Role of **BMP3** in progression of gastric carcinoma in Chinese people

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Abstract

**AIM:** To investigate the relation between gastric cancer and microsatellite instability (MSI), loss of heterozygosity (LOH) and promoter region methylation.

**METHODS:** Fifty primary gastric carcinoma specimens were collected from patients with no family history of cancer. In addition, normal tissues were also collected from patients as controls. DNA was extracted by polymerase chain reaction for single-strand conformation polymorphism, bisulfite DNA sequencing, and methylation-specific band analysis.

**RESULTS:** The positive rate for MSI and LOH in gastric carcinoma was 16% and 20%, respectively. According to the tumor, node and metastasis staging system, the LOH frequency was higher in gastric carcinoma at stages III and IV than in gastric carcinoma at stages I and II ($P = 0.01$), which was also significantly correlated with lymph node metastasis and clinicopathological characteristics of gastric carcinoma. Methylation of bone morphogenetic protein 3 (**BMP3**) gene promoter was detected in 64.44% of gastric carcinoma tissue samples. However, no statistical significance was observed between promoter region methylation and carcinoma differentiation. Interestingly, the **BMP3** gene methylation rate was 71.05% and 28.58%, respectively, in MSI positive and negative cases ($P = 0.031$), suggesting that **BMP3** genetic instability and promoter methylation are initiated during gastric carcinogenesis. LOH was detected mostly in the late stages of gastric carcinoma, indicating that gastric carcinoma at late stages has a higher infiltration and a poorer prognosis.

**CONCLUSION:** Promotor region methylation of the **BMP3** gene may cause gastric carcinoma in Chinese people.

**Key words:** Bone morphogenetic protein 3 gene; Gastric carcinoma; Microsatellite instability; Loss of heterozygosity; Methylation

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INTRODUCTION
Gastric carcinoma is one of the leading causes of cancer-related death in China. Its incidence and mortality are increasing in Chinese people. Research has been focused on the development of cancer and its progression, by studying epigenetics and tumor suppressor genes. It was reported that microsatellite instability (MSI) and loss of heterozygosity (LOH) are associated with gastric cancer[1-5]. In addition, gene promoter region hypermethylation can activate certain tumor suppressor genes. Most of the MSI and LOH studies have been focused on p53[6,7], p16 and fragile histidine triad triple genes[7] with less efforts made on the role of bone morphogenetic protein (BMP) 3 epigenetics in tumor suppressor genes.

BMP3, belonging to the transforming growth factor β superfamily proteins, plays an important role in embryonic development by inducing and patterning early skeletal formation. It has been recently reported that BMP3 is associated with tumor development and progression[8]. However, no report is available on the role of BMP3 in gastric carcinoma development. The present study was to investigate the D4S2922 and D4S2964 loci in 4q21 region of the BMP3 gene by examining their MSI, LOH as well as the promoter region methylation and to reveal the relation between BMP3 gene and gastric carcinogenesis.

MATERIALS AND METHODS
Gastric carcinoma specimens
Fifty gastric carcinoma samples were obtained from patients with no family history of cancer and normal tissue samples were also collected from patients as controls. The study was approved by the Ethics Committee and patient families before chemotherapy and radiotherapy.

DNA extraction and polymerase chain reaction (PCR) analysis
DNA was extracted from gastric carcinoma and normal tissue specimens using a QIAGEN kit according to its manufacturer’s instructions. PCR for D4S2922 was performed using the primers (sense: 5’-TGCTTATGCAAGAGGTTGTTC-3’ and antisense: 5’-ATCAACTCCCAACATCCTACA-3’) for the nonmethylated specific BMP3 gene, yielding a PCR product of 73 bp. Primers (sense: 5’-GTCGACTCCCGAGTCGTACG-3’) and (antisense: 5’-GTCGACTCCCGAGTCGTAGC-3’) were used for the methylated specific BMP3 gene, yielding a PCR product of 70 bp. After an initial preheating step at 94°C for 10 min, 40 cycles of PCR were performed at 94°C for 30 s, at 64°C for 45 s, at 72°C for 30 s and a final extension at 72°C for 10 min. The amplified PCR products were examined on 8% agarose gels.

Band analysis
PCR-SSCP showed one main band of the D17S396 locus in normal lymph node genomic DNA. The presence of two main bands indicated that a sample could be further evaluated in LOH analysis. LOH was considered when less than 50% bands or a lower band density was found in tumor tissue samples than in normal tissue samples, while MSI was considered when more than 50% bands or a band migration was observed in tumor tissue samples.

Statistical analysis
All data were analyzed with SPSS version 16.0 by one-way ANOVA and t-test. P < 0.05 was considered statistically significant.

