

RAPID COMMUNICATION

Loss of heterozygosity and microsatellite instabilities of fragile histidine triad gene in gastric carcinoma

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Abstract

AIM: To detect the loss of heterozygosity (LOH) and microsatellite instabilities (MSI) of fragile histidine triad (FHIT) gene in gastric carcinoma and to study their association with the clinical pathological characteristics of gastric carcinoma.

METHODS: LOH and MSI of FHIT gene were detected at four microsatellite loci D3S13H, D3S4I03, D3S148I and D3S1234 using PCR in matched normal and cancerous tissues from 50 patients with primary gastric cancer.

RESULTS: The average frequency of LOH and MSI of FHIT gene in gastric cancer was 32.4% and 26.4% respectively. LOH and MSI of FHIT gene in gastric cancer had no association with histological, Borrmann, and Lauren's classification. LOH of FHIT gene in gastric cancer was related to invasive depth. The frequency of FHIT LOH in gastric cancer with serosa-penetration was obviously higher than that in gastric cancer without serosa-penetration (73.5% vs 37.5%, $P < 0.05$). MSI of FHIT gene in gastric cancer was associated with the lymph node metastasis. The frequency of MSI in gastric cancer without lymph node metastasis was significantly higher than that in gastric cancer with lymph node metastasis (66.7% vs 34.3%, $P < 0.05$).

CONCLUSION: LOH of FHIT gene is correlated with invasive depth of gastric carcinoma. MSI of FHIT gene is correlated with lymph node metastases. LOH and MSI of FHIT gene play an important role in carcinogenesis of gastric cancer.

Key words: Gastric cancer; FHIT gene; Microsatellite instabilities

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INTRODUCTION

Gastric carcinoma is the most frequent malignancy and the leading cause of cancer-related deaths in China^[1-4]. The specific oncogenes and tumor suppressor genes correlated with gastric cancer have not been found. In recent years, many genes have been found to be related to gastric carcinogenesis, such as fragile histidine triad gene (FHIT), RUNX3 and PTEN, among which FHIT gene is a tumor suppressor gene^[5] which is located in chromosome 3p14.2, and comprises 10 exons. Exons 1-4 and 10 are non-coding exons, and exons 5-9 are coding exons. Recent studies indicate that FHIT abnormality mostly results from loss of exons 5 and 8^[6-9]. Exon 5 contains the start codon (ATG) of opening read frame (ORF), exon 8 contains core unit of coding histidine triplet. Chromosome 3p14.2 containing the common aphidicolin-induced fragile site (FRA3B)^[10-13], is the non-randomly fragmental or fissured site by its spontaneous or induced actions, and susceptible to chromosome loss and rearrangement because of the frequent fragmentation or fissure, which might be related to tumor-genesis. In this study, we detected the loss of heterozygosity (LOH) and microsatellite instabilities (MSI) of microsatellite alleles close to FHIT gene in 50 cases of gastric cancer.

MATERIALS AND METHODS

Specimens

All specimens of gastric cancerous and matched distal normal mucosal tissues were surgically resected at No.1 Hospital of China Medical University without preoperative chemotherapy and radiotherapy. Specimens were put immediately into liquid nitrogen after removal, stored at -70°C, and classified respectively by Borrmann, WHO histological classification, Lauren classification, invasive depth and lymph node metastasis, etc.

Table 1 Primer sequences of FHIT gene at different sites

MSM		Sequence	Product length
D3S1300	F	5'-ATAAACACTACAAAGGAAC-3'	49 bp
	R	5'-TATCTTAAAATTGCACCC-3'	
D3S1234	F	5'-ACGGTGGACGAGAAGAAC-3'	612 bp
	R	5'-TTTGAACAAGAAGTGGCATA-3'	
D3S1481	F	5'-CTGTGGGTTCGTCTGTTA-3'	489 bp
	R	5'-CTGGAGGCATCTCATTAC-3'	
D3S4103	F	5'-TGGTTGGTGCTATTCIC-3'	226 bp
	R	5'-TTATTCGGCTCATTTGTC-3'	

MSM : Microsatellite marker.

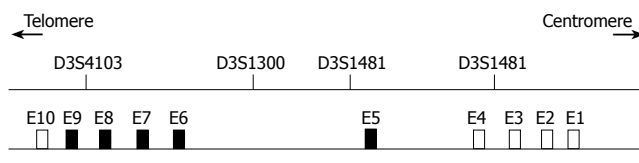


Figure 1 FHIT gene cDNA at different sites.

