

Original Article

Detection of microsatellite instability in gastric cancer and dysplasia tissues

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Abstract: Objective: We aimed to investigate the association between gastric cancer and microsatellite instability (MSI) in the present study. Method: Phenol-chloroform method was employed for DNA extraction from the cancer tissues of 65 gastric cancer patients and the dysplasia tissues and normal control tissues of 32 non-gastric cancer patients. The microsatellite loci Bat25, Bat26, D2S123, D5S346 and D17S250 were detected by using PCR-SSCP silver staining technique, and the MSI of the gastric cancer tissues and the precancerous tissues was analyzed. Results: Of 65 gastric cancer cases, MSI was detected in 43 cases, with the detection rate of 66.2%. There were 13 cases showing MSI-H and 30 cases showing MSI-L, accounting for 30.2% and 69.8%, respectively. Among 32 cases of dysplasia tissues, MSI was detected in 10 cases, with the detection rate of 31.3%. Two cases of dysplasia tissues showed MSI-H and 8 cases showed MSI-L, accounting for 20.0% and 80.0%, respectively. Conclusion: Gastric cancer patients had a high detection rate of MSI. It is speculated that MSI is another molecular mechanism of carcinogenesis and may serve as a sensitive diagnostic indicator of gastric cancer.

Keywords: Gastric cancer, microsatellite instability, PCR-SSCP analysis

Introduction

Studies in molecular biology have shown that the occurrence and progress of gastric cancer is a multi-step process with multiple factors involved. A series of molecular genetic changes occur during this process, including activation of oncogenes, deactivation of tumor suppressor genes and changes of telomerase activity [1-3]. The latest research indicates that microsatellite instability (MSI) induced by defect in DNA mismatch repair gene plays an important role in gastric cancer [4, 5]. This proves to be another carcinogenetic mechanism besides activation of oncogenes and deactivation of tumor suppressor genes. We performed PCR-SSCP technique in the present research to detect 5 MSI loci (including 2 single-nucleotide repeats, Bat 25 and Bat 26, and 3 dinucleotide repeats, D2S123, D5S346 and D17S250). The detection of MSI loci was recommended for the diagnosis of all tumors by National Cancer Institute in 1998 [6]. MSI in gastric cancer tissues and precancerous tissues from a total of

97 cases, and the pathogenic mechanism of gastric cancer was investigated. Some valuable data were provided for early diagnosis, prognosis and treatment of gastric cancer.

