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Review Article

The folate puzzle. Part I: the Janus face of folate in cancer medicine

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CONTENTS

Abstract

Tetrahydrofolate is derived from folate and serves as a backbone for single-carbon transfer reactions. The donation of one-carbon units, such as methyl and methylene, is crucial for nucleotide synthesis and consequently for proper DNA formation and gene regulation. The biochemical pathways of dietary methionine produce *S*-adenosylmethionine, which is the universal methyl donor and a precursor for homocysteine. To save methionine for the body, homocysteine can be converted to methionine. This bioreaction shares the reaction process with the folate bioreaction cycle. Thus, dietary folate and methionine are biochemically linked and folate intake may reduce homocysteine levels. This explains why homocysteine is a sensitive marker for folate deficiency and reduced biomethylation. The results of various epidemiological studies suggest a link between low folate intake and an increased risk of various malignancies, such as cancer of the breast, ovary, esophagus, pancreas, lung, cervix and, notably, colorectal cancer. There is ample preclinical evidence that folate depletion induces hypomethylation in essential coding regions, which may result in dysfunctional tumor suppressor genes and DNA repair mechanisms. In addition to hypomethylation, hypermethylation is also frequently associated with cancer, which suggests that aberrant methylation creates suitable conditions for malignant cells to proliferate. This brings about a valid concern that both folate deficiency and excessive use of folic acid supplementation may increase the risk of cancer. This article reviews the present knowledge on the relationship between folate intake and the risk of cancer.

Introduction

Several epidemiological studies suggest a protective effect of folate intake in relation to cancer risk. These results follow the large body of evidence for the protective effect of periconceptional folic acid supplementation against neural tube disorders (spina bifida) (1). A decreased risk has been demonstrated especially for sporadic and ulcerative colitis-associated colorectal cancer (2). An inverse relationship has also been shown for other malignancies, such as cancer of the pancreas, lung, stomach, cervix, ovary and esophagus (3). It should, however, be noted that the results of various epidemiological studies are not consistent, and some studies have shown results that point to an increased risk for, *e.g.,* breast cancer (4). Obviously, there is both "good" and "not so good" news about folic acid intake and it is the purpose of this article to shed light on these controversies by summarizing the available data. In addition, this summary may help to justify mandatory food supplementation with folic acid, a policy that has been adopted by many countries, including the United States and Canada. Before this, however, in order to understand the effects of folate on cellular processes, we first review the pathways involved in folic acid metabolism.

Folate and methylation

Folate is a water-soluble B vitamin (usually referred to as vitamin B_9 or B_{11}). Tetrahydrofolate (THF) is a biologically active compound derived from folate, and is a chemical compound containing a pteridine ring to which *para*-aminobenzoic acid and glutamic acid are connected (Fig. 1). Tetrahydrofolate may carry two C1 units, such as $–CH_3$ (methyl-THF), $–CH_2$ (methylene-THF) or $–CH_1$ (formyltetrahydrofolate). This feature is important, as the activated C1 unit may be donated to a substrate as part of a single-carbon transfer reaction. The donation of one-carbon units is important for bioreactions in which methylation plays a role, such as nucleotide synthesis and, consequently, DNA formation.

The process of methylation is schematically depicted in Figure 2. The enzyme methylenetetrahydrofolate reductase (MTHFR) converts methylene-THF to methyl-

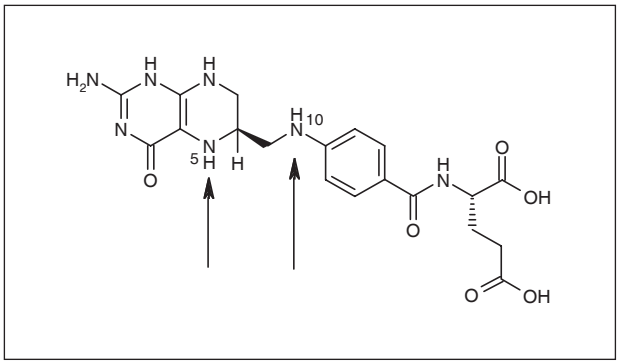

Fig. 1. Tetrahydrofolate.