RESULTS
Correlation between BMP3 genetic instability and clinicopathological features of gastric carcinoma
Both D4S2922 and D4S2964 from tumor and normal tissue samples were successfully amplified and the bands on gel images were heterozygous for the allele. Com-
Figure 1 Polyacrylamide gel electrophoresis (PAGE) of bone morphogenetic protein 3 (BMP3) gene. No difference was found between tumor tissue (1C) and normal tissue (1N). Compared with normal tissue (2N), positive MSI was represented as an added allele band (arrow) in tumor tissue (2C). Compared with normal tissue (3N), positive LOH was represented as lacking an allele band (arrow) in tumor tissue (3C).

| Clinopathological features                  | MSI (% (+/−)) | LOH (% (+/−)) |
|--------------------------------------------|---------------|---------------|
| Differentiation degree                     | 16.00 (8/50)  | 20.00 (10/50) |
| High differentiation                       | 25.00 (2/8)   | 0.00 (0/8)    |
| Middle differentiation                     | 8.69 (2/23)   | 21.74 (5/23)  |
| Low differentiation                        | 21.05 (4/19)  | 26.32 (5/19)  |
| Serosa infiltration                        |               |               |
| Positive                                   | 17.15 (6/35)  | 22.86 (8/35)  |
| Negative                                   | 13.33 (2/15)  | 13.33 (2/15)  |
| Lymph node metastasis                      |               |               |
| Positive                                   | 12.12 (4/33)  | 30.30 (10/33) |
| Negative                                   | 23.53 (4/17)  | 0.00 (0/17)   |
| TNM stage                                  |               |               |
| I + II                                     | 15.16 (5/33)  | 6.06 (2/33)   |
| III + IV                                   | 17.65 (3/17)  | 47.05 (8/17)  |

*P < 0.001 vs positive lymph node metastasis; *P = 0.01 vs TNM stages I + II. MSI: Microsatellite instability; LOH: Loss of heterozygosity; BMP3: Bone morphogenetic protein 3; TNM: Tumor, node and metastasis.

Table 1 Relation of genetic instability and methylation of BMP3 gene with clinicopathological features of gastric cancer

Table 2 Relation between BMP3 gene methylation and clinicopathological features of gastric cancer

| Clinicopathological features | Methylation frequency | P value |
|------------------------------|-----------------------|---------|
| Differentiation degree       | 64.44 (29/45)         |         |
| High                         | 75.00 (6/8)           |         |
| Middle                       | 68.18 (15/22)         | 0.273   |
| Low                          | 53.33 (8/15)          |         |
| Serosa infiltration          |                       |         |
| Positive                     | 59.38 (19/32)         | 0.275   |
| Negative                     | 76.92 (10/13)         |         |
| Lymph node metastasis        |                       |         |
| Positive                     | 62.07 (18/29)         | 0.663   |
| Negative                     | 68.75 (11/16)         |         |
| TNM stage                    |                       |         |
| I + II                       | 62.50 (10/16)         | 0.844   |
| III + IV                     | 65.52 (19/29)         |         |
| MSI                          |                       |         |
| Negative                     | 71.05 (27/38)         | 0.031*  |
| Positive                     | 28.58 (2/7)           |         |
| LOH                          |                       |         |
| Negative                     | 68.57 (24/35)         | 0.290   |
| Positive                     | 50.00 (5/10)          |         |

*P < 0.05.

Relation between BMP3 gene promoter region methylation and clinicopathological features of gastric carcinoma

The methylation status in promoter region of BMP3 gene was analyzed by methylation-specific PCR (Table 1). Positively methylated BMP3 promoter region was found in 45 of the 50 gastric carcinoma tissue samples, with a methylation rate of 64.44%. The frequency of methylation in normal tissue was 53.49% (43/50) with no statistical difference between gastric carcinoma and normal tissue samples (P > 0.05). In addition, BMP3 gene promoter region methylation was not correlated with tumor differentiation, infiltration, lymphatic metastasis and clinical TNM staging.

Correlation between gastric carcinoma gene BMP3 instability and its promoter region methylation

The frequency of BMP3 gene promoter region methylation was much lower in MSI negative than in MSI positive cases (28.58% vs 71.05%, P < 0.05, Table 2). However, the frequency of BMP3 gene promoter region methylation was 68.75% and 50.00%, respectively, in LOH negative and positive cases with no statistical difference.

DISCUSSION

Microsatellites are the short sequences of DNA, usually 2-6 base pairs in a row along a DNA molecule. MSI is mutations in genes, whereby repair of damaged DNA causes microsatellite-associated regions to become longer or shorter. LOH in a cell represents the loss of normal function in one allele of a gene in which the other alleles have been inactivated. MSI and LOH play an
important role in tumor development and progression. LOH occurs when the remaining functional allele in a somatic cell of the offspring becomes inactivated due to mutation. No normal tumor suppressor is produced, thus resulting in tumorigenesis[9].

