Microsatellite instability in gastric cancer and pre-cancerous lesions

Ping Liu, Xiao-Yong Zhang, Yun Shao, Dao-Fu Zhang

AIM: To investigate the microsatellite instability (MSI) in cancer and pre-cancerous lesions of the stomach and its mechanisms underlying the development of gastric cancer.

METHODS: Thirty-six gastric cancer samples were obtained from patients undergoing surgery. Forty-one gastric mucosa samples with dysplasia and 51 with intestinal metaplasia (IM) were obtained from patients with chronic gastritis undergoing gastro-endoscopy. Genomic DNA was extracted from the samples. Silver staining single strand conformation polymorphism-polymerize chain reaction (SSCP-PCR) was used to screen MSI markers at 5 loci (Bat-25, Bat-26, D5S346, D17S250, and D2S123) in fresh tissues and formalin-fixed, paraffin-embedded samples and their corresponding normal gastric mucosa.

RESULTS: The abnormal shifting of the single-strand DNA (MSI) was identified in 21 out of 36 (58.3%) gastric cancers. Seven cases showed high-level MSI (two or more loci altered) and 14 showed low-level MSI (one locus altered). Gastric cancer with MSI had a tendency to be located in the distal stomach. MSI was also detected in 11 out of 41 (26.8%) dysplasia samples and in 9 of 51 (17.6%) IM samples respectively. Three cases of dysplasia and one case of IM showed high-level MSI. Eight cases of dysplasia and 8 cases of IM displayed low-level MSI. MIS in IM was found only in moderate or severe-grade IM. No association was detected between MSI and dysplasia grade.

CONCLUSION: Accumulation of MSI in dysplasia and intestinal metaplasia of gastric mucosa may be an early molecular event during gastric carcinogenesis and may contribute to the acquisition of transformed cell phenotype and the development of gastric cancer.
Tissue DNA extraction
Serial 5-µm-thick sections were obtained from formalin-fixed and paraffin-embedded tissue blocks. After tissue deparaffinization, genomic DNA was isolated by standard proteinase-K digestion and phenol-chloroform extraction protocol as previously described[20].

PCR-SSCP analysis
All samples were analyzed using five markers (Bat-25, Bat-26, D5S346, D17S250, and D2S123) recommended by America National Cancer Institute (NCI) workshop on MSI[21]. Oligonucleotides of five pair primers were synthesized by Sangon Technologies, Shanghai, China. The primer sequences are listed in Table 1.

Polymerase chain reaction (PCR) was performed as described by Fleisher et al.[11] with some modifications. In brief, 25 µL reaction mixture containing 200 ng of DNA, 2.5 µL 10× PCR buffer, 1 µmol/L primer, 1.5-2.0 mmol/L MgCl₂, 200 µmol/L dNTPs, and 0.5 U Taq DNA polymerase (Takara, Japan), was amplified for one cycle at 95 °C for 5 min followed by 35 cycles at 94 °C for 30 s, at 55-58 °C for 30 s, at 72 °C for 15 s, and 72 °C for 1 min.

Single strand conformation polymorphism (SSCP) was carried out. In brief, 12 µL of each PCR product was mixed with 12 µL denaturing buffer, denatured at 97 °C for 7 min, loaded onto a non-denaturing 7% polyacrylamide gel and electrophoresed for 3 h at 20 °C. The bands were visualized by silver staining.

Existence of MSI was defined as an band mobility shift from either alleles or as appearance of a new band with a different size in the testing sample compared to the control one. The analysis was performed once more in samples displaying MSI for confirmation of the results. High-level MSI (MSI-H) was recognized, when more than 30% of the markers showed instability and low-level MSI (MSI-L) was recognized, if less than 30% of the markers displayed instability. None of the markers showing MSI indicated microsatellite stability (MSS)[21].

Statistical analysis
Statistical analysis was performed using the t test or Fisher’s exact test. P<0.05 was considered statistically significant.

