Drinking from the fountain of promise: biomarkers in the surveillance of Barrett's oesophagus—the glass is half full!

S L Preston, J A Jankowski

Biomarkers for prognostication need large randomised controlled trials such as AspECT and BOSS. These trials will deliver surrogates which fulfil the validation criteria necessary for their introduction into the clinic

Barrett's oesophagus (BO) is an acquired premalignant condition that is the only known precursor to oesophageal adenocarcinoma (OA); a malignancy whose incidence has risen steadily in the Western world in the last 20–30 years.¹ The lifetime risk of developing an adenocarcinoma in the context of BO is 2–5%,² and despite recent advances in its treatment the associated morbidity and mortality remain high.³ BO advances through a series of morphological stages: from metaplasia through dysplasia of different grades and finally to adenocarcinoma—the metaplasia-dysplasia-adenocarcinoma sequence (MCS).^{4,5} Although this genetic process is not as well outlined as in the colon,⁴ it is thought to be a similar multistep process consisting of genetic and epigenetic mutations, involving the same or a similar group of genes, which over many years leads to increasing genomic instability and ultimately to the formation of an autonomous clone of cells with invasive and metastatic properties.⁵

Knowledge of the natural history of the adenoma:carcinoma sequence in the colon has translated to clinical medicine, prompting the removal of adenomatous polyps at the premalignant stage, with an associated reduction in the incidence of colorectal cancer.^{6,7} Regrettably, thus far, such gains have not been made in dysplasia in the context of BO, with the effectiveness of current surveillance strategies yet to be conclusively proven; a matter soon to be addressed by the BOSS (Barrett's Oesophagus Surveillance Study), a study integrated with the AspECT (Aspirin and Esomprazole Chemoprevention Trial of Cancer in Barrett's Oesophagus) (7500 patients in total).⁸ At present, a patient with BO can only be risk stratified on the

basis of either endoscopically visible ulceration or by grade of dysplasia (negative, indefinite, low grade, high grade, or cancer) within the endoscopic biopsies.⁹ Neither of these methods is satisfactory: the inter and intra pathologist interpretation of dysplasia has proven hard to reproduce, with one study demonstrating only a 48% agreement in the grade of dysplasia in BO between expert pathologists; this, however, was increased to 86% when high grade dysplasia was compared with all of the preceding three grades.¹⁰ In today's global health service, specialist pathologists are a scarce commodity, and a second opinion in all cases of dysplasia in BO stretches available resources, necessitating other methods by which patients with BO can be risk stratified.

In BO, the right point of intervention in the disease process is unclear, with the intervention itself associated with a significant morbidity and mortality. The current surveillance programme of endoscopy and biopsies is extremely labour intensive, and not without suffering to the patient. What is needed, therefore, is an indicator capable of identifying those patients at greatest risk of neoplastic progression and consequently requiring closer monitoring and earlier surgical intervention—a “biomarker”. Ideally, this tool would be sensitive and specific to BO, identifiable in the premalignant phase, and increase with malignant progression; it would be as non-invasive as possible (preferably detectable in blood, cytology, urine, or faeces) and it would be inexpensive. It could be used as a stand alone measurement but it could interface with existing techniques such as narrow band imaging or high magnification chromoendoscopy—its sole purpose may be to identify a cohort of

patients who would benefit from the current screening programmes, with or without novel endoscopic techniques. As well as predicting neoplastic progression, it would also be beneficial if the biomarker identified those patients with dysplasia who would benefit from surgery, excluding those in whom surgery was too late. The urologists have prostate specific antigen, itself not without its limitations, and every gastroenterologist that has ever undertaken BO surveillance would now like a similar, or preferably better, tool.

In this issue of *Gut*, Murray and colleagues¹¹ describe the potential use of p53 immunostaining in oesophageal biopsies as such a biomarker (see page 1390). Accepting the unsatisfactory relationship between immunostaining for the protein and sequencing for mutations,¹² this study correlates with our knowledge of the molecular events in the progression from metaplasia to adenocarcinoma and presses the case for p53 one day being endorsed as a biomarker in BO. However, the findings of their paper indicate that for routine and pragmatic purposes this case is far from proven. Many other markers, both in BO and other areas of cancer, have been suggested but few if any have realised their initial promise: poor trial design, type of tissue selected, and technical processing of the material been shown to be biased in one way or another, or lacked reproducibility,¹³ have all played their part in discrediting potential candidates.¹⁴ Any such tools identified in this role need to be subjected to the highest quality control, and to this end the National Cancer Institute's Early Detection Research Network (EDRN)¹⁵ outline a system for validation. It is comprised of five separate phases: from laboratory identification of promising markers in the preclinical phase 1, validation studies in phases 2 and 3, through the prospective clinical screening, sensitivity and specificity values in phase 4, and finally the impact on reduction of disease burden as part of a screening programme on the population in phase 5—the only true point at which clinical success can be quantified. Due to the natural history of neoplastic progression in the field of BO and the length of time taken to progress to adenocarcinoma, phase 5 studies would have to be large scale studies and to date no biomarker in BO has been evaluated at this level although the AspECT RCT is such a vehicle for this purpose. For this reason, in the field of BO, we feel it necessary to add phase 6 to this validation process; proven use in multiple centres and in multiple populations (table 1). Only when a biomarker has met these

Table 1 Phase of translational study and validation of a biomarker in Barrett's oesophagus (BO)

Description	Aim
Phase 1 Preclinical and exploratory	Establish biomarker levels are higher in adenomatous than the metaplastic BO tissue
Phase 2 Retrospective validation of clinical assay	Optimise a minimally invasive assay; correlate with dysplastic progression; relate levels to age, sex, etc
Phase 3 Retrospective and longitudinal	Early detection of MCS; define minimal "positive" level in phase 4
Phase 4 Prospective screening of large numbers	Determine the biomarker's ability to predict progression of MCS; establish false referral rate
Phase 5 Cancer reduction	Verify the impact on cancer mortality by interventions initiated by detection of the biomarker
Phase 6 Multiple populations	Ascertain whether the biomarker can be applied to all populations with BO

MCS, metaplasia-dysplasia-adenocarcinoma sequence.

standards could it be confidently used in daily clinical practice. Murray and colleagues¹¹ show p53 to have qualified at best to phase 3 or 4, but no further!

The vast majority of phase 1 and 2 studies result from application of high throughput transcriptional profiling and proteomics applied to the molecular knowledge of neoplastic progression in BO. The list of candidates is vast but with numerous mutations taking place in the setting of genomic instability the most challenging task is filtering out the "hitchhikers"—those markers which are just bystanders and do not impact on subsequent clonal expansion. Of the molecular events that take place in the MCS, those that have showed most potential as biomarkers, in addition to alterations in p53, are DNA ploidy and proliferation. P53 (17p) and P16 (9p) are both tumour suppressor genes and contribute to G1/S phase control of the cell cycle. The former regulates damage checkpoints in the cell cycle and therefore prevents cells with damaged DNA from dividing, in addition to maintaining chromosomal order at the time of mitosis. At a laboratory based level, previous studies have shown promise, with phase 1 and 2 studies demonstrating that the number of p53 lesions increases as the MCS sequence progresses,^{16–17} and at a clinical level, a single phase 4 study¹⁸ has shown that 17p loss of heterozygosity (LOH) is a good predictor of progression, which together place p53 as one of the biomarkers with the most potential in this field (table 2). Within BO, a p16 mutation is one of the earliest mutations to take place, enabling clonal expansion to take place throughout the

segment (table 2)^{19–20}: 85–90% of BO contain one or more p16 lesions (a mutation, LOH, or methylation of the promoter)²⁰ but although it has been evaluated at phases 1 and 2, its role needs to be validated clinically in phases 3–5.

