of laryngeal cancer. *Oral Oncol* 41: 956–965.

Balaghi M, Wagner C (1993) DNA methylation in folate deficiency: Use of CpG methylase. *Biochem Biophys Res Commun* 193: 1184–1190.

Barak AJ, Kemmy RJ (1982) Methotrexate effects on hepatic betaine levels in choline-supplemented and choline-deficient rats. *Drug Nutr Interact* 1: 275–278.

Benavides MA, Oelschlager DK, Zhang HG et al. (2007) Methionine inhibits cellular growth dependent on the p53 status of cells. *Am J Surg* 193: 274–283.

Benbrahim-Tallaa L, Waalkes MP (2008) Inorganic arsenic and human prostate cancer. *Environ Health Perspect* 116: 158–164.

Benbrahim-Tallaa L, Waterland RA, Styblo M et al. (2005) Molecular events associated with arsenic-induced malignant transformation of human prostatic epithelial cells: Aberrant genomic DNA methylation and K-ras oncogene activation. *Toxicol Appl Pharmacol* 206: 288–298.

Bernardino-Sgherri J, Flagiello D, Dutrillaux B (2002) Overall DNA methylation and chromatin structure of normal and abnormal X chromosomes. *Cytogenet Genome Res* 99: 85–91.

Bestor TH, Laudano A, Mttialiano R et al. (1988) Cloning and sequencing of a cDNA encoding DNA methyl transferase of mouse cells: The carboxyl-terminal domain of the mammalian enzyme is related to the bacterial methyl transferase. *J Mol Biol* 203: 971–983.

Bianco T, Chenevix-Trench G, Walsh DC, Cooper JE, Dobrovic A (2000) Tumour-specific distribution of BRCA1 promoter region methylation supports a pathogenetic role in breast and ovarian cancer. *Carcinogenesis* 21(2): 147–151.

Bielawski DM, Zaher FM, Svinarich DM et al. (2002) Paternal alcohol exposure affects sperm cytosine methyltransferase messenger RNA levels. *Alcohol Clin Exp Res* 26: 347–351.

Bird AP (1986) CpG-rich islands and the function of DNA methylation. *Nature* 321: 209–213.
Bonsch D, Lenz B, Fiszer R et al. (2006) Lowered DNA methyltransferase (DNMT-3b) mRNA expression is associated with genomic DNA hypermethylation in patients with chronic alcoholism. *J Neural Transm* 113: 1299–1304.
Bosetti C, Gallus S, Franceschi S et al. (2002) Cancer of the larynx in non-smoking alcohol drinkers and in non-drinking tobacco smokers. *Br J Cancer* 87: 516–518.
Boulton SJ (2006) Cellular functions of the BRCA tumour-suppressor proteins. *Biochem Soc Trans* 34(Pt 5): 633–645.
Brooks PJ, Enoch MA, Goldman D et al. (2009) The alcohol flushing response: An unrecognized risk factor for esophageal cancer from alcohol consumption. *PLoS Med* 6: 258–263.
Brunaud L, Alberto JM, Ayav A et al. (2003) Effects of vitamin B12 and folate deficiencies on DNA methylation and carcinogenesis in rat. *Clin Chem Lab Med* 41: 1012–1019.
Bryan AD, Magnan RE, Hooper AE, Harlaar N, Hutchison KE. (2013) Physical activity and differential methylation of breast cancer genes assayed from saliva: A preliminary investigation. *Ann Behav Med* 45(1): 89–98.
Bujnicki MJ, Radlinska M (1999) Molecular evolution of DNA-(cytosine-N4) methyltransferases: Evidence for their polyphyletic. *Nucleic Acids Res* 27: 4501–4509.
Catteau A, Harris WH, Xu CF, Solomon E (1999) Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: Correlation with disease characteristics. *Oncogene* 18: 1957–1965.
Chanda S, Dasgupta UB, Guhamazumder D et al. (2006) DNA Hypermethylation of promoter of gene *p53* and *p16* in arsenic-exposed people with and without malignancy. *Toxicol Sci* 89: 431–437.
Chen X, Bonnefoi H, Diebold-Berger S, Lyautey J, Lederrey C, Faltin-Traub E, Stroun M, Anker P (1999) Detecting tumor-related alterations in plasma or serum DNA of patients diagnosed with breast cancer. *Clin Cancer Res* 5(9): 2297–2303.
Choi SW, Mason JB (2002) Folate status: Effects on pathways of colorectal carcinogenesis. *J Nutr* 132: 2413S–2418S.
Choi SW, Stickel F, Baik HW et al. (1999) Chronic alcohol consumption induces genomic but not *p53*-specific DNA hypomethylation in rat colon. *J Nutr* 129: 1945–1950.
Costa M (2002) Molecular mechanisms of nickel carcinogenesis. *Biol Chem* 383: 961–967.
Cravo M, Gloria L, Camilo ME et al. (1997) DNA methylation and subclinical vitamin deficiency of folate, pyridoxal-Phosphate and vitamin B12 in chronic alcoholic. *Clin Nutr* 16: 29–35.
Davis CD, Uthus EO, Finley JW (2000) 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.
Dobrovic A, Simpfendorfer D (1997) Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res* 57: 3347–3350.
Dominguez-Salas P, Moore SE, Cole D, da Costa KA, Cox SE, Dyer RA, Fulford AJ et al. (2013) DNA methylation potential: Dietary intake and blood concentrations of one-carbon metabolites and cofactors in rural African women. *Am J Clin Nutr* 97(6): 1217–1227.
Dulaimi E, Hillinck J, Ibanez de Caceres I, Al-Saleem T, Cairns P (2004) Tumor suppressor gene promoter hypermethylation in serum of breast cancer patients. *Clin Cancer Res* 10(18 Pt 1): 6189–6193.
Ehrlich M (2002) DNA methylation in cancer: Too much, but also too little. *Oncogene* 21: 5400–5413.
Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG (2000) Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J Natl Cancer Inst* 92: 564–569.
Fatemi M, Hermann A, Pradhan S et al. (2001) The activity of the murine DNA methyltransferase Dnmt1 is controlled by interaction of the catalytic domain with the N-terminal part of the enzyme leading to an allosteric activation of the enzyme after binding to methylated DNA. *J Mol Biol* 309: 1189–1199.
Freedman ND, Schatzkin A, Leitzmann MF et al. (2007) Alcohol and head and neck cancer risk in a prospective study. *Br J Cancer* 96: 1469–1474.

