Point/Counterpoint

Folic Acid Supplementation and Cancer Risk: Point

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Folate, a water-soluble B vitamin that is present naturally in foods (e.g., green leafy vegetables, asparagus, broccoli, Brussels sprouts, citrus fruit, legumes, dry cereals, whole grain, yeast, lima beans, liver, and other organ meats), and folic acid, the synthetic form of this vitamin that is used commercially in supplements and in fortified foods, have been considered an ideal nutritional factor for the prevention of cancers, especially colorectal cancer. Before the 1980s and early 1990s when a flurry of publications of epidemiologic and clinical observations that suggested an inverse association between folate status and the risk of several human malignancies began to appear (1-5), however, the role of folate in cancer was entirely related to antifolate-based cancer chemotherapy. In the 1940s, shortly after folic acid was discovered, Sidney Farber and colleagues (6) administered folic acid polyglutamate conjugates in children with leukemia based on the observation by Lewisohn et al. (7) that ''folic acid concentrate'' caused regression of mammary tumors in mice.

To their surprise, the administration of folic acid accelerated the progression of leukemia (6). Making good of this discovery, which suggested that the proliferation of leukemia cells might be limited by the supply of folic acid, Sidney Farber went on to show that one of folic acid antagonists, aminopterin, produced complete remissions in children with acute leukemia (8). This discovery thus heralded the beginning of the modern era of antifolate-based chemotherapy. The concept that folate may be a cancer preventive agent therefore seems to be highly provocative and counterintuitive at first glance.

In contrary to the inhibitory effect of antifolate (or folate depletion) on tumors, an accumulating body of epidemiologic, clinical, and experimental evidence suggests that folate deficiency in normal tissues may predispose them to neoplastic transformation, and folate supplementation may suppress the development of tumors in normal tissues (9). Epidemiologic studies suggest an inverse association (in some cases, dose dependent) between folate status (measured by dietary and supplemental intake or blood levels) and the risk of several malignancies including cancer of the colorectum, oropharynx, esophagus, stomach, pancreas, lungs, cervix, ovary, and breast and neuroblastoma and leukemia (9). The precise nature and magnitude of the inverse relationship between folate status and the risk of these malignancies, however, have not been clearly established because of inconsistent results. The relationship between folate status and cancer risk has been best studied for colorectal cancer and there is a reasonable body of evidence that supports the purported inverse association (9). Although not uniformly consistent, the portfolio of retrospectively and prospectively conducted epidemiologic studies, including 2 recent meta-analyses (10, 11), suggest a 20% to 40% reduction in the risk of colorectal cancer or its precursor, adenomas, in subjects with the highest folate status compared with those with the lowest status (9). Furthermore, the Nurses' Health Study reported a 75% reduction in colorectal cancer risk in women using multivitamin supplements containing $\geq$400 μg folic acid for $\geq$15 years compared with those not taking folic acid (12). Some, and not all, molecular epidemiologic studies have reported that certain genetic polymorphisms in the folate metabolic pathway (e.g., MTHFR C677T) modify colorectal cancer risk and interact with folate and other nutritional factors involved in the folate metabolic and one-carbon transfer pathways to further modulate the risk, thereby supporting the role of folate in colorectal carcinogenesis (13-15).

Small human intervention studies have also reported that folic acid supplementation (400 μg-10 mg/day for 3 months to 2 years) can improve or reverse several functional biomarkers of folate and one-carbon metabolism and colorectal cancer (9). However, it is difficult to draw any definitive conclusions about the chemopreventive role of folic acid supplementation in colorectal carcinogenesis from these trials because the number of subjected studied was too small, the duration of follow-up was short, and most of these trials used less well-established biomarkers of colorectal cancer (9). Folic acid chemoprevention trials using colorectal adenoma as the end point have also been conducted. Investigators from Greece reported a study involving 60 subjects with colorectal adenomas: folic acid supplementation (1 mg/day for 2 years) after polypectomy decreased adenoma recurrence by 46% compared with placebo, although this difference was not statistically significant (16).