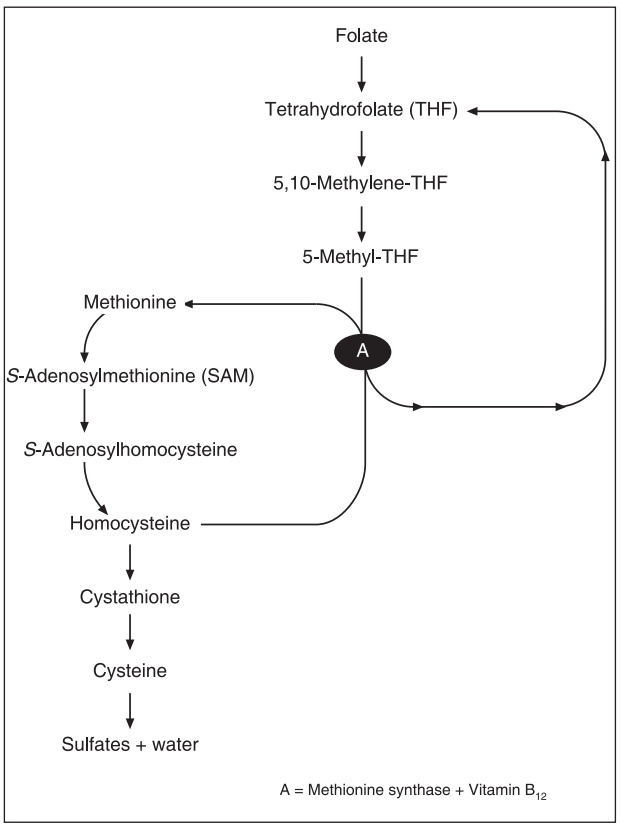

Fig. 2. Methionine and tetrahydrofolate pathways.

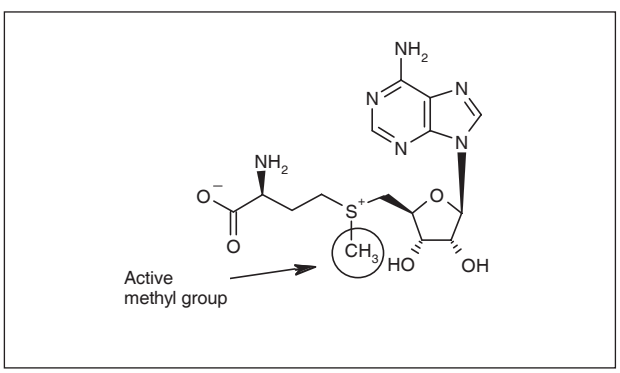

Fig. 3. *S*-Adenosylmethionine (SAM).

THF in a unidirectional reaction. Homocysteine, a thiol-containing amino acid, and methyl-THF are reactants that produce methionine by the addition of a $–CH_3$ group catalyzed by methionine synthase (also called methyltetrahydrofolate—homocysteine methyltransferase). The latter enzyme carries cobalamin (vitamin B_{12}) as co-enzyme. In this way, homocysteine links the methionine cycle with the folate cycle, which starts with dietary folate intake. This explains why homocysteine plasma concentrations are a sensitive marker for folate and vitamin B_{12} deficiency.

The universal methyl donor *S*-adenosylmethionine (SAM) is synthesized from methionine using adenine triphosphate (ATP). SAM carries a positively charged trivalent sulfur atom that activates the adjacent methyl group (Fig. 3). From these pathways it is evident that folate is an essential compound for biological methylation reactions. There is good evidence to support the idea that proper functioning of the folate cycle is also associated with co-enzymes such as riboflavin (vitamin B_2) and pyridoxine (vitamin B_6), as low levels of these vitamins interfere with folate metabolism (5).

Methylation and carcinogenesis

The methylation of DNA is important for gene regulation. The methylation of CpG islands (DNA parts with a high cytosine-guanine content, usually located in gene promoter regions) is a critical factor in the regulation of cell proliferation. Loss of control of proliferation has been attributed to the silencing of tumor suppressor genes, as well as genes involved in the regulation of the cell cycle, gene repair, angiogenisis and apoptosis (6). Alteration of methylation patterns plays a key role in these processes, as is evident from the fact that in human tumor tissue hypermethylation of CpG islands occurs frequently. In a tumor genome, up to 10% of the total 45,000 CpG islands can be hypermethylated (7). Interestingly, this abberant CpG island methylation occurs in a nonrandom and a fairly tumor type-specific manner. This suggests that certain CpG islands are more susceptible to hypermethylation than others. In addition to the hypermethylation of promoter regions, the hypermethylation of exonic CpG islands may also take place, even without affecting the promoter region itself. Various studies suggest that the *de novo* methylation of exonic CpG islands may spread to promoter regions (8). Subsequently, cells with silenced tumor growth defense genes may turn into malignant cancer tissue.

