Expression pattern and clinical significance of DNA methyltransferase 3B variants in gastric carcinoma

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Abstract. The aim of this study was to detect the expression pattern of DNA methyltransferase 3B (*DNMT3B*) variants in primary gastric cancer (GC) and to explore the clinical significance of *DNMT3B* variants in gastric carcinogenesis. Specific polymerase chain reaction (PCR) primer sets were designed to distinguish individual *DNMT3B* variants according to their splicing patterns. Expression levels of *DNMT3B* variants were assessed by quantitative real-time RT-PCR in gastric cancer tissue, normal gastric mucosae and GC cell lines. The relationship between the expression patterns of the *DNMT3B* variants and corresponding clinical information was analyzed by observing the expression levels of different variants in the tumors. These results demonstrate that *DNMT3B* overexpression is related to late phase invasion (P=0.029) and intestinal type (P=0.012) in GC. DNMT3B3 expression was higher in normal tissue, compared to tumor tissue (P=0.033). In contrast, only 18, 32 and 35% of the patient tumors overexpressed *DNMT3B1*, *DNMT3B4* and *DNMT3B5*, respectively. While taking into account environmental factors (*H. pylori*, Epstein-Barr virus infection), *H. pylori* infection elevated *DNMT3B1* and *DNMT3B3* variants in tumors, while increasing *DNMT3B4* in both tumor and non-cancerous tissues. Our findings indicated that the expression of *DNMT3B3* is the major splice variant in normal gastric mucosae and may be affected by *H. pylori* infection. Elevated *DNMT3B* variants may influence the progression of gastric cancer and may possibly be a powerful indicator for the disease.

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Introduction

Gastric carcinoma (GC) is a prevalent cancer and is one of the main causes of death in the Chinese population. Despite advances in the detection and treatment of this disease, the mortality rate of GC remains high. In gastric carcinomas, tumor suppressor genes (TSGs) are more frequently inactivated by aberrant DNA methylation than by mutations (1). DNA methyltransferases (DNMTs), DNMT1, DNMT3A and DNMT3B have been identified as DNA methylating enzymes in eukaryotic cells (2,3). DNMT1 has been identified as a maintenance methyltransferase, which is responsible for maintaining pre-existing methylation patterns during DNA replication (4,5). Dnmt3a and Dnmt3b are essential for *de novo* methylation in embryonic stem (ES) cells and during embryonic development in mice (6). Several splice variants of DNMT3B have been identified both in mouse and human tissues (3,7,8). These splice variants have the potential to encode proteins with altered activities. Numerous studies have demonstrated overexpression of DNMTs in a variety of cancers, suggesting these proteins may be involved in carcinogenesis (8,9). Specifically, the depletion of DNMT1 and DNMT3B in tumor cells indicates that they may play an important role in tumorigenesis (10,11). Overexpression of DNMT3B, but not DNMT1 or DNMT3A, has been found in several cancer types (8,12,13), suggesting that DNMT3B plays an important role in the development of aberrant promoter methylation during tumorigenesis. However, DNMT3B isoforms show different biochemical properties and expression patterns and therefore, may have distinct functions in tumorigenesis.

To the best of our knowledge, six alternatively spliced forms of DNMT3B have been identified. DNMT3B1 and 3B2 share the same translation initiation site and both contain all of the highly conserved motifs (I, IV, VI, VIII, IX and X), as well as the target recognition domain (TRD) in the catalytic domain (8). DNMT3B1 is the major isoform detected in ES cells and it is likely to have a redundant function in carrying out *de novo* methylation of provirus DNA (6). The DNMT3B2 variant lacks exon 10 (7), but it has been shown to be catalytically active (14). DNMT3B3-6 are the result of alternative splicing (15). DNMT3B3 lacks exons 10, 21, and 22, as well

as the less conserved motif VII, the more conserved motif VIII, the target recognition domain TRD and the nine amino acids of motif IX. However, it still may correlate with D4Z4 and satellites 2 and 3 repeat sequences. All of the identified DNMT3B target sequences were methylated in cells that predominantly expressed DNMT3B3 (8,15). DNMT3B4 and DNMT3B5, without conserved methyl-transferase motifs IX and X, probably lack DNA methyl-transferase activity; nevertheless, overexpression of DNMT3B4 may result in DNA methylation on satellite 2 in pericentromeric heterochromatin (8,12). DNMT3B6 is missing exon 10, but has an extra 12 amino acids in the N-terminal compared to DNMT3B1. It is weakly associated with the overall frequency of methylation (16).

