Folate: Could We Live Without It? A Novel Epigenetic Connection

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Abstract

Folate is an essential nutrient obtained through diet and supplements. The term folate is used interchangeably with folic acid, its synthetic form. Folate is metabolized in the one-carbon pathway, and its metabolites are used for a number of biological processes. Metabolites of folate are used in nucleotide synthesis and

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methylation. In fact, the one-carbon pathway produces the major methyl donor used in methylation, *S*-adenosylmethionine (SAM). Folate and DNA methylation are, therefore, closely entwined. Folate deficiency is associated with a number of diseases including congenital disabilities. In the last couple of decades, folate or folic acid supplementation is highly promoted in pregnancy because folate deficiency leads to neural tube defects with a wide range of consequences in children. Folate deficiency is also associated with gastric and colorectal cancers, cardiovascular disease, and liver disease. Alterations in global DNA methylation and disease-specific gene methylation patterns are implicated in the development and progression of these diseases. Folate is an important nutrient to understand the epigenetic regulation of disease.

Keywords

Folate · DNA methylation · Epigenetics · Diabetes · Cancer · Folic acid · DNA methyltransferase · DNMT · Cardiovascular disease · Methyl donor

List of Abbreviations

CDC	Centers for Disease Control
CIMP	CpG island methylator phenotype
CpG Island	Cytosine–phosphate–Guanine Island
CVD	Cardiovascular disease
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
dTMP	Deoxythymidine monophosphate
dTTP	Deoxythymidine triphosphate
dUMP	Deoxyuridine monophosphate
dUTP	Deoxyuridine triphosphate
5-MTHF	5-Methyltetrahydrofolate
MTHFR	Methylenetetrahydrofolate reductase
PABA	Para-aminobenzoic acid
SAM	*S*-adenosylmethionine
TET	Ten–eleven translocation proteins
THF	Tetrahydrofolate
WHO	World Health Organization

Introduction

Proper nutrition with the correct levels of vitamins and minerals are essential to lead a healthy lifestyle. Nutrient deficiency not only has the potential to make a person feel ill but may also change the epigenome, a regulatory mechanism that can alter gene expression without altering the genetic code (Choi and Friso 2010). Ramifications of changes to the epigenome can affect the individual and, with its heritable trait, subsequent generations of children. In this chapter, we will focus on

folate, an essential nutrient that when deficient leads to a host of complications and alterations in DNA methylation.

Folate

Folate or folic acid is a water-soluble vitamin B (also known as vitamin B_9) with an extensive role in human metabolism (Liew 2016). The chemical structure of folate contains a heterobicyclic pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid (Lindzon and O'Connor 2007) (Fig. 1). Folate is the naturally occurring form first discovered in foliated (green leafy) vegetables. Plants and vegetables such as broccoli, asparagus, celery, lentils, beans, and Brussels sprouts have high levels of folate (NIH 2016) (Fig. 2). On the other hand, folic acid is the synthetic form of folate added to fortified foods (such as cereals, flour, grains, and bread) as of 1998 and found in supplements (Liew 2016; Crider et al. 2011; US Department of Health and Human Services FaDA 1996). The term "folate" is used interchangeably between the natural and synthetic forms.

Folate is essential for the synthesis of nucleic acids, amino acids, and methyl donors necessary for a range of biological processes from DNA synthesis to gene or epigene regulation (Liew 2016; Scaglione and Panzavolta 2014). Folate is metabolized through the one-carbon pathway; this metabolism pathway produces precursors for nucleotide synthesis and methyl donors (Bailey and Gregory 1999). In the one-carbon donor pathway, folate is converted to tetrahydrofolate (THF) and further metabolized to its biologically active form 5-methyltetrahydrofolate (5-MTHF) (Bailey and Gregory 1999). However, synthetic folic acid requires additional enzymatic steps including through dihydrofolate reductase (DHFR), an enzyme that has the potential for drug inhibition that is avoidable with natural sources of folate (Scaglione and Panzavolta 2014). Metabolism of synthetic folic acid can be inhibited by drug interactions with DHFR, potentially leading to downstream metabolite deficiencies (Scaglione and Panzavolta 2014). 5-MTHF is involved in an enzymatic reaction to convert homocysteine to methionine, which is then converted to *S*-adenosylmethionine (SAM), a major methyl-donating molecule (Liew 2016; Bailey and Gregory 1999; Blom and Smulders 2011). SAM, in turn, donates its methyl group via methyltransferase enzymes to DNA, RNA, proteins, and lipids (Shorter et al. 2015) (Figs. 1 and 3).