Table 1 Sequences of primers for PCR analysis

| Repeat sequence | Primers | Amplicated fragment(bp) |
|----------------|---------|------------------------|
| BAT-25         | 5‘-TCGCCCTAAGAATGTAAAGT-3’ | -90 |
|                | 5‘-TCTGCATTTTAACTATGGCTC-3’ | |
| BAT-26         | 5‘-TGACTACTTTGGACCTCAGCC-3’ | 80-100 |
|                | 5‘-AAACATCAAATTTAAACC-3’ | |
| D5S346         | 5‘-ACTCAGTCATGTAATATCC-3’ | 96-122 |
|                | 5‘-ACGAGATAAAGAGAGTTGTAATAG-3’ | |
| D17S250        | 5‘-GGATGAAGATGTAATAGC-3’ | 150 |
|                | 5‘-GCCGCCCATATATTATGACC-3’ | |
| D2S123         | 5‘-AACAGAGATGCTCCATTCTA-3’ | 197-227 |
|                | 5‘-GGACTTTTACACCTATGGGAC-3’ | |
RESULTS

**MSI in gastric cancer**

None of the 36 gastric cancer patients had a family history of gastric cancer. MSI was observed in 21 out of 36 (58.3%) gastric cancers. Among the 21 MSI+ cases, 7 showed MSI-H and 14 showed MSI-L. Figure 1 displays a representative MSI band compared to control counterpart. No association was observed between MSI status and age, gender, tumor grade, tumor location or lymph node spread. Gastric cancer with MSI had a tendency to be located in the distal stomach compared to gastric cancer with MSS (Table 2).

**Table 2** Characteristics of 36 gastric cancer patients

| Characteristic                  | MSS (n = 15) | MSI-L (n = 14) | MSI-H (n = 7) |
|---------------------------------|--------------|----------------|--------------|
| Average age                     | 58           | 62             | 58           |
| Sex                             |              |                |              |
| Male                            | 10           | 11             | 4            |
| Female                          | 5            | 3              | 3            |
| Differentiation grade           |              |                |              |
| well-moderate                   | 7            | 9              | 6            |
| poor                            | 8            | 5              | 1            |
| Tumor location                  |              |                |              |
| Distal                          | 8            | 8              | 5            |
| Proximal                        | 7            | 6              | 2            |
| Lymph node spread               |              |                |              |
| Absent                          | 9            | 7              | 4            |
| Present                         | 6            | 8              | 3            |

**MSI in dysplasia**

MSI was detected in 11 out of 41 (26.8%) dysplasia samples. Among the 11 MSI+ samples, three showed MSI-H and 8 displayed MSI-L. The frequency of MSI in moderate to severe dysplasia was higher (33.3%) than that in mild dysplasia (20%), but the difference was not significant. Notably, four out of five severe dysplasia samples presented MSI, suggesting MSI tended to develop frequently in mucosa with severe dysplasia (Table 3).

**Table 3** Characteristics of 41 patients with dysplasia

| Characteristic | MSS (n = 30) | MSI-L (n = 8) | MSI-H (n = 3) |
|----------------|--------------|---------------|--------------|
| Histological grade |            |                |              |
| Mild            | 16           | 3             | 1            |
| Moderate        | 13           | 2             | 1            |
| Poor            | 1            | 3             | 1            |

**MSI in intestinal metaplasia**

MSI was detected in 9 of 51 (17.6%) intestinal metaplasia samples. Among the nine samples, one showed MSI-H and the other eight showed MSI-L. Notably, MSI was found only in moderate or severe-grade IM (9/14) other than in mild-grade IM (0/28, Z = 3.630, P = 0.001). Moreover, IM samples from female patients had a higher frequency of MSI compared to IM tissues from males (29.2% vs 7.4%), but the P value was more than 0.05 (Table 4).

**Table 4** Characteristics of 51 patients with intestinal metaplasia

| Characteristic | MSS (n = 42) | MSI-L (n = 8) | MSI-H (n = 1) |
|----------------|--------------|---------------|--------------|
| Sex            |              |                |              |
| Male           | 25           | 2             | 0            |
| Female         | 17           | 6             | 1            |
| Histological grade |         |                |              |
| Mild           | 28           | 0             | 0            |
| Moderate       | 13           | 7             | 0            |
| Poor           | 1            | 0             | 1            |

**DISCUSSION**

The mechanisms of carcinogenesis in gastric mucosa remain unclear, and may involve multiple genetic and epigenetic changes in susceptible cells of the stomach. Genetic instability including chromosomal instability and microsatellite instability is an important factor for the accumulation of these genetic changes.

