Commentary

Folate Supplementation: Too Much of a Good Thing?

Cornelia M. Ulrich and John D. Potter

Fred Hutchinson Cancer Research Center, Cancer Prevention Program, Seattle, Washington

Folate is critical for nucleotide synthesis and methylation reactions and has been associated with a number of health benefits. The accumulation of good news—ranging from the established reduction of neural tube defects to the putative prevention of several types of cancer, cardiovascular disease, and possibly dementia—has made it seem a ''wonder drug'' that is not only inexpensive, but also safe for use as a chemopreventive agent. Although research to date supports several of these claims, some warning lights have appeared recently, challenging us to adopt a more nuanced view of folate use and raising the need for investigations of the potential health hazards of excessive intakes. Recent data also suggest the need to distinguish between naturally occurring folates and folic acid, the synthetic form added to supplements and fortified foods, further complicating the already complex story of a potent vitamin.

History of Folate and Fortification

Folate was isolated in 1941 from spinach and named after the Latin word *folium* (= leaf; ref. 1). Although the initial impetus for research on folate metabolism was to find a cure for anemia, it was soon recognized that the administration of folate enhanced the growth of existing tumors and that folate metabolism may be a promising target for anticancer drug design (2). Already developed by the late 1940s, folate antagonists, such as methotrexate, have rapidly become a mainstay of cancer chemotherapy (3). Because of the role of folate in nucleotide synthesis, deficiency affects primarily rapidly dividing tissues, such as the epithelium of the gastrointestinal tract, hematopoietic cells, and tumors. This increased need of proliferating tissues for folate explains why macrocytic anemia is one of the clinical manifestations of folate deficiency. Similarly, the crucial role of folate in preventing neural tube defects may be attributed to the high fetal requirement for folate to sustain rapid cell division; clinical trials have shown a clear reduction in neural tube defects with periconceptional folic acid administration (4-6). This success story led policy makers, including the USPHS in 1992 and the Institute of Medicine in 1998, to recommend that all women of reproductive age consume 400 μg folic acid daily from supplements or fortified foods (7, 8). Unfortunately, health promotion efforts targeting childbearing women have been largely unsuccessful in achieving this level of intake. Therefore, policy makers opted for generalized folic acid fortification of flour and uncooked cereal grain products at a targeted level of 140 μg/100 g. This level of fortification was mandatory in the United States by January 1998 and was also implemented in Canada (9, 10). Initial data suggest that this public health measure was successful, resulting in a substantial reduction in the prevalence of neural tube defects (11-14).

Biomarker studies postfortification showed dramatic increases in blood measurements of folate and a concurrent decrease in plasma homocysteine (which is inversely associated with folate status; refs. 15, 16). Although these reports were reassuring in that the population fraction with low serum folate was minimized (from 16% to 0.5%; ref. 16), they also raised concerns that fortification exceeded the original daily intake target by as much as 2-fold (17-19). This intake of folic acid from fortified food (~100 to 200 μg/d) coincides today with high consumption of nutritional supplements in the U.S. population (~400 μg/standard multivitamin; ref. 20) as well as increased availability and marketing of nutrition bars, drinks, and other fortified foods (often supplemented at 400 μg/serving), resulting in a markedly elevated intake of folic acid in the population from multiple sources.

Folic Acid in Supplements—Above the Tolerable Upper Level?

The safety of chronic very high intakes of folic acid is largely unknown. The Institute of Medicine recommends a tolerable upper intake level for folic acid from supplements or fortified foods of 1,000 μg/d for adults and between 300 and 400 μg/d for children between the ages 1 and 8 (21). These upper intake levels were developed primarily to avoid masking the anemia and missing the neuropathy of vitamin B12 deficiency because very few data were available on other possible adverse effects of chronic high intakes (21). Putting the upper intake level in context, an adult who consumes two standard multivitamins daily (at 400 μg each) can easily exceed the daily upper intake level, as can a child who consumes substantial amounts of breakfast cereals (which are often supplemented at a level of 400 μg/serving). However, the clinical significance of the upper intake levels is not well established (see research needs below) and they were instituted as a first attempt to raise awareness that not all levels may be safe.

