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Folate, DNA methylation and colo-rectal cancer

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Prospective cohort and case–control studies suggest an association between low folate intake and increased risk of colo-rectal adenoma and cancer. Some, but not all, animal studies indicate that folate supplementation protects against the development of colo-rectal neoplasms, although supraphysiological folate doses have been shown to enhance tumour growth. Folate is a methyl donor for nucleotide synthesis and biological methylation reactions, including DNA methylation. A low dietary folate intake may increase the risk of colo-rectal neoplasia by inducing genomic DNA hypomethylation, which can affect the expression of proto-oncogenes and tumour suppressor genes associated with the development of cancer. Common polymorphisms in genes involved in the methylation pathway, such as methylenetetrahydrofolate reductase and methionine synthase, have been shown to influence risk of colo-rectal neoplasia, with interactions dependent on folate status and/or alcohol intake, which is known to antagonise methyl group availability. There is some evidence to show that DNA from normal-appearing colo-rectal mucosa in individuals with colo-rectal cancer is hypomethylated. In a case–control study DNA methylation in normal-appearing colo-rectal mucosa has been shown to be lower in individuals with colo-rectal cancer ($P = 0{\cdot}08$) and colo-rectal adenoma ($P = 0{\cdot}009$) than in controls free of colo-rectal abnormalities. Human intervention trials to date suggest that supraphysiological doses of folate can reverse DNA hypomethylation in colo-rectal mucosa of individuals with colo-rectal neoplasia. In a double-blind randomised placebo-controlled study folate supplementation at physiological doses has been shown to increase DNA methylation in leucocytes ($P = 0{\cdot}05$) and colonic mucosa ($P = 0{\cdot}09$). Further studies are required to confirm these findings in larger populations and to define abnormal ranges of DNA methylation.

Folate: DNA methylation: Colo-rectal neoplasia

Diet and colo-rectal neoplasia

Colo-rectal cancer (CC) is the second most common cancer in Western societies (Muir *et al*. 1987). There is marked variation in incidence throughout the world, with high rates in industrialised regions such as Australia, New Zealand, USA, Japan and Western Europe, and low rates in regions such as Africa, China and other parts of Asia (Parkin *et al*. 1999). The majority of CC are believed to be sporadic, and only about 15 % are due to dominantly inherited mutations in susceptibility genes (Cannon-Albright *et al*. 1988; Houlston *et al*. 1992). Neoplastic transformation of the colonic mucosa is characterised by increased cell proliferation, adenomatous polyp formation and growth, malignant transformation and invasion (Fearon & Vogelstein, 1990). This transformation process is accompanied by many genetic and epigenetic changes occurring at various stages of disease progression.

Diet is thought to play a role in the development of CC. Ecological studies suggest that the adoption of a Western diet, characterised by high intakes of fat and lower intakes of starchy foods, is associated with increased risk. There is now moderately consistent evidence that diets containing high intakes of red meat and low intakes of vegetables are associated with increased risk (Department of Health, 1998). In a recent study in which a food-frequency questionnaire specifically designed to assess folate intake was validated against a 7 d weighed food intake record and biomarkers of folate status was validated, vegetables were shown to be a major source of folate in the diet (Pufulete *et al*. 2002*b*). Among the components in vegetables that may be responsible for the observed protective effect is the

Abbreviations: CA, colo-rectal adenoma; CBS, cystathionine-β-synthase; CC, colo-rectal cancer; MS, methionine synthase; MTHFR, methylene-tetrahydrofolate reductase.