Several studies have shown DNMT3B variant expression in a variety of cancers, suggesting that they may be involved in tumorigenesis. *DNMT3B1*, *DNMT3B2* and *DNMT3B4* were the most frequently detected transcripts in primary non-small cell lung cancer (17). *DNMT3B3* and *DNMT3B4* are major splice variants in hepatocellular carcinoma (12). DNMT3B2 and DNMT3B3 perform an essential role in cancer cell survival in colon cancer (11). These results implied that DNMT3B may be a carcinogenic factor. Gastric mucosa infected by *Helicobacter pylori* (*H. pylori*) is a common event and is shown to induce aberrant methylation in gastric carcinoma (18). Epstein-Barr (EB) virus infection is another important carcinogenic factor in gastric carcinogenesis (19). To clarify the molecular mechanisms underlying the role of DNA methyltransferases during human gastric carcinogenesis, we examined the expression levels of splice variants of *DNMT3B* in gastric tumors and non-cancerous gastric mucosae. This is a first attempt to acquire insight into the possible relationship between the expression pattern of DNA methyltransferase 3B variants and gastric carcinoma progression.

Materials and methods

Tissue samples and cell lines. The five studied human cell lines were obtained from the cell bank of the Chinese Academy of Science and cultured as recommended. Four human gastric carcinoma cell lines (AGS, MCG803, SGC-7901 and BGC-823) and one immortalized human gastric normal cell line (GES-1) were cultured in RPMI-1640 (Life Technologies, Inc., Rockville, MD) containing 10% fetal bovine serum in 5% CO_2 incubation at 37˚C. Thirty-four cases of surgically resected GC and adjacent non-cancerous tissue specimens were obtained from the First Hospital of Nanjing, affiliated with Nanjing Medical University, from May 2008 to March 2009. The clinicopathological features are shown in Table II. All the GC samples were diagnosed by a single pathologist and appropriate consent was obtained from each patient. Paired samples of primary GC and non-cancerous normal tissues were obtained from each patient. Ten women and 24 men were included and their ages ranged from 41 to 83 years. Intestinal and diffuse types were histologically typed according to the Lauren's classification (20). Tumor differentiation, depth of invasion, lymph node metastasis, vessel invasion and nerve invasion were judged by routine pathological diagnosis.

Reverse-transcription (RT)-PCR. Total RNA was isolated using TRIzol Reagent (Invitrogen, USA) and first-strand cDNA was prepared from total RNA with Oligo $(dT)_{18}$ primer and AMV reverse transcriptase (BioFlux, Japan) according to the manufacturer's instructions. cDNA derived from human normal gastric mucosae cell lines (GES-1) was used as a control. Subsequent PCR with the primer sets (forward, 5'-CCT GCT GAA TTA CTC ACG CCC C-3' and reverse, 5'-GTC TGT GTA GTG CAC AGG AAA GCC-3') (8) amplified all of the splice variants in the C-terminal catalytic domain of the *DNMT3B* gene. For visual size confirmation, PCR products were separated electrophoretically on 2% agarose gel.

Splice variant-specific quantitative RT-PCR. Complementary DNA (prepared as previously described) was subject to quantitative real-time polymerase chain reaction (qPCR) for *DNMT3B* variants expression using a SYBR-Green PCR kit (Takara, Japan) under the following cycling conditions: PCR reactions were performed in a 50 μl volume with 5 U polymerase (Takara) and cDNA samples equivalent to 1 ng of RNA. SYBR-Green at a dilution of 1:20,000 was included in each reaction for the relative quantification using the ABI 7300 sequence detection system (Applied Biosystems, USA). To normalize the cDNA input among samples, ß-actin was quantified and used as an endogenous standard. The relative level of expression of each variant among the different tissues was then calculated in relation to the amount of ß-actin (ABI PRISM 7300 Detection System, USA). Oligonucleotide primer sets specific for *DNMT3B* variants were designed. Each primer spanned the splice variant-specific exon-exon boundary (see Table I). Quantitative PCRs were performed in duplicate for each sample-primer set and the mean of the two experiments was used as the relative quantification value. At the end of 40 amplification cycles, the reaction products were electrophoresed on a 3% agarose gel, to confirm that non-specific products were not obtained during amplification.

Detection of H. pylori and EB virus in GC tissues. The presence of *H. pylori* and EB virus infection was determined by PCR for *H. pylori* 16S rDNA and the EBNA-1 region, respectively. The primers for *H. pylori* were as follows: sense: 5'-GCC AAT GGT AAA TTA GTT-3', antisense: 5'-CTC CTT AAT TGT TTT TAC-3'. The primers for EB virus were as follows: sense: 5'-GTC ATC ATC ATC CGG GTC TC-3', antisense: 5'-TTC GGG TTG GAA CCT CCT TG-3'. The DNA templates from the samples obtained from the GC patients were extracted using the Biospin Tissue Genomic DNA Extraction Kit (BioFlux). *H. pylori* infection was also determined by Giemsa-stained histological staining. Cases that showed positivity in any assay were regarded as *H. pylori*-positive or EB virus-positive.