Proliferative markers, such as Ki-67, should ostensibly be good indices for progression but good data from phase 3, 4, or 5 translational studies are lacking (table 2). The evidence base is greater for the potential use of cyclin D1, a promoter of cell division, and hence integral in the hyperproliferative state; levels have been shown to increase in a prospective phase 3, indicating risk of adenocarcinoma progression,²¹ as well as being overexpressed in phase 1 and 2 studies in OAs within BO²² but this has not been widely reproduced (table 2). Another biomarker that increases with the progression from metaplasia to dysplasia is DNA ploidy abnormalities (aneuploidy and tetraploidy), as a result of increased genomic instability (table 2). This can be detected with flow cytometry and has been shown in the context of BO, including in phase 4 studies, to be predictive of high grade dysplasia and adenocarcinoma,^{18–23} Most likely because of technical difficulties, these results have also not been reproduced in other studies,²⁴ and as a result flow cytometry has not progressed to phase 5 trials.

The holy grail is not yet round the corner. Until then, we will continue using dysplasia, accepting that phase 4 studies of high grade dysplasia have shown it to be imprecise for identifying those likely to progress to carcinoma, with some demonstrating that most of

these patients die of unrelated causes.²⁵ P53 shows some promise but clinicians will require large, prospective, preferably multicentre trials before its use is integrated to surveillance strategies in BO. Only 5% of patients who present with OA already have a diagnosis of Barrett's metaplasia²⁶; the perfect biomarker should therefore be more accessible than through endoscopic biopsies—interestingly, antibodies to our prime candidate p53 can be detected in the serum of patients with OA.²⁷

The list of candidates being generated from phase translational 1 and 2 trials continues at an exponential rate, yet at present, as clinicians, we are left only with histopathology as a way to risk stratify our patients with BO. Translational science has failed to take these promising markers and deliver a robust tool to determine which patients we should be focusing our attentions on and which we should leave well alone. Why have none made the grade? What has been lacking so far is a strategic plan to identify the most promising biomarkers, and take them, and only them, through the different phases of validation on a large scale in a coordinated fashion. For this to occur, a well defined infrastructure of directed and optimised research is needed to allow the important scientific discoveries to be translated into large, population based, integrated, multicentre clinical trials as efficiently as possible.²⁸ Both the AsPECT and BOSS trials offer this infrastructure. Half full? At least the level appears to be rising!

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Table 2 Current status of prognostic biomarkers in Barrett's oesophagus

Biomarker	Phase 1/2	Phase 3	Phase 4	Phase 5/6	Ready for clinical use?	Comments
Dysplasia	✓	✓	✓	X	Diagnostic but poorly prognostic	Ideally two or more specialist pathologists. <i>Current gold standard</i> but poor reproducibility;
P53	✓	✓	✓	X	No	Single phase 4 study showing 17p LOH is a good marker of progression
P16	✓	X	X	X	No	Hypothetically good but lacks scientific data
Aneuploidy	✓	X	✓	X	No	Difficult technique to reproduce
Cyclin D1	✓	✓	X	X	No	Possible role in combination with p16
Ki-67	✓	X	X	X	No	Single phase 4 trial found no evidence that S phase was a predictor of MCS progression

LOH, loss of heterozygosity.

Authors' affiliations

S L Preston, Department of Gastroenterology, Concord Repatriation General Hospital, Sydney, Australia, and Histopathology Unit, Cancer Research UK, London, UK
J A Jankowski, Department of Clinical Pharmacology, University of Oxford, Oxford, UK, Digestive Diseases Centre, University Hospitals of Leicester, Leicester, UK, and Histopathology Unit, Cancer Research UK, London, UK

Correspondence to: Professor J A Jankowski, Department of Clinical Pharmacology, University of Oxford, Woodstock Rd, Oxford OX2 6HE, UK; janusz.jankowski@clinpharm.ox.ac.uk

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Oesophagus

Impedance-pH monitoring in proton pump inhibitor resistant patients: ready for clinical application?

J P Galmiche

Impedance-pH monitoring represents a useful tool for studies and clinical research in oesophageal disorders, and may hold promise for the group of patients dissatisfied with their antireflux treatment

Gastro-oesophageal reflux disease (GORD) is a very common disorder in which the reflux of gastric (or gastroduodenal) contents causes troublesome symptoms and/or lesions of the oesophageal mucosa. The harmful effects of acid injury on the oesophageal mucosa have been established for decades by experimental and clinical studies.¹ From a clinical and therapeutic viewpoint, the central role of acid in the pathogenesis of reflux

symptoms and lesions of the oesophagus was most convincingly confirmed by the introduction of acid suppressive therapy; the development of proton pump inhibitors (PPIs) achieved a level of efficacy not previously reached by antacids or H₂ blockers.²

Despite the progress made with PPIs, it has now however become clear that up to 40% of patients with GORD symptoms are either not completely satisfied with their acid suppressive

therapy or may be resistant to PPIs.³ Several reasons may underlie an individual's resistance to his or her PPI regimen. Firstly, it may be that symptoms are not related to acid reflux; this may be the case when atypical and non-specific symptoms are reported and endoscopy does not show mucosal breaks, for example. In addition, acid injury may not be completely eliminated by PPI treatment and acid exposure remains abnormal. The level of acidity necessary to cause symptoms is not precisely defined and is likely to vary from subject to subject or even in the same individual. Several studies have shown that "weakly acidic" or short lasting reflux episodes may cause symptoms similar to those reported in classical GORD despite an overall normal 24 hour oesophageal acid exposure time.^{4,5} In these patients with an apparently hypersensitive oesophagus, an increased dose of PPI (for example, omeprazole twice daily) proved to be effective in the subgroup of individuals with a significant association between symptoms and acid reflux episodes.⁶ In practice, demonstration of such a temporal relationship requires analysis of symptoms and acid events recorded

during 24 hour oesophageal pH monitoring. Different symptom indices and probability calculations are commonly used to express the strength of this association between acid reflux and symptomatic events.⁷⁻⁹

However, despite the significant role of acid in GORD pathogenesis, other components of the reflux material can also exert a deleterious effect on the oesophageal mucosa; they can act either alone or synergistically with acid. These molecules include biliary acids and enzymes such as trypsin, normally present in the duodenum, which can reflux into the stomach and then the oesophagus (duodenogastro-oesophageal reflux (DGOR)).¹ Non-chemical stimuli such as mechanical distension of the oesophagus by the reflux bolus can also trigger symptoms, which are likely to include heartburn and regurgitation. Finally, demonstration of the role of reflux in the pathogenesis of symptoms is frequently a difficult issue. Although 24 hour pH monitoring in patients off therapy may provide some clues, the sensitivity of this technique is relatively poor in non-erosive GORD, and probably does not exceed 60–70% at best.¹⁰ With respect to pH monitoring, the problem is further complicated in patients who continue to report symptoms while receiving acid suppression; the classical threshold of acid exposure (that is, per cent time pH less than 4) may be too stringent for the detection of abnormalities when monitoring patients who continue to receive PPI therapy.¹¹ In fact, an oesophageal pH less than 4 cannot be considered a reliable marker for the detection of reflux episodes in a patient on PPI therapy or in those with spontaneously low gastric acidity, such as frequently fed infants.

