Low Colonocyte Folate Is Associated with Uracil Misincorporation and Global DNA Hypomethylation in Human Colorectum\textsuperscript{1,2}

Angela P. McGlynn,\textsuperscript{3} Gillian R. Wasson,\textsuperscript{3} Sharleen L. O'Reilly,\textsuperscript{4} Helene McNulty,\textsuperscript{3}* C. Stephen Downes,\textsuperscript{3} Chin-Kuo Chang,\textsuperscript{3} Leane Hoey,\textsuperscript{3} Anne M. Molloy,\textsuperscript{4} Mary Ward,\textsuperscript{3} J. J. Strain,\textsuperscript{3} George McKerr,\textsuperscript{3} Donald G. Weir,\textsuperscript{3} and John M. Scott\textsuperscript{4}

\textsuperscript{3}Centre for Molecular Biosciences, University of Ulster, Coleraine, Northern Ireland; and \textsuperscript{4}School of Biochemistry and Immunology, and \textsuperscript{5}School of Clinical Medicine, Trinity College, Dublin, Ireland

Abstract

Low folate status is a risk factor for colon carcinogenesis; mechanisms proposed to account for this relationship include uracil misincorporation into DNA and global DNA hypomethylation. We investigated whether such biomarkers are related to folate status in isolated colonocytes from colonoscopy patients. In cases with adenomatous polyps (n = 40) or hyperplastic polyps (n = 16), colonocytes were isolated from biopsies from the polyp, from a site adjacent to the polyp, and from normal mucosa 10–15 cm distal to the polyp. In polyp-free controls (n = 53), biopsies were taken from ascending, transverse, and descending areas of colon. Within adenoma cases, there was a trend (P\textsuperscript{-trend} < 0.001) of decreasing colonocyte folate (pg/10\textsuperscript{7} cells, mean ± CI) from the site distal to the polyp (16.9 ± 2.4), to the site adjacent to the polyp (14.7 ± 2.3), to the polyp (12.8 ± 2.0). Correspondingly, there were increases in uracil misincorporation (P\textsuperscript{-trend} < 0.001) and global DNA hypomethylation (P\textsuperscript{-trend} = 0.012) across the 3 sites. Colonocyte folate concentrations were significantly correlated with RBC folate concentrations, but only in individuals with generally lower (<484 μg/L) RBC folate status (r = 0.54; P = 0.006; n = 24), and were also significantly lower in normal mucosa of cases with adenomatous polyps than in controls matched for colonic segment. In conclusion, localized folate deficiency in specific areas of colon might create carcinogenic fields and affect the development of colorectal polyps through uracil misincorporation and DNA hypomethylation; alternatively, the polyp itself might deplete folate in the surrounding tissue. Folate supplementation trials aimed at colon cancer prevention should target individuals with suboptimal folate status. \textit{J. Nutr.} doi: 10.3945/jn.112.167148.

Introduction

A considerable body of evidence from epidemiologic, clinical, and animal studies shows that folate status is inversely associated with colorectal cancer risk (1). One recent meta-analysis of 27 published case-control and cohort studies estimated that a high folate intake could decrease colorectal cancer risk by 8–15% (2). Controversially, however, recent concerns have been raised regarding potential adverse effects of folic acid (the synthetic form of the vitamin) at high intakes, at least for certain carcinogenic fields and affect the development of colorectal polyps through uracil misincorporation and DNA hypomethylation; alternatively, the polyp itself might deplete folate in the surrounding tissue. Folate supplementation trials aimed at colon cancer prevention should target individuals with suboptimal folate status. *To whom correspondence should be addressed. E-mail: h.mcnulty@ulster.ac.uk.

