Identification of a new target region on the long arm of chromosome 7 in gastric carcinoma by loss of heterozygosity

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Abstract

AIM: To define the common deleted region on the long arm of human chromosome 7q linked to primary gastric carcinomas in Chinese by loss of heterozygosity (LOH) and its clinical significance.

METHODS: Nine microsatellite markers distributed over chromosome 7q with an average marker density of 10cM were used to examine 70 primary gastric carcinomas for LOH by PCR amplification. The PCR products were separated by electrophoresis on polyacrylamide gel. Genescan and Genotyper softwares were used to analyze LOH.

RESULTS: LOH with at least one marker on 7q occurred in 34.3% (12/50) of the tumors. Among them, LOH at D7S486 and D7S798 was higher in 24.0% (24/70) and 19.2% (5/26), respectively. By statistical analysis we also observed an obvious genotype-phenotype correlation on 7q (P < 0.05). The frequency of LOH at D7S486 in patients with lymph node metastasis was significantly higher than that in those without lymph node metastasis (P = 0.015).

CONCLUSION: The high incidence of LOH at D7S486 and its correlation with poorer prognosis suggest that there might be putative tumor suppressor genes in this region involved in the tumorigenesis and progression of gastric carcinoma.
MATERIALS AND METHODS

Materials

Primary gastric cancer and its corresponding normal tissue were obtained from 70 patients resected at the Affiliated Cancer Center of Zhongshan University during December 2000 to January 2002. All the patients did not receive any radiotherapy or chemotherapy before operation. The tissues were taken immediately after excision. One part of the samples was preserved at -80°C for DNA extraction, the others were treated with routine protocol, embedded with paraffin and stained with HE.

Seventy patients (52 men and 18 women) aged 19 - 76 years (mean age 52.9 years) were enrolled in this study. The histological types included 1 papillary adenocarcinoma, 2 mucinous adenocarcinomas, 20 signet-ring cell adenocarcinomas, 6 undifferentiated adenocarcinomas, and 1 adenosquamous carcinoma, which were diagnosed according to the WHO classification of gastric carcinoma (1990). Based on the UICC TNM classification of gastric carcinoma, the patients were divided into 7 cases of stage I (3 of I A and 4 of I B), 13 cases of stage II, 24 cases of stage III (14 of III A and 10 of III B) and 26 cases of stage IV.

DNA extraction

Genomic DNA samples of cryo-preserved gastric carcinomas and matched normal gastric mucosa were isolated by phenol chloroform extraction using standard protocols. DNA purification and concentration were detected by gel electrophoresis.

Selection of microsatellite markers and PCR amplification

A total of 9 microsatellite markers distributed over the whole chromosome 7q were selected from Genethon human genetic linkage map: D7S515, D7S486, D7S530, D7S640, D7S684, D7S661, D7S636, D7S798 and D7S2465. The frequencies of heterozygosity of the markers were over 75% and the average marker density was about 10cM. Synthesized microsatellite primers were respectively labeled with three different color fluorescent dyes: FAM, NED and HEX (purchased from PE Company). DNA samples were divided into 2 groups according to molecular size of the products and color of the fluorescent dyes. Primers (including 4 to 5 pairs) of each group were mixed in a tube for amplification. Each multiplex PCR reaction volume (5 μL in total) contained 0.5 μL 10 × buffer, 0.2 μL 10 mmol/L dNTP, 0.3 μL 25 mmol/L MgCl2, 0.05 μL 10 pmol/L primers, 0.2 U hot start Taq™ DNA polymerase (Gene Company, USA) and 45 ng genomic DNA. PCR amplifications were carried out in Gene Amp PCR system 9700 thermal cycler (Perkin-Elmer Co, Norwalk, USA). The reactive conditions were as follows: initial denaturation at 95°C for 12 min followed by 10 cycles at 94°C for 30 s, at 63°C for 60 s (decreasing by 0.5°C per cycle), at 72°C for 90 s, and 26 cycles at 94°C for 30 s, at 58°C for 60 s, at 72°C for 90 s and a final extension at 72°C for 15 min.

