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Folate levels in mucosal tissue but not methylenetetrahydrofolate reductase polymorphisms are associated with gastric carcinogenesis

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Abstract

AIM: To evaluate whether folate levels in mucosal tissue and some common methylenetetrahydrofolate reductase (MTHFR) variants are associated with the risk of gastric cancer through DNA methylation.

METHODS: Real-time PCR was used to study the expression of tumor related genes in 76 mucosal tissue samples from 38 patients with gastric cancer. Samples from the gastroscopic biopsy tissues of 34 patients with chronic superficial gastritis (CSG) were used as controls. Folate concentrations in these tissues were detected by the FOL ACS: 180 automated chemiluminescence system. MTHFR polymorphisms were analyzed by PCR-RFLP, and the promoter methylation of tumor-related genes was determined by methylation-specific PCR (MSP).

RESULTS: Folate concentrations were significantly higher in CSG than in cancerous tissues. Decreased expression and methylation of c-myc accompanied higher folate concentrations. Promoter hypermethylation and loss of $p16^{INK4A}$ in samples with MTHFR 677CC were more frequent than in samples with the 677TT or 677CT genotype. And the promoter hypermethylation and loss of $p21^{WAF1}$ in samples with MTHFR 677CT were more frequent than when 677CC or 677TT was present. The 677CT genotype showed a non-significant higher risk for gastric cancer as compared with the 677CC genotype.

CONCLUSION: Lower folate levels in gastric mucosal tissue may confer a higher risk of gastric carcinogenesis through hypomethylation and overexpression of c-myc.

Key words: Folate; Methylenetetrahydrofolate reductase; Polymorphism; DNA methylation; Gastric cancer

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INTRODUCTION

Methylation of gene regulatory elements is a well-documented epigenetic change that can lead to gene inactivation. Human gastric carcinogenesis is suggested to be associated with the decrease of total genomic DNA methylation, hypomethylation of certain specific oncogenes such as c-myc, and hypermethylation of promoter of some tumor suppressor genes containing $p16^{INK4A}$ and *hMLH1* gene[1]. Folate (or folic acid) is essential for normal DNA methylation and synthesis. We have performed a series of studies to investigate the interrelationship between DNA methylation and folate status in plasma of patients with gastric cancer[2,3]. The plasma folic acid concentration in patients who showed hypomethylation of c-myc was lower than that in patients showing normal methylation. Low plasma levels of folate have been associated with an increased risk for gastric cancers[4,5].

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism that regulates the intracellular folate pool. Two MTHFR polymorphisms, C677T and A1298C, are known to be risk factors for gastric cancer in Chinese[6], but not in Korean[7]. The MTHFR 677T allele was significantly associated with gastric cancer risk with an odds ratio (OR) of 2.49 [95% confidence interval (CI): 1.48-4.20] in heterozygous MTHFR 677CT carriers and an OR of 2.85 (95% CI: 1.52-5.35) in homozygous MTHFR 677TT carriers in a high risk Italian population[8]. These findings suggest that common variants of MTHFR may play a role in the etiology of gastric cancer, particularly

gastric cardia adenocarcinoma. Future studies using large sample sizes and incorporated detailed data on dietary folate intake and related serological measurements are needed to confirm these findings[9].

The extent to which tissue folate levels and MTHFR 677 (C→T) polymorphism interact to affect DNA methylation in gastric carcinogenesis is uncertain. It is even not clear that there is a relationship between folate concentrations and DNA methylation in gastric mucosal tissue. In the current study, we hypothesized that folate levels and some common MTHFR variants are associated with the risk of gastric cancer through DNA methylation. Our data show that decreased folate in tissues is associated with a higher risk of gastric cancer. However, MTHFR gene polymorphisms are not independent risk factors for initiation and progression of gastric cancer, although the 677CT genotype shows a non-significant higher risk for gastric cancer as compared with the 677CC genotype.