1 Supported by contract N05028 from the Food Standards Agency, UK, awarded to H. McNulty and C. S. Downes.


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novel synthesis of thymidine monophosphate (from deoxyuridine monophosphate) on the other. Thus, low folate status could alter gene expression through defective cytosine methylation or could lead to catastrophic cycles of aberrant DNA repair following increased uracil incorporation into DNA in place of thymine (10). In fact, the generation of abasic sites by the removal of uracil has been shown to be, in itself, mutagenic in yeast (11) and uracil misincorporation increases the formation of chromosome breaks in human lymphocytes in vitro (12). Low folate status was associated with DNA hypomethylation (13,14) and uracil misincorporation (13,15) in human lymphocytes and was also shown to reduce DNA stability in immortalized human colonocytes in vitro (16).

In the current study, we conducted a detailed examination of colonic folate concentrations and potential folate-related DNA biomarkers in normal and abnormal human colon. We measured uracil misincorporation and global DNA hypomethylation in colonocytes of patients with either hyperplastic or adenomatous polyps (cases) and patients without polyps in the colonic mucosa (controls) and related these biomarkers to colonic folate concentrations.

Materials and Methods

Patient details

The Joint Research Ethics Committee of the Federated Dublin Voluntary Hospitals and St. James’ Hospital approved the study. Written informed consent was obtained from all patients. Patients scheduled for colonoscopy were recruited at St. James’ Hospital, Dublin. Exclusion criteria included active inflammatory bowel disease or diagnosis of celiac disease, intestinal malabsorption syndrome, liver or renal disease, pregnancy, epilepsy, pernicious anemia, or the use of anti-folate medication (e.g., sulphasalazine or methotrexate). All patients were prepared for colonoscopy by taking the same orally administered colonic lavage solution (Kleanprep; Norgine). Colonoscopy patients were defined as cases (i.e., those harboring at least one adenomatous or hyperplastic polyp) or controls (i.e., those without polyps and histologically normal colonoscopy). General medical and lifestyle data, together with weight and height, were recorded for all patients. Patients were identified as folic acid supplement users if they reported taking folic acid or multivitamin preparations containing folic acid at any time in the previous year.

Sample collection and epithelial cell isolation

A venous blood sample was collected from fasting participants into either an EDTA-containing vacutainer for DNA extraction, RBC folate, and total plasma homocysteine determination or a vacutainer without anticoagulant for the determination of serum vitamin B-12 concentrations. For homocysteine determination, plasma was separated within 1 h of collection and frozen at −80°C.

In the control patients, 6 colonic biopsies were taken in sets of 2 from 3 anatomic segments (ascending, transverse, and descending) of colon. In cases, 2 biopsies from the poly, 2 biopsies from an area immediately adjacent to the poly, and 2 biopsies from an area 10–15 cm distal from the poly were taken. Biopsy tissue was examined for histology to confirm that tissues from the adjacent and distal sites were histologically normal. The biopsies were processed and the epithelial layer separated off using the isolation methodology previously described in detail (17,18). We previously found no detectable loss of cell folates during this extraction procedure (17). The resulting cell yield (~2 × 10^5 cells/2 pooled biopsies) was washed in HBSS after assessment of viability by ethidium bromide and acridine orange staining. The resulting epithelial cell suspension was split into 2 fractions: one for colonicocyte folate analysis was stored in 1% sodium ascorbate PBS (pH 6.5) and the other was resuspended at a concentration of 1 × 10^5 cells/mL PBS on ice prior to immediate Comet slide preparation. The purity of the cell preparation was confirmed by flow cytometry with detection of the expression of epithelial cell antigen (Ber-Ep-4; antibody from Dako).

Modified alkaline comet assays

For the current study, the traditional alkaline Comet assay, which measures DNA strand breaks in single cells (19), was modified to specifically measure strand breakage caused by enzymatic digestion of misincorporated uracil (15) or hypomethylated DNA (20).