Folate metabolites are also involved in the production of nucleotides. In DNA synthesis, folate metabolism leads to deoxyuridine monophosphate (dUMP) methylation forming deoxythymidine monophosphate (dTMP) (Liew 2016; Stover 2009). dTMP is then converted to thymidine triphosphate (dTTP), which is one of the four deoxynucleotides needed for DNA synthesis as well as DNA repair (Liew 2016). The low availability of folate can lead to the decreased production of dTMP from dUMP and ultimately a decreased supply of dTTP available for DNA replication and repair. Excess dUMP will then be converted to deoxyuridine triphosphate (dUTP) (Scaglione and Panzavolta 2014; Bailey and Gregory 1999). Due to its inability to distinguish between dTTP and dUTP, DNA polymerases can incorrectly

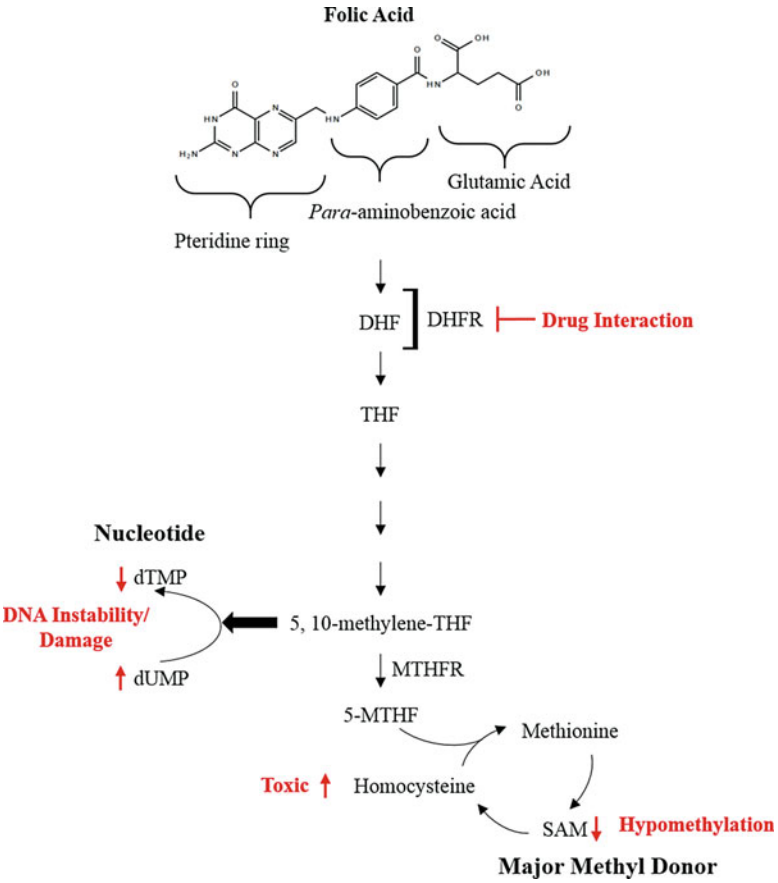

Fig. 1 Folate metabolism to metabolites involved in DNA synthesis and methylation. Folate metabolism via the one-carbon metabolism pathway. Folate is converted to DHF and then tetrahydrofolate (*THF*) by DHF reductase. THF is further metabolized to 5,10-methylene-THF which enters into nucleotide synthesis to convert deoxyuridine monophosphate (*dUMP*) to deoxythymidine monophosphate (*dTMP*) which is then incorporated as a nucleotide into DNA replication. 5,10-Methylene-THF is metabolized to 5-methyltetrahydrofolate (*5-MTFH*) by methyltetrahydrofolate reductase (*MTHFR*). 5-MTHF enters into the pathway to convert homocysteine to methionine, which can then be converted to *S*-adenosylmethionine (*SAM*). SAM is a major methyl donor in DNA methylation and other methylation processes. Figure adapted from Crider et al. (2012) with permissions

incorporate uracil in place of thymidine in the DNA synthesis, which can lead to an overabundance of uracil in the DNA (Blount et al. 1997). Excessive uracil incorporation from folate deficiency can lead to DNA instability, chromosome breaks, and increased risk for certain diseases such as cancer (Liew 2016; Blount et al. 1997; Duthie 1999) (Fig. 1). Folate deficiency is associated with a number of diseases including increased risk of certain cancers, cardiovascular disease, and neurological defects (Blount et al. 1997; Duthie 1999; Mattson et al. 2002; Ward 2001). The

