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REVIEW

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Beneficial role for folate in the prevention of colorectal and breast cancer

Summary Folate is involved in the synthesis of nucleotides and amino acid metabolism such as methylation of homocysteine to methionine. Methionine is activated by adenosine triphosphate (ATP) to produce S-adenosylmethionine (SAM), the primary intracellular methyl donor. Thus, folate is essential for the synthesis, methylation, and repair of DNA. With regard to its biochemical function it has been hypothesized that a diminished folate status may contribute to carcinogenesis by alteration of gene expression and increased DNA damage. Animal and human studies support this hypothesis, particularly with respect to colorectal cancer. Epidemiological evidence for the association between folate status and cancer was first observed among ulcerative colitis patients. Several case-control studies demonstrated reduction in colorectal cancer risk with better folate status. Two large, prospective cohort studies support the concept that high folate intake is protective against colon cancer. In contrast to colorectal cancer, the potential association of folate status and risk has been less investigated in breast cancer. Recently, convincing epidemiological data establishing a positive effect of folate status on breast cancer risk were published.

This review summarizes the epidemiological evidence for the association between folate status and colorectal and breast cancer risk. In addition, a short overview is given on the discussed mechanism(s) by which folate might be involved in carcinogenesis.

Key words Folate – Folic acid – Colorectal cancer – Breast cancer – Risk reduction – Prevention

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Introduction

The role of the B-vitamin folate was originally thought to be restricted to the prevention of megaloblastic anaemia, but folate has now received a great deal of attention because it has been shown to prevent fetal malformations such as spina bifida and to be possibly involved in the risk reduction of cardiovascular disease as mediated by homocysteinemia. More recently, a diminished folate status has been discussed as a risk factor in cancerogenesis. Diets high in vegetables and fruits are related to a decreased incidence of cancer. This association is often assigned to the large quantity of antioxidants present in such a diet, which, however, is also rich in folate. Epidemiological studies have observed that mild folate depletion is linked to increased risk of cancer, particularly of colorectal cancer.

Folate is a generic term for compounds that have vitamin activity and includes folic acid, the synthetic form of the vitamin used in drugs, supplements and fortified food products (Fig. 1), and a wide variety of derivatives, which differ from folic acid by the level of reduction of the pteridine ring, one-carbon substitutions at the N-5 and N-10 positions and additional glutamate moieties [1, 2].

Dietary folate is present as a mixture of pteroylmonoglutamate and pteroylpolyglutamate forms. Prior to transport across the intestinal mucosa polyglutamates are hydrolyzed to monoglutamyl derivatives by

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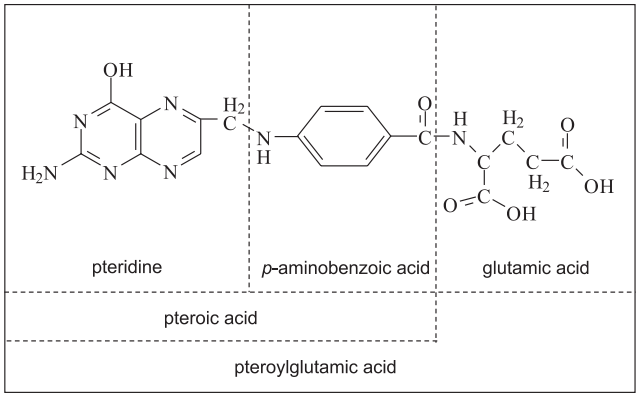

Fig. 1 Structure of folic acid

the enzyme γ-glutamylhydrolase [3, 4]. Because of the required splitting polyglutamates are less available than monoglutamates. The term *Dietary Folate Equivalent* (DFE) which is used in the US and German Dietary Reference Intakes [2, 5] accounts for the lower bioavailability of natural folate compared to folic acid (1 µg DFE = 1 µg of food folate = 0.5 µg of folic acid). After absorption much of the folate is taken up by the liver, where it is stored or released into blood or bile [6, 7]. Folate excreted with bile can be reabsorbed and is thus subject to enterohepatic circulation [8]. Transport in plasma occurs mainly in the form of 5-methyl-tetrahydrofolate (5-MTHF) bound to low-affinity plasma proteins and specific high-affinity binding proteins. The folate level in plasma is much lower than in red blood cells (RBC) in which folate is incorporated only during erythropoiesis. Folate concentration less than 6.8 nmol/L in serum and less than 317 nmol/L in red blood cells indicate folate deficiency [1]. Renal excretion of folate and its metabolites is limited because of effective reabsorption.

