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Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis

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Abstract Inappropriate diet may contribute to one third of cancer deaths. Folates, a group of water-soluble B vitamins present in high concentrations in green, leafy vegetables, maintain DNA stability through their ability to donate one-carbon units for cellular metabolism. Folate deficiency has been implicated in the development of several cancers, including cancer of the colorectum, breast, ovary, pancreas, brain, lung and cervix. Generally, data from the majority of human studies suggest that people who habitually consume the highest level of folate, or with the highest blood folate concentrations, have a significantly reduced risk of developing colon polyps or cancer. However, an entirely protective role for folate against carcinogenesis has been questioned, and recent data indicate that an excessive intake of synthetic folic acid (from high-dose supplements or fortified foods) may increase human cancers by accelerating growth of precancerous lesions. Nonetheless, on balance, evidence from the majority of human studies indicates that dietary folate is genoprotective against colon cancer. Suboptimal folate status in humans is widespread. Folate maintains genomic stability by regulating DNA biosynthesis, repair and methylation. Folate deficiency induces and accelerates carcinogenesis by perturbing each of these processes. This review presents recent evidence describing how these mechanisms act, and interact, to modify colon cancer risk.

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Folate status and colon cancer risk

Diet and lifestyle play crucial roles in cancer aetiology, and it has been estimated that inappropriate nutrition could account for more than one third of cancer deaths (Doll and Peto 1981; WCRF 2007). Whereas there is no real consensus as to the positive dietary risk factors for cancer, high fruit and vegetable consumption has been shown to be consistently associated with a decreased risk of cancer (WCRF 2007). Folates, a group of water-soluble B vitamins found in high concentrations in green leafy vegetables, maintain DNA stability through their ability to donate one-carbon units for cellular metabolism. Mammals are unable to synthesise folates de novo and so must obtain them either directly from the diet or from microbial breakdown produced during digestion.

Folate deficiency has been implicated in the development of several human epithelial cell malignancies, including cancer of the breast, ovary, pancreas, brain, lung and cervix (Glynn and Albanes 1994; Kim 2007; Yang et al. 2009). However, the evidence linking low folate status with an increased risk of colorectal cancer (CRC) is strongest. Generally, data from the majority of human studies (retrospective, case-control and prospective) suggest that people who habitually consume the highest level of folate, or with the highest blood folate concentrations, have a significantly reduced relative risk (RR) of developing colon polyps or cancer (Giovannucci 2002; Sanjoaquin et al. 2005; Kim 2007). A recent large-scale meta-analysis of prospective studies (Sanjoaquin et al. 2005) reported a

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reduced risk for CRC in individuals with a high dietary folate intake compared with low intake [RR 0.75; 95% confidence interval (CI)=0.64–0.89]. Folate maintains genomic stability by regulating DNA biosynthesis, repair and methylation (Fig. 1). Within the methionine cycle, 5-methyltetrahydrofolate (5-methyl THF) remethylates homocysteine to methionine, which is further metabolised to s-adenosylmethionine (SAM). SAM, the principal methyl donor in most cellular reactions, controls gene transcription and ultimately protein expression through its ability to methylate cytosine in the DNA molecule. Similarly, folate is essential for the synthesis of both purines and the pyrimidine nucleoside thymidine. Deoxyuridine monophosphate (dUMP) is converted to thymidine monophosphate (TMP) by thymidylate synthase (TS) using 5,10-methylenetetrahydrofolate (5,10-methylene THF) as methyl donor. Subsequently, 5,10-formyltetrahydrofolate (5,10-formyl THF) is involved in the production of both adenosine and guanosine. Continual production of these DNA precursors is essential for normal DNA synthesis and repair (Fig. 1).

Folate deficiency induces and accelerates carcinogenesis by perturbing each of these processes. If dietary folate is limited, the balance of purine and pyrimidine DNA precursors is altered, and normal DNA repair is inhibited. Moreover, uracil, which is not normally present in DNA, is misincorporated into the DNA molecule in place of thymidine, resulting in DNA strand breakage, chromosomal damage and malignant transformation. Furthermore, cytosine methylation is altered, leading to global DNA hypomethylation and/or changes in gene-specific methylation and inappropriate protooncogene activation (Fig. 1). Evidence in support of each of these mechanisms is described in detail below.

