Frequent Loss of Heterozygosity at the DCC Locus in Gastric Cancer

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ABSTRACT

We examined 28 cases of surgically resected gastric cancer, excluding the diffuse type, for loss of heterozygosity (LOH) on 12 chromosomal arms using polymorphic DNA markers. LOH on chromosome 18q was detected in 61% (14 of 23) of the cases by the probes OLVIIA8, OLVIIE10, p15-65, SAM 1.1, and OS-4, and a putative common region showing LOH included the locus of the DCC tumor suppressor gene. LOH on chromosome 17p was also frequently found (8 of 19 or 42% of the cases) by the probes p10-3 and pHF12-1, and in 5 of these 6 cases the LOH on chromosome 17p was accompanied by LOH on chromosome 18q. On the other hand, the incidence of LOH was 30% or less using probes pH2ES, pHF12-65, p-c-mybE2.6, NJ3 3.2, pHF12-8, pHINS6.11, p9D1I, hp2-a, pCMM6, and PI A5 on chromosomes 1q, 5, 6q, 7q, 9, 11p, 13q, 16q, 20, and 22q, respectively. LOH on chromosome 18q was frequent irrespective of the depth of tumor invasion, whereas the incidence of LOH on chromosome 17p was higher in the cases in which the tumor invaded beyond the muscularis propria than in those in which tumor invasion was limited to the submucosa and muscularis propria.

These results suggest that LOH on chromosome 18q occurs at an earlier stage than LOH on chromosome 17p and that the inactivation of tumor suppressor genes located on chromosome 17p and 18q (e.g., the p53 and DCC genes) is critically involved in the development of the majority of gastric cancers. While alteration of the p53 gene is observed in various human cancers, that of the DCC gene is considered to occur more selectively in gastrointestinal cancers.

INTRODUCTION

Tumor suppressor genes are thought to play an important role in carcinogenesis by the mechanism of inactivation consisting of loss of one chromosomal allele containing the tumor suppressor gene and mutation of the gene in the remaining allele (1). Up to the present, several tumor suppressor genes have been discovered (2).

The accumulation of multiple genetic alterations such as ras mutation, 5q deletion, 18q deletion, and 17p deletion is thought to contribute to the development of colorectal carcinoma (3,4). In this process, inactivations of the MCC or APC gene on chromosome 5q (5-7), the DCC gene on chromosome 18q (8), and the p53 gene on chromosome 17p (9) are considered to play an important role. The presence of tumor suppressor genes on chromosomes 5q and 18q is also supported by chromosome transfer experiments which showed that the introduction of normal human chromosomes 5q and 18q into a colon carcinoma cell line changed its morphology and reduced its tumorigenicity (10). Thus, the MCC or APC gene located on chromosome 5q and the DCC gene located on chromosome 18q are thought to operate as tumor suppressor genes.

The p53 gene, which is also considered to be a tumor suppressor gene, is present on chromosome 17p13, and LOH on chromosome 17p has also been frequently observed in various cancers (11). It has been shown that many tumors including colorectal carcinoma have allelic deletion of 17p have point mutations of the p53 gene in the retained allele (12).

Stomach cancer is one of the most common cancers in the world and is the leading cause of cancer death in Japan in both males and females. Compared with cancers occurring in the other organs, we had little information about the genetic alterations which occur in gastric cancer until a few years ago (13). But recent extensive study has elucidated many genetic alterations which occur relatively frequently in gastric cancer, e.g., amplification of the c-erbB-2 gene, amplification of K-sam, point mutation of c-Ki-ras (13), mutation of p53 (14), and TPR-MET rearrangement (15). RFLP analysis in gastric cancers has also been performed in several laboratories, and LOH on chromosomes 13q (16), 1q, and 12q (17) was found to be frequent. Furthermore, Sano et al. (18) recently reported a high incidence of LOH on chromosomes 17p and 5q. Since gastric cancer tissue often has abundant nonneoplastic cells in the cancer stroma, it is very important to select tissues that have predominantly cancer cells, especially for RFLP analysis. There is a possibility that in some of the previous RFLP analyses of gastric cancer the incidence of LOH was underestimated due to the contamination of nonneoplastic cells. Therefore, we studied LOH using DNAs extracted from tissues carefully selected by the cryostat section technique to avoid the problem caused by heavy contamination with nonneoplastic cells.