MATERIALS AND METHODS

Subjects

Thirty-eight consecutive patients with gastric cancer underwent resection at Shanghai Renji Hospital between May and December 2004. Clinicopathological factors, tumor histologies and disease stages were evaluated according to the General Rules for Clinical and Pathological Studies on gastric cancer. Paired samples (76) of histologically verified primary gastric cancer and corresponding non-cancerous gastric mucosa (> 5 cm away from cancerous margin) of 38 patients were obtained immediately after surgical resection. HE-stained sections were examined for pathological diagnoses, and were categorized according to the WHO histological classifications of gastric cancer. The histological characteristics of non-cancerous tissues were chronic atrophic gastritis, intestinal metaplasia, or dysplasia. All of tumors were located in gastric antrum or corpus, and not in fundus or cardia. There were 23 cases of tubular adenocarcinoma, 4 cases of mucinous adenocarcinoma and 11 cases of tubular-papillary adenocarcinoma. The mean age of the patients at resection was 61 (range 31-81) years and it included 25 men and 13 women. A portion of each tissue (approximately 3-5 g) was snap-frozen on dry ice and kept in liquid nitrogen until use for DNA or RNA extraction. Another 34 patients with chronic superficial gastritis (CSG) were studied as sex, age and *H pylori* infection (by histology, urease test or breath test, as well as alcohol and tobacco intake matched controls to the gastric cancer group). Three endoscopic biopsy tissue samples were obtained from each control. All controls were subjected to clinical assessment, upper gastrointestinal endoscopy, histopathology of antral mucosa. No chronic atrophic gastritis, intestinal metaplasia or dysplasia was detected in any of the controls. Complete written consent was obtained from all patients and controls.

Determination of folate concentrations in tissues

One milliliter of PBS was added to 10 mg of mucosal tissues. Lysates were sonicated, and the debris was removed from samples by centrifugation for 10 min at 15 000 × *g*, 4℃ in a microcentrifuge. Folate levels in gastric mucosa were measured with an ACS: 180 automated chemiluminescence analyzer (Chiron Diagnostics Corporation, East Walpole, MA). The ACS: 180 Folate assay is a direct chemiluminescence competitive immunoassay. Folates in the patient sample competed with acridinium ester-labeled folates in the Lite Reagent for a limited amount of biotin-labeled folate binding proteins. Biotin-labeled folate binding proteins bind to avidin that is covalently coupled to paramagnetic particles in the Solid Phase. The sample was pretreated to release the folates from endogenous binding proteins.

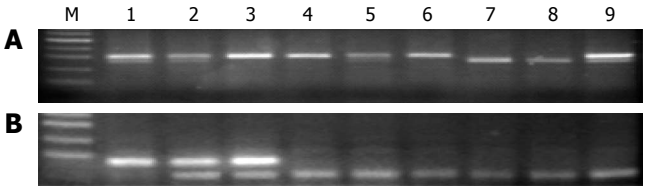

Figure 1 **A**: *Hinf* I distinguishes MTHFR 677 (C→T) PCR products. M: 50 bp Ladder; L1, 2, 5 and 9: CT genotype; L3, 4 and 6: CC genotype; L7 and 8: TT genotype; **B**: *Mbo* II distinguishes MTHFR (A→C) PCR products. M: 50 bp ladder; L1: CC genotype; L2 and 3: AC genotype; L4-9: AA genotype.

Analysis of MTHFR polymorphisms using PCR-RFLP

Genomic DNA was isolated from gastric mucosal tissue using QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. MTHFR C677T and A1298C mutations were detected after PCR amplification with the corresponding primers. The restriction enzyme *Hinf* I (New England Biolabs, Beverly, MA) was used to distinguish the 677 (C→T) polymorphism. The variant allele (677TT) gained an *Hinf* I restriction site and resulted in two fragments of 175 bp and 23 bp after digestion. The wild-type allele (677CC) had a single band representing the entire 198 bp fragment (Figure 1A). The restriction enzyme *Mbo* II (New England Biolabs) was used to distinguish the 1298 (A→C) polymorphism. The restriction site was absent in the variant allele (1298CC), and digestion yielded four fragments of 84, 31, 30 and 18 bp, whereas the wild-type allele (1298AA) generated 56, 31, 30, 28 and 18 bp bands (Figure 1B). The restriction products were analyzed by electrophoresis in a 3% agarose gel stained with ethidium bromide. PCR amplifications were run for 35 cycles, with each amplification cycle consisting of 30 s at 94℃, 30 s at 62℃ or 51℃ (for polymorphic sites at positions 677 and 1298, respectively), and 45 s at 72℃. PCR products were visualized on 3% agarose gels[10].