Fujimaki Y (1926) Formation of gastric carcinoma in albino rats fed on deficient diets. *J Cancer Res* 10: 469.

Goll MG, Kirpekar F, Maggert KA et al. (2006) Methylation of t-RNA-Asp by the DNA methyltransferase homolog Dnmt2. *Science* 311: 395–398.

Gowher H, Jeltsch A (2001) Enzymatic properties of recombinant Dnmt3a DNA methyltransferase from mouse: The enzyme modifies DNA in a non-processive manner and also methylates non-CpG sites. *J Mol Biol* 309: 1201–1208.

Handa V, Jeltsch A (2005) Profound flanking sequence preference of Dnmt3a and Dnmt3b mammalian DNA methyltransferases shape the human epigenome. *J Mol Biol* 348: 1103–1112.

Hansmann T, Pliushch G, Leubner M, Kroll P, Endt D, Gehrig A, Preisler-Adams S, Wieacker P, Haaf T (2012) Constitutive promoter methylation of BRCA1 and RAD51C in patients with familial ovarian cancer and early-onset sporadic breast cancer. *Hum Mol Genet* 21(21): 4669–4679.

Hayslip J, Montero A (2006) Tumor suppressor gene methylation in follicular lymphoma: A comprehensive review. *Mol Cancer* 5: 44.

Hermann A, Gowher H, Jeltsch A (2004) Biochemistry and biology of mammalian DNA methyltransferases. *Cell Mol Life Sci* 61: 2571–2587.

Horne WD, Cook RJ, Wagner C (1989) Effect of dietary methyl group deficiency on folate metabolism in rats. *J Nutr* 119: 618–621.

Hotchkiss RD (1948) The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. *J Biol Chem* 175: 315–332.

Hu S, Ewertz M, Tufano RP, Brait M, Carvalho AL, Liu D, Tufaro AP et al. (2006) Detection of serum deoxyribonucleic acid methylation markers: A novel diagnostic tool for thyroid cancer. *J Clin Endocrinol Metab* 91(1): 98–104.

Ikoma H, Ichikawa D, Koike H, Ikoma D, Tani N, Okamoto K, Ochiai T, Ueda Y, Otsuji E, Yamagishi H. (2006) Correlation between serum DNA methylation and prognosis in gastric cancer patients. *Anticancer Res* 26(3B): 2313–2316.

