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Folate, DNA stability and colo-rectal neoplasia

Susan J. Duthie[1]*, Sabrina Narayanan[1], Linda Sharp[2], Julian Little[2], Graham Basten[3] and Hilary Powers[3]

[1]*Division of Cellular Integrity, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK*
[2]*Epidemiology Group, Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB25 2DZ, UK*
[3]*Centre for Human Nutrition, University of Sheffield, Sheffield S5 7AU, UK*

Lower levels of dietary folate are associated with the development of epithelial cell tumours in man, particularly colo-rectal cancer. In the majority of epidemiological studies blood folate or reported folate intake have been shown to be inversely related to colo-rectal cancer risk. Folate, via its pivotal role in C_1 metabolism, is crucial both for DNA synthesis and repair, and for DNA methylation. This function is compromised when vitamin B_{12} is low. Vitamin B_{12} deficiency has been shown to increase biomarkers of DNA damage in man but there is no evidence directly linking low vitamin B_{12} with cancer. Disturbingly, folate and vitamin B_{12} deficiencies are common in the general population, particularly in the underprivileged and the elderly. How folate and/or vitamin B_{12} deficiency influence carcinogenesis remains to be established, but it is currently believed that they may act to decrease DNA methylation, resulting in proto-oncogene activation, and/or to induce instability in the DNA molecule via a futile cycle of uracil misincorporation and removal. The relative importance of these two pathways may become clear by determining both DNA stability and cytosine methylation in individuals with different polymorphic variants of key folate-metabolising enzymes. 5,10-Methylenetetrahydrofolate reductase converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and thereby controls whether folate is employed for DNA synthesis or DNA methylation. Colo-rectal cancer risk is decreased in subjects homozygous for a common variant (C677T) of the gene coding for this enzyme, suggesting that DNA synthesis and repair may be 'enhanced' in these individuals. Evidence from animal and human studies is presented here in support of folate acting to maintain genomic stability through both these mechanisms.

Folate: Vitamin B_{12}: DNA stability: DNA methylation: MTHFR: Colon cancer

Folate and colo-rectal cancer risk

Inadequate dietary folate has been implicated in the development of several epithelial-cell cancers, including cancer of the cervix, lung and breast (Glynn & Albanes, 1994). However, the most convincing evidence linking low folate intake with an increased risk of malignancy relates to colo-rectal cancer (Prinz-Langenohl *et al*. 2001; Giovannucci, 2002; Potter, 2002). Cohort and case–control studies have consistently shown an inverse relationship between colo-rectal cancer incidence, reported intake of folate and blood-cell folate concentrations (Benito *et al*. 1991; Giovannucci *et al*. 1995; Giovannucci, 2002; Konings *et al*. 2002; Potter, 2002). Plasma and erythrocyte folate levels are lower in colo-rectal cancer patients than in normal subjects (Porcelli *et al*. 1996). Conversely, supplementation with folic acid protects against the development of colo-rectal neoplasia in high-risk patients with ulcerative colitis (Lashner *et al*. 1997).

Folate, vitamin B_{12} and genomic stability

Folate has a fundamental role in DNA metabolism and function through its ability to methylate cytosine and regulate gene expression, and via its role in nucleotide synthesis and DNA repair (Fig. 1). It is currently believed that folate deficiency may increase the risk of malignant transformation by disrupting both these functions. 5-Methyltetrahydrofolate, the major circulating form of folate, acts as a cofactor in the conversion of homocysteine to methionine. Methionine is subsequently metabolised to *S*-adenosylmethionine, the principal methyl donor in the majority of biochemical reactions, including cytosine

Abbreviation: MTHFR, 5,10-methylenetetrahydrofolate reductase.