Microsatellites were first reported in 1981 by Miesfeld et al[10] and further elucidated in 1993 by Aaltonen et al[11] who revealed a higher MSI frequency in hereditary non-polyposis colorectal cancer cells. MSI has been reported in colon, gastric, cervical, breast, prostate and pancreatic carcinomas[12-17].

Alexander et al[18] investigated the sporadic colon carcinoma cases and found that MSI occurs in early stages with a better prognosis, suggesting that MSI can serve as an early diagnostic index for gastric and colon carcinomas. Our previous report also demonstrated that the frequency of MSI is higher in D17S396 loci of the nm23H1 gene in gastric and colon carcinomas at stages I and II with a better prognosis than in those at stages III and IV with a worse prognosis, suggesting that MSI can serve as an early diagnostic index for gastric and colon carcinomas[9]. In this study, however, no statistical significance was found between MSI of BMP3 gene and tumor differentiation, infiltration, lymphatic metastasis and tumor TNM staging.

Berney et al[20] showed that the LOH frequency of nm23H1 gene is significantly correlated with tumor infiltration and metastasis. Candusso et al[21] reported that LOH is more frequently found in late stage tumors, often with lymphatic metastasis. In our study, the LOH frequency of BMP3 gene was much higher in gastric carcinomas at stages III and IV without lymphatic metastasis than in those at stages I and II with lymphatic metastasis, suggesting that LOH occurs more often in late tumor stages with lymphatic metastasis and can thus serve as an index for the evaluation of malignancy, metastasis and prognosis of gastric carcinomas.

DNA methylation is believed to be closely associated with tumor development and progression. Reduced methylation and hypermethylation in gene promoter regions can inactivate tumor suppressor genes, leading to tumor development. In the present study, the methylation level in promoter region of BMP3 gene in gastric carcinomas was high, leading to the occurrence of gastric carcinoma. Loh et al[8] reported that BMP3 gene methylation is associated with MSI in colon cancer, indicating that the higher the methylation is, the higher the MSI frequency is. In this study, the level of methylation was higher in MSI negative than in MSI positive gastric carcinoma cases, which is not consistent with the findings of Loh et al[8]. Further study is needed to verify the differences.

In conclusion, genetic instability of the BMP3 gene and promoter region methylation occur in gastric carcinomas, thus affecting tumor characteristics through different pathways. LOH is observed more frequently in late stage tumors with infiltration. Hypermethylation in promoter region of BMP3 gene may result in gastric carcinoma in Chinese people.

**COMMENTS**

**Background**

Gastric carcinoma is one of the leading causes of cancer-related death in China. Its incidence and mortality are increasing in Chinese people. Microsatellite instability (MSI) and loss of heterozygosity (LOH) are known to be associated with gastric cancer. Moreover, gene promoter region hypermethylation can inactivate certain tumor suppressor genes.

**Research frontiers**

Genetic instability and gene promoter region methylation play an important role in gene mutation, gene inactivation, and carcinogenesis. However, the role and mechanism of bone morphogenetic protein 3 (BMP3) in cancer development are poorly characterized. The relation between the development of gastric cancer and the number of altered microsatellite loci (D4S2922 and D4S2964), LOH, and promoter region methylation was elucidated in the present study.

**Innovations and breakthroughs**

In the present study, the methylation levels in promoter region of BMP3 gene in gastric carcinoma was high, which may lead to gastric carcinoma and is not consistent with the reported findings. The methylation level was higher in MSI negative than in MSI positive gastric carcinoma.

**Applications**

MSI and LOH of the BMP3 gene can serve as an index for the evaluation of malignancy, metastasis and prognosis of gastric carcinoma.

**Terminology**

MSI is a condition manifested as damaged DNA due to defects in normal DNA repair process. LOH in a cell represents the loss of normal function in one allele of a gene in which the other allele has been inactivated.

**Peer review**

The paper is well-written and interesting. BMP3 gene, MIS, LOH and promoter region methylation were evaluated in patients with gastric adenocarcinoma, thus making an additional contribution to studies on the role of MIS and LOH in carcinogenesis.