Microsatellites are ubiquitous, short, repetitive DNA sequences widely and randomly distributed throughout the human genome, with unknown function. MSI is a form of genetic instability characterized by expansions and contractions of simple sequence repeats in DNA. It represents an important form of genomic instability associated with defective DNA mismatch repair in tumors. MSI can be identified in tumors when alleles of novel sizes are detected in microsatellite sequences derived from cancer DNA that are not present in normal tissues of the same individual. MSI has been observed in a subset of gastric carcinoma ranging from 13% to 44%, even more than 70% in some individual reports[10-14]. The discrepancy in different researches is probably due to different types and numbers of microsatellite markers used in different studies.

In the present study, we have screened three groups of patients with the reference set of five markers (two mono- and three di-nucleotide repeats) recommended by America National Cancer Institute (NCI) and the criteria for identification of MSI. In our study, the incidence of MSI in gastric cancer was 58.3%. Leung et al[13], reported, that MSI-H gastric tumors are statistically associated with location of the tumor (distal area of the stomach), fewer lymph node metastases and better prognosis. Wirtz et al[23], reported, that no association is observed between MSI and gender, tumor invasion, pathological grade, lymph node metastases, Lauren’s classification and prognosis, which is similar to our results.

The association between MSI and clinicopathologic characteristics of gastric cancer remains unknown. Wu et al[23], reported, that MSI-H gastric tumors are statistically associated with location of the tumor (distal area of the stomach), fewer lymph node metastases and better prognosis. Wirtz et al[23], reported, that no association is observed between MSI and gender, tumor invasion, pathological grade, lymph node metastases, Lauren’s classification and prognosis, which is similar to our results.

IM and dysplasia are considered as early phenotypic changes in cascade of events leading to gastric cancer. Development of some gastric cancer may be the result of an accumulation of abnormal gene change in these pre-cancerous lesions[12, 16-19]. In our study, the MSI incidence of IM was 17.6%, lower than that previously reported (30-44.5%)[18,19]. The discrepancy might be due to the IM samples
collected from the patients with chronic gastritis, other than gastric cancer and the histological grade of IM. Different markers used in different studies may be another explanation for the discrepancy. MSI is connected with moderate-grade, which is of importance in grading of IM. We detected MSI in 26.8% samples with dysplasia in the present study. Lee et al[24], reported, that there is no significant association between MSI and histological grade of dysplasia. Investigating the occurrence of MSI in gastric cancer and pre-cancerous lesions may help us to explore the mechanisms of gastric carcinogenesis. Being detected in gastric cancer, IM and dysplastic mucosa, MSI might play a role in the multistep process of carcinogenesis of the stomach. It was reported that a well-differentiated adenocarcinoma develops 3 years later at the IM mucosa displaying MSI[25]. In conclusion, early involvement and continuous accumulation of MSI in susceptible cells of the stomach may trigger the multi-step carcinogenesis pathway. Detection of MSI in pre-cancerous lesions may help us to investigate the stomach carcinogenesis and to identify patients at risk of developing gastric malignancies.