The use of nutritional supplements in the United States is high and probably increasing (20, 22, 23). Supplement use is greatest among older individuals, and more common among women and those who are White or Asian, with distinct regional differences (20, 22, 24). National Health and Nutrition Examination Survey reports that 63% of individuals over age 60 years take a dietary supplement: 40% take folic acid–containing multivitamins, 7% B-complex vitamins, and 2% folic acid supplements (20). Among female supplement users, 55% take multiple supplements and 17% take four or more supplements (20). The INTERMAP study of middle-aged participants reports that folate intakes among supplement users are ~2-fold higher than among non–supplement users (~600 versus ~300 μg/d; ref. 24). Thus, it is not surprising that more than half of women over age 60 years (the group with highest supplement use) in National Health and Nutrition Examination Survey have serum folate levels in excess of 40 nmol/L (16). The potential health effects of these high concentrations are

Cancer Epidemiol Biomarkers Prev 2006;15(2):189–93

Grant support: NIH grants CA105437 and CA59045 (C.M. Ulrich).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Neli Ulrich, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, P.O. Box 19024, M4-B402, Seattle, WA 98109-1024. Phone: 206-667-7617; Fax: 206-667-7850. E-mail: nulrich@fhcrc.org

doi:10.1158/1055-9965.EPI-152CO

unclear; even more unclear is whether such high intakes prevent cancer, especially among the elderly.

A Dual Role for Folate in Carcinogenesis?

An increasing body of evidence suggests that folate plays a dual role in carcinogenesis, involving both the prevention of early lesions and potential harm once preneoplastic lesions have developed. Observational studies suggest that high folate intakes reduce cancer risk, particularly for malignancies of the colon, pancreas, esophagus, stomach, and possibly cervix and breast. However, folate can promote the growth of existing cancers and, as noted above, antifolate drugs are potent chemotherapeutic agents. Initial experimental studies have suggested that folate supplementation can enhance the carcinogenic progression of mammary tumors (25). So where is the line between benefit and possible harm? Elegant work by Kim et al. (26) has shown that the dose and timing of folate interventions may be critical. For example, in two colorectal cancer mouse models, modest doses of folate supplementation suppressed the development and progression of colorectal cancer. However, this beneficial effect was observed only for folate given before the establishment of neoplastic foci in the intestine (27, 28). Once preneoplastic lesions were present, dietary folate enhanced the development and progression of colorectal cancer (27, 28). Similar results in a rat model of breast cancer suggest that folate deficiency inhibited, rather than enhanced, cancer development (25, 29). Preliminary results from the first randomized-controlled trial of folic acid for chemoprevention of colorectal polyps have been recently reported at a national meeting (30). More than 1,000 participants with a recent history of colorectal adenomas were randomly assigned to 1 mg folic acid daily, with or without aspirin. Follow-up colonoscopies were scheduled ~3 years after the initial endoscopy and supplementation continued until a second surveillance exam. In this trial, folic acid use did not prevent the recurrence of colorectal adenomas (rate ratio, 1.04). However, participants in the folic acid group tended to have greater adenoma multiplicity, with a significant increase among those who continued treatment throughout the second follow-up interval (rate ratio, 1.44; 95% confidence interval, 1.03-2.02; ref. 30). These preliminary findings are consistent with a role of folate in fostering the progression of premalignant lesions. Although participants had the initial polyp removed before study entry, the increased risk of later multiple metachronous polyps suggests that, among a subgroup of individuals who had multiple preneoplastic lesions, folic acid supplementation may have promoted their growth. The results also suggest that this effect may be modest at supplementation below 1,000 μg/d, but do not provide any information about the effects on growth of existing polyps that are not detected during a colonoscopy.