Addition of the detection of bilirubin (used as a marker of DGOR) to pH monitoring has been shown to be useful in patients poorly responsive to a once daily dose of a standard PPI.¹² This method is however hampered by the special monitoring conditions required (liquid diet) and limited access to the technique in clinical practice. In summary, neither standard pH monitoring nor bilirubin monitoring can satisfactorily address the situation of the so-called “non-acid reflux”, especially in patients with persistent symptoms on PPI therapy.

What really is “non-acid reflux”? No definition is universally accepted and this terminology actually regroups a number of different conditions with no well defined corresponding clinical entities.¹³ New concepts have recently emerged with the development of a novel technology combining impedance and pH measurement. Impedance is a

parameter which describes resistance to the flow of an electric current between adjacent pairs of electrodes mounted on an oesophageal catheter; when adjacent electrodes are linked by a liquid bolus, conductivity is high and therefore impedance is low. In contrast, when electrodes are separated by gas, such as during a belch, conductivity is low and impedance increases. In recent years several groups have applied this technique to the detection of reflux episodes in both normal volunteers and reflux patients.¹⁴⁻¹⁶ These studies have provided convincing evidence that impedance-pH monitoring can accurately detect and characterise nearly all reflux episodes. Following this technical progress, a tentative classification of non-acid reflux has been proposed by a panel of experts.¹⁷ According to this technology based description system, the term non-acid reflux includes both “weakly acidic reflux” (reflux events that result in an oesophageal pH between 4 and 7, or 6.5 in reference to physiological neutrality) and “weakly alkaline reflux” (reflux detected by a change in impedance level but with pH remaining above the oesophageal value of 7 or 6.5).

In this issue of *Gut*, Mainie and colleagues¹⁸ report on the clinical application of impedance-pH monitoring to patients with symptoms that persist despite acid suppressive therapy (*see page 1398*). This large series of 168 patients was recruited on the basis of therapeutic failure after at least one month of a twice daily PPI regimen. Interestingly, persistent symptoms were categorised as typical (heartburn, regurgitation, or chest pain) or atypical (for example, cough, hoarseness, abdominal discomfort). The primary endpoint of the study was the symptom index (SI), defined as the number of symptoms associated with reflux episodes divided by the total number of symptomatic events. Symptom association probability (SAP) was also determined according to previously described methodology.⁸ Surprisingly, the time windows used to determine whether or not a symptom was reflux related differed for SI (reflux within five minutes prior to symptom occurrence) and SAP (two minute segments with and without reflux). The most original aspect of the work, for which the authors should be complimented, relies on the fact that impedance-pH was monitored in patients on therapy. This experimental design allowed more appropriate analysis of the mechanisms of symptoms occurring during the average 20 hour recording period. As expected,¹¹ very few patients (11%) continued to have abnormal acid exposure while receiving a PPI twice daily and only 17% of the reflux

episodes detected by impedance were acid reflux (with pH below 4). In contrast, more than 80% of reflux episodes were non-acid, as defined previously, the majority of which (90%) being categorised as weakly acidic and rarely, weakly alkaline (10%). It is of note that of the 144 patients who reported symptoms during the monitoring period, nearly half had a negative SI, suggesting a mechanism other than reflux in the pathogenesis of their symptoms. For those with a positive SI, non-acid reflux was the predominant mechanism underlying residual symptoms. Finally, typical GORD symptoms in patients on PPI therapy were more likely to be associated with a positive SI (primarily non-acid) compared with atypical symptoms which were more frequently associated with the absence of reflux.

The results of the study by Mainie and colleagues¹⁸ are consistent with other studies which have already assessed the additional yield of oesophageal impedance monitoring in patients on and off therapy.¹⁴⁻¹⁹⁻²⁰ It has been confirmed that standard 24 hour pH monitoring alone is of limited value in understanding the mechanism of PPI resistance as the large majority of these patients have normal or even low acid exposure values while receiving PPI therapy.¹¹ It is of note that Mainie and colleagues¹⁸ used especially low thresholds to define abnormal acid exposure times in PPI receiving patients. While the addition of impedance to pH monitoring seems of relatively limited clinical value in patients not receiving therapy,¹⁹⁻²⁰ the diagnostic yield of the combined measurement seems to be substantially improved in treated patients.¹⁸⁻¹⁹ The role of weakly acidic reflux episodes in symptom pathogenesis has now been established beyond doubt, both in typical (for example, regurgitation on therapy) and atypical GORD; for example, an association with chronic cough has now been reported in patients either off²¹ or on therapy.¹⁹ However, as for pH monitoring alone, controversies remain regarding the best method of expression of the strength of association between symptoms and reflux episodes; this issue seems particularly relevant as the aim is to best predict therapeutic response to acid suppression.²² It is indeed again surprising to see that the correlation between the SAP and SI results in the Mainie and colleagues study¹⁸ was so poor, a result that is however consistent with our own experiences.¹⁹

The choice of SI rather than SAP, as well as the time window for symptom analysis (two or five minutes) remains largely based on personal and arbitrary

choices; this may be an area of research that deserves further attention. Standardisation of both the technique and parameters and validation of software required for reducing the analysis time are first necessary to further increase clinical application. Finally, the exact role of pH-impedance monitoring will depend on its diagnostic relevance and ability to influence the management of GORD patients, particularly those with predominant extra-oesophageal symptoms. Data from Mainie and colleagues¹⁸ and other groups concerning the outcome of surgery in PPI resistant patients that are carefully selected on the basis of objective impedance-pH analysis is eagerly awaited. Application of this recent technology will be particularly relevant to the pharmacological assessment of new drugs aimed at reduction of transient lower oesophageal relaxations, such as GABA-b agonists and glutamate ligands.^{23–25}

In conclusion, at its present stage of development, impedance-pH monitoring represents a useful tool for studies and clinical research in oesophageal disorders. It is probably too early for it to be considered as ready for routine clinical application but recent studies hold promise, particularly for the group of patients dissatisfied with their anti-reflux treatment. Further studies should more directly measure the impact of results on patient management.

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Correspondence to: Professor J P Galmiche, Institut des Maladies de l'Appareil Digestif, University Hospital Hôtel-Dieu, 44093 Nantes, France; jeanpaul.galmiche@chu-nantes.fr

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Neurogastroenterology

Hypnosis for non-cardiac chest pain

O S Palsson, W E Whitehead

Hypnosis may be an effective treatment for patients with non-cardiac chest pain that has not responded to acid inhibition

Non-cardiac chest pain (NCCP) is a condition defined by recurrent episodes of substernal chest pain suggestive of coronary artery disease in patients in whom no cardiac cause can be found after a comprehensive evaluation.¹ It is aetiologically heterogeneous,

and the cause in individual patients often remains unclear even after thorough investigation. Patients with NCCP are frequently not reassured by negative investigations and are persistent in their pursuit of further medical evaluations for possible cardiovascular disease. The

consequent impairment in personal and occupational functioning and subjective well being can be substantial. A recent estimate places the annual cost of health care services provided in the USA to treat NCCP at 6.5 billion dollars.²

The most commonly identified cause of NCCP symptoms is gastro-oesophageal reflux disease. Consequently, a therapeutic trial of a proton pump inhibitor is often recommended to confirm the diagnosis and as the firstline treatment.^{3–5} However, a variety of other factors have been suggested as possible causes of NCCP in individual patients, including oesophageal dysmotility, visceral hyperalgesia, musculoskeletal problems, and psychological factors, such as anxiety and somatisation.⁵ When

patients fail to respond to proton pump inhibitors, few treatment options remain and the results of medical management are often unsatisfactory.

