Published in final edited form as:
Gut. 2011 January ; 60(1): 66–72. doi:10.1136/gut.2010.208074.

Concentration of folate in colorectal tissue biopsies predicts prevalence of adenomatous polyps

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Abstract

Background and aims—Folate has been implicated as a potential etiologic factor for colorectal cancer. Prior research has not adequately exploited concentrations of folate in normal colonic mucosal biopsies to examine the issue.

Methods—We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of adenoma according to tissue concentration of folate using asymptomatic average-risk women (40–70 years) in a colorectal cancer screening study. Of the 1,593 eligible women who were offered enrollment, 1,483 (93%) participated. Colonoscopy was complete to the cecum in 98.7% (1,463/1,483) of the subjects and normal colonic tissue biopsies were obtained from 813 (56%) of these, of whom 170 had at least one adenoma.

Results—We observed a marginal reduction in risk for proximal adenomas (OR=0.56, 95% CI 0.29–1.09) but not distal adenomas (OR=1.01, 95% CI 0.43–2.37) among women in the highest quintile of tissue folate concentration. We observed a significant reduction in risk for advanced adenoma for women in the highest quintile of tissue folate concentration (OR=0.24, 95% CI 0.06–0.93). Defining the outcome as proximal adenomatous and/or hyperplastic polyps, we also observed statistically significant inverse associations with tissue concentrations of folate (OR=0.54, 95% CI 0.31–0.95 for Q5 *vs.* Q1).

Conclusions—These findings are consistent with the hypothesis that folate status of colonic mucosa is an exposure that is etiologically important in determining the risk of particular molecular sub-types of colorectal cancer.

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Competing Interests: None.

Keywords

Adenoma; folic acid; DNA microsatellite instability; colon carcinogenesis; methylation

BACKGROUND AND AIMS

Despite decades of investigations on dietary constituents as risk factors for colorectal cancer, a recent comprehensive review of the literature identified few dietary variables that had well-established, likely-causal links to this disease. [1] Not all of the epidemiology has been entirely inconclusive on this question, however. In the case of folate, the bulk of the observational epidemiology does show an inverse association, in a dose-dependent manner, between dietary intake of folate and colorectal cancer, although the possibility of residual confounding by fiber (also abundant in folate-rich foods) and some inconsistency in the results of cohort studies does temper potential conclusions of causality. [1] Moreover, dietary folate may not be the most important measure of folate status, as the concentration of folate in the colon tissue itself is likely to be much more biologically relevant.

The role of folate in colorectal carcinogenesis is complex and likely involves multiple metabolic pathways. These would include the role of folate in conversion of deoxy uracil monophosphate (dUMP) to deoxy thymidine monophosphate (dTMP). Failure to produce adequate supplies of dTMP results in uracil misincorporation, which has been linked to DNA strand breaks, loss of heterozygosity, and other forms of DNA instability. [2]

Additionally, folate is the intermediary carrier molecule for the labile methyl group in S-adenosylmethionine, which is responsible for nearly all methylation reactions in the body, including methylation of DNA. A minimum level of genomic methylation may protect against carcinogenesis as methylation is thought to stabilize the genome. [2, 3] Prior studies have shown that genomic methylation is typically lower in colon tumor tissue than in adjacent normal tissue in the same patient [4] and that genomic methylation in lymphocyte DNA is lower in subjects with adenoma compared to those without. [5] A second role of methylation in carcinogenesis is in regulation of gene activity through methylation in the promoter regions of genes essential to regulation of cell growth and replication. Aberrant methylation of these promoter regions can thus result in disregulation of the controls on cell growth and replication with obvious implications for carcinogenesis.

The classic Vogelstein model of a sequence of genetic alterations from *APC* mutations to *p53* mutations that mark the transition from normal colonic epithelium to adenomatous polyp and then to invasive colorectal cancer describes about two thirds of colorectal cancers, and these tend to be more prevalent in the distal colon. [6, 7] A separate class of colon cancers comprises tumors displaying the CpG island methylator phenotype (CIMP). [8, 9] CIMP cancers are characterized by promoter methylation and hence gene silencing at multiple genes involved in carcinogenesis. CIMP cancers account for 10 to 30% of all colorectal cancers and are concentrated in the proximal colon. Interestingly, CIMP cancers appear to arise not through the classic Vogelstein, *APC* mutation-based tubular adenoma precursor lesion but rather through either a villous or serrated adenoma or a hyperplastic polyp precursor lesion. [10, 11, 12]

The concept that is explored in this study—whether inadequate folate availability in the colon leads to inappropriate hypermethylation of several important tumor suppressor genes--is biologically plausible and supported by work in a pre-clinical model, where folate inadequacy was observed to lead to promoter hypermethylation and reduced expression of a critical tumor suppressor gene. [13] However, the issue of whether the same phenomenon operates in the human colon has not been adequately addressed, and there is little consensus

among the few observational studies that have tried to examine the question. [14, 15, 16] Perhaps the conflicting results relate, in part, to the fact that they have each used blood folate levels, or dietary intake, as a proxy measure of colonic folate status.

Given the centrality of aberrant methylation in the CIMP pathway and the essential nature of folate in methylation reactions, we hypothesized that low colonic tissue folate status would be a risk factor for polyps that are CIMP-related. To test this hypothesis, we examined the association of folate concentration in normal colonic mucosa with adenomatous and hyperplastic polyps in a colonoscopy screening study of average-risk, asymptomatic women.