What are the possible mechanisms of a dual role for folate in carcinogenesis that depends on timing and perhaps dose? The function of folate in nucleotide synthesis may be central. Folate is essential for the synthesis of thymidine via thymidylate synthase, and of purines, as illustrated in Fig. 1. Folate deficiency results in a reduced production of thymidine and misincorporation of uracil into DNA (31, 32). During the excision of uracil, single-strand breaks and, eventually, double-strand breaks can occur (32). Because of the lack of reliable biomarkers, the effects of folate deficiency on purine synthesis and apurinic sites in DNA are less well established. However, some epidemiologic studies suggest that this common form of DNA damage may be a critical mechanism linking folate to cancer risk (33, 34). If we assume that folate deficiency in a rapidly proliferating tissue (such as the colon) enhances genomic instability, then the probability of a loss of function of genes that prevent adenoma formation increases. Although an initial animal study did not observe an effect of folate deficiency on the rate of APC mutations, the sample size may have been too small to obtain reliable results (35). After a small tumor or microadenoma has been established, this tumor may grow more rapidly with folate supplementation, due to the greater provision of nucleotides: Experimental results from Melnyk et al. (36) show that folate repletion to folate-deficient cells results in a promotional stimulus, and that these cells, if injected into nude mice, show greater tumorigenic potential and aggressive growth.

How could one estimate the net effect of these two potentially competing mechanisms of folate supplementation

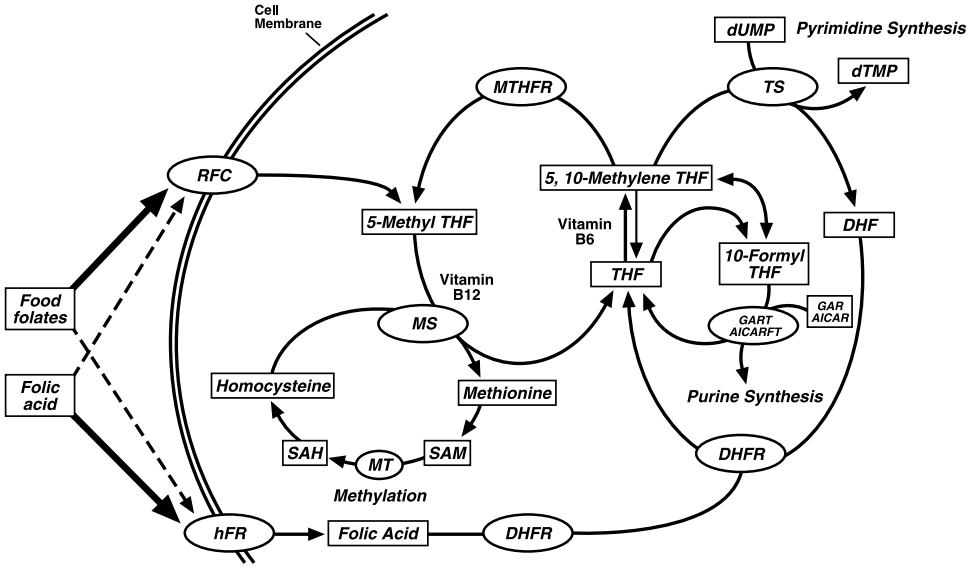

Figure 1. Overview of folate metabolism, entry of folic acid and physiologic folates into the cell, and links to methylation reactions and nucleotide synthesis (modified with permission from ref. 3). *THF,* tetrahydrofolate; *DHF,* dihydrofolate; *RFC,* reduced folate carrier; *hFR,* human folate receptor; *MTHFR,* 5,10-methylenetetrahydrofolate reductase; *DHFR,* dihydrofolate reductase; *GART,* glycinamide ribonucleotide transformylase; *AICARFT,* 5-amino-imidazole-4-carboxamide ribonucleotide transformylase; *AICAR,* 5-aminoimidazole-4-carboxamide ribonucleotide; *GAR,* glycinamide ribonucleotide; *SAM (AdoMet), S*-adenosylmethionine; *SAH (AdoHcy), S*-adenosylhomocysteine; *SHMT,* serine-hydroxy-methyltransferase; *MS,* methionine synthase; *TS,* thymidylate synthase; *MT,* methyltransferases.