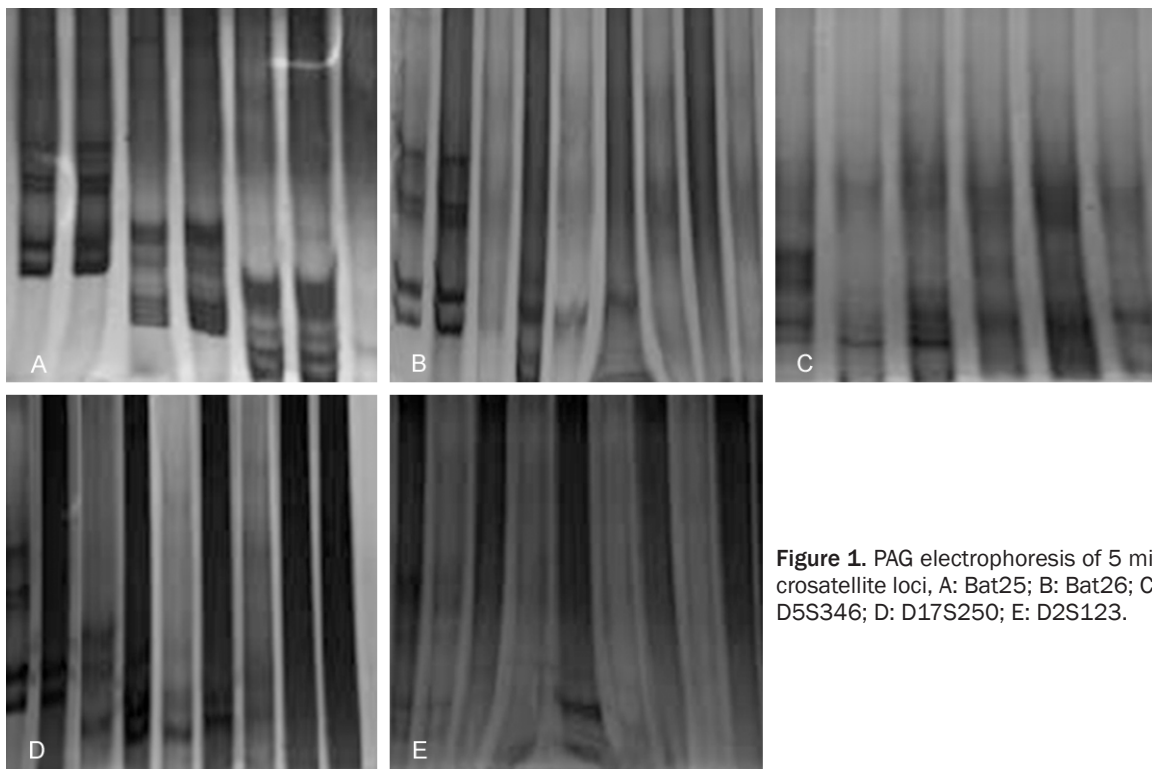
Materials and methods

Materials

All fresh tissues from gastric cancer patients and dysplasia patients totaling 97 were collected from the General Hospital of the People's Liberation Army. The patients and the normal controls had all lived in Beijing for 20 years or above. The postoperative biopsy specimens of 65 gastric cancer patients were collected from Department of Pathology in March 2010 to November 2014. The gastric dysplasia tissues of 32 patients were collected by gastroscopy at Department of Gastroenterology during the same period. All specimens were pathologically confirmed. The control tissues from the gastric cancer patients were collected at 5 cm away from the margin of the cancer tissues. These

Table 1. The primer sequences of each locus

MSI locus	Chromosome	Gene	Primers sequences	Length (bp)
BAT26	2p22-21	hMSH2	5'-TGACTACTTTGACTTCAGCC-3' 5'-AACCATTCAACTTTTAAACC-3'	121~123
BAT25	4q12	c-Kit	5'-TCGCCTCCAAGAATGTAAGT-3' 5'-TCTGCATTTTAACTATGGCTC-3'	123~125
D2S123	2p16	hMSH6	5'-AAACAGGATGCCTGCCTTTA-3' 5'-GGACTTTCCACCTATGGGAC-3'	197~227
D5S346	5p21/22	APC	5'-ACTCACTCTAGTGATAAATCGGG-3 5'-GGAGATAAGACAGTATTACTAGTT-3	96~122
D17S250	17q11.2-q12		5'-GGAAGAATCAAATAGACAAT-3' 5'-GCTGGCCATATATATTTAAACC-3'	151~169

**Figure 1.** PAG electrophoresis of 5 microsatellite loci, A: Bat25; B: Bat26; C: D5S346; D: D17S250; E: D2S123.

tissues contained no cancer cells under microscopic observation. The control tissues from the dysplasia patients were the normal gastric mucosa. Tumor classification was based on WHO's new classification standard (1990) [6]. PCR primers were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). PCR Assay Kit was purchased from Beijing Tianwei Biotech Co., Ltd (Beijing, China). Other experimental equipment included PCR amplifier (PTC-220, USA), vertical electrophoresis system (DYCZ-24D, Beijing), and gel imaging analysis system (SRNGENE-RQ2.04, UK).

Methods

DNA extraction

DNA extraction was performed using phenol-chloroform method. PCR reaction system (25 μ L) consisted of 2 $\times$ Master Mix 12.5 μ L, DNA template 1 μ L, each primer 1 μ L, double-distilled water 9.5 μ L. Reaction conditions were as follows: preheating at 94°C for 3min, denaturation at 94°C for 30s, annealing at 48-55°C for 30s, extension at 72°C for 1 min, 35 cycles, final extension at 72°C for 5min. The quality of

Table 2. General information of gastric cancer patients and MSI detection [n (%)]