Folate in form of tetrahydrofolate (THF) is involved in the transfer of one-carbon units as a coenzyme carrying reversibly a methyl (-CH_3), formyl (-CHO), methenyl (-CH), or methylene (CH_2) group at its N-5 and N-10 positions. They are used in nucleotide biosynthesis (purines, thymidine), generation of formate, and in amino acid metabolism (Fig. 2). The synthesis of methionine from homocysteine requires 5-methyl-THF and vitamin B12 as enzymatic cofactors. Methionine is needed for the synthesis of S-adenosylmethionine (SAM), which is important in intracellular methylation reactions, including methylation of DNA. Thus, folate is required for the proper synthesis and repair as well as indirectly for methylation of DNA [1]. Because of its involvement in these activities, folate has been hypothesized to be associated with carcinogenesis.

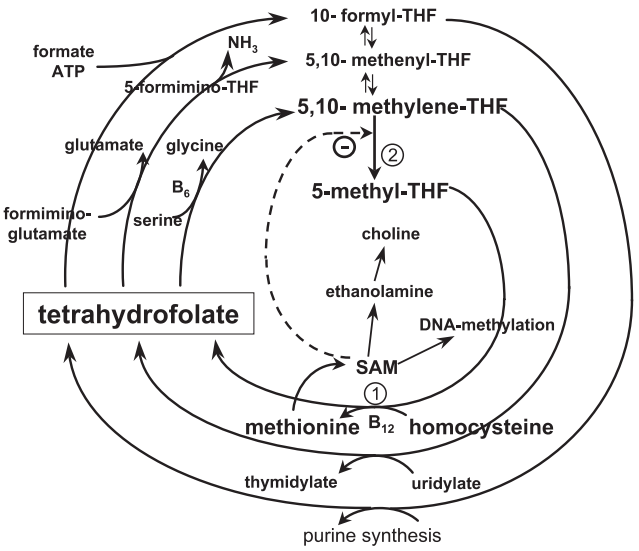

Fig. 2 Metabolism of tetrahydrofolate (THF) derivatives modified from [56]; ① methionine synthase; ② 5,10-methylenetetrahydofolate reductase; SAM = S-adenosylmethionine; THF = tetrahydrofolate

Possible mechanism(s) for folate-related enhancement of carcinogenesis

Several hypotheses were derived to explain, individually or in combination, the role of a folate deficiency in cancerogenesis (Table 1).

Since folate is involved in DNA methylation by generating SAM, research activities center on this aspect. DNA methylation has been suggested as one of the molecular mechanisms relevant to gene expression, stability of the DNA, and sensitivity for mutations [9, 25].

A modified pattern of DNA methylation is a consistent observation in tumor cells. A global DNA hypomethylation as well as a site-specific hypo- or hypermethylation are biochemical characteristics already observed early in carcinogenesis in humans [10–13]. Associations between the global methylation status and premalignant stages of different tumor localizations such as liver [14], stomach [15], ovary [16], and uterine cervix [17, 18] are described.

In the rodent model, the effect of folate deficiency on global DNA hypomethylation produced conflicting results. Hypomethylation in DNA from liver in folate defi-

Table 1 Potential mechanisms for folate-related enhancement of carcinogenesis

Induction of DNA hypomethylation
Secondary choline deficiency
Diminution in natural killer cell surveillance
Increased chromosome fragility or diminished DNA repair
Misincorporation of uridylate for thymidylate in DNA synthesis
Facilitation of tumorigenic virus metabolism

according to [27, 55]

ciency has been observed in some studies [19–21], whereas in others no effect on hepatic and colonic DNA of rats has been reported [22]. Altered gene expression indicated by a rise of mRNA of the protooncogenes *c-fos*, *c*-HA-*ras*, and *c-myc*, was observed as concomitant effect of DNA hypomethylation [21]. A site-specific hypomethylation within the *p53*-suppressor-gene due to severe folate deficiency was shown in rats [9]. Few human studies observed hypomethylation of human lymphocyte DNA in persons consuming a low folate diet [23, 24].