***Corresponding author:** S. Duthie, fax +44 1224 716629, email sd@rri.sari.ac.uk

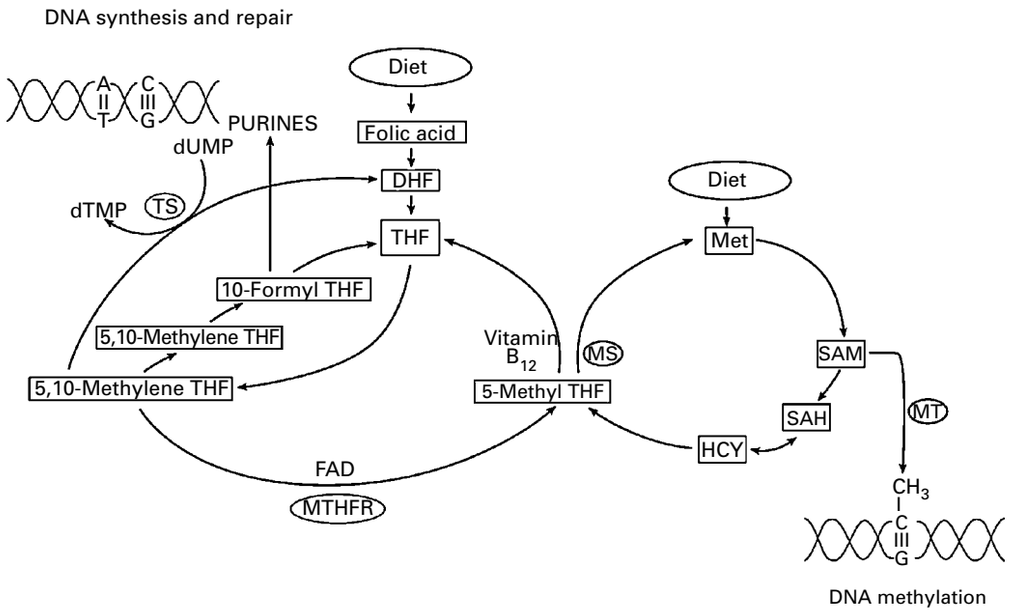

Fig. 1. Simplified diagram showing how folate (and vitamin B_{12}) are strategic cofactors in DNA methylation and DNA synthesis and repair. TS, thymidylate synthase; THF, tetrahydrofolate; DHF, dihydrofolate; MTHFR, 5,10-methylenetetrahydrofolate reductase; MS, methionine synthase; HCY, homocysteine; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; MT, DNA methyltransferase.

methylation in DNA. DNA methylation controls gene expression. Under conditions of folate deficiency *S*-adenosylmethionine is depleted and *S*-adenosylhomocysteine the product of methyltransferase activity is elevated, leading to DNA hypomethylation (Yi *et al*. 2000), inappropriate proto-oncogene activation and transcription, and malignant transformation (Feinberg & Vogelstein, 1983; Fang *et al*. 1996; Fang & Xiao, 2001).

Folic acid is essential for the synthesis of purines and the pyrimidine nucleoside thymidine. dUMP is converted to TMP by thymidylate synthase using 5,10-methylenetetrahydrofolate as a methyl donor. If folate is low dUMP may accumulate, inducing uracil misincorporation into DNA in place of thymine. DNA repair enzymes act to remove misincorporated uracil from the DNA strand, causing a temporary breakage in the DNA molecule that is sealed by DNA ligase. However, if folate is continually limited, uracil misincorporation and repair may occur repeatedly in a 'catastrophic' or 'futile' repair cycle causing frequent breakage of the DNA molecule, chromosomal damage and malignant transformation (Reidy, 1987; Blount & Ames, 1994). Moreover, purine biosynthesis is negatively affected by low folate (10-formyltetrahydrofolate), similarly reducing the availability of nucleotides for DNA synthesis and repair (Fig. 1).