Genomic scanning and analysis of microsatellite instability

PCR products (0.5μL) were diluted with 1.0 μL electrophoresis loading buffer containing 70% formamide, 3.75 mmol/L EDTA, 7.5 mg/ml blue dextran, 1.2 mmol/L Genescan 350. Mixture (1.0 μL) was loaded in urea-containing denaturing Sequagel Tm polyacrylamide gel (Gene Company, USA), and subjected to electrophoresis on a 377 DNA sequencer (ABI PRISM, PE Applied Biosystems). The automatically collected data were analyzed with Genescan Version 3.0 and Genotyper Version 2.0 software. Genotype for a given locus with two peaks of PCR fragments was defined as heterozygosity, and genotype with a single peak was regarded to be homozygosity. Only patients heterozygous for a given locus were regarded to be informative. Comparison of the ratios between tumors (T) and their controls (N) was made using the following formulas for calculations:

\[
\frac{(T_1/N_1) \cdot (T_2/N_2)}{(T_1/N_1) \cdot (T_2/N_2)} \quad (1)
\]

\[
\frac{(T_1/N_1) \cdot (T_2/N_2)}{(T_1/N_1) \cdot (T_2/N_2)} \quad (2)
\]

where \(T_1\) and \(N_1\) are the peak height of the smaller allele, while \(T_2\) and \(N_2\) are the peak height of the larger allele. Formula (1) was used to calculate the ratio of the smaller allele while formula (2) was used to calculate the ratio of the larger allele. For ratios greater than 1, the reciprocal of the ratio was calculated to give a value between 0.00 and 1.00. A value <0.67 was assigned as indicative of LOH[10-12].

Statistical analysis

The data of two-sample ratio were evaluated by chi-square test and Fisher’s exact test. \(P<0.05\) was considered statistically significant.

RESULTS

Distribution and frequency of LOH in primary gastric carcinoma

Genomic DNA was extracted from 70 primary gastric carcinomas and paired normal gastric mucosa tissues, 9 microsatellite fragments were amplified. Subsequent genotype analysis of the reliable amplification fragments was performed. LOH at all 9 loci was found in 70 primary gastric carcinoma cases (Table 1). The highest frequency of LOH was 24.0% at D7S486 and 19.2% at D7S798, respectively. LOH was detected in 24/70 (34.3%) of

| Table 1 Distribution and frequency of LOH at 9 microsatellite loci in primary gastric carcinomas |
|-----------------------------------------------|
| Microsatellite loci | Location on chromosome | LOH cases | Heterozygous cases | Positive rate of LOH (%) |
|---------------------|--------------------------|-----------|-------------------|-------------------------|
| D7S515              | 7q22.1                   | 6         | 53                | 11.3                    |
| D7S486              | 7q31.2                   | 12        | 50                | 24                      |
| D7S530              | 7q32.1                   | 4         | 50                | 6.2                     |
| D7S640              | 7q33                     | 7         | 63                | 4.8                     |
| D7S684              | 7q34                     | 4         | 58                | 6.9                     |
| D7S661              | 7q35                     | 3         | 27                | 11.1                    |
| D7S636              | 7q36.1                   | 3         | 60                | 5.9                     |
| D7S798              | 7q36.2                   | 5         | 26                | 19.2                    |
| D7S2465             | 7q36.3                   | 5         | 52                | 9.6                     |
primary gastric carcinomas, 15/24 cases (62.5%) showed LOH at only one locus, 9/24 (37.5%) cases had LOH at two loci, and LOH occurred at more than three loci in 5/24 cases (25.0%).

### Correlations between LOH and clinicopathology

Relations between LOH frequency and clinicopathology in 70 primary gastric carcinomas are shown in Table 2. There were statistical correlations between LOH frequencies at 7q and clinical stages. The frequency increased with poorer clinical stages ($P = 0.046$). LOH at all 9 loci was detected in 13/26 (50.0%) cases with clinical stage IV, of which 6 cases (46.1%) were found to have LOH at D7S486. Five cases with LOH at more than 3 loci were all in stage IV. The frequency of LOH at D7S486 was related with lymph node metastasis. It was significantly higher in cases with lymph node metastasis than in those without metastasis ($P = 0.015$).

