Null Results in Brief

A Prospective Study of Dietary Folate and Vitamin B and Colon Cancer According to Microsatellite Instability and *KRAS* Mutational Status

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Abstract

Sporadic microsatellite instability (MSI)-high colon cancers are positively associated with *MLH1* promoter methylation and inversely with *KRAS* mutation. One-carbon metabolism is critical for methylation reactions and nucleotide biosynthesis, but the influence of dietary one-carbon nutrients such as folate and B vitamins on molecular changes in colon cancer is not known. Using the database of two independent prospective cohort studies (88,691 women and 47,371 men), we examined the relation between dietary intake of one-carbon nutrients and the incidence of microsatellite instability and *KRAS* mutation in 669 incident colon cancers. The overall inverse association between folate and colon cancer did not differ significantly according to MSI status [relative ratio (RR), 0.79; 95% confidence interval (95% CI), 0.60-1.03 for microsatellite stable/MSI-low colon cancers; and RR, 0.61, 95% CI, 0.37-1.02 for MSI-high colon cancers; $P_{heterogeneity}$ = 0.53] or *KRAS* status (RR, 0.66; 95% CI, 0.49-0.87 for *KRAS* wild-type colon cancers; and RR, 1.05; 95% CI, 0.68-1.61 for *KRAS* mutated colon cancers; $P_{heterogeneity}$ = 0.12), although our analyses had limited power to preclude an effect of folate on *KRAS* wild-type colon cancers. Similarly, high vitamin B_6 or B_{12} intake was inversely associated with colon cancers, regardless of MSI or *KRAS* status. No significant effect of methionine intake or alcohol consumption was observed for colon cancers with MSI high or *KRAS* mutation. In conclusion, the influence of dietary one-carbon nutrient intake on colon cancer risk does not seem to differ according to MSI or *KRAS* mutational status. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2895–8)

Introduction

A high degree of microsatellite instability (MSI)-high due to defective mismatch repair is one of the mechanisms in colon carcinogenesis. Most sporadic MSI-high colon cancers are positively associated with *MLH1* promoter methylation (1, 2) and inversely associated with *KRAS* mutation (3). Folic acid and related B vitamins (one-carbon nutrients) are essential for DNA methylation and nucleotide biosynthesis, and it is therefore plausible that chronic folate deficiency may be associated with MSI or *KRAS* mutation. Adequate dietary intake of these nutrients has previously been related to a lower colon cancer risk (4-6). However, whether their intake differentially affects molecular subtypes of colon cancer has not extensively been studied. We therefore assessed whether the influence of folate and B vitamin intake on colon cancer risk differed according to the presence of MSI-high or *KRAS* mutation in two prospective cohort studies where folate intake has been inversely associated with the risk of colon cancer (7, 8).

Materials and Methods

Two independent prospective cohort studies, the Nurses' Health Study (121,701 women followed since 1976; ref 9) and the Health Professional Follow-up Study (51,529 men followed since 1986; ref. 10), formed the study population. Information on potential risk factors and newly diagnosed cases of cancer was updated biennially. Dietary intake of various nutrients including folate, vitamin B_6, B_{12}, and methionine were assessed by self-administered semiquantitative food frequency questionnaires (11, 12). All nutrient contributions including those from supplements were added to the specific nutrient intake from foods to calculate a daily intake for each participant (11). We assumed an ethanol content of 13.1 grams for a 12-ounce (38-dl) can or bottle of beer, 11.0 grams for a 4-ounce (12-dl) glass of wine, and 14.0 grams for a standard portion of spirits.

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All colon cancer cases were confirmed through medical record review by study physicians. We collected paraffin-embedded tissue blocks from hospitals where colon cancer patients underwent resections of primary tumors (10). Based on availability of adequate tissue specimens, we analyzed 669 colon cancers for MSI and *KRAS*. Characteristics of those for whom we did and did not analyze for molecular markers have previously been found to be very similar (10). Genomic DNA from paraffin-embedded tissue was extracted and *KRAS* codons 12 and 13 were sequenced as previously described (13). MSI status was determined using D2S123, D5S346, D17S250, BAT25, BAT26 (14), BAT40, D18S55, D18S56, D18S67, and D18S487 (i.e., 10-marker panel; ref. 15). MSI-high was defined as the presence of instability in ≥30% of the markers.

