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Chromosomal Alterations in Paired Gastric Adenomas and Carcinomas

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Gastric adenoma is a precancerous lesion of the stomach and its malignant transformation is thought to result from accumulative series of gene alterations. The aim of this study was to determine the pattern of chromosomal changes during gastric carcinogenesis. Pairs of adenoma and carcinoma tissues from 15 gastrectomy cases containing both adenomas and carcinomas in the same (adjacent pairs, 6 cases) and different (non-adjacent pairs, 9 cases) lesions, were analyzed for chromosomal alterations of 39 non-acrocentric chromosomal arms by comparative genomic hybridization (CGH). CGH analysis identified frequent chromosomal alterations in most of the gastric adenomas (14/15, 93%) and all of the carcinomas. The mean number of chromosomal alterations was higher in carcinoma (5.5 for adenoma and 11.7 for carcinoma; $P = 0.006$, by nonparametric Wilcoxon's test). Losses on the short arm of chromosome 17 were most common in both adenomas (43%) and carcinomas (67%). The pattern of chromosomal alterations in paired gastric adenomas and carcinomas showed greater similarity compared to the non-case pairs and this similarity was increased in the adjacent pairs. Deletion mapping analysis on chromosome 17p also demonstrated that the conserved deletion area was more frequent in the adjacent pairs. Among these 6 adjacent pairs, all had common deletion areas. In contrast, among the 9 non-adjacent pairs, 2 (22%) had common area of deletion, 5 (56%) showed deletion only in the carcinoma, and the remaining 2 (22%) had no deletion on 17p, suggesting diverse genetic changes might be involved in the multiple tumor formation. Our results that common clonal genetic changes between adjacent pairs of gastric adenomas and carcinomas and accumulated genetic changes in the carcinomas provide evidences for the stepwise mode of gastric carcinogenesis through the accumulation of a series of genetic alterations. *(Am J Pathol 2001, 158:655–662)*

Accumulated evidence has established that carcinogenesis is a multistep process that is associated with alterations in cellular oncogenes and tumor suppressor genes necessary for malignant transformation.[1,2] Two main genetic pathways appear to be involved in gastrointestinal tumors; genomic instability associated with multiple chromosomal alterations, and genomic instability associated with defective DNA mismatch repair in tumors, which is called microsatellite instability (MSI).[1,3,4] Gastric carcinogenesis also displays multiple genetic alterations including oncogenes, tumor suppressor genes, and DNA mismatch repair genes.[5–11] The results of molecular genetic changes related to gastric carcinomas have recently been rapidly accumulating. DNA aneuploidy, proto-oncogene activation, tumor suppressor gene inactivation, and defective DNA mismatch repair genes have been reported in gastric carcinomas. The change in DNA copy numbers is one of the hallmarks of the gastric carcinogenesis and is considered to be related to oncogene activation and tumor suppressor gene inactivation. Although many chromosomal aberrations have been reported in gastric carcinogenesis, there remains disagreements among the previous studies. Frequent nonrandom chromosomal deletions on 1q, 5q, 7q, 9p, 11p, 11q, 13q, 16q, 17p, and 18q were observed in gastric carcinomas.[5,12–17] In addition, recent studies with comparative genomic hybridization (CGH) analysis have demonstrated that chromosomal gains are also frequent in gastric carcinomas.[18–23] These chromosomal gains are found in various combinations with chromosomal losses and may be associated with the overexpression of dominant oncogenes contributing to tumor progression.

Gastric adenoma is a precancerous lesion of the stomach and is associated with intestinal type carcinoma. The adenoma-carcinoma sequence in gastric carcinogenesis is believed to exist in a subset of gastric carcinomas and might develop through accumulative series of genetic alterations similar to that of colorectal cancer.[24,25] Inactivation of *p53* has been reported in gastric adenomas.[26,27] In addition, microsatellite instability (MSI) was reported in a subset of gastric adenomas.[24,28] Little is

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known, however, about the pattern of genetic changes during the progression through the gastric adenoma-carcinoma sequence, because only small numbers of adenomas have been studied, and conflicting results have been reported.[20,29,30] We had previously demonstrated the possible sequence of genetic events in gastric adenomas with MSI,[31] and found that the frequency of MSI in gastric adenomas was similar to that of gastric carcinomas, but frameshift mutations of the target genes were not frequent. The genetic pathways of microsatellite stable gastric adenomas, which account for more than 80% of sporadic tumors, have not been elucidated.