Comet slide preparation.
The colonicocyte suspension was prepared for Comet analysis using neutral lysis as previously described (17). Uracil misincorporation and global DNA hypomethylation-specific Comet assays were then carried out as previously described using uracil glycosylase (15) or digestion using the methylation-specific endonucleases HpaII and HhaI (20) following neutral lysis at a concentration of 1 unit/slide for 1 h followed by alkaline lysis. Alternatively, uracil DNA glycosylase was added to a separate slide at a concentration of 1 unit/slide and incubated at 37°C for 1 h followed by alkaline electrophoresis and staining as described (17). Comet analysis was performed using a final magnification of ×400 (Nikon ×40 Fluor lens) and Komet 5.0 software (Kinetic Imaging). The mean percent DNA in the Comet tail for each of 50 cells/duplicate slide was measured. Results were expressed as the ratio of percent Comet Tail DNA in the enzyme-treated slides to that of slides treated with buffer alone for both uracil misincorporation [Uracil Glycosylase (UG) ratio] and DNA hypomethylation [HpaII / HhaI (HH) ratio]. Values close to 1 indicate minimal uracil misincorporation or hypomethylation, whereas higher values indicate increased DNA damage biomarker levels. The CV of the modified Comet assay we employed was 13.2% (20). Results from these methods are semiquantitative but have the advantage that meaningful data can be obtained from very small, ethically acceptable, human mucosal biopsy samples.

Folate and related measurements

Total folate concentrations (in blood and colonocytes) were measured by a microbiological assay (interassay CV <8.89%) using Lactobacillus rhamnosus (formerly known as Lactobacillus casei; NCIB 10463) received from Torrey Research Station, Scotland) as previously described (21). For colonicocyte folate analysis, the cell suspension was pretreated with chicken pancreas conjugase (EC 3.4.1.79) to convert all the polyglutamated folates to the diglutamate form prior to microbiological assay (18). Results were then expressed as pg folate/10^5 cells (To convert to pmol/10^5 cells, multiply by 2.266). Plasma homocysteine was measured using the Imx Hcy assay (interassay CV <5.57%; Abbott Laboratories) (22). Serum vitamin B-12 was measured by microbiological assay using Lactobacillus leichmannii (interassay CV <9.64%) as previously described (23). MTHFR genotyping was performed by PCR amplification followed by Hinfl restriction digestion (24).

Statistical analysis

All statistical analysis was performed using SPSS 11.5 for PC (SPSS UK). Colonicocyte folate concentrations and DNA biomarkers were examined at 3 colonic sites for each patient. The analyses were carried out using 1-way, within-subjects ANOVA and linear regressions (Figs. 1 and 2). These tests of within-subjects contrasts were used to identify difference of means in the data and pair-wise comparisons were performed using the Bonferroni adjustment for multiple comparisons. Concentrations of colonicocyte folate, blood folate biomarkers, and DNA biomarkers across the 3 colonic sites were examined using linear regressions. P values, where P < 0.05 was deemed significant, and 95% CIs for comparisons are given. Independent samples t tests were performed for comparison of biomarker values between cases (at the distal site) and controls matched for anatomical segment of the colon (Fig. 3). For categorical variables, differences between cases and controls were examined by using chi-square tests (Table 1). Correlations between colonicocyte folate and blood folate biomarkers were performed using Pearson correlation coefficients.

Some patients had incomplete data sets (i.e., a missing value at a particular site for colonicocyte folate, DNA uracil misincorporation, or DNA hypomethylation) accounting for 8.2% of overall values. To deal with the problem of missing data, a value was estimated for each missing particular site for colonocyte folate, DNA uracil misincorporation, or DNA hypomethylation) accounting for 8.2% of overall values. To deal with the problem of missing data, a value was estimated for each missing value based on multiplying the 2 available values for that participant by the mean of the values of all values for the site with the missing value and that of each of the other 2
sites) and then taking the mean of the 2 corrected values, an approach previously adopted and found to be an acceptable means of dealing with lacunae in data (25). For comparative purposes, the data were in addition analyzed using only those patients with complete data sets (i.e., no missing values).