A deficiency in vitamin B_{12} would be expected to induce DNA instability in the same way as folate deficiency. Both 5-methyltetrahydrofolate and vitamin B_{12} are required for the methylation of homocysteine to methionine by methionine synthase (Fig. 1). When vitamin B_{12} is limiting 5-methyltetrahydrofolate is not metabolised to tetrahydrofolate, which in turns reduces the availability of 5,10-methylenetetrahydrofolate in the methylation of dUMP to dTMP for DNA synthesis and repair. Similarly, failure of 5-methyltetrahydrofolate to act in the remethylation of homocysteine to methionine limits the production of *S*-adenosylmethionine, which in turn may result in DNA hypomethylation and proto-oncogene activation. While there is no direct evidence linking vitamin B_{12} deficiency with genomic instability in human cells *in vitro* (Fenech, 2001*a*), vitamin B_{12} deficiency does increase DNA damage in man. Endogenous micronuclei frequency (as an indicator of chromosomal damage) is negatively associated with serum vitamin B_{12} (Fenech, 1997), and can be markedly reduced by vitamin B_{12} supplementation (Fenech *et al*. 1998). Although the geno-protective effect of vitamin B_{12} in these studies is reported to act independently of folate (Fenech, 1997; Fenech *et al*. 1998), there is little evidence of a relationship between vitamin B_{12} and cancer, and this issue will not be discussed further.

Data from *in vitro* experiments and *in vivo* rodent and human studies support the theory that folate (and where appropriate vitamin B_{12}) deficiency can act to induce genomic instability and carcinogenesis. This proposition is discussed in the remainder of the present review.

Folate deficiency and altered DNA methylation

Methylation of genes at specific locations in the DNA molecule either stops or reduces the rate of transcription. In this way site-specific DNA methylation controls gene expression and function. Alterations or disruption either to global or site-specific DNA methylation may increase malignant transformation. Changes in *S*-adenosylmethionine:*S*-adenosylhomocysteine ratio and subsequent global DNA hypomethylation are associated with tumour progression and multiplicity in the *Min* mouse model of colon

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cancer (Sibani *et al*. 2002). Aberrant DNA methylation is one of the most common molecular changes in human cancers. Global DNA hypomethylation is frequently found in human tumours and is an early event in carcinogenesis, while hypermethylation of the promoter region of certain tumour suppressor genes may accelerate the carcinogenic process (Fang & Xiao, 2001; Johanning *et al*. 2002).

It has long been established that extreme methyl depletion alters genomic methylation. For example, global genomic DNA hypomethylation is induced in rats fed a methyl-deficient diet (deficient in methionine, choline, folic acid and vitamin B_{12}), and mRNA for the specific proto-oncogenes c-*myc*, c-*fos* and c-Ha-*ras* is elevated (Wainfain & Poirier, 1992). However, the effect of folate deficiency alone on DNA methylation varies markedly according to the tissue under investigation, the rodent model and the treatment regimen employed. Rats fed a folate-depleted diet for 4 weeks exhibit hypomethylated DNA in the liver (Balaghi & Wagner, 1993), while folate deficiency appears specifically to favour hypomethylation of p53 exons 6 and 7 from rat colon mucosa. Moreover, folate supplementation can reverse chemically-induced hypomethylation in exon 8 of the *p53* proto-oncogene (Kim *et al*. 1996). However, two studies in rats have reported that folate deficiency does not induce hypomethylation, either in total cellular DNA or site-specific methylation in hepatic c-*myc* or colonic *p53* (promoter and exon 6–7; Kim *et al*. 1995; Sohn *et al*. 2003), even though colonic mucosal folate concentrations are markedly depleted (Sohn *et al*. 2003). Additionally, in rats the methylation of hepatic, blood and colonocyte DNA-cytosine is not influenced by folate status (Duthie *et al*. 2000*c*).