In this issue of *Gut*, Jones and colleagues⁶ demonstrated in a small randomised controlled trial that hypnosis is an effective treatment for patients with NCCP that has not responded to acid inhibition (*see page 1403*). Following 12 sessions of individualised treatment with hypnosis, 80% of NCCP patients reported that they were “completely better” or “moderately better” compared with only 23% of patients in a control group. The controls received supportive psychotherapy and a placebo tablet to insure that the findings were not explained by expectancy or increased attention from a health care provider. The greater effectiveness of hypnotherapy was confirmed by the secondary endpoints in this study: hypnotherapy patients, when compared with control patients, showed greater reductions in pain intensity, greater improvements in quality of life, and greater reductions in medication usage. Strengths of this trial were the use of blind raters and an intent to treat analysis to evaluate efficacy.

The Manchester team that conducted this trial, headed by Dr Peter Whorwell, pioneered hypnosis treatment for functional gastrointestinal disorders more than two decades ago, and the current study is but the latest in a series of innovative contributions they have made over the years. They were the first to demonstrate that hypnosis can be of substantial help for refractory irritable bowel syndrome (IBS).⁷⁻⁸ They went on to demonstrate in a large patient series that it is reliably effective for IBS, benefiting more than 70% of treated patients, and that the results last for years.⁹⁻¹⁰ More recently, the Manchester group published a randomised placebo controlled trial in patients with functional dyspepsia showing that hypnotherapy is more effective than supportive therapy plus placebo medication or treatment with ranitidine.¹¹ In this study, they also demonstrated significant reductions in overall health care visits and amount of prescribed medication taken during the year following treatment with hypnosis, showing that the greater cost of treating patients with hypnosis is offset by downstream reductions in health care utilisation. The published studies of the Manchester group have inspired other research groups to test the effectiveness of hypnosis and to independently confirm the value of this type of treatment for severe and refractory IBS cases.¹² Replication of their work on functional dyspepsia is awaited.

Examining the similarities among the functional gastrointestinal disorders for which hypnosis has been found to be effective may provide insights into how hypnosis benefits these patients and how it might be adapted to further improve its efficacy. Each of these disorders is associated with a greater than expected amount of psychological symptoms,¹³⁻¹⁵ and because hypnosis is a psychological intervention, it might be anticipated that it would be more effective in psychologically distressed patients. Consistent with this hypothesis, four published studies that evaluated the efficacy of cognitive behavioural therapy (CBT) for NCCP¹⁶⁻¹⁹ all reported significantly greater improvement in the CBT condition compared with a control condition. However, these were uniformly small studies, and all of them compared CBT to no treatment or to standard care. Such control conditions do not elicit any anticipation of therapeutic change and therefore do not control for the placebo effect. (In this regard, the design of Jones *et al*'s trial is substantially stronger as its double placebo control condition is likely to have produced a considerable expectation of therapeutic effect.) However, psychological distress does not appear to be the explanation for the effectiveness of hypnosis. Unlike other psychological treatments, the benefits of hypnosis, at least in IBS where this has been tested, are no better in patients with anxiety and depression.⁹ Thus hypnosis does not appear to be effective because it reduces psychological distress.

A second similarity between IBS, functional dyspepsia, and NCCP which may make them more amenable to treatment with hypnosis is that all three are defined by symptoms of pain or discomfort, and large subsets of patients with all three disorders have been shown to have lower thresholds for pain induced by intraluminal distension (that is, increased pain sensitivity).²⁰⁻²² While this has been interpreted by some as evidence of a biologically based difference in peripheral receptor sensitivity or spinal cord transmission of pain,²³ possibly related to inflammatory processes,²⁴ other evidence suggests that psychologically based perceptual response bias may explain the phenomenon²⁰⁻²⁵; when techniques that distinguish between biologically based pain sensitivity and perceptual response bias (that is, psychological influences on perception) are used, it has been shown for both IBS²⁵ and NCCP²⁶ that perceptual sensitivity is similar between patients and healthy controls but that psychologically based perceptual response bias is greater in patients and is correlated with pain thresholds. This suggests that patients with IBS and

NCCP, and probably also functional dyspepsia,²⁷ are hypervigilant in noticing pain related sensations and interpreting them as symptoms of disease. This perceptual response bias may account for the fact that these functional gastrointestinal disorders are associated with multiple comorbid complaints. Whorwell's group have reported that hypnosis reduces pain sensitivity in IBS patients whose pain thresholds are abnormally low,²⁸⁻²⁹ and research from our laboratory³⁰ shows that hypnosis substantially reduces somatisation in IBS patients. These findings suggest that hypnosis is perhaps uniquely able to modify hypervigilance for visceral pain sensations, and this may be one of the mechanisms that explains its effectiveness in functional gastrointestinal disorders.

A third similarity between IBS, functional dyspepsia, and NCCP is that all are symptom based diagnoses for which there are no biological markers. In each case, diagnosis requires the presence of a characteristic set of symptoms but also a negative medical evaluation for other diseases that might produce these symptoms.³¹ Absence of objective criteria for diagnosis may increase uncertainty on the part of both the physician and the patient, with the result that anxiety and hypervigilance to symptoms on the part of either of them may play a greater role in the perpetuation of symptoms than might be the case, for example, in a fracture or an infection. One reason for the effectiveness of hypnosis intervention for functional gastrointestinal disorders could be that it focuses on reducing catastrophising cognitions and overattention to symptoms, which is likely to be particularly important in these symptom based conditions. This speculation requires testing.

This first ever study of hypnosis for NCCP has limitations which readers will need to keep in mind. Firstly, the 28 patients who were enrolled in the study were highly selected and may not be representative of all patients with NCCP: 865 patients were considered for the study, but 97% were not enrolled in treatment either because they were found to be ineligible (35%), could not be contacted (11%), listed travel related inconvenience or family and work conflicts (30%), or refused (20%). A second limitation to the study is that there was no follow up. Previous work from this group of investigators has shown that hypnosis produces improvements in IBS symptoms that are sustained for at least five years⁹⁻¹⁰ and improvements in functional dyspepsia that persist for at least a year.¹¹ These durable treatment effects, and the reductions in health care visits

and medication use that accompanied treatment for functional dyspepsia,¹¹ are important to assess in NCCP.