To address these uncertainties about genetic characteristics in the gastric adenoma-carcinoma sequence, we studied the chromosomal alterations in paired adenoma and carcinoma tissues from 15 gastrectomy cases. We evaluated the chromosomal copy number changes by CGH analysis. We also compared the adenomas and carcinomas for the frequency and extent of the chromosomal deletions by deletion mapping study on the short arm of chromosome 17. The results have implications for the understanding of the biology of gastric neoplasia as well as diagnosis and treatment.

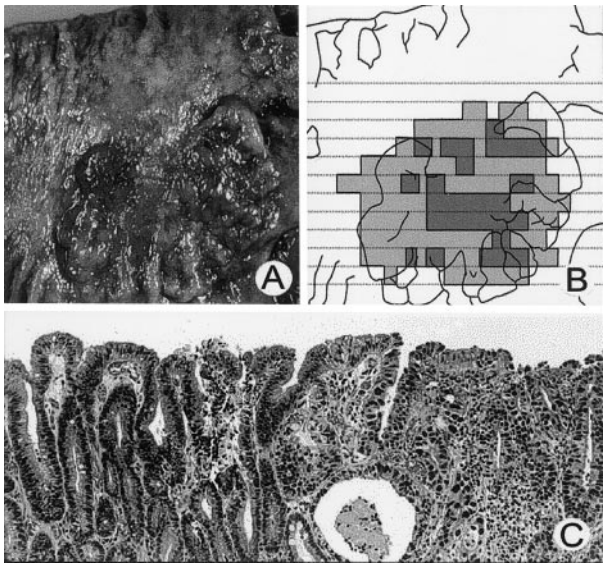

Figure 1. Examples of synchronous gastric adenoma and carcinoma in the same lesion (adjacent pair). Gross features of a tumor in the body (**A**) and schematic histological figure of mapping; **gray box** denotes adenoma and **black box** denotes carcinoma (**B**). Light microscopic findings of adenoma and carcinoma (**C**).

Materials and Methods

Tissue Samples

Pairs of adenoma and carcinoma tissues from 15 gastrectomy cases were included in this study. All cases had synchronous adenoma and carcinoma, which were identified prospectively and consecutively among 626 cases of gastric carcinomas in the Department of Pathology at Yonsei University Medical Center (Seoul, Korea) between September 1995 and November 1999 for a study of molecular markers in gastric carcinomas. Among the 15 adenomas, 6 were present in the periphery of the carcinomas (adjacent pairs, Figure 1) and 9 were present in separate lesions (non-adjacent pairs, Figure 2). Information from chart reviews and clinicians was obtained to determine demographic data and tumor sites. The patients included were 3 females and 12 males, ranging in age from 51 to 82 years.

For DNA extraction, tumors and adjacent nontumorous mucosal tissues were obtained immediately after surgical excision. The selected tissues were rapidly frozen in liquid nitrogen and stored at −70°C until the DNA was isolated. To enrich the tumor cell population, areas containing more than 80% of tumor cells were selected from the hematoxylin-eosin (H&E)-stained slides using a cryostat microdissection technique. Genomic DNA was prepared by the sodium dodecyl sulfate-proteinase K and phenol-chloroform extraction method.

Pathological Analysis

Conventional pathological parameters (tumor size, tumor number, and differentiation) were examined prospectively without knowledge of the molecular data. The gastric adenomas were divided into two groups (low grade and high grade dysplasia), according to the criteria of Lewin.[32] Using these criteria, 6 cases were categorized as low grade dysplasia and 9 cases as high grade dysplasia. Gastric carcinomas were classified according to the Lauren's classification; all cases were categorized as intestinal type.[33]

