

Allelic Loss of the Region of Chromosome 1p35-pter Is Associated with Progression of Human Gastric Carcinoma

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In order to identify the region on distal chromosome 1p that is thought to include one or more tumor suppressor genes for gastric carcinoma, 39 gastric carcinomas were examined for allelic loss using 11 polymorphic microsatellite markers and 1 marker of single strand conformation polymorphism. Loss of heterozygosity (LOH) was found in 18 (46%) of 39 informative patients. The regions with high frequency of loss of heterozygosity were the loci at D1S548 (6/17; 35.3%) and D1S2843 (7/20; 35%), and we found three commonly deleted regions on chromosome 1p35-pter. The frequency of allelic loss in the region of chromosome 1p35-pter was significantly associated with advanced-stage gastric carcinoma, but not with early-stage tumor or with the histology. These results suggest that allelic loss at chromosome 1p35-pter may play a role in the progression of gastric carcinoma.

Key words: Gastric carcinoma — Loss of heterozygosity — 1p35-pter — Tumor progression

The activation of oncogenes and the inactivation of tumor suppressor genes are important mechanisms in the pathogenesis of cancers. Gastric carcinoma is the second most common cause of cancer-related death in the world.¹⁾ Many independent studies have reported relationships between multiple clinicopathological factors and medical treatments in gastric carcinoma. Several genetic alterations have also been reported in gastric carcinoma, e.g. amplifications of the *c-erbB-2*,^{2,3)} and *K-sam*,^{4,5)} genes, and mutations of the *APC*,⁶⁾ *ras*⁷⁾ and *p53*⁸⁾ genes. However, the genetic alterations in gastric carcinogenesis are largely unclear.

Loss of heterozygosity (LOH) studies are useful to define the regions including tumor suppressor genes. Recently, LOH studies have suggested that loci containing tumor suppressor genes associated with gastric carcinogenesis exist on chromosomal arms 1p, 1q, 5q, 7p, 7q, 11p, 11q, 12q, 13q, 17p, and 18q.^{9–13)} Allelic loss on 1p is frequently observed in gastric carcinoma^{11,14)} as well as in hepatocellular carcinoma,¹⁵⁾ colorectal carcinoma,¹⁶⁾ head and neck squamous cell carcinoma,¹⁷⁾ neuroblastoma,¹⁸⁾ glioma,¹⁹⁾ malignant melanoma,²⁰⁾ multiple endocrine neoplasia type 2²¹⁾ and male germ cell tumors.²²⁾ However, there have not been any reports regarding the commonly deleted regions, especially at 1p35-pter, in gastric carcinoma. Therefore, we performed fine-scale deletion mapping of the region by using 39 gastric carcinomas with 11 polymorphic microsatellite markers and a single strand

conformation polymorphism (SSCP) marker. The study has revealed that deletion at 1p35-pter is associated with advanced-stage gastric carcinomas.

MATERIALS AND METHODS

Samples A total of 39 paired samples of tumors and corresponding normal tissues were obtained from Japanese patients with gastric carcinoma during surgery at the Shinshu University Hospital, Nagano. Twenty-three tumors were well differentiated adenocarcinoma and 16 were poorly differentiated adenocarcinomas or signet ring cell carcinomas. Nine cases were early gastric carcinoma in which the depth of tumor invasion was limited to the submucosa, and 30 cases were advanced carcinomas. Tumor tissue and corresponding normal tissue were immediately frozen after the surgical resection and stored at -80°C until use. DNA was extracted using a QIAamp Tissue Kit (QIAGEN, Hilden, Germany).

LOH analysis Ten polymorphic microsatellite markers used in this study are D1S80, D1S214, D1S548, D1S508, D1S450, D1S244, D1S507, D1S2843, D1S458 and MYCL. The sequence information for the PCR primers for those markers were obtained from the Genome Data Base and the sequence of the primer for TNFR2 (SSCP) was previously described.²³⁾ The primer sequences used for amplification of the polymorphic regions of the *p73* gene were described elsewhere.²⁴⁾ PCR was performed with 10 ng of DNA in a reaction volume of 10 μl using 0.25 units of *Taq* polymerase (Expand High Fidelity PCR System, Boehringer-Mannheim, Mannheim, Germany) with 0.25

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mM concentrations each of dNTP. ³²P-dCTP was added before PCR except in the case of the marker of D1S80. The PCR was carried out for 30 cycles and the reaction conditions were optimized (annealing temperature from 51 to 61°C) for each primer. Using the marker of D1S80, PCR products were electrophoresed on a 5% polyacrylamide gel at 200 V·h, stained with ethidium bromide, and viewed under UV light. For all other markers, the PCR products were electrophoresed on a 6% acrylamide gel, and the gel was dried on a sheet of filter paper with a gel dryer and exposed to radiographic film at -80°C. To confirm the results, a BAS 1500 bio-imaging analyzer (Fuji Photo Film Co., Tokyo) was also used. A sample was judged to be LOH when the intensity of one allele in the tumor was 50% less than that in the corresponding normal tissue.

