DNMT3B 579 G>T promoter polymorphism and risk of esophagus carcinoma in Chinese

Hong Fan, Dong-Sheng Liu, Shu-Hong Zhang, Jia-Bo Hu, Feng Zhang, Zhu-Jiang Zhao

AIM: To investigate the relationship between 579 G>T polymorphisms in the DNMT3B gene, which is involved in de novo methylation and associated with the risk of esophagus cancer (EC) in Chinese.

METHODS: DNMT3B 579 G>T genotypes were determined by PCR-RFLP in 194 EC patients and 210 healthy controls matched for age and sex, who did not receive radiotherapy or chemotherapy for newly diagnosed and histopathologically confirmed EC.

RESULTS: In control subjects, the frequency of T/T and G/T genotypes, and T and G alleles was 80.5%, 19.0%, 90.0% and 10.0%, respectively. The distribution of genotypes and allelotypes in the EC patients was not significantly different from that in the controls. When stratified by sex and age, there was still no significant association between the risks of EC and GT and GG genotypes. This study also showed a distinct difference in the distribution of DNMT3B and single nucleotide polymorphism (SNP) between Chinese and Koreans.

CONCLUSION: DNMT3B 579 G>T polymorphism may not be a stratification marker to predict the susceptibility to EC, at least in Chinese. DNMT3B promoter SNP is diverse in ethnic populations.

© 2008 WJG. All rights reserved.

Key words: Esophagus cancer; DNMT3B; Methylation; Polymorphism

INTRODUCTION

Esophagus carcinoma (EC) is one of the most common malignancies and the main cause of cancer-related death in the world. Because symptoms typically remain absent until late in the course of disease, most cancers are detected at an advanced stage when prognosis is poor. Therefore, it is important to investigate the genetic and epigenetic variation in susceptibility to esophagus carcinogenesis and identify the markers that will facilitate identification of individuals at risk of esophagus carcinogenesis.

DNA methylation is a major epigenetic modification involving the addition of a methyl group to the 5' position of a cytosine in a CpG dinucleotide. A number of studies suggested that aberrant DNA cytosine methylation may play an important role in carcinogenesis[1-5]. DNMT3A and DNMT3B are required for the establishment and maintenance of genomic methylation patterns and proper murine development[6-9]. Both genes are up-regulated to different degrees in some malignancies, including colon cancer and EC[10-14]. Recently, several candidate single nucleotide polymorphisms (SNPs) in the DNMT3B gene have been deposited in public databases. Although the functional effects of these polymorphisms have not been elucidated, some studies showed that some of these variants may influence the DNMT3B activity on DNA methylation,
thereby modulating the susceptibility to lung cancer, breast cancer and gastric cardiac adenocarcinoma\textsuperscript{[15-17]}. The DNMT3B gene contains a single G>T SNP in the transcription start site of the promoter region (\textasciitilde 579 bp from exon 1B), and this probably affects gene function\textsuperscript{[18]}. Some studies suggested that DNMT3B -579 G>T may modify susceptibility to tumors. Although conflicting results have been reported in different tumor types, the heterozygous genotypes have a significantly reduced risk of developing lung and colon cancer\textsuperscript{[19-21]}. However, no report on the association between this allele and the development of EC is available. This study was to investigate the association between this polymorphism and EC in Chinese.

**MATERIALS AND METHODS**

**Study population**

This case-control study included 194 EC patients and 210 healthy controls. EC was histopathologically confirmed in the 194 patients during surgery at the Zhongda Hospital of Southeast University and Tumor Hospital, Nanjing, China. The control subjects were selected from cancer-free subjects who visited the same hospital for a regular physical examination and volunteered to participate in the epidemiology survey during the same period. We defined a healthy subject as a person free of disease (including no history of cancer) at health check-up. The controls were matched for age and sex with the patients (Table 1). All patients and controls were ethnically Chinese and resided in Jiangsu Province or in its surrounding regions.

**DNA extraction**

Five milliliters of venous blood was drawn from each subject into vacuum tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within one week after sampling by proteinase K digestion and salted out as previously described\textsuperscript{[19]}. 