The impact of folate deficiency on DNA damage and repair

Low cellular 5,10-methylene THF retards conversion of dUMP to deoxythymidine monophosphate (dTMP), leading to thymidine depletion and elevated uracil concentrations. As uracil and thymidine differ only by a single methyl group, uracil is misincorporated into DNA in place of thymidine during DNA synthesis but is quickly removed by DNA repair enzymes, leaving a single-strand break in the DNA molecule. If folate is persistently limited, this "catastrophic cycle" of DNA breakage and repair continues,

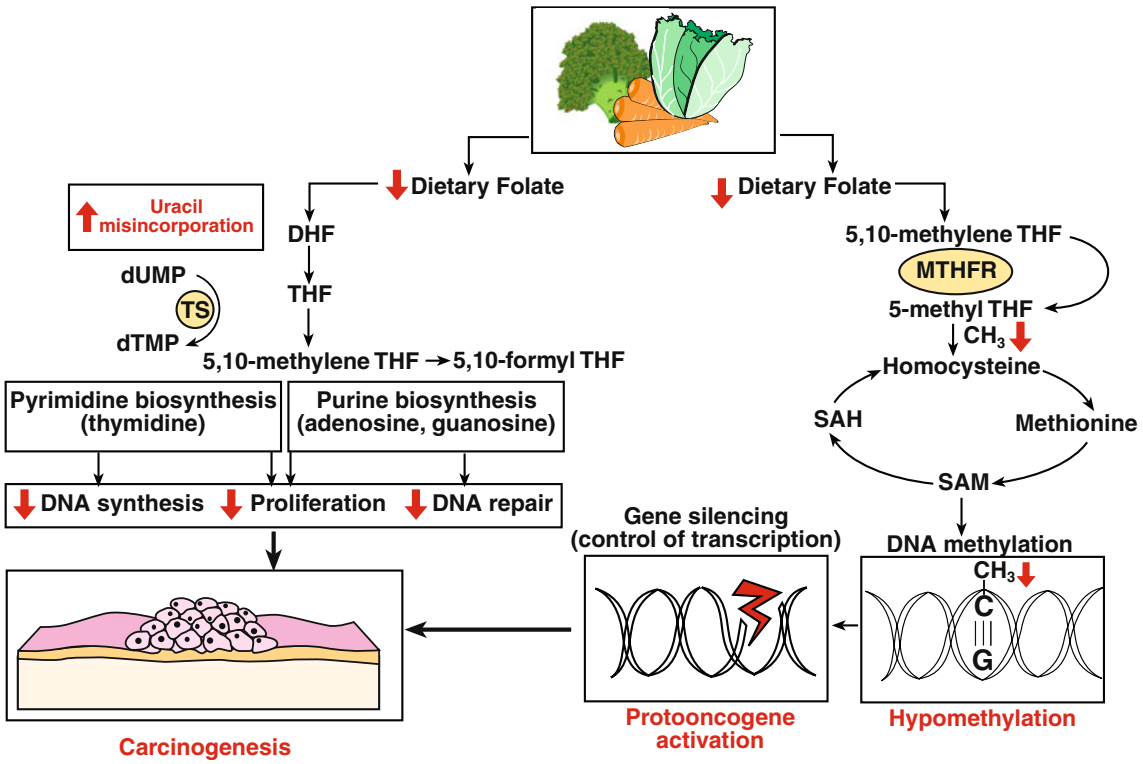

Fig. 1 Folate and one-carbon metabolism: regulation of DNA synthesis, repair and methylation. A simplified scheme describing how dietary and cellular folates mediate normal DNA synthesis, repair and methylation and how folate depletion impacts on these processes. *DHF* dihydrofolate, *THF* tetrahydrofolate, *5,10-methylene THF* 5,10-methylenetetrahydrofolate, *5,10-formyl THF* 5,10-formyltetrahydrofolate, *5-methyl THF* 5-methyltetrahydrofolate, *SAM* s-adenosylmethionine, *SAH* s-adenosylhomocysteine, *MTHFR* methylenetetrahydrofolate reductase, *dUMP* deoxyuridine monophosphate, *TMP* thymidine monophosphate, *TS* thymidylate synthase