METHODS

Study design

Data for these analyses came from the Colorectal Neoplasia Screening with Colonoscopy in Average-Risk Women at Regional Naval Medical Centers (CONCeRN) study. CONCeRN is a screening study with the primary clinical objective of evaluating the efficacy of sigmoidoscopy compared to colonoscopy as a screening method for colorectal cancer in average-risk, asymptomatic women. [17] In addition to this clinical aspect, CONCeRN included an etiologic component with the aim of investigating the associations between a variety of lifestyle factors and colorectal polyps. The etiologic portion of the study commenced after the clinical portion was underway, and hence only a subset of the full CONCeRN study population has data and specimens available for etiological analyses. Subjects were recruited from 4 tertiary-care, military medical centers from July 1, 1999 through December 31, 2002 after referral to those centers for standard colorectal cancer screening. Upon reporting to the medical centers, subjects completed questionnaires designed to assess health history, diet, and lifestyle practices and also provided blood samples for biochemical analyses. All subjects then underwent complete colonoscopy during which all identified polyps were removed and biopsies of normal colonic mucosa were obtained. Further details of the methods appear below. The study protocols were approved by the human rights committee and institutional review boards at each treatment facility and at the National Cancer Institute, and all patients provided written informed consent prior to their participation.

Study participants

Subjects were consecutive female patients aged 50–70 years who did not have lower gastrointestinal tract symptoms and who were referred to one of the 4 tertiary care centers for colorectal cancer screening during the enrolment period. The study also included women aged 40–49 years if they had a history of colorectal cancer in a primary relative (*i.e.*, if they met guidelines for screening colonoscopy for individuals in that age range). Patients were excluded if they had lower gastrointestinal symptoms suggestive of organic gastrointestinal disease, a positive fecal occult blood test within six months of referral, a history of iron deficiency anemia within six months of referral, a history of rectal bleeding or hematochezia within the past 12 months, unintentional weight loss of more than 10 pounds within the previous six months, a personal history of colon adenomas, colorectal cancer, inflammatory bowel disease, hereditary non-polyposis colorectal cancer syndrome or familial adenomatous polyposis or a history of a normal colonoscopy or barium enema within the past 10 years or a normal flexible sigmoidoscopy within the past five years. A total of 1,593 eligible women were offered enrollment in the study and 1,483 (93%) participated. Of these, 813 also participated in the etiological portion of the study and provided tissue biopsies as described below.

Colonoscopy procedures and identification of polyps

Procedures for performing colonoscopy and histologic evaluation have been described in detail previously [17] and are summarized here. Patients completed standard bowel preps for colonoscopy with 4 liters of polyethylene glycol and bisacodyl. Colonoscopy was complete to the cecum in 98.7% (1,463/1,483) of the women. We restricted the present analyses to women who underwent a complete colonoscopic examination. During colonoscopy, the location of all polyps was defined based upon depth of insertion of the colonoscope and anatomic landmarks, including the hepatic flexure, splenic flexure, and sigmoid-descending colon junction. Histologic specimens from every polyp were reviewed by an expert gastrointestinal pathologist who was blinded to the colonoscopy findings and initial pathologic diagnosis. The interpretation of the expert gastrointestinal pathologist was considered final. Advanced adenomas were defined by the presence of any of the following adenoma characteristics: size ≥ 10 mm, any villous histology, high grade dysplasia, or colorectal cancer that was either invasive (through the muscularis mucosa) or in-situ. For analyses of advanced adenoma, patients were classified on the basis of their most advanced pathologic finding.

Tissue biopsies

During colonoscopy, the endoscopist removed three pairs of normal colonic mucosa biopsies in the region of the splenic flexure from each woman enrolled in the etiological portion of the study. The study nurse in the endoscopy suite immediately snap froze the biopsies using liquid nitrogen, placed the frozen specimens on dry ice, and transferred them to a biorepository facility where they were stored at −70° C.

Folate concentration in tissue samples

Folate concentration was determined in the Vitamins & Carcinogenesis Laboratory at Tufts University using a conventional microbiological microtiter plate assay that employed *Lactobacillus Casei*, with some modifications. [18, 19] Briefly, 5 to 20 mg of colon mucosa was put into a tube with 20× μl folate extraction buffer (2% sodium ascorbate, 2% Bis-Tris, and 0.07% 2-mercaptoethanol) per milligram of tissue. This was homogenized and the homogenate was immersed in boiling water for 20 minutes, cooled in an ice water bath, and then centrifuged at 36,000g for 20 minutes at 4°C. The supernatant was then transferred to a vacutainer and stored at -70°C. Prior to the microbiological assay, the sample was incubated with dialyzed chicken pancreas conjugase for 2 hours at 37°C. After conjugase treatment, the sample was put into a 96-well plate with serial dilutions, and incubated with *Lactobacillus casei* for 24 hours. The plate was read at 595 nm. The folate concentration was calculated based on an internal folic acid standard curve created with serial dilutions of a standard solution of folic acid that was established for each plate. The folate concentration was corrected by normalizing the values obtained from each plate to an external-standard plasma sample that was run on each plate.