An alternative mechanism for the involvement of folate in carcinogenesis is less available 5,10-methylene-THF for methylation of the uracil residue of deoxyuridine monophosphate (dUMP) with generation of deoxythymidine monophosphate (dTMP). Due to folate deficiency the synthesis of thymidylate might be decreased leading to an increase of the deoxyuridylate pool. The developing nucleotide imbalance was shown to cause misincorporation of nucleotides into DNA with uracil instead of thymine, since DNA polymerase α can insert either dTMP or dUMP, although the latter less efficiently. The cell is able to remove dUMP from DNA by different repair systems, which are also dependent on a balanced nucleotide pool. Unrepaired DNA results in abnormal replication and might be more susceptible to strand breaks [25]. Blount and Ames [26] showed that folate deficiency increases the uracil content both in human marrow cells and in peripheral leukocytes. Other authors reported that folate deficiency in vitro increases strand breakage and uracil misincorporation in human lymphocytes and colonocytes [27, 28].

Epidemiological studies

Cancer of the colon and rectum

There is a constant increase in the incidence and mortality of colorectal cancer in industrialized countries, but also in urban areas of developing countries. Colorectal cancer is associated with a number of risk factors including genetic predisposition and ulcerative colitis. The risk may also be modified by diet. Alcohol and high body mass (colon only) probably increase the risk of colorectal cancer, whereas diets high in vegetables and fiber seem to decrease the risk [29].

The first evidence of the folate-colorectal cancer association was reported in patients with ulcerative colitis, a disease known for an increased risk of colorectal dysplasia and cancer. Further, these patients often show a lowered and insufficient folate status due to inadequate dietary intake, intestinal losses due to inflammation, and the use of drugs, such as sulfasalazine which is a competitive inhibitor of folate absorption and metabolism.

In a case-control study of individuals afflicted with chronic ulcerative colitis, Lashner et al. [30] observed folic acid supplementation to be associated with a non-significant 62 % reduction in the risk of colonic dysplasia/neoplasia. These observations were supported by another study showing RBC folate concentrations to be lowered in colitis patients with dysplasia or cancer compared to unaffected patients (1028 nmol/L vs. 1178 nmol/L). However, RBC folate concentration was still within the range of values accepted as normal for both groups. Serum folate, food folate intake, and proportion of patients supplemented with folic acid did not differ significantly between the groups [31].

Both studies indicate a role of folate in cancerogenesis and initiated further research in both colorectal adenomas and colorectal cancer.

Colorectal adenomas

Several studies investigated the relationship between dietary folate intake and the risk of colorectal adenomas. A case-control study in patients with adenomas identified dietary folate intake as one protective factor besides fiber, magnesium, zinc, vitamin C, and vitamin B6 [32]. Paspatis et al. [33] reported significantly lower RBC folate concentrations in individuals with adenomas compared to those without (1214 nmol/L vs. 1683 nmol/L, $p < 0.01$) suggesting that decreased RBC folate may be associated with development of colonic adenomas. Data from two large prospective cohort studies (n=25,474) – the *Nurses' Health Study* and the *Health Professionals Follow-Up Study* – support the hypothesis that folate status is inversely associated with the risk of colorectal adenoma [34]. Folate intake (diet and supplement) was assessed by using a semiquantitative food frequency questionnaire. After adjusting for possible confounders, the risk reduction for all adenomas was about 35 % (RR = 0.66, 95 % CI = 0.46–0.95 in women; RR = 0.63, 95 % CI = 0.41–0.98 in men) comparing the highest quintile of folate intake (median: 711 µg/day in women, 847 µg/day in men) with the lowest quintile (median: 166 µg/day in women, 241 µg/day in men). Intake of dietary folate alone (excluding supplements with folic acid) revealed only a weak, non-significant inverse relationship with the risk of adenoma. Alcohol consumption increased the risk (≥ 30 g/day vs. abstinence, RR = 1.64, 95 % CI = 0.92–2.93 in men, RR = 1.84, 95 % CI = 1.19–2.86 in women). Individuals with the lowest dietary methionine intake had also an elevated risk of adenomas ≥ 1cm (RR = 0.62, 95 % CI = 0.46–0.85, combining both sexes).

Colorectal cancer

In a case-control study, Freudenheim et al. [35] assessed the potential association between nutrient intake and

colonic or rectal cancer. After adjusting for energy, an inverse relationship was observed between dietary folate intake and risk of rectal cancer. Two further case-control studies, which were conducted by Benito et al. [36] in patients with colorectal cancer and by Ferraroni et al. [37] in patients with colon cancer, confirmed the trend for a protective role of dietary folate intake on the risk of colorectal cancer. In contrast, Meyer and White [38] could not observe such an association in their study, whereas dietary fiber was identified to be protective and alcohol to be harmful.