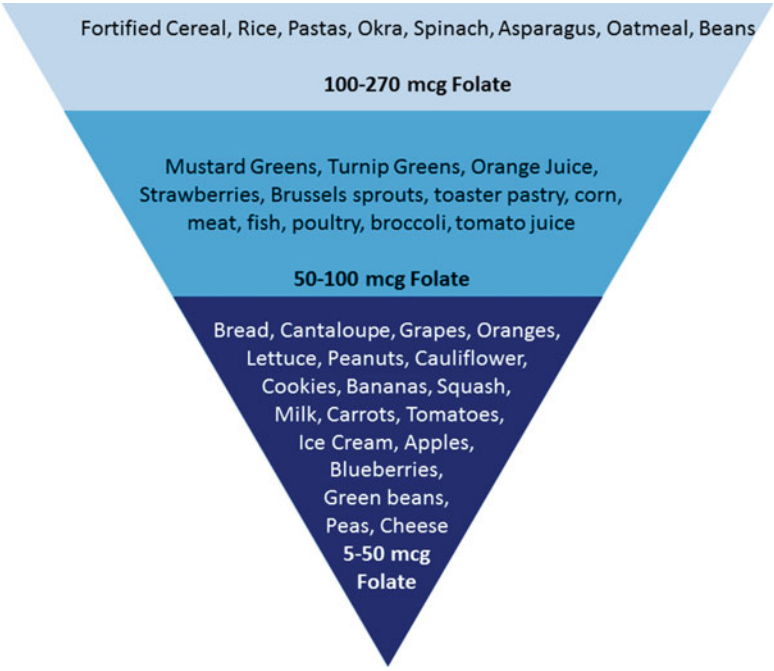

Fig. 2 Folate levels in foods. Common foods are stratified by the amount of folate or folic acid each contains. Foods are displayed by ranking of folate content in micrograms (mcg) from the USDA Food Composition Database (U.S. Department of Agriculture, Agricultural Research Service 2017). The highest folate containing foods are those supplemented with folic acid such as fortified cereals, rice, and pasta as well as some natural occurring high folate containing foods such as asparagus, Brussels sprouts, spinach, broccoli, and beans. Fruits, some vegetables, and dairy contain lower amounts of folate

mechanism underlying folate deficiency and disease risk may provide key diagnostic and therapeutic strategies in the future.

DNA Methylation

DNA methylation is one of the most studied and most well-understood epigenetic marks (Smith and Meissner 2013). It is a repressive mark leading to decreased expression of targeted genes without altering the genetic code (Choi and Friso 2010). During the process of DNA methylation, the addition of a methyl group occurs at the C5 position of the pyrimidine ring of the cytosine nucleotides via DNA methyltransferase (DNMT) enzymes (Kruman and Fowler 2014; Pacchierotti and Spano 2015). Three DNMTs have been identified as DNMT1, DNMT3a, and DNMT3b. DNMT1 is used commonly in maintaining DNA methylation patterns. Whereas DNMT 3a and 3b are involved in *de novo* DNA methylation which is the methylation of DNA during the embryonic stage of development (Qu et al. 2013;

