The B-vitamin folate is essential for one-carbon transfer reactions. These include methylation reactions, such as the methylation of DNA or other substrates (by use of S-adenosylmethionine, SAM, which is concurrently converted to S-adenosylhomocysteine), and also the synthesis of thymidine and purines, which are needed for DNA synthesis and repair (see Figure 1). A protective role of folate early in carcinogenesis is supported by epidemiologic observations of inverse associations with the intakes of folate or biomarkers of folate status, by animal experimental studies, and by molecular epidemiologic studies that show associations between folate-related polymorphisms and cancer risk, particularly for colorectal and hematopoietic malignancies. However, after the establishment of pre-neoplastic lesions, folate may foster carcinogenesis.

The biological mechanisms for the folate-cancer relationship are not yet well defined. The link of folate deficiency to reduced provision of nucleotides causing DNA damage subsequently is well established. However, the relationship between folate status and epigenetic changes is less clear. DNA methylation disturbances that occur during carcinogenic processes involve both global DNA methylation, largely at repeat or satellite regions of DNA, as well as concurrent promoter-specific DNA hypermethylation, which is associated with gene silencing. Global DNA hypomethylation may be linked to carcinogenesis by causing genomic instability, but may also, when present to a modest degree, reduce the rate of C > T transition mutations. These C > T transitions are the most common type of mutation in the genome, and methylated CpG sites are considered “mutational hotspots” for C > T changes. For example, C > T transitions are common in the p53 tumor suppressor gene. Our group has investigated the 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in relation to p53 mutation spectra and reported that the 677 TT genotype, which reduces enzyme activity to about 30% of wild type and is associated with reduced levels of genomic DNA methylation, is associated with a reduced risk of C > T transition mutations in colorectal cancers, specifically at CpG sites. Although these results were based on a limited number of cancer cases with these specific type of mutations, they support a role of mild DNA hypomethylation in reducing risk of C > T mutations.

Observations from human intervention studies suggest that folate status can affect the degree of global DNA methylation, as measured by methyl-acceptance assays, probably more so among individuals with the MTHFR 677 TT genotype. The role of folate status in determining promoter methylation and associated gene silencing is less clear: Epigenetic gene silencing during development is modifiable by one-carbon nutrients, yet epigenetic silencing during adulthood may be caused by other mechanisms, independent of folate status. Few epidemiologic or intervention studies have investigated the relationship between folate status and promoter hypermethylation/gene silencing or the CpG island methylator phenotype (CIMP), which is characterized by widespread promoter hypermethylation and present in about 18% of colorectal cancer cases. Slattery et al. did not observe an association between dietary folate and CIMP or the associated BRAF mutations in a large case-control study. However, in the same study population, we observed that some associations between polymorphisms in folate-metabolism and colorectal cancer may differ by CIMP status. For example, the MTHFR 677 TT genotype was associated with a reduced risk of CIMP-negative tumors [odds ratio (OR) 0.6, 95% confidence interval (CI) 0.5–0.9], yet not of
CIMP-positive tumors (OR 1.0, 95%CI 0.6–1.6). However, these studies require confirmation in other study populations. A limitation of this work was also that most of the polymorphisms investigated were not directly related to the methionine cycle or DNA methylation. For example, we had not genotyped the study population for a polymorphism in the gene encoding the maintenance DNA methyltransferase, \textit{DNMT3b}, \textit{–149C>T}, that had been previously associated with cancer risk. In a parallel case-control study of colorectal adenoma and polyp-free controls (as confirmed by colonoscopy), we observed that the \textit{DNMT3b–149C>T} variant increases risk of colorectal adenoma in individuals with a diet low in one-carbon nutrients.\textsuperscript{26}

New insights into the complex nature of folate and DNA methylation in carcinogenesis can be obtained by mathematical modeling. Our group has developed and validated a mathematical model based on the biochemical properties of folate-mediated one-carbon metabolism (see Figure 1).\textsuperscript{27} The model uses differential equations based on known enzyme kinetics and biochemical properties, including biochemical regulatory mechanisms. The model can be used to simulate gene-gene or gene-environment interactions, as well as specific experimental conditions. Predictions from this “\textit{in silico} metabolomics” approach can help target or inform epidemiologic and experimental research by providing mechanistic information and extending studies to experiments that are not feasible. It may also provide an avenue for pathway-based epidemiologic data analyses.\textsuperscript{28} Software from this model will shortly be available publicly (for more information, please contact the authors).