The evidence demonstrating a role for folate deficiency in the modulation of either global or gene-specific DNA methylation in man is equally inconsistent. DNA hypomethylation (measured in colon tissue) and low folate status are associated with an increased risk of colo-rectal neoplasia (Pufulete *et al*. 2003*a*). Few studies have measured DNA methylation in normal colo-rectal mucosa, although a recent study suggests that genomic DNA methylation in this tissue is inversely associated with folate status (Pufulete *et al*. 2003*b*). DNA hypomethylation is induced in lymphocytes isolated from healthy post-menopausal women with subclinical folate deficiency (mean plasma folate 9·3 nmol/l compared with 19·5 nmol/l at baseline) following supplementation with folic acid at 56 µg/d for 5 weeks and 111 µg/d for 4 weeks, and is reversible following intervention with folate at 286–516 µg/d (Jacob *et al*. 1998). These effects on methylation have been confirmed in a study of elderly women consuming a moderately-folate-depleted diet (Rampersaud *et al*. 2000). In contrast, in subjects with normal folate status, lymphocyte genomic DNA methylation is unaffected by folate status, and supplementation with folate does not alter methylation status (Fenech *et al*. 1998). This finding has been confirmed in a recent placebo-controlled study in which healthy subjects were supplemented with folic acid (1·2 mg/d) for 12 weeks (GB Basten, MH Hill, N Vaughan, SJ Duthie and HJ Powers, unpublished results). Global DNA methylation remains unchanged throughout the study period (Fig. 2).

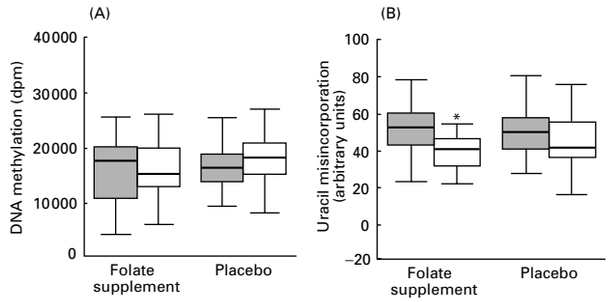

Fig. 2. The effect of folate supplementation on global cytosine methylation (A) and endogenous uracil misincorporation (B) in human lymphocytes *in vivo*. Volunteers were given 1·2 mg folic acid/d (*n* 28) or placebo (*n* 33) for 12 weeks. Uracil was measured in isolated lymphocytes by single-cell gel electrophoresis (Duthie & Hawdon, 1998). Global DNA methylation was determined by measuring the incorporation of $C^3{}_3H_3$ groups into DNA (Balaghi & Wagner, 1993). Values are medians (—), interquartile ranges and outliers, represented by vertical bars, at week 0 (▨) or week 12 (□). Median value was significantly lower than the corresponding value at week 0: $^*P<0{\cdot}05$.

Gene-specific DNA hypermethylation occurs during tumour development (Jones & Laird, 1999). A recent *in vitro* study to determine the impact of folate deficiency on global gene expression in a human naso-pharyngeal carcinoma cell line (KB) has shown that mRNA from only eight genes (sampled from >2000) are altered by folate deficiency, with three genes being up regulated and five genes being down regulated (Jhaveri *et al*. 2001). DNA from one of the down-regulated genes, H-cadherin, a protein that maintains cell-to-cell adhesion and tissue structure, is hypermethylated. Dysfunction of H-cadherin has been implicated in tumorigenesis (Takeichi, 1993). Folate and methyl deficiencies also increase site-specific DNA methylation (hypermethylation) within the p53 gene in rat liver (Pogribny *et al*. 1995) and *de novo* methylation of the p16 tumour suppressor gene promoter (Pogribny & James, 2002). Hypermethylation of *p16* is an early pre-neoplastic event that precedes tumorigenesis (Pogribny & James, 2002).

While an increased risk for cancer might be explained by DNA hypermethylation with subsequent silencing of tumour suppressor genes (Esteller *et al*. 1999), the role of low folate levels in this mechanism is still unclear.

Folate deficiency, DNA synthesis and repair

In vitro, folate deficiency negatively affects intracellular DNA nucleotide precursor pools (James *et al*. 1994) and dose-dependently increases uracil misincorporation, chromosomal breakage (measured as micronuclei frequency) and chromosomal abnormalities in human lymphocytes (Duthie & Hawdon, 1998; Crott *et al*. 2001*a*). Moreover, proliferation is abnormal in these cells and they are unable to repair oxidative DNA damage efficiently (Duthie & Hawdon, 1998). Similar detrimental effects (increased DNA strand breakage, uracil misincorporation and altered DNA base excision repair) are observed in immortalised human colonocytes grown under folate-deficient conditions (Duthie *et al*. 2000*b*). Folate deficiency has also been

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shown to increase mutagenesis and malignant transformation in cultured rodent cells (Branda *et al*. 1997; Melnyk *et al*. 1999).

