Colorectal cancer

Folate: a magic bullet or a double edged sword for colorectal cancer prevention?

Y-I Kim

Low folate status might inhibit colorectal carcinogenesis and high folate status may promote colorectal carcinogenesis, contradicting findings from epidemiological studies showing an inverse relationship between folate status and risk of colorectal cancer

Folate is a water soluble B vitamin, deficiency of which appears to play an important pathogenetic role in the development of anaemia, atherosclerosis, neural tube defects (NTDs), adverse pregnancy outcomes, neuropsychiatric disorders, and cancer.¹ Folic acid is the fully oxidised monoglutamyl form of this vitamin that is used commercially in supplements and in fortified foods. Folate is generally regarded as safe and has long been presumed to be purely beneficial and an ideal functional food component for disease prevention.^{2,3} For example, an overwhelming body of evidence for a protective effect of periconceptional folic acid supplementation against NTDs led to mandatory folic acid fortification in the USA and Canada in 1998.⁴ The effectiveness of folic acid fortification in improving folate status has already been shown to be quite striking, with a dramatic increase in blood measurements of folate concentrations in the USA and Canada.⁴ Preliminary reports also suggest a significant reduction (~15–50%) in the incidence of NTDs in the USA and Canada.⁴

Perhaps one of the most speculative and provocative new medical applications of folate nutrition is the potential role of folate as a cancer preventive agent.^{1,5} The concept that folate deficiency enhances, whereas folate supplementation reduces, the risk of neoplastic transformation appears counterintuitive and contradictory to our conventional understanding of folate biochemistry. Folate is an essential cofactor for the *de novo* biosynthesis of purines and thymidylate, and in this role folate plays an important role in DNA synthesis and replication.^{5,6} Consequently, folate deficiency in tissues with rapidly replicating cells results in ineffective DNA synthesis. In neoplastic cells where DNA replication and cell division are occurring at an accelerated rate, interruption of folate

metabolism causes ineffective DNA synthesis, resulting in inhibition of tumour growth.^{5,6} Indeed, this has been the basis for cancer chemotherapy using a number of antifolate agents (for example, methotrexate) and 5-fluorouracil.^{5,6} Furthermore, folate deficiency has been shown to induce regression and suppress progression of pre-existing neoplasms in experimental models.^{7–9} In contrast with the inhibitory and promoting effect of folate deficiency and supplementation, respectively, on established neoplasms, however, folate status appears to have an opposite effect in normal tissues. An accumulating body of epidemiological, clinical, and experimental evidence suggests that folate deficiency in normal tissues appears to predispose them to neoplastic transformation, and folate supplementation suppresses the development of tumours in normal tissues.^{1,5}

Epidemiological studies have suggested an inverse association between folate and the risk of cancer of the colorectum, oropharynx, oesophagus, stomach, pancreas, lungs, breast, cervix, ovary, and breast, and neuroblastoma and leukaemia.^{1,5} The role of folate in carcinogenesis has been best studied for colorectal cancer (CRC).^{1,5,10,11} Collectively, more than 20 case control studies have shown either a significant or equivocal inverse relationship that was not statistically significant, that became non-significant after adjustment, or that could not be distinguished from other factors in their relation to risk between folate status (assessed by dietary folate intake or by measurement of blood folate levels) and the risk of CRC or its precursor, adenoma, in the general population and in individuals with chronic ulcerative colitis.^{1,5,10,11} Several large prospective studies also suggest a 40% reduction in the risk of CRC and adenomas in those with the highest intake of folate compared with those with the lowest intake.¹¹ Two

recent meta-analyses of epidemiological studies have confirmed a significant inverse association between folate intake and the risk of CRC, with a 20–25% reduction in the risk of CRC in subjects with the highest folate intake compared with those with the lowest intake.¹² Some epidemiological studies have shown a beneficial effect of multivitamin supplements containing ≥400 µg folic acid for ≥15 years on CRC risk and mortality.^{13,14} In addition, several small clinical trials have demonstrated that folic acid supplementation can improve or reverse surrogate endpoint biomarkers of CRC.^{1,5,15}

There exist several biologically plausible mechanisms by which folate deficiency increases, whereas folate supplementation reduces, the risk of CRC in normal colorectal epithelial cells.^{5,6,15,16} As an essential cofactor for the *de novo* biosynthesis of purines and thymidylate, folate plays an important role in DNA synthesis, stability and integrity, and repair, aberrations of which have been implicated in colorectal carcinogenesis.^{5,6,16} Folate may also modulate DNA methylation, which is an important epigenetic determinant in gene expression, in the maintenance of DNA integrity and stability, in chromosomal modifications, and in the development of mutations.^{5,6,15} A growing body of evidence from *in vitro*, animal, and human studies indicates that folate deficiency is associated with DNA strand breaks, impaired DNA repair, increased mutations, and aberrant DNA methylation, and that folate supplementation can correct some of these defects induced by folate deficiency.^{5,6,15}

