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BRIEF ARTICLE

CpG island methylator phenotype and *Helicobacter pylori* infection associated with gastric cancer

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Abstract

AIM: To investigate the association between the CpG island methylator phenotype (CIMP) and serum *Helicobacter pylori* (*H. pylori*) levels for clinical prediction of gastric cancer (GC) progression.

METHODS: We analyzed the serum CIMP status of 75 patients with GC using a methylation marker panel and a methylation-specific polymerase chain reaction. Serum samples from 40 healthy persons were examined at the same time. The genes examined were *APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK* and *E-cad*. *H. pylori* infection in serum was assayed with an anti-*H. pylori* immunoglobulin G antibody test and a rapid urease test.

RESULTS: The frequencies of high-level methylation in GC tissues for the seven genes were: 48% for *APC*, 57.33% for *WIF-1*, 56% for *RUNX-3*, 50.67% for *DLC-1*, 52% for *SFRP-1*, 54.67% for *DKK*, and 48% for *E-cad*. The frequencies in GC serum were 30.67% for *APC*, 34.67% for *WIF-1*, 37.33% for *RUNX-3*, 29.33% for *DLC-1*, 33.33% for *SFRP-1*, 32% for *DKK*, and 26.67% for *E-cad*. CIMP+ (defined as $\geqslant$ 3 methylated genes) was associated with 47 (62.67%) GC tissue samples and 44 (58.67%) GC serum samples. CIMP+ was not associated with non-neoplastic mucosal tissues or the serum of healthy persons. Of the 75 GC cases, 51 (68%) were *H. pylori*+, and 24 (32%) were *H. pylori*-. Of the 51 *H. pylori*+ cases, 36 were CIMP+ and 15 were CIMP-. In contrast, for the 24 *H. pylori*- cases, 11 were CIMP+, and 13 were CIMP-. The difference was significant between the *H. pylori*+ and *H. pylori*- groups (χ^2 = 4.27, P < 0.05). Of the 51 *H. pylori*+ GC patients, 34 were CIMP+ and 17 were CIMP-, while among the 24 *H. pylori*- GC cases, 10 were CIMP+ and 14 were CIMP-. The difference was significant between the *H. pylori*+ and *H. pylori*- groups (χ^2 = 4.21, P < 0.05). A 2-year follow-up showed significant difference in the rates of metastasis and recurrence between *H. pylori*+/CIMP+ cases and the *H. pylori*+/CIMP- cases or CIMP- cases associated with *H. pylori* assayed in serum (P < 0.05). However, there were no significant differences in survival rates between the two groups.

CONCLUSION: *H. pylori*+/CIMP+ cases are associated with higher rates of metastasis and recurrence than *H. pylori*+/CIMP- cases. Serum may be useful for examining CIMP status.

Key words: CpG island methylator phenotype; *Helicobacter pylori*; Serum; Prognosis; Gastric cancer

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INTRODUCTION

Gastric cancer (GC) is one of the most common human tumors and is the second-leading cause of cancer-related deaths worldwide[1]. GC remains a major clinical challenge because it has a poor prognosis and limited treatment options due to its relative resistance to radiotherapy and chemotherapy[2].

DNA methylation is an enzyme-mediated chemical modification that occurs in cytosine-guanine dinucleotide-rich areas (CpG islands) in gene promoter regions. Aberrant promoter hypermethylation leads to loss of gene function in tumors[3]. In several types of cancers, including GC, global hypomethylation and promoter hypermethylation in specific genes are associated with genomic instability and inactivation of tumor-suppressor genes[4]. The methylation pattern(s) of multiple genes can provide useful information regarding global epigenetic alterations[5]. The hypermethylated subtype associated with tumors, denoted that the CpG island methylator phenotype (CIMP) for which multiple genes are concurrently methylated, is a novel marker of tumor progression[6-8].