**DNMT 3B genotyping**

Transition from G to T of the DNMT3B SNP creates a PvuII restriction site, which can be exploited for genotyping by PCR and subsequent restriction fragment length polymorphism (RFLP) analysis. PCR was performed in a volume of 25 \( \mu \text{L} \) containing 100 ng of DNA template, 10 \( \times \) PCR master mix (Promega, USA), and 10 pmol/L each of sense primer (5'-GAGGTCTCAATATGCCTAGG-3') and antisense primer (5'-GGGAGCCTACCTTCTAGGAA-3'). For PCR amplification, an initial denaturation at 94°C for 5 min was followed by 30 cycles at 94°C for 30 s, at 57°C for 30 s, at 72°C for 30 s, and a final extension at 72°C for 7 min. The PCR products were digested overnight with 5 units of PvuII (New England Biolabs, Beverly, Mass.) at 37°C and separated on 2% agarose gels. RFLP bands were visualized under UV light with ethidium bromide staining. The DNMT3B T/T genotype was expected to show two DNA bands at the positions of 225 bp and 132 bp, whereas the G/G genotype was expected to show a single band (225 bp), and the heterozygote was expected to have three bands (225 bp, 132 bp, 93 bp). For quality control, genotyping analysis was performed blindly with respect to case/control status and repeated twice for all subjects.

**Table 1 Distribution of selected variables in esophagus cancer patients and control subjects (n (%))**

| Variables | Patients (\( n = 194 \)) | Control (\( n = 210 \)) | \( P \) |
|-----------|---------------------|---------------------|------|
| Age (yr)  |                     |                     | > 0.05 |
| < 40      | 1 (0.5)             | 2 (0.9)             |      |
| 40-60     | 117 (60.3)          | 110 (52.4)          |      |
| 61-80     | 76 (39.2)           | 98 (46.7)           |      |
| Sex       |                     |                     | > 0.05 |
| Male      | 150 (77.3)          | 146 (69.5)          |      |
| Female    | 44 (22.7)           | 64 (30.5)           |      |

**DNA sequencing analysis**

To confirm the genotyping results, selected PCR-amplified DNA samples were examined by DNA sequencing. The PCR fragments were recovered from agarose gel followed by purification with a DNA clean-up kit (Wizard SV Gel and PCR Clean-up System, Promega). DNA sequences of the PCR products were determined using the PCR sense primer with an Applied Biosystems model 377 sequencer (PE Applied Biosystems, Warrington, UK). The results of genotyping were 100% concordant.

**Statistical analysis**

Patients and controls were compared using Student’s \( t \)-test for continuous variables and chi square (\( \chi^2 \)) test for categorical variables. Hardy-Weinberg equilibrium was tested with a goodness-of-fit \( \chi^2 \) test with one degree of freedom to compare the observed genotype frequencies with the expected genotype frequencies among the subjects. Comparison of the DNMT3B genotype and allele distribution in the study groups was performed by means of two-sided contingency tables using \( \chi^2 \) test or Fischer’s exact test. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model and adjusted for age and gender accordingly. \( P \leq 0.05 \) was considered statistically significant.

**RESULTS**

The demographics of the cases and controls enrolled in this study are shown in Table 1. There were no significant differences in the mean age and sex distribution between cases and controls, suggesting that the matching based on these two variables was adequate. There was no evidence of a deviation from Hardy-Weinberg equilibrium among the cases or controls. The mean age of the patients and controls was 59.6 years (± 10.2 years; range, 34-80 years) and 59.6 years (± 10.2 years; range, 34-80 years), respectively.

All the patients and controls were successfully genotyped for the DNMT3B polymorphism (Figure 1). The genotyping by PCR-RFLP analysis was completely confirmed by DNA sequencing analysis, and the results of PCR-RFLP genotyping and sequencing analysis were also 100% concordant (Figure 2). The distribution of DNMT3B 579 G>T polymorphism was in Hardy-Weinberg equilibrium. The frequency of G allele in control subjects (0.10) was different from that in the previous
The distributions of DNMT3B 579 G>T genotypes in Chinese and Koreans
are shown in Table 2.

The distributions of  DNMT3B 579 G>T genotypes in controls and patients are shown in Table 3. The genotype distributions of both polymorphisms in the controls were in Hardy-Weinberg equilibrium. No significant deviation was observed in the genotype distributions of both polymorphisms between overall esophagus cancer patients and controls. Then we stratified the results by sex and age, patients and controls were found to be slightly different with respect to the genotype distribution (Table 4). Combined GG and GT genotypes were found to have a little higher OR in male esophagus cancer patients and the group under the age of 59 years (males: OR 1.35; 95% CI, 0.76-2.39; under 59: OR 1.47; 95% CI, 0.74-2.90). However, combined GG and GT genotypes showed no significant association between DNMT3B 579 G>T polymorphism and the risk of esophagus cancer.