Folate deficiency similarly alters nucleotide synthesis, genomic stability and DNA repair in animal models. In splenic lymphocytes isolated from folate-deficient rats DNA strand breakage is increased and intracellular NAD, the substrate for poly(ADP ribose) polymerase, is depleted, indicating up-regulation of DNA repair activity (James & Yin, 1989; James *et al*. 1992). DNA strand breakage is increased progressively within exons 5–8 of the tumour suppressor gene p53 in folate-deficient rat colon. Conversely, supplementation with folate markedly increases p53 integrity (Kim *et al*. 2000). In these experiments DNA strand breakage is correlated with colonic mucosal folate levels (Kim *et al*. 2000). Uracil misincorporation is increased in the liver of rats fed a combined methyl-deficient diet (folate-, methionine- and choline-free diets; Pogribny *et al*. 1997) and in the liver of partially-hepatectomised rats treated with methotrexate (to reduce thymine synthesis; Blount & Ames, 1994). A similar increase in uracil misincorporation (2–3-fold) and DNA strand breakage occurs in lymphocytes isolated from rats fed only a folate-deficient diet for 8 weeks (Duthie *et al*. 2000*a*).

Folate status influences genomic stability in man. Uracil misincorporation is elevated in bone marrow cells from subjects with megaloblastic anaemia (as a result of severe folate deficiency), while thymidine levels are considerably depleted (Wickramasinghe & Fida, 1994). Bone marrow and blood DNA from folate-deficient splenectomised subjects (erythrocyte folate <140 ng/ml) contain eight to nine times more uracil and there are three times as many micronucleated reticulocytes and erythrocytes compared with subjects with normal (>140 ng/ml) erythrocyte folate (Blount *et al*. 1997). Furthermore, supplementation with folic acid (5 mg/d for 8 weeks) markedly decreases uracil misincorporation and micronuclei frequency. These data support the hypothesis that folate deficiency can induce genomic instability in man, and that intervention with folate, under these conditions, can reverse this process.

However, the impact of folate on DNA stability in individuals who do not have frank folate deficiency is less clear. Folate status does not influence endogenous micronuclei frequency in young adults with normal blood folate levels (Fenech *et al*. 1998). Moreover, while supplementation with folate and vitamin B_{12} (3·5 times the recommended dietary intake for each vitamin for 12 weeks followed by 10 times the recommended dietary intake for a further 12 weeks) decreases micronuclei frequency by 25% in subjects in the high 50th percentile for this biomarker; this decrease is associated only with elevated blood vitamin B_{12} levels and not erythrocyte folate levels. However, it has recently been shown that supplementing healthy volunteers with folic acid (1·2 mg/d) for 3 months significantly ($P<0·05$) reduces endogenous uracil levels in isolated lymphocytes (Fig. 2). This positive effect of folate on DNA stability is specific against uracil misincorporation, as DNA strand breakage and total cytosine methylation remain unchanged (Fig. 2; GB Basten, MH Hill, N Vaughan, SJ Duthie and HJ Powers, unpublished results).

