Research Article

Cancer Prevention Research

Folic Acid Prevents the Initial Occurrence of Sporadic Colorectal Adenoma in Chinese Older than 50 Years of Age: A Randomized Clinical Trial

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Abstract

Colorectal adenoma (CRA) is the precursor lesion of colorectal cancer (CRC). Several agents have been shown to be effective in the chemoprevention of CRA recurrence, but there has been little research on its primary prevention. Participants older than 50 years with no adenomas were recruited for our study and randomized to receive either 1 mg/day folic acid supplement or treatment without folic acid. After 3 years of follow-up, plasma folate and colonoscopy were evaluated. Seven hundred ninety-one participants (91.98%) completed the study. CRA occurred in 64 (14.88%) participants in the folic acid group and 132 (30.70%) in the control group [unadjusted risk ratio (RR), 0.49; 95% confidence interval (CI), 0.37–0.63; $P < 0.01$]; left-sided adenoma (unadjusted RR, 0.54; 95% CI, 0.38–0.76; $P = 0.001$) and advanced CRA (unadjusted RR, 0.36; 95% CI, 0.16–0.81; $P = 0.01$) were most common. There was no significance difference in the occurrence of three or more adenomas (unadjusted RR, 0.70; 95% CI, 0.36–1.77; $P = 0.38$) or right-sided adenoma (unadjusted RR, 0.55; 95% CI, 0.30–1.00; $P = 0.07$) between the two groups. Participants with low plasma folate may have a high risk of CRA. In conclusion, primary prevention with 1 mg/day folic acid supplementation could reduce the incidence of CRA, especially left-sided and advanced disease in those with no previous adenomas. People with differing baseline plasma folate levels should be given individualized treatment. Those with low plasma folate should be encouraged to take adequate supplements; plasma folate should be elevated to an effective therapeutic level, which may reduce the incidence of CRA. *Cancer Prev Res; 6(7); 744–52. ©2013 AACR.*

Introduction

Almost 90% of cases of colorectal cancer (CRC) develop from colorectal adenoma (CRA; refs. 1–4). CRA, the precursor lesion of CRCs in a sequence that may last 10 to 15 years (5), is common in older people, especially those above 50 years of age (6–8).

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Many Asian countries, including China, have experienced an increase of 2 to 4 times in the incidence of CRCs during the past few decades (9). CRC was ranked as the third most prevalent malignancy in Shanghai urban area (10, 11). Data from the CancerBase of the international Agency for Research on Cancer (IARC) show that the incidence in many affluent Asian countries is similar to that in the West (9).

It is recognized that the combination of colonoscopy screening and polypectomy for CRA could reduce the incidence and mortality of CRCs (3, 8, 12, 13). However, the recurrence rate is high (14, 15). Interest has recently been focused on the relationship between chemoprevention and recurrence of CRA. Several agents, including nonsteroidal anti-inflammatory drugs (NSAID) such as aspirin and COX-2–selective inhibitors such as celecoxib, have been shown to be effective for chemoprevention but have not gained general acceptance due to side effects (16, 17). Therefore, the use of nutritional compounds, which usually have fewer severe side effects, is an emerging field in CRA prevention and health maintenance.

The effectiveness of folic acid and its derivatives (folate) in the prevention of CRCs and CRA recurrence has been investigated. However, clinical trials evaluating the chemopreventive effect of folic acid in humans have yielded

conflicting results (18–22). However, these above studies do not provide information on primary prevention by folic acid.

There has been little research on primary prevention of CRA, especially in China. Here, we report a randomized, controlled, large number, open-label cohort trial investigating the effect of supplementation with folic acid on CRA primary prevention in multiple centers, in patients older than 50 years.

Materials and Methods

Design and setting

The trial had a prospective, randomized, controlled design comparing daily supplementation with 1 mg folic acid with controls (who did not receive folic acid folic acid). Patients were randomized after a run-in period to receive either 1 mg folic acid daily supplements or treatment without folic acid over 3 years. The trial involved 5 clinical centers (Ren-Ji Hospital, Shanghai Jiao-Tong University School of Medicine; Military General Hospital of Beijing, PLA; Southwest Hospital, Third Military Medical University; Shanghai 1st Hospital, Shanghai Jiao-Tong University; and Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School). All participants were older than 50 years.