Statistical analysis. Correlation between the frequency of *DNMT3B* splice variants and pathological features were analyzed with the Chi-square test, using SPSS 13.0 software for Windows. Differences were analyzed by the Fisher's exact test. The results for the *DNMT3B* variants mRNA expression level are presented as mean ± SE, association were tested

Table I. Primers and amplicon size of genes analyzed by real-time quantitative PCR.

DNMT3B variants	Primers sequence	Amplicon size bp
DNMT3Bs	5'-GACTTGGTGATTGGCGGAA-3' (sense) 5'-GGCCCTGTGAGCAGCAGA-3' (antisense)	270
DNMT3B1	5'-ATAAGTCGAAGGTGCGTCGT-3' (sense) 5'-GGCAACATCTGAAGCCATTT-3' (antisense)	203
DNMT3B3	5'-GATGAACAGGATCTTTGGCTTT-3' (sense) (12) 5'-GCCTGGCTGGAACTATTCACA-3' (antisense)	163
DNMT3B4	5'-CGGGATGAACAGTTAAAGAAAGTAC-3' (sense) (12) 5'-CCAAAGATCCTTTCGAGCTC-3' (antisense)	140
DNMT3B5	5'-CCAAGGATCTTTGGCTTTC-3' (sense) 5'-GAGGGAACTGAGGTACACG-3' (antisense)	277
ß-actin	5'-AAAGACCTGTACGCCAACAC-3' (sense) 5'-GTCATACTCCTGCTTGCTGAT-3' (antisense)	220

using Student's t-test. Results were considered statistically significant at $P<0.05$.

Results

Identification of DNMT3B splicing variants in gastric carcinoma cell lines and tissues. To examine the potential mechanisms of aberrant *de novo* DNA methylation in gastric carcinogenesis, we amplified the cDNA encoding *DNMT3B* from a normal gastric mucosae cell line and several gastric cancer cell lines. Using a set of primers specific for the alternative splicing position (Fig. 1A), we amplified *DNMT3B* variant mRNA by semi-quantitative RT-PCR. Based on the RT-PCR analysis, *DNMT3B1*, *3B3*, *3B4* and *3B5* were detected as major variants (Fig. 1B), consistent with a previous study that reported these four splice variants were significantly expressed in human testis tissue (8). *DNMT3B6* was not detected after >35 cycles of amplification (except AGS cell line) and *DNMT3B2* could not be distinguished by the set of PCR conditions due to the use of primers that were non-specific (Fig. 1A). We then detected a *DNMT3B* variant expression pattern in gastric carcinoma and adjacent non-tumor tissues. These results are consistent with those obtained using the GC cell lines (Fig. 1C). To explore whether the expression pattern and expression level of *DNMT3B* variants is involved in gastric carcinogenesis, *DNMT3B*, *3B1*, *3B3*, *3B4*, *3B5* were evaluated by qPCR in GC cases.

Expression of DNMT3B variants in GCs and the corresponding normal gastric mucosae. The mRNA expression levels of the *DNMT3B* variants were detected by qPCR on 34 pairs of matched tumor and non-tumor tissues specimens, as well as on 5 normal gastric tissues samples. The frequency of over-expression (≥2-fold) for *DNMT3B* was 55.9% in GCs. The mRNA expression levels of *DNMT3B* in GC were higher than that in non-tumor tissues ($P=0.002$) and normal gastric tissue ($P=0.003$) (Fig. 2). Because *DNMT3B* variant expression in human tumor tissue may contribute to tumorigenesis, we evaluated the frequency and level of expression of *DNMT3B* variants in the patient samples. The frequency of over-expression (≥2-fold) of the variants *DNMT3B1*, *3B3*, *3B4* and *3B5* was 17.6, 32.4, 32.4 and 35.3%, respectively, in tumor tissues compared to matched non-tumor tissues. Importantly,

Table II. Correlation of tumor *DNMT3B* variant expression level with clinicopathological features of patients in 34 GC cases.

Parameters	Cases	T>N	T≤N	P-value
Age				0.724
≤60	12	6	6	
>60	22	13	9	
Gender				1
Male	24	13	11	
Female	10	6	4	
Differentiation				0.091
Poorly	19	8	11	
Moderately/Well	15	11	4	
Lauren type				0.012[a]
Intestine	22	16	6	
Diffuse	12	3	9	
Invasive degree				0.029[a]
Early stage	4	0	4	
Progression	30	19	11	
Lymphnode metastasis				0.724
Yes	22	13	9	
No	12	6	6	

[a]Significant difference.