IMPLICATIONS FOR TREATMENT OF NCCP WITH HYPNOSIS

Should further studies on hypnosis treatment for NCCP show equally dramatic beneficial effects of hypnosis, and especially if the long term benefits prove favourable, there is little question that hypnosis has much to offer chronic patients with this diagnosis. It must be acknowledged however that several obstacles presently prevent widespread practical application of hypnosis for gastrointestinal problems. Firstly, the number of health care providers trained in hypnosis is limited. Knowledge in a special gut directed approach to hypnosis, rather than general hypnotherapy, is required for good success in treating gastrointestinal disorders, and training in this method is hard to come by. Efforts to overcome this barrier include the development of gut focused hypnosis scripts which make it easier for health care providers to learn how to deliver effective treatment.³²

A second barrier is that this approach to treatment is costly and requires multiple visits; this may deter use of this adjunctive treatment option. There are ongoing research studies to determine whether self hypnosis with the use of tape recordings may be equally effective.³³

Finally, scepticism by some patients (and some physicians) due to lack of face validity of using a psychological therapy to treat a gut problem, especially a psychological method that has traditionally carried an aura of magic and mystery, may deter the treatment. However, educational efforts and growing interest due to the accumulating body of research indicative of the effectiveness of hypnosis treatment for functional gastrointestinal disorders are resulting in more and more patients and physicians becoming interested in hypnosis as a reasonable treatment alternative for refractory functional gastrointestinal symptoms, and increasing numbers of medical and mental health professionals are attending training workshops in gut directed hypnosis. For example, in the USA, several hundred clinicians nationwide now offer hypnosis treatment specifically for IBS, according to an empirically tested protocol.³²

Much still remains to be done to enable patients with functional gastrointestinal disorders to benefit from hypnotherapy as a matter of course. Two changes in the way healthcare systems currently care for patients with these disorders will be important in this regard. One is the formal addition of

hypnotherapy services to the scope of clinical services offered to patients seen in gastroenterology and primary care settings where functional gastrointestinal patients are treated, either through an established referral mechanism or preferably with on site delivery of hypnotherapy in medical clinics. Dr Whorwell and colleagues have convincingly demonstrated, through the example of their own clinic in Manchester,⁹⁻¹⁰ that hypnotherapy can be incorporated successfully into clinical gastroenterology. For many years, they have employed several full time hypnotherapists (non-physicians) who routinely treat those of their functional gastrointestinal patients who prove unresponsive to more conventional treatment. They report a high rate of success in patients who in many other gastrointestinal settings would be left without further treatment options. While the integration of psychological services into ambulatory care clinics would seem optimal in light of the complex biopsychosocial nature of NCCP and other functional gastrointestinal disorders, it is a rare exception in today's healthcare delivery.

The other system change required for hypnosis to enter mainstream healthcare for gastrointestinal problems is improved coverage of the cost of hypnosis by health care systems or insurance providers. Such coverage seems reasonable in light of the accumulating evidence that hypnotherapy reduces healthcare utilisation and medication needs long term.¹¹ As long as this expense must be borne by the patients, as is predominantly the case in the USA and in some other countries, individuals with chronic functional gastrointestinal disorders will be deprived of the potential benefit of this treatment option.

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Authors' affiliations

O S Palsson, Center for Functional Gastrointestinal and Motility Disorders, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

W E Whitehead, Division of Gastroenterology and Hepatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Correspondence to: Professor W E Whitehead, Campus Box 7555, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7555, USA; William.Whitehead@med.unc.edu

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Colorectal cancer

Another important function for an old friend! The role of iron in colorectal carcinogenesis

J R Butterworth

Epidemiological data strongly support a role for dietary and haem iron in colorectal carcinogenesis through multiple pathways

The aetiopathogenesis of colorectal carcinoma (CRC) remains the holy grail for researchers in the field. CRC arises from benign neoplasms and evolves into adenocarcinoma through a stepwise histological progression sequence, proceeding from either adenomas or hyperplastic polyps/serrated adenomas. Genetic alterations are associated with specific steps in this polyp-adenocarcinoma sequence and are believed to drive the histological progression towards colon cancer. Approximately 50% of CRCs are attributed to dietary factors and about 15–20% to genetic factors, including the high risk familial syndromes.¹ Large prospectively collected epidemiological data have suggested that iron may confer an increased risk for CRC.²

WHAT ARE THE MECHANISMS BY WHICH IRON CONFERS AN INCREASED RISK OF CRC?

An apparent dose-response for serum ferritin level and adenoma risk suggests that exposure to dietary iron may be involved in the development of colorectal adenomas (and particularly proximal adenomas).³ Dietary haem iron (through its effect on epithelial proliferation) is associated with an increased risk of proximal colon cancer, especially in women who drink alcohol.⁴ In the dextran sodium sulphate model of mouse colitis, a twofold increase in dietary iron increased iron accumulation

in colonic luminal contents at the colonic mucosal surface and in superficial epithelial cells with a concomitant increase in colitis associated CRC incidence.⁵ High dietary iron decreases tocopherol levels in rat colonocytes, promotes oxidative stress (through generation of lipid peroxides and reactive oxygen species (ROS)) in faeces and colonocytes,^{6–8} and decreases the activity of the colonic antioxidant enzyme manganese superoxide dismutase.⁹ ROS activation of activator protein 1 and nuclear factor κB signal transduction pathways leads to transcription of genes involved in cell growth regulatory pathways.¹⁰

In addition, some studies suggest that possession of HFE gene mutations are statistically associated with increased rates of CRC,¹¹ but other studies have not confirmed this finding.¹² Dietary haem also promotes the development of aberrant crypt foci (ACF),¹³ the earliest identifiable neoplastic lesions in the colon cancer model. However, only a small fraction of ACF evolves to form cancer.¹⁴ Progression from ACF through adenomatous polyp to cancer results from an accumulation of proteomic abnormalities (for example, B-catenin, E-cadherin, inducible nitric oxide synthase, cyclooxygenase (COX-2), and P16(INK4a)); genetic mutations (for example, K-ras, APC, p53); genomic instabilities; microsatellite instability; loss of heterozygosity; and defects in mismatch repair systems.

In summary, dietary iron is taken up by colon cells and participates in the induction of oxidative DNA damage. Its capacity to catalyse the formation of reactive oxygen species is an important risk factor for CRC.

Work conducted by Brookes and colleagues¹⁵ in this issue of *Gut* begins to shed some light on a putative role of iron and the iron cognate proteins in colon carcinogenesis (*see page 1449*). Since identification of the HFE gene by Feder *et al* in 1996,¹⁶ the last 10 years has seen an unprecedented advance in our understanding of iron physiology. A number of iron related proteins have been identified and their role characterised.¹⁷ Brookes and colleagues¹⁵ have shown that “progression to colorectal cancer is associated with increased expression in iron import proteins and a block in iron export due to decreased expression and aberrant localisation of HEPH (hephaestin) and FPN (ferroportin-1) respectively, resulting in increased intracellular iron which may induce proliferation and repress cell adhesion”.

A major finding of the study was that a difference in the expression of the iron related proteins appeared to be evident only at the carcinoma stage of epithelial cell dedifferentiation. Intuitively, if iron is related to the process of colorectal carcinogenesis then one would have expected to find a gradation of abnormalities from normal colorectal mucosa through dysplasia to carcinoma. However, there was no statistically significant difference between expression of the iron cognate proteins in normal tissue compared with colorectal adenomas with histological high grade dysplasia. It could be inferred from this that expression of these iron proteins is merely an epiphenomena related to accumulation of multiple genetic abnormalities but that iron itself is not involved in any meaningful aetiopathological manner to the process of colorectal carcinogenesis. However, would this be a correct interpretation?

There are a number of pathways by which iron may be involved in epithelial

cell carcinogenesis. Some are outlined below, but there are potentially many more.