The MTHFR 677 (C→T) allele in gastric cancer and CSG tissues, but not the MTHFR 1298 (A→C) allele in gastric cancer samples, was in Hardy-Weinberg equilibrium ($P > 0.05$).

Real-time RT-PCR for tumor-related genes

The transcription levels of tumor-suppressor genes, $p16^{INK4A}$ and $p21^{WAF1}$; proto-oncogene, c-myc; and mismatch repair (MMR) genes, *hMLH1* and *hMSH2*; were detected using real-time RT-PCR. Total RNA was isolated using a commercial kit (Trizol) according to the manufacturer's instructions (Invitrogen Gibco BRL, Carlsbad, CA).

Table 1 Sequences of primers and probes for real-time PCR, and primers for MSP

Gene	Primer (forward) (5'-3')	Primer (reverse) (5'-3')	Probe	Genbank number
β-*actin*	CTG GCA CCC AGC ACA ATG	GGA CAG CGA GGC CAG GAT	ATC ATT GCT CCT CCT GAG	BC016045
$p16^{INK4A}$	CAT AGA TGC CGC GGA AGG T	CAG AGC CTC TCT GGT TCT TTC AA	CCT CAG ACA TCC CCG	NM_058197
$p21^{WAF1}$	CTG GAG ACT CTC AGG GTC GAA	GGA TTA GGG CTT CCT CTT GGA	ACG GCG GCA GAC CAG CAT GA	NM_078467
c-myc	ACA CCG CCC ACC ACC AG	CCA CAG AAA CAA CAT CGA TTT CTT	AGC GAC TCT GAG GAG G	V00568
hMLH1	GGC CAG CTA ATG CTA TCA AAG AG	CTT TAA CAA TCA CTT GAA TAC TTG TGG A	ATT GAG AAC TGT TTA GAT GCA	U07418
hMSH2	ATC CAA GGA GAA TGA TTG GTA TTT G	CAA AGA GAA TGT CTT CAA ACT GAG AGA	CAT ATA AGG CTT CTC CTG GC	U04045
$p16^{INK4A}$(M)	TTA TTA GAG GGT GGG GCG GAT CGC	GAC CCC GAA CCG CGA CCG TAA		X94154
$p16^{INK4A}$(U)	GGG GGA GAT TTA ATT TGG	CCC TCC TCT TTC TTC CTC		X94154
$p21^{WAF1}$(M)	TGT AGT ACG CGA GGT TTC G	TCA ACT AAC GCA ACT CAA CG		NM_007592
$p21^{WAF1}$(U)	TTT TTG TAG TAT GTG AGG TTT TGG	AAC ACA ACT CAA CAC AAC CCT A		NM_007592
c-myc (M)	TAG AAT TGG ATC GGG GTA AA	CGA CCG AAA ATC AAC GCG AAT		AF002859
c-myc (U)	TAG AAT TGG ATT GGG GTA AA	CCA ACC AAA AAT CAA CAT GAA T		AF002859
hMLH1 (M)	ACG TAG ACG TTT TAT TAG GGT CGC	CCT CAT CGT AAC TAC CCG CG		AB017806
hMLH1 (U)	TTT TGA TGT AGA TGT TTT ATT AGG GTT GT	ACC ACC TCA TCA TAA CTA CCC ACA		AB017806
hMSH2 (M)	TCG TGG TCG GAC GTC GTT C	CAA CGT CTC CTT CGA CTA CAC CG		AB006445
hMSH2 (U)	GGT TGT TGT GGT TGG ATG TTG TTT	CAA CTA CAA CAT CTC CTT CAA CTA CAC CA		AB006445

M: Methylation PCR primers; U: Unmethylation PCR primers.

Reverse transcription reactions using 5 μg of total RNA in a total reaction volume of 20 μL were performed with Superscript Ⅱ reverse transcriptase (Invitrogen Life Technologies, Inc.). Relative quantitation using the comparative Ct method with data from the ABI PRISM 7700 Sequence Detection System (version 1.6 software) was performed according to the manufacturer's protocol. The primers and fluorogenic probes for these genes were provided by Shenyou Company, Shanghai. The sequences of the probes and forward and reverse primers are shown in Table 1. Real-time PCR was also performed with primers and a probe for *β-actin* to normalize each of the extracts for amplifiable human DNA. The results were expressed as the ratio of copies of target genes to *β-actin*. Ct values were measured, and the average Ct of triplicate samples was calculated. An alteration of mRNA expression was defined as a 3-fold difference in expression level[11].