Study outcomes

Primary outcome. The primary outcome parameter was the incidence of any type of CRA (tubulovillous, tubular, villous, or serrated lesions) at 3-year follow-up colonoscopy in both groups. The occurrence, number, location, size, and histologic subtype of adenoma and invasive growth were assessed in both groups.

Secondary outcome. Plasma folate was evaluated as a secondary outcome parameter at the beginning and end of the follow-up period. Serum samples were stored at −20°C until needed. We used an automated chemiluminescence system (ACS: 180; Chiron Diagnostics Corporation) to determine folic acid concentrations in plasma. The relationship between initial plasma folate level and the incidence of CRA was determined as well as the effective therapeutic folic acid level.

Sample population, randomization, and interventions

Recruitment was conducted between June 2008 and May 2012. A sample size of 800 participants was selected to provide power of at least 80% to detect a risk reduction with folic acid (40% reduction) using a 2-sided statistical significance level of $P < 0.05$ (19). This assumed an adenoma occurrence rate of 30% in the control group (7, 23) and a follow-up rate of 80%. Potential participants were identified by clinical center staff from colonoscopy and pathology reports. Eligible participants were ages 50 to 80 years and had undergone complete colonoscopy with no adenoma found at least twice in the past 5 years, the last colonoscopy being conducted within 1 year before recruitment. Exclusion criteria for participation in the study were a history of hereditary nonpolyposis CRC or familial adenomatous polyposis, inflammatory bowel disease, CRC, malabsorption syndromes, any condition that could be worsened by supplemental folic acid, and any condition commonly treated with folate (e.g., arthritis, megaloblastic anemia, atrophic gastritis) or NSAIDs (e.g., rheumatoid arthritis, vasculitis, some connective tissue disorders).

After baseline colonoscopy and a run-in period, we used computer-generated randomization to allocate participants in a 1:1 ratio to 1 mg/day folic acid or treatment without folic acid (Multivitamin Tablets; ref. 6); Sino-American Shanghai Squibb Pharmaceutical Co., Ltd; a standard vitamin supplement containing no folate). The study start date was the first day of folic acid intake.

Follow-up and study visits

During the 3-year follow-up period, participants in the folic acid supplementation group were asked to visit their medical centers every 3 months to check for any adverse events or medication changes and to confirm that they had been taking the study medication regularly. Participants in the control group were followed-up by telephone or letter at the same intervals. Data on health status and complaints about the folic acid supplement were collected and, after each visit, the study medication for the next study period was dispensed. The final visit, 3 years after the start of the study, included colonoscopy.

All colonoscopies had to have been conducted by skilled endoscopists who were unaware of the clinical details (especially whether the subject was taking the folic acid supplement), using standard colonoscopes (CF 200L, 240L or 260L; Olympus Optical Co., Ltd.). Bowel preparation included 2 to 5 L polyethylene glycol electrolyte solution administered in the morning before an afternoon examination or the previous evening in patients undergoing examination in the morning. If the colonoscopy did not reach the cecum, it was not included for further analysis and the patient was required to undergo colonoscopy by another endoscopist on the next occasion. The mean colonoscopy withdrawal time was required to be 6 minutes or more. During the examination, the location and size of all polypoid lesions were recorded. The size of each polyp was measured *in vitro* and all retrieved polypoid lesions were sent to local pathology laboratories for histologic evaluation. The results of the colonoscopy as well as histologic confirmation were documented, as were clinical data on health status and complaints about and adherence to the study medication.

Plasma folate was evaluated at the end of the follow-up period to determine its relationship with the incidence of CRA and to ascertain adherence to treatment.