**DISCUSSION**

Single nucleotide polymorphism (SNP) is the most common form of human genetic variation, and may contribute to an individual’s susceptibility to cancer. Studies suggested that some variants in the promoter region of genes may affect either the expression or activity levels of enzymes[1,18,22] and therefore may be mechanistically associated with cancer risk. It has been recently shown that SNP of the DNMT3B promoter 579 G>T (from exon 1B transcription start site) decreases the susceptibility of an individual to lung and colon cancer[20,21], suggesting that DNMT3B promoter 579 G>T polymorphism can be used as a risk factor for cancer to evaluate the population susceptible to tumors. However, it was also reported that there is no association between polymorphism of 579 G>T and head and neck squamous cell carcinoma[23]. However, to the best of our knowledge, the relative significance of SNP in the genetic susceptibility to esophagus cancer has not yet been disclosed. In the

Table 2  DNMT3B genotype and allele frequency in Chinese and Koreans

|        | TT    | GT    | GG    | G allele frequency (%) |
|--------|-------|-------|-------|------------------------|
| Chinese| 169 (80.5) | 40 (19.0) | 1 (0.5) | 10.0 |
| Korean | 153 (61.7) | 91 (36.7) | 4 (1.6) | 20 |

\[ P < 0.01 \text{ vs Chinese.} \]

Table 3  DNMT3B genotype and allele frequency in patients and control subjects and their association with esophagus cancer

| Variable | Case patients \((n = 194)\) | Control subjects \((n = 210)\) | OR (95% CI) |
|----------|-----------------------------|-----------------------------|-------------|
| DNMT3B 579 G>T |                              |                             |             |
| TT       | 151 (77.8)                  | 169 (80.5)                  | 1.17 (0.73-1.90) |
| GT       | 43 (22.2)                   | 40 (19.0)                   |             |
| GG       | 0 (0)                       | 1 (0.50)                    |             |
| G allele | 11.1                        | 10.0                        |             |

Table 4  Stratification analysis of DNMT3B 579 G>T genotype frequencies in esophagus cancer patients and controls, adjusted OR (95% CI)

| Variable | TT genotype Case/control | GT + GG genotype Case/control | Odds ratios of GT + GG genotype |
|----------|--------------------------|-------------------------------|-------------------------------|
| Age < 60 | 81/89                    | 24/18                         | 1.47 (0.74-2.90)              |
| ≥ 60     | 70/80                    | 19/23                         | 0.94 (0.48-1.88)              |
| Sex      |                          |                               |                               |
| Male     | 116/120                  | 34/26                         | 1.35 (0.76-2.39)              |
| Female   | 35/49                    | 9/15                          | 0.84 (0.33-2.14)              |
current study, we investigated the influence of DNMT3B polymorphisms on the risk of esophage cancer in a hospital-based case-control study.

This is the first study of DNMT3B polymorphism in esophage cancer. We investigated the influence of 579 G>T polymorphism in the DNMT3B gene on the risk of esophage cancer. Individuals carrying G allele in the DNMT3B gene were found to have a nearly consistent risk of EC compared with those carrying T allele. Then we stratified the results by sex and age, patients and controls. Combined GG and GT genotypes showed no significant association between DNMT3B 579 G>T polymorphism and risk of esophage cancer, suggesting that 579 G>T polymorphism in the DNMT3B gene cannot be used as a marker of genetic susceptibility to esophage cancer even in young individuals. Our study showed that DNMT3B polymorphism was not associated with the risk of esophage carcinoma, at least in the study population, although other studies reported a decreased risk of lung and colon cancer in those harboring G allele. Since the different variants of DNMT3B may alter catalytic activity and are expressed in a tissue specific manner[24–27] and the repression of DNMT3B activity does not result in re-expression of all hypermethylated tumor suppressor genes in some cell systems[28–31], it is therefore important to explore the complex interplay of DNMTs in different tumor types.

In this study, a distinct difference was found in the distribution of DNMT3B SNP between Chinese and Koreans. However, few G/G genotypes were found in both populations. Additionally, the frequency of G/T genotype in Chinese was lower than that in Koreans. The great diversity in DNMT3B SNP distribution in different ethnic populations remains unknown.

In conclusion, the DNMT3B gene may not be involved in the development of esophage cancer. Further studies with a larger sample are required to confirm our findings, to understand the role of DNMT3B polymorphisms in determining the risk of esophage cancer, and to clarify the association of DNMT3B polymorphism with esophage cancer in different ethnic populations.