Bisulfite modification and methylation-specific PCR of promoters of tumor-related genes

To address whether DNA methylation of tumor-related genes is associated with the folate level in mucosal tissue and MTHFR polymorphisms, methylation-specific PCR (MSP) was performed in CpG-rich regions of $p16^{INK4A}$ and $p21^{WAF1}$, c-myc, and *hMLH1* and *hMSH2*, in order to detect changes in DNA methylation of the genes due to drug treatments. Bisulfite modification protocols were adopted as described by Xiong and Laird[12]. Genomic DNA treated with bisulfite was amplified with promoter specific primers of each gene (Table 1). The primers were designed without CpG dinucleotides to enable the amplification of both methylated and unmethylated alleles.

The 50 μL PCR reactions consisted of 100 ng of bisulfite-treated DNA, 0.1 mmol/L dNTPs, 2.0 mmol/L $MgCl_2$, and 0.5 μmol/L of each primer. PCR products were directly loaded onto 3% agarose gels and electrophoresed. The gel was stained with ethidium bromide and directly visualized under UV illumination. Wild-type $p16^{INK4A}$ and $p21^{WAF1}$ primers were used to verify that complete conversion of the DNA occurred in the bisulfite reaction. A positive control for complete methylation was also amplified.

Statistical analysis

Data were presented as means ± SD. Comparisons between groups were made using Student's paired *t* test. The differences between cancerous, non-cancerous and CSG tissues, and their relationships were analyzed by Fisher's exact test using SAS v.6.12. All statistical tests were two tailed and considered significant at $P = 0.05$. A case-control study was performed and the allelic frequency of the polymorphism was calculated for both cases and controls. Odds ratios (OR) and 95% CI were calculated to evaluate the association between CSG or cancer and the presence of MTHFR C677T polymorphism. The Mantel Haenszel χ^2 procedure was used to assess the linear trend between lesion severity and magnitude of the association with MTHFR C677T genotype.

Table 2 Folate level and transcription and methylation of c-myc

Methylation status	Transcription	Case number (*n*)	*P*[1]	Average folate concentration (μg/L)	*P*[2]
Hypomethylated	Up-regulated	6		2.25 ± 1.23	
	Unchanged	0	0.02		0.06
Methylated	Up-regulated	13		3.91 ± 2.01	
	Unchanged	19			

[1]Transcription and DNA methylation of c-myc; [2]Folate concentration and DNA methylation of c-myc.

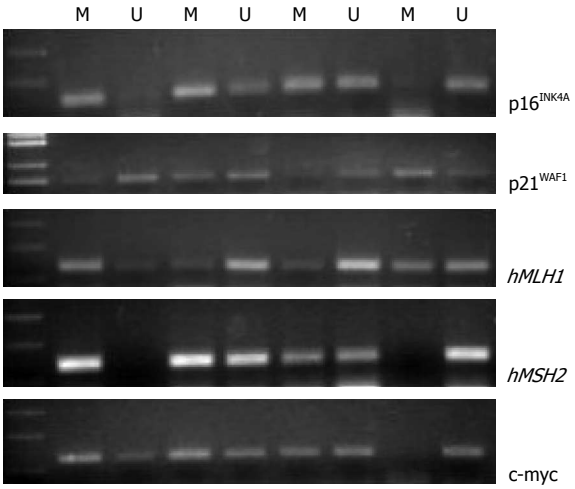

Figure 2 Methylation-specific PCR (MSP) for tumor-related genes. MSP was performed on DNA from gastric cancerous tissue with primers designed to specifically detect methylated and unmethylated promoter regions. The data shown are representative of three replicate MSP experiments. Marker: 100 bp ladder; U: Unmethylated-MSP; M: Methylated-MSP.