on carcinogenesis? The epidemiologic evidence to date suggests that higher folate intakes generally correlate with a reduced risk, thus implying an overall reduction in risk. (Nonetheless, it needs to be remembered that folate intake is highly correlated with supplementary vitamin intake in general, which raises the potential for confounding.) Building on a previously developed mathematical model of colorectal carcinogenesis (37), we have investigated the question of a ''net effect'' of the putative opposing mechanisms by simulating the effect of a folic acid intervention on reduction of mutations and increase in tumor growth simultaneously. Initial results suggest that the net effect of folic acid fortification on individual colon cancer risk is modified by age: Whereas children may be more likely to experience reduced colorectal cancer rates in the future, rates among middle-aged adults are likely to increase.[1] The findings of this mathematical model clearly illustrate the need for a better quantification of the molecular effects of folate (see below).

Folate and Carcinogenesis—Yet Another Discrepancy between Observational Studies and Clinical Trials?

When the results from the folate polyp-prevention trial (30) are published, it will be important to interpret them carefully and to avoid rejecting folate as a chemoprevention agent altogether. The discrepancy between results from observational studies and randomized controlled studies has recently been discussed in this journal (38). The story of folate adds yet another possible explanation for such disparate findings: differences in biological effects of potent agents depending on the stage of carcinogenesis. Almost all chemoprevention trials aim at prevention among those with a history of earlier lesions (precancer or cancer), largely because of the higher risk of subsequent tumors in such patients, and thus reduced cost and time and increased power. However, we should not lose sight of the limitations of this approach: As discussed above, for folate, the experimental evidence suggests that administration is beneficial before the appearance of preneoplastic lesions, but potentially harmful after. Whereas a randomized trial that evaluates the influence of folic acid administration on recurrent polyps after a primary diagnosis will be able to answer exactly that question, it is unlikely to provide relevant information regarding folic acid supplementation early in life. Primary prevention trials of folate (intervention before early lesions) would be expensive and of long duration. We may need to continue to rely on less explicit evidence, including that from ecologic studies of folic acid fortification by age cohort, observational epidemiologic studies, and animal experiments.

Folate and Cardiovascular Disease

The discrepancy between findings from observational studies and randomized controlled trials is now also apparent for another possible health benefit attributed to folate. Because of the unequivocal homocysteine-lowering effects of folic acid supplementation and experimental data suggesting a role of homocysteine in endothelial damage, the prevention of cardiovascular disease was assumed to be another health benefit of increased folate intakes (39). Unfortunately, the first results from randomized controlled trials with actual disease outcomes provide no evidence for such an effect (40). We may need to be more cautious of biomarkers of disease until they are established as being in the causal pathway.

[1]E.G. Luebeck, et al., unpublished data.

Other Potential Deleterious Effects of High Folic Acid

Two recent studies raise concern about the implications of very high folic acid intakes on other health outcomes. A prospective cohort of 3,718 elderly individuals (>65 years) who participated in the Chicago Health and Aging Project investigated cognitive decline over the course of 6 years in relation to dietary intakes of folate and vitamin B12. Unexpectedly, high folate intake was associated with a faster rate of cognitive decline. Those in the highest quintile of folate intake (comprised largely of supplement users with a mean intake of 742 μg/d) had a statistically significantly more pronounced decline, and this association was particularly strong for supplement use in excess of 400 μg/d. Limitations of the study were its lack of biomarker assessments and the potential for confounding by indication. Nevertheless, these unexpected findings urge us toward further research on the cognitive implications of high levels of folic acid in older individuals.