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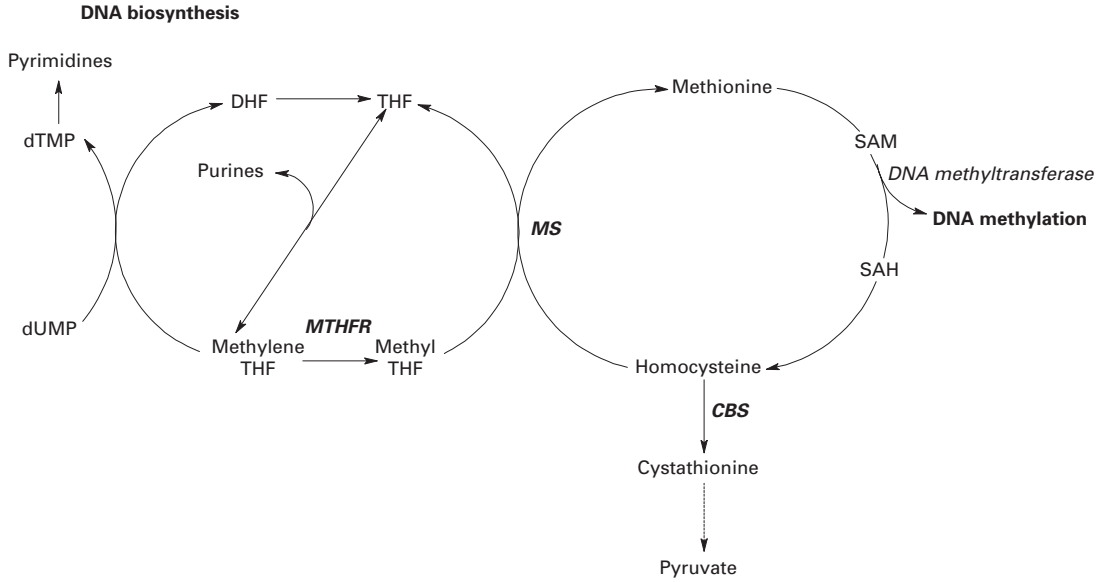

Fig. 1. Competing pathways in folate metabolism. dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; DHF, dihydrofolate; THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; CBS, cystathionine-β-synthase.

water-soluble B-vitamin, folate, which has recently attracted a lot of attention because of its important role in DNA metabolism, which is markedly altered in carcinogenesis.

Biochemical function of folate

Folate donates and accepts C_1 units, a reaction that is essential for the synthesis of DNA, RNA and glycine, and maintaining the methylation cycle (Fig. 1). Deficiency results in ineffective DNA synthesis, reduced cell proliferation and impaired cellular physiology.

Folate, in the form of 5,10-methylenetetrahydrofolate is required for purine synthesis and the *de novo* synthesis of thymidylate from deoxyuridylate. The regeneration of methionine from homocysteine in the methylation cycle requires a methyl group from 5-methyltetrahydrofolate, in a reaction catalysed by the vitamin B_{12}-dependent enzyme, methionine synthase (MS). The activated form of methionine, *S*-adenosylmethionine, donates the methyl group derived from folate in over eighty methylation reactions, including DNA methylation, which is a fundamental mechanism for the epigenetic control of gene expression and maintenance of genomic integrity.

At first glance, folate seems an unlikely candidate as a protective agent against cancer. It has been known for some time that folate can promote tumour growth, an observation that has led to the development of anti-folate agents such as methotrexate, which is used in cancer chemotherapy (Kamen, 1997). Folate plays a key role in DNA replication and cell division; therefore, reducing the amount of folate in tissues with rapidly replicating cells will impair these processes. However, healthy tissues also require an adequate folate pool to maintain normal DNA synthesis and function, and a disruption of these processes can lead to genomic instability, creating an environment that potentiates risk factors for carcinogenesis.

Folate intake and risk of colo-rectal neoplasia

Prospective cohort (Giovannucci *et al*. 1995, 1998; Glynn *et al*. 1996; Su & Arab 2001; Fuchs *et al*. 2002; Konings *et al*. 2002; Terry *et al*. 2002) and case–control (Benito *et al*. 1991; Freudenheim *et al*. 1991; Meyer & White, 1993; Ferraroni *et al*. 1994; La Vecchia *et al*. 1997; White *et al*. 1997) studies that have investigated the effect of dietary folate intake on CC risk generally show a reduction in risk (about 35% overall) in subjects with the highest dietary folate intake compared with those with the lowest intake. These effects appeared to be greatest in subjects with the lowest alcohol intake (Giovannucci *et al*. 1995; Glynn *et al*. 1996). In some studies regular use of folic acid supplements was associated with the protective effect (Giovannucci *et al*. 1998; Fuchs *et al*. 2002). Only four studies showed no protective effect of folate (Boutron-Ruault *et al*. 1996; Kato *et al*. 1999; Levi *et al*. 2000; Flood *et al*. 2002). A high folate intake has also been associated with decreased risk of colo-rectal adenoma (CA; Giovannucci *et al*. 1993; Boutron-Ruault *et al*. 1996; Tseng *et al*. 1996; Baron *et al*. 1998). CA is believed to be a precursor of CC (Fenoglio & Lane, 1974) and is considered an intermediary stage of colo-rectal neoplasia. Folate supplementation (1 mg/d) has been shown to decrease CA recurrence by 46% in thirty-one subjects with previously resected CA (Paspatis & Karamanolis, 1994).