Another case-control study nested within the Alpha-Tocopherol Beta-Carotene (ATBC) cohort of male smokers demonstrated a decreasing risk of colorectal cancer with increasing dietary folate intake. Furthermore, a high-folate, high-methione, and low-alcohol containing diet resulted in a lower risk compared to a low-folate, low-methionine, and high-alcohol diet (OR = 4.79, 95% CI = 1.36–16.93). The same study failed to show any significant difference of serum folate concentration between individuals with or without colorectal cancer [39]. Unlike these authors, Ma et al. [40] observed in a case-control study, which was a part of the *Physicians Health Study*, an increased risk for colorectal cancer (OR = 1.78, 95% CI = 0.93–3.42) among subjects with deficient plasma folate concentrations (< 6.8 nmol/L) compared to individuals with higher plasma folate concentrations.

Further epidemiological evidence on the role of dietary folate in colorectal cancer is provided by the two large prospective cohort studies previously mentioned in the adenoma section. More than 47,000 men were enrolled in the *Health Professionals Follow-Up Study* [41]. During 6 years of follow-up, 205 cases of colonic cancer were diagnosed. The authors observed that alcohol consumption doubled the risk of this malignancy (RR = 2.07, 95% CI = 1.29–3.32; > 2 drinks/day vs. 0.25 drink/day). The combination of high alcohol consumption, low dietary folate, and low methionine intake increased the risk (colon cancer: RR = 3.30, 95% CI = 1.58–6.88; distal colon cancer: RR = 7.44, 95% CI = 1.72–32.1). Smoking, fat or fiber intake, physical activity, BMI, or the use of multivitamins or aspirin did not affect risk.

In the *Nurses' Health Study* the correlation between dietary folate and colon cancer was evaluated in more than 88,000 female participants [42]. During 14 years of follow-up, 442 cases of colon cancer and 143 cases of rectal cancer were identified. After adjusting for potential confounders an approximately 30% lower risk of colon cancer was observed with higher folate intake at the onset of the study (RR = 0.69, 95% CI = 0.52–0.93, > 400 µg folate/day vs. 200 µg/day). Total folate intake > 400 µg/day included supplemental sources. Use of supplements containing 400 µg/day or more of folic acid resulted in a significant benefit with respect to colon cancer after at least 15 years of treatment (RR = 0.25, 95% CI = 0.13–0.51, use of supplements vs. non-use). Dietary folate alone was not correlated to a substantial risk reduction, but no association could be observed between dietary folate intake and reduction of the risk of rectal cancer (Table 2).

The therapeutic effect of folic acid supplementation has only been examined in some small, prospective intervention trials. Cravo et al. [43] reported that daily supplementation with 10 mg folic acid over 6 months of subjects with resected colonic adenoma and colonic cancer was found to be associated with a reduced rate of global DNA hypomethylation in the colorectal epithelium. In a later trial of this author, patients received 5 mg PGA/d for 3 months [44]. The result of the first study was confirmed only in patients after removal of one single polyp.

Current research is focusing on a gene-nutrient interaction. The 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylene-THF to 5-MTHF, which converts homocysteine to methionine (see Fig. 2). A polymorphism in this gene (C677T) causes thermolability and reduced activity of this enzyme. Data from the *Physicians Health Study* [40] and of a case-control study [45] indicate that homozygosity for the C677T polymorphism in the MTHFR gene (T/T genotype) results in a 50% lower risk for colorectal cancer when folate intake is adequate. This protection was no longer evident, however, when alcohol was consumed and folate intake was low. Chen et al. [46] could not confirm MTHFR polymorphism as a protective factor in individuals with colorectal adenoma. These authors concluded that this defect may be of significance only in a late stage of cancerogenesis and/or may contribute to protection of malignant transformation of adenomas.

Breast cancer

Similar to colorectal cancer a growing body of epidemiological evidence support a role of nutritional factors such as diets high/low in vegetables and fruits, alcohol consumption, and weight in the risk of developing breast cancer [29].