RESULTS

Folate levels were lower in cancerous tissue than in non-cancerous tissue or CSG

Folate concentrations in CSG tissue (5.48 ± 2.15 ng/mL) were significantly higher ($P < 0.05$) than in the other two groups (gastric cancerous tissue, 3.65 ± 1.97 μg/L and corresponding non-cancerous gastric mucosa, 4.01 ± 2.11 μg/L). There were no significant differences in folate concentrations between different sites of the stomach including antrum and corpus. No correlation was found between folate levels and the degree of infiltration, lymph node metastasis, tumor size, or TNM clinical stage of gastric cancer (data not shown).

c-myc expression and methylation were associated with folate concentrations

As shown in Table 2 and Figure 2, decreased expression of c-myc accompanied higher folate levels. However, there was no definite association between folate levels and different expression levels of tumor suppressor genes including $p16^{INK4A}$, $p21^{WAF1}$, *hMLH1* and *hMSH2* in mucosal tissue (data not shown). Furthermore, hypomethylation of c-myc was found in cancerous tissues, which showed up-regulated expression of c-myc, and folate levels in tissues with a hypomethylated c-myc gene showed a downward trend compared with folates in unmethylated samples in advanced gastric cancer (tubular-, mucinous-, and tubular-papillary adenocarcinoma).

Table 3 MTHFR gene polymorphisms and the transcription of tumor-related genes

Genes	MTHFR	Up-regulation *n* (%)	Normal (*n*)	Down-regulation *n* (%)	mRNA ratio of cancerous to non-cancerous tissue (mean ± SD)	*P*
$p16^{INK4A}$	CC	1	3	10 (71.43)	0.63 ± 1.05	0.84
	CT	1	8	10 (52.63)	0.7 ± 0.88	0.69
	TT	0	2	3 (60)	0.85 ± 0.96	-
	AA	1	9	16 (61.54)	0.57 ± 0.96	0.51
	AC	1	4	7 (58.33)	0.81 ± 1.15	-
$p21^{WAF1}$	CC	1	7	6 (42.86)	1.06 ± 1.06	-
	CT	1	5	13 (68.42)	0.47 ± 0.69	0.062
	TT	0	2	3 (60)	0.55 ± 0.55	0.32
	AA	2	10	14 (53.85)	0.75 ± 0.85	0.57
	AC	0	4	8 (66.67)	0.58 ± 0.85	-
c-myc	CC	6 (42.86)	7	1	2.26 ± 2.17	0.048
	CT	11 (57.89)	6	2	4.32 ± 3.25	-
	TT	2 (40)	2	1	2.15 ± 1.8	0.17
	AA	15 (57.69)	8	3	3.84 ± 3.17	0.076
	AC	4 (33.33)	7	1	2.06 ± 1.64	-
hMLH1	CC	0	3	11 (78.57)	0.46 ± 0.67	0.053
	CT	1	8	10 (52.63)	0.57 ± 0.82	0.13
	TT	0	3	1 (25)	1.3 ± 1.28	-
	AA	2	10	14 (53.85)	0.72 ± 0.96	0.28
	AC	0	4	8 (66.67)	0.39 ± 0.55	-
hMSH2	CC	0	5	9 (64.29)	0.31 ± 0.32	-
	CT	1	10	8 (42.11)	0.88 ± 0.97	0.04
	TT	0	3	2 (40)	0.94 ± 1.12	0.064
	AA	1	13	12 (46.15)	0.8 ± 0.98	0.19
	AC	0	5	7 (58.33)	0.4 ± 0.42	-

MTHFR polymorphisms might affect the methylation status of tumor-suppressor gene promoters

As indicated in Table 3, the 677CC, CT, CC, CT and CC genotypes of MTHFR were most frequently detected with aberrant methylation of $p16^{INK4A}$, $p21^{WAF1}$, *hMLH1*, *hMSH2* and c-myc, respectively. The 1298AA genotype was found associated with aberrant methylation of all tumor-related genes studied.

Compared to the 677CC genotype, expression of *hMSH2* was significantly down-regulated in cases with the 677CT genotype, and showed a large but non-significant decrease in the presence of 677TT. Cases with the 677CT genotype showed a lower level of $p21^{WAF1}$ expression than did cases with the wild-type 677CC genotype, while *hMLH1* coupled with the 677CC genotype displayed a trend of decreased expression compared with the 677TT genotype. No differences were observed in $p16^{INK4}$ mRNA levels between 677CC, 677CT or 677TT. c-myc transcription in cases with the 677CT genotype was significantly higher than when the 677CC genotype was present.