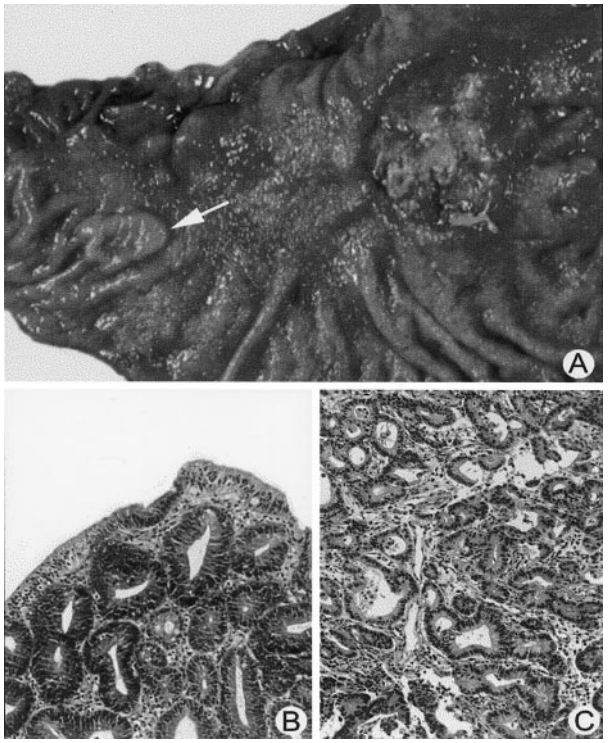

Figure 2. Examples of synchronous gastric adenoma and carcinoma in the different lesion (non-adjacent pair). Gross features of a flat adenoma (**white arrow**) in the distal antrum and ulcerating carcinoma in the body (**A**). Light microscopic findings of adenoma (**B**) and carcinoma (**C**).

Comparative Genomic Hybridization and Digital Image Analysis

Genomic DNA samples from tumors were labeled with Spectrum Green dUTP (Vysis Inc., Downers Grove, IL), and normal reference genomic DNA was labeled with Spectrum Red dUTP (Vysis) using the nick translation technique. Labeled tumor and reference DNA (200–400 ng), as well as 10 μg of unlabeled human Cot-1 DNA (Vysis) were dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, and 2× SSC) and denatured at 72°C for 2 minutes. Hybridization was performed at 37°C on denatured normal metaphase spreads. After hybridization for 3 days, the slides were washed and counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) in antifade solution. CGH hybridizations were analyzed using an Olympus fluorescent microscope and the Cytovision image analysis system (Applied Imaging, Sunderland, Tyne & Wear, UK). Three digital images (DAPI, Spectrum Green, and Spectrum Red) were acquired from 10 to 20 metaphases in each hybridization. Normal male DNA and DNA from tumor cell lines with known aberrations were used as control test DNA. Green-to-red intensity ratio profiles were calculated for each chromosome and threshold values defining gains and losses were set at 1.25 and 0.75, respectively. High level increase in copy number (amplicon) was defined as ratio of tumor/reference greater than 1.5.

Deletion Mapping on the Chromosomal Arm of 17p

Fifteen pairs of DNA from gastric adenomas and carcinomas and matched normal DNAs were PCR amplified at 9 microsatellite loci of 17p (D17S786, D17S796, D17S921, D17S947, D17S969, D17S1871, D17S1879, D17S20 14, and D17S2027) to evaluate the frequency and extent of the deletion area. PCR reactions were carried out in a mixture of 20 μl containing 1.5 mmol/L $MgCl_2$, 20 pmol primer, 0.2 mmol/L each dATP, dGTP, and dTTP, 5 μmol/L dCTP, 1 μCi of [α-^{32}P]dCTP (3000 Ci/mmol; NEN DuPont, Boston, MA), 50 ng of sample DNA, 1× PCR buffer, and 1.25 U *Taq* polymerase (Gibco-BRL, Grand Island, NY). After denaturation at 95°C for 5 minutes, DNA amplification was performed in 25 cycles consisting of denaturation at 95°C for 30 seconds, primer annealing at 55–60°C for 30 seconds, and elongation at 72°C for 15 seconds. PCR products were separated in 6% polyacrylamide gel containing 5.6 mol/L urea, followed by autoradiography. Allelic deletion was scored when the band intensity of one marker was significantly decreased (>70% reduction) in tumor DNA compared with that in normal DNA. MSI was determined by the mobility shift of products from PCR. In tumors with MSI, additional bands were found in the normal allele regions.

