Folate, DNA methylation, and gene expression: factors of nature and nurture[1,2]

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Folate provides the one-carbon units required for purine and thymidylate syntheses and for methylation of a wide variety of essential biological substances, including phospholipids, proteins, DNA, and neurotransmitters. In the activated methyl cycle, folate, as N^5-methyltetrahydrofolate, supplies a methyl group to convert homocysteine to methionine, which is then converted to the universal methyl donor, S-adenosylmethionine. Although nutrients other than folate supply or transport methyl groups (methionine, choline, and vitamin B-12), only folate is capable of de novo generation of one-carbon groups. The functional importance of folate for in vivo methylation reactions was shown in the many studies indicating an inverse relation between folate nutriture and plasma homocysteine, an associative or possibly causal risk factor for vascular disease.

The report by Rampersaud et al (1) in this issue of the Journal, which shows an effect of folate deficiency on methylation of lymphocyte DNA in healthy elderly women, helps document a new functional role of folate that has potentially important implications. The findings reported confirm and extend previous research showing that lack of folate or methyl group nutrients in the diet may cause DNA hypomethylation in both rats and humans (2, 3). In the human studies, DNA hypomethylation was observed with use of an in vitro test that measured the uptake of [^{3}H]methyl groups from S-adenosylmethionine into certain CpG dinucleotide sequences of genomic DNA isolated from lymphocytes (1, 3). Somewhat surprisingly, significant DNA hypomethylation occurred during moderate folate depletion without clinical signs of folate deficiency. That lymphocyte DNA hypomethylation occurred over a period of 7–9 wk is also surprising because the mean residence time for total body folate has been estimated by kinetic modeling as $\approx$200 d (4). Apparently, the dynamics of some folate-dependent methylation reactions are much more sensitive to changes in folate intake than are alterations of total body folate pools.

Among the most significant implications of the new information on folate and DNA methylation are the hypothesized mechanisms that underlie the links between folate nutriture and carcinogenesis (5). The most attractive of these hypotheses centers on the role of DNA methylation as a reversible signal for gene expression (6). Recent work has shown that methylation silences gene expression by inducing histone acetylation, which is involved in transcriptional repression, and that the repression can be alleviated by demethylation of the DNA (7). Evidence of links between DNA methylation, gene expression, and carcinogenesis is substantial.

The evidence includes the common findings of genomic hypomethylation of DNA in tumor tissues and effects of site-specific alterations in DNA methylation on expression of protective enzymes, tumor suppressor proteins, and oncogenes (5, 6).

Although the new evidence linking folate, DNA methylation, and gene expression has many important implications, caution is needed in the interpretation of the data because of the complex pathways and sometimes inconsistent observations that may interrelate folate nutriture, DNA methylation, and carcinogenesis. Because DNA methylation is a potent suppressor of gene expression, alterations in DNA methylation secondary to folate deficiency may affect the expression of both oncogenes and tumor suppressor genes. Before developing hepatic tumors, rats fed methyl-deficient diets show hypomethylation of DNA at specific sites within the protooncogenes c-*myc*, c-*fos*, and c-Ha-*ras* along with elevated amounts of the corresponding messenger RNAs (8). Although DNA hypomethylation may lead to overexpression of oncogenes, other evidence indicates that inactivation of tumor suppressor genes by hypermethylation of promoter regions is associated with a variety of human neoplasms and tumors (6, 9). In rats, chronic folate or methyl deficiency was shown to induce site-specific methylation within the p53 gene associated with reduced amounts of messenger RNA (2).

Even though epidemiologic studies support an inverse association between folate status and human cancer risk, results from experimental animal studies are conflicting (10). Depending on the time of dietary intervention and the pathophysiologic state of the cells, folate deficiency can act as a procarcinogen or an anticarcinogen. For example, DNA damage secondary to folate deficiency can promote neoplastic transformation in normal cells but by the same mechanism induce apoptosis and tumor regression in established tumor cells. Similarly, folate supplementation can be preventive before neoplastic transformation but act as a promoter in neoplastic cells. Although the conflicting results from animal studies may be due to different study conditions, it is also important to note that the effect of chronic folate deficiency on

DNA methylation patterns varies with the stage of carcinogenesis by inducing global hypomethylation in preneoplastic cells and regional hypermethylation in tumor cells (2). Thus, it is apparent that an important in vivo methylating agent such as folate may have diverse effects on the expression of genes related to carcinogenesis and that much more information is needed to define the specific roles and concentration ranges of folate involved in the various stages of carcinogenesis. In addition to the differences between genomic and site-specific DNA methylation alluded to above, the authors of the present report (1) caution that the assessment of DNA methylation status has not been standardized and that the relation of the observed DNA hypomethylation in leukocytes to the methylation status of DNA in other body tissues is not known.

DNA methylation may also affect carcinogenesis by affecting DNA repair and chromosome stability. The promoter region of the mismatch repair gene *hMLH1* was found to be frequently hypermethylated and silenced in cases of sporadic colorectal tumors, and other evidence indicates that the proficiency of *hMLH1* in several cell lines is related to the methylation patterns of several cellular genes (6). Evidence suggests that DNA methylation regulates gene expression through reversible alterations of histone acetylation that affect chromatin structure and accessibility of transcription factors to their binding sites (7). The above results suggest that DNA methylation may play a role in the findings that folate and vitamin B-12 status affect DNA and chromosome fragility in humans (11, 12). A recent folate and vitamin B-12 supplementation study showed correlations of lymphocyte chromosome damage markers with plasma vitamin B-12 (inverse) and homocysteine (direct). No relation of chromosome damage to folate or DNA methylation status was observed in this study; however, the subjects were not folate deficient or given a low-folate diet (13).

The findings that in vivo folate and methyl deficiencies result in DNA hypomethylation, aberrant gene expression, and chromosomal instability suggest that folate-related methylation deficits may play a role in the occurrence of certain birth defects. This hypothesis was tested by James et al (14), who assessed folate and methyl metabolism in mothers of children with Down syndrome and in age-matched controls. Plasma homocysteine concentrations and the frequency of the common C-to-T substitution at nucleotide 677 of the methylenetetrahydrofolate reductase gene were both greater in the mothers of children with Down syndrome, suggesting that the syndrome may be partially explained by a diet-genotype interaction that results in abnormal folate metabolism. In the same report, some evidence is presented that single nucleotide polymorphisms of other genes involved in the methyl cycle may contribute to the occurrence of both Down syndrome and neural tube defects. The recent identification of numerous polymorphisms of genes involved in methylation pathways, and the important roles of folate, vitamin B-12, and methionine in these pathways, suggests that many complex gene-environment interactions may affect DNA methylation, gene expression, and a variety of clinical outcomes.

The recent data suggest that for in vivo methylation processes that may relate to the occurrence of birth defects and chronic disease, factors of both nature and nurture are important. Further research in this area will require attention to a multitude of these factors but holds great promise for sorting out their relative importance.

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