General information	n	MSI-H	MSI-L	MSI	MSS
Tumor site					
Cardia	21	5 (23.8)	8 (38.1)	13 (61.9)	8 (38.1)
Fundus	3	0 (0.0)	2 (66.7)	2 (66.7)	1 (33.3)
Gastric body	17	4 (27.3)	3 (18.2)	7 (45.5)	10 (54.5)
Antrum	20	3 (15.0)	14 (70.0)	17 (85.0)	3 (15.0)
Multiple sites	4	1 (25.0)	3 (75.0)	4 (100.0)	0 (0.0)
Degree of differentiation					
Low differentiation	33	5 (15.2)	19 (57.6)	24 (72.7)	9 (27.3)
Medium and high differentiation	32	8 (25.0)	11 (33.4)	19 (59.4)	13 (40.6)
Lymph node metastasis					
No	21	5 (23.8)	7 (33.3)	12 (57.1)	9 (42.9)
Yes	30	4 (13.3)	15 (50.0)	19 (63.3)	11 (36.7)
Unclear	14	4 (28.6)	8 (57.1)	12 (85.7)	2 (14.3)
Total	65	13 (20.0)	30 (46.2)	43 (66.2) ^a	22 (33.8)

^aP<0.05 compared with control (MSS).

PCR products was evaluated by agarose gel electrophoresis.

SSCP

10 µL of the PCR products was added with 10 µL of denaturing gel loading buffer (980 mL/L formamide deionized, 20 mmol/L EDTA, 0.1 g/L bromophenol blue, 0.1 g/L xylene cyanol) and 30 µL of paraffin. After boiling for 10 min, the specimens were taken out and embedded in crushed ice for over 20 min. The water phase was loaded for 80 g/L native-PAGE (containing 50 g/L glycerol, proportion of acrylic amide to methylene-bisacrylamide 29:1). Electrophoresis conditions were room temperature 20°C-25°C, voltage 80V, and reaction time about 3 h. The electrophoresis was followed by silver staining and imaging. PCR amplification, electrophoresis and silver staining were all performed under the same conditions for the cancer tissues and the control tissues.

Criteria for positive MSI

Compared with the control group, the shift of abnormal bands or bands after electrophoresis was considered as positive MSI. High-frequency MSI (MSI-H) was defined as 30%-40% unstable loci or above. Low-frequency MSI (MSI-L) was defined as less than unstable loci. MS-stable (MSS) was defined as no MSI.

Statistical process

All statistical analyses were done using SPSS10.0 software. The detection rate was

analyzed by χ^2 test, and P<0.05 was considered as statistically significant.

Results

MSI detection of gastric cancer tissues

The primers of the detection of 5 MSI loci are shown in **Table 1**. PAGE of positive MSI is shown in **Figure 1**. Of 65 gastric cancer cases, 43 cases were MSI positive, with the detection rate of 66.2%. There were 13 MSI-H cases, accounting for 30.2% of MSI-positive cancer cases, and the detection rate was 20.0%. There were 30 MSI-L cases, accounting for 69.8% of MSI-positive cancer cases, and the detection rate was 80.0%. 22 cases showed MSS, with the detection rate of 33.8% (**Table 2**).

MSI detection in dysplasia tissues

MSI was detected in 10 out of 32 dysplasia tissues, with the detection rate of 31.3%. Among them, 2 cases showed MSI-H, and the detection rate was 6.3%; 8 cases showed MSI-L, accounting for 25.0%; 22 cases showed MSS, accounting for 68.7%.

Relationship between MSI and the site, staging and pathological type of gastric cancer

Tumor site: Compared with MSI-H, MSI-L was mostly found in fundus, but the two types of cancer showed no significant difference in tumor site (P<0.05, **Table 2**).

Degree of differentiation: Compared with MSI-H, more MSI-L cancers were low-differentiated adenocarcinoma ($P < 0.05$, **Table 2**).

Lymph node metastasis: Although there were more MSI-L cases with lymph node metastasis than MSI-H cases with lymph node metastasis, the difference was of no statistical significance ($P > 0.05$, **Table 2**).

MSI detection in gastric cancer tissues and dysplasia tissues

MSI-H was detected in 13 out of 65 gastric cancer cases (20.0%), and MSI-L was detected in 30 cases (46.2%). Among dysplasia cases, 2 showed MSI-H (6.3%) and 8 showed MSI-L (25.0%). Significance test indicated that the occurrence of MSI-H and MSI-L was significantly different between gastric cancer tissues and dysplasia tissues ($P < 0.05$). There were 43 gastric cancer cases (66.2%) and 10 dysplasia cases (31.3%) showing positive MSI, with significant difference ($P < 0.05$).