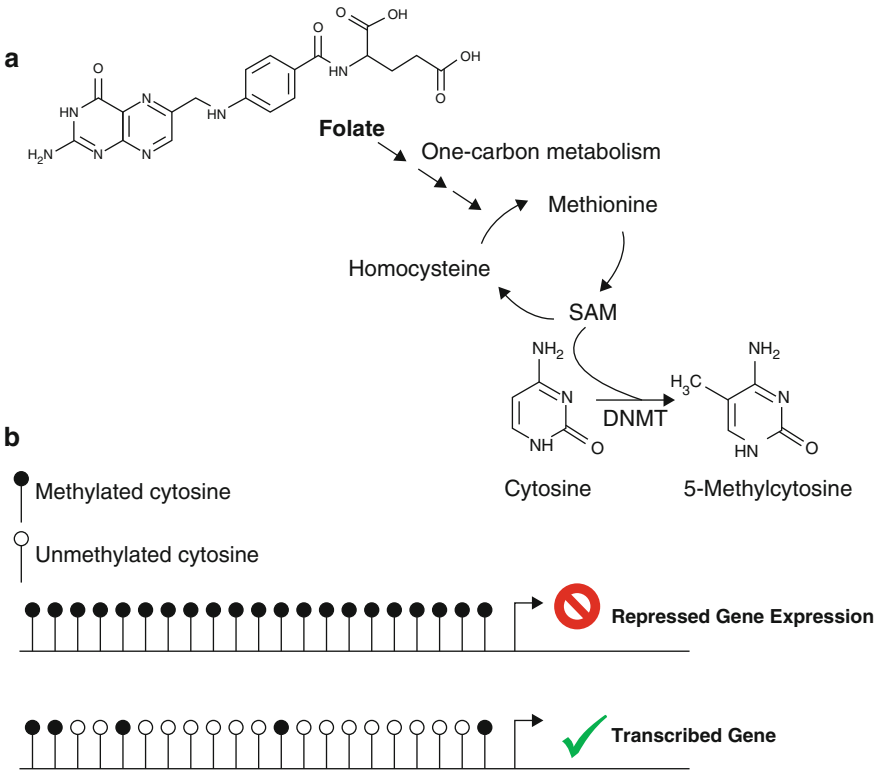

Fig. 3 Overview of DNA methylation by folate metabolite, SAM. (**a**) Folate is metabolized through the one-carbon pathway leading to the generation of SAM, a major methyl donor. DNA methylation occurs when DNMTs (DNMT1, DNMT3a, or DNMT3b) transfer a methyl group from SAM to the 5-position on cytosine generating 5-methylcytosine, a repressive mark. (**b**) Methylated cytosines (solid ball and stick) are repressive marks and prevent gene transcription. Hypomethylated genes or genomic regions where cytosines do not have a methyl group at the 5-position (open ball and stick) can be transcribed, and genes are expressed

Quintero-Ronderos and Montoya-Ortiz 2012). DNA methylation does not occur at random cytosine nucleotides. Instead, methylation occurs at CpG islands (areas of rich CG dinucleotide base pairs) near the promotor region. However, CpG islands found around transcriptional start sites of endogenous and regulatory genes often remain hypomethylated allowing for the binding of transcription factors (Kruman and Fowler 2014). Studies have also shown that methylation occurs at other CpG islands distal to the promoter region which magnifies the role of DNA methylation beyond regulation of gene expression to a more genome-wide function (Kruman and Fowler 2014; Kandi and Vadakedath 2015).

DNA methylation patterns are commonly set during development. As such, DNA methylation is stable in somatic cells, and gene expression is not rapidly altered by DNA methylation as in other epigenetic mechanisms, such as histone modifications

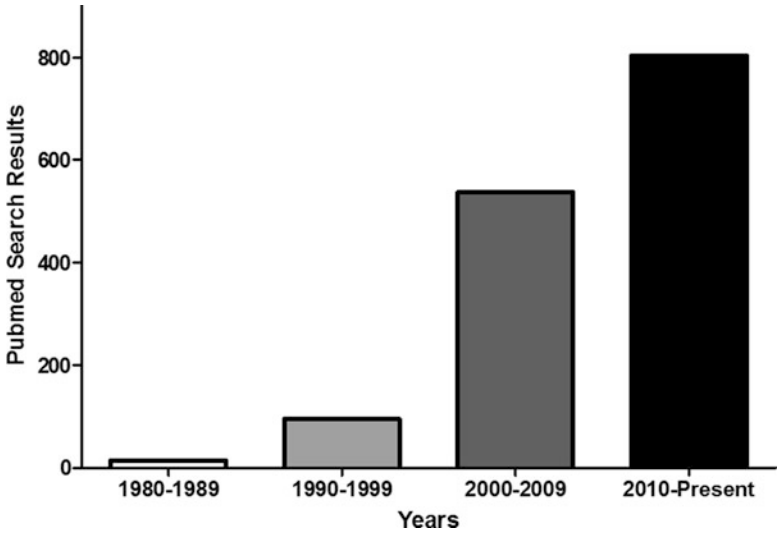

Fig. 4 Publications on folate and DNA methylation by decade since 1980. PubMed search results for the terms "Folate" and "DNA Methylation." Search results are displayed by decade from 1980 to 1989, 1990 to 1999, 2000 to 2009, and 2010 to present (2017)

(Smith and Meissner 2013; Robertson 2005). However, once DNA methylation patterns have been set during embryogenesis, they must be maintained throughout life during DNA replication. Folate is necessary to provide methyl donor pools of its metabolite SAM continually. Folate deficiency, and therefore SAM deficiency, has the potential to lead to a form of passive DNA demethylation where methylation is not maintained on newly synthesized DNA and subsequent cellular generations (Crider et al. 2012) (Fig. 3). Folate deficiency at the onset of development or later in life could have a substantial impact on the future health of the individual by altering DNA methylation patterns (Irwin et al. 2016). Thirty years ago, folate and DNA methylation research was almost nonexistent (Fig. 4). However, as the epigenetics field has emerged, studies into folate and DNA methylation also increased and is still on the rise (Fig. 4).

DNA Demethylation

DNA demethylation is also vital in certain stages of development and programming (Wu and Zhang 2014; Messerschmidt et al. 2014). For instance, DNA methylation marks are cleared from the genome in early stages of the embryogenesis and germline cells, erasing parental marks and priming the cells for pluripotent potential and new genome DNA methylation patterns (Wu and Zhang 2014). This reprogramming step is necessary to create a totipotent state that will allow for sex-determination and germ-line-specific differentiation of the embryo (Messerschmidt et al. 2014).