A recent report of another double-blind, placebo-controlled trial showed that folic acid supplementation at 5 mg/day for 3 years in subjects with resected adenomas (n = 94) significantly reduced the number of recurrent adenomas (0.36$\pm$0.69 per patient in the folic acid supplemented group versus 0.82$\pm$1.17 per patient in

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the placebo group; $P < 0.05$; ref. 17). However, clinical significance of this magnitude of reduction is highly questionable. Another recently published multicenter, randomized, double-blind trial (The United Kingdom Colorectal Adenoma Prevention Trial) of aspirin (300 mg/day) and folic acid supplementation (0.5 mg/day) in a 2×2 factorial design showed no significant effect of folic acid supplementation on the recurrence of adenomas [relative risk, 1.07; 95% confidence interval (CI), 0.85-1.34] or advanced adenomas (defined as having a diameter of $\geq$1 cm; villous or tubulovillous features; or severe dysplasia; relative risk, 0.98; 95% CI, 0.68-1.40) in 945 patients who had had an adenoma ($\geq$0.5 cm) removed in the 6 months before recruitment (18). In contrast, aspirin significantly reduced the recurrence of adenoma and advanced adenomas by 21% and 37%, respectively (18).

In contrast to either the protective or null effect of folic acid supplementation on surrogate end point biomarkers of colorectal cancer in the previously published trials, the Aspirin/Folate Polyp Prevention Study has recently reported a potential tumor-promoting effect of folic acid supplementation (19). The Aspirin/Folate Polyp Prevention Study is a double-blind, placebo-controlled, 2-factor, phase 3, randomized chemoprevention trial conducted at 9 clinical centers in the United States and Canada (19). Using a 3×2 factorial design, this trial compared 81 mg/day and 325 mg/day of aspirin with placebo and 1 mg/day of folic acid with placebo in persons with a history of colorectal adenomas. The findings regarding aspirin were reported in 2003: low-dose aspirin (81 mg/day) had a moderate, statistically significant chemopreventive effect, reducing the risk of colorectal adenomas by 19%, whereas high-dose aspirin (325 mg/day) provided no significant benefit (20). With regard to folic acid, participants were randomized to receive 1 mg/day of folic acid ($n = 516$) or placebo ($n = 505$) and followed with 2 colonoscopies (the first was at 3 years and the second was at 3-5 years later).

Overall, there was no effect of folic acid supplementation on the recurrence of adenomas, with relative risk of 1.04 (95% CI, 0.90-1.20) at 3 years ($n = 987$) and 1.13 (95% CI, 0.93-1.37) at the second follow-up ($n = 607$). Unexpectedly, however, at the second follow-up, there was a 67% *increased* risk of advanced lesions with a high malignant potential, defined as $\geq$25% villous features, high-grade dysplasia, size of $\geq$1 cm, or invasive adenocarcinoma, (relative risk, 1.67; 95% CI, 1.00-2.80), along with a >2-fold *increased* risk of having at least 3 adenomas (relative risk, 2.32; 95% CI, 1.23-4.35). There was no significant effect modification by sex, age, smoking, alcohol use, body mass index, baseline plasma folate, or aspirin allocation. Another unexpected secondary finding from this trial was that the risk of cancers other than colorectal cancer was significantly increased in the folic acid supplemented group ($P = 0.02$). This was largely due to an excess of prostate cancer in the folic acid group ($P = 0.01$).

The potential tumor-promoting effect of folic acid supplementation observed in this trial is not entirely surprising and is consistent with the observations made in animal models (9). Animal studies have collectively provided critical information concerning dual modulatory effects of folate on colorectal cancer development and progression depending on the timing and dose of folic acid intervention (9). Folate deficiency has an inhibitory effect, whereas folic acid supplementation has a promoting effect on the progression of established colorectal neoplasms (9, 21-23). In contrast, folate deficiency in normal colorectal mucosa seems to predispose it to neoplastic transformation, and modest levels of folic acid supplementation (4-10 times above the basal dietary requirement) suppress, whereas supraphysiologic supplemental doses enhance the development of colorectal cancer in normal colorectal mucosa (9, 21, 22, 24-29).