- (1) c-Myc induced cell transformation.
- (2) E-cadherin gene silencing.
- (3) Hypermethylation of CpG islands of target genes involved in carcinogenesis.
- (4) Cyclin dependent control of cell cycle.
- (5) CDX2 regulated expression of iron transport proteins.

(1) C-MYC OVER EXPRESSION AND INCREASED FREE CYTOSOLIC IRON

The proto-oncogene c-Myc is over-expressed in a wide variety of human cancers with 80% of breast cancers, 70% of colon cancers, 90% of gynaecological cancers, 50% of hepatocellular carcinomas, and a variety of haematological tumours possessing abnormal Myc expression. Myc proteins act as transcription factors, regulating gene expression. c-Myc protein is capable of repressing the expression of the heavy subunit of the protein ferritin (H-ferritin), stimulating expression of the iron regulatory protein 2,¹⁸ and increasing the expression of transferrin receptor (CD71).¹⁹ These effects combined result in intracellular accumulation of iron. Indeed, c-Myc induced cell transformation requires repression of H-ferritin, implying that intracellular iron concentrations are essential for control of cell proliferation and transformation by c-Myc. Interestingly, c-MYC expression also represses natural resistance associated macrophage protein 1 promoter function leading to an increase in iron in the cytosol.²⁰

(2) E-CADHERIN GENE SILENCING

A striking feature of the work published by Brookes and colleagues¹⁵ in this month's issue of *Gut* is the significant downregulation of E-cadherin mRNA expression following iron loading of the Caco-2 and SW480 cell lines. E-cadherin is a transmembrane glycoprotein that mediates epithelial cell to cell adhesion. Loss of E-cadherin can result in disruption of cell clusters and has been shown to be an independent predictor in disease progression in several cancers. E-cadherin was originally viewed exclusively as a structural protein mediating cell-cell adhesion. However, more recently, its signalling functions have been recognised. Loss or downregulation of E-cadherin releases proteins, such as β -catenin and p120 catenin, from a membrane bound state into the cytoplasm, which are known to regulate transcriptional activity. The

repression effect on E-cadherin may be mediated by the Snail family of transcription factors which are implicated in the differentiation of epithelial cells into mesenchymal cells (epithelial-mesenchymal transition). Functional perturbations of E-cadherin have been associated with the transition from adenomas to invasive carcinomas.²¹ Snail transcription factor appears in the mouse model to be a strong repressor of E-cadherin gene transcription.²² Loss of E-cadherin is considered to be diagnostic of a poor prognosis in CRC and blocking E-cadherin downregulation in tumours may be an important future approach in gene therapy for this disease. To target this molecule is the logical path to prevent the metastasising potential of almost any epithelial tumour.

(3) IRON INDUCED HYPERMETHYLATION OF CPG ISLANDS OF TARGET GENES INVOLVED IN CARCINOGENESIS

Aberrant methylation or hypermethylation is an important epigenetic alteration occurring early in human cancer and resulting in transcriptional silencing. Methylation profile of promoter CpG islands of a number of genes that might play an aetiological role in colon carcinogenesis reveals that genes demonstrating moderate or high methylation intensity include O-6-methylguanine-DNA methyltransferase, hMLH1, and p16(INK4a). The highest methylation intensity is seen in the COX-2, cadherin 13, H-cadherin, p73, and Wilms tumour 1 genes.²³ The role of iron in influencing the methylation status of these genes has yet to be determined. Interestingly, E-cadherin possesses a region from exon 1 to exon 2 which contains a high density CpG island of approximately 1500 base pairs and may be a possible site of aberrant methylation resulting in gene silencing.²⁴ In addition, demethylation of promoter regions within E-cadherin suppresses expression of E-cadherin mRNA, leading to tumour metastasis in certain cancers.²⁵

(4) IRON DEPENDENT CONTROL OF CELL CYCLE PROGRESSION

Iron is also closely involved in cell cycle control, although research into this aspect of iron metabolism is still in its infancy. The best studied molecules include the cyclins and the cyclin dependent kinases (cdks) which associate to form complexes which phosphorylate substrates such as the retinoblastoma protein (rRb) which facilitates cell cycle progression. Treatment of cells with iron chelators has been shown to result in a marked

reduction in expression of cyclins D1, D2, and D3,²⁶⁻²⁸ cdk2,²⁶ cdk1,²⁹ and the hypophosphorylation of pRb.²⁶⁻²⁷ Changes in expression of cdk2 and cyclins D1, D2, and D3 may result in the G1/S arrest observed after iron deprivation.³⁰ The cyclin-cdk2 complexes are modulated by cyclin dependent kinase inhibitors, the most important of which is the p21^{CIP1/WAF1}. Marked transactivation of the WAF1 gene which encodes p21 is also seen after iron chelation and this finding may suggest a potential role for p21 in the G1/S arrest after iron chelation.³¹ Therefore, it is postulated that intracellular iron would promote progression of the cell cycle beyond G1/S by means of rRb or a similar molecule and thus facilitate cellular proliferation.

(5) CDX2 REGULATED EXPRESSION OF IRON TRANSPORT PROTEINS

CDX1 and CDX2 are homeobox transcription factors that demonstrate intestine specific expression and maintain the intestine phenotype in adult tissues.³² They have a key role in regulating proliferation and intestinal cell fate. Cdx2 mutations result in the development of multiple polyps in the proximal colon.³³ In humans, loss of Cdx1 and/or Cdx2 is found in a subset of CRCs.³⁴ Recent work undertaken to define CDX2 regulated genes revealed that CDX2 and the gene for hephaestin (*HEPH*) are strongly correlated in colon cancer cells³⁵ and that the *HEPH* gene is the primary target of CDX2 activity. In addition, the 5' flanking region of the *HEPH* gene contains a CDX2 responsive element. In the colon cancer cell line HT-29, CDX2 activation resulted in modest inhibition of iron uptake rates and marked inhibitory effects on the steady state concentration of intracellular iron. These findings support the hypothesis that activation of *HEPH* expression by CDX2 leads to an increase in iron export from cells. However, further work by Hinoi and colleagues³⁵ did not find a significant change in expression of the iron cognate protein ferroportin with CDX activation. Allelic mutations within the CDX2 gene may be expected to reduce *HEPH* activation and subsequent basolateral membrane ferroportin expression with a subsequent block on cellular iron export and increase in intracellular iron concentrations. Clearly, the role of CDX2 in colon carcinogenesis requires further detailed clarification.

CONCLUSION

Epidemiological data strongly support a role for dietary and haem iron in colorectal carcinogenesis. Although, the

study reported by Brookes and colleagues¹⁵ appears to demonstrate a significant difference in iron staining and protein expression of the iron cognate proteins in colon cancer tissue only, it is highly probable that iron is intimately involved in the process of epithelial cell dedifferentiation in the colon (and by extension in gastric, oesophageal, and pancreatic tissue). The next few years will reveal in more detail the precise role of iron in either initiating or perpetuating this process leading eventually to the develop of carcinoma.

Potentially, iron is involved in the development of colorectal carcinoma through multiple pathways and a number of questions remain unanswered. The work published by Brookes and colleagues¹⁵ is the beginning of another exciting journey in elucidating the role of iron in colon cancer pathogenesis. The field of iron metabolism has enjoyed unprecedented success over the past 10 years since the seminal discovery of the HFE gene by Feder and colleagues.¹⁶ As attention is turned to studying the effects of iron and associated proteins in the colon, it is to be hoped that similar success will result.