Helicobacter pylori (*H. pylori*) infection of the stomach is associated with an increased risk for gastric carcinoma[9]. Several studies have demonstrated that *H. pylori* infection is associated with gene promoter hypermethylation and gene-type-specific methylation profiles involved in the multistep process of carcinogenesis[10,11]. *H. pylori* infection may induce methylation of the Trefoil factor family 2 and E-cadherin promoters in GC[12]. Certain studies have shown that methylation levels in GC patients are one to two orders of magnitude greater in *H. pylori*+ individuals than in *H. pylori*- individuals[13]. In infected mucosae, aberrant methylation and subsequent silencing of tumor suppressor genes, including *p16*, *E-cadherin*, and *hMLH1*, are strongly correlated with subsequent cancer risk[14].

In the present study, we focused on the relationship between *H. pylori* infection and CIMP status in GC risk. We did not specifically study the relationship between CIMP and *H. pylori* infection or the clinical value of evaluating CIMP associated with *H. pylori* infection.

We analyzed the relationship between serum CIMP of 75 GC patients and *H. pylori* infection in GC. The examined genes were *APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK*, and *E-cad*, which were selected because they had been found to be frequently methylated in GC and other malignancies The aim of this study was to evaluate the clinical significance of serum CIMP and *H. pylori*-infection levels for the prediction of GC progression.

MATERIALS AND METHODS

Patients and specimens

Between 2008 and 2009, tumor samples, adjacent non-neoplastic tissues, and sera from 75 Chinese GC patients were collected during surgical resection at the Nantong Tumor Hospital. Samples from 40 healthy people were used as matched controls. The controls had no self-reported history of cancer and could frequently be matched to the cases in age (± 5 years), gender, and residential area. After resection, tissue specimens were immediately frozen in liquid nitrogen and stored at -70 ℃. Patients consisted of 53 men and 22 women, ranging in age from 31 to 76 (52 ± 7) years. Questionnaire data and blood samples were also collected from all patients and controls. After centrifugation, isolated serum samples were stored at -70 ℃. The diagnosis of GC was confirmed by pathological examination and magnetic resonance imaging and/or computerized tomography. Tumor-node-metastasis (TNM) stage was classified according to the 6th edition TNM classification of the American Joint Committee on Cancer. Written informed consent was obtained from each patient and healthy control, and the local ethics committee approved the study protocol.

DNA extraction and sodium bisulfite

DNA from serum (200 μL per sample) and tissue samples was treated with proteinase K and then extracted with phenol-chloroform according to the manufacturer's instructions (Shanghai ShineGene Molecular Biotech, Inc., Shanghai, China) and stored at -20 ℃. A final elution volume of 50 μL was collected. The extracted DNA was treated with sodium bisulfite using EZ DNA Methylation kit reagents (Zymo Research, Orange, CA, United States) to convert all unmethylated cytosines to uracils. Bisulfite-modified DNA was suspended in 10 μL of elution buffer and stored at -20 ℃ until methylation-specific polymerase chain reaction (PCR) (MSP) was performed.

MSP

MSP was used to determine the methylation status of CpG islands in genes after bisulfate treatment[15,16]. The methylation status of the promoters of *APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK*, and *E-cad* was determined with a two-step amplification/detection MSP protocol. PCR products were electrophoresed through 2% agarose gels, stained with ethidium bromide, and visualized with ultraviolet illumination. All experiments were performed in duplicate. Table 1 lists the sequences of the PCR primers used.

H. pylori assay

H. pylori infection was assayed with the reagents of a serum anti-*H. pylori* immunoglobulin G (IgG) antibody test (PBM, PRINCETON, United States) and a rapid urease test (Fujian Sanqiang Biological and Chemical Co., Ltd,