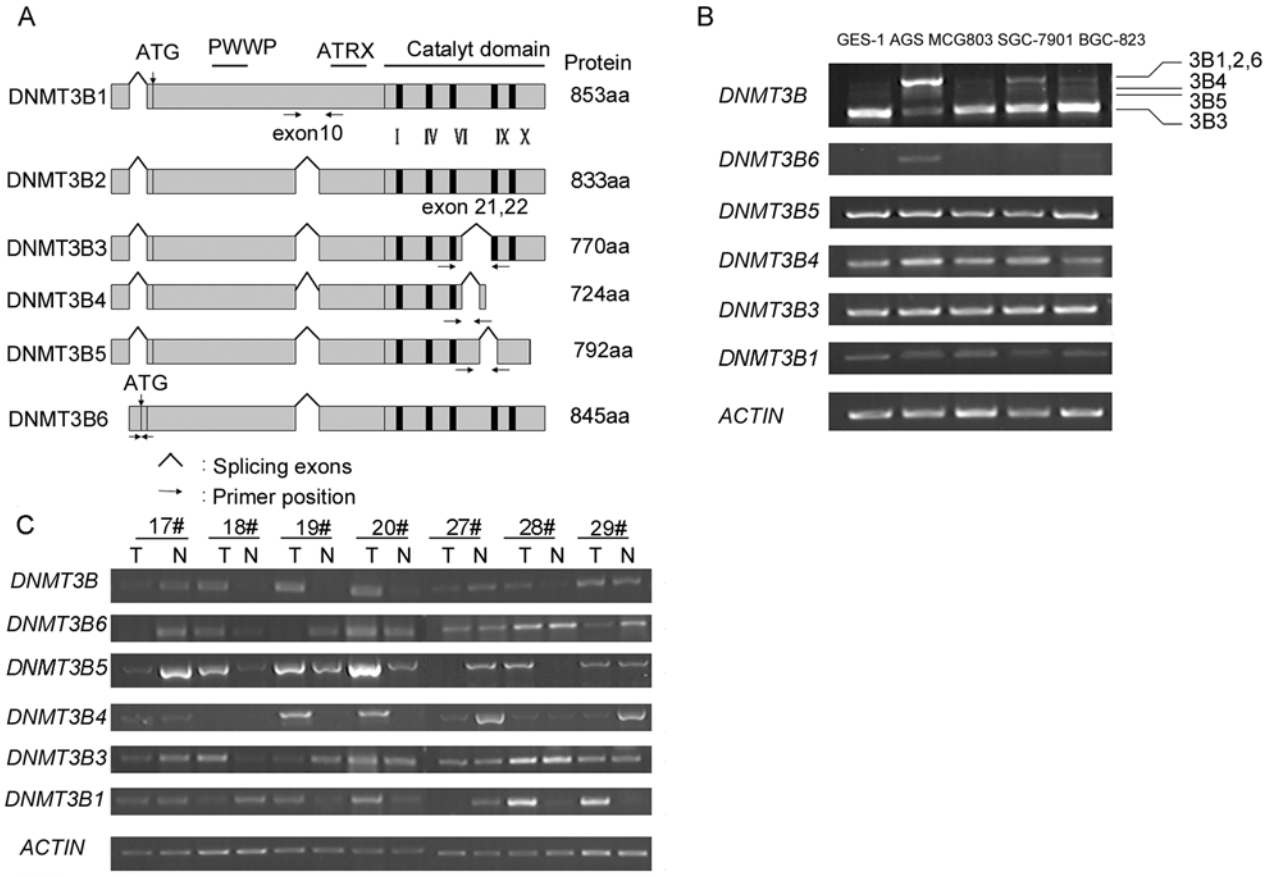

Figure 1. *DNMT3B* variant expression analyzed in gastric cell lines, gastric cancer tissue, and adjacent non-tumor samples. (A) Schematic diagrams of the human *DNMT3B* variants: the position of the conserved PWWP domain, ATRX homology domain, the methyltransferase motifs (I, IV, VI, and IX, X) and sites of alternative splicing are shown. Numbers represent amino acids. The arrow represents the position where we design the specific primers for the different variants. (B) RT-PCR of 5 gastric cell lines (including one normal gastric cell line GES-1, four GC cell lines). Upper panel, *DNMT3B* variants were amplified in four specific band as is shown on the left using the special primer. The upper band is derived from *DNMT3B1*, *3B2*, *3B6*, the middle two bands are derived from *DNMT3B4*, *DNMT3B5*, respectively; the bottom band is derived from *DNMT3B3*. The middle panels, five variants (*DNMT3B1*, *3B3*, *3B4*, *3B5 3B6*) were amplified by the specific primers (35 cycles in PCR condition). Amplification of the *ACTIN* cDNA served as a loading control, demonstrating equal amounts of input cDNA from each cDNA source (bottom panel). (C) RT-PCR of gastric cancer tissue (T) and non-tumor tissue (N). *DNMT3B* and 5 variants were amplified in 35 cycles (except *DNMT3B6*, >40 cycles). Amplification of the *ACTIN* cDNA served as a loading control, demonstrating equal amounts of input cDNA from each cDNA source (bottom panel).