Compared with the 1298AC genotype, c-myc

transcription showed an increase in cases with the 1298AA genotype. There was no difference in the expression of $p16^{INK4A}$, $p21^{WAF1}$, *hMLH1* or *hMSH2* between the three MTHFR genotypes.

MTHFR polymorphisms might not be an independent factor affecting initiation and progression as well as biological characteristics of gastric cancer

There were no differences in either the genotype distribution or allele frequency for alleles 677T between gastric cancerous tissue and CSG ($P > 0.05$), although the 677CT genotype showed a non-significant higher risk for advanced gastric cancer as compared with the 677CC genotype (Table 4). In addition, due to the limitation of sample size, we failed to find any significant association between MTHFR polymorphisms, 677 (C→T) and 1298 (A→C), and the degree of infiltration, lymph node metastasis, tumor size, or clinical stage of gastric cancer (Table 5).

MTHFR polymorphisms were not associated with folate levels in gastric cancerous tissues

The genotypes of 677 and 1298 sites were not associated with folate concentrations in gastric cancerous tissue and CSG (data not shown).

Table 4 MTHFR polymorphisms of gastric cancerous tissue and CSG

Polymorphic site	MTHFR genotype	Cancerous tissue *n* (%)	CSG *n* (%)	OR (95% CI)	*P*
C677T	CC	14 (36.84)	15 (44.12)	1 (reference)	-
	CT	19 (50.00)	11 (32.35)	1.85 (0.65-5.24)	0.24
	TT	5 (13.16)	8 (23.53)	0.67 (0.17-2.57)	0.55
A1298C					
	AA	26 (68.42)	22 (64.71)	1 (reference)	-
	AC	12 (31.58)	11 (32.35)	0.92 (0.34-2.52)	0.87
	CC	0	1 (2.94)	-	-

DISCUSSION

Gastric cancer is a common malignant tumor worldwide, with a much higher incidence in Asian than in Western countries. Multiple genetic and epigenetic alterations are involved in gastric carcinogenesis.

Folate is an important constituent of fruits and vegetables and may confer protection against cancer. An important biological function of folate is to provide methyl groups required for intracellular methylation reactions and *de novo* deoxynucleoside triphosphate synthesis; therefore, folate deficiency is thought to be carcinogenic through disruption of DNA methylation and synthesis and impaired DNA repair[13]. However, folate requires metabolic transformations catalyzed by several enzymes including MTHFR, which irreversibly converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The MTHFR gene is highly polymorphic, of which the two most common variants are located at nucleotides 677 (C→T) and 1298 (A→T). Low MTHFR activity may prevent the shunting of methyl groups from *de novo* dTMP synthesis, a rate-limiting step for DNA synthesis, to methylation pathways.

It has been suggested that the cancer risk associated with MTHFR polymorphisms may be modulated by folate intake[14]. When folate intake is sufficient, individuals carrying the variant MTHFR genotypes may have a decreased risk because under these conditions, while adequate provision of methyl donors is still ensured, enhanced genomic integrity would be achieved *via* conservation of folate within a cyclic intracellular pathway by shunting methyl groups toward nucleotide synthesis due to diminished MTHFR activity. However, when folate intake is low, both DNA methylation and DNA synthesis and repair might be impaired in carriers of variant MTHFR genotypes, which, in turn, results in increased risk of carcinogenesis. This hypothesis of a gene-nutrient interaction may explain the conflicting reports showing either reduced[10,15,16] or elevated[17,18] risk of cancers.

Many previous studies have investigated the relationship between folate status and MTHFR gene polymorphisms in carcinogenesis. However, because they were mainly focused on blood plasma and blood cells, little is known about folate status in mucosal tissues. Gastric cancer originates from epithelial cells; therefore, a study performed in mucosal tissues is more accurate and effective than those performed using blood. In the present study, we examined folate concentrations and MTHFR gene polymorphisms in gastric mucosal tissues, and found that folate levels were significantly lower in cancer cases (including cancerous and corresponding non-cancerous tissues) than that in the CSG controls (no chronic atrophic gastritis, intestinal metaplasia or dysplasia), suggesting that folate deficiency may increase the risk of cancer[4]. However, we failed to find the association between folate levels and the degree of infiltration, lymph node metastasis, tumor size, or clinical stage of gastric cancer. Possibly folate levels in mucosal tissue influence the initiation but not the progression of gastric cancer.