Biomarkers of folate status and risk of colo-rectal neoplasia

The relationship between biomarkers of folate status and risk of colo-rectal neoplasia is less well defined. Two nested case–control studies provide conflicting information; the first showed that serum folate concentrations were significantly lower ($P < 0{\cdot}001$) in women with CC

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compared with controls (Kato *et al*. 1999), while the second showed no differences in serum folate concentrations between male smokers with and without CC (Glynn *et al*. 1996). In a large case–control study investigating the relationship between biomarkers of folate status and risk of CA, serum and erythrocyte folate concentrations were significantly different ($P < 0{\cdot}05$) between cases and controls, but only in men (Bird *et al*. 1995). On the other hand, Paspatis *et al*. (1995) showed that erythrocyte (but not serum) folate concentrations were significantly lower in subjects with CA compared with controls ($P < 0{\cdot}01$).

Two studies have reported data on the relationship between plasma homocysteine concentration, which rises in folate depletion and is thought to be a sensitive indicator of cellular folate depletion, and risk of colo-rectal neoplasia. The first study showed that plasma homocysteine concentrations were significantly higher in women with CC compared with controls ($P = 0{\cdot}04$; Kato *et al*. 1999), while a smaller study showed that although plasma homocysteine concentration was higher in subjects with CA compared with controls ($P = 0{\cdot}04$), blood folate concentrations were similar in both groups (Kim *et al*. 1998).

Polymorphisms of enzymes involved in the methylation cycle and risk of colo-rectal neoplasia

The effect of common polymorphisms in key enzymes involved in the methylation pathway (methylenetetrahydrofolate reductase (MTHFR) 677C→T, MS 2756A→G and cystathionine-β-synthase (CBS) 844ins68) on CC risk has been investigated in several studies. MTHFR is a critical enzyme in folate metabolism (Fig. 1), catalysing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The 677C→T mutation decreases MTHFR activity (Frosst *et al*. 1995), leading to reduced plasma folate and increased plasma homocysteine.

Three studies have reported a decreased risk of CC in individuals homozygous for the MTHFR 677C→T mutation compared with those not carrying the mutation, although the protective effect was absent in those with folate deficiency or high alcohol intake (Chen *et al*. 1996; Ma *et al*. 1997; Slattery *et al*. 1999). Studies in subjects with CA show similar interactions between the MTHFR 677C→T mutation, folate status and alcohol intake (Ulrich *et al*. 1999; Levine *et al*. 2000; Ulvik *et al*. 2001), suggesting that the homozygous mutation increases CA risk under conditions of low folate status or high alcohol intake. The MS 2756A→G mutation has also been associated with a small non-significant increase in risk of developing CC and CA (Chen *et al*. 1998; Ma *et al*. 1999). MS is the vitamin B_{12}-dependent enzyme that catalyses the conversion of homocysteine to methionine in the methylation cycle (Fig. 1). This mutation can also lead to increased levels of plasma homocysteine (Harmon *et al*. 1999). One study has also reported a lower frequency of the CBS 844ins68 mutation in CC subjects compared with controls, particularly in cases with proximal colon tumours (Shannon *et al*. 2002). CBS irreversibly removes homocysteine from the methylation cycle by transulfuration to cystathionine, a pathway that may become more important when folate supply is limited.