An additional concern has arisen in relation to immune function: In a preliminary cross-sectional study of 104 postmenopausal women, we reported an inverse U-shaped relationship between folate from dietary sources and supplements and natural killer cell cytotoxicity (41). Natural killer cells are part of the innate immune response and low cytotoxicity may increase cancer risk (42). Unmetabolized folic acid was detected in 78% of fasting plasma samples from the participants. This is the first study reporting the presence of this compound in healthy individuals who are not subjected to pharmacologic doses of folic acid. The presence of unmetabolized folic acid was associated with decreased natural killer cytotoxicity, and a trend toward lower natural killer cytotoxicity with greater amounts of folic acid in plasma was observed among older women (>60 years). Although the study should be considered preliminary, it highlights the need for a better understanding of the relation of folate to immune function. No clear mechanism for the association is established. It is possible that a low capacity to metabolize large amounts of folic acid, perhaps caused by polymorphisms in the *dihydrofolate reductase* (*DHFR*) gene (43), may play a role. As shown in Fig. 1, DHFR is critical for reducing folic acid for entry into folate metabolism.

Folic acid is inexpensive to produce and is characterized by greater bioavailability than the natural folates (44). Nevertheless, it is not equivalent to the polyglutamated forms of food folates and enters primarily via a different carrier system (Fig. 1); thus, it may have different effects on folate-binding proteins and transporters (45). Another explanation for the possible adverse effects of excessive intakes of folate is derived from a mathematical simulation model of folate metabolism (46). Several enzymes in this pathway function as folate-binding proteins, which simultaneously inhibits their enzyme activity. The biochemical properties of the pathway, as modeled, suggest that there is an optimum folate concentration above which folate-reaction velocities decline. This theoretical work requires confirmation in experimental studies, yet illustrates how an interdisciplinary approach can help understand different aspects of this complicated pathway.

Future Research Needs

It is clear that the relationships between folate and health outcomes are complex; further, we need a better understanding of the relevant biological mechanisms to avoid misinterpretation. For solid tumor carcinogenesis, we need experimental studies that quantify possible effects of folate on growth of aberrant crypt foci, polyps, and similar early lesions in other tissues. The role of folate in hematopoietic malignancies also needs further research: Hematopoietic cells are particularly sensitive to folate deficiency and pediatric leukemias and

lymphomas are commonly treated with antifolate drugs (47). For malignancies treated with antifolates, we also need to understand better whether treatment efficacy is altered by excessive supplement use.

Folate not only plays a role in nucleotide synthesis but is also critical for the provision of *S*-adenosylmethionine (Fig. 1), the universal methyl donor. DNA methylation of promoter regions has been established as one mechanism of gene regulation (48). A recent study of agouti mice shows that methyl supplementation (including folic acid) can alter the epigenetic gene regulation of offspring (49). Pennisi (50) subsequently reported on folate supplementation as a means of modifying morphology in tails of mice with a transposable element in the *axin* gene. Whether there are other effects of excessive perinatal folic acid supplementation on epigenetic mechanisms, less benign than changes in coat color or unkinking of tails, defines another area of research need.

We also need more research on the safety of folic acid per se. Because this compound is now found in the bloodstream, data are needed on whether there are implications for the transport of natural folates. Human studies of folic acid at multiple levels, lasting several months or even years, and monitoring all beneficial and adverse effects described to date are a critical step. To understand the health effects of more chronic long-term intakes of doses at the upper intake level, studies ancillary to the recently completed randomized controlled trials of cancer chemoprevention are needed, targeting the specific outcomes of the animal and human studies described above, particularly cognitive and immune function. This is perhaps a unique opportunity to settle the issue of safety of long-term high intakes.