Table 1 Primer sets for nested methylation-specific polymerase chain reaction

Gene			Primer sequence (5'→3')
APC	U	F	GTGTTTTATTGTGGAGTGTGGGTT
		R	CCAATCAACAAACTCCCAACAA
	M	F	TATTGCGGAGTGCGGGTC
		R	TCGACGAACTCCCGACGA
WIF-1	U	F	GGGTGTTTTATTGGGTGTATTGT
		R	AAAAAAACTAACACAAACAAAATACAAAC
	M	F	CGTTTTATTGGGCGTATCGT
		R	ACTAACGCGAACGAAATACGA
RUNX-3	U	F	TTATGAGGGGTGGTTGTATGTGGG
		R	AAAACAACCAACACAAACACCTCC
	M	F	TTACGAGGGGCGGTCGTACGCGGG
		R	AAAACGACCGACGCGAACGCCTCC
DLC-1	U	F	AAACCCAACAAAAAAACCCAACTAACA
		R	TTTTTTAAAGATTGAAATGAGGGAGTG
	M	F	CCCAACGAAAAAACCCGACTAACG
		R	TTTAAAGATCGAAACGAGCGAGCG
SFRP-1	U	F	GAGTTAGTGTTGTGTGTTTGTTGTTTTGT
		R	CCCAACATTACCCAACTCCACAACCA
	M	F	GTGTCGCGCGTTCGTCGTTTCGC
		R	AACGTTACCCGACTCCGCGACCG
DKK	U	F	TTAGGGGTGGGTGGTGGGGT
		R	CTACATCTCCACTCTACACCCA
	M	F	GGGGCGGGCGGCGGGGC
		R	ACATCTCCGCTCTACGCCCG
E-cad	U	F	TAATTTTAGGTTAGAGGGTTATTGT
		R	CCACCCCAATACTAAATCACAACA
	M	F	TTAGGTTAGAGGGTTATCGCGT
		R	TAACTAAAAATTCACCTACCGAC

M: Methylated sequence; U: Unmethylated sequence; F: Forward sequence; R: Reverse sequence.

Sanming, China). The sensitivities of the serum anti-*H. pylori* IgG antibody test and rapid urease test have been reported to be ≥ 90% of the culture test[13].

Follow-up examination

Patients were followed up to 2 years after surgery. The last follow-up was on September 12, 2010. Patients had not received chemotherapy prior to surgery. The median follow-up period was 17.5 mo (range, 8-28 mo). Patients were given a physical examination, abdominal ultrasonography, and chest X-ray, and serum was collected and tested for associated tumor markers. During the first year, local recurrence and the development of distant metastasis were monitored every 3 mo with computerized tomography and/or magnetic resonance imaging. Of the 75 patients, 13 (18.67%) died from cancer-related causes. We tracked all but three of the remaining patients during the entire study period. Seventeen patients (22.67%) had GC recurrence found on computerized tomography Fourteen patients (18.67%) had GC metastases. Sixty-two patients were still alive at the time of the last follow-up report.

Statistical analysis

The SPSS 13.0 software package (SPSS, Inc., Chicago, IL, United States) was used for statistical analysis. Values for the clinical and biological characteristics of the patients are expressed as mean ± SD. Comparison was done with the Student's *t* test. The χ^2 test was used to compare the incidence of methylation. All *P* values are two-sided, and a *P* value < 0.05 was considered statistically significant.

Table 2 Number of samples with methylated genes for gastric cancer, adjacent non-neoplastic tissue, and serum from gastric cancer patients and healthy controls

Gene	Tumor tissue (*n* = 75)	Serum from GC patients (*n* = 75)	Non-neoplastic tissues (*n* = 72)	Serum from healthy controls (*n* = 40)	χ^2
APC	36[a]	23	3	0	36.21
WIF-1	43[a]	26	4	0	45.28
DLC-1	38[a]	22	3	0	39.49
DKK	41[a]	24	2	0	47.79
SFRP-1	39[a]	25	4	0	38.29
E-cad-1	36[a]	20	3	0	36.21
RUNX-3	42[a]	28	5	0	40.64

[a]*P* < 0.05 *vs* the number of methylated gene promoters for tumor and non-neoplastic tissue samples with the χ^2 test. GC: Gastric cancer.

RESULTS

Clinical data

The median tumor size was 3.8 cm (range 2.5-9.6 cm). According to the Edmondson-Steiner classification system, six cases were classified as grade Ⅰ, 22 as grade Ⅱ, 40 as grade Ⅲ, and seven as grade Ⅳ. According to the 6th edition TNM classification of the American Joint Committee on Cancer, 24 cases were classified as grade Ⅰ, 31 as grade Ⅱ, and 20 as grade Ⅲ.