Data management and statistical analysis

Data collection was conducted using paper Case Report Form (CRFs), and the data were entered and stored in a validated database that conformed with Good Clinical Practice (GCP) requirements. The CRFs were monitored regularly. The predefined primary statistical analysis was the

χ^2 test with a significance level of 5% comparing the risk of CRA between the 2 groups. The trial results were evaluated by intention-to-treat and per-protocol analysis. Risk ratios (RR) and 95% confidence intervals (CI) were also used compare the folic acid supplementation and control groups by Cox regression methods.

Independent sample and paired *t* tests were used for comparisons between the groups to evaluate the relationship between the initial plasma folate level and the incidence of CRA. All statistical analyses were conducted using SPSS 17.0.

Ethics

The study was conducted according to the study protocol, was approved by the ethics committee of each medical center, and was registered with the Chinese Clinical Trial Registry (ChiCTR-PRC-08000123). Each participant was required to sign an informed consent form.

Results

Participants and follow-up

Nine hundred eighty participants who were confirmed to have no adenomas by colonoscopy entered the run-in period; after a 2-week run-in period, 860 underwent randomization for the study (Fig. 1). Of the 120 participants who were not randomized, 25 (20.83%) were unable to avoid taking medication or supplements prohibited by the study, 20 (16.67%) were nonadherent and 46 (38.33%) declined to continue the study. Twenty-nine (24.17%) were excluded for other reasons (most were found to have a concurrent illness, such as dysfunction of the liver or