The Aspirin/Folate Polyp Prevention Study was designed to address secondary rather than primary prevention of colorectal adenomas. The participants had previous colorectal adenomas removed before entry into the trial. These predisposed individuals were at high risk of developing colorectal adenomas and cancer and might have harbored microscopic precursors of colorectal cancer (e.g., aberrant crypt foci or microscopic adenomas). Therefore, folic acid supplementation might have promoted the progression of these already existing, undiagnosed preneoplastic lesions. Although there were no important differences in the baseline characteristics between the folic acid and placebo groups, the folic acid group had a higher proportion of subjects with large adenomas ($\geq$1 cm in diameter) removed $\leq$16 months before recruitment ($P = 0.06$). Large adenomas are considered to be advanced lesions with a higher malignant potential (30) and as such, the subjects in the folic acid group might have been more predisposed to colorectal carcinogenesis than those in the placebo group. Another possibility, albeit unlikely, is that folic acid supplementation might have promoted the progression of adenomas missed on initial and first follow-up colonoscopies.

The observed higher incidence of prostate cancer associated with folic acid supplementation is not surprising either and can be readily explained. The mean age of the study participants was 57 years ($\sim$64% were men), and it is therefore highly likely that some of the male participants might have harbored precursor lesions in the prostate, which were allowed to progress more rapidly with folic acid supplementation. The take-home message from this important trial is that folic acid supplementation should not be given to individuals with previous colorectal adenomas because their colons may already be predisposed to neoplastic transformation and to those suspected of harboring precursor lesions of colorectal cancer in the colorectum. However, this study does not rule out a possibility that folic acid supplementation may prevent the development of *de novo* colorectal adenomas or cancer, and this issue can be addressed only by a primary prevention trial.

Data from animal studies and clinical observations suggest that folate possesses dual modulatory effects on colorectal cancer development and progression and wields a ''double-edged sword'' depending on the timing and dose of folic acid intervention (9, 31). There exist several biologically plausible mechanistic explanations for these seemingly paradoxical effects of folate relating to its essential role in mediating the transfer of one-carbon moieties necessary for DNA synthesis, stability and integrity, and repair (9, 31). The accumulating body of *in vitro* and *in vivo* evidence indicates that in normal colorectal epithelial cells, folate deficiency

induces DNA strand breaks, chromosomal and genomic instability, uracil misincorporation, impaired DNA repair, and increased mutations, and that folic acid supplementation can correct some of these defects (9, 32). In contrast, in preneoplastic or neoplastic colorectal epithelial cells where DNA replication and cell division are occurring at an accelerated rate, folate deficiency causes ineffective DNA synthesis, resulting in inhibition of tumor growth and progression, which is the basis for cancer chemotherapy using antifolate agents and 5-fluorouracil (9). Mechanistically, the most likely mechanism by which folic acid supplementation may promote the progression of established preneoplastic and neoplastic lesions in the colorectum is the provision of nucleotide precursors to rapidly replicating cells for accelerated proliferation and progression (9). Several other potential biological mechanisms for the dual effects of folate on colorectal carcinogenesis, including that relating to DNA methylation, have not yet been well established (9, 33).