Assessment of covariates

Each of the women in the etiologic portion of the study completed a series of questionnaires prior to her endoscopic procedure in order to assess demographics, height, weight, medical history, risk factor exposures and usual dietary intake. Diet was assessed using the 137-item NCI Diet History Questionnaire (DHQ). Its design and validation have been described previously. [20] The Diet*Calc software package used DHQ responses to generate nutrient and food group intake estimates for the study subjects. Intake of folate from food sources (including from fortification), from multivitamins and other nutrient supplements, as well as intake of natural folate and synthetic folate were included in the nutrient estimates. All values for folate intake were in mcg/day.

Statistical analysis

To describe baseline characteristics of the study population, we calculated mean values (and standard errors) for continuous variables and proportions for categorical variables across quintiles of tissue folate concentration. We used logistic regression (PROC LOGISTIC in SAS statistical software, version 9.1) to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to assess the association between tissue concentration of folate and polyps. We used several definitions of the outcome. First, we considered all adenomas and advanced or non-advanced adenomas. Second, we considered anatomic location of the adenoma with separate categories for subjects having only proximal adenomas (up to and including the hepatic flexure), only distal adenomas (descending colon to rectum), or both distal and proximal adenomas. Finally, in an effort to identify the lesions most likely to be CIMP-related, we devised several categories of endpoints that included hyperplastic polyps and incorporated anatomic subsite since hyperplastic polyps and serrated adenomas in the proximal colon have been identified as potential precursor lesions for CIMP-related cancers.

Initial models controlled only for age. We also considered potential confounding by the following factors: diabetes status, regular aspirin use, education, smoking, menopausal hormone therapy, physical activity, alcohol, red meat intake, dietary fiber, dietary calcium, calcium from supplements, and body mass index (BMI). Dietary variables were energy adjusted using the nutrient density method (unless otherwise specified). Adjusting for these factors made no material difference in the results (*i.e.*, in no case did the ORs change by more than 10%), therefore we have presented only the results from the age-adjusted models.

RESULTS

Baseline characteristics by quintile of tissue folate concentration for the CONCeRN subjects who were enrolled in the etiologic portion of the study appear in Table 1. Not surprisingly, there was a modest increase in mean dietary folate (from 223 to 248 μg/1000 kcals/day) and a more substantial increase in intake of folate from supplements (from 124 to 276 μg/day) and hence in total folate intake (from a mean of 468 to 658 μg/day, adjusted for energy using the residual method for the dietary portion of that total) across quintiles of tissue folate. Also showing positive associations with tissue folate concentration were age, physical activity, alcohol intake (though alcohol intake in this population was quite modest), total calcium intake, fiber consumption, and educational attainment. Red meat intake and BMI were inversely related to tissue folate concentration.

Among the 813 women with tissue folate information available in this study, 170 had at least one prevalent adenomatous polyp, and 37 of these women had at least one advanced adenoma. We observed a modest, non-significant inverse association between tissue folate concentration and prevalent adenomas overall (OR = 0.68, 95% CI 0.40–1.16 for Q5 *vs.* Ql; p trend = 0.53). However for advanced adenomas, the most clinically relevant outcome other than invasive colorectal cancer, women in the top quintile of tissue folate concentration had a statistically significant lower risk (OR = 0.24, 95% CI 0.06–0.93; p trend = 0.049).

Analyses by anatomic location indicate a subsite specificity for the association between tissue folate concentration and adenomas (Table 3). In the proximal colon the OR for Q5 *vs.* Q1 was 0.56 (although the 95% CI did include 1.0), and furthermore, in Q3 and Q4, the point estimates, 0.48 for Q3 and 0.49 for Q4, were statistically distinct from the null. By contrast, there was no evidence of association between tissue folate concentration and prevalent adenomas in the distal colon. These results suggest that tissue folate may be important in the etiology of adenomas that arise in the proximal colon, but not in those arising in the distal colon.

When we defined the outcome as one or more prevalent hyperplastic polyps (and no adenomas), without consideration of subsite, there was no association with tissue concentration of folate (Table 4). Similarly, if we defined the outcome as having either prevalent hyperplastic polyps or prevalent adenomas, without consideration of subsite, there was no association. But when we restricted the outcome definition to include only proximal hyperplastic polyps and/or proximal adenoma, then there was a statistically significant, inverse association (OR = 0.54, 95% CI 0.31–0.95 for Q5 *vs.* Q1 of tissue folate concentration).

DISCUSSION

A growing body of evidence supports the notion that at least three primary pathways to colorectal cancer exist, each with a unique molecular pathology, and each with distinct clinical features related to presentation, anatomic location, and prognosis. [11] Risk factors for proximal colon cancers are distinct from those that arise in the distal colon. Distal cancers are more frequent in younger men while proximal cancers occur most commonly in older women [21]. Familial adenomatous polyposis (germline mutation of the *APC* gene) patients develop distal tumors, and hereditary non-polyposis colon cancer (germline mutation of mismatch repair genes, often *MSH2* or *hMLH-1*) patients develop proximal tumors [21]. Of particular relevance to this study, long term use of folate-containing supplements was associated with reduced risk of proximal, but not distal, colon cancer in a cohort of U.S. nurses [22]. Furthermore, in a case-control study from Australia, a polymorphism in the folate-dependent enzyme 5,10-methylenetetrahydrofolate reductase (*MTHFR* C677T) was associated with an increased risk of proximal colon cancer, especially among those with low folate intake [23].