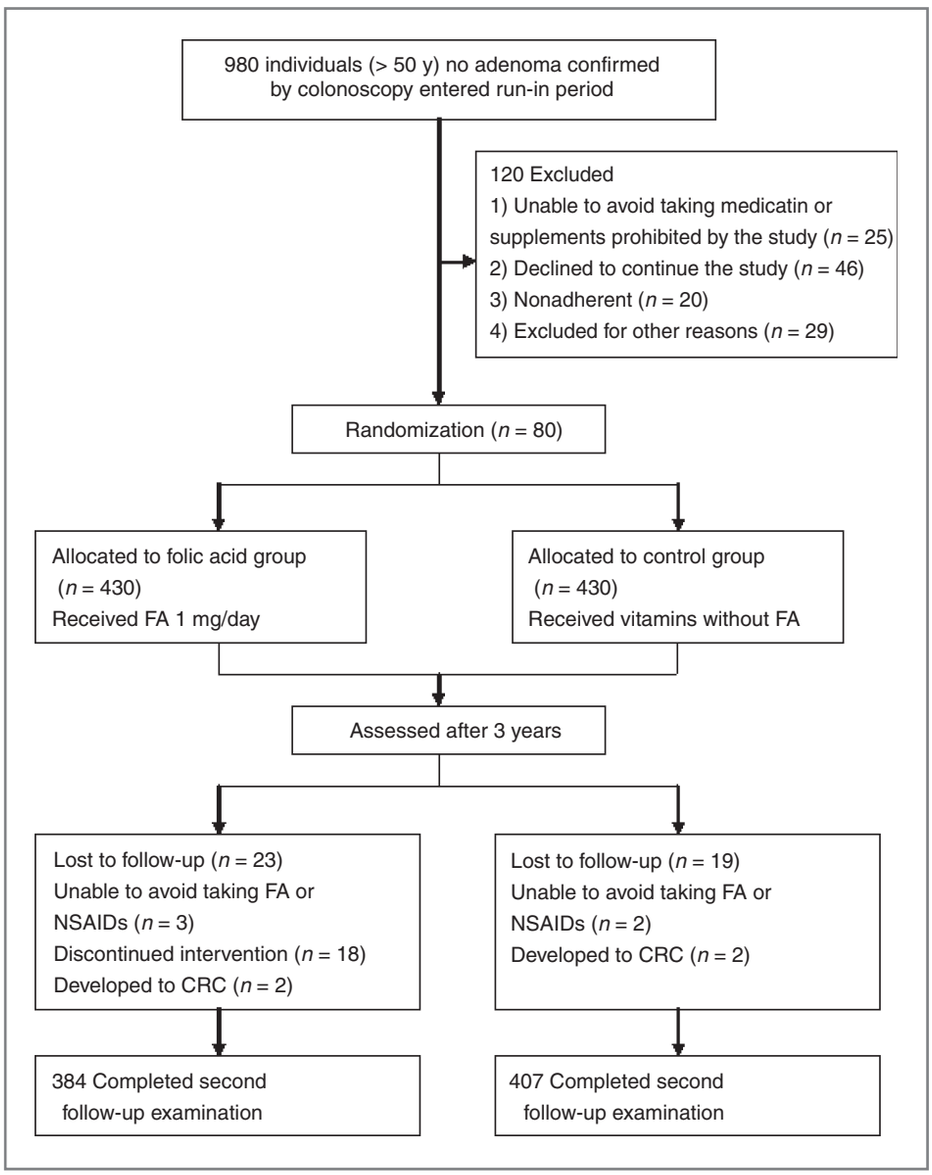

Figure 1. Flow diagram of the phases of the study. FA, folic acid.

Table 1. Baseline characteristics of the participants

Characteristic	Folic acid group ($n = 430$)	Control group ($n = 430$)	*P*
Age (±SD), y	60.79 ± 7.53	60.26 ± 7.11	0.92
Male (%)	50.92%	49.75%	0.85
Plasma folate, ng/mL	5.04	5.56	0.09
Cigarette smoke			
Smoker	19.3%	15.35%	0.15
Ex-smoker	2.79%	1.86%	0.49
Alcohol consumption			
Yes	17.44%	14.88%	0.35
Weekends only	8.37%	11.16%	0.21
Tea consumption	11.86%	8.6%	0.14
Coffee consumption	3.72%	5.81%	0.20
Grain/cereals (±SD), g/d	352.1 ± 8.7	361.7 ± 10.6	0.75
Meat (±SD), g/d	55.6 ± 3.1	59.3 ± 2.8	0.29
Fish (±SD), g/d	104.7 ± 4.0	110.4 ± 3.1	0.26
Citrus fruit			
Usually	33.02%	39.3%	0.07
Seldom	2.56%	1.86%	0.64
Milk			
Usually	53.72%	48.14%	0.12
Seldom	7.21%	6.51%	0.68
Leafy green vegetables (±SD), g/d	85.7 ± 3.2	79.5 ± 4.9	0.53
Other vegetables (±SD), g/d	143.2 ± 4.7	135.6 ± 6.0	0.31
Soybean products (±SD), g/d	113.5 ± 4.1	110.7 ± 5.4	0.68

kidney). Four hundred thirty participants were randomized to the 1 mg/day folic acid group and 430 to the control group. The first participant was enrolled in June 2008 and the last at the end of 2008. Seven hundred ninety-one participants (91.98%) underwent colonoscopy after 3 years of follow-up. The remaining 69 participants were lost to follow-up ($n = 42$), discontinued the intervention ($n = 18$), or were unable to avoid regularly taking folic acid or NSAIDs for more than 2 weeks during the follow-up period ($n = 5$) and 2 in each group developed CRCs. The mean time from randomization to completion of follow-up was 37.8 ± 3.7 months. There were no differences in baseline characteristics between the folic acid and the control groups (Table 1).

Primary outcome measure

After 3 years of follow-up, we found that folic acid supplementation may decrease the risk of CRA. In the intention-to-treat population, CRA occurred in 64 (14.88%) participants in the folic acid group and 132 (30.70%) in the control group (unadjusted RR, 0.49; 95% CI, 0.37–0.63; $P < 0.01$; Table 2). Among the participants for whom follow-up information was available, 3 or more adenomas occurred in 14 (3.26%) participants in the folic acid group and 20 (4.65%) participants in the control group (unadjusted RR, 0.70; 95% CI, 0.36–1.77; $P = 0.38$). Adenoma occurred on the left side of the colon in 42 participants in folic acid group and 78 participants in the control group (9.77% vs.18.14%); this was a significant difference (unadjusted RR, 0.54; 95% CI, 0.38–0.76; $P = 0.001$). However, there was no such trend in right-sided adenoma and the difference did not reach significance (16 participants in the folic acid group and 29 participants in the control group; unadjusted RR, 0.55; 95% CI, 0.30–1.00; $P = 0.07$).