leading to DNA double-strand breaks, chromosomal aberrations and malignant transformation (Reidy 1988; Blount et al. 1997). Uracil misincorporation, genome-wide DNA strand breakage and chromosomal instability in response to experimental folate deficiency have been observed in various cell culture models, including human lymphocytes, human colonocytes and Chinese hamster ovary (CHO) cells (Duthie and McMillan 1997; Melnyk et al. 1999; Duthie et al. 2000a; Beetstra et al. 2005; Duthie et al. 2008). Moreover, human lymphocytes grown under conditions of low folate display increased gene-specific DNA strand breaks in the p53 tumour suppressor gene and a three fold increase in micronuclei frequency, a marker of chromosomal instability (Crott et al. 2007). Folate deficiency has also been shown to increase the susceptibility of mammalian cells and cultured rodent splenocytes to mutagens (Branda et al. 1997, 2007) and irradiation (Beetstra et al. 2005) and to promote malignant transformation in CHO cells inoculated in mice (Melnyk et al. 1999).

Folate deficiency likewise induces genomic instability in animal models. Uracil misincorporation and DNA strand breakage are increased in lymphocytes, liver and colon tissue either from rats fed a folate-depleted diet for up to 24 weeks (James and Yin 1989; Pogribny et al. 1997; Duthie et al. 2000b, c; Kim et al. 2000; Duthie et al. 2010) or given methotrexate to disrupt folate metabolism (Blount and Ames 1994). Most importantly, folate status is associated with uracil misincorporation, DNA strand breakage and chromosome instability in humans. Bone marrow from megaloblastic anaemia patients contains elevated uracil and correspondingly low levels of thymidine (Wickramasinghe and Fida 1994). Uracil and micronuclei frequency are increased eight and three fold, respectively, in folate-deficient splenectomised individuals (Blount et al. 1997). Moreover, uracil misincorporation and chromosomal abnormalities are reversed in severely deficient individuals by high dose synthetic folic acid (5 mg/day for 8 weeks; Blount et al. 1997). Uracil is also present in DNA from normal healthy individuals (Narayanan et al. 2004; Basten et al. 2006) and is responsive to folate supplementation (Basten et al. 2006). Uracil misincorporation in isolated lymphocytes is decreased by folic acid supplementation (1.2 mg/day for 12 weeks), even in volunteers with adequate folate status (Basten et al. 2006). However, intervention did not alter DNA strand breakage in this study (Basten et al. 2006). Similarly, micronuclei frequency is not improved by substantially increasing folate intake in young adults with normal blood folate levels (Fenech et al. 1998). In contrast to the above reports of a cytoprotective role for folate, very-high-dose folic acid and vitamin B12 supplementation (5 mg and 1.25 mg daily for 6 months) increased uracil concentrations in rectal biopsies from colorectal adenoma patients (van den Donk et al. 2007). The potential detrimental effects of high-dose folic acid intervention will be covered later in this review.

Folate deficiency impacts on DNA repair by inhibiting production of thymidine, adenosine and guanosine. DNA is under continual attack from environmental pollutants, drugs, radiation, cigarette smoke and endogenous agents such as reactive oxygen and alkylative species generated by cellular metabolism (De Bont and van Larebeke 2004). Safeguarding genomic integrity is complex, as many types of damage occur in DNA, including mismatched and oxidised bases, "bulky" adducts, photoproducts, crosslinks between DNA or protein and single- and double-strand breaks. These various forms of DNA damage are processed by diverse repair pathways, including damage reversal (DR), base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR). The complexity of these systems varies enormously (Christmann et al. 2003). Defective DNA repair is linked to human cancer development. Mutations in specific *MMR* genes are associated with heritable colon cancers (Whitehouse et al. 1998). Polymorphisms in DNA repair genes (e.g. *hOGG1*, *XRCC1* and *PARP1*) in the general population correlate with altered cancer risk and disease progression in colorectal adenoma (Bigler et al. 2006), and mutations in DNA repair genes are responsible for syndromes such as xeroderma pigmentosum that carry an increased risk of cancer (De Boer and Hoeijmakers 2000).