Methylation of tumor-associated genes in GC

We examined the methylation status of seven tumor-associated genes (*APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK* and *E-cad*) from tissue and serum samples of GC patients and from serum samples of controls. The overall results are summarized in Table 2. No methylated gene promoters from control serum were detected. Methylation occurred more frequently in the GC DNA than in adjacent non-neoplastic mucosal tissues, and the same relationship was observed for serum samples and non-neoplastic tissues. Methylation was detected in one or more of the genes in 69 (92%) of the 75 cases. The frequencies of high-level methylation in GC tissue and serum were ≥ 15% for the seven genes: 48% for *APC*, 57.33% for *WIF-1*, 56% for *RUNX-3*, 50.67% for *DLC-1*, 52% for *SFRP-1*, 54.67% for *DKK*, and 48% for *E-cad* in tissue. In serum, the frequencies were 30.67% for *APC*, 34.67% for *WIF-1*, 37.33% for *RUNX-3*, 29.33% for *DLC-1*, 33.33% for *SFRP-1*, 32% for *DKK*, and 26.67% for *E-cad*.

According to the criteria used in a related study[17], the CIMP status of our 75 GC samples was classified as CIMP+ (≥ 3 methylated genes) or CIMP- (two or fewer methylated genes). In this study, because the cutoff value was 3, the average number of methylated genes found

Table 3 CpG island methylator phenotype associated with *Helicobacter pylori* infection in 75 gastric cancer tissue and serum samples

CIMP	*H. pylori* infection in tissue samples		*H. pylori* infection in serum samples	
	Negative (-)	Positive (+)	Negative (-)	Positive (+)
Negative (-)	13	15	14	17
Positive (+)	11	36[a]	10	34[a]

[a]$P < 0.05$ *vs* the frequencies of CIMP between *H. pylori*+ and *H. pylori*- samples in serum with the χ^2 test. *H. pylori*: *Helicobacter pylori*; CIMP: CpG island methylator phenotype.

was 3.6 in tumor tissue and 2.0 in serum. For tumor tissue samples, 47 (62.67%) were classified as CIMP+, and 28 (37.33%) were classified as CIMP-. For serum samples, 44 (58.67%) were classified as CIMP+, and 31 (41.33%) were classified as CIMP-. No non-neoplastic tissues or serum from healthy controls were classified as CIMP+.

H. pylori in GC

We determined how many of the 75 GC patients were infected with *H. pylori* and found 51 (68%) to be *H. pylori*+ and 24 (32%) to *H. pylori*-.

Of the 51 *H. pylori*+ GC cases examined in tissues, 36 were CIMP+ and 15 were CIMP-. In contrast, of the 24 *H. pylori*- GC cases, 11 cases were CIMP+ and 13 were CIMP-. There was a significant difference between the *H. pylori*+ and *H. pylori*- groups ($\chi^2 = 4.27$, $P < 0.05$). When the sera from the 51 *H. pylori*+ GC cases were examined, 34 were CIMP+ and 17 were CIMP-. In contrast, of the 24 *H. pylori*- GC cases, 10 were CIMP+ and 14 were CIMP-. There was a significant difference between the two groups ($\chi^2 = 4.21$, $P < 0.05$). The overall results are summarized in Table 3.

Prognostic significance of CIMP associated with H. pylori infection in GC

After the two-year follow-up, the metastasis rates were found significantly different between the *H. pylori*+/CIMP+ and the *H. pylori*+/CIMP- cases when analyzed in serum ($P < 0.05$). The tumors of *H. pylori*+/CIMP+ patients frequently metastasized. The recurrence rates were significantly higher in the *H. pylori*+/CIMP+ group than in the *H. pylori*+/CIMP- group when serum was examined ($P < 0.05$). There were no differences in survival rates between *H. pylori*+/CIMP+ cases and *H. pylori*+/CIMP- cases examined in serum ($P > 0.05$). The overall results are summarized in Table 4.