We also evaluated the impact of folic acid on the occurrence of advanced CRA, which was defined as an adenoma with a diameter of 10 mm or more, a villous adenoma (≥25% villous) or an adenoma with high-grade dysplasia (7, 23). Advanced CRA occurred in 8 (1.86%) participants in the folic acid group and 22 (5.17%) participants in the control group, which was a significant difference (unadjusted RR, 0.36; 95% CI, 0.16–0.81; $P = 0.01$). The results of the per-protocol analysis were similar to those of the intention-to-treat analysis. Adjustment for age, sex, clinical center, and duration of follow-up at baseline did not substantially affect the results.

Secondary outcome measures

Association of baseline plasma folate with adenoma risk. We evaluated the relationship between plasma folate level and the occurrence of CRA. Baseline folic acid levels did not differ between the 2 groups but did show a difference between participants who developed CRA and those who did not (Table 3). In the folic acid group, baseline folic acid was lower in participants who developed CRA (4.07 ± 2.73 ng/mL) than in those who had no adenoma

Table 2. Risk of adenoma after randomization in the intention-to-treat population

	No. (%) of participants			
	Folic acid ($n = 430$)	Control ($n = 430$)	RR (95% CI)	*P*
Any adenoma	64 (14.88%)	132 (30.70%)	0.49 (0.37–0.63) 0.74[a] (0.65–0.85)	<0.001
No. of adenomas ≥ 3	14 (3.26%)	20 (4.65%)	0.70 (0.36–1.77) 1.01[a] (0.87–1.16)	0.38
Left-sided adenoma	42 (9.77%)	78 (18.14%)	0.54 (0.38–0.76) 0.40[a] (0.33–0.49)	0.001
Right-sided adenoma	16 (3.72%)	29 (6.74%)	0.55 (0.30–1.00) 0.77[a] (0.55–1.09)	0.07
Advanced adenoma	8 (1.86%)	22 (5.17%)	0.36 (0.16–0.81) 0.67[a] (0.58–0.76)	0.01
CRCs	2 (0.47%)	2 (0.47%)	1.00 (0.14–7.07) 1.00[a] (0.87–1.15)	1.00

NOTE: The intention-to-treat population comprised all randomized participants with 3 years of follow-up data, including those participants who discontinued. *P* values are based on the χ^2 test.

[a]Adjustment for age, sex, clinical center, and duration of follow-up at baseline.

(5.36 ± 3.94 ng/mL). The same tendency was found in the control group (4.28 ± 2.79 vs. 5.85 ± 2.78 ng/mL). These results show that baseline plasma folate was inversely associated with CRA risk, which means that people with low plasma folate may be at high risk of CRA ($P < 0.05$ in folic acid group, $P < 0.01$ in control group).

Effective therapeutic folic acid level and adenoma risk in participants with differing baseline plasma folate. Although 1 mg/day folic acid supplements were provided to all participants randomized to the folic acid group, their plasma folate levels varied. As Table 3 shows, after 3 years of folic acid supplementation, plasma folate increased, especially in participants who did not develop adenoma (7.70 ± 3.06 ng/mL, $P < 0.01$). However, although plasma folate was increased above baseline in the participants who had developed CRA by the end of the 3-year follow-up period, the difference was not significant (5.44 ± 2.19 ng/mL, $P = 0.05$). These results indicate that there may be an effective therapeutic level of folic acid and that people who do not achieve this level may still be at risk of CRA.