It has been proposed that the MTHFR 677C→T mutation reduces CC risk by influencing the DNA synthesis pathway (Fig. 1). As the mutation decreases MTHFR activity, there is inefficient conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Increased levels of 5,10-methylenetetrahydrofolate, which is a cofactor for nucleotide synthesis, may increase nucleotide precursor pools resulting in more efficient DNA synthesis and repair. When there is an adequate supply of methyl groups from folate the negative effects of these polymorphisms on DNA methylation may be overcome. Conversely, when the supply of methyl groups is low, both DNA methylation and synthesis may be impaired, increasing the risk of neoplasia. A high alcohol intake can also overcome the apparent protective effect of the mutations because it reduces the availability of methyl groups (Finkelstein *et al*. 1974) and can cleave folate (Shaw *et al*. 1989), impair folate absorption (Romero *et al*. 1981) and increase folate excretion (Eichner & Hillman, 1971). Alcohol has also been shown to interfere with MS activity (Barak *et al*. 2001).

Animal studies

Studies in animal models of cancer generally complement the epidemiological evidence, although results have not been entirely consistent. In controlled experiments using the dimethylhydrazine rodent model of CC, folate depletion increased tumour incidence following injection with dimethylhydrazine, a colo-rectal carcinogen (Cravo *et al*. 1992; Kim *et al*. 1996*b*). In the study by Kim *et al*. (1996*b*) folate supplementation of up to four times the dietary requirement was associated with reduced incidence of tumour growth. Studies that have used the azoxymethane rodent model of CC have shown no effect of folate supplementation on tumour incidence and aberrant crypt foci (an early precursor lesion and well-established intermediate biomarker of CC; Shivapurkar *et al*. 1995; Reddy *et al*. 1996). In some studies pharmacological doses of folate increased aberrant crypt foci (Wargovich *et al*. 1996) and tumour growth (Kim *et al*. 1996*b*), suggesting that in a strongly carcinogenic environment folate promotes tumour growth. This outcome has been observed in animals with well-established cancers (Baggott *et al*. 1992; Bills *et al*. 1992).

Studies have also assessed the role of folate intake on the development of intestinal polyps in a murine model of intestinal tumorigenesis that carries a heterozygous mutation in the adenomatous polyposis coli gene. The mutation predisposes to polyp formation throughout the small intestine and colon. These studies suggest that folate supplementation only suppresses polyp formation in the early stages of development (Song *et al*. 2000*a,b*). At later time points, folate supplementation was associated with an increase in the number of polyps, emphasising the protective role of folate only at the initiation stages of carcinogenesis.

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Potential mechanisms by which folate may influence cancer risk

A low folate status is thought to increase the risk of colo-rectal neoplasia by affecting both the DNA synthesis and methylation pathways (Mason & Choi, 2000). Folate depletion can induce deoxynucleotide pool imbalance and uracil misincorporation in DNA in place of thymidine, which leads to abnormal DNA synthesis and repair. As the main focus of the present paper is on the role of folate in DNA methylation, it is beyond its scope to review the literature concerning the role of folate in DNA synthesis and repair.

Alterations in DNA methylation

DNA methylation is thought to play crucial roles in the regulation of gene expression and gene integrity. Of the four bases that make up DNA, only cytosine has the potential to be methylated in man and most mammals. Methylation occurs at the 5′ position of cytosine residues that form cytosine–guanine sequences (CpG dinucleotides). The pattern of methylation in these sequences is a heritable, tissue- and species-specific change (Razin & Szyf, 1984). Most CpG dinucleotides are clustered in small stretches of DNA known as CpG islands. As CpG islands are mainly found at the active sequences of genes (promoter regions), DNA methylation in these regions is an important determinant of gene expression. CpG islands are not usually methylated and *de novo* methylation in these regions is associated with gene silencing (Bird, 1986; Razin & Cedar, 1991). Elsewhere in the genome, approximately 70–90% of CpG dinucleotides are methylated (Razin & Szyf, 1984).

Apart from altering gene expression, DNA methylation is important in the conformational configuration and structural stability of DNA (Antequera *et al*. 1990; Keshet *et al*. 1986; Lewis & Bird, 1991), binding of transcription factors and other proteins (Keshet *et al*. 1986; Boyes & Bird, 1991; Levine *et al*. 1991), genomic imprinting (differential expression of parental alleles in normal development; Li *et al*. 1993) and mutations (Jones *et al*. 1992).