Folate, colo-rectal cancer and genetic susceptibility

It had been anticipated that the relative importance of the two previously mentioned mechanisms in the aetiology of folate deficiency and malignant transformation would become apparent by determining DNA stability and cytosine methylation in individuals with specific polymorphisms in critical folate-metabolising genes. Methylenetetrahydrofolate reductase (MTHFR) is an important regulatory enzyme in the metabolism of folate. MTHFR converts 5,10-methylenetetrahydrofolate irreversibly to 5-methyltetrahydrofolate, the principal circulating form of folate and the methyl donor in the remethylation of homocysteine to methionine. This key protein, therefore, controls whether folate is employed for DNA synthesis or DNA methylation. The most common variant of the MTHFR gene is located at nucleotide 677 (C677T) and causes a valine for alanine substitution in the protein that is associated with increased thermolability and decreased enzyme activity (Frosst *et al*. 1995). Heterozygotes (CT) or homozygotes (TT) have a markedly reduced *in vitro* enzyme activity (65 and 30% of the wild type (CC) respectively; Frosst *et al*. 1995). Homozygosity is associated with changes in the normal distribution of blood folates and homocysteine. Erythrocytes from individuals homozygous for the TT variant have decreased 5-methylfolate levels with a concomitant increase in formylfolate derivatives (Bagley & Selhub, 1998). Similarly, plasma folate is decreased and homocysteine elevated in TT subjects (Jacques *et al*. 1996; Narayanan *et al*. 2004). The frequency of homozygosity for the C677T variant of the MTHFR gene varies between geographic areas and by ethnic origin, but is generally reported as approximately 12% in northern Europe and in white populations in North America (Bailley & Gregory, 1999).

Evidence is now emerging as to how DNA stability and carcinogenesis are affected by polymorphisms in the MTHFR gene. Impaired MTHFR activity might be expected to increase cancer risk as a result of reduced 5-methyltetrahydrofolate levels, with associated DNA hypomethylation and proto-oncogene activation (Fig. 3). However,

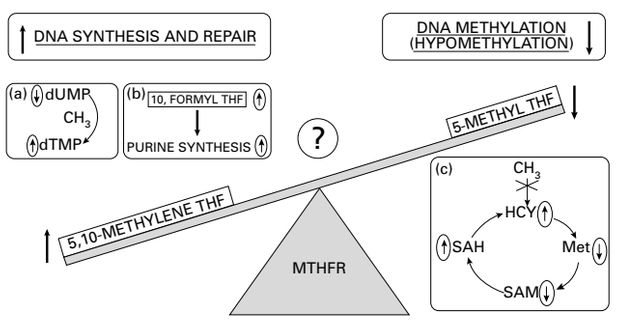

Fig. 3. Schematic diagram highlighting the pivotal role that the 5,10-methylenetetrahydrofolate reductase (MTHFR) protein plays in genomic stability and the potential imbalance that polymorphisms in the gene may cause. (a), Thymidine synthesis; (b), purine synthesis; (c), DNA methylation. THF, tetrahydrofolate; HCY, homocysteine: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

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homozygosity for the C677T variant is actually associated, at least in the majority of studies, with a decrease in colorectal cancer risk (Chen *et al*. 1996; Ma *et al*. 1997; Sharp & Little, 2004). Individuals homozygous for the variant (TT) generally have a reduced risk of developing colorectal cancer compared with heterozygotes or wild type (the relative risks are generally in the range 0·45–0·9), at least in subjects with normal plasma folate levels (Sharp & Little, 2004). The relationship between MTHFR genotype and risk of colon cancer appears to be profoundly influenced by diet and environment, with low folate and methyl donor status and high alcohol intake (which adversely affects folate metabolism) either negating the inverse association or increasing risk (Sharp & Little, 2004). The discovery that this polymorphism in the MTHFR gene in fact decreases colon cancer risk is surprising, given that impaired MTHFR enzyme activity and low blood folate would, based on current epidemiological evidence, be expected to increase risk of malignancy. However, low MTHFR activity may increase plasma 5,10-methylenetetrahydrofolate, which as discussed earlier is crucial in the production of thymidine and purines for DNA synthesis and repair, and thereby may actually enhance DNA stability by preventing uracil misincorporation and chromosomal breakage (Fig. 3).