REFERENCES

1 Fang DC. Role of genetic instability in development of gastric cancer. Shi jie Huaren Xiaohua Zazhi 2003; 11: 1-5
2 Yasui W, Yokozaki H, Fujimoto J, Naka K, Kuniyasu H, Tahara E. Genetic and epigenetic alterations in multistep carcinogenesis of the stomach. J Gastroenterol 2000; 35 (Suppl 12): 111-115
3 Tamura G. Genetic and epigenetic alterations of tumor suppressor and tumor-related genes in gastric cancer. Histol Histopathol 2002; 17: 323-329
4 Wang RQ, Fang DC, Liu WW. MUC2 gene expression in gastric cancer and preneoplastic lesion tissues. Shi jie Huaren Xiaohua Zazhi 2000; 8: 285-288
5 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759-767
6 Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylvakanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. Science 1993; 260: 812-816
7 Thibodeau SN, Bren G, Scha id D. Microsatellite instability in cancer of the proximal colon. Science 1993; 260: 816-819
8 Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perou M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993; 363: 558-561
9 Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. Nature 1997; 368: 623-627
10 Fang DC, Jass JR, Wang DX, Zhou XD, Luo TH, Young J. Infrequent loss of heterozygosity of APC/MCC and DCC genes in gastric cancer showing DNA microsatellite instability. J Clin Pathol 1999; 52: 504-508
11 Fleisher AS, Esteller M, Tamura G, Rashid A, Stine OC, Yin J, Zou TT, Abraham JM, Kong D, Nishizuka S, James SF, Wilson KT, Herman JG, Meltzer SJ. Hypermethylation of the hMLH1 gene promoter is associated with microsatellite instability in early human gastric neoplasia. Oncogene 2001; 20: 329-335
12 Leung WK, Kim JJ, Kim JC, Graham DY, Sepulveda AR. Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. Am J Pathol 2000; 56: 537-543
13 Wang YX, Ke Y, Ning T, Feng LY, Lu GR, Liu WL. Studies of microsatellite instability in Chinese gastric cancer tissues. Chinese J Med Genet 1998; 15: 155-157
14 Halling KC, Harper H, Moskaluk CA, Thibodeau SN, Petroni GR, Yustein AS, Tosi P, Minacci C, Roviello F, Piva P, Hamilton SR, Jackson CE, Powell SM. Origin of microsatellite instability in gastric cancer. Am J Pathol 1999; 155: 205-211
15 Semba S, Yokozaki H, Yamamoto S, Yasui W, Tahara E. Microsatellite instability in pre-cancerous lesions and adenocarcinomas of the stomach. Cancer 1996; 77(Suppl 8): 1620-1627
16 Hao DM, Sun XJ, Zheng ZH, He G, Ma MC, Xu HM, Wang MX, Sun KL. Screening and expression of associated genes in gastric dysplasia. Shi jie Huaren Xiaohua Zazhi 2003; 11: 6-9
17 Chen SY, Wang JY, Ji Y, Zhang XD, Zhu CW. Effects of Helicobacter pylori and protein kinase C on gene mutation in gastric cancer and pre-cancerous lesions. Shi jie Huaren Xiaohua Zazhi 2001; 9: 302-307
18 Ruol A, Parenti A, Zaninotto G, Merigliano S, Costantini M, Cagol M, Alfieri R, Bonavina L, Perachchia A, Ancona E. Intestinal metaplasia is the probable common precursor of adenocarcinoma in Barrett esophagus and adenocarcinoma of the gastric cardia. Cancer 2000; 88: 2520-2528
19 Kobayashi K, Okamoto T, Takayama S, Akiyama M, Ohno T, Yamada H. Genetic instability in intestinal metaplasia is a frequent event leading to well-differentiated early adenocarcinoma of the stomach. Er J Cancer 2000; 36: 1113-1119
20 Burton MP, Schneider BG, Brown R, Escamilla-Ponce N, Gulley ML. Comparison of histologic stains of use in PCR analysis of microdissected paraffin-embedded tissues. Biotechniques 1998; 24: 86-92
21 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58: 5248-5257
22 Wu MS, Lee CW, Chun CT, Wang HP, Lee WJ, Chang MC, Shen JC, Lin JT. Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes. Genes Chromosomes Cancer 2000; 27: 403-411
23 Wirtz HC, Muller W, Noguchi T, Scheven M, Ruschoff J, Hommel G, Gabbert HE. Prognostic value and clinicopathologic profile of microsatellite instability in gastric cancer. Clin Cancer Res 1998; 4: 1749-1754
24 Lee JH, Abraham SC, Kim HS, Nam JH, Choi C, Lee MC, Park CS, Juhung SW, Rachid A, Hamilton SR, Wu TT. Inverse relationship between APC gene mutatio in gastric adenomas and development of adenocarcinoma. Am J Pathol 2002; 161: 611-618
25 Kashiwagi K, Watanabe M, Ezaki T, Kanai T, Ishii H, Mukai M, Hibi T. Clinical usefulness of microsatellite instability for the prediction of gastric adenoma or adenocarcinoma in patients with chronic gastritis. Br J Cancer 2000; 82: 1814-1818