To determine the therapeutic level of folic acid in subjects with differing baseline folate levels, we divided all of the participants who received folic acid supplements into 2 groups: those with low baseline plasma folate [<4.27 ng/mL, the value was determined by receiver operating characteristic (ROC) curve; ref. 24] and those with high baseline plasma folate (≥4.27 ng/mL). The results are shown in Table 4. Most of the participants who received folic acid supplements exhibited a significant increase of plasma folate, but participants with high baseline plasma folate did not achieve an increase to a protective level. In contrast, some subjects achieved a protective level but CRA still occurred. This phenomenon indicates that there is a threshold level for effective therapeutic folic acid. Plasma folate remained low (4.65 ± 1.75 ng/mL) after supplementation in the group in whom CRA occurred with low baseline levels. Doubling of folic acid (4.65/2.61) may not satisfy the requirements of these participants; folic acid may need to be elevated by more than 3 times (9.22/2.49) to avoid CRA.

Side effects and contraindications. Side effects are seldom reported for folic acid supplementation, especially with normal doses. In the present study, allergic reactions (1, 0.23%), gastrointestinal discomfort (4, 0.93%), diarrhea (1, 0.23%), and constipation (2, 0.47%) were reported. Similar rates were reported by those in the control group, who experienced gastrointestinal discomfort (3,

Table 3. Plasma folate before and after follow-up

	Folic acid group, ng/mL			Control group, ng/mL		
	No CRA	CRA occurred	*P*	No CRA	CRA occurred	*P*
Plasma folate in baseline	5.36 ± 3.94	4.07 ± 2.73	<0.05	5.85 ± 2.78	4.28 ± 2.79	<0.01
Plasma folate after follow-up	7.70 ± 3.06	5.44 ± 2.19		5.90 ± 2.18	5.32 ± 2.33	
P	<0.01	0.05		>0.05	>0.05	

NOTE: All data were shown in mean ± SD.

Table 4. The benefit of folic acid supplementation and adenoma risk in participants with differing baseline plasma folate levels ($N = 384$)

	Baseline plasma folate[a] (<4.27 ng/mL)		Baseline plasma folate (≥4.27 ng/mL)	
	$n = 118$		$n = 266$	
	No CRA ($n = 76$)	CRA occurred ($n = 42$)	No CRA ($n = 244$)	CRA occurred ($n = 22$)
Plasma folate in baseline	2.49 ± 1.51	2.61 ± 1.34	7.07 ± 3.78	6.08 ± 2.57
Plasma folate after follow-up	9.22 ± 2.96	4.65 ± 1.75	8.14 ± 3.10	6.86 ± 2.24
P value	<0.001	<0.001	<0.01	0.85

NOTE: All data are shown in mean ± SD.

Abbreviation: *n*, number of participants.

[a]RR, 6.13; 95% CI, 3.44–10.91, which means participants with low baseline plasma folate may have a high risk of CRA.

0.70%), diarrhea (1, 0.23%), and constipation (3, 0.70%) but no allergic reactions.

Discussion

The initiation and development of CRCs is a complex process involving multiple molecular pathways; from the occurrence of adenoma to the development of carcinoma, the process can last 2 decades (5). Both aspirin and COX-2 inhibitors can reduce the incidence of CRA and may have an effect on the development of CRCs, but safe doses and durations of treatment remain to be determined and may limit their use in healthy individuals. Use of dietary agents that may delay the onset and progression of this disease is likely to have significant health benefits.

Folates are important cofactors in key DNA synthesis and methylation pathways (25). Many observational studies of folate and CRCs in large populations have indicated that deficiency of dietary folate may be correlated with increased occurrence of CRCs, but results differ between reports. We believe that carcinogenesis is a multistep process in which cancers arise as the result of interactions over time between genetic alterations and the environment, and that the timing of intervention may be of major importance. However, there have been no systematic studies to evaluate the effect of folate on the primary prevention of CRA.