DNA extraction

DNA was extracted from the frozen gastric cancerous and normal mucosal tissues by standard phenol-chloroform method^[3,4], dissolved and stored in TE buffer. Finally, purity and concentration of the extracted DNA were analyzed by gel electrophoresis and ultraviolet spectrophotometry.

Microsatellite marker selection and PCR amplification

Four microsatellite marker sites (D3S1300, D3S1234, D3S4103 and D3S1481) in chromosome 3p14.2 were selected to detect LOH and MSI of FHIT gene. PCR primers (sequences in Table 1 and sites in Figure 1) were synthesized by Beijing AoKe Biology Company. PCR amplification was carried out in a final volume of 50 μ L containing 50 ng DNA, 0.5 μ mol/L of each primer, 200 μ mol/L of each dNTP, and 1U Taq DNA polymerase (TaKaRa Ex TaqTM). The amplification conditions were an initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, at 57°C for 1min, at 72°C for 40 s, and a final extension at 72°C for 5 min.

Polyacrylamide gel electrophoresis (PAGE)

The amplified PCR products were denatured at 95°C for 5 min and run on 8% denaturation polyacrylamide gel (1 \times TBE buffer, circulatory water) at a voltage 500 V, power 45 W for 3.5 h. Silver staining was performed as previously described^[14].

Determination of FHIT LOH and MSI

Genomic allele with heterozygote was considered as target of LOH analysis information. LOH was defined as complete loss or up to 50% decreased relative density of silver staining bands of PCR products in primary gastric cancerous tissues compared to distal normal mucosal tissues, and MSI was defined as the increased or mobile bands of PCR products of gastric cancerous tissues

Table 2 FHIT gene LOH and MSI at different microsatellite sites *n* (%)

MSM	<i>n</i>	AIT	MSI	LOH
D3S1300	50	38 (76.0)	14 (36.8)	10 (26.3)
D3S1234	50	42 (80.4)	9 (21.4)	13 (31.0)
D3S1481	50	29 (54.5)	6 (20.7)	8 (27.6)
D3S4103	50	39 (74.5)	10 (25.6)	17 (43.6)

MSM: Microsatellite marker; AIT: Analysis information target; MSI: Microsatellite instabilities; LOH: Loss of heterozygosity.

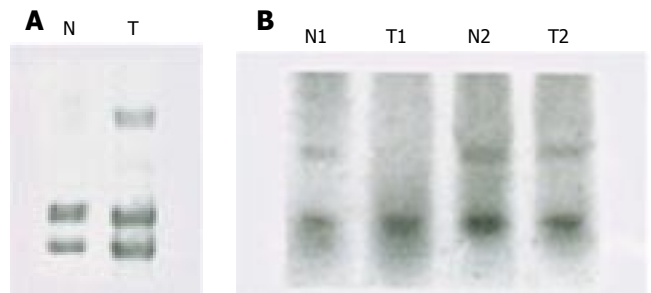


Figure 2 MSI (A) and LOH (B) at D3S4103. T: Gastric cancer tissues; N: Normal gastric mucosal tissues.

compared to normal tissues.

Statistic analysis

Sample rate between different groups was analyzed with chi square test. $P < 0.05$ was considered statistically significant.

RESULTS

Detection of FHIT gene LOH and MSI in gastric cancers

The frequencies of LOH and MSI of FHIT gene in four microsatellite sites are shown in Table 2. The average frequency of FHIT gene LOH in gastric cancer was 32.4%, and the most frequent site was D3S4103, accounting for 43.6% (Figure 2A). All samples had different degrees of LOH at four sites. The average frequency of FHIT gene MSI in gastric cancer was 26.4%, the most frequent site was D3S1300, accounting for 36.8% (Figure 2B), and 11 cases (22.0%) had MSI at two or more microsatellite sites.