Ilyas M, Tomlinson IP (1997) The interactions of APC, E-cadherin and beta-catenin in tumour development and progression. *J Pathol* 182:128–137.

Institute of Medicine and National Academy of Sciences USA. *Dietary Reference Intakes for Folate, Thiamin, Riboflavin, Niacin, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press, 1998.

Jeltsch A, Jurkowska RJ, Jurkowski TP (2007) Application of DNA methyltransferases in targeted DNA methylation. *Appl Microbiol Biotechnol* 75: 1233–1240.

Jiang G, Xu L, Song S et al. (2008) Effects of long-term low-dose cadmium exposure on genomic DNA methylation in human embryo lung fibroblast cells. *Toxicology* 244: 49–55.

Jing F, Jun L, Yong Z, Wang Y, Fei X, Zhang J, Hu L. (2008) Multigene methylation in serum of sporadic Chinese female breast cancer patients as a prognostic biomarker. *Oncology* 75(1–2): 60–66.

Johansson M, Appleby PN, Allen NE et al. (2008) Circulating concentrations of folate and vitamin B12 in relation to prostate cancer risk: Results from the European prospective investigation into cancer and nutrition study. *Cancer Epidemiol Biomarkers Prev* 17: 279–285.

Jung AY, Poole EM, Bigler J et al. (2008) DNA methyltransferase and alcohol dehydrogenase: Gene-nutrient interactions in relation to risk of colorectal polyps. *Cancer Epidemiol Biomarkers Prev* 17: 330–338.

Keshet I, Lieman-Hurwitz J, Cedar H (1986) DNA methylation affects the formation of active chromatin. *Cell* 44: 535–543.

Kim HH, Park CS (2003) Methionine cytotoxicity in the human breast cancer cell line MCF-7, in vitro cell. *Dev Biol Anim* 39: 117–119.

Kim JM, Hong K, Lee JH et al. (2009) Effect of folate deficiency on placental DNA methylation in hyperhomocysteinemic rats. *J Nutr Biochem* 20: 172–176.

Kim M, Long TI, Arakawa K, Wang R, Yu MC, Laird PW (2010) DNA methylation as a biomarker for cardiovascular disease risk. *PLoS One* 5(3): e9692.

Kim Y (2004) Folate and DNA methylation: A mechanistic link between folate deficiency and colorectal cancer? *Cancer Epidemiol Biomarkers Prev* 13: 511–519.

King-Batoon A, Leszczynska JM, Klein CB (2008) Modulation of gene methylation by genistein or lycopene in breast cancer cells. *Environ Mol Mutagen* 49: 36–45.

Kovacheva VP, Mellott TJ, Davison JM et al. (2007) Gestational choline deficiency causes global and Igf2 gene- DNA hypermethylation by upregulation. *J Biol Chem* 282: 31777–31788.

Larsson SC, Giovannucci E, Wolk A (2007) Methionine and vitamin B_6 intake and risk of pancreatic cancer: A prospective study of Swedish women and men. *Gastroenterology* 132: 113–118.

Lee YW, Broday L, Costa M (1998) Effects of nickel on DNA methyltransferase activity and genomic DNA methylation levels. *Mutat Res* 415: 213–218.

Lee YW, Klein CB, Kargacin B et al. (1995) Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: A new model for epigenetic carcinogens. *Mol Cell Biol* 15: 2547–2557.

Lehmann U, Langer F, Feist H et al. (2002) Quantitative assessment of promoter hypermethylation during breast cancer development. *Am J Pathol* 160: 605–612.

Li S, Rong M, Iacopetta B (2006) DNA hypermethylation in breast cancer and its association with clinicopathological features. *Cancer Lett* 237(2): 272–280.

Lorincz MC, Dickerson DR, Schmitt M et al. (2004) Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. *Nat Struct Mol Biol* 11: 1068–1075.

Lu S, Hoestje SM, Choo EM et al. (2002) Methionine restriction induces apoptosis of prostate cancer cells via the c-Jun N-terminal kinase-mediated signaling pathway. *Cancer Lett* 179: 51–58.

Lu SC, Alvarez L, Huang ZZ et al. (2001) Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc Natl Acad Sci USA* 98: 5560–5565.