Epidemiologic studies should expand their investigations toward the high end of folate intakes and carefully evaluate the potential for confounding in the interpretation of results. Finally, there are multiple genetic polymorphisms in folate metabolism that result in interindividual differences in response (51). A full genetic screen of mutations in this biological pathway has not yet been undertaken. Exploration of the effect of multiple genetic variants, under different dietary conditions, on critical biomarkers relevant to carcinogenesis and other health outcomes is essential.

It remains unclear whether the possible deleterious effects of high folic acid outweigh the known and potential benefits. Further, this balance may differ across individuals and populations, by genetic characteristics and by life stage. Because of the high intake of folic acid from supplements and fortified foods in a large fraction of the population, these questions need answers soon.

Acknowledgments

We thank colleagues, postdoctoral fellows, and students who have worked with us on research related to folate and health.

References

1. Mitchell HK, Snell EE, Williams RJ. The concentration of ''folic acid''. J Am Chem Soc 1941;63:2284–1.
2. Hoffbrand AV, Weir DG. The history of folic acid. Br J Haematol 2001;113:579–89.
3. Ulrich CM, Robien K, Sparks R. Pharmacogenetics and folate metabolism—a promising direction. Pharmacogenomics 2002;3:299–313.
4. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation [see comment]. N Engl J Med 1832;327:1832–5.
5. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet 1991;338:131–7.
6. Berry RJ, Li Z, Erickson JD, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention [see comment][erratum appears in N Engl J Med. 1999 Dec 9;341(24):1864]. N Engl J Med 1485;341:1485–90.
7. Centers for Disease Control and Prevention. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Morb Mortal Wkly Rep 1992;41:1.
8. Institute of Medicine. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (District of Columbia): National Academy Press; 1998.
9. US Department of Health and Human Services Food and Drug Administration. Food standards: amendment of the standards of identity for enriched grain product to require addition of folic acid. Fed Regist 1996;61:8781.
10. Health Canada. Regulations amending the Food and Drug Regulations (1066). Can Gaz Part 1 1997;131:3702–37.
11. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA 2001;285:2981–6.
12. Williams LJ, Mai CT, Edmonds LD, et al. Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. Teratology 2002;66:33–9.
13. Gucciardi E, Pietrusiak MA, Reynolds DL, Rouleau J. Incidence of neural tube defects in Ontario, 1986-1999. CMAJ 2002;167:237–40.
14. Persad VL, Van den Hof MC, Dube JM, Zimmer P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. CMAJ 2002;167:241–5.
15. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340:1449–54.
16. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. Am J Clin Nutr 2005;82:442–50.
17. Choumenkovitch SF, Selhub J, Wilson PW, Rader JI, Rosenberg IH, Jacques PF. Folic acid intake from fortification in United States exceeds predictions. J Nutr 2002;132:2792–8.
18. Shane B. Folate fortification: enough already? Am J Clin Nutr 2003;77:8–9.
19. Quinlivan EP, Gregory JF III. Effect of food fortification on folic acid intake in the United States. Am J Clin Nutr 2003;77:221–5.
20. Radimer K, Bindewald B, Hughes J, Ervin B, Swanson C, Picciano MF. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999-2000. Am J Epidemiol 2004;160:339–49.
21. Institute of Medicine. Dietary Reference Intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (District of Columbia): National Academy Press; 1998.
22. Shikany JM, Patterson RE, Agurs-Collins T, Anderson G. Antioxidant supplement use in Women's Health Initiative participants. Prev Med 2003;36:379–87.
23. Brownie S, Myers S. Wading through the quagmire: making sense of dietary supplement utilization. Nutr Rev 2004;62:276–82.
24. Archer SL, Stamler J, Moag-Stahlberg A, et al. Association of dietary supplement use with specific micronutrient intakes among middle-aged American men and women: the INTERMAP Study. J Am Diet Assoc 2005;105:1106–14.
25. Baggott JE, Vaughn WH, Juliana MM, Eto I, Krumdieck CL, Grubbs CJ. Effects of folate deficiency and supplementation on methylnitrosourea-induced rat mammary tumors. J Natl Cancer Inst 1992;84:1740–4.
26. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 2004;80:1123–8.
27. Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Res 2000;60:5434–40.
28. Song J, Sohn KJ, Medline A, Ash C, Gallinger S, Kim YI. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/−Msh2−/− mice. Cancer Res 2000;60:3191–9.
29. Kotsopoulos J, Sohn KJ, Martin R, et al. Dietary folate deficiency suppresses *N*-methyl-*N*-nitrosourea-induced mammary tumorigenesis in rats. Carcinogenesis 2003;24:937–44.
30. Cole BF, Baron JA, Sandler RS, et al. A randomized trial of folic acid to prevent colorectal adenomas [abstract]. AACR 96th Annual Meeting; 2005.
31. Duthie SJ, Grant G, Narayanan S. Increased uracil misincorporation in lymphocytes from folate-deficient rats. Br J Cancer 2000;83:1532–7.
32. Duthie SJ, Narayanan S, Brand GM, Pirie L, Grant G. Impact of folate deficiency on DNA stability. J Nutr 2002;132:2444–9S.
33. Ulrich CM, Bigler J, Bostick R, Fosdick L, Potter JD. Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenomas. Cancer Res 2002;62:3361–4.
34. Ulrich CM, Curtin K, Potter JD, Bigler J, Caan B, Slattery ML. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2509–16.
35. Sohn KJ, Puchyr M, Salomon RN, et al. The effect of dietary folate on Apc and p53 mutations in the dimethylhydrazine rat model of colorectal cancer. Carcinogenesis 1999;20:2345–50.
36. Melnyk S, Pogribna M, Miller BJ, Basnakian AG, Pogribny IP, James SJ. Uracil misincorporation, DNA strand breaks, and gene amplification are associated with tumorigenic cell transformation in folate deficient/repleted Chinese hamster ovary cells. Cancer Lett 1999;146:35–44.
37. Luebeck EG, Moolgavkar SH. Multistage carcinogenesis and the incidence of colorectal cancer. Proc Natl Acad Sci U S A 2002;99:15095–100.