Table 4 Prognosis of CpG island methylator phenotype cases associated with *Helicobacter pylori* infection in gastric cancer

Prognosis	*H. pylori*+		*H. pylori*-		χ^2
	CIMP+	CIMP-	CIMP+	CIMP-	
Metastasis					
Yes	9	2	2	1	3.593[a]
No	22	15	8	13	
Recurrence					
Yes	10	3	2	2	2.369[a]
No	21	14	8	12	
Survival					
Yes	24	15	8	12	1.043
No	7	2	2	2	

[a]$P < 0.05$ *vs* the prognosis between *H. pylori*+/CIMP+ and *H. pylori*+/CIMP- in serum with the χ^2 test. *H. pylori*: *Helicobacter pylori*; CIMP: CpG island methylator phenotype.

DISCUSSION

Epigenetic alterations have been suggested to be significant initiating events in tumorigenesis[18]. Aberrant methylation of promoter DNA regions rich in CpG islands is the key step in epigenetic gene silencing. Abnormal gene expression may be an early event in tumorigenesis and a potential biomarker for early detection[19]. In several studies, aberrant DNA methylation in gastric biopsies from *H. pylori*+ patients was found to be correlated with a greater gastric cancer risk[13,20]. Methylation level of promoters in several genes in the gastric mucosa is 5-300 times higher in infected than in uninfected individuals. In infected mucosae, the aberrant methylation and subsequent silencing of tumor suppressor genes has been strongly correlated with subsequent cancer risk[14].

Notably, the term "CIMP" has been used in a variety of ways in the context of gastric carcinoma[21]. The hypermethylator phenotype may be related to patient-specific factors, such as exposure to carcinogens or genetic predisposition[22]. The relationship between *H. pylori* and methylation of multiple genes has been demonstrated, but little attention has been paid to the association of CIMP and *H. pylori* with respect to gastric cancer or the relationship between CIMP in serum and the presence of *H. pylori* in gastric cancer.

We collected tissue and serum samples from GC patients and healthy controls to examine the methylation status of seven tumor-associated genes (*APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK*, and *E-cad*). We found that the promoters of these genes from healthy control serum were not methylated. The frequencies of high-level methylation in GC tissue and serum were at least 15% for these seven genes. Thus, examining the methylation status of multiple genes may help diagnose early GC. We also found that promoter methylation in serum DNA was highly specific for the cancer-related genes and that the results in serum samples were similar to tissue samples. We also determined the CIMP status of 75 GC samples and found a good concordance between serum and tumor CIMP status.

Of the 75 GC cases, 51 (68%) were *H. pylori*+ and 24 (32%) were *H. pylori*-. Thus, *H. pylori* infection was substantial in GC, suggesting that eradicating *H. pylori* infection may be essential to prevent or treat GC.

The prognostic roles of CIMP status have been evaluated in several cancer types. In esophageal adenocarcinoma, CIMP is associated with a poor prognosis[16]. In GC, concordant methylation of multiple genes/loci (CIMP-H)

is associated with a better survival, but is not an independent predictor of prognosis in resected GC[2].

H. pylori infection is also a factor in the prognosis of GC. We found that CIMP was associated with *H. pylori* infection. At the end of the 2-year follow-up, we found that the rates of metastasis differed significantly between *H. pylori*+/CIMP+ and *H. pylori*+/CIMP- cases when examining the serum ($P < 0.05$). The tumors of *H. pylori*+/CIMP+ cases frequently metastasized. The recurrence rates were also significantly higher in the *H. pylori*+/CIMP+ cases than in the *H. pylori*+/CIMP- cases when serum was examined ($P < 0.05$). There were no significant differences in survival rates between *H. pylori*+/CIMP+ and *H. pylori*+/CIMP- cases examined in serum ($P > 0.05$). Therefore, aberrant DNA hypermethylation may be frequently associated with chronic inflammation, but not with *H. pylori* infection. Modulation levels of pleiotropic regulators suggested that DNA methylation plays an important role in host response to *H. pylori* infection. *H. pylori* promotes genetic instability via decreasing expression of DNA repairing genes[22,23]. We observed that CIMP+ in conjunction with *H. pylori* infection was associated with metastasis and recurrence of GC, but not the survival rate after the 2-year follow-up period.