**COMMENTS**

**Background**

DNA methylation is a major epigenetic modification involving the addition of a methyl group to the 5' position of a cytosine in CpG dinucleotide. DNMT3A and DNMT3B are required for the establishment and maintenance of genominc methylation patterns. Single nucleotide polymorphisms (SNPs) in the DNMT3B gene may influence DNMT3B activity on DNA methylation, thereby modulating the susceptibility to some cancer.

**Research frontiers**

Some variants in the promoter region of genes may affect either the expression or activity levels of enzymes and therefore may be mechanistically associated with cancer risk. It has been recently reported that SNP of the DNMT3B promoter 579 G>T (from exon 1B transcription start site) decreases the susceptibility of an individual to lung and colon cancer. However, it was also reported that there is no association between polymorphism of 579 G>T and head and neck squamous cell carcinoma. The relative significance of SNP in genetic susceptibility of an individual to cancer is diverse in different populations.

**Innovations and breakthroughs**

It is important to investigate the genetic and epigenetic variation in susceptibility to esophagus carcinogenesis and identify markers that will facilitate identification of individuals at risk of esophagus carcinogenesis. Although no significant association was found between DNMT3B 579 G>T polymorphism and risk of esophage cancer, this is the first study of DNMT3B polymorphism in esophage cancer. This study also showed the significance of great diversity in DNMT3B SNP distribution in different ethnic populations and their susceptibility to cancer.

**Applications**

A distinct difference was found in the distribution of DNMT3B SNP between Chinese and Koreans in this study. The significance of great diversity in DNMT3B SNP distribution in different ethnic populations remains unknown. These results suggest that the DNMT3B gene may not be involved in the development of esophage cancer. Future studies of other DNMT3B sequence variants and their biologic function are needed to understand the role of DNMT3B polymorphisms in determining the risk of esophage cancer.

**Peer review**

The association between DNMT3B 579 G>T polymorphism and the risk of esophage cancer in Chinese was studied. The results show that the DNMT3B 579 G>T polymorphism was not associated with the risk of esophage carcinoma, at least in the study population. This study also showed the significance of great diversity in DNMT3B SNP distribution in different ethnic populations and their susceptibility to cancer.

**REFERENCES**

1. Momparler RL, Bovenzi V. DNA methylation and cancer. J Cell Physiol 2000; 183: 145-154
2. Beaulieu N, Morin S, Chute IC, Robert MF, Nguyen H, MacLeod AR. An essential role for DNA methyltransferase DNMT3B in cancer cell survival. J Biol Chem 2002; 277: 28176-28181
3. Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet 1999; 21: 163-167
4. Johnson KA, Lerner CP, Di Lacio LC, Laird PW, Sharpe AH, Simpson EM. Transgenic mice for the preparation of hygromycin-resistant primary embryonic fibroblast feeder layers for embryonic stem cell selections. Nucleic Acids Res 1995; 23: 1273-1275
5. Cooper DN, Yooussoufian H. The CpG dinucleotide and human genetic disease. Hum Genet 1988; 78: 151-155
6. Bachman KE, Rouuntree MR, Baylin SB. Dnmt2a and Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. J Biol Chem 2001; 276: 32282-32287
7. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999; 99: 247-257
8. Gowher H, Jeltsch A. Molecular enzymology of the catalytic domains of the Dmnt3a and Dnmt3b DNA methyltransferases. J Biol Chem 2002; 277: 20409-20414
9. Robertson KD. DNA methylation, methyltransferases, and cancer. Oncogene 2001; 20: 3139-3155
10. Xu W, Fan H, He X, Zhang J, Xie W. LOI of IGF2 is associated with esophageal cancer and linked to methylation status of IGF2 DMR. J Exp Clin Cancer Res 2006; 25: 543-547
11. Simao Tde A, Simoes GL, Ribeiro FS, Cidade DA, Andreollo NA, Lopes LR, Macedo JM, Acatauassu R, Teixeira AM, Felzenszwalb I, Pinto LF, Albano RM. Lower expression of p14ARF and p16INK4a correlates with higher DNMT3B expression in human oesophageal squamous cell carcinomas. Hum Exp Toxicol 2006; 25: 515-522
12. Takeshima H, Matsuoka I, Shimahara H, Ura K, Tate S, Tajima S. Distinct DNA methylation activity of Dmnt3a and Dnmt3b towards naked and nucleosomal DNA. J Biochem 2006; 139: 503-515
13. Robertson KD, Keyomarsi K, Gonzales FA, Velicescu M, Jones PA. Differential mRNA expression of the human DNA methyltransferases (DNMTs) 1, 3a and 3b during G(0)/G(1) to S phase transition in normal and tumor cells. Nucleic Acids Res 2000; 28: 2108-2113
Robertson KD, Jones PA. DNA methylation: past, present and future directions. *Carcinogenesis* 2000; 21: 461-467