Our results show for the first time that 1 mg folic acid supplements taken over a period of 3 years can decrease the risk of sporadic CRA in patients older than 50 years. More importantly, folic acid supplements can significantly reduce the incidence of advanced CRA, which may reduce carcinogenesis. A similar trend was observed for left-sided adenomas but not for right-sided or multiple (≥3) adenomas. Because folic acid plays an important role in DNA methylation and cellular homeostasis, folate deficiency might impair these processes and cause chromosomal breaks, as well as deleterious changes in gene expression. All of these factors might induce genomic instability and aberrant DNA methylation, which contribute to carcinogenesis (22, 26, 27). Data from our clinical trial clearly support a chemopreventive role for folic acid in primary prevention of CRA, especially in patients older than 50 years. We believe that the mechanism of primary prevention of adenoma may differ from the effects of folic acid on progression from adenoma to carcinoma. Many preclinical carcinogenesis studies in animal models suggest that periods of tumor initiation and tumor progression or promotion are dissimilar (20). The timing of folic acid administration is important if it is to have a protective effect on normal mucosa in animals exposed to carcinogens or in mice bearing mutations in genes known to be involved in carcinogenesis (28). Our previous study showed that folic acid supplementation was significantly associated with decreased risk of CRCs in a mouse model and that folic acid was more effective in a subgroup without precancerous lesions than in mice with precancerous lesions (29). Thus, we suggest that, to reduce the incidence of CRCs, it is better to provide folic acid before the initiation of CRA.

Our study shows that low plasma folate is associated with an increased risk of CRA, and folic acid supplementation at a dose of 1 mg/day can significantly reduce the adenoma risk. That means the baseline folate level might predict the risk of CRA, but this might be partly risk because it can be altered by further supplementation. We believe the effects of a chemopreventive agent, especially one based on a dietary component, may vary depending on the baseline state of sufficiency. Previous results showed that folate supplementation may protect against adenoma or cancer only in those with low baseline folate levels (30). Our data strongly support this theory; therefore, people with low plasma folate should be encouraged to take adequate folate supplements based on the potential perceived risk of CRCs.

Interestingly and importantly, a threshold level for effective therapeutic folic acid may exist, which suggests that the degree plasma folate increase is more important than the dose of folic acid supplementation (Table 4). Our analysis shows that primary prevention of CRA should be individualized. Participants with differing baseline plasma folate levels should be given folic acid supplementation at different doses or for different durations to achieve therapeutic levels. Subjects with high baseline folate will obtain limited benefit from folic acid supplements and only if their plasma folate is elevated significantly above baseline. However,

subjects with low baseline folate should be encouraged to take folic acid supplements to achieve the therapeutic level. Those who achieve only limited increases of plasma folate may need higher doses or longer durations of supplementation to reach the therapeutic level. The reason why there remained some in whom therapeutic levels were not achieved after 3 years of folic acid supplementation may be that the synthetic folic acid used in this study differs from natural folates in its chemopreventive properties, which may influence its absorption and utilization. Also, the participants in our study had a mean age of 60 years, and degeneration of gastrointestinal function with age may have led to malabsorption of folic acid. MTHFR polymorphisms, which may influence folate status, may also affect plasma folate levels; further studies are needed.

Nine (14.07%) participants in the folic acid group achieved therapeutic folic acid levels but developed CRA. Mean baseline plasma folic acid levels in these patients were quite high (7.15 $\pm$ 2.49 ng/mL) and 3 achieved effective increases of folic acid, which indicates that the mechanism of the development of CRA in these people was not principally due to low plasma folate. Other bioactive food components such as fiber, short-chain fatty acids, methionine, or calcium might be involved. Further studies are needed.

Although several studies have suggested that high-dose folic acid might increase the recurrence and progression of CRCs (31, 32), only 2 participants in our folic acid group developed CRCs during 3 years of follow-up, and there was no significant difference with the control group. We believe that 1 mg/day folic acid supplementation is safe and has few side effects.

In our study, 14 participants developed multiple CRA in the folic acid group, most of whom (71.43%) did not reach therapeutic folic acid levels. A similar trend occurred in right-sided adenoma, so we hypothesize that higher doses and longer durations of folic acid supplementation may significantly reduce the incidence of multiple CRA or right-sided adenoma. folic acid supplementation is relatively safe, and further studies of higher doses may be needed.