The relationship between dietary folate and breast cancer has mainly been evaluated using case-control designed studies. Graham et al. [47] reported that folate intake is inversely associated with the risk of breast cancer in postmenopausal women and that this effect is confined to the highest quartile of folate intake. Freudenheim et al. [48] showed that folate intake was significantly lower among cases than controls in premenopausal women, whereas another case-control study did not confirm such an association [49]. The EURAMIC study could not provide evidence that a high in-

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Table 2 Epidemiological studies of folate and colorectal neoplasia

Study Design	Diagnosis	N	RR/OR*	95 % CI	Ref.
Case-control	dysplasia and cancer	99	0.38	0.12–1.20	30
Case-control	dysplasia and cancer	67	0.82	0.68–0.99	31
Case-control	colorectal adenomas	343	0.27	NA	32
Prospective cohort	colorectal adenomas, males	9,490	0.63	0.41–0.98	34
Prospective cohort	colorectal adenomas, females	15,984	0.66	0.46–0.95	34
Case-control	cancer				35
	colon, males	410	1.03	0.56–1.89	
	rectum, males	570	0.31	0.16–0.59	
	colon, females	446	0.69	0.36–1.30	
	rectum, females	297	0.50	0.24–1.03	
Case-control	colorectal cancer	3,350	0.52	0.40–0.68	37
	colon cancer				38
	males	462	1.24	0.81–1.24	
	females	376	0.54	0.66–1.00	
Nested case-control	cancer, males				39
	colon	245	0.51	0.20–1.31	
	rectum	140	1.13	0.37–3.41	
Prospective cohort	colon cancer, males	47,931	0.86	0.50–1.47	41
Prospective cohort	colon cancer, females	88,756	0.69	0.52–0.93	42

* Relative Risk/Odds Ratio for high folate intake/status compared to low intake/status adjusted for various confounding factors; RR = Relative Risk, OR = Odds Ratio, CI = Confidence Interval, Ref. = Reference, NA = not available

take of folate in postmenopausal women reduces the risk of breast cancer [50]. Alcohol consumption was inversely but not significantly associated with breast cancer, possibly due to the relatively low alcohol intake in the study population, which averaged at 2.3 g/day in the affected individuals and 1.7 g/day in controls.

Wu et al. [51] conducted a case-control study in 27,075 blood donors (time of blood donation: 1974, n=12,450; 1989, n=14,625) to assess the possible association between breast cancer and serum micronutrient concentration. Serum folate concentration did not correlate with risk of breast cancer. However, a protective effect was shown for vitamin B12. Postmenopausal women with a low vitamin B12 status (lowest quintile of serum cobalamin) had a 4.0 times (1974 cohort RR = 4.0, 95 % CI = 1.05–15.2) and 2.25 times (1989 cohort RR = 2.25, 95 % CI = 0.86–5.91) higher risk of breast cancer than women in the highest quintile of serum cobalamin. Since vitamin B12 is responsible for methylation reactions in co-operation with folate, this observation seemed reasonable to the authors.

Zhang et al. [52] assessed dietary influences on the risk of breast cancer in the *Nurses' Health Study*. A total of 3,483 cases of breast cancer were documented among 88,818 participants. Folate intake was not associated with the overall risk of breast cancer. Premenopausal women with an alcohol consumption of ≥ 15 g/day had a significantly lower risk (–35 %) of breast cancer (RR = 0.65, 95 % CI = 0.33–1.28) when in the highest folate intake quintile (> 600 µg/day) than women with the same alcohol consumption but lower folate intake (150–299 µg/day). This significant association was also observed in women after the menopause (RR = 0.49, 95 % CI = 0.33–0.74). Possible confounders such as age, total energy intake, intake of antioxidative vitamins, familiar occurrence of breast cancer, and postmenopausal hormone therapy, were taken into account by a multivariate analysis. Since the higher folate intake is often due to use of folic acid containing supplements, this aspect was examined separately. Women, taking supplements regularly and consuming > 15 g alcohol/day had a significantly reduced risk (–26 %) of breast cancer (RR = 0.74; 95 % CI = 0.59–0.93) compared to those never taking vitamins.

Another recent large epidemiologic study also suggested that the protective effect of folate may occur in subgroups of women, only. In the *Canadian National Breast Screening Study*, a prospective study with more than 50,000 women followed for 8–13 years, Rohan et al. [53] observed similar associations like Zhang et al. [52]. Women consuming > 14 g/day of alcohol and assigned to the highest quintile of folate intake had a clearly reduced risk (incidence rate ratio IRR = 0.34; 95 % CI = 0.18–0.61) compared to women with the same alcohol consumption but lower folate intake (lowest quintile). The difference was even more pronounced when only data of postmenopausal women were considered (IRR = 0.28; 95 % CI = 0.14–0.55) (Table 3).

Conclusions

Using the case-control design studies resulted in inconsistent findings with regard to folate status/intake and cancer risk [35–40, 47–51] possibly due to the retrospective design which renders it difficult to consider all potential confounders or to record accurately dietary intake of folate or folic acid supplements.