Folate intake and blood measurements of folate in the United States and Canada have dramatically increased over the past decade, owing to the drastic increase in dietary folate intake from mandatory folic acid fortification (providing a daily average of 100-200 μg) in 1998 (34, 35) and a large proportion (up to 30-40%) of the North American population consuming supplemental folic acid (400 μg in standard multivitamin or 1 to 5 mg in special preparations) for several possible but as yet unproven health benefits (36). In the United States and Canada, the average total folate intake postfortification is estimated to be ~400 μg/day in supplement nonusers with ~200 μg/day as folic acid provided in enriched products. For those taking multivitamins containing folic acid, the estimated total intake is ~800 μg/day. However, these estimates of folate intake are likely underestimates, and indeed, several studies have suggested that the increased folate intake postfortification in the US population may be about twice that originally anticipated (34). In the National Health and Nutrition Examination Survey 1999 to 2000, after folic acid fortification began, 23% of the US population, 43% of children ages ≤5 years and 38% of the elderly persons had high serum folate concentrations (>45.3 nmol/L; ref. 35). However, a more recent National Health and Nutrition Examination Survey extending to 2003 to 2004 has shown that serum and RBC folate concentrations declined significantly from those seen in 1999 to 2000, with the prevalence of children and elderly with high serum folate concentrations dropping to 19% and 32%, respectively (37), probably owing to the decreased amount of folic acid added to enriched cereal grain products by food industry and to the reduced consumption of cereal grain products resulting from the low-carbohydrate diet trend (38). Nevertheless, given the potential tumor-promoting effect of folate on preneoplastic and neoplastic cells, the effect of the dramatically increased folate status resulting from mandatory folic acid fortification and supplementation on cancer incidence in the United States and Canada is of great concern.

To address this important public health concern, Mason et al. (39) have examined a temporal trend of colorectal cancer incidence in the United States and Canada postfortification using two data sets from these countries, the Surveillance, Epidemiology and End Result registry and Canadian Cancer Statistics (by the Canadian Cancer Society, National Cancer Institute of Canada, and Statistics Canada), respectively. Their analysis shows that concurrent with folic acid fortification, the United States and Canada experienced abrupt reversals of the downward trend in colorectal cancer incidence that the two countries had enjoyed in the preceding decades (39). Absolute rates of colorectal cancer began to increase in 1996 (United States) and 1998 (Canada), peaked in 1998 (United States) and 2000 (Canada), and have continued to exceed the pre-1996/1997 trends by 4 to 6 additional cases per 100,000 individuals (39). These investigators hypothesized that the institution of folic acid fortification may have been wholly or partly responsible for the observed increase in colorectal cancer rates in the mid-1990s (39). Changes in the rate of colorectal cancer screening by endoscopic procedures do not seem to account for this increase in colorectal cancer incidence (39). However, because of the lack of complete control of potential confounders inherent in the two data sets, these observations do not prove a causal link between folic acid fortification and increased rates of colorectal cancer in North America in the mid-1990s. Nevertheless, these observations provide a highly provocative impetus for further discussion, debate, and research aimed at elucidating potential deleterious effects of folic acid fortification and supplementation.

However, there is an emerging body of evidence that suggests that folic acid fortification and periconceptional maternal use of folic acid supplementation may prevent the development of cancers in a site-specific manner. A Canadian study (40) reported that folic acid fortification was associated with a significant 60% reduction in the incidence of neuroblastoma among children ages ≤17 years (from 1.57 cases per 10,000 births in 1996 to 0.62 cases per 10,000 births after 1997, when folic acid fortification became mandatory in Canada) using the Pediatric Oncology Group of Ontario. However, the incidence of infant acute lymphoblastic leukemia and hepatoblastoma remained almost the same in this study (40). The results from this study corroborate those of previous epidemiologic studies, which reported an inverse association between periconceptional maternal use of folic acid and the incidence of brain tumors in the offspring (41). Furthermore, a recent meta-analysis of seven articles selected out of 61 articles that investigated the effect of periconceptional multivitamin supplements containing folic acid on several pediatric cancers showed a protective effect for childhood leukemia (odds ratio, 0.64; 95% CI, 0.53-0.78), in particular, acute lymphocytic leukemia (odds ratio, 0.61; 95% CI, 0.50-0.74), and for pediatric brain tumors (odds ratio, 0.73; 95% CI, 0.60-0.88), especially neuroblastoma (odds ratio, 0.53; 95% CI, 0.42-0.68; ref. 42). Interestingly, however, a recent population-based case-control study from Germany that included 1,867 cases and 2,057 controls has shown that although maternal use of vitamin, folic acid, or iron supplementation is associated with a reduced risk of non–Hodgkin's lymphoma and certain leukemia, these supplements are associated with an increased risk of neuroblastoma (43). Furthermore, there is a growing body of evidence from animal studies that suggests that folic acid supplementation *in utero* may have a permanent and heritable epigenetic effect on the

offspring with permanent changes in certain phenotypes (44-46). Because aberrant and dysregulation of epigenetics are mechanistically related to the development of cancer (47), whether or not folic acid fortification and periconceptional folic acid supplementation influence epigenetics and subsequent cancer risk in the offspring needs to be determined in humans.