Discussion

MS is the simple sequence repeat (SSR) composed of less than 10 nucleotides in human genome [7-10]. Also known as short tandem repeat (STR), it has 2-6 base pair repeats such as (CA) $_n$, (GT) $_n$ and (CAG) $_n$, with (CA) $_n$ being the most common [11, 12]. The value of n , which is usually 10 to 60, depends on the copy number of the repeating units and serves as the marker of high polymorphism [13]. It is generally believed that MS is closely related to gene recombination and is the source of gene recombination and variation [14]. MS performs the function of gene regulation by changing DNA structure or binding to specific proteins. MSI refers to the gain or loss of SSR due to DNA replication error. It is found that the occurrence of gastric cancer involves 2 different genetic pathways [15]. One is the classical pathway of tumor inhibition, and the other is MSI pathway. The former is associated with most gastric cancers showing MSI-L and MSS. Heterozygosity (LOH) and mutation of tumor suppressor genes APC/MCC, DCC and p53 play important roles in gastric cancer [16]. The latter pathway is associated with the gastric cancer showing MSI-H. Defect in DNA mismatch repair gene can lead to the increase of single-nucleotide mutation rate of TGF β RII, BAX and hMSH6 genes and extensive MSI [17-19].

Among the gastric cancer patients in this study, the detection rate of MSI was 66.2%, as opposed to 30%-41.5% reported in domestic literatures [20-22] and 16%-59% in foreign literatures [23, 24]. In some foreign cases, the positive detection rate of MSI was 76.7% [25]. In the present study, the detection rate of MSI in dysplasia tissues was 31.3%, which agreed with the results by other domestic researches under comparable conditions [20-22]. The main reason for the inconsistent detection rate of MSI is the lack of uniform standard for MSI detection for the diagnosis of cancers. Different type and number of MS loci, sample size and criteria for positive MSI may be chosen. Besides, the positive rate of MSI is also related to genetic background and geographical location [26].

So far the relationship between positive MSI and the clinical and pathological features of MSI is not fully understood. Some scholars [27, 28] believed that MSI-H gastric cancer mainly occurs in gastric antrum with less lymph node metastasis and good prognosis. Wirtz et al. [29] showed that MSI is independent from sex, age, depth of tumor invasion, degree of cellular differentiation, lymph node metastasis, Lauren classification and prognosis. According to our findings, the cases with MSI-L gastric cancer tended to have earlier lymph node metastasis, and their prognosis was worse than those showing MSI-H and MSS. Moreover, MSI-L gastric cancer was mostly lowly differentiated compared with MSI-H gastric cancer. However, statistical analysis indicated that there was no significant difference in terms of the degree of cellular differentiation and lymph node metastasis between the two types of gastric cancer. Further analysis did not lead to the conclusion that MSI-H gastric cancer mainly occurs in gastric antrum with less lymph node metastasis and good prognosis as previously found. We propose two reasons for this. One is the small sample size, and the other is the factor of geological location. All cases come from Beijing, China, where the frequency of MSI may differ from that of elsewhere. As shown by the results, most gastric cancers show MSI-L and MSS, with a few showing MSI-H. Intestinal metaplasia in gastric mucosa and dysplasia are considered to be the precancerous lesions in gastric cancer. The occurrence of some types of gastric cancer is the result of accumulated gene mutations in precancerous tissues. There are

evidences showing that MSI is involved in the progression from intestinal metaplasia to gastric cancer. In some literatures, the incidence of MSI in intestinal metaplasia tissues is 30%-44.15% [20-22]. Due to the lack of intestinal metaplasia cases, the results obtained may have certain limitations. Studies with larger sample size are needed to confirm the findings. Some foreign scholars carried out investigation over the relationship between MSI and prognosis of gastric cancer. It was believed that the patients with high positive rate of MSI were more sensitive to cisplatin chemotherapy. Nevertheless, the mutation rate of p53 and the positive rate of MSI did not have a significant impact on the efficacy of cisplatin chemotherapy [30].

MSI is prevalent in precancerous tissues of gastric cancer. Gene instability already occurs at this stage as an abnormal molecular event, showing the potential to trigger the multi-step process towards gastric cancer. MSI is an important factor in the occurrence of gastric cancer. MSI detection of precancerous tissues of gastric mucosa can be used to predict the risk of gastric cancer, and those showing MSI need to be followed up regularly for early diagnosis. The positive rate of MSI is very high in gastric cancer patients in our study. MSI may be another molecular mechanism involved in the multi-step process of the development of gastric cancer at such a staggeringly high incidence in China. Therefore MSI can serve as a sensitive indicator of genetic instability in gastric cancer.

Disclosure of conflict of interest

None.

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