The overall average level of plasma folate in the present study seems lower than that reported in previous studies (18, 19). We believe this may be due to the participants in our study being older (>50 years). B vitamin status is frequently inadequate in the elderly (33), and studies have shown that the elderly should be a particular concern because of age-related declines in vitamin absorption and extraction of vitamin B12 from protein (34), which may lead to subclinical folate deficiency (35). Ethnic differences should also be considered. Studies of genetic polymorphisms in folate metabolism suggest a causal role for folate in cancer prevention. The availability of 5-methyltetrahydrofolate (5-methylTHF), the main folate coenzyme in the circulation, is modified by a common single-nucleotide polymorphism, 677C→T, in the MTHFR gene (36). The presence of this mutation is known to correlate with suboptimal folate status (37) and its prevalence has been shown to vary between populations (38). A recent study showed that genetic variations in exons of the FR-α gene, FOLR1, can cause severe folate deficiency in Eurasians (39). These gene mutations may cause the average level of plasma folate to be lower than those observed in previous studies. Further studies are needed.

Our results show that about 30.70% of participants in the control group had developed CRA after 3 years; this incidence was quite high. We believe there may several reasons. First, previous studies have shown that the incidence of CRA is high in elderly people (7, 8, 13, 40), and the incidence of neoplastic lesions can reach 37.5% in asymptomatic patients (8). It is worth noting that, after the follow-up period, the incidence of CRA can reach 16% to 26% in patients with no adenomas on baseline screening, especially in those with hyperplastic polyps (41–43). The enrolled patients in our study were older than 50 years, which may have increased the incidence of CRA. Second, optimal bowel preparation as well as ongoing efforts to improve the technology of colonoscopy, such as the use of high-definition endoscopy, could minimize the risk of missing important lesions in clinical practice. The endoscopists' skill may also have contributed to our findings. According to experts, 6 minutes is the minimum time required for adequate inspection during instrument withdrawal (44), and this requirement may ensure the validity of CRA detection.

Because this was a clinical study, we cannot exclude the possibility of residual confounding, although we tried to avoid this; participants who were unable to avoid taking medication prohibited by the study (e.g., NSAIDs for more than 2 weeks during follow-up) were required to quit the study. We could not restrict the participants' diets, but no folate-fortified foods are currently available for purchase in China. Also, baseline folate levels showed no difference between the groups, and it is well-known that the elderly seldom change their eating habits or food preferences, which may have minimized the influence of these factors.

Our study is the first to explore the relationship between folate intake and primary prevention of CRA in a Chinese population. The data were gathered from multiple centers, thereby lessening the potential for observation bias and measurement errors. Despite these strengths, however, the study also had some limitations. For example, there is the possibility of differential recall between cases and controls. Furthermore, this was an open-label cohort study that did not contain a blank placebo group and did not use double-blind methods. However, the endoscopists were unaware of the clinical details (especially whether the subject was taking the folic acid supplement), which may have ensured the objectivity of the findings. Third, the folic acid level of most of the subjects enrolled in our study seems to be lower than that recorded in other studies (18, 19), which suggests that those with lower folate levels may benefit from folic acid supplements. Because the enrolled patients in our study were Chinese, we still lack of the material about the effectiveness of folic acid supplementation in the non-Chinese population. Therefore, to popularize the usage of folic acid supplementation may still need more evidences. We should enlarge our sample size in further studies,

especially including different ethnic population and those with higher basal folate levels to evaluate the impact of folic acid supplementation without observation bias. Finally, we only provide a simple stratified result, which may have limited the power of the study.

In conclusion, we found a statistically significant relationship between folic acid supplementation and reduced risk of CRA in those older than 50 years with no previous adenomas. Primary prevention with 1 mg/day folic acid reduced the incidence of CRA (RR, 0.485), especially advanced CRA and left-sided adenoma. People with differing baseline plasma folate levels should be given individualized treatment. Those with low plasma folate should be encouraged to take adequate supplements, and plasma folate should be elevated to an effective therapeutic level, which may reduce the incidence of CRA.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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