Methyl Supply, Methyl Metabolizing Enzymes and Colorectal Neoplasia

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ABSTRACT A low intake of vegetables (but not fruit) is established as a risk factor for colon cancer. Although there are a multitude of active agents that may explain this, one important candidate is folate. Among studies specifically examining intake of folate derived from food and supplements, higher intake is generally associated with lower risk of both adenomas and cancer. Other nutrients associated with the folate pathway—methionine, vitamin B-6, vitamin B-12—or that impact the pathway—alcohol—have also been shown to influence risk in predictable ways. Polymorphisms in enzymes involved in the metabolism of folate also are associated with modification in risk, but essentially only in the presence of low intakes of folate and related nutrients. The consistency of the above evidence suggests that folate is an active agent, not just a marker for the intake of other effectors found in vegetables and multivitamin preparations. There are at least two mechanisms that may explain these findings: folate is central both to the provision of S-adenosylmethionine, the universal methyl donor, and to the provision of nucleotides for DNA synthesis and repair. Fortification of food with folate, as well as intake from multivitamin and pharmacological sources, may increasingly contribute to the primary prevention of colorectal neoplasia although it is possible that there is such a condition as having too much folate. J. Nutr. 132: 2410S–2412S, 2002.

KEY WORDS: • colorectal cancer • adenomatous polyps • folate diet • vegetables • methyl groups • MTHFR

Colorectal cancer is the fourth most common incident cancer and the second most common cause of cancer death in the United States, with approximately 130,000 new cases and 55,000 deaths per year. Colon and rectal cancers share many environmental risk factors and are both found in individuals with specific genetic syndromes; however, there are some differences in etiology. Worldwide, an estimated 875,000 cases of colorectal cancer occurred in 1996, accounting for 8.5% of all new cases of cancer (1). Incidence rates vary approximately 20-fold around the world, with the highest rates seen in the developed world and the lowest in India (2,3). Colon cancer is the only cancer that occurs with approximately equal frequency in men and women (4); however, in high-incidence areas such as North America and Australia, as well as in Japan and Italy where rates are rising rapidly, rates in men now exceed those in women by as much as 20%. Rectal cancer is up to twice as common in men as in women. Five-year relative survival following diagnosis of colon cancer is around 55% in the United States (5). Rectal cancer may have a better overall survival where screening is more common.

DISCUSSION

Known risk factors

There are a variety of established risk modulators for colorectal neoplasia (6,7). Although some potentially protective factors have been identified for other cancers, colorectal cancer may be the cancer where a variety of preventive strategies are likely to be beneficial. These include higher physical activity, use of nonsteroidal anti-inflammatory drugs (3), in women, hormone replacement therapy, and a diet rich in vegetables. For the last of these, there are several classes of potentially beneficial agents—antioxidant vitamins and minerals, other bioactive agents, dietary fiber, etc. Of late, interest has been particularly focused on folate. The interest has extended beyond the folate content of plant foods to include the use of supplements. The relevant hypotheses embrace the possible role of alcohol in increasing risk and cofactors in folate metabolism, such as methionine, vitamin B-6, and vitamin B-12, in reducing risk. Finally, attention is also directed at enzymes in the folate pathway that are polymorphic. Variation in enzyme func-

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Folate lies at the intersection of several metabolic pathways: the formation of nucleotide pools for DNA synthesis and the production of S-adenosylmethionine, the universal methyl donor, and thus the provision of methyl groups that, among many other functions, modify DNA expression by methylation of cytosine-guanine dinucleotide islands, particularly in the promoter regions of genes. Thus, folate is related to DNA structure and function in crucial ways. This is discussed in detail elsewhere in the proceedings. This report is concerned specifically with the evidence for the relationship between the availability of folate and genetic variation in folate metabolism, on the one hand, and risk of colorectal neoplasia, on the other.

**Vegetables and colorectal cancer**

Evidence that vegetable intake is associated with a lower risk of colon cancer has accumulated over several decades. The association with fruit is much weaker. Much of that evidence is summarized in the World Cancer Research Fund Report of 1997 (7). Although not every study shows a lower risk with higher intake, a majority of studies do so. The most recent evidence comes from the Swedish Mammography Screening Cohort, which reported a 30% lower risk of colorectal cancer in women in the highest vs. lowest quartiles of intake of both fruit and vegetables (8).