In summary, our study shows that promoter methylation in serum DNA was highly specific and that serum and tissue samples yielded similar results. Therefore, CIMP detection in serum, rather than in tumor tissues, may be used as a reliable index for predicting the clinical course of patients with GC.

This study is limited by a relatively small number of patients, cancer-related genes, as well as follow-up period of less than 2 years. Thus, larger-scale multi-gene studies with an extended follow-up period are needed to validate our results.

COMMENTS

Background

Gastric cancer (GC) is one of the most common human tumors and is the second-leading cause of cancer-related deaths worldwide. GC remains a major clinical challenge because it has a poor prognosis and limited treatment options due to its relative resistance to radiotherapy and chemotherapy. Aberrant DNA methylation in gastric biopsies from *Helicobacter pylori* (*H. pylori*)+ patients has been shown to be correlated with a greater GC risk. The relationship between *H. pylori* and methylation of multiple genes has been demonstrated, but little attention has been paid to the association of the CpG island methylator phenotype (CIMP) and *H. pylori* with respect to GC or the relationship between CIMP in serum and the presence of *H. pylori* in GC.

Research frontiers

DNA methylation is an enzyme-induced chemical modification that occurs in cytosine-guanine dinucleotide-rich areas (CpG islands) in the gene promoter regions. Aberrant promoter hypermethylation is an important mechanism for loss of gene function in tumors including GC. The methylation pattern of multiple genes can provide useful information and an overall picture of epigenetic alterations. The hypermethylated subtype in tumors is termed as the CIMP, where multiple genes are concurrently methylated. *H. pylori* infection is found associated with gene promoter hypermethylation and gene-type-specific methylation profiles that are involved in the multistep process of carcinogenesis. Methylation levels in patients with GC are one to two orders of magnitude greater in *H. pylori*+ individuals than in *H. pylori*- individuals.

Innovations and breakthroughs

In the present study, the authors focused on the relationship between *H. pylori* infection and CIMP status in GC risk. The authors analyzed the relationship between serum CIMP of 75 patients with GC and *H. pylori* infection in GC. The examined genes were *APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK*, and *E-cad*, which were selected because they had been found to be frequently methylated in GC and other malignancies. This study evaluated the clinical significance of serum CIMP and *H. pylori*-infection levels for the prediction of GC progression.

Applications

It was found in this study that *H. pylori*+/CIMP+ cases were associated with higher metastasis and recurrence rates than *H. pylori*+/CIMP- cases. CIMP detection in serum, rather than in tumor tissues, may be used as a reliable index for predicting the clinical course of patients with GC.

Terminology

Epigenetic changes: Heritable changes in gene structure without changing the gene sequence; CpG islands: CpG rich areas located in the promoter regions of many genes; CpG island methylation: The addition of a methyl group to a cytosine residue that lies next to guanine within CpG dinucleotides; CIMP: The hypermethylated subtype in tumors, where multiple genes are concurrently methylated.

Peer review

This manuscript was well designed and executed, and data were summarized well.

REFERENCES

1 **Hohenberger P**, Gretschel S. Gastric cancer. *Lancet* 2003; **362**: 305-315

2 **An C**, Choi IS, Yao JC, Worah S, Xie K, Mansfield PF, Ajani JA, Rashid A, Hamilton SR, Wu TT. Prognostic significance of CpG island methylator phenotype and microsatellite instability in gastric carcinoma. *Clin Cancer Res* 2005; **11**: 656-663

3 **Baylin SB**. DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol* 2005; **2** Suppl 1: S4-11

4 **Park SY**, Yoo EJ, Cho NY, Kim N, Kang GH. Comparison of CpG island hypermethylation and repetitive DNA hypomethylation in premalignant stages of gastric cancer, stratified for Helicobacter pylori infection. *J Pathol* 2009; **219**: 410-416

5 **Costello JF**, Frühwald MC, Smiraglia DJ, Rush LJ, Robertson GP, Gao X, Wright FA, Feramisco JD, Peltomäki P, Lang JC, Schuller DE, Yu L, Bloomfield CD, Caligiuri MA, Yates A, Nishikawa R, Su Huang H, Petrelli NJ, Zhang X, O'Dorisio MS, Held WA, Cavenee WK, Plass C. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nat Genet* 2000; **24**: 132-138