Statistical Analysis

Differences in DNA copy number aberrations between the adenomas and carcinomas were compared using the Wilcoxon's signed rank test and Fisher's exact test by contingency table analysis. For the evaluation of nonrandom similarities between adenomas and carcinomas, summary statistical analysis was performed to compare the gains and losses among pairs of tumors, at each of the chromosome arms as described previously. 34 Comparisons were performed on two types of pairs: (i) adjacent pairs of adenomas and carcinomas, and (ii) nonadjacent pairs of adenomas and carcinomas.

Results

Chromosomal Copy Number Aberrations in Gastric Adenomas by CGH Analysis

Chromosomal alterations were found in 14 cases (93%) among the 15 gastric adenomas: 7 cases showed both chromosomal losses and gains, 5 cases showed only chromosomal losses, and the remaining 2 cases showed only chromosomal gains. A schematic summary of all chromosomal copy number aberrations is shown in Table 1 and Figure 3. The chromosomal losses were more frequent than the gains. The mean number of chromosomal losses was 3.7 and of gains, 1.9. Frequent chromosomal losses (>40%) were detected in 17p (47%). Several other chromosomal arms also showed segmental losses or gains as presented in the Figure 4A.

Chromosomal Copy Number Aberrations in Gastric Carcinomas by CGH Analysis

All of the 15 gastric carcinomas showed both chromosomal losses and gains for at least one of the chromosomal arms and all of the 39 evaluated chromosomal arms showed chromosomal aberrations for at least 1 patient. The mean number of chromosomal losses was 6.7 and for the gains was 4.9. The mean number of chromosomal alterations was not related to the tumor stage: 19 in stages I and II, 14.5 in stages III and IV ($P = 0.33$). A schematic summary of copy number aberrations is shown in Table 1 and Figure 3. The chromosomal arms with frequent losses (>40%) were 12q (40%), 14q (53%), 15q (40%), 16p (40%), and 17p (67%). Chromosomal gains were also frequent, and were observed in 8q (80%), 13q (40%), and 20p (40%). Most of the chromosomal gains on 8q showed a wide scope of alterations usually covering the entire chromosomal arm, whereas gains of the other chromosomal arms usually involved small segmental areas (Figure 4B). In contrast to the gastric adenomas, several amplicons were present in gastric carcinomas. Among the chromosomal arms with gains, amplicons were present in 8q (3 cases), 20p and 20q (2 cases), and 8p, 13q, and 15q (1 case), as shown in Figure 4B.

Table 1. Summary of Pathological Data and DNA Copy Number Changes in 15 Paired Gastric Adenomas and Carcinomas