Results

Demographic data. From a total of 148 colonoscopy patients approached for participation in the study, 135 consented and were biopsied. Of these, biopsies from 15 patients were deemed unavailable for analysis (for reasons such as the biopsy being too small to generate a sufficient cell yield or the biopsy being lost in the process of removal); therefore, 120 patients took part in the study. Biopsies from 3 colonic sites from 53 controls and 56 cases with at least one polyp (adenomatous, n = 40; hyperplastic, n = 16) were analyzed for both colonocyte folate and DNA biomarkers. Some biopsies from the 120 patients were unsuitable for investigation. Reasons for this random loss of values included unsuitability of biopsies for disaggregation or Comet analysis owing to loss of cells during isolation, breakage of slides, and loss of Comet gels during transport or electrophoresis. The final analysis included 109 patients (53 controls, 40 adenoma...
cases, and 16 hyperplastic cases). In total, 8.2% of the values used in the statistical analysis were values that were estimated to deal with missing values (see “Statistical analysis” above). Participant characteristics for the cases and controls included in the final analysis are given in Table 1. One-way ANOVA with a Bonferroni post hoc test showed a difference in age between groups (P < 0.001). Differences were therefore adjusted for age in all further analysis using ANCOVA (Table 1). For categorical variables, differences between cases and controls were examined by using chi-square tests (Table 1). No significant differences in systemic folate-related variables were observed between the 3 groups. No difference in the distribution of the 677C → T (T allele) of the MTHFR gene was found between cases and controls (P = 0.68).

Analysis of colonocyte folate and DNA biomarkers in cases. The majority of polyps in adenoma cases (i.e., in 27 patients) were isolated from the descending colon (including the sigmoid colon and rectum); polyps were isolated from the ascending colon (including the cecum) in 8, and from the transverse colon in 5 adenoma cases. Among adenoma cases, colonocyte folate concentrations were lowest in polyp biopsies and increased significantly in a step-wise manner from the polyp to the histologically normal site adjacent to the polyp and from the adjacent site to the normal site distal to the polyp (Fig. 1). Similar results were found for the hyperplastic cases (Fig. 2). Although these results were generated using calculated values for any missing data (see “Statistical analysis”), sensitivity analysis of the same data including only those cases with no missing values showed identical outcomes. Correspondingly, both adenoma and hyperplastic cases showed a significant trend for a decrease in colonocyte folate concentrations in the distal (“normal”) site in adenoma cases compared with controls matched with the cases for anatomic segment of the colon (Fig. 3). Colonocyte folate concentrations were significantly lower in adenoma cases than in controls in both the ascending and descending colon. Significantly lower colonocyte folate was not observed in hyperplastic cases compared with controls (not shown). There were no significant differences in uracil misincorporation or DNA hypomethylation at various anatomical segments of the colon within controls or between controls and adenoma cases (normal site), again matched for anatomical segment of the colon (results not shown).

**Case-control comparisons of colonocyte folate, uracil misincorporation, and global DNA hypomethylation.** Colonocyte folate concentrations were examined in the distal (i.e., “normal”) site in adenoma cases compared with controls matched with the cases for anatomic segment of the colon (Fig. 3). Colonocyte folate concentrations were significantly lower in adenoma cases than in controls in both the ascending and descending colon.

**Correlation analysis of blood folate, colonocyte folate, and DNA biomarkers.** Colonocyte folate concentrations in the normal site of adenoma cases were found to correlate with the functional folate biomarker total plasma homocysteine (r = −0.41; P = 0.009; n = 30) and with RBC folate, albeit with borderline significance (r = 0.31; P = 0.05; n = 30). Similarly, in hyperplastic cases.