However, frequencies of LOH showed no statistically differences in tubular adenocarcinomas, poorly-differentiated adenocarcinomas, signet-cell carcinomas and undifferentiated adenocarcinomas. Four cases showed LOH in 6 undifferentiated adenocarcinomas.

### DISCUSSION

In our previous study, chromosome aberrations and their roles in the genesis and development of primary gastric cancer were investigated using direct G-banding analysis and FISH. The deletion of chromosome 7q is the most consistent aberration, and 7q31-qter is the commonly lost segment. LOH of this region is a very common occurrence in many kinds of human malignancies including cancers of breast, prostate, colon and ovary, as well as primary squamous cell carcinoma of the head and neck. Taken together, a critical TSG probably exists in this region with activation in a broad range of tissues. Some putative TSGs in this region such as ST7, Caveolin-1, ING3, and PPP1R3 have been reported.

However, no further researches provide reliable evidence for the correlation between these candidate genes and primary gastric carcinomas.

Tumor occurrence and progression involve multi-genes and multi-steps. Different genetic alterations participate in tumor occurrence and progression, and genetic alteration plays a vital role in different tumors. Several chromosomal amplifications and deletions have been reported in primary gastric carcinomas. Kuniyasu et al. have reported LOH at 5 microsatellite markers on 7q in 32% (26/82) of 98 gastric carcinomas. D7S95 on 7q31-35 is the most frequent change locus. Similar results were also reported by Nishizuka et al. Our findings are consistent with these previous studies. In our study, the total LOH at 7q was 34.3% in gastric carcinomas, and the frequency of LOH at D7S486 reached 24.0%. The higher frequencies of LOH at D7S486 and D7S798 than at the other 7 loci indicate the presence of tumor suppressor genes in these regions, particularly near D7S486.

Different results about correlations between LOH and clinical factors of chromosome 7q have been reported in various studies. Kuniyasu et al. found that LOH at D7S95 on 7q31-35 is much higher in stage IV gastric carcinomas and that patients with LOH at D7S95 show celiac metastasis compared with those without LOH ($P < 0.05$). Moreover, patients of stage III-IV with LOH at D7S95 survive shorter than those without LOH ($P < 0.05$). Thus, LOH at D7S95 is likely involved in gastric carcinoma progression and prognosis. In our 70 gastric carcinomas, the frequency of LOH at any locus on 7q increased obviously with the rising of clinical stage ($P = 0.046$), and reached 50.0% (13/26) in patients with clinical stage IV. Moreover, 5 cases with LOH at more than 3 loci were all in stage IV. The frequency of LOH at D7S486 in patients with lymph node metastasis was obviously higher than that in those without lymph node metastasis ($P = 0.015$). There was no significant correlation between LOH and histological types. This lack of correlation may be related to the small number of undifferentiated tumors. Our results suggest
that one or more tumor suppressor genes associated with gastric carcinomas might situate on chromosome 7q and D7S486. Loss of restraining effects on tumor proliferation, infiltration and metastasis of these candidate genes might promote gastric carcinoma progression.

The region around the marker D7S486 may contain a fragile site. In fact, a 7q31.2 fragile site (FRA7G) of 300 kb is located between markers D7S486 and D7S522\(^\text{[18]}\). FRA7G is a common aphidicolin-inducible fragile site at 7q31.2, showing LOH in human malignancies. Common fragile sites are specific regions in mammalian chromosomes that are prone to breakage and rearrangements. This genetic instability can lead to disease manifestations and may play a role in oncogenesis\(^\text{[19]}\). The present study delineated a breakpoint of putative TSG near the marker D7S486. Tatarelli et al\(^\text{[20]}\) investigated the structure of FRA7G spanning the region between marker D7S486 and Met H and have identified a gene encoding a 421-amino-acid protein with three LIM domains with 89% identity to murine Testin. These findings suggest that TESTIN may represent a candidate tumor suppressor gene at 7q31.2.

The genetic intervals of microsatellite markers in our study were relatively wide (10cM). Additional studies are needed to narrow these regions on D7S486 and identify potential tumor suppressor genes.

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