Data on the influence of the MTHFR C677T polymorphism on global or site-specific DNA methylation status is inconsistent. Global DNA methylation status in lymphocytes isolated from approximately 200 healthy subjects is similar for all genotypes, and independent of folate status (Narayanan *et al*. 2004). In contrast, global DNA hypomethylation has been reported in leucocytes from nine TT individuals compared with ten subjects with the CC genotype (Choi *et al*. 1999; Stern *et al*. 2000), yet methylation at exon 5–8 of the p53 gene is similar for all variants (Choi *et al*. 1999). The results of a larger study have shown that genomic DNA methylation in peripheral blood mononuclear cells is lower in TT compared with CC individuals when plasma folate concentrations are low (Friso *et al*. 2002). 5-Methylcytosine levels are lower in normal human colon, breast and lung tissue from CT and TT individuals (Paz *et al*. 2002). Contrary to expectations, this relationship between genotype and global DNA methylation is not apparent in primary tumour samples from the same subjects. Moreover, the level of CpG island hypermethylation in specific tumour suppressor genes is similar for all three MTHFR variants (Paz *et al*. 2002).

In contrast, *in vitro* and *in vivo* studies have consistently found that DNA stability (measured using a variety of biomarkers) is unaffected by genotype. The ability of lymphocyte DNA *in vitro* to resist uracil misincorporation and chromosomal damage is comparable for all genotypes (Crott *et al*. 2001*a*,*b*). *In vivo*, endogenous DNA strand breakage, sister chromatid exchange and micronuclei formation in blood cells is similar for all MTHFR C677T variants (Zijno *et al*. 2003). Moreover, endogenous uracil misincorporation in lymphocyte DNA from healthy control subjects is unaffected by C677T genotype, and is independent of folate status and smoking habit (Narayanan *et al*. 2004).

Thus, there is little support for the hypothesis that the C677T MTHFR variant reduces the risk of malignancy by increasing the availability of 5,10-methylenetetrahydrofolate for thymidine and purine synthesis, decreasing uracil misincorporation into DNA and thereby increasing genomic stability, which is disappointing. However, necessarily the majority of data has been collected in studies using surrogate tissues such as peripheral blood lymphocytes, and the effect genotype on uracil misincorporation and DNA stability in the human colon remains unknown. Moreover, the influence that other dietary micronutrients such as vitamin B_6 and riboflavin have on MTHFR activity, DNA stability and cancer risk has not been established (McNulty *et al*. 2002; Moat *et al*. 2003).

As discussed previously, MTHFR activity (and colorectal cancer risk) is profoundly influenced by folate status. A recent human study investigating the associations between the C677T MTHFR polymorphism, smoking and folate status on colonic dysplasia has reported some startling findings (Ulvik *et al*. 2001). Folate status and genotype act to modulate the procarcinogenic effect of smoking. In smokers with the T allele (both CT and TT individuals) low folate intake is associated with an increased risk of developing high-risk adenomas. This finding could be explained by the induction of DNA hypomethylation by low folate and reduced enzyme activity (see earlier, p. 574). However, high folate status and smoking in subjects with the homozygous wild-type genotype (CC) also increases the risk of malignancy, presumably by accelerating the growth of already initiated (by smoking) colon epithelial cells (Ulvik *et al*. 2001). While this complex study could be criticised for lacking statistical power after stratifying subjects according to genotype, folate status and smoking habit, data from animal models also show that folate can act either to reduce malignant transformation or to accelerate carcinogenesis. This differential effect is critically dependent on the time of intervention and the model system employed. Rats treated with the colon carcinogen azoxymethane and made folate deficient have markedly fewer aberrant crypt foci (an early indicator of malignant transformation) in the colon than control animals (Le Leu *et al*. 2000). Similarly, folate given before establishment of neoplastic foci lowers the number of colonic aberrant crypt foci and adenomas in a mouse model of familial colo-rectal cancer combined with a deletion in mismatch repair (Apc+/−Msh2−/− mice; Song *et al*. 2000). Conversely, when administered after the development of neoplastic lesions in the same model elevated folate acts to increase formation of intestinal adenomas (Song *et al*. 2000).

Thus, folate may be protective against initiation of carcinogenesis, but may act to accelerate progression of the cancer in pre-existing neoplastic cells.

Is it possible to set an RDA for folate and vitamin B_{12} to ensure optimum DNA stability?