Folic acid has been generally regarded as safe (48) and has long been presumed to be purely beneficial and an ideal functional food component for disease prevention including coronary artery disease, stroke, neural tube defects (NTD), and cognitive impairment. In contrast to the largely supportive evidence from observational epidemiologic studies, however, results from several large folic acid intervention trials in humans have been inconsistent and generally have not been supportive of the cardioprotective effect of folic acid supplementation (49-51). However, folic acid supplementation seems to reduce the risk of stroke (52) and NTDs (53-55). In fact, the overwhelming evidence for the protective effect of periconceptional folic acid supplementation on the development of NTDs (53-55) led to the eventual mandatory folic acid fortification in the United States and Canada in 1998 (55-57), resulting in a significant 15% to 50% reduction in the rate of NTDs (58-63). Despite the unequivocal success in reducing NTDs rates, the debate on mandatory folic acid fortification has not ceased, and as a matter of fact, the controversy over this public health policy has intensified, partly because of an uncertain role of folate in cancer development and progression (64, 65). The major concern with mandatory folic acid fortification has been that although it protects against the development of NTDs, certain segments of the exposed population may benefit less and may even experience some adverse effects from an increased folic acid intake (66, 67).

What can we conclude about the effect of folic acid fortification and supplementation on cancer risk? From the discussion above, it seems that folic acid fortification and periconceptional supplementation may reduce the risk of certain childhood cancers in the offspring (40-42). Furthermore, folic acid supplementation may prevent the development of cancers in normal tissues (9, 21, 22, 24-29). However, folic acid supplementation and fortification may promote the progression of already existing preneoplastic and neoplastic lesions (9, 19, 21-23, 39, 66, 68). However, the threshold level above which folic acid supplementation may exert the tumor-promoting effect on preneopalstic and neoplastic lesions as well as dose-response of such a effect associated with folic acid supplementation have not been clearly established in humans nor can they be extrapolated from animal studies because inherent differences in folate absorption and metabolism between humans and rodents. Furthermore, it is unclear whether the potential tumor-promoting effect is limited to folic acid, the synthetic form of folate, and is generalizable to naturally occurring folate present in foods and to other synthetic form of this vitamin such [6S]-5-methyltetrahydrofolate (69).

At present, based on the lack of compelling supportive evidence, on the potential tumor-promoting effect, and on the almost impossible task of determining the presence of preneoplastic or neoplastic foci in the general population, folic acid supplementation should not be recommended as a chemopreventive measure against colorectal cancer or other cancers. Furthermore, safety and adverse effects, with a particular attention to cancer incidence and mortality, of the dramatically increased folate status in the Untied States and Canada resulting from folic acid supplementation and fortification should be careful monitored. More specifically for colorectal cancer, folic acid supplementation should not be given to individuals with previous colorectal adenomas because their colons may already be predisposed to neoplastic transformation and to those suspected of harboring precursor lesions of colorectal cancer in the colorectum. This of course applies to the large segment of the North American population as it has been estimated that ~25% to 50% of people by ages 50 years in the United States (> 60 millions) and Canada (>10 millions) harbor asymptomatic colorectal adenomas, and the prevalence increases with age (30). This translates to 16 to 32 millions of the Americans ages ≥50 years who might be susceptible to the tumor-promoting effect of folic acid supplementation. Even a greater number of the North Americans likely harbor aberrant crypt foci (the probable earliest precursor of colorectal cancer; ref. 70) or microscopic adenomas in the colon and folic acid supplementation may accelerate the progression of these early precursor lesions to colorectal adenomas and cancer. In this regard, a recent animal study has shown that folic acid supplementation promotes the progression of established aberrant crypt foci to colorectal cancer (23).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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