Collective evidence suggests that aberrations in DNA methylation are a cause rather than a consequence of carcinogenesis. Genomic and gene-specific DNA hypomethylation has been observed in cancer cells, including CC (Feinberg & Vogelstein, 1983*a*; Goelz *et al*. 1985). DNA hypomethylation in proto-oncogenes may lead to their increased expression and has been reported in *ras* oncogenes (Feinberg & Vogelstein, 1983*b*) and the *c-myc* oncogene (Sharrard *et al*. 1992). DNA hypomethylation is also associated with changes in interactions between DNA and methyl-specific proteins and changes in chromatin conformation, both of which can enhance the accessibility of specific sequences to DNA-damaging agents or endonucleases, thereby promoting genomic instability (Wolf & Migeon, 1985; Keshet *et al*. 1986; Antequera *et al*. 1990; Lewis & Bird, 1991). In cancer cells these hypomethylation events are frequently accompanied by hypermethylation in promoter regions of genes. DNA hypermethylation can silence tumour suppressor genes, including adenomatous polyposis coli, *p*16 and *h*MLH1, and has been reported in CA and CC (Sharrard *et al*. 1992; Hiltunen *et al*. 1997; Rashid *et al*. 2001; Yi *et al*. 2001).

Some CpG sequences, e.g. in genes such as the *p*53 tumour suppressor gene, are not only sites of DNA methylation, but also mutational hotspots for cancers (Hollstein *et al*. 1991; Jones *et al*. 1992; Greenblatt *et al*. 1994). Most mutations that occur in CpG sequences are cytosine to thymine transitions. Several mechanisms have been proposed for this transition, including spontaneous deamination of methylated cytosine to thymine (Rideout *et al*. 1990), enzymic deamination of methylated cytosine to thymine (Jones *et al*. 1992; Yebra & Bhagwat 1995) and enzymic deamination of unmethylated cytosine to uracil followed by methylation to thymine by methyltransferase, which binds to DNA and blocks repair of DNA mismatches (Jones *et al*. 1992; Shen *et al*. 1992; Yang *et al*. 1995).

Animal studies on DNA methylation

Diets deficient in methyl group donors (choline, folate, methionine and vitamin B_{12}) have been shown to increase the risk of spontaneous and chemically-induced liver cancer in animals (Locker *et al*. 1986; Jones *et al*. 1992; Shen *et al*. 1992). Genomic and gene-specific (*c*-myc, *c*-fos and *c*-ha-ras proto-oncogenes and the *p*53 tumour suppressor gene) DNA hypomethylation occurs well before tumour growth (Wilson *et al*. 1984; Locker *et al*. 1986; Dizik *et al*. 1991; Cravo *et al*. 1992; Wainfan & Poirier, 1992; Zapisek *et al*. 1992; Pogribny *et al*. 1995; Kim *et al*. 1996*b*; Pogribny *et al*. 1997). Studies have also reported altered levels of hepatic *S*-adenosylmethionine during methyl-group depletion or folate deficiency (Henning *et al*. 1989; Balaghi & Wagner, 1993; Kim *et al*. 1994; Miller *et al*. 1994). It is not clear whether DNA hypomethylation occurs secondary to reduced levels of *S*-adenosylmethionine.

One study has shown that isolated folate deficiency leads to genomic DNA hypomethylation in rat liver (Balaghi & Wagner, 1993), although data from subsequent studies suggest that folate deficiency alone does not induce genomic DNA hypomethylation in liver and colon DNA (Kim *et al*. 1995; Duthie *et al*. 2000; Le Leu *et al*. 2000). The reason could be that decreased *S*-adenosylmethionine availability enhances the activity of DNA methyltransferase, the enzyme responsible for DNA methylation (Shivapurkar & Poirier, 1983; Henning *et al*. 1989).

However, isolated folate deficiency has been shown to induce hypomethylation at critical loci, which is thought to be more important in carcinogenesis than genomic DNA hypomethylation. The *p*53 tumour suppressor gene is critically involved in carcinogenesis (Hollstein *et al*. 1991; Greenblatt *et al*. 1994); exons 5–8 of this gene contain large numbers of CpG nucleotides and are regarded as a mutational hotspot. Isolated folate deficiency has been shown to cause hypomethylation within exons 6 and 7 of the *p*53 tumour suppressor gene in rat colon (Kim *et al*. 1996*a*, 1997). Dimethylhydrazine treatment of the folate-depleted rats also caused DNA hypomethylation in exon 8, which was effectively overcome by increasing levels of dietary folate in a dose-responsive manner (Kim *et al*. 1996*a*). Two studies in rats have shown a parallel increase in strand breaks with decreasing DNA methylation in this gene

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during folate deficiency (Pogribny *et al*. 1995; Kim *et al*. 1997). Folic acid supplementation has also been observed to prevent *p*53 mutations in subjects with chronic ulcerative colitis, a disease associated with increased risk of CC (Shapiro *et al*. 1997).