Transcriptional silencing of tumor suppressor genes by DNA hypermethylation and over-expression of proto-oncogenes by DNA hypomethylation play crucial roles in the progression of gastric cancer. Many genes involved in the regulation of cell cycle, tissue invasion, DNA repair and apoptosis have been shown to be inactivated by this type of epigenetic mechanism. The loss of $p16^{INK4A}$ expression, and hypermethylation in the promoter region is the mechanism of loss of $p21^{WAF1}$, and hypermethylation of the *hMLH1* gene promoter has been associated with a transcriptional blockade. The fact that the blockade is reversible with demethylation suggests that an epigenetic mechanism underlies h*MLH1* gene inactivation and MMR genes deficiency[19]. However, the role of DNA methylation in the loss of *hMSH2* expression has been controversial[20,21]. Aberrant methylation of c-myc can induce over-expression of the gene, and participate in the development of tumors. Hypomethylation of c-myc has been detected in gastric carcinogenesis[22], and it has been reported that folate[23] and MTHFR gene polymorphisms[24] are associated with aberrant methylation of some tumor-related genes.

Table 5 MTHFR polymorphism and biological characters of patients with gastric cancer (*n*)

Biological character		677CC	677CT	677TT	1298AA	1298AC
Degree of infiltration	Muscularis mucosae	2	2	1	3	2
	Muscular layer	1	3	2	5	1
	Serosa	7	9	1	12	5
	Out-serosa	4	5	1	6	4
Lymph node metastasis	Yes	7	11	2	14	6
	No	7	8	3	12	6
Tumor size	< 5 cm	9	7	2	11	7
	⩾ 5 cm	5	12	3	15	5
TNM classification	Ⅰ	2	2	1	3	2
	Ⅱ	7	12	2	16	5
	Ⅲ	5	5	2	7	5

Miao *et al*[25] revealed that the 677TT genotype is associated with an increased risk of gastric cardia cancer. However, our data showed that MTHFR polymorphisms may not be an independent factor affecting initiation and progression of gastric cancers, including antrum and corpus cancers. In addition, due to the limit of sample size, we could not find a significant association between the MTHFR polymorphisms [677 (C→T) and 1298 (A→C)] and the degree of infiltration, lymph node metastasis, tumor size, or clinical stage of gastric cancer. Folate levels but not MTHFR polymorphisms affect the methylation and expression of proto-oncogene or tumor suppressor genes related to human gastric carcinogenesis, although it is unclear how low folate levels lead to c-myc hypomethylation and its down-regulated expression.

In summary, a folate level reduction was observed in gastric cancer tissues. This change, but not methylenetetr ahydrofolate reductase polymorphisms, is associated with upregulation of c-myc expression and hypomethylation of its promoter region.

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COMMENTS

Background

Multiple genetic and epigenetic alterations are involved in gastric carcinogenesis. Folate deficiency is thought to be carcinogenic through disruption of DNA methylation and synthesis and impaired DNA repair. However, folate requires metabolic transformations catalyzed by several enzymes including MTHFR, which is highly polymorphic.

Research frontiers

The present study discussed the relationship between folate concentrations in mucosal tissues, MTHFR gene polymorphisms and expression of tumor-related genes, as well as DNA methylation in human gastric carcinogenesis.

Innovations and breakthroughs

Gastric cancer originates from epithelial cells, therefore, a study performed in mucosal tissues is more accurate and effective than those performed in blood. The extent to which folate tissue levels and MTHFR polymorphisms interact to affect DNA methylation in gastric carcinogenesis is uncertain.

Applications

The present study investigated the etiology of gastric carcinogenesis, which may provide the evidence for prevention and treatment of gastric cancer.

Terminology

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism that regulates the intracellular folate pool. The MTHFR gene is highly polymorphic, of which the two most common variants are located at nucleotides 677 (C→T) and 1298 (A→T).

Peer review

The authors evaluated whether folate level in mucosal tissue and some common MTHFR variants are associated with the risk of gastric cancer through an effect on DNA methylation. The figures are clear. However, the title and conclusion need to be more concise and exact.

S- Editor Wang GP **L- Editor** Zhu LH **E- Editor** Bai SH