		Adenoma			Carcinoma	
Case no.	Location of adenoma and carcinoma	DNA copy number over-representations	DNA copy number under-representations	Tumor stage‡	DNA copy number over-representations	DNA copy number under-representations
1	adjacent*	6q, 13q	1p, 2p, 2q, 4p, 4q, 10q, 11q, 12q, 14q, 15q, 16p, 17p, 17q, 20q	I	5p, 6q, 7p, 7q, 8q, 13q	1p, 2p, 2q, 4p, 4q, 11q, 12q, 14q, 15q, 16p, 17p, 17q, 20p, 20q
2	adjacent	8q, 12q, 13q	1p, 15q, 16p, 17p	I	4q, 8q, 12q, 13q	1p, 9q, 11q, 14q, 15q, 16p, 16q, 17p, 19p, 19q
3	adjacent	No change	No change	I	8q	14q
4	adjacent	2q, 3q, 6q, 7q, 8q, 13q, 14q, 20p	4p, 6p, 12q, 14q, 16p, 17p	I	2q, 3q, 6q, 7q, 8p, 8q, 13q, 20p	4p, 4q, 9q, 12q, 14q, 16p, 17p, 17q, 18p, 18q
5	adjacent	3q	13q, 17p, 18q	I	3q, 6q, 8q, 10p, 11p, 17q, 20p	13q, 15q, 17p, 18q
6	adjacent	No change	4q, 6p, 17p, 17q	I	8q	4q, 6p, 13q, 17p, 17q
7	non-adjacent†	11q, 13q, 18q	1p, 9q, 12q, 15q, 20q	III	11q, 12p, 13q	1p, 2p, 2q, 3q, 6q, 12q, 15q
8	non-adjacent	20p	8q, 11q	I	1p, 2p, 2q, 3p, 3q, 5p, 6p, 7p, 8q, 10q, 11p, 15q, 20p, 20q	4p, 5q, 8p, 9p, 11q, 16p, 17p, 18p, 18q
9	non-adjacent	No change	1p, 9q, 11q, 15q, 16p, 16q, 17p	III	1q, 2q, 7p, 8q	1p, 4p, 9q, 11q, 12q, 14q, 15q, 16p, 17p, 17q, 21q, 22q
10	non-adjacent	8q	No change	II	1q, 3q, 5p, 6p, 7p, 7q, 8q, 10p, 13q, 16p, 18q, 20p, 20q	3p, 4p, 4q, 5q, 6q, 9p, 14q
11	non-adjacent	3q, 8p, 12q, 19p, 19q, 20p	18p	IV	3q, 8p, 13q, 19p, 19q, 20p	17p
12	non-adjacent	No change	10q, 12q, 16p, 17p	III	8p, 8q	3p, 12q, 14q, 17p
13	non-adjacent	4q, 8p, 8q	No change	III	4q, 8p, 8q	9q
14	non-adjacent	No change	9p, 20p, 20q	III	5p, 8q, 20p	1p, 4q, 9p, 14q, 15q, 16p, 16q, 17p, 18q
15	non-adjacent	No change	10p, 11p	II	No change	2q, 8p, 11p, 11q, 12q, 22q

*Adjacent denotes paired adenoma and carcinoma in the same lesion.
†Non-adjacent denotes paired adenoma and carcinoma in the different lesion.
‡UICC tumor staging classification.[48]

Comparison of Chromosomal Aberrations in Gastric Adenomas and Carcinomas

In our gastric adenomas and carcinomas, both tumors showed gains and losses on several chromosomal arms. The number of chromosomal alterations, however, was significantly higher in carcinomas and the mean number of chromosomal alterations was 5.5 in adenoma and 11.7 in carcinoma ($P = 0.006$). When the chromosomal alterations of gastric adenomas and carcinomas were compared within case pairs, common alterations were found. All of the 14 gastric adenomas with chromosomal alterations had common chromosomal changes with the paired carcinomas in more than one chromosomal arm. Additional analysis was performed using summary statistics,[34] which was applied to the adjacent and non-adjacent type pairs of tumors. The distributions of these pairings are shown in Figure 5. These plots showed that when the pairs were from the same patient, the summary statistics were greater than when the pairs were from different patient. In our gastric adenomas and carcinomas, the chromosomal changes within the case pairs showed greater similarities than between the non-case pairs. These similarities were more significant in adjacent pairs of gastric adenomas and carcinomas: similarities were found in all of the adjacent pairs and only in some of the non-adjacent pairs (Figure 5).

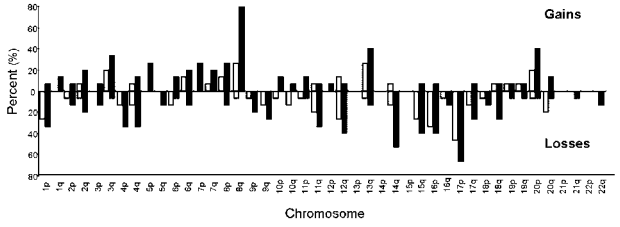

Figure 3. The rate of chromosomal loss and gain observed on a designated 39 non-acrocentric chromosomal arms of paired gastric adenomas and carcinomas in graphic form. Each bar represents the percentage of loss (**lower**) or gain (**upper**) of a chromosomal arm; the **white bar** represents adenoma and the **black bar** represents carcinoma.