Moore LE, Pfeiffer RM, Poscablo C et al. (2008) Genomic DNA hypomethylation as a biomarker for bladder cancer susceptibility in the Spanish bladder cancer study: A case—Control study. *Lancet Oncol* 9: 359–366.

Müller HM, Widschwendter A, Fiegl H, Ivarsson L, Goebel G, Perkmann E, Marth C, Widschwendter M (2003) DNA methylation in serum of breast cancer patients: An independent prognostic marker. *Cancer Res* 63(22): 7641–7645.

Nass SJ, Herman JG, Gabrielson E, Iversen PW, Parl FF, Davidson NE, Graff JR (2000) Aberrant methylation of the estrogen receptor and E-cadherin 5′ CpG islands increases with malignant progression in human breast cancer. *Cancer Res* 60: 4346–4348.

Perry C, Yu S, Chen J et al. (2007) Effect of vitamin B6 availability on serine hydroxymethyltransferase in MCF-7 cells. *Arch Biochem Biophys* 462: 21–27.

Pilsner JR, Liu X, Ahsan H et al. (2007) Genomic methylation of peripheral blood leukocyte DNA: Influences of arsenic and folate in Bangladeshi adults. *Am J Clin Nutr* 86: 1179–1186.

Privat M, Aubel C, Arnould S et al. (2009) Breast cancer cell response to genistein is conditioned by *BRCA1* mutations. *Biochem Biophys Res Commun* 379: 785–789.

Reichard JF, Schnekenburger M, Puga A (2007) Long term low-dose arsenic exposure induces loss of DNA methylation. *Biochem Biophys Res Commun* 352: 188–192.

Reither S, Li F, Gowher H et al. (2003) Catalytic mechanism of DNA-(cytosine-C5)-methyltransferases revisited: Covalent intermediate formation is not essential for methyl group transfer by the murine Dnmt3a enzyme. *J Mol Biol* 329: 675–684.

Richardson B, Yung R (2002) Role of DNA methylation in the regulation of cell function. *J Lab Clin Med* 134: 333–340.

Roman-Gomez J, Jimenez-Velasco A, Agirre X et al. (2005) Promoter hypomethylation of the LINE-1 retrotransposable elements activates sense/antisense transcription and marks the progression of chronic myeloid leukemia. *Oncogene* 24: 7213–7223.

Rowling MJ, McMullen MH, Schalinske KL (2002) Vitamin A and its derivatives induce hepatic glycine *N*-methyltransferase and hypomethylation of DNA in rats. *J Nutr* 132: 365–369.

Saxonov S, Berg P, Brutlag DL (2006) A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc Natl Acad Sci* 103: 1412–1417.

Seitz HK, Stickel F (2007) Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 7: 599–612.

Seniski GG, Camargo AA, Ierardi DF, Ramos EA, Grochoski M, Ribeiro ES, Cavalli IJ et al. (2009) ADAM33 gene silencing by promoter hypermethylation as a molecular marker in breast invasive lobular carcinoma. *BMC Cancer* 9: 80. doi: 10.1186/1471-2407-9-80.

Shutoh M, Oue N, Aung PP et al. (2005) DNA methylation of genes linked with retinoid signaling in gastric carcinoma: Expression of the retinoid acid receptor beta, cellular retinol-binding protein 1, and tazarotene-induced gene 1 genes is associated with DNA methylation. *Cancer* 104: 1609–1619.

Sporn MB, Roberts AB (1983) Role of retinoids in differentiation and carcinogenesis. *Cancer Res* 43: 3034–3040.

Takiguchi M, Achanzar WE, Qu W et al. (2003) Effect of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp Cell Res* 286: 355–365.

Tao MH, Marian, C, Shields PG et al. (2011) Alcohol consumption in relation to aberrant DNA methylation in breast tumors. *Alcohol* 45(7): 689–699.

Vainio H, Rautalahti M (1999) An international evaluation of the cancer preventive potential of vitamin A. *Cancer Epidemiol Biomarkers Prev* 8: 107–109.

Van De Voorde L, Speeckaert R, Van Gestel D, Bracke M, De Neve W, Delanghe J, Speeckaert M (2012) DNA methylation-based biomarkers in serum of patients with breast cancer. *Mutat Res* 751(2): 304–325.

van den Donk M, van Engeland M, Pellis L et al. (2007) Dietary folate intake in combination with *MTHFR* C677T genotype and promoter methylation of tumor suppressor and DNA repair genes in sporadic colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 16: 1562–1566.