After excluding participants who did not complete the baseline dietary questionnaire, or reported a baseline history of cancer (except nonmelanoma skin cancer), inflammatory bowel disease, hereditary non-polyposis colon cancer, or a familial polyposis syndrome, 88,691 women and 47,371 men were eligible for analysis. We used a previously described method of competing risk analysis using duplication method Cox regression to compare the specific effect of intake of folate and other nutrients on colon cancer risk according to MSI (or *KRAS* status; refs. 16, 17). We assessed the statistical significance of the difference between the risk estimates according to tumor type using a likelihood ratio test that compared the model that allowed for separate associations of folate and other nutrients according to MSI (or *KRAS* status) with a model that assumed a common association. Established or suspected risk factors for colon cancer were included in the multivariate models. We used SAS version 9.1.3 for all analyses.

Results

Among all 88,691 women and 47,371 men included in these analyses, those with a baseline folate intake of <200 μg/day were slightly more likely to eat meat, smoke and less likely to exercise or report multivitamin use (Table 1).

We documented 669 incident cases of colon cancer accessible for MSI or *KRAS* mutation data during 2,566,968 person-years. Of these, 127 (19%) tumors were MSI high and 242 (36%) tumors were *KRAS* mutated.

As in our previous studies (7, 8, 18, 19), we observed an inverse association between folate and vitamin B_6 intake and colon cancer risk among all cases in our

Table 1. Baseline characteristics of the Nurses' Health Study and Health Professional Follow-up cohort

Characteristic	Energy-adjusted folate intake, μg/d							
	Women				Men			
	<200	200-299	300-399	≥400	<200	200-299	300-399	≥400
	n = 20,907	*n* = 28,882	*n* = 12,997	*n* = 25,905	*n* = 1,512	*N* = 10,122	*n* = 13,425	*n* = 22,312
Dietary intake*								
Folate (μg/d)	159	246	341	677	173	258	347	682
Vitamin B_6 (mg/d)	1.59	2.05	2.76	5.15	3.29	3.73	4.80	13.6
Vitamin B_{12} (mg/d)	5.55	6.45	7.78	15.1	7.89	8.79	9.78	16.4
Alcohol (g/d)	6.7	6.4	6.0	6.3	13.4	13.0	11.4	10.5
Methionine (mg/d)	1.74	1.86	1.95	1.93	2.03	2.13	2.20	2.21
Calcium (mg/d)	574	710	797	796	577	743	858	987
Beef, pork, or lamb as a main dish (servings/wk)	3.1	2.6	2.3	2.3	2.4	2.1	1.8	1.5
Other characteristics								
Median age (y)	46.6	46.8	46.8	46.6	54.4	54.4	54.4	54.4
Former or current smoker (%)	60	56	54	55	60	55	51	50
Pack-yr †	23.3	20.4	18.7	19.2	31.7	27.2	24.1	23.1
Regular aspirin user	31	32	32	35	26	27	28	32
Body mass index (kg/m^2) ‡	24.4	24.5	24.3	24.0	25.8	25.9	25.6	25.3
Physical activity, METS/wk (%)§	11.1	13.8	15.8	15.6	12.9	17.0	20.5	23.7
Postmenopausal (%)‖	44	44	44	44	—	—	—	—
Never used hormones (%)	64	62	61	59	—	—	—	—
Past use of hormones (%)	18	19	19	19	—	—	—	—
Current use of hormones (%)	18	19	20	22	—	—	—	—
Current multivitamin use (%)	8	13	24	84	12	15	23	67
Prior lower endoscopy (%)	2	2	2	2	22	24	26	27
Colorectal cancer in a parent or sibling (%)	8	8	7	8	9	8	8	9

NOTE: Dietary intake and other characteristics at baseline questionnaire in 1980 (Nurses' Health Study) and 1986 (Health Professionals Follow-up Study). Mean value, unless otherwise indicated. All values have been directly standardized according to the age distribution of the cohort.