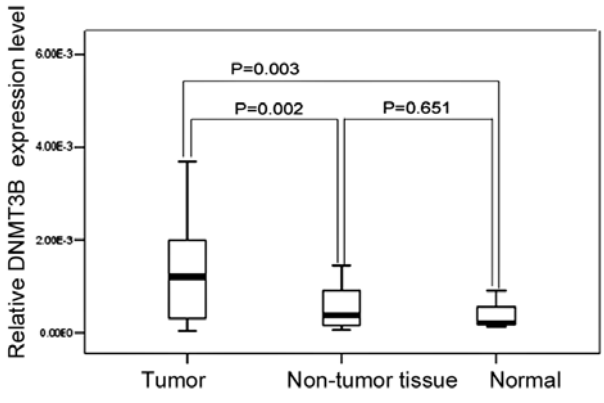

Figure 2. Relative *DNMT3B* mRNA expression levels were determined by qPCR. The top and bottom horizontal lines of the box indicate the 25th and 75th percentiles, respectively. The bold lines within the box indicate the median values. The top and bottom horizontal bars indicate the maximum and minmum values, respectively. The level of *DNMT3B* in gastric tumor tissues was significantly higher when compared with their non-tumor counterpart (P=0.002) and normal gastric mucosae from healthy individuals (P=0.003). There was no statistical significance between the level of DNMT3B in non-tumor specimen obtained from patients with gastric cancer and that in gastric mucosae from healthy individuals (P=0.651).

DNMT3B3 was the major expressed variant in the normal gastric tissues. The expression level of *DNMT3B3* was higher in normal tissue than in tumor (P=0.033) and non-tumor tissue (P=0.037); however, there was no significant difference in the expression levels among the other variants (*DNMT3B1*, *DNMT3B4* and *DNMT3B5*) in tumor tissue, non-tumor tissue or normal tissue (Fig. 3).

Correlation between the frequency of DNMT3B variants and clinicopathological variables. In order to correlate the expression pattern of *DNMT3B* variants and clinicopathological features of patients with gastric cancer, patients were categorized into two groups according to the expression level difference (≥2-fold) of *DNMT3B* variants. Thirty-four patients were classified into 'up-regulated' and 'down-regulated' groups based on the variants expression levels. The correlation between the frequency of *DNMT3B* variants and clinicopathological variables of the patients in the two groups were analyzed. Overexpression of *DNMT3B* was significantly correlated with lymph node metastasis (pTNM) stage (P=0.045), Lauren type (P=0.012) and depth of invasion (P=0.029) (Table II). Most of the patients in the advanced