Apart from the hypermethylation process, another distinct DNA methylation abnormality has been observed in cancer. This concerns the genome-wide reduction in DNA methylation (global hypomethylation). This aberrant methylation pattern was one of the earliest changes observed in human cancers. Although the underlying mechanisms are unclear, global hypomethylation is likely to induce proto-oncogene activation and chromosomal breakage and rearrangements. This phenomenon has been studied in patients with the ICF syndrome (immuno-

deficiency, centromeric instability, facial dysmorphy) characterized by, among other things, DNA rearrangements showing DNA hypomethylation. In these patients prone to cancer development, mutations in the methyltransferase enzyme lead to hypomethylation (9). Thus, paradoxically, both hyper- and hypomethylation are associated with carcinogenesis and in clincal research the alteration of DNA methylation has become an indicator of carcinogenic risk and early diagnosis of malignancy (10).

There is also accumulating evidence that precancerous lesions showing aberrant methylation may progress rapidly to a malignant state (11). This expains why DNA methylation is being explored as a biomarker for tumor classification and prognosis (12, 13). In this context, it is interesting to note that mutations of DNA methyltransferase result in altered methylation patterns (14). Whether this leads to the silencing of tumor suppressor genes is uncertain. At the same time, the process of methylation itself may also lead to mutations (15). Methylated cytosine may deaminate spontaneously, resulting in random cytosine to thymine transitions (Fig. 4). This occurs especially in CpG zones, which are therefore called "mutational hotspots". Furthermore, the conversion of deoxyuridylate to deoxythymidylate is essential for the fidelity of DNA sythesis (16). It is known that folate deficiency results in the misincorporation of uracil into DNA, which needs to be excised by repair glycocilane. During this process, single- or even double-strand breaks may occur, which fuels carcinogenesis (17).

Methylation and RNA

The universal methyl donor SAM also plays a role in the methylation of RNA. Various sites of methylation in the RNA molecule are crucial for its integrity and functionality. RNA methylation involves highly conserved aptamer (specific oligonucleotide sequences) domains responding to SAM with high affinity and specificity (18). These structures are called SAM riboswitches and are regulatory elements that control gene expression through a mechanism recognizing the levels of specific small molecules, and employing the binding energy for its control function (19).

Folate deficiency results in the improper methylation of RNA through SAM depletion (see mechanism in Figure 2). It has only recently been recognized that undermethylation of these aptamer domains of riboswitches leads to miscommunication to the "expression platform", another RNA domain which directs the transcription or translation of messenger RNA (20). Thus, precise genetic control is in the hands of SAM, making it one of the most pertinent compounds in life as a spider in a biochemical (methylation) net (21).

Clinical links to cancer

Aberrant DNA methylation can mediate gene transcriptional silencing, giving rise to loss of key gene functions during carcinogenesis. Dysregulated methylation may also cause disturbances in nucleotide synthesis, followed by DNA strand breaks. Interestingly, some DNA abnormalities induced by folate deficiency can be reversed by folate supplementation (22). Therefore, one-carbon metabolism has increasingly received attention based on epidemiological studies linking low folate intake to an increased risk of various malignancies. This has been suggested in early clinical studies and in more recent meta-analyses, as in cases of cancers of the breast (23), ovary (24), esophagus, stomach and pancreas (25), lung (26) and cervix (27). The most convincing evidence has been obtained for colorectal cancer: low folate intake has been associated with an increased risk of the development of both the precursor lesion colorectal adenoma and malignant colorectal cancer (28).