Deficiencies in micronutrients that are either required as cofactors for DNA metabolism and repair (like folate and vitamin B_{12}) or form an integral part of the DNA molecule (like Zn) may have as destructive an effect on DNA as

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radiation or chemical carcinogens (Ames, 2001). This situation has led to the concept of creating a RDA to ensure optimum genomic stability in man (Ames, 2001; Fenech, 2001*b*; for review, see Fenech, 2003). Currently, RDA for vitamins and minerals are based on the prevention of acute overt deficiencies. A RDA allowing optimum genomic stability would require redefinition based on the prevention of cancer (Fenech, 2003). It is clear that folate (and vitamin B_{12}) is crucial for normal DNA metabolism and repair and for controlling appropriate gene expression, and that deficiencies in both B vitamins cause genomic instability *in vitro* and *in vivo*. Is it possible to determine what level of B vitamins may be optimal for preventing damage to DNA by further examining data from experimental studies? Uracil misincorporation and DNA strand breakage are negatively correlated with folic acid levels, and decrease dose-dependently in cultured human lymphocytes and colonocytes supplemented with increasing concentrations of folic acid (Duthie & Hawdon, 1998; Duthie *et al*. 2000*b*). Both biomarkers of DNA damage are minimised after *in vitro* intervention with 100 nM-folic acid (Duthie & Hawdon, 1998; Duthie *et al*. 2000*b*). Similarly, uracil misincorporation and micronuclei frequency are at their lowest in cultured human lymphocytes supplemented with 120 nM-folic acid (Crott *et al*. 2001*a*,*b*). In a placebo-controlled human study intervention with folic acid (700 μg) and vitamin B_{12} (2·5 μg) for several months has been found to reduce lymphocyte micronuclei frequency by 25 % (Fenech *et al*. 1998). In a recent review of data from human trials Fenech (2003) has proposed that genomic instability can be minimised when plasma folate is >34 nM, erythrocyte folate is >700 nM and vitamin B_{12} is >300 pM. Studies using a decrease in micronuclei frequency as an indicator of enhanced genomic stability indicate that a RDA of 700 μg/d for folic acid and 7 μg/d for vitamin B_{12} would be sufficient to 'ensure' genomic stability in young adults (Fenech, 2001*b*). Increasing folate intake can markedly decrease endogenous uracil misincorporation in lymphocyte DNA from volunteers taking 1·2 mg folic acid/d for 3 months (GB Basten, MH Hill, N Vaughan, SJ Duthie and HJ Powers, unpublished results). It remains to be established whether a lower intake of folate would have a similar beneficial effect.

The earlier data suggest that genomic stability can be improved by increasing intake of folate (and vitamin B_{12}), above the current RDA, and it is likely that this increase can only be achieved through supplementation or food fortification. This method might also have additional benefits in terms of decreased incidence of neural-tube defects, heart disease and ageing-associated degenerative disorders such as cognitive impairment.

Summary

In addition to exogenous and endogenous mutagens and carcinogens, inappropriate nutrition can induce DNA damage and ultimately cancer.

Data from human observational and supplementation studies, animal models and *in vitro* cell-culture experiments strongly support a role for folate in maintaining genomic stability, acting either through its ability to maintain normal DNA synthesis and repair or through its ability to control gene expression via DNA methylation. Currently, the relative importance of these pathways is unknown.

Risk from cancer appears to be influenced by polymorphisms in key folate-metabolising genes, although the mechanism of cytoprotection remains elusive. However, while it is clear that folate protects against DNA damage and initiation of carcinogenesis, recent data from several animal experiments and one human population study suggest that high folate intakes may be detrimental to individuals with specific genetic polymorphisms who also smoke, by accelerating progression of cancer. This disturbing finding, together with the data from human intervention studies suggesting that the dietary intake of folate required to optimise DNA stability is substantially higher than the current RDA, obviously raises important safety and ethical concerns in the debate over the potential benefits to human health of mandatory fortification of certain foods with folate.

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