BER is compromised in folate-deficient cultured cells. Excision of oxidative and alkylation damage is retarded in human lymphocytes (Duthie and Hawdon 1998) and colonocytes (Duthie et al. 2000a, 2008) grown in folate-deplete medium and is independent of initial DNA damage that incurred in the cells. BER of the oxidised base, 8-oxo-7,8-dihydroguanine (8-oxodG), is similarly inhibited in folate-depleted rat hippocampal neurons in vitro (Krugman et al. 2002) and in colon cells isolated from rats fed a folate-free diet (Choi et al. 1998). Folate deficiency also affects DNA repair in animal models. Intracellular nicotinamide adenine dinucleotide (NAD) is elevated in lymphocytes from folate-deficient rats, indicating upregulation of DNA repair enzymes, possibly in response to increased DNA strand breakage (James et al. 1992). We recently reported that moderate but prolonged folate deficiency in rats effects repair of two lesions implicated in human cancer. The strongly mutagenic oxidised base, 8-oxo-dG, occurs in significant quantities in human DNA and induces G:C to T:A transitions (Fortini et al. 2003); 8-oxoguanine-DNA glycosylase (OGG1) repairs this lesion. O^6-methylguanine is detectable at high levels in tumour-prone regions of the human bowel (Povey et al. 2002) and is cytotoxic, mutagenic (causing G:C to A:T transitions and recombinations) and carcinogenic unless repaired by O^6-methylguanine-DNA methyltransferase (MGMT). Decreased activity

of MGMT is associated with G:C to A:T mutations in the K-ras protooncogene in both normal human colorectal tissue and cancerous tissue (Povey et al. 2002). In this study, folate deficiency significantly increased the activity of both these incision repair proteins (by approximately 25%) in rat liver (Duthie et al. 2010). Using proteomic analysis to determine global protein expression in human colon cells in vitro, we have also shown that folate deficiency upregulates specific DNA incision repair enzymes, including MSH2 and XRCC5 (involved in MMR and double-strand-break repair) (Duthie et al. 2008). Induction of DNA repair proteins probably reflects increased DNA damage due to folate deficiency, as described earlier in this review.

Few studies have investigated whether DNA repair activity can be modified by folate status in humans. NER (measured using the host-cell reactivation ex vivo assay) is impaired in lymphocytes from individuals with poor folate status (Wei et al 2003). Here, individuals in the lowest tertile of total dietary folate intake (<170 μg/1,000 kcal per day) had significantly lower NER repair capacity compared with those in the upper tertile (>225 μg/1,000 kcal per day). MGMT activity, measured in normal human colon biopsies is inversely linked to vegetable consumption, whereas low dietary folate intake is specifically associated with high levels of alkylation damage (Billson et al. 2009). Whereas folate deficiency appears to affect DNA repair detrimentally, it is unclear whether increasing folate intake can improve repair. We recently compared the ability of lymphocytes from folate-supplemented and -unsupplemented individuals to incise 8-oxodG from DNA. Despite significant increases in blood and lymphocyte folate, and a corresponding decrease in misincorporated uracil, folic acid supplementation did not significantly alter DNA BER capacity (Basten et al. 2006). Individuals in this study had adequate folate levels prior to intervention. Conversely, microsatellite instability (a type of DNA damage) is decreased by very-high-dose folic acid supplementation (5 mg daily for 6 months) in patients with ulcerative colitis, a condition predisposing to CRC and associated with low folate status (Cravo et al. 1998a). These data suggest that DNA repair activity is suboptimal in people with low folate intake but may not be improved in individuals with satisfactory folate status.

Folate status, altered DNA methylation and cancer risk

In addition to inducing DNA damage and compromising DNA repair, folate deficiency also modulates the epigenome. DNA methylation is an important determinant of genomic stability, mutagenesis and gene expression. Generally, genes methylated at specific sites, e.g. upstream of a promoter, are either not transcribed or are transcribed at a reduced rate, and translation into the protein for which the gene codes is decreased. Epigenetic site-specific DNA methylation therefore contributes to the control of gene, and ultimately, protein expression (Costello and Plass 2001). Folate deficiency, by attenuating remethylation of s-adenosylhomocysteine (SAH) to SAM in the methionine cycle, may disrupt this function (Fig. 1). Under conditions of low dietary folate, SAM is reduced whereas SAH is elevated, resulting in hypomethylation of newly synthesised DNA and, potentially, increased proto-oncogene expression (reviewed in Kim 2007). Surprisingly, folate deficiency is also associated with hypermethylation in specific gene regions, notably in tumour suppressor genes. Folate deficiency may induce both gene-specific DNA hypermethylation and global DNA hypomethylation by its DNA-damaging effect. DNA methyltransferase (DNMT), an ancestral DNA repair protein, is sequestered away from the DNA replication fork to regions of folate-deficiency-induced DNA damage, resulting in both genomic hypomethylation and site-specific hypermethylation in gene promoter regions (James et al. 2003). Global and gene-specific DNA hypomethylation and site-specific hypermethylation are common features in tumorigenesis (Arasaradam et al. 2008). The influence of folate on DNA hypomethylation is covered later in this review.