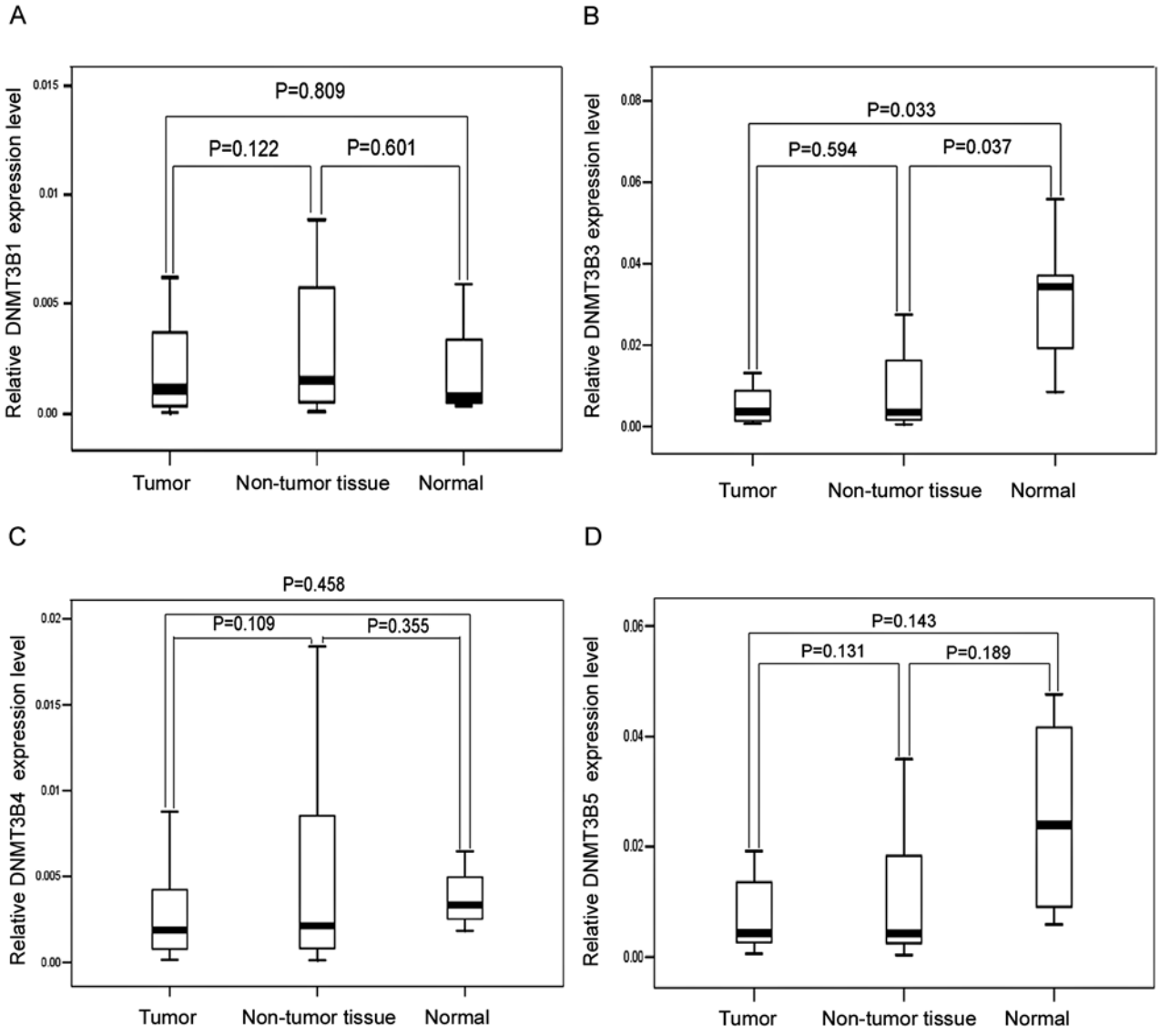

Figure 3. Relative *DNMT3B1*, *3B3*, *3B4*, *3B5* expression levels were determined by qPCR from RNA preparation obtained from 34 pairs of gastric cancer samples, their matched adjacent non-tumor samples and 5 normal gastric tissues from healthy donors. (A) Relative *DNMT3B1* expression level. (B) Relative *DNMT3B3* expression level. (C) Relative *DNMT3B4* expression level. (D) Relative *DNMT3B5* expression level.

pTNM stage (75% cases, 12/16) and those with intestinal type gastric cancer (72.72% cases, 16/22) showed high *DNMT3B* expression levels, whereas no patients in the early stage of invasive degree exhibited high *DNMT3B* expression. These data suggested that *DNMT3B* may play an important role in the progression of gastric cancer. In addition to this, female patients carried a higher frequency of *DNMT3B3* expression than that of male (P=0.045). Also, the expression of *DNMT3B4* was more frequent in lymph vessel invasion tumors (P=0.045) (Table III). However, there was no significant relationship between the other variants (*DNMT3B1* and *DNMT3B5*) and pathological features.

Correlation between expression of DNMT3B variants and environment factors (H. pylori and EB virus infection). We then analyzed the *H. pylori* and EB virus infection status of patients and evaluated the relationship between these two carcinogenic factors and the level of *DNMT3B* variant in GC cases. Of the 34 GC cases, 13 samples were *H. pylori*-positive and 21 samples were *H. pylori*-negative; 18 samples were EB virus-positive and 16 samples were EB virus-negative. The expression level of *DNMT3B1*, *3B3* and *3B4* was higher in GC tumor tissues with *H. pylori* infection than in non-tumor tissues without *H. pylori* infection (Fig. 4A). The expression level of *DNMT3B4* was higher in cases with *H. pylori* infection than in those without *H. pylori* infection (Fig. 4B). The *DNMT3B* expression was lower in both tumor tissue and non-tumor tissue infected with EB virus infection than in these tissues without EB virus infection (Fig. 4C and D), although there was no statistical correlation between *DNMT3B1*, *3B3*, *3B4* and *3B5* expression and EB virus factor.

Discussion

Aberrant DNA methylation is a common epigenetic event in gastric carcinogenesis; however, DNA methyltransferases play an important role in the initiation and maintenance of cytosine methylation in the human genome. Given that the role

Table III. Correlation of *DNMT3B1*, *3B3*, *3B4*, *3B5* expression level with clinicopathological features in 34 GC cases.

	DNMT3B1		*DNMT3B3*		*DNMT3B4*		*DNMT3B5*	
Parameters	T>N	P-value	T>N	P-value	T>N	P-value	T>N	P-value
Gender		1		0.045[a]		0.692		0.714
Male	4		5		7		8	
Female	2		6		4		4	
Differentiation		1		0.151		0.475		0.288
Poorly	3		4		5		5	
Moderately/Well	3		7		6		7	
Lauren type		0.389		0.252		0.705		0.14
Intestine	5		9		8		10	
Diffuse	1		2		3		2	
Lymph node metastasis		0.641		1		0.705		0.711
Yes	3		7		8		7	
No	3		4		3		5	
Lymph vessel invasion		0.328		0.232		0.045[a]		0.714
Yes	3		5		6		4	
No	3		6		5		8	

[a]Significant difference.