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**TABLE 1** Characteristics of patients harboring polyps and polyp-free controls

<table>
<thead>
<tr>
<th>General characteristic</th>
<th>Polyp case</th>
<th>Control (n = 53)</th>
<th>Hyperplastic (n = 16)</th>
<th>Adenoma (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td></td>
<td>26 (49)</td>
<td>9 (56)</td>
<td>22 (55)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td>54 ± 16</td>
<td>59 ± 15</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td>25 ± 5</td>
<td>29 ± 5</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td></td>
<td>13 (25)</td>
<td>7 (44)</td>
<td>14 (35)</td>
</tr>
<tr>
<td>Alcohol, kg units/wk</td>
<td></td>
<td>14 (10–22)</td>
<td>27 (3–52)</td>
<td>16 (6–24)</td>
</tr>
<tr>
<td>Folic acid supplement, n (%)</td>
<td>7 (13)</td>
<td>0</td>
<td>10 (25)</td>
<td></td>
</tr>
<tr>
<td>MTHFR 677C → T genotype, % CC:CT:TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% CC:CT:TT</td>
<td></td>
<td>46:46:8</td>
<td>47:40:13</td>
<td>38:50:12</td>
</tr>
</tbody>
</table>

**B vitamin status**

- RBC folate, µg/L: 524 ± 285 (561 ± 280) 474 ± 234
- Plasma homocysteine, µmol/L: 9.4 ± 2.4 (10.2 ± 2.4) 11.9 ± 5.5
- Serum vitamin B12, ng/L: 383 ± 168 (446 ± 184) 356 ± 162

**Clinical details, n (%)**

- Indications of colonoscopy
  - Altered bowel habit: 12 (23) 4 (25) 6 (11)
  - Rectal bleeding: 12 (23) 2 (13) 6 (11)
  - Abdominal pain: 10 (19) 0 1 (2)
  - Weight loss: 4 (8) 0 4 (7)
  - Anemia: 17 (32) 9 (57) 22 (40)
  - Family history of colorectal cancer: 5 (9) 0 2 (38)
  - Patient history of polyps: 7 (13) 1 (6) 0

**Polyph location**

- Ascending: n/a 8 (50) 8 (20)
- Transverse: n/a 2 (13) 5 (13)
- Descending: n/a 6 (38) 27 (68)

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1 Values are mean ± SD unless otherwise indicated. n (%) refers to patient numbers with percentage of total patients in parenthesis. To convert folate to pmol/10⁶ cells, multiply by 2.266; to convert vitamin B-12 to pmol/L, multiply by 0.738.

2 One-way ANOVA with Bonferroni post hoc test showed a difference in age between the groups, P < 0.001. Differences were therefore adjusted for age using ANCOVA.

3 One unit of alcohol = 10 g pure alcohol/ethanol; values are mean (95% CI).

4 Reported regular use of multivitamins containing folic acid during the previous year.

5 Wild-type (CC), heterozygous (CT), and homozygous (TT) genotypes for 677C → T polymorphism in MTHFR.

6 Some individuals presented with multiple symptoms.
cases, significant correlations were observed between colonocyte folate (at the normal site) and both folate biomarkers, homocysteine \((r = -0.52; P = 0.047; n = 14)\) and RBC folate \((r = 0.68; P = 0.005; n = 14)\). In contrast, among controls, colonocyte folate was not correlated with either blood folate biomarker. However, when the control group was split into those with generally lower or generally higher blood folate status (using the median RBC folate value of 484 μg/L as a cutoff point), a correlation between colonocyte folate and RBC folate was observed in those with lower folate status \((r = 0.54; P = 0.006; n = 24)\) but not in those with higher status \((r = -0.25; P = 0.38; n = 25)\).

Uracil misincorporation correlated with global DNA hypomethylation in adenomatous \((P < 0.001)\) and hyperplastic \((P = 0.005)\) cases and in controls \((P < 0.001)\). Neither uracil misincorporation nor global DNA hypomethylation correlated with blood folate biomarkers in either cases or controls.