*Nutrient values (folate, vitamin B_6, B_{12}, methionine, and calcium) represent the mean of energy-adjusted intake.

† Pack-years were calculated for former and current smokers only.

‡ The body mass index is the weight in kilograms divided by the square of the height in meters.

§METS are metabolic equivalents. This was calculated based on the frequency of a range of physical activities (such as jogging) in 1986.

‖Hormones are defined as postmenopausal estrogen or estrogen/progesterone preparations. Percent of never, past, and current use was calculated among postmenopausal women only.

Table 2. Relative risk of folate intake and colon cancer according to MSI status and *KRAS* mutation among 88,691 women from the Nurses' Health Study and 47,371 men from the Health Professionals Follow-up Study

	Energy-adjusted folate intake, μg/d				P_{trend}
	<200	200-300	301-400	>400	
All cancer cases					
No of cases/person-years	114/461,476	182/757,168	139/473,380	231/874,861	
Age-adjusted RR (95% CI)	1.0	0.82 (0.65-1.03)	0.84 (0.66-1.08)	0.76 (0.60-0.95)	0.03
Multivariate RR (95% CI)*	1.0	0.80 (0.63-1.01)	0.80 (0.61-1.04)	0.75 (0.58-0.96)	0.08
MSI-low/MSS cancer cases †					
No of cases/Person-years	89/461,496	144/757,201	117/473,401	189/874,904	
Age-adjusted RR (95% CI)	1.0	1.21 (0.93-1.58)	0.91 (0.67-1.20)	0.80 (0.62-1.03)	0.05
Multivariate RR (95% CI)*	1.0	1.24 (0.95-1.62)	0.86 (0.64-1.15)	0.79 (0.60-1.03)	0.11
MSI-high cancer cases †					
No of cases/Person-years	25/461,549	38/757,294	22/473,478	42/875,029	
Age-adjusted RR (95% CI)	1.0	0.77 (0.47-1.28)	0.60 (0.34-1.07)	0.62 (0.38-1.02)	0.38
Multivariate RR (95% CI)*	1.0	0.75 (0.45-1.01)	0.57 (0.32-1.01)	0.61 (0.37-1.02)	0.45
All cancer cases					
No of cases/Person-years	113/461,476	182/757,197	142/473,402	232/874,893	
Age-adjusted RR (95% CI)	1.0	0.82 (0.65-1.04)	0.87 (0.68-1.12)	0.77 (0.61-0.97)	0.04
Multivariate RR (95% CI)*	1.0	0.81 (0.63-1.02)	0.82 (0.63-1.07)	0.76 (0.59-0.97)	0.08
KRAS-wild-type cancer cases †					
No of cases/Person-years	83/461,503	114/757,261	84/473,452	146/874,968	
Age-adjusted RR (95% CI)	1.0	1.42 (1.07-1.89)	0.70 (0.52-0.96)	0.66 (0.50-0.87)	0.04
Multivariate RR (95% CI)*	1.0	1.45 (1.09-1.93)	0.67 (0.49-0.92)	0.66 (0.49-0.87)	0.06
KRAS-mutated cancer cases †					
No of cases/Person-years	30/461,543	68/757,290	58/473,476	86/875,027	
Age-adjusted RR (95% CI)	1.0	1.16 (0.75-1.78)	1.33 (0.85-2.06)	1.06 (0.70-1.62)	0.46
Multivariate RR (95% CI)*	1.0	1.13 (0.73-1.74)	1.25 (0.80-1.97)	1.05 (0.68-1.61)	0.55