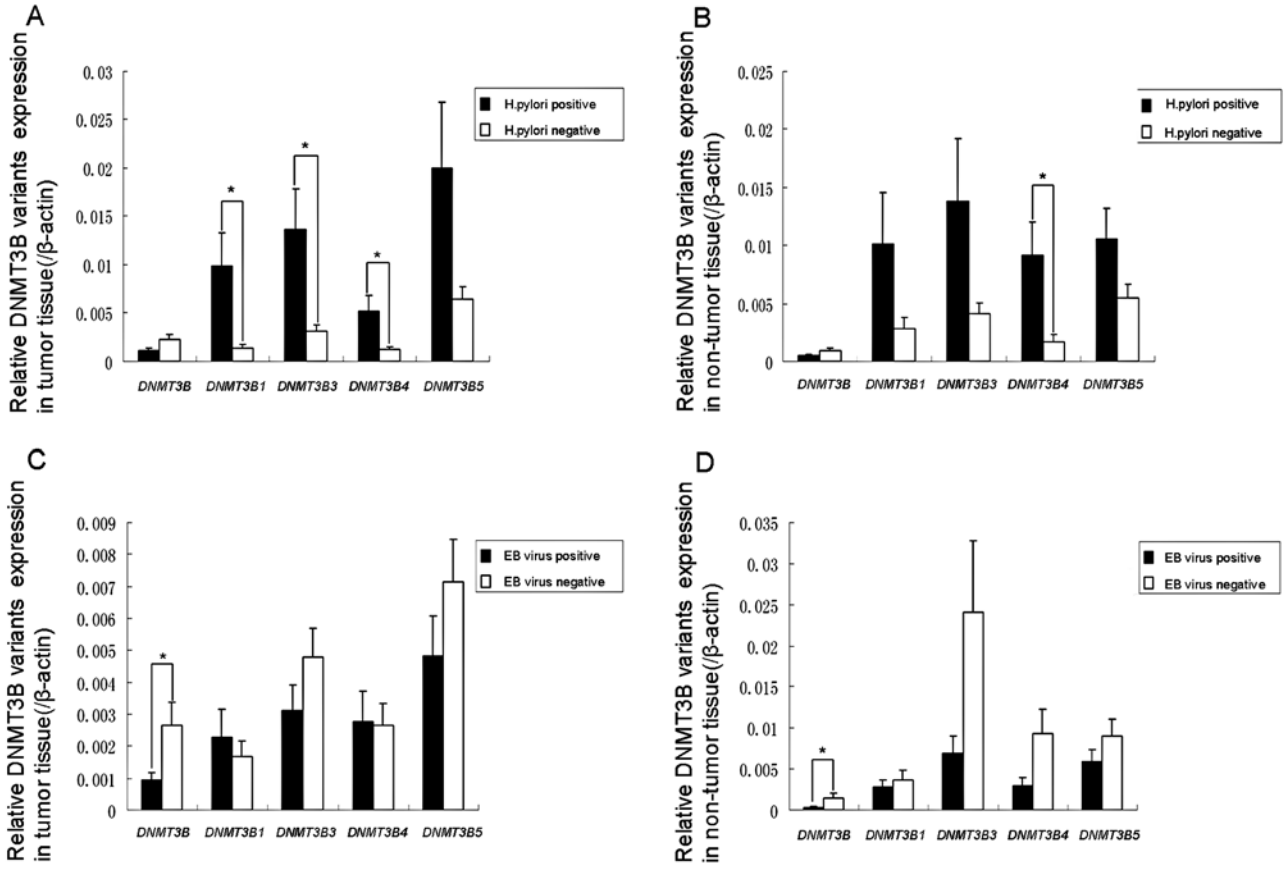

Figure 4. The mRNA expression levels of *DNMT3B* variants (*DNMT3B*, *3B1*, *3B3*, *3B4*, *3B5*) in gastric cancer tissue and non-cancerous gastric mucosae of GC patients with and without *H. pylori* and EB virus infection. (A) mRNA expression levels of *DMNT3B* variants in gastric tissue with and without *H. pylori* infection. (B) mRNA expression levels of *DMNT3B* variants in non-cancerous tissue with and without *H. pylori* infection. (C) mRNA expression levels of *DMNT3B* variants in gastric tissue with and without EB virus infection. (D) mRNA expression levels of *DMNT3B* variants in non-canerous tissue GC patients with and without EB virus infection. *Statistical significance (P<0.05).

of DNMT3B variants relates to *de novo* DNA methylation, these enzymes not only possess distinct activities (3,21), but also interact with each other in complex biological processes to regulate patterns of DNA methylation in the human genome (22-24). Ostler *et al* suggested that the abnormal patterns of DNA methylation present in nearly all cancer cells may be regulated in part by the presence of catalytically inactive DNMT3B proteins (25). Levels of DNMT3B protein is significantly elevated in hypermethylated breast cancer cell lines and these cells exhibit aberrantly increased DNMT activity, implying that DNMT3B overexpression contributes to a hypermethylated phenotype (26). *DNMT3B* expression is also essential for a fully manifested neoplastic phenotype in lung epithelial cells (27) and mouse embryonic fibroblasts (MEFs) (28). To characterize the expression of the *DNMT3B* gene within gastric cancers, we systematically amplified *DNMT3B* variants from tumor, non-cancerous tissues and normal gastric mucosae.