Human studies on DNA methylation

Methylation abnormalities are not confined entirely to the neoplasm. One small study has shown that DNA from normal-appearing colo-rectal mucosa was significantly ($P < 0{\cdot}005$) hypomethylated in twelve subjects with cancer compared with eight controls (Cravo *et al*. 1994). A study was carried out to investigate whether genomic DNA methylation in normal-appearing colo-rectal mucosa differed between thirty-five subjects with CA, twenty-eight subjects with CC and seventy-six controls free of any colo-rectal abnormality. DNA methylation was determined by measuring [^{3}H]methyl incorporation into DNA (Balaghi & Wagner, 1993). Thus, an increase in [^{3}H]methyl incorporation reflects a decrease in DNA methylation. After adjusting for various lifestyle factors (gender, age, BMI, smoking and alcohol intake) and polymorphisms in MTHFR, MS and CBS genes, DNA methylation in normal-appearing colo-rectal mucosa was 26 and 30 % lower in subjects with CA ($P = 0{\cdot}009$) and CC ($P = 0{\cdot}08$) respectively compared with controls (Pufulete *et al*. 2003; Fig. 2).

Leucocyte DNA methylation has been shown to decrease in response to moderate folate depletion in post-menopausal women (Jacob *et al*. 1998; Rampersaud *et al*. 2000). DNA methylation was positively correlated with dietary folate intake and plasma folate (Jacob *et al*. 1998) and negatively correlated with plasma homocysteine (Rampersaud *et al*. 2000). This relationship was not confirmed in a study in younger subjects (Fenech *et al*. 1998). Two studies have also shown interactions between the MTHFR 677C→T mutation, folate status and DNA methylation in leucocytes (Stern *et al*. 2000; Friso *et al*. 2002).

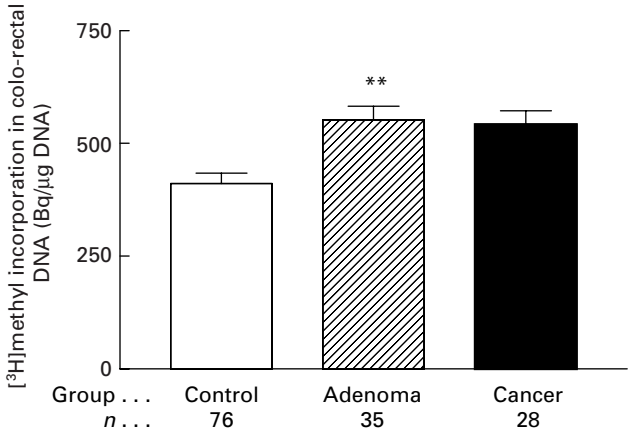

Fig. 2. Differences in C^3H_3 incorporation into DNA from normal-appearing colo-rectal mucosa in subjects with colo-rectal adenoma (▨) and colo-rectal cancer (▬) and in control subjects (□). Values are means with their standard errors represented by vertical bars. Mean value was significantly different from the control value: $^{**}P = 0{\cdot}009$. (From Pufulete *et al*. 2003.)

To date, there are no published studies that have assessed the influence of folate status on DNA methylation in colo-rectal mucosa. This relationship was investigated in a cross-sectional study of sixty-one subjects (twenty-nine men and thirty-two women, 38–78 years) free from CA or CC. A score based on estimates of dietary intake, serum and erythrocyte folate concentrations was used to assess folate status. DNA hypomethylation was negatively correlated with folate status ($P = 0{\cdot}01$) and positively correlated with plasma homocysteine ($P = 0{\cdot}03$; M Pufulete, P Emery and TAB Sanders, unpublished results).