The etiologic mechanisms underlying the basis for the three primary molecular subtypes of colorectal cancer remain incompletely understood, though it is clear that methylation of specific genes (*e.g.*, *hMLH1*), of the genome generally (*i.e.*, genomic methylation), and of classes of genes (*i.e.*, methylation profiles) are all involved. In particular, the CIMP cancers are characterized by aberrant methylation of promoter regions in multiple genes related to regulation of cell growth and differentiation. Although it is important to emphasize that they do not prove an association between tissue folate concentration and a specific molecular subtype of colorectal cancer, our findings from women enrolled in the CONCeRN study do suggest that folate status in the colonic epithelial tissue may be linked to the aberrant methylation patterns that can result in cancers developing from one of these three molecular pathways, the CIMP pathway.

Prior research in this area has not adequately exploited concentrations of folate in normal colonic biopsies to examine the potential etiologic role of folate in colorectal carcinogenesis. Our study was distinct in that we were able to measure folate status directly in the target tissue of interest, colonic mucosa, whereas nearly all previous research in humans has relied on markers of folate status (*i.e.*, dietary intake or blood concentration) that were in effect proxy measures of folate concentration in the colon.

Our design also allowed us to use tissue obtained from asymptomatic, average-risk women referred for standard colorectal cancer screening meaning we were not reliant on a small case series as previous studies of tissue folate concentration have been. [24, 25, 26] Furthermore, the colonoscopic procedure ensured that we were able to identify polyps at all anatomic locations in the colon and rectum, a critical design feature given the different anatomic distribution of the various molecular subtypes of colorectal cancer. In combination, the availability of direct measures for tissue folate concentration, the enrolment of 813 asymptomatic women, and the identification of neoplasia at all anatomic

subsites via complete colonoscopy (resulting in the classification of women as having healthy colons, adenomatous polyps, and/or hyperplastic polyps) provided a robust and unique resource to study this question.

The value of our effort to focus on the association of folate status with one specific molecular subtype of colorectal neoplasia, the CIMP subtype, is apparent when we contrast our results to those from one of the few prior studies using human tissue specimens. Meenan and colleagues found that folate concentration in adjacent normal tissue in subjects with adenomas or cancer was similar to that in normal epithelial tissue from subjects with healthy colons, [24] a result that would appear to be in contradiction to our findings. All case subjects in the earlier analysis, however, had either distal cancer or distal adenomas, and, as our analysis suggests, the association in the distal colon would be expected to be null, just as they observed. In contrast, we were able to consider proximal location and to include hyperplastic polyps (both relevant to CIMP-related cancers) in defining our outcomes thus allowing us some ability to isolate the CIMP-related polyps. After doing so, we were able to observe a significant association that was not found if we included polyps of histologic type or anatomic location that made them unlikely to be CIMP-related.

A recent report from Levine and colleagues adds additional evidence in support of a site-specific and molecular subtype-specific effect of folate and methylation on colorectal cancer etiology. [27] In a large family-based case-control study, the *MTHFR 677 TT* genotype was associated with a decreased risk of MSI-S/MSI-L tumors but an increased risk of MSI-H tumors, and it was further associated with decreased risk of distal and rectal tumors but an increased risk of proximal tumors. Together with our results, these findings provide evidence that the effects of folate metabolism on colorectal carcinogenesis are specific in nature to the type of tumor involved. The effects of folate metabolism and folate status on proximal, microsatellite-unstable tumors are quite distinct from the effects on distal tumors.

The wide range of colonic folate concentrations that were observed is perhaps of importance. The mean concentration of colonic folate among those in the highest quintile was six times greater than that observed in the lowest quintile (range: 0.273 – 1.676 μg/g for mean of Q1 to mean of Q5). Moreover, this wide range of concentrations was present even though the total mean folate intake of two extreme quintiles only differed by ~40%. Thus, considerable biological variability in colonic folate concentrations exists within a relatively healthy, ambulatory single-gender population and the extent of variability extends far beyond the variation in habitual intake of the vitamin, suggesting other factors play important roles in determining colonic folate levels. If mucosal folate levels are indeed a causal factor in determining the risk of neoplastic transformation, such a pronounced variation between individuals makes it easier to understand why some individuals would be at higher risk than others due to this factor.

It is estimated that far less than 10% of adenomas will ever go on to develop into cancers, and therefore, the recognition of the ‘advanced adenoma’ as a lesion that is much more likely to progress to cancer [28, 29] was an important one since it is therefore likely to be a more accurate biomarker of cancer risk. Consequently it is of considerable interest that colonic folate concentrations were observed to be significantly associated (in an inverse fashion) with advanced adenomas, but not with other types. This suggests that colonic folate concentrations are more closely linked with those lesions that are most likely to progress to cancer than with those that are most likely to remain indolent and benign.