DNMT3B variants mRNA levels were evaluated by quantitative real-time PCR and showed different expression patterns between GCs and their matching non-cancerous mucosae. Overexpression of *DNMT3B* was observed in 56% of the 34 patients, demonstrating that the expression of *DNMT3B* is related to gastric tumorigenesis. In contrast, only 18% of the patient tumors overexpressed *DNMT3B1* and *DNMT3B4* and *DNMT3B5* were overexpressed in 32 and 35% patients, respectively. Conversely, 68% of the 34 patients showed the common expression of *DNMT3B3* in normal gastric mucosae. Searching for the relationships between *DNMT3B* variant expression level and clinical, pathological and biological parameters, we found significant relationships between *DNMT3B* overexpression and histological grade III-IV (P=0.045), invasion late phase (P=0.029) and intestinal type (P=0.012), suggesting that *DNMT3B* may be a marker of both tumor aggressiveness and proliferation in GC. Further evaluation of detectable variants of *DNMT3B* failed to explore the clinicopathological significance of *DNMT3B1*, *DNMT3B4* and *DNMT3B5*. Expression levels of *DNMT3B6* mRNA in all tissue samples are too low to be detected by this quantitative RT-PCR system. Significantly, we demonstrated that the expression of *DNMT3B3* represents the majority of the *DNMT3B* transcripts in normal gastric mucosae.

Previous studies have reported that *DNMT3B3* is ubiquitously expressed in normal human tissues (8) and is the major variant in normal liver tissues (12). Structurally, *DNMT3B3* possesses conserved methyltransferase motifs I, IV, VI, IX and X, providing a clue to its potential DNA methyltransferase activity; however, murine DNMT3B3 did not show DNA methylation in an *in vitro* study (14). Because the expression of variant 3 of *DNM3B* is associated with DNA methylation on pericentromeric satellite regions in human liver tissues via competition with *DNMT3B4* for targeting to pericentromeric satellite regions (12), we hypothesized that *DNMT3B3*, which is a common variant of *DNMT3B*, is necessary for methylating certain specific site in normal gastric tissues. In addition to *DNMT3B3*, others variants, including *3B1*, *3B4* and *3B5*, have no significantly different expression pattern among group tissues, non-cancerous and normal gastric mucosae.

H. pylori infection status is a significant risk factor for both intestinal and diffuse cancer types in China. Findings of Nakajima *et al* indicated that the methylation profile induced by *H. pylori* infection can persist even after clearance of *H. pylori* infection and eradication of *H. pylori* decreased the incidence of individuals with methylation (18). We hypothesize that this abnormal methylation may be due to the increased activity of *de novo DNMT3B* by a carcinogenic factor. To gain insight into how *H. pylori* infection induces aberrant methylation via *de novo* methyltransferases, we analyzed mRNA expression levels of *DNMT3B* variants in gastric mucosa samples both with and without *H. pylori* infection. The expression level of *DNMT3B1* and *3B3* was higher in tumors than in matched non-cancerous gastric tissues. Epstein-Barr virus infection is considered to be another important carcinogenic factor in gastric carcinogenesis (29). Recent studies showed that the CpG island methylator phenotype (CIMP) status is related to EB virus infection in gastric mucosae (30). In these studied GC cases with EB virus infections, however, there was no statistical correlation between the expression of *DNMT3B* variants and EB virus factor, including *DNMT3B* and, *3B3*, which show increased expression in cases of *H. pylori* infection. In EB virus infection cases, decreased *DNMT3B* expression was observed in both tumor and non-tumor tissue. These data implied that abnormalities in epigenetic regulation induced by *H. pylori* infection also need to be investigated. *DNMT3B3* is the major variant and is affected significantly by *H. pylori* infection, implying that *DNMT3B3* may play an important role in DNA methylation.

In this study, we provided a comprehensive overview of the expression profiles of several *DNMT3B* variants in a panel of GC tumors. We found that *DNMT3B* was most frequently overexpressed in primary gastric tumors and expression of this variant was related to clinicopathological significance. *H. pylori* infection may be a carcinogenic risk factor that contributes to the elevated expression of *DNMT3B* variants. Further study of expression patterns of different variants involved in the DNMT3B pathway and their relationship with gene silencing or specific methylated sites during gastric tumorigenesis, is necessary.

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