6 **Toyota M**, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999; **96**: 8681-8686

7 **Toyota M**, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 1999; **59**: 5438-5442

8 **Wang YC**, Yu ZH, Liu C, Xu LZ, Yu W, Lu J, Zhu RM, Li GL, Xia XY, Wei XW, Ji HZ, Lu H, Gao Y, Gao WM, Chen LB. Detection of RASSF1A promoter hypermethylation in serum from gastric and colorectal adenocarcinoma patients. *World J Gastroenterol* 2008; **14**: 3074-3080

9 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131

10 **Shin CM**, Kim N, Jung Y, Park JH, Kang GH, Kim JS, Jung HC, Song IS. Role of Helicobacter pylori infection in aberrant DNA methylation along multistep gastric carcinogenesis. *Cancer Sci* 2010; **101**: 1337-1346

11 **Nakajima T**, Yamashita S, Maekita T, Niwa T, Nakazawa K, Ushijima T. The presence of a methylation fingerprint of

Helicobacter pylori infection in human gastric mucosae. *Int J Cancer* 2009; **124**: 905-910
12 **Peterson AJ**, Menheniott TR, O'Connor L, Walduck AK, Fox JG, Kawakami K, Minamoto T, Ong EK, Wang TC, Judd LM, Giraud AS. Helicobacter pylori infection promotes methylation and silencing of trefoil factor 2, leading to gastric tumor development in mice and humans. *Gastroenterology* 2010; **139**: 2005-2017
13 **Maekita T**, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, Arii K, Kaneda A, Tsukamoto T, Tatematsu M, Tamura G, Saito D, Sugimura T, Ichinose M, Ushijima T. High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res* 2006; **12**: 989-995
14 **Niwa T**, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, Ichinose M, Tatematsu M, Ushijima T. Inflammatory processes triggered by Helicobacter pylori infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res* 2010; **70**: 1430-1440
15 **Chan AO**, Issa JP, Morris JS, Hamilton SR, Rashid A. Concordant CpG island methylation in hyperplastic polyposis. *Am J Pathol* 2002; **160**: 529-536
16 **Eads CA**, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, Peters JH, DeMeester SR, DeMeester TR, Skinner KA, Laird PW. Epigenetic patterns in the progression of esophageal adenocarcinoma. *Cancer Res* 2001; **61**: 3410-3418
17 **Weisenberger DJ**, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, Kang GH, Widschwendter M, Weener D, Buchanan D, Koh H, Simms L, Barker M, Leggett B, Levine J, Kim M, French AJ, Thibodeau SN, Jass J, Haile R, Laird PW. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006; **38**: 787-793
18 **Feinberg AP**, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 2006; **7**: 21-33
19 **Zhou X**, Popescu NC, Klein G, Imreh S. The interferon-alpha responsive gene TMEM7 suppresses cell proliferation and is downregulated in human hepatocellular carcinoma. *Cancer Genet Cytogenet* 2007; **177**: 6-15
20 **Nakajima T**, Maekita T, Oda I, Gotoda T, Yamamoto S, Umemura S, Ichinose M, Sugimura T, Ushijima T, Saito D. Higher methylation levels in gastric mucosae significantly correlate with higher risk of gastric cancers. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2317-2321
21 **Kusano M**, Toyota M, Suzuki H, Akino K, Aoki F, Fujita M, Hosokawa M, Shinomura Y, Imai K, Tokino T. Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with the CpG island methylator phenotype and an association with Epstein-Barr virus. *Cancer* 2006; **106**: 1467-1479
22 **Sepulveda AR**, Yao Y, Yan W, Park DI, Kim JJ, Gooding W, Abudayyeh S, Graham DY. CpG methylation and reduced expression of O6-methylguanine DNA methyltransferase is associated with Helicobacter pylori infection. *Gastroenterology* 2010; **138**: 1836-1844
23 **Touati E**. When bacteria become mutagenic and carcinogenic: lessons from H. pylori. *Mutat Res* 2010; **703**: 66-70

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