Although transferring a methyl group involves a covalent bond, DNA methylation is a reversible process (Wu and Zhang 2014). However, not in the same way as other epigenetic modifications such as histone acetylation and deacetylation, which is a coordinated balance between histone acetyltransferases and histone deacetyltransferases. DNA demethylation is mediated through both active and passive mechanisms. Active mechanisms of demethylation are oxidation of the 5-methylcytosine via ten–eleven translocation proteins (TET) and base excision of the methylated cytosine (Wu and Zhang 2014). When DNMT1, responsible for maintaining methylation patterns, does not methylate a strand of replicating DNA, those marks will disappear in subsequent rounds of DNA replication leading to passive demethylation (Wu and Zhang 2014). In conclusion, exploring DNA demethylation mechanisms has the potential to expand the current therapeutic knowledge of diseases.

Folate, DNA Methylation, and Disease

Folate deficiency results from either insufficient dietary intake or genetic defects in the metabolism of folate. This deficiency is prevalent in the population, and the resulting strain on the methyl donor pool may have deleterious effects on overall human health (Niculescu and Zeisel 2002). As for folate deficiency in the one-carbon donor pathway, low levels of folate results in low levels of SAM and may alter global DNA methylation patterns leading to changes in gene expression involved in various diseases including oncogenes (Liew 2016; Niculescu and Zeisel 2002) (Fig. 3).

Pregnancy Complications

Folate supplementation is imperative during pregnancy. The CDC recommends that women who may become pregnant, even before conception, take 400 micrograms of folate daily (CDC 2016). Folate is required for the proper neural tube closure during embryogenesis and development; this process occurs in the first few weeks of pregnancy (Cordero et al. 2015). Therefore, folate deficiencies during pregnancy can lead to a number of pathogeneses resulting from neural tube defects (NTD), including spina bifida (Liew 2016; Irwin et al. 2016; Pitkin 2007; Beaudin and Stover 2007). Up to 70% of neural tube closure defects are thought to be treatable with proper folate supplementation, the reason being that proper supplementation before conception ensures enough folate for neural tube formation (CDC 2016; Cordero et al. 2015). Therefore, it is recommended women who are planning to become or are pregnant take a folate supplement. Another mechanism for folate-associated pregnancy complications is accumulation of homocysteine, the precursor to methionine and SAM in the methyl donor pathway. Homocysteine levels may also affect fetal health, with an accumulation of homocysteine (a possible side effect of folate deficiency) competing for and blocking SAM binding to DNMTs leading to DNA hypomethylation (Iacobazzi et al. 2014). It is suggested that Down syndrome

where mitochondrial DNA is hypomethylated, cleft palates, and congenital heart defects are complications for imbalanced homocysteine and abnormal folate levels and metabolism (Blom and Smulders 2011) (Fig. 5).

Cancer

Alterations in DNA methylation of oncogenes and tumor suppressors can have a profound effect on cancer susceptibility (Clark and Melki 2002; Ehrlich 2002). In a hypermethylation state, tumor suppressor gene expressions are inhibited, and on the other hand during a hypomethylated state, oncogenes are overexpressed (Kandi and Vadakedath 2015). DNA methylation becomes an issue when it occurs abnormally. Anything that leads to this abnormality is of absolute importance in cancer. There