An important limitation to our analysis is the lack of specific molecular subtype information for our outcomes. Our use of a proxy variable (what we described as lesions most likely to be CIMP-related rather lesions with clinically-determined CIMP pathology) depends on an

assumption that CIMP and non-CIMP tumors, and their precursor lesions, have an anatomic specificity for proximal and distal location, respectively. This is clearly an oversimplification. In truth, the low levels of folate could be associated with the non-CIMP tumors that develop in the right colon from advanced adenomas. Furthermore, not all methylation that occurs in colorectal neoplasms is CIMP-related. However, the available evidence suggests this is not likely to be an oversimplification that yields inappropriate conclusions. Using prospectively collected specimens from a hospital-based frozen tumor bank containing 879 colorectal cancer cases, Whitehall and colleagues [30] found that 88% of cancers classified as having a high degree of CIMP characteristics and that were also MSI-H occurred in the proximal colon. Among those cancers that were CIMP high but were not MSI-H, 50% were in the proximal colon. By contrast, only 17% of non-CIMP cancers occurred in the proximal colon. With respect to hyperplastic polyps, a recent paper from Vaughn and colleagues [31] reported that while BRAF mutations (hypothesized to be the initiating mutation for the hyperplastic polyp-CIMP tumor pathway [32]) were very common in both proximal and distal hyperplastic polyps, 48% of proximal hyperplastic polyps had CIMP characteristics in contrast to distal hyperplastic polyps where only 4% did so. Thus while the classification we used is certainly imperfect, it is clear that the vast majority of CIMP tumors arise in the proximal colon, and that a large fraction, though admittedly not all, of the lesions in the proximal colon are CIMP-related (*i.e.*, lesions that arise from the same molecular pathway that will produce CIMP cancers).

Nonetheless, what we describe as likely to be CIMP-related polyps are in fact proxies for the true outcome of interest, and hence they necessarily involve some degree of misclassification. While it is impossible to quantify the degree of misclassification, the most likely result of this misclassification would be to bias the results toward the null. Yet despite this, we were still able to observe an inverse association between tissue concentration of folate and prevalence of polyps crudely classified as most likely to be CIMP-related. Certainly a more-precise and more-direct determination of CIMP status would be a design feature that future investigations would do well to employ, but unfortunately this was not available for the CONCeRN study.

The analysis we conducted used tissue biopsies obtained from the region of the splenic flexure, but the polyps we found were distributed across the entire length of the colon and rectum. In other words, the normal tissue biopsies in which we measured folate concentration were not necessarily adjacent to the polyps that defined our endpoints. In fact, the site where the association of polyps with the tissue folate concentration was strongest, the proximal colon, was by definition distant from the location of the tissue biopsies. This suggests that the association we observed was the result a generalized field effect, that low concentration of folate in the normal mucosa of the colon in general predisposed the proximal colon to risk of adenoma or hyperplastic polyp.

One complication to this conclusion of a field effect is the cross-sectional nature of the study design. Given that tissue folate concentration was assessed at the same time as the polyps were identified, it is impossible to determine conclusively which preceded the other. That said, it seems unlikely that these small lesions in asymptomatic women at average risk of colorectal cancer could produce changes in folate concentration in normal tissue elsewhere in the colon.

Finally, results from some recent supplement trials have raised concern about the possibility of a paradoxical cancer-promoting effect of high-dose folate, and that issue may have relevance in the present study. [33] We considered this possibility in our analyses but found no evidence that such an effect was present in this study population (data not shown). Its absence in our dataset was, in retrospect, not surprising since more than 95% of the subjects

were consuming less than 940 μg of folate per day. Given that the paradoxical cancer-promoting effect has been observed only in people who are receiving exceptionally high amounts of folic acid on a habitual basis, the level of intake in the CONCeRN Study population is likely far below the threshold at which we might expect to observe this cancer-promoting effect.

In summary, we found that concentrations of folate in normal colonic mucosa were inversely though modestly associated with prevalent adenomatous polyps in asymptomatic women undergoing screening colonoscopy. Even more interestingly, if we defined the outcome to include those polyps most likely to be CIMP-related, and hence to polyps where aberrant methylation is most directly relevant, we also saw significant inverse associations with tissue concentrations of folate. Any other definitions of the outcome, definitions that included large numbers of polyps that were unlikely to be CIMP-related, resulted in null associations. These findings, though they are not definitive, are consistent with the hypothesis that risk factors for colorectal cancer may be specific to molecular subtype and in particular that folate status in colonic mucosa is an exposure that is etiologically important to CIMP cancers as distinct for other molecular subtypes.

Acknowledgments

Funding: Research funded by National Institutes of Health grant K07 CA108910-01A1 (AF), National Institute of Health grant K23-DK-60040 (PSS), and an American Society for Gastrointestinal Endoscopy Career Development Award (PSS). The CONCeRN Study was funded by research grants from the American College of Gastroenterology and the American Society for Gastrointestinal Endoscopy as well as Intramural Research Program funds from the National Cancer Institute, Bethesda, MD.

Abbreviations

OR	odds ratio
CI	confidence interval
CIMP	CpG Island Methylator Phenotype
MSI	microsatellite instability
MSI-H	MSI-high
MIS-L	MIS-low
MSS	microsatellite stable
dUMP	deoxy uracil monophosphate
dTMP	deoxy thymidine monophosphate
DHQ	Diet History Questionnaire
BMI	body mass index
LOH	loss of heterozygosity
MTHFR	5, 10-methylenetetrahydrofolate reductase

References

1. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: 2007.
2. Eden A, Gaudet F, Waghmare A, et al. Chromosomal instability and tumors promoted by DNA hypomethylation. Science. 2003; 300:455. [PubMed: 12702868]

3. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. JNutr. 2000; 130:129–132. [PubMed: 10720158]
4. Costello JF, Plass C. Methylation matters. J Med Genet. 2001; 38:285–303. [PubMed: 11333864]
5. Lim U, Flood A, Choi SW, et al. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. Gastroenterology. 2008; 134:47–55. [PubMed: 18166347]
6. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990; 61:759–767. [PubMed: 2188735]
7. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996; 87:159–170. [PubMed: 8861899]
8. Ahuja N, Mohan AL, Li Q, et al. Association between CpG island methylation and microsatellite instability in colorectal cancer. Cancer Res. 1997; 57:3370–3374. [PubMed: 9269998]
9. Toyota M, Ahuja N, Ohe-Toyota M, et al. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A. 1999; 96:8681–8686. [PubMed: 10411935]
10. Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer. 2004; 4:988–993. [PubMed: 15573120]
11. Issa JP. Colon cancer: it's CIN or CIMP. Clin Cancer Res. 2008; 14:5939–5940. [PubMed: 18829469]
12. Jass JR, Whitehall VL, Young J, et al. Emerging concepts in colorectal neoplasia. Gastroenterology. 2002; 123:862–876. [PubMed: 12198712]
13. Jhaveri MS, Wagner C, Trepel JB. Impact of extracellular folate levels on global gene expression. Mol Pharmacol. 2001; 60:1288–1295. [PubMed: 11723236]
14. de Vogel S, Bongaerts BW, Wouters KA, et al. Associations of dietary methyl donor intake with MLH1 promoter hypermethylation and related molecular phenotypes in sporadic colorectal cancer. Carcinogenesis. 2008; 29:1765–1773. [PubMed: 18339680]
15. Jensen LH, Lindebjerg J, Cruger DG, et al. Microsatellite instability and the association with plasma homocysteine and thymidylate synthase in colorectal cancer. Cancer Invest. 2008; 26:583–589. [PubMed: 18584349]
16. Mokarram P, Naghibalhossaini F, Saberi Firoozi M, et al. Methylenetetrahydrofolate reductase C677T genotype affects promoter methylation of tumor-specific genes in sporadic colorectal cancer through an interaction with folate/vitamin B12 status. World J Gastroenterol. 2008; 14:3662–3671. [PubMed: 18595133]
17. Schoenfeld P, Cash B, Flood A, et al. Colonoscopic screening of average-risk women for colorectal neoplasia. N Engl J Med. 2005; 352:2061–2068. [PubMed: 15901859]
18. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol. 1992; 45:344–347. [PubMed: 1577973]
19. Tamura, T. Microbiologic assay of folate. In: Picciano, MF.; Stockstad, ELR.; Gregory, JF., editors. Folic Acid Metabolism in Health and Disease. New York: Wiley-Liss; 1990. p. 121-137.
20. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires : the Eating at America's Table Study. AmJEpidemiol. 2001; 154:1089–1099.
21. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer. 2002; 101:403–408. [PubMed: 12216066]
22. Giovannucci E, Stampfer MJ, Colditz GA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. AnnInternMed. 1998; 129:517–524.
23. Iacopetta B, Heyworth J, Girschik J, et al. The MTHFR C677T and DeltaDNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. Int J Cancer. 2009; 125:84–90. [PubMed: 19326430]
24. Meenan J, O'Hallinan E, Scott J, et al. Epithelial cell folate depletion occurs in neoplastic but not adjacent normal colon mucosa. Gastroenterology. 1997; 112:1163–1168. [PubMed: 9097999]
25. Kim YI, Fawaz K, Knox T, et al. Colonic mucosal concentrations of folate correlate well with blood measurements of folate status in persons with colorectal polyps. AmJClinNutr. 1998; 68:866–872.

26. Kim YI, Fawaz K, Knox T, et al. Colonic mucosal concentrations of folate are accurately predicted by blood measurements of folate status among individuals ingesting physiologic quantities of folate. Cancer EpidemiolBiomarkers Prev. 2001; 10:715–719.

27. Levine AJ, Figueiredo JC, Lee W, et al. Genetic variability in the MTHFR gene and colorectal cancer risk using the Colorectal Cancer Family Registry. Cancer Epidemiol Biomarkers Prev. 2010; 19:89–100. [PubMed: 20056627]

28. Stryker SJ, Wolff BG, Culp CE, et al. Natural history of untreated colonic polyps. Gastroenterology. 1987; 93:1009–1013. [PubMed: 3653628]

29. Brenner H, Hoffmeister M, Stegmaier C, et al. Risk of progression of advanced adenomas to colorectal cancer by age and sex: estimates based on 840,149 screening colonoscopies. Gut. 2007; 56:1585–1589. [PubMed: 17591622]

30. Whitehall VL, Wynter CV, Walsh MD, et al. Morphological and molecular heterogeneity within nonmicrosatellite instability-high colorectal cancer. Cancer Res. 2002; 62:6011–6014. [PubMed: 12414620]

31. Vaughn CP, Wilson AR, Samowitz WS. Quantitative evaluation of CpG island methylation in hyperplastic polyps. Mod Pathol. 23:151–156. [PubMed: 19855373]

32. O'Brien MJ, Yang S, Mack C, et al. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. Am J Surg Pathol. 2006; 30:1491–1501. [PubMed: 17122504]

33. Mason JB. Folate, cancer risk, and the Greek god, Proteus: a tale of two chameleons. Nutr Rev. 2009; 67:206–212. [PubMed: 19335714]

SUMMARY BOX

What is known

- The bulk of the observational epidemiology shows an inverse association between dietary folate and colorectal cancer, but dietary folate may not be the relevant indicator of folate status; tissue concentration may be more informative.
- Folate is the source of the methyl group for S-adenosylmethionine which is responsible for all methylation reactions in the body, including DNA methylation, and aberrant methylation is implicated in CIMP, MSI-H and CIMP MSI-L/MSS pathways of colorectal carcinogenesis.
- CIMP, MSI-H and CIMP MSI-L/MSS tumors are concentrated in the proximal colon, and hyperplastic polyps that progress to serrated adenomas in the proximal colon are the precursor lesions for CIMP, MSI-H tumors (distal hyperplastic polyps are not).