**Discussion**

We used novel methods to examine whether localized folate status was related to molecular effects within colonocytes in vivo. We showed that colonic folate concentrations were significantly lower in the normal mucosa of colonoscopy patients harboring adenomatous polyps compared with controls without polyps. Of greater note, within cases, there was a stepwise decrease in colonocyte folate from the normal tissue to the tissue adjacent to a polyp to the polyp itself. In general agreement with the latter observation, significantly lower folate concentrations were previously reported in malignant tissue compared with normal tissue in colorectal adenocarcinoma patients (26). The current results therefore suggest that in cases with adenomatous polyps, there is a colon-wide reduction in folate concentration, with further folate depletion in the area surrounding the polyp.

Examination of the molecular effects of folate deficiency showed results generally consistent with the biologic mechanisms that are widely considered to link folate status with colorectal cancer risk, based on the central role of folate in 1-carbon metabolism (10). Specifically, with folate depletion, the conversion of deoxyuridine monophosphate to thymidine monophosphate is impaired, leading to uracil being inappropriately incorporated into DNA (12) while hypomethylation of DNA can arise with folate deficiency owing to lower levels of S-adenosylmethionine-independent methylation (13,14). In the current study, the colonocyte folate concentration was mirrored in biomarkers of DNA damage within the same biopsies. In cases with adenomatous polyps, we observed higher rates of uracil misincorporation into DNA in the polyp and the histologically normal site immediately adjacent to the polyp than in mucosa distal to the polyp, corresponding to the sites with lowest and highest folate concentrations. Uracil misincorporation into DNA can, in turn, lead to carcinogenesis through increased DNA strand breakage and chromosomal damage (12). Using immortalized normal human colonocytes grown in folate-sufficient and -deficient conditions in vitro, Duthie et al. (16) showed uracil misincorporation and inhibition of DNA excision repair in response to folate depletion. The current results also showed significantly decreased rates of DNA hypomethylation progressing from the polyp to the polyp-adjacent site to the distal tissue in adenoma cases, corresponding to increasing concentrations of colonocyte folate across the 3 sites. Using the current methodology in an in vitro model, we previously reported lower levels of both global and p53 gene region-specific DNA methylation in colon carcinoma cells grown in folate-depleted conditions than in folate-replete cells (20). Hypomethylation of genomic DNA was also previously reported in human colon tumor tissue (27,28), and DNA methylation was found to be responsive to folic acid supplementation in rectal mucosa from patients with colorectal neoplasms (29). Likewise, healthy older women consuming a moderately folate-deficient diet had decreased global DNA methylation (30), and in a prospective human study of 20 patients with resected colonic adenomas, folate supplementation increased DNA methylation in patients with a single polyp, albeit this effect was not found in those with multiple lesions (31). In addition, an increase in colonic mucosal DNA methylation (though nonsignificant; \(P = 0.09\)) was reported with folic acid supplementation of patients with colorectal adenoma (32).

The current findings that a lower colonic folate concentration is associated with increases in both uracil misincorporation and global hypomethylation in human colonic mucosal DNA therefore offer experimental evidence to support both biologic mechanisms linking folate status with carcinogenesis. Each polyp thus appears to be set in a field of folate-depleted normal mucosa with malfunctioning DNA metabolism. Whether the polyp or the tissue field is the causal factor cannot at this stage be confirmed in this study. It is possible that the polyp itself depletes folate in the surrounding tissue, though it is not clear how that might happen. It is equally likely that changes in regions of histologically normal tissue make the development of cancers more likely, an old concept (33) that was more recently refined to postulate epigenetic defects in aspects of DNA metabolism that predispose cells (commonly of the gastrointestinal tract) in a carcinogenic field to mutation (34,35). A similar epigenetic field effect has been put forward as an explanation for the polyclonality of urothelial cancers (36). Our data are generally consistent with low localized folate concentrations and consequent disturbances in DNA metabolism contributing to such field effects, but only a randomized controlled trial can confirm a causative association.