**REFERENCES**

1. Cai YC, So CK, Nie AY, Song Y, Yang GY, Wang LD, Zhao X, Kinzy TG, Yang CS. Characterization of genetic alteration patterns in human esophageal squamous cell carcinoma using selected microsatellite markers spanning multiple loci. Int J Oncol 2007; 30: 1059-1067
2. Chakrabarti S, Sengupta S, Sengupta A, Basak SN, Roy A, Panda C, Roychoudhury S. Genomic instabilities in squamous cell carcinoma of head and neck from the Indian population. Mutagenesis 2006; 45: 270-277
3. Inoue Y, Miki C, Watanabe H, Ojima E, Kusunoki M. Genomic instability and tissue expression of angiogenic growth factors in sporadic colorectal cancer. Surgery 2006; 139: 305-311
4. Nowacka-Zawisza M, Bryś M, Hanna RM, Zadrozny M, Kulig A, Krajewska WM. Loss of heterozygosity and microsatellite instability at RAD52 and RAD54 loci in breast cancer. Pol J Pathol 2006; 57: 83-89
5. Yoshida K, Miki Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. Cancer Sci 2004; 95: 866-871
6. Juwan R, Hudler F, Gazvoda B, Repse S, Bracko M, Komel R. Significance of genetic abnormalities of p53 protein in Slovenian patients with gastric carcinoma. Croat Med J 2007; 48: 207-217
7. Xiao YP, Wu DY, Xu L, Xin Y. Loss of heterozygosity and microsatellite instabilities of fragile histidine triad gene in gastric carcinoma. World J Gastroenterol 2006; 12: 3766-3769
8. Loh K, Chia JA, Greco S, Cozzi SJ, Buttenshaw RL, Bond CE, Simms LA, Pike T, Young JP, Jass JR, Spring KJ, Leggett BA, Whitehall VL. Bone morphogenetic protein 3 inactivation is an early and frequent event in colorectal cancer development. Genes Chromosomes Cancer 2008; 47: 449-460
9. Storchova Z, Pellman D. From polyplody to aneuploidy,
genome instability and cancer. Nat Rev Mol Cell Biol 2004; 5: 45-54

10 Miesfeld R, Krystal M, Arnheim N. A member of a new repeated sequence family which is conserved throughout eucaryotic evolution is found between the human delta and beta globin genes. Nucleic Acids Res 1981; 9: 5931-5947

11 Aaltonen LA, Peltohämä P, Leach FS, Sistonen P, Pylkkänen L, Mecklin JP, Järvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. Science 1993; 260: 812-816

12 Svrcek M, El-Bchiri J, Chalastanis A, Capel E, Dumont S, Buhard O, Oliveira C, Seruca R, Bossard C, Mosnier JF, Berger F, Leteurtre E, Lavergne-Slove A, Chenard MP, Hamelin R, Cosnes J, Beaugerie L, Tiret E, Duval A, Fléjou JF. Specific clinical and biological features characterize inflammatory bowel disease associated colorectal cancers showing microsatellite instability. J Clin Oncol 2007; 25: 4231-4238

13 Sakurai M, Zhao Y, Oki E, Kakeji Y, Oda S, Maehara Y. High-resolution fluorescent analysis of microsatellite instability in gastric cancer. Eur J Gastroenterol Hepatol 2007; 19: 701-709

14 An HJ, Kim KL, Kim JY, Shim JY, Kang H, Kim TH, Kim JK, Jeong JK, Lee SY, Kim SJ. Microsatellite instability in endometrioid type endometrial adenocarcinoma is associated with poor prognostic indicators. Am J Surg Pathol 2007; 31: 846-853

15 Pizzì C, Di Maio M, Daniele S, Mastranrosso P, Spagnolletti I, Limite G, Pettinato G, Monticelli A, Coccozza S, Contegio-como A. Triplet repeat instability correlates with dinucleotide instability in primary breast cancer. Oncol Rep 2007; 17: 193-199

16 Burger M, Denzinger S, Hammerschmied CG, Tannapfel A, Obermann IC, Wieland WF, Hartmann A, Stoehr R. Elevated microsatellite alterations at selected tetranucleotides (EMAST) and mismatch repair gene expression in prostate cancer. J Mol Med 2006; 84: 833-841

17 House MG, Herman JG, Guo MZ, Hooker CM, Schulick RD, Cameron JL, Hruban RH, Maitra A, Yeo CJ. Prognostic value of hMLH1 methylation and microsatellite instability in pancreatic endocrine neoplasms. Surgery 2003; 134: 902-908; discussion 909

18 Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 2001; 158: 527-535

19 Yang YQ, Wu L, Chen JX, Sun JZ, Li M, Li DM, Lu HY, Su ZH, Lin XQ, Li JC. Relationship between nm23H1 genetic instability and clinical pathological characteristics in Chinese digestive system cancer patients. World J Gastroenterol 2008; 14: 5549-5556; discussion 5555

20 Berney CR, Fisher RJ, Yang J, Russell PJ, Crowe PJ. Genomic alterations (LOH, MI) on chromosome 17q21-23 and prognosis of sporadic colorectal cancer. Int J Cancer 2000; 89: 1-7

21 Candonusso ME, Luinetti O, Villani L, Alberizzi P, Klersy C, Fiocca R, Ranzani GN, Solcia E. Loss of heterozygosity at 18q21 region in gastric cancer involves a number of cancer-related genes and correlates with stage and histology, but lacks independent prognostic value. J Pathol 2002; 197: 44-50

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