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Correspondence to: Dr J R Butterworth, Department of Gastroenterology, Shrewsbury and Telford Hospital NHS Trust, Shrewsbury, Shropshire SY3 8XQ, UK; j.r.butterworth@btinternet.com

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Colorectal cancer

Folate: a magic bullet or a double edged sword for colorectal cancer prevention?

Y-I Kim

Low folate status might inhibit colorectal carcinogenesis and high folate status may promote colorectal carcinogenesis, contradicting findings from epidemiological studies showing an inverse relationship between folate status and risk of colorectal cancer

Folate is a water soluble B vitamin, deficiency of which appears to play an important pathogenetic role in the development of anaemia, atherosclerosis, neural tube defects (NTDs), adverse pregnancy outcomes, neuropsychiatric disorders, and cancer.¹ Folic acid is the fully oxidised monoglutamyl form of this vitamin that is used commercially in supplements and in fortified foods. Folate is generally regarded as safe and has long been presumed to be purely beneficial and an ideal functional food component for disease prevention.^{2,3} For example, an overwhelming body of evidence for a protective effect of periconceptional folic acid supplementation against NTDs led to mandatory folic acid fortification in the USA and Canada in 1998.⁴ The effectiveness of folic acid fortification in improving folate status has already been shown to be quite striking, with a dramatic increase in blood measurements of folate concentrations in the USA and Canada.⁴ Preliminary reports also suggest a significant reduction (~15–50%) in the incidence of NTDs in the USA and Canada.⁴

Perhaps one of the most speculative and provocative new medical applications of folate nutrition is the potential role of folate as a cancer preventive agent.^{1,5} The concept that folate deficiency enhances, whereas folate supplementation reduces, the risk of neoplastic transformation appears counterintuitive and contradictory to our conventional understanding of folate biochemistry. Folate is an essential cofactor for the *de novo* biosynthesis of purines and thymidylate, and in this role folate plays an important role in DNA synthesis and replication.^{5,6} Consequently, folate deficiency in tissues with rapidly replicating cells results in ineffective DNA synthesis. In neoplastic cells where DNA replication and cell division are occurring at an accelerated rate, interruption of folate

metabolism causes ineffective DNA synthesis, resulting in inhibition of tumour growth.^{5,6} Indeed, this has been the basis for cancer chemotherapy using a number of antifolate agents (for example, methotrexate) and 5-fluorouracil.^{5,6} Furthermore, folate deficiency has been shown to induce regression and suppress progression of pre-existing neoplasms in experimental models.^{7–9} In contrast with the inhibitory and promoting effect of folate deficiency and supplementation, respectively, on established neoplasms, however, folate status appears to have an opposite effect in normal tissues. An accumulating body of epidemiological, clinical, and experimental evidence suggests that folate deficiency in normal tissues appears to predispose them to neoplastic transformation, and folate supplementation suppresses the development of tumours in normal tissues.^{1,5}

Epidemiological studies have suggested an inverse association between folate and the risk of cancer of the colorectum, oropharynx, oesophagus, stomach, pancreas, lungs, breast, cervix, ovary, and breast, and neuroblastoma and leukaemia.^{1,5} The role of folate in carcinogenesis has been best studied for colorectal cancer (CRC).^{1,5,10,11} Collectively, more than 20 case control studies have shown either a significant or equivocal inverse relationship that was not statistically significant, that became non-significant after adjustment, or that could not be distinguished from other factors in their relation to risk between folate status (assessed by dietary folate intake or by measurement of blood folate levels) and the risk of CRC or its precursor, adenoma, in the general population and in individuals with chronic ulcerative colitis.^{1,5,10,11} Several large prospective studies also suggest a 40% reduction in the risk of CRC and adenomas in those with the highest intake of folate compared with those with the lowest intake.¹¹ Two

recent meta-analyses of epidemiological studies have confirmed a significant inverse association between folate intake and the risk of CRC, with a 20–25% reduction in the risk of CRC in subjects with the highest folate intake compared with those with the lowest intake.¹² Some epidemiological studies have shown a beneficial effect of multivitamin supplements containing $\geq 400 \mu\text{g}$ folic acid for ≥ 15 years on CRC risk and mortality.^{13,14} In addition, several small clinical trials have demonstrated that folic acid supplementation can improve or reverse surrogate endpoint biomarkers of CRC.^{1,5,15}

There exist several biologically plausible mechanisms by which folate deficiency increases, whereas folate supplementation reduces, the risk of CRC in normal colorectal epithelial cells.^{5,6,15,16} As an essential cofactor for the *de novo* biosynthesis of purines and thymidylate, folate plays an important role in DNA synthesis, stability and integrity, and repair, aberrations of which have been implicated in colorectal carcinogenesis.^{5,6,16} Folate may also modulate DNA methylation, which is an important epigenetic determinant in gene expression, in the maintenance of DNA integrity and stability, in chromosomal modifications, and in the development of mutations.^{5,6,15} A growing body of evidence from *in vitro*, animal, and human studies indicates that folate deficiency is associated with DNA strand breaks, impaired DNA repair, increased mutations, and aberrant DNA methylation, and that folate supplementation can correct some of these defects induced by folate deficiency.^{5,6,15}

Folate therefore appears to be an ideal nutritional factor for CRC prevention. However, recent animal studies and intervention trials have dampened the enthusiasm for the potential role of folate supplementation in CRC prevention.^{4,17} Data from animal studies generally support a causal relationship between folate depletion and CRC risk and an inhibitory effect of modest levels of folate supplementation on colorectal carcinogenesis.^{1,5} However, these animal studies have also shown that the dose and timing of folate intervention are critical in providing safe and effective chemoprevention; exceptionally high supplemental folate levels and folate intervention after microscopic neoplastic foci are established in the colorectal mucosa promote rather than suppress colorectal carcinogenesis.^{1,5} For example, in a standard chemical carcinogen rodent model of CRC, supraphysiological levels of folic acid supplementation (>20 times the basal daily dietary requirement) have been shown to

increase the development and progression of CRC.^{18–20} Furthermore, in two genetic models of CRC (*Apc^{Min}* and *Apc⁺/– x Msh2^{–/–}* mice), moderate dietary folate deficiency enhanced, whereas modest levels of folic acid supplementation (4–10 times the basal daily dietary requirement) suppressed, the development and progression of CRC, if folate intervention was started before the establishment of neoplastic foci in the intestine.^{21–22} If however folate intervention was started after the establishment of neoplastic foci, dietary folate had an opposite effect on the development and progression of CRC.^{21–22}