Folate therefore appears to be an ideal nutritional factor for CRC prevention. However, recent animal studies and intervention trials have dampened the enthusiasm for the potential role of folate supplementation in CRC prevention.^{4,17} Data from animal studies generally support a causal relationship between folate depletion and CRC risk and an inhibitory effect of modest levels of folate supplementation on colorectal carcinogenesis.^{1,5} However, these animal studies have also shown that the dose and timing of folate intervention are critical in providing safe and effective chemoprevention; exceptionally high supplemental folate levels and folate intervention after microscopic neoplastic foci are established in the colorectal mucosa promote rather than suppress colorectal carcinogenesis.^{1,5} For example, in a standard chemical carcinogen rodent model of CRC, supraphysiological levels of folic acid supplementation (>20 times the basal daily dietary requirement) have been shown to

increase the development and progression of CRC.^{18–20} Furthermore, in two genetic models of CRC (*Apc^{Min}* and *Apc⁺–x Msh2–/–* mice), moderate dietary folate deficiency enhanced, whereas modest levels of folic acid supplementation (4–10 times the basal daily dietary requirement) suppressed, the development and progression of CRC, if folate intervention was started before the establishment of neoplastic foci in the intestine.^{21–22} If however folate intervention was started after the establishment of neoplastic foci, dietary folate had an opposite effect on the development and progression of CRC.^{21–22}

Furthermore, the Aspirin-Folate Polyp Prevention Study (n = 1021) reported that folic acid supplementation (1 mg/day) for up to six years in subjects with previous colorectal adenomas did not significantly prevent the recurrence of colorectal adenomas (rate ratio (RR) 1.04).²³ However, folic acid supplementation significantly increased the number of adenomas by 44% (RR 1.44 (95% confidence interval (CI) 1.03–2.02)) and non-significantly increased the incidence of advanced adenomas with a high malignant potential compared with placebo.²³ One explanation for this unexpected observation is that folic acid supplementation might have promoted the progression of already existing undiagnosed preneoplastic lesions (for example, aberrant crypt foci (ACF), probably the earliest precursor of CRC, or microscopic adenomas) or adenomas missed on initial colonoscopy in these genetically predisposed patients at high risk of developing CRC. This hypothesis is supported by prior observations that addition of folate to established tumours causes an “acceleration phenomenon” in humans; children with acute leukaemia treated with folate supplementation experienced accelerated progression of leukaemia.²⁴ Two recently published large, randomised, placebo controlled intervention trials designed to test the effect of folic acid supplementation in conjunction with other B vitamins on primary and secondary prevention of cardiovascular events have reported a non-significant trend towards an increased risk of total cancer (RR 1.22 (95% CI 0.88–1.70)) in the Norwegian Vitamin trials (n = 3749; 800 µg folic acid/day for 40 months)²⁵ and of colon cancer (RR 1.36 (95% CI 0.89–2.08)) in the Heart Outcomes Prevention Evaluation 2 trial (n = 5522; 2.5 mg folic acid/day for five years).²⁶

Although all of the published epidemiological studies to date have not reported a harmful effect of folate on CRC risk,¹¹ the study by Van Guelpen and colleagues²⁷ in this issue of *Gut* suggests for the first time a potential

harmful effect of high folate status on CRC risk (see page 1461).²⁷ This population based nested case control study in the Northern Sweden Health and Disease Cohort reports that plasma folate concentrations are significantly related to the risk of CRC in a bell shaped manner; multivariate odds ratios (OR) were 2.00 (95% CI 1.13–3.56) for the middle versus lowest quintile and 1.34 (95% CI 0.72–2.50) for the highest versus the lowest quintile. Furthermore, in subjects followed for longer than the median of 4.2 years, plasma folate concentrations were strongly positively related to CRC risk; multivariate OR for the highest versus lowest quintile was 3.87 (95% CI 1.52–9.87; p trend = 0.007). The main and novel finding of this study is that low folate status might inhibit colorectal carcinogenesis and that high folate status may promote colorectal carcinogenesis. Therefore, this study contradicts the findings of other epidemiologic studies that showed the inverse relationship between folate status and CRC risk.¹¹ However, this study supports the findings from previously discussed animal studies and clinical observations that suggest that folate status might promote colorectal carcinogenesis depending on the dose and timing of folate intervention. Although several animal studies have suggested a potential harmful effect of high folate status, these studies have largely been ignored by epidemiologists and public health policy makers.^{4–17} This type of study can no longer be performed in North America because of the mandatory folic acid fortification implemented in 1998 and hence the findings from this study provide very important information regarding the safety of folic acid supplementation.