The mathematical model of folate-mediated one-carbon metabolism currently reflects the “methylation reaction rate” and thus not the actual rate at which specific \textit{CpG} sites are methylated. It also does not take into account specific regulatory mechanisms, including histone configuration. Thus, its outputs may best be interpreted as “methylation capacity” and it will most likely reflect global genomic DNA methylation.

We have validated this model extensively and have shown that it replicates clinical conditions, such as vitamin B\textsubscript{12} deficiency, as well as observed genotype-biomarker relationships.\textsuperscript{27} Because of the very high levels

\textbf{Figure 1 Overview of folate-mediated one-carbon metabolism (simplified) links to methylation reactions and nucleotide synthesis.}

\textit{Abbreviations:} AICAR, 5-aminomimidazole-4-carboxamide ribonucleotide; AICARFT, 5-aminomimidazole-4-carboxamide ribonucleotide transformylase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dUMP, deoxyuridine monophosphate; GAR, glycaminide ribonucleotide; GART, glycaminide ribonucleotide transformylase; hFR, human folate receptor; MS, methionine synthase; MT, methyltransferases; MTHFR, 5,10-methylene tetrahydrofolate reductase; RFC, reduced folate carrier; SAH (AdoHcy), S-adenosylhomocysteine; SAM (AdoMet), S-adenosylmethionine; THF, tetrahydrofolate; dTMP, deoxythymidine monophosphate; TS, thymidylate synthase; X, a variety of substrates for methylation.

Adapted from Ulrich et al. (2003)\textsuperscript{35} with permission.
of folate intakes in subgroups of the population, particularly among supplement users, and in light of recent concerns about excessive folate possibly fostering carcinogenesis,\textsuperscript{26,28,30} we have modeled the effects of excessive folate levels on the biochemical properties of folate metabolism. Intriguingly, we observed that the reaction velocities of most enzymes in the folate cycle decrease at high intracellular folate concentrations.\textsuperscript{31} This observation can best be explained by the role of folate enzymes as folate-binding proteins; folate binding inhibits the enzymes at very high folate levels.\textsuperscript{27}

A second important observation from the model is that many of the longer-range regulatory mechanisms in folate-metabolism have apparently evolved to protect the cellular "methylation rate" against fluctuations in methionine and folate inputs.\textsuperscript{32} In addition to models of hepatic and epithelial cellular folate-mediated one-carbon metabolism, we have modeled the interplay between cytosolic and mitochondrial folate metabolism\textsuperscript{33} and performed an exploration of sensitivity and robustness in folate-mediated one-carbon metabolism towards large variations in system inputs. Results indicate that daily fluctuations in amino-acid input from meals cause large fluctuations in some metabolite concentrations and reaction velocities while others (specifically those involved in nucleotide synthesis and DNA methylation) remain quite stable because of regulatory mechanisms.\textsuperscript{34} In the future, we are planning to expand this interdisciplinary modeling work towards whole-body folate status, and we intend to incorporate a variety of methylation reactions to support and complement experimental research.

Overall, the role of folate in DNA methylation and its impact on carcinogenesis requires more research before definite conclusions can be drawn. This includes epidemiologic studies with a comprehensive assessment of folate-related biomarkers, as well as of genetic variability in folate-mediated one-carbon metabolism, particularly in genes related to methylation, such as the DNA methyltransferases and methionine adenosyltransferases. These assays need to be performed in conjunction with state-of-the-art assays for the assessment of DNA methylation changes in target tissues.

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