Furthermore, the Aspirin-Folate Polyp Prevention Study (n = 1021) reported that folic acid supplementation (1 mg/day) for up to six years in subjects with previous colorectal adenomas did not significantly prevent the recurrence of colorectal adenomas (rate ratio (RR) 1.04).²³ However, folic acid supplementation significantly increased the number of adenomas by 44% (RR 1.44 (95% confidence interval (CI) 1.03–2.02)) and non-significantly increased the incidence of advanced adenomas with a high malignant potential compared with placebo.²³ One explanation for this unexpected observation is that folic acid supplementation might have promoted the progression of already existing undiagnosed preneoplastic lesions (for example, aberrant crypt foci (ACF), probably the earliest precursor of CRC, or microscopic adenomas) or adenomas missed on initial colonoscopy in these genetically predisposed patients at high risk of developing CRC. This hypothesis is supported by prior observations that addition of folate to established tumours causes an “acceleration phenomenon” in humans; children with acute leukaemia treated with folate supplementation experienced accelerated progression of leukaemia.²⁴ Two recently published large, randomised, placebo controlled intervention trials designed to test the effect of folic acid supplementation in conjunction with other B vitamins on primary and secondary prevention of cardiovascular events have reported a non-significant trend towards an increased risk of total cancer (RR 1.22 (95% CI 0.88–1.70)) in the Norwegian Vitamin trials (n = 3749; 800 µg folic acid/day for 40 months)²⁵ and of colon cancer (RR 1.36 (95% CI 0.89–2.08)) in the Heart Outcomes Prevention Evaluation 2 trial (n = 5522; 2.5 mg folic acid/day for five years).²⁶

Although all of the published epidemiological studies to date have not reported a harmful effect of folate on CRC risk,¹¹ the study by Van Guelpen and colleagues²⁷ in this issue of *Gut* suggests for the first time a potential

harmful effect of high folate status on CRC risk (see page 1461).²⁷ This population based nested case control study in the Northern Sweden Health and Disease Cohort reports that plasma folate concentrations are significantly related to the risk of CRC in a bell shaped manner; multivariate odds ratios (OR) were 2.00 (95% CI 1.13–3.56) for the middle versus lowest quintile and 1.34 (95% CI 0.72–2.50) for the highest versus the lowest quintile. Furthermore, in subjects followed for longer than the median of 4.2 years, plasma folate concentrations were strongly positively related to CRC risk; multivariate OR for the highest versus lowest quintile was 3.87 (95% CI 1.52–9.87; p trend = 0.007). The main and novel finding of this study is that low folate status might inhibit colorectal carcinogenesis and that high folate status may promote colorectal carcinogenesis. Therefore, this study contradicts the findings of other epidemiologic studies that showed the inverse relationship between folate status and CRC risk.¹¹ However, this study supports the findings from previously discussed animal studies and clinical observations that suggest that folate status might promote colorectal carcinogenesis depending on the dose and timing of folate intervention. Although several animal studies have suggested a potential harmful effect of high folate status, these studies have largely been ignored by epidemiologists and public health policy makers.^{4–17} This type of study can no longer be performed in North America because of the mandatory folic acid fortification implemented in 1998 and hence the findings from this study provide very important information regarding the safety of folic acid supplementation.

Also, the widely accepted notion that folate status is inversely related to the risk of developing other cancers has begun to be challenged in recent epidemiological studies. For example, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (n = 25 400 postmenopausal women) has reported that women consuming supplemental folic acid ≥400 µg/day have a 20% increased risk of developing breast cancer compared with subjects reporting no supplemental intake (hazard ratio (HR) 1.19; 95% CI 1.01–1.41; p trend = 0.04).²⁸ Although food folate intake was not significantly related to breast cancer risk (HR 1.04; 95% CI 0.83–1.31; p trend = 0.56), total folate intake, mainly from folic acid supplementation, significantly increased the risk by 32% (HR 1.32; 95% CI 1.04–1.68; p trend = 0.03).²⁸

What can we conclude about the role of folate in CRC from these seemingly

paradoxical and contradictory epidemiological, animal, and clinical observations? Folate appears to possess dual modulatory effects on colorectal carcinogenesis depending on the timing and dose of folate intervention.^{1–4–5} Folate deficiency has an inhibitory effect whereas folate supplementation has a promoting effect on the progression of established neoplasms.^{1–4–5} In contrast, folate deficiency in normal colorectal epithelial cells appears to predispose them to neoplastic transformation, and modest supplemental levels (4–10 times above the basal dietary requirement) suppress whereas supraphysiological supplemental doses enhance the development of tumours in normal mucosa.^{1–4–5} An obvious inference from these observations is that for folate to be a safe and effective chemopreventive agent against CRC, modest doses of folic acid supplementation should be implemented before the development of precursor lesions in the colorectum or in persons free of any evidence of neoplastic foci. However, determining the presence of neoplastic foci in the general population is obviously an almost impossible task. Furthermore, folate might prevent the progression of certain precursor or preneoplastic lesions to frank malignancy but promote the progression of other lesions. What constitutes safe precursor or preneoplastic lesions on which folate may exert a protective effect has not yet been established. For example, should folate chemoprevention be started before there is evidence of established premalignant lesions, such as ACF or microscope adenomas in the colorectum, or should folate chemoprevention be started even after these lesions are present? In this regard, animal studies investigating the effects of folic acid supplementation on the progression of ACF, microscopic adenomas, and adenomas are urgently needed.

Mechanistically, the most likely mechanism by which folic acid supplementation may promote the progression of established preneoplastic and precursor lesions of CRC is provision of nucleotide precursors to rapidly replicating neoplastic cells for accelerated proliferation and growth.^{1–4–5} Another mechanism may be de novo methylation of promoter CpG islands of tumour suppressor genes with consequent gene inactivation leading to tumour progression.^{4–15} This potential epigenetic mechanism of tumour progression is supported by recent animal studies using viable yellow agouti mice that unequivocally have demonstrated that maternal dietary methyl group supplementation, including a modest amount of folic acid, permanently alters

phenotypic coat colour of the offspring via increased methylation at the promoter CpG site of the agouti gene.^{29–31}

Whether or not possible deleterious effects of folic acid supplementation (for example, cancer promoting effect on established preneoplastic and neoplastic lesions) outweigh the known and potential health benefits (for example, prevention of atherosclerosis and NTDs; improvement of cognitive function; cancer prevention in normal tissues free of preneoplastic and neoplastic foci) is largely unknown at present. An emerging body of evidence suggests that folate may possess potentially serious adverse effects, including masking of B12 deficiency especially in the elderly; the occurrence of resistance or tolerance to antifolate based chemotherapy and anti-inflammatory and anti-seizure drugs; epigenetic instability; decreased natural killer cell cytotoxicity; and genetic selections of disease alleles (for example, MTHFR C677T).^{3 4 17 32–35} Some proponents of mandatory folic acid fortification have labelled the delay in folic acid fortification in European countries as public health malpractice.³⁶ However, a reasonable conclusion from the above discussion is that inertia on folic acid fortification in these European countries should not be construed as public health malpractice but should be regarded as public health prudence. Mandatory folic acid fortification is probably the most important science drive intervention in nutrition and public health in decades.³⁷ However, the possibility remains that certain segments of the exposed population may benefit less and may even experience some adverse effects from increased folic acid intake. In addition to the drastic increase in dietary folate intake from mandatory folic acid fortification, 30–40% of the North American population consume supplemental folic acid for several possible but as yet unproven health benefits.³⁸ Therefore, long term follow up studies are urgently warranted to determine the effect of folic acid fortification and supplementation on the incidence of cancer and other potential adverse effects. The potential cancer promoting effect of folic acid fortification and supplementation in the vast majority of the North American population who are not at risk of NTDs but have been unintentionally exposed

to high levels of folic acid is a legitimate public health concern and needs careful monitoring.

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Correspondence to: Dr Y-I Kim, Room 7258, Medical Sciences Building, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada, M5S 1A8; youngin.kim@utoronto.ca

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