Relationship between FHIT gene LOH, MSI and clinicopathological characteristics of gastric cancer

There was no statistical relationship between FHIT gene LOH and Borrmann's, WHO's, Lauren's classification and lymph node metastasis of gastric cancer ($P > 0.05$), while FHIT gene LOH was significantly associated with invasive depth of gastric cancer. LOH frequency in gastric cancers penetrating serosa was significantly higher than that in gastric cancers not penetrating serosa ($P < 0.05$). There was no statistical relationship between FHIT gene MSI and Borrmann's, WHO's, Lauren's classification and invasive depth of gastric cancer ($P > 0.05$), while FHIT gene MSI was significantly associated with lymph node metastasis

Table 3 Relationship between FHIT gene LOH, MSI and clinicopathological characteristics of gastric cancer *n* (%)

	<i>n</i>	MSI ¹	LOH ²
Histological type			
pap.ade & wel.ade	6	3 (50)	4 (66.7)
mod.ade	9	5 (55.6)	5 (55.6)
por.ade	26	13 (50.0)	17 (65.4)
muc.ade	7	3 (42.9)	3 (42.9)
SRC	2	1 (50.0)	1 (50.0)
Lauren			
Diffusive type	35	17 (48.6)	21 (60.0)
Intestinal type	15	8 (53.3)	9 (60.0)
Bormann			
I	5	3 (60.0)	3 (60.0)
II	10	6 (60.0)	7 (70.0)
III	15	7 (46.7)	10 (66.7)
IV	10	5 (50.0)	7 (70.0)
Invasive depth			
without serosa penetration	16	7 (43.8)	6 (37.5)
with serosa penetration	34	18 (52.9)	25 (73.5) ^a
LN metastasis			
+	35	12 (34.3)	21 (60.0)
-	15	10 (66.7) ^a	9 (60.0)

¹ MSI occurring at least one microsatellite site; ² LOH occurring at least one microsatellite site; ^a*P* < 0.05 vs without serosa penetration and with LN metastasis.

of gastric cancer. MSI frequency in gastric cancers with lymph node metastasis was significantly higher than that in gastric cancers without lymph node metastasis (*P* < 0.05, Table 3).

DISCUSSION

FHIT gene fragile site containing instable repeated sequence of tris-nucleotide (CGG or CCG) or binucleotide (AT), is subjected to easy breakage because of its susceptibility to external environment carcinogen and mutagen. There are many Alu repeated sequences in proximal telomere of fragile site FRA3B. Susceptible fragile site has highly instable repeated sequence (TAA)₁₅^[11,12,15-20]. It can be inferred that under the action of external carcinogen, breakage and crevice may occur at FRA3B site, following DNA abnormal repair and rearrangement, thus making complex diversity of FHIT gene^[21-24].

Our study showed that the average frequency of FHIT gene LOH and MSI was 32.4% and 26.4% respectively in gastric cancer. There was no significant discrepancy between different microsatellite sites. The most frequent site of LOH and MSI was D3S4103 (43.6%) and D3S1300 (36.8%) respectively located in the first coding exon 5 near the FHIT gene, suggesting that FHIT gene loss is common in gastric cancer. Moreover, many cases had both LOH and MSI at two or more sites, suggesting that variant range of FHIT gene is broader. We did not discover that FHIT gene LOH was associated with Borrmann, histological and Lauren's classification as well as lymph node metastasis. But significant relationship was found between FHIT LOH and invasive depth of gastric cancer. Huiping *et al*^[6] reported that gastric cancer is concomitant with frequent

FHIT gene LOH and MSI (84% and 27% respectively), and loss of FHIT protein is closely associated with lymph node metastasis. Cappuzzi *et al*^[7] also reported that loss of FHIT protein is positively correlated with clinicopathological stage and histological grade of gastric cancer, but negatively with poor prognosis. Noguchi *et al*^[25] showed that LOH of FHIT gene is not related to progression and prognosis of gastric cancer.

There were no significant differences in FHIT gene LOH and MSI among various gastric cancers, suggesting that FHIT gene loss might not be associated with different gastric cancer. FHIT gene LOH was significantly associated with invasive depth of gastric cancer. LOH frequency in gastric cancers penetrating serosa was significantly higher than that in gastric cancer not penetrating serosa, suggesting that FHIT gene LOH is correlated with infiltration and diffusion of gastric cancer. FHIT gene MSI was significantly associated with lymph node metastasis of gastric cancer. MSI frequency in gastric cancers with lymph node metastasis was significantly higher than that in gastric cancers without lymph node metastasis, suggesting that FHIT gene mainly participates in early gastric carcinogenesis. The mechanism of down-regulated expression of FHIT post-transcription needs further study.

In conclusion, FHIT LOH and MSI reflect the genomic instability of gastric cancer and play an important role in gastric carcinogenesis. Abnormal FHIT gene may provide some new clues to malignant biological behavior and molecular mechanism of gastric carcinogenesis.

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