In experiments in rats, folate depletion has been demonstrated to induce hypomethylation in the coding region of *p53.* Needless to say, *p53* is both an important tumor suppressor gene essential for DNA repair and an important regulator of the cell cycle. Hypomethylation due to folate depletion was shown to result in strand breaks within the *p53* gene, a major cause of oncogenesis. Suprisingly, when tumor mass was evident in the liver, exon-specific hypomethylation in the hepatic *p53* gene was followed by a rebound hypermethylation during the course of the experiments (29). Whether this switch from hypo- to hypermethylation results in gene and gene product repair is not known. One might hypothesize that –in their efforts to proliferate– tumor cell biomechanisms use the effects of both hypo- and hypermethylation. In human colorectal carcinoma, the promotor regions of various tumor suppressor genes (*p16, p53*) are frequently found to be hypermethylated, contributing to the evolution of the cancer (30). Obviously, there are preferred genomic sites of aberrant methylation which create conditions for malig-

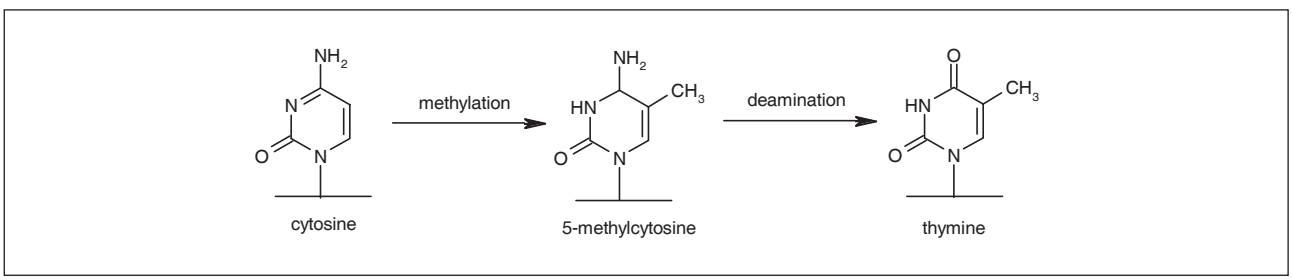

Fig. 4. Deamination of cytosine.

nant cells to proliferate. These results are consistent with the findings of Jahveri *et al*. (31) in human nasopharyngeal tumor cells, suggesting that folate depletion produces gene-specific rather than global hypomethylation instability of the DNA, making it susceptible to breakage, enhancing the chances for cellular transformation and increasing the risk of cancer.

A closer look at colorectal cancer

Epidemiological studies have provided evidence that folate intake is inversely related to the development of colorectal cancer. In his review, Giovannucci (28) points out that the results of epidemiological studies and clinical interventional studies are not uniformly consistent, but the majority of these studies suggest a moderately lower risk of colorectal adenoma or cancer associated with higher folate intake or plasma folate levels (32-34).

Animal studies generally support the results of human studies, and indicate that folic acid intake has a preventive effect on the development of precancerous and cancerous tissue in the colorectal area. In rats, dietary deficiency of folate increased the incidence of colonic premalignant and malignant lesions. A reverse effect was observed when increasing amounts of dietary folate were fed to the animals (35).

However, a different picture arises from very recent studies in humans. Cole *et al*. (36) performed a placebo-controlled, randomized clinical trial in 1,021 men and women with colorectal adenoma. Each person took 1 mg/day folic acid for up to 6 years. It was found that this supplemental dose did not lower the incidence of subsequent colorectal adenomas. On the contrary, at the first follow-up 3 years after the start of the study, the relative risk of at least one new adenoma was 1.04 (95% CI = 0.90-1.20). At the second follow-up 3-5 years after the first follow-up, the relative risk had increased to 1.13 (95% CI = 0.93-1.37). At that stage, the incidence of at least one advanced lesion was 11.6% for the folic acid and 6.9% for the placebo groups.

This disturbing result was corroborated by other recent findings (37). Cho *et al*. conducted the first epidemiological study of choline and betaine intake and the risk of distal colorectal adenoma in women. Betaine is a metabolite of choline and can transfer a one-carbon unit to homocysteine in order to produce methionine, followed by the generation of the universal methyl donor SAM. Dietary choline and folate can substitute for each other and one can compensate for deficiency of the other. This study was performed among 39,246 women who were initally free of cancer or adenoma and who had at least one endoscopy in the period 1984-2002. Unexpectedly, increasing choline intake was associated with an elevated risk of colorectal adenoma. For the highest quintile of intake, the relative risk amounted to 1.45 (95% CI = 1.27-1.67).