New findings

- The concentration of total folate in normal colon epithelial tissue was marginally associated with adenomas overall, but the association was confined to subjects with proximal adenomas only.
- The association was strongest if the outcome was defined to focus on those neoplastic lesions that were most likely to arise from the CIMP, MSI-H pathway (i.e., hyperplastic and/or adenomatous polyps in the proximal colon).
- These results provide support to the notion that increasing folate concentration in tissue reduces risk of precursor lesions to CIMP, MSI-H tumors, the molecular subtype most distinctly characterized by aberrant methylation, and they provide no evidence that tissue folate is related to risk in the distal colon (where tumors having LOH as their defining feature are concentrated).

Impact on clinical practice

This study provides evidence that folate status may be related to colon cancers of a particular subtype: CIMP, MSI-H tumors. In doing so it further informs our understanding of colorectal cancer etiology and our understanding of the different molecular pathways that define the tumors – pathways that have distinct clinical presentation and prognoses. These results also suggest that improved folate status, despite recent clinical trial results showing an apparent paradoxical cancer promoting effect among those taking high-dose folate supplements, is inversely associated with polyps of the CIMP, MSI-H molecular subtype, and does not increase risk of other types of polyps (*i.e.*, distal adenomas), among those consuming folate at levels other than what we would associate with high-dose supplement use.

Table 1

Baseline characteristics among 813 women in the CONCeRN Study by quintile of folate concentration in normal colonic epithelial tissue. All values are quintile means (±std. error) in units listed, counts, or % of subjects in listed category, as indicated.

	Quintile of Tissue Folate Concentration				
	Q1 < 0.442 µg/g wet tissue	**Q2 0.442–0.679 µg/g wet tissue**	**Q3 0.680–0.917 µg/g wet tissue**	**Q4 0.918–1.261 µg/g wet tissue**	**Q5 ≥ 1.262 µg/g wet tissue**
Age (years)	57.0 (±0.64)	57.1 (±0.59)	58.4 (±0.64)	59.0 (±0.64)	59.5 (±0.68)
Greater than high school education (%)	61.0	68.7	65.6	73.6	74.0
Height (inches)	63.9 (±0.22)	64.2 (±0.21)	64.0 (±0.19)	64.2 (±0.20)	64.4 (±0.20)
BMI (kg/m^2)	27.9 (±0.52)	27.2 (±0.45)	26.5 (±0.42)	26.8 (±0.45)	26.2 (±0.36)
Physical activity (% regular vigorous)	29.0	30.0	41.1	36.8	43.2
Smokers (% current and former)	45.7	31.9	45.4	38.0	43.8
NSAIDs (% regular users)	33.3	26.4	34.4	33.7	31.5
Dietary alcohol (g/day)	6.2 (±0.84)	4.0 (±0.56)	6.2 (±0.76)	8.3 (±1.23)	8.7 (±1.38)
Total energy intake (kcals/day)	1598 (±55)	1544 (±53)	1508 (±49)	1574 (±41)	1559 (±50)
Total fat (% of energy)	32.1 (±0.58)	32.5 (±0.61)	30.8 (±0.64)	31.1 (±0.66)	31.1 (±0.63)
Fiber (g/1000 kcals/day)	10.4 (±0.29)	11.1 (±0.29)	11.8 (±0.33)	11.4 (±0.30)	11.6 (±0.30)
Red meat (g/1000 kcals/day)	27.5 (±1.4)	27.7 (±1.3)	26.0 (±1.4)	26.1 (±1.3)	24.2 (±1.2)
Total calcium[a] (mg/day)	926 (±37.6)	1012 (±35.0)	1112 (±37.6)	1150 (±37.8)	1159 (±36.7)
Dietary folate (µg/1000 kcals/day)[b]	223 (±6.1)	241 (±5.4)	242 (±5.9)	242 (±5.6)	248 (±6.5)
Supplemental folate (µg/day)	124 (±13.9)	212 (±14.1)	279 (±14.1)	237 (±14.9)	276 (±14.5)
Total folate (µg/day)[a]	468 (±17.3)	583 (±15.8)	651 (±18.3)	610 (±18.2)	658 (±18.3)

[a] Energy adjusted values for total folate and total calcium calculated by residual method and use mean energy intake for total study population.

[b] Dietary folate includes natural folates found in foodstuffs and folic acid added to foodstuffs as voluntary or mandatory fortification.

Table 2

Age-adjusted odds ratios for prevalent colorectal adenoma by quintile of tissue folate concentration among 813 women in the CONCeRN Study.