38. Meyskens FL, Jr., Szabo E. Diet and cancer: the disconnect between epidemiology and randomized clinical trials. Cancer Epidemiol Biomarkers Prev 2005;14:1366–9.
39. Beresford SA, Boushey CJ. Homocysteine, folic acid and cardiovascular disease risk. Preventive nutrition. Totowa (New Jersey): The Humana Press; 1997.
40. Davey Smith G, Ebrahim S. Folate supplementation and cardiovascular disease. Lancet 2005;366:1679–81.
41. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136:189–94.
42. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795–9.
43. Johnson WG, Scholl TO, Spychala JR, Buyske S, Stenroos ES, Chen X. Common dihydrofolate reductase 19-base pair deletion allele: a novel risk factor for preterm delivery. Am J Clin Nutr 2005;81:664–8.
44. Rosenberg IH. Virtual folate: virtual success? Am J Clin Nutr 1999;70:177–8.
45. Rosenberg IH. Science-based micronutrient fortification: which nutrients, how much, and how to know? Am J Clin Nutr 2005;82:279–80.
46. Nijhout HF, Reed MC, Budu P, Ulrich CM. A mathematical model of the folate cycle: new insights into folate homeostasis. J Biol Chem 2004;279:55008–16.
47. Robien K, Boynton A, Ulrich CM. Pharmacogenetics of folate-related drug targets in cancer treatment. Pharmacogenomics 2005;6:673–89.
48. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3:415–28.
49. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23:5293–300.
50. Pennisi E. Environmental epigenomics meeting: supplements restore gene function via methylation. Science 2005;310:1761.
51. Ulrich CM. Genetic variability in folate-mediated one-carbon metabolism and cancer risk. In: Choi SW, Friso S, editors. Nutrient-gene interactions in cancer. Boca Raton (Florida): Taylor & Francis LLC. In press 2006.