Whereas extreme methyl donor depletion in animal models undoubtedly alters DNA methylation (most notably in the liver) and is associated with hepatocarcinogenesis (Wainfan and Poirier, 1992; Pogribny et al. 2004), the effect of folate deficiency alone on cytosine methylation in vitro and in vivo is highly variable and profoundly dependent upon treatment regime, tissue and genes examined (reviewed in Kim 2007). Folate depletion in vitro decreases DNA methylation (global and gene-specific) in certain human and animal cells but not in others. Folate deficiency induced DNA hypomethylation in untransformed NIH/3T3 mouse fibroblast cells and CHO-K1 cells after 12 days of culture but did not effect cytosine methylation in human colon adenocarcinoma cell lines HCT116 and Caco-2 cells, even after 20 days (Stempak et al. 2005). Conversely, global and region-specific p53 tumour suppressor gene DNA methylation was decreased in SW620 human colon adenoma cancer cells grown in folate-free medium and restored following repletion with folic acid (Wasson et al. 2006). We have shown that global cytosine methylation is decreased in SV40-immortalised human colonocytes grown in folate-free medium for 14 days (Duthie et al. 2000a) but not in NCM460 nonmalignantly transformed human colon cells cultured under the same conditions (Duthie et al. 2008). These data demonstrate that the effect of folate status on DNA methylation in vitro is inconsistent and independent on malignant phenotype.

Generally, severe and prolonged folate deficiency in rodents causes global DNA hypomethylation in the liver

(Balaghi and Wagner 1993; James et al. 2003) and in certain regions of the colon (Kim et al. 1996a, b). Conversely, moderate deficiency does not alter genome-wide cytosine methylation in blood, liver and colon despite a progressive reduction in blood and tissue folate concentrations, an increase in plasma homocysteine and a decrease in liver SAM to SAH ratio (Sohn et al. 2003; Duthie et al. 2000c; Duthie et al. 2010). Little association between folate and genome-wide DNA methylation status is evident in healthy individuals with adequate blood folate (Fenech et al. 1998; Ingrosso et al. 2003; Kim 2007), and whereas suboptimal folate status is associated with DNA hypomethylation in both healthy and diseased tissue in certain studies, the evidence is conflicting. Global DNA is hypomethylated in lymphocytes from women who were made folate deficient over several weeks (Jacob et al. 1998; Rampersaud et al. 2000), whereas low dietary folate intake (<200 μg/day) is associated with an increased frequency of hypomethylated long-interspersed nucleotide element repeats (LINE-1; a marker of genome-wide DNA methylation) in human colon tumours (Schernhammer et al. 2009). Conversely, LINE-1 methylation was found not to be related either to dietary folate intake or blood folate status in colon biopsy samples from the large-scale aspirin/folate polyp prevention study (Figueiredo et al. 2009a). The effect of intervention with folic acid on DNA methylation is equally inconsistent and highly dependent on initial folate status, level and duration of supplementation, tissues examined and whether the study individual has cancer. Supplementing healthy volunteers, with systemic folate levels within the normal range, has little impact on blood cell DNA methylation (Fenech et al. 1998). Similarly, giving healthy individuals 1.2 mg folic acid daily for 12 weeks does not alter lymphocyte DNA methylation status despite significantly elevated whole blood, plasma and lymphocyte folate (Basten et al. 2006). Conversely, white blood cell DNA methylation is increased in folate-deficient individuals repleted with synthetic folic acid (Jacob et al. 1998; Rampersaud et al. 2000) and in leucocytes from colorectal adenoma patients given 400 μg of folic acid daily for 10 weeks, although DNA methylation was not altered in the colonic mucosa of these individuals (Pufulete et al. 2005). Pharmacological rather than nutritional levels of folic acid (5–10 mg/day for 3–6 months) can increase DNA methylation in the colorectum of humans (Cravo et al. 1994; Cravo et al. 1998b). However, supplementing colon adenoma patients with a more moderate dose of 1 mg folic acid daily did not alter LINE-1 methylation in colon biopsy samples at 3-years' follow-up (Figueiredo et al. 2009a). The positive impact that supplementation may have is, therefore, highly dependent on dose and timing of intervention and on health status (Cravo et al. 1994, 1998a; Pufulete et al. 2005).