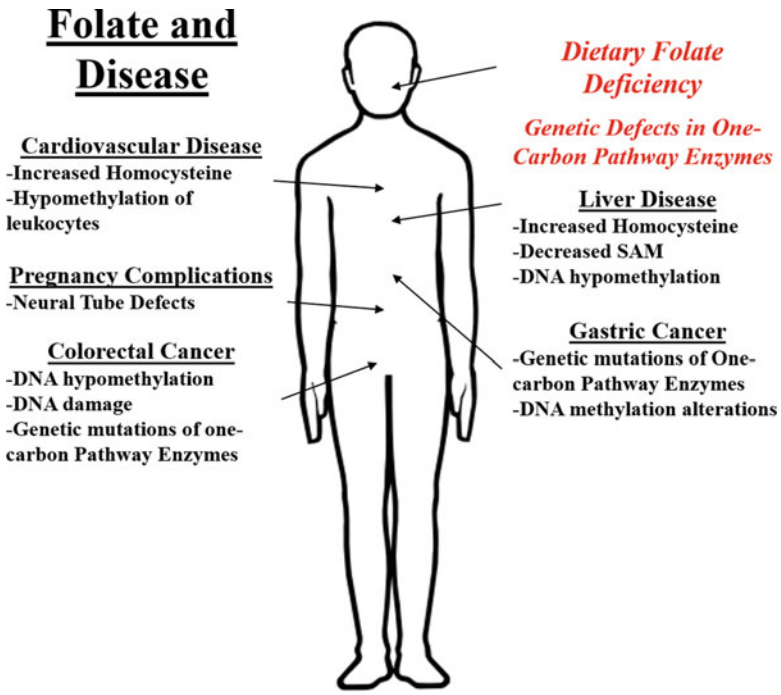

Fig. 5 Overview of folate and disease mechanisms. Folate deficiency or perturbations in folate metabolism have been linked to numerous diseases. Folate deficiency leads to increased homocysteine levels, which is toxic and detrimentally affects the cardiovascular system and liver contributing to a disease state. Folate deficiency produces less SAM, a methyl donor, which in turn results in DNA hypomethylation. DNA hypomethylation may alter oncogene and tumor suppressor gene expressions in cancers, leukocytes in cardiovascular disease, and hepatic genes that regulate liver disease. DNA damage due to DNA hypomethylation as well as nucleotide pool imbalance caused by folate deficiency is a factor in the development of cancer. While a person may take in the correct amount of dietary folate, metabolism of folate may be disrupted by polymorphisms or mutations in key enzymes in the one-carbon metabolism pathway, which contributes to cancer development

has been evidence that chemical pollutants, dietary components, and other factors are involved in changing epigenetic mechanisms such as DNA methylation (Qu et al. 2013). Folate deficiency has been shown to result in hypomethylation due to the low availability of methyl group donors, such as SAM (Qu et al. 2013). Even though methylation states of cancer cells vary, hypomethylation is common and a source of altered gene expression (Duthie 1999) (Fig. 5).

Gastric and Colorectal Cancer

Recent scientific research shows that folate deficiency either by low folate intake or due to a genetic component is associated with increased risk for gastric cancer (Gao et al. 2013; Zhao et al. 2012). Low serum folate levels have been associated with gastric cancer development and invasiveness (Lee et al. 2014). Folate deficiency has also been associated with the susceptibility of colorectal cancer by folate deficiency-induced DNA hypomethylation (Kim 2004; Pufulete et al. 2003). Although the mechanisms between folate and gastric and colorectal cancers are still being elucidated, studies suggest DNA methylation may be playing a role. As with many cancers, methylated promoter sites and aberrant methylation are present in both gastric cancer and colorectal cancer (Qu et al. 2013; Zhao et al. 2012; Bardhan and Liu 2013; Esteller 2002; Wajed et al. 2001). Diagnosis of gastric and colorectal cancers is commonly based on gastrointestinal endoscopy, colonoscopy, and fluoroscopy, which are invasive and not comfortable for the patient (Winawer et al. 2003; Pasechnikov et al. 2014). Thus, using DNA methylation as a possible diagnostic screening tool to detect cancer is becoming important. DNA methylation as a screening tool uses gastric fluids (serum or gastric washes) or biopsy specimens, and it identifies the prevalence of methylation in DNA within the specimen (Nakamura et al. 2014; Ye et al. 2010; Kalnina et al. 2015). Such DNA is the secreted product from gastric cancer cells; thus, the methylation patterns observed in the specimen are thought to be correlated with those in cancer cells (Nakamura et al. 2014; Ye et al. 2010; Kalnina et al. 2015). Numerous genes have been identified as having altered DNA methylation patterns in cancers versus normal tissue. In colorectal cancer, CpG island methylator phenotype (CIMP) is used to classify colorectal cancers (Bardhan and Liu 2013). These genes, which are hypermethylated in colorectal cancer, include MLH1, p16, MINT1, MINT2, and MINT31, common genes in DNA mismatch repair mechanisms and tumor suppressors (Bardhan and Liu 2013). CIMP panels can further be expanded to include a number of other genes, including RUNX3, IGF2, and WRN (Bardhan and Liu 2013). Furthermore, DNA methylation of serum RUNX3 and serum RASSF1A used to diagnose gastric cancer with the latter having high specificity, but low sensitivity (Qu et al. 2013). The promoters of RUNX3 and RASSF1A are hypermethylated in gastric cancer tissue compared to normal tissue (Qu et al. 2013; Chen et al. 2010; Kim et al. 2004; Waki et al. 2003; Wang et al. 2008; Ye et al. 2007) (Table 1). These hypermethylation marks can be used as biomarkers of disease risk and state. A significant difference between cancer and normal DNA is the methylation of the promoter site for gene p16, so it has become of

Table 1 Alterations in genes leading to colorectal and gastric cancer susceptibility. Changes in gene methylation or gene polymorphisms involved in colorectal or gastric cancers. Changes in DNA methylation or polymorphism increase the risk for colorectal and gastric cancers. Certain markers are used as biomarkers in disease to sub-classify cancers