MATERIALS AND METHODS

Samples. Thirty-eight primary gastric cancers surgically resected at the National Cancer Center Hospital, Tokyo, Japan, were studied. Small pieces of cancer and noncancerous tissues excised from the resected specimen were immersed in O.C.T. compound (Miles Scientific) immediately after surgery and were frozen in acetone with dry ice. These blocks were sliced with a cryostat, and 3-μm sections were stained with hematoxylin and eosin in order to ascertain whether the cancer cells in the tissues were predominant or not. Carcinomas of 28 cases contained more than 50% cancer cells compared to normal cells and were judged to be cancer cell rich. These cases consisted of one case of early gastric cancer, in which the depth of tumor invasion was limited to the submucosa, and 27 cases of advanced gastric cancer, in which the depth of tumor invasion reached the muscularis propria in 5 cases and was beyond the muscularis propria in 22 cases. Histopathological classification was performed according to the General Rules for the Gastric Cancer Study by the Japanese Research Society for Gastric Cancer (19). Four cases were diagnosed as papillary adenocarcinoma, 7 as well-differentiated tubular adenocarcinoma, 7 as moderately differentiated tubular adenocarcinoma, and 10 as poorly differentiated adenocarcinoma. All of the poorly differentiated adenocarcinomas showed cohesive cancer cell nests. Then 500-μm sections of these tissues were cut with a scalpel, and the area composed predominantly of the p53 gene, which is also considered to be a tumor suppressor gene, is present on chromosome 17p13, and LOH on chromosome 17p has also been frequently observed in various cancers (11). It has been shown that many tumors including colorectal carcinoma have allelic deletion of 17p have point mutations of the p53 gene in the retained allele (12).
The incidence of LOH is shown in Table 1. LOH occurred at several chromosomal loci and was detected in 23 (82%) of the 28 cases at one or more loci. We found a markedly high incidence of LOH on chromosome 18q (14 of 23, or 61%), followed by that on chromosome 17p (8 of 19, or 42%). Fig. 1 shows representative results of Southern blot analysis using probes p10-3 and pHF12-1 on chromosome 17p and OLVIIE10, OLVIIE10, and OS-4 on chromosome 18q. The incidence of LOH on chromosome 18q shown by the probes was 47% (8 of 17), 71% (5 of 7), 71% (5 of 7), 50% (5 of 10), 67% (10 of 15) by probes OLVIIE10, OLVIIE10, p15-65, SAM1.1, and OS-4, respectively. OLVIIE10 and p15-65 detected the same MspI polymorphisms and gave identical results. Fig. 2 shows the map of deletion on chromosome 18q together with LOH on chromosome 17p. The putative common region showing LOH on chromosome 18q is the region 18q21.3-qter, and this region includes the locus of the DCC gene. However, there was no case in which we could detect rearrangement or partial loss of the DCC gene with probes OLVIIE10, p15-65, SAM1.1, and OS-4.

Seventeen cases were informative with the probes on both chromosomes 17p and 18q. LOH on chromosome 17p or 18q was detected in 12 (71%) of these informative cases (Fig. 2). Interestingly, the majority of carcinomas with allele loss on chromosome 17p also showed allele loss on chromosome 18q (5 of 6, or 83%), whereas among 11 cases which showed allele loss on chromosome 18q, 5 (45%) demonstrated LOH on chromosome 17p. As shown in Table 2, the incidence of allele loss on chromosome 18q in carcinomas in which the depth of tumor invasion was limited to the submucosa or muscularis propria was very high. On the other hand, allele loss on chromosome 17p was only 20% in carcinomas in which the depth of tumor invasion was limited to the submucosa or muscularis propria, while it increased to 50% in the carcinomas in which the depth of tumor invasion was beyond the muscularis propria. One case of early gastric cancer showed allele loss on chromosome 18q and retention of alleles on chromosome 17p together with chromosomes 9 and 16q. LOH on chromosome 18q was frequently seen in the cases in which the depth of tumor invasion was limited to the submucosa or muscularis propria, but LOH on chromosome 17p was seen more frequently in the cases in which the depth of tumor invasion was beyond the muscularis propria than in the cases in which the depth of tumor invasion was limited to the submucosa or muscularis propria.

The incidence of LOH on chromosome 5 (probe pHF12-65) was 20% (1 of 5) and that on chromosome 22q (probe PIA5) was 29% (2 of 7). Since only a small number of cases showed constitutional heterozygosity, we could not determine if there was a correlation between LOHs on chromosomes 17p and 18q and LOHs on chromosomes 5 and 22q.

**DISCUSSION**

LOH on chromosome 18q was frequently detected (61%) in gastric cancers in this study. All carcinomas showing LOH on...
18q allele loss in gastric cancer

Chromosome 17p

Case 6

p10-3

NT

OLVIIA8

NT

OLVIIE10

NT

OS-4

NT

9.4-

A1-

A2-

6.6-

3.8-

3.1-

11.0-

8.2-

6.6-

8.6-

8.7-

Case 7

pHF12-1

NT

OLVIIA8

NT

OLVIIE10

NT

SAM1.1

NT

9.0-

11.0-

8.2-

1.1-

Fig. 1. LOH on chromosomes 18q and 17p in gastric cancer. DNA from the tumor (Lane T) and corresponding normal tissue (Lane N) from case 6 (A) and case 7 (B) of Fig. 2. Ten μg of DNA were digested with the appropriate enzyme, electrophoresed, and analyzed by Southern blot hybridization as described in the text. Filters were hybridized with 32P-labeled probes: p10-3 and pHF12-1 on chromosome 17p and OLVIIA8, OLVIIE10, SAM1.1, and OS-4 on chromosome 18q. Case 6 showed constitutional allelic loss in the tumor tissue at OLVIIA8, OLVIIE10, and SAM1.1. Case 7 showed LOH at pHF12-1, OLVIIA8, OLVIIE10, and OS-4. Left ordinate, molecular size of the polymorphic alleles in kilobases. I1 and I2, two polymorphic alleles.