Folate–gene interactions and cancer risk: MTHFR activity, genomic stability and DNA methylation

It has been suggested that under conditions of limiting folate or methionine, SAM synthesis has metabolic priority over DNA biosynthesis, and 1-carbon units are directed preferentially through the methionine cycle to facilitate methylation reactions (including DNA) at the expense of DNA synthesis and repair (reviewed in Kim 2007). The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyses the irreversible conversion of 5,10-methylene THF (the methyl donor in the conversion of dUMP to dTMP) into 5-methyl THF, which remethylates homocysteine to methionine (Fig. 1). This key protein, therefore, controls whether folate is partitioned towards DNA precursor synthesis or DNA methylation. Polymorphisms in the *MTHFR* gene modulate the risk of human cancers. The relative importance of DNA synthesis and methylation in cancer development may therefore be gauged by examining these two processes in individuals with *MTHFR* gene variants. The most common variant of the *MTHFR* gene, *C677T*, causes a valine for alanine substitution in the protein and reduced enzyme activity in the heterozygotes (CT; 35%) and homozygotes (TT; 70%) (Frosst et al. 1995). Plasma total folate is decreased and homocysteine is elevated in TT individuals (Jacques et al. 1996; Narayanan et al. 2004). Moreover, homozygosity is associated with changes in the distribution of blood folates. Red cells from TT individuals have low 5-methyl THF but high formyl THF levels (Bagley and Selhub 1998). Mechanistically, impaired MTHFR activity would be expected to increase cancer risk due to low blood 5-methyl THF, DNA hypomethylation and protooncogene activation (Fig. 1). Surprisingly, the TT variant is in fact associated with reduced CRC risk (Chen et al. 1996; Ma et al. 1997; Sharp and Little 2004), although this effect is profoundly dependent on folate status. It is hypothesised that low MTHFR activity (associated with the T allele) may reduce CRC risk by increasing the availability of 5,10-methylene THF (and subsequently formyl THF) for thymidine and purine production, thereby providing nucleoside precursors for normal DNA synthesis and repair and preventing uracil misincorporation and chromosomal breakage (Kono and Chen 2005; Fig. 1). Evidence for the MTHFR C677T polymorphism strongly influencing either of these processes is weak. DNA methylation is high in human colonocytes expressing the *MTHFR* TT mutation cultured in adequate or high folic acid but is hypomethylated in folate-deficient cells (Sohn et al. 2009). DNA is hypomethylated in white cells from TT individuals compared with CC wild types (Choi et al. 1999; Stern et al. 2000), yet gene-specific methylation of p53 is similar between genotypes (Choi et al. 1999; Friso et al. 2002). DNA hypomethylation is

evident in normal human colon, breast and lung tissue from CT and TT individuals but not in tumour tissue from the same individuals (Paz et al. 2002). In contrast, genome-wide DNA methylation is comparable between all genotypes in lymphocytes isolated from healthy individuals (Narayanan et al. 2004). Evidence in support of the *MTHFR* TT genotype improving genomic stability is equally inconsistent. Uracil misincorporation and chromosomal damage in human lymphocytes and colon cells (in response to folate depletion) is comparable between genotypes (Crott et al. 2001a, b; Sohn et al. 2009). In the majority of human studies, uracil misincorporation, DNA strand breakage, sister chromatid exchange and micronuclei formation in blood cells is similar for all *MTHFR* variants (Zijno et al. 2003; Narayanan et al. 2004), whereas in others, the TT genotype is associated with lower uracil concentrations (DeVos et al. 2008). Kinetic studies, using ^{13}C-labelled 1-carbon precursors suggest that adenine and thymidine synthesis is greater in monocytes from TT compared with CC variants (Quinlivan et al. 2005). Volunteers in this study were fed a low folate diet for several weeks prior to tracer infusion, and it remains to be established whether similar effects would have been observed in individuals with adequate folate status. Overall, there is little evidence in support of the hypothesis that having the *MTHFR* TT variant reduces risk of malignancy by increasing the availability of 5,10-methylene THF for thymidine and purine synthesis. However, the majority of studies have used surrogate tissues, such as lymphocytes, and it remains unknown what affect genotype has on DNA stability in normal human colon tissue.