Statistics The statistical significance of differences was analyzed by the χ^2 and Fisher's exact tests.

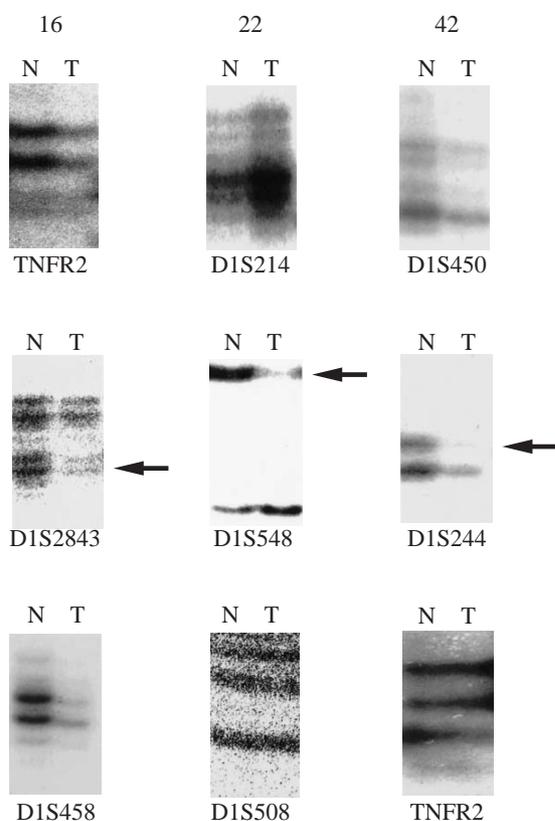


Fig. 1. Representative results of normal and tumor genotypes that determined the three commonly deleted regions at 1p35-pter in primary gastric carcinoma. The patient numbers are shown above the images. The microsatellite markers yielding these results are indicated beneath the respective autoradiographic images. N and T denote DNA from normal and carcinoma tissue, respectively. Arrows indicate the allelic loss.

RESULTS

All 39 tumors were informative at one or more of the loci tested. LOH was detected in 18 (46%) out of 39 cases at one or more loci. Typical patterns of LOH are indicated in Fig. 1. The marker loci and their frequencies of LOH are shown in Table I, in descending order from the telomere to centromere according to the human comprehensive linkage map. We found high incidences of LOH at D1S548 (6/17, 35.3%) and D1S2843 (7/20, 35%). The losses or retentions of alleles in all the tumors with LOH are summarized in Fig. 2. In case 22, a small interstitial deletion was detected between D1S214 and D1S508. Similarly, in case 16, it was detected between TNFR2 and D1S458. Furthermore, in both case 5 and case 42, the interstitial deletion was observed between D1S244 and TNFR2. Larger interstitial deletions involving all three small regions were observed in cases 9, 28, 29 and 45.

Allelic losses were observed in 7 of 16 (43.8%) poorly differentiated adenocarcinoma and 11 of 23 (47.8%) well differentiated adenocarcinoma. There was no significant relationship between the allelic loss and the histological type.

Interestingly, allelic losses on 1p35-pter were found in 17 of 30 advanced cancers, whereas they were detected only in 1 of 9 early cancers. Thus, LOH was significantly correlated with tumor progression ($P=0.02$, by Fisher's exact test) (Table II).

Lymph node metastasis was observed in 26 of 39 tumors (66.7%) and in 14 out of 18 tumors with LOH (77.8%). There was no correlation between allelic loss and lymph node metastasis.

Table I. Frequencies of LOH Detected at 12 Polymorphic Loci on Distal Chromosome 1p in 39 Primary Gastric Carcinomas

Name	Number of tumors	
	Informative	LOH (%) ^{a)}
D1S80	29	2 (6.9)
p73	22	1 (4.5)
D1S214	18	1 (6)
D1S548	17	6 (35.3)
D1S508	32	3 (9.4)
D1S450	25	4 (16)
D1S244	19	3 (15.8)
TNFR2	23	5 (21.7)
D1S507	20	3 (15)
D1S2843	20	7 (35)
D1S458	23	3 (13)
MYCL	31	3 (9.7)
Total	39	18 (46)

a) Loss of heterozygosity.