*Multivariate models are adjusted for age (continuous), gender, energy intake (kcal), screening sigmoidoscopy (yes/no), family history of colorectal cancer (yes/no), aspirin use (≥2 tablets/wk or less), smoking (pack-years), physical activity in METs (quintiles), body mass index in five categories (<21, 21-22.9, 23-24.9, 25-29.9, 30+), colon polyps (yes/no), beef intake (quintiles), calcium intake (quintiles), multivitamin use (yes/no), alcohol use (none, <5, 5-14.9, ≥15 g/d), and intake of vitamin B_6, B_{12}, and methionine (quintiles).
† MSS, microsatelllite stable. *KRAS* mutation in codon 12 or 13.

cohort (Table 2). The multivariate relative risk of colon cancer was 0.75 [95% confidence interval (95% CI), 0.58-0.96] for a total folate intake of ≥400 μg, compared with <200 μg folate per day (Table 2). The influence of total folate intake did not differ according to MSI status; comparing extreme categories, the relative ratio (RR) was 0.79 (95% CI, 0.60-1.03) for microsatellite stable/MSI-low colon cancer and 0.61 (95% CI, 0.37-1.02) for MSI-high tumors ($P_{heterogeneity}$ = 0.53). In contrast, the inverse relation between total folate intake seemed to be limited to *KRAS* wild-type cancer, although the tests for heterogeneity did not reach statistical significance ($P_{heterogeneity}$ = 0.12). Results remained virtually unchanged when we limited our examination to cases that occurred before 1998 (before folate fortification became mandatory; data not shown).

We further examined the influence of intake of folate, vitamin B_6, B_{12} and methionine (in quintiles) as well as alcohol intake, but the effects on cancer did not seem to differ by MSI or *KRAS* status (Supplementary Tables S1 and S2).

Discussion

In this large prospective cohort study, we found that both low folate and vitamin B_6 intakes were associated with an increased risk of colon cancer, but these effects did not differ significantly by MSI or *KRAS* mutational status. Few studies have assessed the influence of one-carbon nutrients on colon cancer according to MSI or *KRAS* status and the results have been inconsistent (20-22). It is plausible that chronic folate deficiency may be associated with MSI or *KRAS* mutation, given the importance of folate in DNA methylation and synthesis. Martinez et al. (20) reported a lower incidence of *KRAS*-mutated colon adenomas in individuals with higher folate intake, but others have not found such an effect (21). Only one study has evaluated the association between dietary methyl donor intake and MSI and did not describe an important interaction (22). The absence of a significant association between one-carbon nutrients and MSI or *KRAS* mutational status in the current analysis suggests that more work is still needed to fully delineate the influence of one-carbon nutrients on colon carcinogenesis.

Our study has several important strengths. First, because we collected detailed, updated information on a number of dietary and life-style covariates relevant to colon carcinogenesis over up to 22 years of follow-up and with high follow-up rates, we were able to examine long-term exposures to one-carbon nutrients and to take into consideration important confounding factors. Second, our study is prospective, eliminating concerns about differential recall bias, particularly with regard to our dietary assessments. Any remaining bias from exposure misclassification would thus be nondifferential by nature, biasing our results toward the null.

Limitations of note relate to folate fortification, which became mandatory in 1998 (23). We did obtain multiple assessments of one-carbon nutrient intakes before fortification. In addition, because the development of

colon cancer likely requires some induction period before the onset of a clinically apparent tumor, it is unlikely that the postfortification folate exposure would substantially influence colon cancer risk through 2002. Another potential limitation is that we were unable to obtain tumor tissue from all cases of confirmed colon cancer detected in the two cohorts. However, risk factors in cases unavailable for tissue analysis did not appreciably differ from those in cases with tumor tissue available.

In conclusion, our results show that the reduced risk of colon cancer associated with replete folate status does not seem to vary by MSI or *KRAS* mutational status. Additional studies are needed to elucidate the mechanisms underlying the preventive effect of one-carbon nutrients on colon cancer.

Disclosure of Potential Conflicts of Interest

All of the authors declare no relevant conflict of interest.

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