**Folate, adenomas, and carcinomas**

When individual studies have reported on folate intake specifically and risk of either colorectal cancer or adenoma, the story has been less clear. Notable has been the inconsistency in the findings between the sexes. Although some studies show a reduced risk with higher intake and sometimes in a dose-response fashion, the lower risk is more often seen in women than in men. Is this a real sex difference or are men poorer at reporting their diets? This inconsistency between the sexes also is seen for the relationship between dietary folate and adenoma, although here there are associations seen in men but not women (9) and vice versa (10,11).

Although there are some studies that have used serum or red blood cell levels as a measure of folate, these have been case-control studies. The results are not readily interpretable because it remains unclear whether the low folate is a cause or result of the diagnosed cancer. Prospectively collected data are needed.

**Methyl groups, alcohol, and colorectal cancer**

Clearer evidence of the role of dietary methyl groups more generally comes from studies where multiple dietary sources of methyl groups (e.g., folate, methionine) and of the folate “antagonist” alcohol were considered together. The evidence suggests that low methyl-group intake and high alcohol intake as a combination may increase risk of colon cancer approximately 2.5-fold over those with a high methyl-group intake and low alcohol consumption (12). Again, however, there are suggestions of possible differences between the sexes (13).

**Folate, methylenetetrahydrofolate reductase (MTHFR)², and colorectal neoplasia**

When polymorphisms in MTHFR (EC 1.5.1.20), a key enzyme in folate metabolism, also are considered, additional clarity emerges. Several studies have shown that the cytosine-to-thymine transition at position 677 (C677T) polymorphism often is associated with modification of risk of colorectal neoplasia, but most show that this is only when folate intake or status is low. In both colorectal cancer and adenomatous polyp studies, the pattern seen is that the highest risk is found among those with a homozygous variant MTHFR genotype (low enzyme activity) and a low intake both of folate and of other cofactors in the folate pathway such as vitamin B-12 and vitamin B-6. This is the pattern seen, for instance, in our study of adenomas, and the risk is particularly elevated among those over 60 y of age (14).

For cancers of the breast, pancreas, esophagus, and possibly lung, and for some leukemias, there also is some evidence of increased risk with reduced folate intake or in the presence of low MTHFR activity (15–20). To date, there are no published results from large randomized trials of folate in reducing risk of adenoma, adenoma recurrence, or cancer, but several such studies are currently underway.

**Folate and DNA methylation**

There are some human studies that have shown that folate supplementation may alter DNA methylation in rectal mucosa (21) and weaker evidence on reduction of DNA damage (22). There is one small study to suggest that, in relation to DNA methylation, individuals with the low activity MTHFR homozygous variant genotype may be more susceptible to folate depletion (23).

**Folate and neoplasia in animals**

There are several animal studies that raise important questions regarding the role of the timing of folate supplementation. In mice heterozygous for the adenomatosis polyposis coli gene and homozygous deleted for the DNA mismatch repair gene Msh2 (Apc+/−; Msh2−/−), increased folate intake reduced small bowel adenomas, colonic aberrant crypt foci (ACF), and colonic adenomas, but only if given before neoplastic foci developed. The later reduction of folate intake actually reduced the number of ileal adenomas (24). Similarly, elevated folate intakes reduced the formation of ileal adenomas and colonic ACF in the Apc+/− mouse only if given early in life; when administered later, the elevated folate intakes led to increased formation of ileal adenomas (25). In azoxymethane-treated rats, 12 wk of folate depletion plus succinylsulfathiazole resulted in a reduction in colonic ACF (26). Thus, in all three experiments, timing mattered and suggested that folate supplementation late in the progression of neoplasia may be deleterious. The experience of the role of β-carotene in the Carotene and Retinol Efficacy Trial and Alpha-Tocopherol, 36-Carotene Cancer Prevention Study may be relevant here: β-carotene supplementation in those at risk of lung cancer resulted in elevated, not reduced, risks of lung cancer (27). Furthermore, we have unpublished data to support high folate may have a deleterious effect on immune function.

In human observational studies, folate sufficiency and de-
iciency seem to influence risk of colorectal neoplasia in predictable ways. Similarly, the low-activity MTHFR thymine/thymine dinucleotide polymorphism is associated with an elevated risk of colon neoplasia in the presence of a low folate intake. Human data on supplementation are not yet available, but the animal data suggest that timing may be important. Thus, we need data from human trials before drawing further conclusions. Nonetheless, widespread fortification and self-medication are already underway. As a final thought, it is worth remembering that some of our most effective chemotherapeutic agents are antifolate drugs. Could widespread supplementation and fortification abrogate the effectiveness of these drugs?

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