Both studies cast doubt on the earlier findings indicating that the intake of methyl group donors is associated with a reduced risk of colorectal adenomas (and possibly colorectal cancer), and urge reconsideration of previously formulated goals concerning the reduction of cancer incidence.

What is good and what is bad?

DNA fidelity is largely assured by proper *de novo* synthesis of thymidine and purines. Folate facilitates this biosynthesis and is essential for the production of the methyl donor SAM. However, there are two sides to every coin. Cancer cells have the propensity to upregulate folate receptors (folate receptor antagonists such as methotrexate are important drugs in cancer chemotherapy). In order to proliferate, the cancer cell takes advantage of the role of folate in nucleotide synthesis to support DNA formation. Therefore, folate not only prevents cancer, but it may also stimulate its growth. Moreover, it may enhance the progression of precancerous lesions by meeting the requirements for rapidly dividing tissues. This brings about a valid concern about the fact that excessive folic acid supplementation may increase the risk of cancer. As the bioavailability of folic acid is higher than that of folate, there is even more reason to question the beneficial effect of supplementation with folic acid (38).

In view of these opposing effects, the dose and the timing of folate intake are likely to be crucial for its overall clinical effect (39, 40). In a recent commentary, Ulrich and Potter (38) argue that the line between benefit and possible harm is not known, and that the net effect on carcinogenesis requires further research. These authors indicate that randomized, controlled trials may provide more appropriate answers, and that at present the scientific and clinical community has to rely on less explicit evidence, which suggests that “higher folate intakes generally correlate with a reduced risk”. In this context, it should be mentioned that a recent study hypothesized that the introduction of folic acid into food may be responsible for an increase in the colorectal cancer rate observed in the mid-1990s in the U.S. and Canada (41).

Randomized, controlled trials are also needed for other reasons. Recently, data have become available on the side effects of folate intake with regard to cognitive and immune function. As far as cognitive function is concerned, there is conflicting information about its decline (42) and improvement (43). Another reason to carry out more detailed epidemiological tests is to evaluate the role of alcohol and its interference with one-carbon metabolism. The chronic or acute effects of alcohol on folate metabolism have been reviewed by Mason and Choi (44). This subject is beyond the scope of this paper, but it should be mentioned that a causal relationship between alcohol consumption and altered folate metabolism on the one hand, and an increased cancer risk on the other, has been established (45).

Since the approval of folic acid fortification of cereal and grain products in 1998 in the U.S., tremendous progress has been made in understanding the biological effects of methylation in both animal and human studies. There is little doubt that folate supplementation can have a beneficial effect on the risk of colorectal cancer. If, how-

ever, modest levels of folate supplementation are exceeded, the risk of colorectal cancer may increase, and even accelerated cancer progression may occur (46).

Another phenomenon has also appeared: a more detailed analysis of epidemiological (case-control) studies has revealed that individuals with the variant genotype 677 TT and low folate intake show an increased risk for colorectal carcinoma, especially the elderly (47). This finding illustrates the well-known fact that polymorphisms may have a functional impact on protein function (see Box). Recently, in a Dutch case-control study it was found that in TT homozygotes with the *MTHFR* C677T gene variation, the occurrence of adenomas without promoter methylation was increased (48). Kono and Chen (49) have reviewed the consistency of the reported associations of polymorphisms with colorectal cancer and adenoma. There was a fairly consistent association between *MTHFR* C677T polymorphism and a decreased risk of colorectal cancer. The authors report that this decrease was observed in people with either high or low folate status. These confusing data strongly suggest that genetic polymorphism in folate metabolism results in interindividual differences in response.

The general conclusion is that an adequate folate status (corresponding to a daily chronic intake not exceeding 400 μg/day; see Ref. 28), together with adequate vitamin B_{12} plasma concentrations, confers protection against the development and progression of colorectal cancer. There is certainly a role for new disciplines such as molecular epidemiology, pharmacogenetics and nutrigenetics to elucidate gene-diet interactions, and to investigate the possibility of establishing individualized and targeted nutrient supplementation.