Data from prospective epidemiological studies, however, suggest that folate may influence a person's risk of developing colorectal and breast cancer. The evidence for this relationship seems to be strongest in colorectal

Table 3 Epidemiological studies of folate and breast cancer

Study design	menopausal status	N	RROR*	95 % CI	Ref.
Case-control	postmenopausal	933	0.70	0.48–1.02	47
Case-control	premenopausal	608	0.50	0.31–0.82	48
Case-control	premenopausal	2,019	1.11	0.8–1.5	49
Case-control	postmenopausal	149	1.14	0.73–1.79	50
Nested case-control#	1974 cohort				51
	pre-post[1]	114	1.57	0.49–4.96	
	post-post[2]	126	0.66	0.17–2.60	
	1989 cohort				
	pre-pre[3]	44	0.89	0.10–7.70	
	post-post[2]	170	0.67	0.26–1.72	
Prospective cohort	pre- and postmenopausal	3,483	0.91	0.52–1.01	52
	pre- and postmenopausal and alcohol intake > 14 g/day	530	0.55	0.39–0.78	
Prospective cohort	pre- and postmenopausal	1,336	0.99°	0.79–1.25	53
	pre- and postmenopausal and alcohol intake > 14 g/day	298	0.34°	0.18–0.61	

* Relative Risk/Odds Ratio for high folate intake/status compared to low intake/status adjusted for various confounding factors, # IRR = incidence rate ratio, [1] pre-post = premenopausal at blood donation and postmenopausal at diagnosis, [2] post-post = postmenopausal at blood donation and postmenopausal at diagnosis, [3] pre-pre = premenopausal at blood donation and premenopausal at diagnosis; RR = Relative Risk, OR = Odds Ratio, CI = Confidence Interval, Ref. = Reference

cancer. Three large cohort studies, the *Nurses' Health Study*, the *Health Professionals Follow-Up Study* and the *Canadian National Breast Screening Study* resulted in positive findings [41, 42, 52, 53]. They revealed a positive relationship between folate intake/status and risk of colon and breast cancer, respectively. The risk for colon cancer was 30 % lower in individuals consuming > 400 µg folate/day. Further risk reduction (RR = 02.25) was observed in long-term use of supplements containing folic acid [42]. Similar results were reported with respect to breast cancer [52, 53]. Although all human studies have limitations, these data linking folate intake/status with the risk of colon and breast cancer are convincing. The major strengths of these studies are the large sample size, the prospective design, the duration of the follow-up, and the adjustment for various confounding factors. Additionally, in these studies cases were only considered after histopathological confirmation.

Several studies showed that moderate or alcohol consumption combined with low folate intake is associated with an increased risk of cancer [39, 41, 52]. This observation may be explained by an altered folate metabolism caused by alcohol as such. Alcohol interferes with absorption and renal conservation of folate and accelerates folate breakdown by acetaldehyde produced radicals [54].

Recently, the role of gene-nutrient interaction has been investigated. Studies have shown that the MTHFR polymorphism may influence the risk for colon cancer depending on the level of folate status [40, 45]. Individuals with the T/T genotype and an adequate folate status may have a decreased risk possibly due to increased availability of 5,10-methylene-THF needed for the nucleotides synthesis during DNA synthesis/repair. However, at the present time, data are insufficient to make any definitive statement about this interaction.

Prospective, controlled intervention studies with folic acid (or folate) are still scarce. Two intervention trials in patients with adenoma [43, 44] provide promising results although some methodological problems such as small sample size, lack of placebo control, and use of an intermediary endpoint (hypomethylation of DNA) do not allow to classify folic acid (or folate) as a chemopreventive substance. A relationship between a methyl deficient diet and global DNA and gene-specific hypomethylation was shown in animal experiments [19–21]. Inadequate folate intake may cause hypomethylation of human lymphocyte DNA [23, 24], but the relation of this phenomenon to the methylation status of DNA in other tissues is to be clarified.

In summary, epidemiological data strongly suggest that folate may be of interest with regard to a potential role in cancer prevention, particularly colon cancer. Since it is likely that micronutrients exert their protective effects in a concerted action rather than independently, consumption of a diet high in vegetables, fruits and whole grain products is advisable. Such diet provides not only folate, but also other potentially protective compounds such as fiber, minerals, and antioxidants. A folic acid-containing preparation could be used to adjust for a nutritional deficit if adequate supply cannot be achieved by means of the diet.

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