Also, the widely accepted notion that folate status is inversely related to the risk of developing other cancers has begun to be challenged in recent epidemiological studies. For example, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (n = 25 400 postmenopausal women) has reported that women consuming supplemental folic acid ≥400 µg/day have a 20% increased risk of developing breast cancer compared with subjects reporting no supplemental intake (hazard ratio (HR) 1.19; 95% CI 1.01–1.41; p trend = 0.04).²⁸ Although food folate intake was not significantly related to breast cancer risk (HR 1.04; 95% CI 0.83–1.31; p trend = 0.56), total folate intake, mainly from folic acid supplementation, significantly increased the risk by 32% (HR 1.32; 95% CI 1.04–1.68; p trend = 0.03).²⁸

What can we conclude about the role of folate in CRC from these seemingly

paradoxical and contradictory epidemiological, animal, and clinical observations? Folate appears to possess dual modulatory effects on colorectal carcinogenesis depending on the timing and dose of folate intervention.^{1–4–5} Folate deficiency has an inhibitory effect whereas folate supplementation has a promoting effect on the progression of established neoplasms.^{1–4–5} In contrast, folate deficiency in normal colorectal epithelial cells appears to predispose them to neoplastic transformation, and modest supplemental levels (4–10 times above the basal dietary requirement) suppress whereas supraphysiological supplemental doses enhance the development of tumours in normal mucosa.^{1–4–5} An obvious inference from these observations is that for folate to be a safe and effective chemopreventive agent against CRC, modest doses of folic acid supplementation should be implemented before the development of precursor lesions in the colorectum or in persons free of any evidence of neoplastic foci. However, determining the presence of neoplastic foci in the general population is obviously an almost impossible task. Furthermore, folate might prevent the progression of certain precursor or preneoplastic lesions to frank malignancy but promote the progression of other lesions. What constitutes safe precursor or preneoplastic lesions on which folate may exert a protective effect has not yet been established. For example, should folate chemoprevention be started before there is evidence of established premalignant lesions, such as ACF or microscope adenomas in the colorectum, or should folate chemoprevention be started even after these lesions are present? In this regard, animal studies investigating the effects of folic acid supplementation on the progression of ACF, microscopic adenomas, and adenomas are urgently needed.

Mechanistically, the most likely mechanism by which folic acid supplementation may promote the progression of established preneoplastic and precursor lesions of CRC is provision of nucleotide precursors to rapidly replicating neoplastic cells for accelerated proliferation and growth.^{1–4–5} Another mechanism may be de novo methylation of promoter CpG islands of tumour suppressor genes with consequent gene inactivation leading to tumour progression.^{4–15} This potential epigenetic mechanism of tumour progression is supported by recent animal studies using viable yellow agouti mice that unequivocally have demonstrated that maternal dietary methyl group supplementation, including a modest amount of folic acid, permanently alters

phenotypic coat colour of the offspring via increased methylation at the promoter CpG site of the agouti gene.^{29–31}

Whether or not possible deleterious effects of folic acid supplementation (for example, cancer promoting effect on established preneoplastic and neoplastic lesions) outweigh the known and potential health benefits (for example, prevention of atherosclerosis and NTDs; improvement of cognitive function; cancer prevention in normal tissues free of preneoplastic and neoplastic foci) is largely unknown at present. An emerging body of evidence suggests that folate may possess potentially serious adverse effects, including masking of B12 deficiency especially in the elderly; the occurrence of resistance or tolerance to antifolate based chemotherapy and anti-inflammatory and anti-seizure drugs; epigenetic instability; decreased natural killer cell cytotoxicity; and genetic selections of disease alleles (for example, MTHFR C677T).^{3 4 17 32–35} Some proponents of mandatory folic acid fortification have labelled the delay in folic acid fortification in European countries as public health malpractice.³⁶ However, a reasonable conclusion from the above discussion is that inertia on folic acid fortification in these European countries should not be construed as public health malpractice but should be regarded as public health prudence. Mandatory folic acid fortification is probably the most important science drive intervention in nutrition and public health in decades.³⁷ However, the possibility remains that certain segments of the exposed population may benefit less and may even experience some adverse effects from increased folic acid intake. In addition to the drastic increase in dietary folate intake from mandatory folic acid fortification, 30–40% of the North American population consume supplemental folic acid for several possible but as yet unproven health benefits.³⁸ Therefore, long term follow up studies are urgently warranted to determine the effect of folic acid fortification and supplementation on the incidence of cancer and other potential adverse effects. The potential cancer promoting effect of folic acid fortification and supplementation in the vast majority of the North American population who are not at risk of NTDs but have been unintentionally exposed

to high levels of folic acid is a legitimate public health concern and needs careful monitoring.

Gut 2006;55:1387–1389.

doi: 10.1136/gut.2006.095463

Correspondence to: Dr Y-I Kim, Room 7258, Medical Sciences Building, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada, M5S 1A8; youngin.kim@utoronto.ca

Conflict of interest: None declared.

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