Cancer	Property	Outcome or marker	References
Gastric	RUNX3 hypermethylation	Increased risk, biomarker	Qu et al. (2013), Chen et al. (2010), Kim et al. (2004), and Waki et al. (2003)
	RASSF1A hypermethylation	Increased risk, biomarker	Qu et al. (2013), Wang et al. (2008), and Ye et al. (2007)
	p16 hypermethylation	Increased risk, biomarker	Qu et al. (2013)
	MTHFR polymorphism	Increased risk	Gao et al. (2013)
Colorectal	DNA hypomethylation	Increased susceptibility	Kim (2004) and Pufulete et al. (2003)
	CIMP hypermethylation	Used to subtype	Bardhan and Liu (2013)

special interest to use this as a biomarker for gastric cancer (Qu et al. 2013) (Table 1). As for potential treatment, the idea of demethylation of tumor suppressor genes has gained much appeal since demethylation of these genes leads to cell apoptosis (cell death) (Qu et al. 2013). Folate deficiency or disruptions in the folate metabolism pathway may lead to alterations in DNA methylation patterns, and impact the development of these cancers. However, further studies into the connection between folate status, DNA methylation of these genes, and gastric and colorectal cancer is needed. Elucidating a connection between folate and DNA methylation patterns will hold diagnostic value in gastric and colorectal cancers.

Defects in the folate metabolism pathway may also markedly impact cancer development. Polymorphisms in an essential enzyme in folate metabolism, methylenetetrahydrofolate reductase (MTHFR), correlated with increased risk for gastric cancer and when associated with folate intake, decreased folate intake and further increased the risk of gastric cancer above the MTHFR polymorphism and folate deficiency alone (Gao et al. 2013) (Table 1 and Fig. 5). Deficient folate intake and the resulting DNA hypomethylation in colorectal cancer leads to damage- and mutation-prone DNA and insufficient repair mechanisms, some of which is reversible with proper folate supplementation (Kim 2004; Pufulete et al. 2003). In addition, in several cases, colorectal cancers have polymorphisms in the enzymes in the one-carbon pathway where regardless of folate intake, metabolism to methyl donors is affected due to genetics rather than environment (Zhao et al. 2012; Pufulete et al. 2003). It is thought that the reactivation of these enzymes may be possible, and whose function may trigger tumor suppression (Zhao et al. 2012) (Table 1 and Fig. 5).

Cardiovascular Disease

The evidence demonstrated a connection between DNA methylation in cardiovascular diseases (CVD) such as atherosclerosis, heart failure, myocardial infarction, and cardiac hypertrophy (Blom and Smulders 2011; Ward 2001; Li et al. 2016). Although evidence for DNA methylation and folate in CVD are at its initial stages, researchers are actively exploring the field in hopes to use epigenetics in the treatment of patients with CVD (Voelter-Mahlknecht 2016). Folate deficiency contributes to CVD in that it causes high levels of homocysteine in the body, which is damaging to the cardiovascular system (Mattson et al. 2002; Winder 1998). High levels of homocysteine are due to folate deficiency in which levels of folate are inadequate to methylate homocysteine to methionine (Mattson et al. 2002; Winder 1998; Castro et al. 2003; Rosenquist 2013). Folate supplementation lowers homocysteine levels, but its beneficial effects vary in disease states; folate supplementation has shown benefits in stroke prevention while it has shown no benefit in CVD (Voelter-Mahlknecht 2016). Although the mechanism is unknown, leukocytes in the serum of patients with vascular disease are globally hypomethylated compared to healthy controls (Kandi and Vadakedath 2015; Castro et al. 2003). This hypomethylation correlates with increased homocysteine levels in the serum; this pathway is consistent with characteristics of folate deficiency and one-carbon pathway alterations (Castro et al. 2003). Scientists speculate that the folate supplementation would help bring homocysteine levels back to safe levels, a major contributor to the development of various heart-related diseases (Li et al. 2016) (Fig. 5).

Liver Disease

Medici and Halsted demonstrated that folate deficiency is associated with the development of alcoholic liver disease through numerous mechanisms (Medici and Halsted 2013). The group showed that low levels of folate, thiamine, and vitamin B6 play a role in the development of alcoholic liver disease. It is thought that the low availability of folate and similar compounds in the human body will lead to hypomethylation of DNA and DNA instability in the liver and will cause expression of harmful liver enzymes that result in liver damage (Medici and Halsted 2013). Furthermore, folate feeds into metabolism of methionine and affects homocysteine levels, antioxidant effects, and lipid mobilization (Medici and Halsted 2013). Chronic alcohol intake disturbs the one-carbon metabolism; its hallmark effect is elevated levels of homocysteine (Kruman and Fowler 2014). Since the one-carbon metabolism is disturbed by chronic alcohol intake, it is safe to say that the effects also lead to changes in DNA methylation and subsequent gene expression, not only at the liver but multiple organs as well (Kruman and Fowler 2014). The mechanism of how chronic alcohol intake affects is not entirely understood, but it may be a result from two possible pathways: the direct interference of enzyme function from ethanol inhibition, or the decreased levels of folate due to the poor diet as seen in alcoholics (Kruman and Fowler 2014). Either way, one-carbon metabolism dysfunction leads to low levels of SAM which leads to hypomethylation of DNA (Kruman and Fowler 2014). Additionally,

increased alcohol intake strongly associates with low folate intake and colorectal cancer via mechanisms of promoter hypomethylation and elevated homocysteine levels (van Engeland et al. 2003; Giovannucci et al. 1995; Kato et al. 1999) (Fig. 5).