Folate supplementation and DNA methylation

A small number of randomised double-blind placebo-controlled folate intervention trials in subjects with CC and CA have shown that folate supplementation can alter genomic DNA methylation. DNA hypomethylation in colo-rectal mucosa was reversed following 6 months of supplementation with folate (10 mg/d) in eleven subjects with either CA or CC following the removal of these lesions (Cravo *et al*. 1994). This outcome was not observed in the eleven subjects receiving placebo. A 3-month crossover study in twenty subjects with resected CA, using half the dose of folate (5 mg/d), showed a 40% increase in genomic DNA methylation in colo-rectal mucosa in subjects with a single adenoma ($P = 0{\cdot}05$), although subjects with multiple adenomas did not respond (Cravo *et al*. 1998). In another study both folate supplementation (5 mg/d) and placebo for 1 year in twenty subjects with CA increased genomic DNA methylation and decreased strand breaks in exons 5–8 of the *p*53 tumour suppressor gene (Kim *et al*. 2001). The corresponding changes in the placebo group suggest that factors other than folate were responsible for the observed improvements.

A randomised placebo-controlled study was conducted in order to determine whether short-term (10 weeks) supplementation with a physiological dose of folate (400 μg/d) could increase DNA methylation in leucocytes and colo-rectal mucosa in thirty-one subjects with CA. The results showed that after adjusting for various lifestyle factors (gender, age, BMI, smoking and alcohol intake) and polymorphisms in MTHFR 677C→T and MS 2756A→G folate supplementation increased genomic DNA methylation by 31% ($P = 0{\cdot}05$ for folate *v*. placebo; Fig. 3) and 25% ($P = 0{\cdot}09$ for folate *v*. placebo) respectively in leucocytes and colo-rectal mucosa (Pufulete *et al*. 2002*a*).

Conclusion

There is a growing body of evidence linking a low folate status to DNA hypomethylation and colo-rectal neoplasia. Further research is needed to clarify the mechanisms through which genomic DNA hypomethylation increases the risk of carcinogenesis, and to determine the between- and within-person variation in DNA methylation and to define normal ranges of DNA methylation, thus providing a definition of 'hypomethylation'.

The proposed fortification programme in the UK is likely to improve folate status and lower plasma homocysteine concentration in the population, but more rigorous studies

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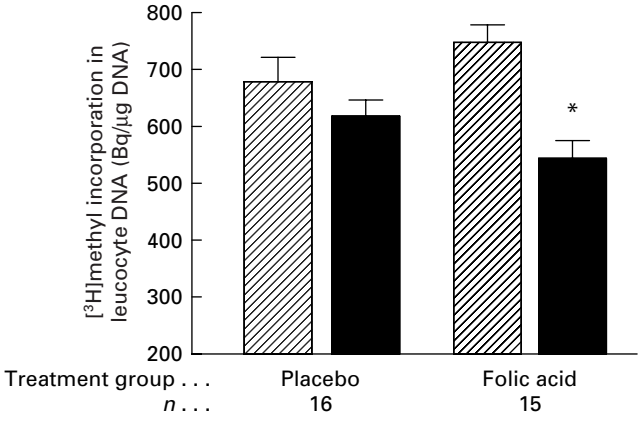

Fig. 3. Changes in [^{3}H]methyl incorporation into leucocyte DNA following a 10-week period of supplementation with folic acid or placebo in thirty-one subjects with colo-rectal adenoma. (▨), week 0; (▭), week 10. Values are means with their standard errors represented by vertical bars. Change from baseline was significantly different from that for the placebo: *P=0·05. (From Pufulete *et al.* 2002*a*.)

are required before speculating on any possible benefits on CC risk. There are currently three randomised double-blind placebo-controlled multi-centre folate chemo-prevention trials ongoing in the USA (New England Medical Centre Multicentre Study, Darmouth Medical Centre Multicentre Study and the Nurses' Health Study and Health Professionals Follow-up Study). All three studies are using supraphysiological doses of folate (1–5 mg/d) and the latter two studies have CA recurrence as an end point. It is likely that the folate fortification programme that began in the USA after some of these trials commenced will influence their outcome, so it is open to question whether these studies will clarify the effect of folate supplementation on colo-rectal neoplasia.

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