Chromosome 18q

Case 1

2.9-

2.1-

3.8-

3.1-

1.1-

Fig. 2. Loss of heterozygosity on chromosomes 17p and 18q in gastric cancer. Data are from RFLP analysis of gastric cancer patients with the probes on both chromosomes 17p and 18q and who had LOH of one or more loci. 0, LOH; 0, no LOH; —, not informative. a, papillary adenocarcinoma; b, poorly differentiated adenocarcinoma; c, well-differentiated tubular adenocarcinoma; d, moderately differentiated tubular adenocarcinoma. The map of chromosome 18 was delineated according to Refs. 8 and 22.

Table 2 Association of allelic loss on chromosome 18q and 17p with the depth of tumor invasion in gastric cancer

<table>
<thead>
<tr>
<th>Depth of tumor invasion</th>
<th>Chromosome 18q</th>
<th>Chromosome 17p</th>
</tr>
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<tbody>
<tr>
<td>Limited to the submucosa or muscularis propria</td>
<td>5 (80)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Beyond the muscularis propria</td>
<td>18 (55)</td>
<td>14 (70)</td>
</tr>
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chromosome 18q had allelic loss, including the region of the DCC gene. These data suggest that the DCC gene is a tumor suppressor gene and plays an important role in carcinogenesis not only in colorectal cancer but also in gastric cancer. The high incidence of allelic loss on chromosome 18q suggests the possibility that it is one of the critical genetic alterations in the pathway of carcinogenesis in gastric cancer. Gastric cancers were previously examined for LOH on chromosome 18q with probe OS-4 by several researchers, but a significant high incidence of LOH on this locus was not demonstrated (16, 23). In the present study, however, we extracted DNAs from tissues carefully selected by the cryostat section technique and detected a high incidence of LOH on chromosome 18q by using five probes.

We also demonstrated that LOH on chromosome 17p13, near the p53 gene locus, occurred in 42% of gastric cancers. Furthermore, we found a higher incidence of LOH on chromosome 17p in the cases with tumor invasion beyond the muscularis propria. Recently we found, by using a combination of cell sorting and the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method, that mutations of the p53 gene occurred only in aneuploid gastric carcinoma cells (14). LOH on chromosome 17p may be involved in the progression in the grade of malignancy and be correlated with the depth of tumor invasion or the ploidy pattern of carcinoma cells.

Among the cases which were informative for loci on both chromosomes 17p and 18q, carcinomas with allelic deletion of chromosome 17p were more likely to show LOH on chromosome 18q (5 of 6, or 83%). However, 5 of 11 cases which showed allelic loss on chromosome 18q demonstrated LOH on chromosome 17p. These data suggest that allelic loss on chromosome 18q precedes allelic loss on chromosome 17p.

In colorectal carcinoma, the accumulation of multiple genetic alterations such as ras mutation, 5q deletion, 18q deletion, and 17p deletion is believed to contribute to multistage carcinogenesis through colorectal adenoma to carcinoma (3). In gastric cancers, we previously detected c-Ki-ras point mutation in tubular adenoma and differentiated type adenocarcinoma by using DNA samples from formalin-fixed and paraffin-embedded tissues (13), and Sano et al. (18) revealed that LOH on chromosome 17p and 5q was frequently detected in gastric cancers. From these findings, together with the results of the present investigation on gastric cancer excluding the diffuse type, the genetic pathway of the development of the majority...
of gastric cancers is considered to be similar to that of colorectal cancer.

Many gastric cancers other than diffuse carcinomas are often associated with chronic atrophic gastritis and intestinal metaplasia, which are considered to be background conditions possibly contributing to carcinogenesis. Lesions indicating an adenoma-carcinoma sequence similar to that in the colorectum are also observed in the stomach. Similarities in genetic alterations between colorectal carcinoma and gastric carcinoma may reflect a carcinogenetic pathway common to colorectal carcinoma and gastric carcinoma.

LOH on chromosome 17p and mutation of the p53 gene are found in various human carcinomas, indicating that the p53 gene may normally play an important role in the regulation of various cells of different embryonic lineage. On the other hand, the high incidence of LOH on chromosome 18q found in gastric cancer was only second to that of colorectal cancer, despite extensive investigations with the same RFLP probes (24–27). Therefore, alterations of the DCC gene may have selectivity in gastrointestinal tract cancers. When the function of the DCC gene product is elucidated, it may turn out that the DCC gene plays an important role in the embryonic development or maintenance of the gastrointestinal epithelium.

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