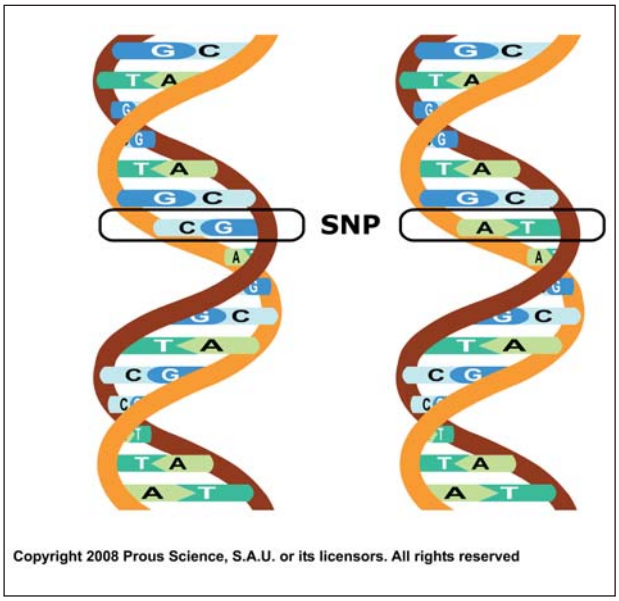

Fig. 5. C/T (cytosine/thymine) polymorphism (see BOX).

BOX: Polymorphism and cancer.

The cell nucleus contains chromatin granules composed of DNA. Chromosomes are made of, among other things, this DNA. The chromosome set differs from one species to another. DNA is a long chain of nucleotides known as a polynucleotide. Each nucleotide is composed of three compounds, phosphoric acid, deoxyribose and a base, linked together. DNA contains only four chemical bases, often referred to as building blocks, divided into two classes: purines and pyrimidines. In DNA, the purines are adenine (A) and guanine (G) and the pyrimidines are cytosine (C) and thymine (T). In the human genome, there are about 3.2 billion bases. DNA is made of two long, complementary chains (the famous double-helix structure made of two strands) in which A is always paired with T, and C with G, joined together by hydrogen bonds.

All information for protein synthesis is coded in DNA. A coding region contains genes which are unique DNA sequences within a chromosome. In order to synthesize a protein, another polynucleotide chain is formed on a DNA strand, which is called messenger RNA (mRNA) and which differs from DNA by having ribose rather than deoxyribose and uracil instead of thymine. mRNA brings the genetic code for protein synthesis to the cytoplasm, where protein synthesis takes place in the ribosomes. The genetic code dictates the synthesis of a specific protein with a specific function. This is achieved by molecular triplets, consisting of the RNA bases. For instance, UUU uniquely provides instructions for the incorporation of phenylalanine and CCC for proline. The process of transferring the information by DNA into RNA is called transcription. The transcription is initiated by a regulatory region close to a gene and is called a promoter. Surprisingly, only 3% of the human genome is used for instructions. The other remaining 97% of the genome has no known function and is called “junk DNA” containing noncoding regions.

It is amazing that there is very little (about 0.1%) variation in the DNA sequence of one person compared to another. This 0.1% variation determines, however, much of the individual characteristics. A variation occurring in a DNA sequence is called a polymorphism. A single nucleotide polymorphism occurs in a single nucleotide, *e.g.,* the C-G pair in DNA strand 1, may be changed to a T-A pair (called a C/T polymorphism; Fig. 5). In this case, there are two alleles, an alternative form of a gene, at the same locus in a chromosomal pair. These variations in DNA sequences may be present within the coding sequences of genes, the noncoding regions or the intergenic regions. Not all variations seem to have an effect, probably because they fall in the noncoding regions. If, however, polymorphism occurs in a coding or regulatory region, it may cause changes, either harmless (*e.g.*, the color of eyes or height) or harmful (causing diseases or susceptibility to diseases such as diabetes, cardiac disorders and cancer). Variations, also called mutations, may only cause malignant disease when many variations have occurred in different genes in the same cell.

In the context of this article, the common polymorphism in the gene coding for MTHFR affects DNA methylation and synthesis. In this gene, polymorphisms are associated with colorectal cancer and adenoma. Promoter methylation of the tumor suppressor and DNA repair genes in these genotypes is important due to possible silencing of gene transcription.

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