In adenoma cases, colonocyte folate in the normal (distal) mucosa was significantly correlated with both RBC folate and plasma homocysteine concentrations. However, in controls, there was no correlation between colonocyte folate and either of these blood folate biomarkers. Likewise, Meenan et al. (37) reported that RBC folate did not predict colonic folate concentration in folate-replete patients. Furthermore, although Kim et al. (38) reported a positive relation between blood folate and colonic tissue folate in unsupplemented individuals, this association was not observed in individuals receiving supraphysiological doses of folic acid for 1 y. Interestingly, when we further examined these associations in our controls by splitting the sample using the median RBC folate value as a cutoff point, a significant correlation between colonocyte folate and RBC folate concentration was observed in those with lower RBC folate, but this relationship was not found with higher folate concentrations. The finding of an association between blood folate and colonocyte folate only among individuals with generally lower status, in this and aforementioned studies, implies that increasing dietary folate intake may enhance the folate supply to colonocytes only in people with generally low folate status and not where folate is optimal. Furthermore, high-dose folic acid supplementation in an already replete population in one recent study did not result in any significant effect on rates of uracil misincorporation in rectal mucosa cells (39), suggesting that any beneficial effects of increased folate intake in reducing colorectal cancer risk are also likely to be found only where folate status is suboptimal. This will predominantly include those with limited access to (or intake of) folic acid-fortified foods who are therefore dependent on natural food sources for their folate supply. Natural folates have a limited potential (compared with folic acid, the synthetic vitamin form...
found in fortified foods) to optimize blood folate status because of the well-recognized poor stability and bioavailability of the reduced-folate forms found naturally in foods (40).

This study is not without some limitations. Cases with adenomatous polyps were found to be significantly older than controls. Where we do compare data between cases and controls, however, we adjusted for the effect of age in the analysis. Furthermore, the most relevant findings reported here relate to our results within cases rather than to case-control comparisons. Also, although results from the Comet assay we employed could be considered somewhat limited in that they are semiquantitative, this study highlights the value of investigating very small, ethically acceptable, human mucosal biopsy samples with the potential for further study to investigate the response to folate intervention to determine whether the relationships observed here are causative.

In conclusion, this study shows that lower colonocyte folate is associated with both uracil misincorporation and global DNA hypomethylation. Although we cannot confirm a cause and effect relation between a lower folate concentration and the molecular effects leading to polyp formation, our study offers mechanisms that are relevant within the target tissue. Our data are consistent with the hypothesis that localized folate deficiency in precancerous fields of the colon contributes to colorectal carcinogenesis mediated through uracil misincorporation and DNA hypomethylation; thus, optimization of folate status could play a preventative role. Given that colonic folate concentrations appear to reflect folate intake only when the status is generally low, however, suggests that further folate increases in folate-replete individuals are unlikely to be of benefit in preventing colon cancer. Thus, future randomized trials of folate supplementation aimed at colon cancer prevention should target those with suboptimal folate status and, given some concerns regarding potential adverse effects of overexposure to folic acid (4–8), should use low-dose folic acid (i.e., within the dietary folate range).

Acknowledgments

The authors thank Shauna Harte, Centre for Molecular Biosciences, University of Ulster Coleraine, for her valued administrative assistance. C.S.D., J.M.S., and H.M. designed the study; A.P.M., G.R.W., and S.O.R. conducted the research; A.P.M., G.R.W., S.O.R., H.M., and J.M.S. were responsible for analysis and interpretation of data; A.P.M., G.R.W., and H.M. drafted the manuscript; A.P.M., G.R.W., S.O.R., H.M., C.-K.C., C.S.D., A.M.M., J.J.S., M.W., G.M., D.G.W., and J.M.S. provided technical and material support; C.S.D., H.M., J.M.S., and D.G.W. supervised the study; and H.M. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