	Q1 < 0.442 µg/g wet tissue	Q2 0.442–0.679 µg/g wet tissue	Q3 0.680–0.917 µg/g wet tissue	Q4 0.918–1.261 µg/g wet tissue	Q5 ≥ 1.262 µg/g wet tissue	p trend
Any adenoma (N=170 cases)						
Cases	40	33	30	34	33	
OR (95% CI)	1.00 (reference)	0.77 (0.45–1.31)	0.63 (0.37–1.09)	0.72 (0.42–1.22)	0.68 (0.40–1.16)	0.53
Non-advanced adenoma (N=133 cases)						
Cases	31	23	23	26	30	
OR (95% CI)	1.00 (reference)	0.69 (0.38–1.26)	0.64 (0.35–1.16)	0.73 (0.41–1.30)	0.82 (0.46–1.44)	0.83
Advanced adenoma (N=37 cases)						
Cases	9	10	7	8	3	
OR (95% CI)	1.00 (reference)	1.05 (0.41–2.73)	0.62 (0.22–1.74)	0.69 (0.25–1.89)	0.24 (0.06–0.93)	0.049

[1]All models adjust for age. Adjusting for diabetes status, regular aspirin use, education, smoking, menopausal hormone therapy, physical activity, alcohol, dietary calcium, calcium from supplements, dietary fiber, red meat intake, or body mass index did not substantially modify these results (*i.e.,* no point estimate differed by more than 10% from the age-adjusted odds ratio).

Table 3

Age-adjusted odds ratios for prevalent colorectal adenoma at specific anatomic locations by quintile of tissue folate concentration among 813 women in the CONCeRN Study.

	OR (95% CI)[1]					
	Q1 < 0.442 µg/g wet tissue	**Q2 0.442–0.679 µg/g wet tissue**	**Q3 0.680–0.917 µg/g wet tissue**	**Q4 0.918–1.261 µg/g wet tissue**	**Q5 ≥ 1.262 µg/g wet tissue**	**p trend**
Distal adenoma (N=60 cases)[2]						
Cases	11	10	11	15	13	
OR (95% CI)	1.00 (reference)	0.85 (0.35–2.07)	0.86 (0.36–2.07)	1.19 (0.53–2.72)	1.01 (0.43–2.37)	0.61
Proximal adenoma (N=95 cases)[2]						
Cases	26	21	15	15	18	
OR (95% CI)	1.00 (reference)	0.75 (0.40–1.42)	0.48 (0.24–0.96)	0.49 (0.24–0.97)	0.56 (0.29–1.09)	0.11
Both proximal and distal adenoma (N=15 cases)[2]						
Cases	3	2	4	4	2	
OR (95% CI)	1.00 (reference)	0.63 (0.10–3.87)	1.05 (0.23–4.85)	1.02 (0.22–4.74)	0.47 (0.08–2.94)	0.42

[1] All models adjust for age. Adjusting for diabetes status, regular aspirin use, education, smoking, menopausal hormone therapy, physical activity, alcohol, dietary calcium, calcium from supplements, dietary fiber, red meat intake, or body mass index did not substantially modify these results (*i.e.*, no point estimate differed by more than 10% from the age-adjusted odds ratio).

[2] In models of distal adenomas, case subjects were defined as those with only distal adenomas. Similarly, in models of proximal adenomas, case subjects were defined as those with only proximal adenomas. Subjects with both distal and proximal adenomas were categorized separately.

Table 4

Age-adjusted odds ratios for prevalent hyperplastic polyps and/or colorectal adenoma at specific anatomic locations by quintile of tissue folate concentration among 813 women in the CONCeRN Study.

	OR (95% CI)[1]					
	Q1 < 0.442 µg/g wet tissue	**Q2 0.442–0.679 µg/g wet tissue**	**Q3 0.680–0.917 µg/g wet tissue**	**Q4 0.918–1.261 µg/g wet tissue**	**Q5 ≥ 1.262 µg/g wet tissue**	**p trend**
Hyperplastic polyps only (any subsite) (N= 95 cases)[2]						
Cases	19	12	20	22	22	
OR (95% CI)	1.00 (reference)	0.60 (0.28–1.28)	1.02 (0.52–2.00)	1.13 (0.58–2.18)	1.12 (0.58–2.17)	0.51
Hyperplastic polyps (any subsite); may also have adenoma (any subsite) (N=140 cases)[2]						
Cases	31	20	27	32	30	
OR (95% CI)	1.00 (reference)	0.60 (0.32–1.09)	0.80 (0.45–1.42)	0.97 (0.56–1.69)	0.89 (0.51–1.56)	0.45
Hyperplastic polyps (any subsite) and/or proximal adenoma (N=215 cases)						
Cases	50	35	41	44	45	
OR (95% CI)	1.00 (reference)	0.60 (0.36–1.01)	0.70 (0.42–1.14)	0.75 (0.46–1.22)	0.76 (0.47–1.24)	0.89
Hyperplastic polyps (proximal location) and/or proximal adenoma (N=147 cases)						
Cases	39	27	27	27	27	
OR (95% CI)	1.00 (reference)	0.62 (0.36–1.08)	0.57 (0.33–1.00)	0.55 (0.32–0.97)	0.54 (0.31–0.95)	0.18

[1] All models adjust for age. Adjusting for diabetes status, regular aspirin use, education, smoking, menopausal hormone therapy, physical activity, alcohol, dietary calcium, calcium from supplements, dietary fiber, red meat intake, or body mass index did not substantially modify these results (*i.e.*, no point estimate differed by more than 10% from the age-adjusted odds ratio).