Synthetic folic acid intervention, cancer prevention and cancer promotion

Whereas high folate status (based on dietary intake and blood levels) is generally positively associated both with biomarkers of genomic stability and a decreased risk of CRC, the effect of intervention with folic acid to prevent or reduce cancer recurrence in large-scale human trials is disappointing (reviewed in Kim 2007; Cole et al. 2007). Moreover, there is now concern over the potential harmful effects of long-term intervention with high doses of synthetic folic acid. Data from animal models, human intervention trials and analyses of cancer incidence data suggest that supplementation with synthetic folic acid may promote growth of initiated cancer cells (Cole et al. 2007, Mason et al. 2007, Figueiredo et al. 2009b). Rodent studies report a reduction in early markers of colon cancer, such as aberrant crypt foci (ACF), when folic acid is given prior to initiation of lesions (Kim et al. 1996a, b; Song et al. 2000a; Kim 2007). However, carcinogenesis is accelerated if folic acid is given after the emergence of lesions, presumably through provision of DNA precursors for cancer cell growth (Song et al. 2000a, b). Recent findings from several large-scale human observational or placebo-controlled intervention trials indicate that supplemental folic acid increases risk of cancer at several sites, including the breast (Stolzenberg-Solomon et al. 2006), colon (Cole et al. 2007), lung (Ebbing et al. 2009) and prostate (Figueiredo et al. 2009b). Overall cancer mortality may also be increased (Ebbing et al. 2009). Synthetic folic acid supplementation has also been shown to act detrimentally on biomarkers of genomic stability. Combined high-dose folic acid and vitamin B_{12} supplementation (5 mg and 1.25 mg daily for 6 months, respectively), increased uracil misincorporation and tumour suppressor gene promoter methylation in rectal biopsies from colorectal adenoma patients (van den Donk et al. 2007). Similarly, a nonsignificant increase in cancer incidence has been reported in cardiovascular disease patients given folic acid to decrease homocysteine (Bonaa et al. 2006, Ebbing et al. 2008). Moreover, there are recent indications that population-wide mandatory folic acid fortification has had a detrimental effect on colon cancer incidence. Fortification of flour and uncooked cereal-grain products with folic acid was introduced in the United States and Canada in 1998 in an attempt to reduce the incidence of neural tube defects. The level of fortification was agreed upon on the basis that it would not increase intakes above the upper safe limit of 1 mg per day in any societal group. The actual increase in folate intake is higher than anticipated. An analysis of cancer statistics in the USA suggests that augmenting synthetic folic acid intake through mandatory fortification has increased colon cancer risk (Mason et al. 2007). A similar effect has been described in Chile, where fortification was introduced around the same time (Hirsch et al. 2009).

It should be noted that in certain of the intervention studies described here, cancer incidence was either not the primary outcome or the studies were underpowered to detect significant changes in cancer risk at those sites. Moreover, it is not possible to demonstrate causality when analysing colon cancer rates postfortification. Nonetheless, the potential detrimental effects of high-dose synthetic folic acid intervention remain to be established.

Conclusion

High dietary folate intake and high blood folate status are generally associated with a decreased risk of certain malignancies, including colorectal cancer, although an entirely protective role for folate against carcinogenesis has been questioned recently. Folates have a critical role in maintaining DNA stability by donating one-carbon moieties. In vitro, animal and human studies demonstrate that folate deficiency

induces epigenetic changes by attenuating remethylation of SAH to SAM in the methionine cycle, leading to cytosine demethylation, global DNA hypomethylation, protooncogene activation and chromosomal instability. Moreover, inadequate dietary folate increases uracil misincorporation, DNA strand breakage, chromosomal breakage and malignant transformation. In addition to its effects on DNA damage, folate deficiency impacts on DNA repair by inhibiting thymidine and purine biosynthesis. The effects of folate status on DNA methylation and potentially on colon carcinogenesis are profoundly dependent not only on the severity and duration of the folate depletion, but on the gene, tissue and stage of malignant transformation. On balance, the evidence available does not strongly support the hypothesis that altered genome-wide DNA methylation, as a direct consequence of low folate status, increases human colon cancer risk. Similarly, whereas the evidence for folate deficiency decreasing genomic instability by inducing DNA damage, inhibiting DNA repair and increasing malignant transformation are more consistent across in vitro, rodent and human studies, strong definitive evidence demonstrating a causal association between these biomarkers of genomic stability and cancer risk is limited.

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