Conclusions

In summary, folate is an important dietary requirement, especially during pregnancy. Folate deficiency during pregnancy leads to detrimental neural tube defects, but more, folate deficiency correlates with gastric and colorectal cancer, as well as, cardiovascular and liver disease later in life. Its metabolism pathway, the one-carbon pathway, generates the methyl donor SAM for DNA and other methylation processes. Folate deficiency may be leading to unstable DNA by disrupting nucleotide synthesis and increasing susceptibility to disease. Further, folate deficiency may deplete methyl donor pools affecting DNA methylation and altering expression of genes associated with disease. Epigenetics and epigenetic regulation of disease is an emerging and fast developing field and folate's role in epigenetic regulation of disease is still unfolding. Therefore, more attention needs to be focused on the epigenetic impact of folate in human health and disease. Folate and its metabolites have potential to be used in preventative, diagnostic, and therapeutic measures in a number of diseases. Determining folate status and folate supplementation may be beneficial in treating diseases associated with elevated homocysteine levels in the blood or tissue, as in CVD and liver disease. Further, evaluating folate levels and DNA methylation in a disease context, globally or disease gene-specific, may provide valuable information on the underlying mechanism of disease, and the potential for folate supplementation in restoring proper DNA methylation. Therefore, maintaining proper folate supplementation throughout life, not just at critical stages such as pregnancy, should be evaluated in reducing the risks of developing diseases such as cancer, CVD, and liver disease.

Policies and Protocols

The World Health Organization (WHO) issued guidelines in 2015 reflecting the appropriate folate levels in red blood cells in women of reproductive age. Folate levels must exceed 400 ng/mL (906 nmol/L) in RBCs in women of reproductive age to reduce the chances of fetuses being affected by neural tube defects during development (Cordero et al. 2015). The CDC recommends women of reproductive age to consume 400 mcg of folate daily (CDC 2016).

Dictionary of Terms

- **Epigenetics** – The study of how environmental pressures, such as nutrient deficiency, alter the expression of genes without altering the genetic code.

- **DNA methylation** – An epigenetic mechanism where a methyl group is added to the 5-carbon of cysteine in CpG islands and canonically represses gene transcription.
- **DNA methyltransferase** – An epigenetic enzyme family consisting of DNMT1, DNMT3a, and DNMT3b that adds a methyl group to the C5 position of cytosine by de novo either methylation (DNMT3a and DNMT3b) or maintenance of already methylated DNA (DNMT1).
- **Folate** – An essential dietary nutrient commonly found in broccoli, celery, asparagus, beans, and Brussels sprouts and its synthetic form, folic acid, that is added to fortified foods since 1998 and found in supplements.
- **One-carbon metabolism** – Folate participates in this pathway where it is metabolized into tetrahydrofolate and undergoes further metabolism to produce nucleotides or 5-methyltetrahydrofolate, which is further metabolized into the primary methyl donor in methylation processes, *S*-adenosylmethionine.

Summary Points

- This chapter discusses the dietary nutrient, folate, and its connection to epigenetics.
- Folate is metabolized through the one-carbon metabolism pathway, and its metabolites are used in DNA synthesis and methylation processes.
- One such metabolite is *S*-adenosylmethionine, the major methyl donor in methylation processes.
- Folate deficiency leads to pregnancy complications leading to neural tube defects in children and disease such as cancer, cardiovascular diseases, and cancer in mothers and children.
- Folate deficiency may lead to insufficient methyl donor pools to maintain proper methylation of the DNA.
- Hypomethylation, or decreased methylation, of the DNA, can lead to DNA instability leading to DNA damage.
- In addition, folate deficiency and resulting hypomethylation can lead to the expression of oncogenes leading to the development of cancers such as colorectal and gastric cancers.
- Folate deficiency can also alter the balance in one-carbon metabolism to an accumulation of homocysteine, which causes damage to the cardiovascular system associated with a number of cardiovascular diseases.
- Proper supplementation of folate (400 mcg/day) has shown to prevent pregnancy complications.
- Folate levels and methylation have diagnostic and therapeutic potential; in fact, supplementation of folate has shown improvement in certain diseases.

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