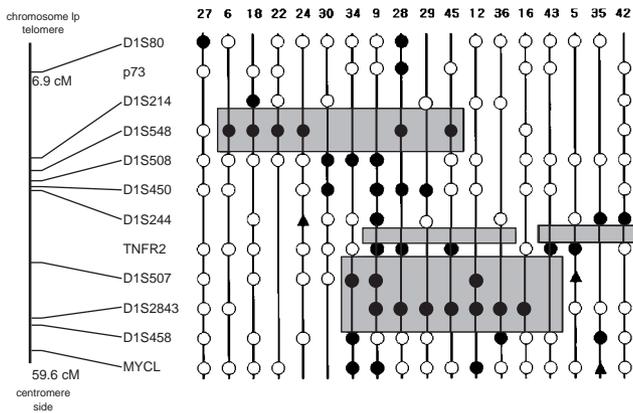


Fig. 2. Deletion mapping in 18 gastric carcinoma. Locations and order of the polymorphic markers (left) were derived from published linkage information. Distances between loci are indicated in centimorgans. Tumor identification numbers are at the top of each column. White circles indicate retention of heterozygosity, black circles indicate loss of heterozygosity, black triangles indicate replication error, and gaps indicate uninformativeness for specific markers. Three commonly deleted regions are indicated as a shaded bar. ○ LOH (-), ● LOH (+), ▲ RER.

Table II. Correlation between LOH on Distal Chromosome 1p and Tumor Progression in Gastric Carcinomas

	Total	LOH ^{a)}		
		(-) (%)	(+) (%)	
Early ^{b)}	9	8 (88.9)	1 (11.1)] P=0.02
Advanced ^{c)}	30	13 (43.3)	17 (56.7)	

- a) Loss of heterozygosity: -, negative; +, positive.
- b) Early gastric carcinomas.
- c) Advanced gastric carcinomas.

Replication error (RER) was found in 7 cases (17.9%), and at multiple loci in 2 of the 7 cases.

DISCUSSION

In the present study, we demonstrated the presence of at least three commonly deleted regions at 1p35-pter in gastric carcinomas. Allelic losses were frequent at D1S548, although only 1 allelic loss (6%) was observed at D1S214. Similarly, a high frequency of allelic losses was observed at D1S2843, though only 3 of 23 informative cases (13%) at D1S458 had allelic losses. The former region which lies between D1S214 and D1S508, overlapped with regions of LOH reported in tumors of breast carcinoma^{25, 26)} and neuroblastoma.¹⁸⁾ The latter region, which was reduced between TNFR2 and D1S458, lay within the regions of LOH reported in tumors of hepatocellular carcinoma,¹⁵⁾

colorectal cancer¹⁶⁾ and breast carcinoma.^{25, 26)} Furthermore, we detected a third small deletion between D1S244 and TNFR2. This region also overlapped with LOH regions in breast carcinoma.^{25, 26)} These data suggest that at least three common deleted regions exist on 1p35-pter, and the putative tumor suppressor genes present in these regions would be lost or inactivated in a variety of human carcinomas. In gastric carcinoma, Koizumi *et al.*²⁷⁾ indicated that 18% (6 of 33 cases) showed a DNA copy number decrease at 1p34.2-p36.2 using comparative genomic hybridization and Ezaki *et al.*¹⁴⁾ reported 13% (1 of 8 cases) allelic loss at the D1S228 locus, which exists between D1S244 and D1S507. It is very interesting that no one has reported more than 30% allelic loss or a common deleted region on 1p35-pter in gastric carcinoma.

We have shown that LOH for 1p35-p36 is present in 18 (46%) of 39 primary gastric cancers and except for one tumor, all of them were advanced carcinomas. Sano *et al.*¹¹⁾ reported a higher incidence of 1p LOH in advanced carcinoma than early carcinoma. Moreover, Tahara²⁸⁾ found that 1p LOH was related to the change of early cancer into advanced cancer in poorly differentiated adenocarcinoma. Therefore, any tumor suppressor gene on 1p35-pter may play a role in the progression of gastric carcinoma.

Sano *et al.*¹¹⁾ also indicated a higher incidence of 1p LOH in poorly than in well-differentiated adenocarcinoma. However, in our study, no significant difference was detected between the two histologic grades. This discrepancy may be due to the number and locations of the markers used in this study. However, Ezaki *et al.*¹⁴⁾ reported 1p LOH in a high proportion of well-differentiated adenocarcinomas of the stomach, which supports our observation.

Tsukamoto *et al.*²⁶⁾ found a correlation between lymph node metastasis and 1p LOH in breast carcinomas. They reported a significant correlation between LOH at 1p22-p31 and lymph node metastasis, whereas no relation was observed between LOH at 1p36 and lymph node metastasis. In gastric carcinoma, no one has examined the correlation between 1p LOH and lymph node metastasis. We found no significant correlation between LOH and lymph node metastasis.

We detected RERs at several microsatellite loci. RER is a phenomenon thought to reflect any defect in the DNA mismatch repair system.²⁹⁾ Although we excluded RER from this LOH study, it may well have some connection with the progression of gastric carcinomas.

In conclusion, the data presented here suggest that at least three common deletion regions, which may have candidate tumor suppression genes, are present on chromosome 1p35-pter in gastric carcinoma and allele losses on chromosome 1p35-pter seem to be involved in the progression of gastric carcinoma regardless of histological type.

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