Van Speybroeck L (2002) From epigenesis to epigenetics: The case of C. H. Waddington. *Ann N Y Acad Sci* 981: 61–81.

Varela-Moreiras G, Selhub J, da Costa K et al. (1992) Effect of chronic choline deficiency in rats on liver folate content and distribution. *J Nutr Biochem* 3: 519–522.

Viet CT, Schmidt BL (2008) Methylation array analysis of preoperative and postoperative saliva DNA in oral cancer patients. *Cancer Epidemiol Biomarkers Prev* 17(12): 3603–3611.

Waddington CH (1942) The epigenotype. *Endeavour* 1: 18–20.

Wakefield MJ, Alsop AE (2006) Assignment of BReast Cancer Associated 1 (BRCA1) to tammar wallaby (Macropus eugenii) chromosome 2q3 by in situ hybridization. *Cytogenet Genome Res* 112(1–2): 180C.

Wallwork JC, Duerre JA (1985) Effect of zinc deficiency on methionine metabolism, methylation reactions and protein synthesis in isolated perfused rat liver. *J Nutr* 115: 252–262.

Waterland RA (2006) Assessing the effects of high methionine intake on DNA methylation. *J Nutr* 136: 1706S–1710S.

Whitman SP, Hackanson B, Liyanarachchi S et al. (2008) DNA hypermethylation and epigenetic silencing of the tumor suppressor gene, *SLC5A8*, in acute myeloid leukemia with the *MLL* partial tandem duplication. *Blood* 112: 2013–2016.

WHO. *Arsenic and Arsenic Compounds*. 2nd edn. Geneva, Switzerland: Inter-Organization Programme for the Sound Management of Chemicals, World Health Organization 2001.

Widschwendter M, Jiang G, Woods C, Müller HM, Fiegl H, Goebel G, Marth C et al. (2004) DNA hypomethylation and ovarian cancer biology. *Cancer Res* 64(13): 4472–4480.

Wynder EL, Bross IJ (1957) Aetiological factors in mouth cancer; an approach to its prevention. *Br Med J* 18: 1137–1143.

Xu X, Gammon MD, Zeisel SH et al. (2008) Choline metabolism and risk of breast cancer in a population-based study. *FASEB J* 22: 2045–2052.

Yoder JA, Soman N, Verdin GV et al. (1997) DNA methyltransferase in mouse tissues and cells: Studied with a mechanism based probe. *J Mol Biol* 270: 385–395.

Yokoyama A, Omori T, Yokoyama T et al. (2006) Risk of squamous cell carcinoma of the upper aerodigestive tract in cancer-free alcoholic Japanese men: An endoscopic follow-up study. *Cancer Epidemiol Biomarkers Prev* 15: 2209–2215.

Zapisek WF, Cronin GM, Lyn-Cook BD et al. (1992) The onset of oncogene hypomethylation in the livers of rats fed methyl-deficient, amino acid-defined diets. *Carcinogenesis* 13: 1869–1872.

Zeng H, Combs Jr GF (2008) Selenium as an anticancer nutrient: Roles in cell proliferation and tumor cell invasion. *J Nutr Biochem* 19: 1–7.

Zhang J, Fu Y, Li J et al. (2009) Effects of subchronic cadmium poisoning on DNA methylation in hens. *Environ Toxicol Pharmacol* 27: 345–349.

Zhang SM, Cook NR, Albert CM et al. (2008) Effect of combined folic acid, vitamin B_6, and vitamin B_{12} on cancer risk in women. *JAMA* 300: 2012–2021.

Zhang SM, Moore SC, Lin J et al. (2006) Folate, Vitamin B6, multivitamin supplements, and colorectal cancer risk in women. *Am J Epidemiol* 163: 108–115.

Zhang SM, Willett WC, Selhub J et al. (2003) Plasma folate, vitamin B_6, vitamin B_{12}, homocysteine, and risk of breast cancer. *J Natl Cancer Inst* 95: 373–380.

Zheng S, Wang Z, Pan Y (2008) Oral intake of genistein changes DNA methylation at the promoters of rat colon genes. *FASEB J* 22: 885–886.

Zhou X, Li Q, Arita A et al. (2009) Effects of nickel, chromate, and arsenite on histone 3 lysine methylation. *Toxicol Appl Pharmacol* 236: 78–84.