Simao Tde A, Simoes GL, Ribeiro FS, Cidade DA, Andreollo NA, Lopes LR, Macedo JM, Acatauassu R, Teixeira AM, Felzenszwalb I, Pinto LF, Albano RM. Lower expression of p14ARF and p16INK4a correlates with higher DNMT3B expression in human oesophageal squamous cell carcinomas. *Hum Exp Toxicol* 2006; 25: 515-522

Wang YM, Wang R, Wen DG, Li Y, Guo W, Wang N, Wei LZ, He YT, Chen ZF, Zhang XF, Zhang JH. Single nucleotide polymorphism in DNA methyltransferase 3B promoter and its association with gastric cardiac adenocarcinoma in North China. *World J Gastroenterol* 2005; 11: 3623-3627

Cebrian A, Pharoah PD, Ahmed S, Ropero S, Fraga MF, Smith PL, Conroy D, Luben R, Perkins B, Easton DF, Luben R, Perkins B, Easton DF, Dunning AM, Esteller M, Ponder BA. Genetic variants in epigenetic genes and breast cancer risk. *Carcinogenesis* 2006; 27: 1661-1669

Shen H, Wang L, Spitz MR, Hong WK, Mao L, Wei Q. A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. *Nucleic Acids Res* 1988; 16: 1215

Lee SJ, Jeon HS, Jang JS, Park SH, Lee GY, Lee BH, Kim CH, Kang YM, Lee WK, Kam S, Park RW, Kim JS, Cho YL, Jung TH, Park JY. DNMT3B polymorphisms and risk of primary lung cancer. *Carcinogenesis* 2005; 26: 403-409

Hong YS, Lee HJ, You CH, Roh MS, Kwak JY, Lee MJ, Kim JY. DNMT3B 39179GT polymorphism and the risk of adenocarcinoma of the colon in Koreans. *Biochem Genet* 2007; 45: 155-163

Skoog T, van't Hooft FM, Kallin B, Jovinge S, Boquist S, Nilsson J, Eriksson P, Hamsten A. A common functional polymorphism (C->A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. *Hum Mol Genet* 1999; 8: 1443-1449

Chang KP, Hao SP, Liu CT, Cheng MH, Chang YL, Lee YS, Wang TH, Tsai CN. Promoter polymorphisms of DNMT3B and the risk of head and neck squamous cell carcinoma in Taiwan: a case-control study. *Oral Oncol* 2007; 43: 345-351

Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; 99: 247-257

Wang J, Walsh G, Liu DD, Lee JJ, Mao L. Expression of Delta DNMT3B variants and its association with promoter methylation of p16 and RASSF1A in primary non-small cell lung cancer. *Cancer Res* 2006; 66: 8361-8366

Saito Y, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S. Overexpression of a splice variant of DNA methyltransferase 3b, DNMT3B4, associated with DNA hypomethylation on pericentromeric satellite regions during human hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2002; 99: 10060-10065

Chen T, Ueda Y, Xie S, Li E. A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. *J Biol Chem* 2002; 277: 38746-38754

Soejima K, Fang W, Rollins BJ. DNA methyltransferase 3b contributes to oncogenic transformation induced by SV40T antigen and activated Ras. *Oncogene* 2003; 22: 4723-4733

Weisenberger DJ, Velicescu M, Chen JC, Gonzales FA, Liang G, Jones PA. Role of the DNA methyltransferase variant DNMT3B3 in DNA methylation. *Mol Cancer Res* 2004; 2: 62-72

Yu Z, Kone BC. Hypermethylation of the inducible nitric-oxide synthase gene promoter inhibits its transcription. *J Biol Chem* 2004; 279: 46954-46961

Kim SH, Park J, Choi MC, Kim HP, Park JH, Jung Y, Lee JH, Oh DY, Im SA, Bang YJ, Kim TY. Zinc-fingers and homeoboxes 1 (ZHX1) binds DNA methyltransferase (DNMT) 3B to enhance DNMT3B-mediated transcriptional repression. *Biochem Biophys Res Commun* 2007; 355: 318-323

S- Editor Zhong XY  L- Editor Wang XL  E- Editor Yin DH