Deletion Mapping on the Chromosomal Arm of 17p

A deletion mapping study on the short arm of chromosome 17 by using 9 microsatellite markers was carried out in 15 cases of paired gastric adenomas and carcinomas. For each case, genomic DNAs from the tumor and matched normal tissue were analyzed by polymerase chain reaction based loss of heterozygosity (PCR-LOH) method. Representative PCR-LOH results are shown in

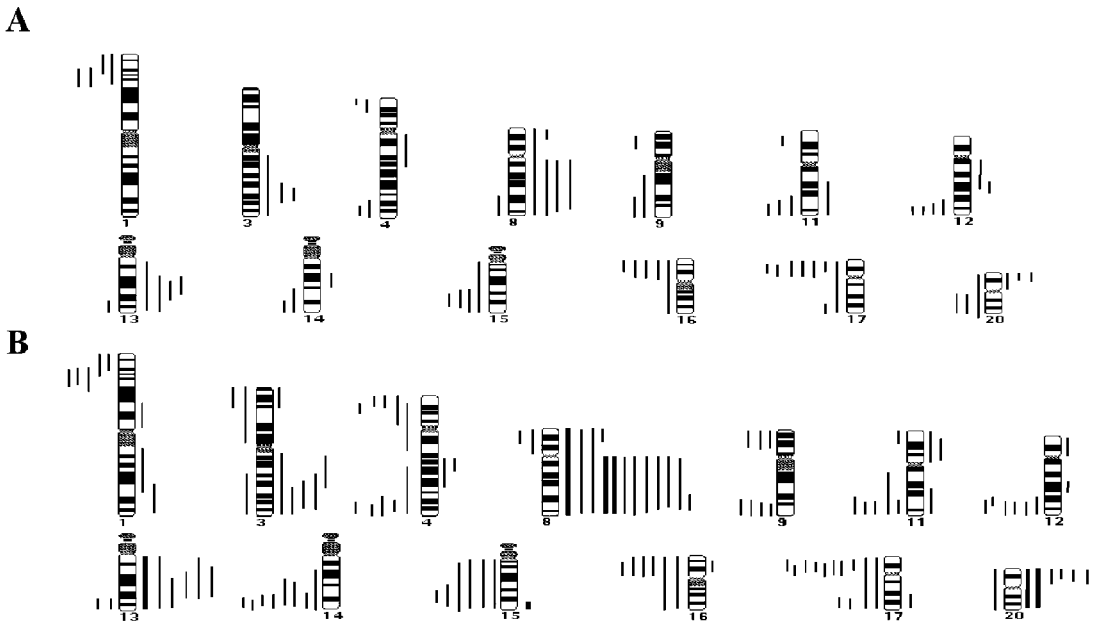

Figure 4. Summary of CGH imbalance detected in 15 paired gastric adenomas (**A**) and carcinomas (**B**). Vertical lines on the left of each chromosome idiogram represent chromosomal losses, whereas vertical lines on the right correspond to chromosomal gains. Amplicon is demonstrated as thick vertical lines on right.

Figure 6. Among the 15 pairs of gastric adenomas and carcinomas, 3 adenomas (cases 9, 12, and 13) and 2 carcinomas (cases 13 and 15) showed MSI on multiple loci and these cases were also categorized as high MSI (MSI-H) with the 5 markers proposed by the National Cancer Institute.[35] The overall LOH on 17p was 8 in adenomas and 13 in carcinomas. Among the 8 adenomas and 13 carcinomas with 17p deletion, 4 (50%) adenomas and 7 carcinomas (54%) showed LOH in most of the foci, suggesting entire deletion on the short arm of the chromosome 17, whereas the remaining 4 adenomas (50%) and 6 carcinomas (46%) showed partial LOH. Detailed deletion mapping identified two independent commonly deleted regions on chromosome 17p. The first region was between D17S2014 and D17S796, encompassing approximately 8cM region and defined by D17S2027 locus. The second region could be defined by the D17S947, D17S921, and D17S1871 locus. Comparison of the deletion area between adenomas and carcinomas on chromosome 17p demonstrated carcinoma as having a wider area of deletion, and the conserved deletion area was more frequent in the adjacent pairs of adenomas and carcinomas. Among the 6 pairs of adjacent adenomas and carcinomas, all had common deletion areas. In contrast, of the 9 non-adjacent pairs of gastric adenomas and carcinomas, 2 (22%) had common areas of deletion, 5 (56%) showed deletion only in the carcinoma, and the remaining 2 (22%) had no deletion on 17p (Figure 6).

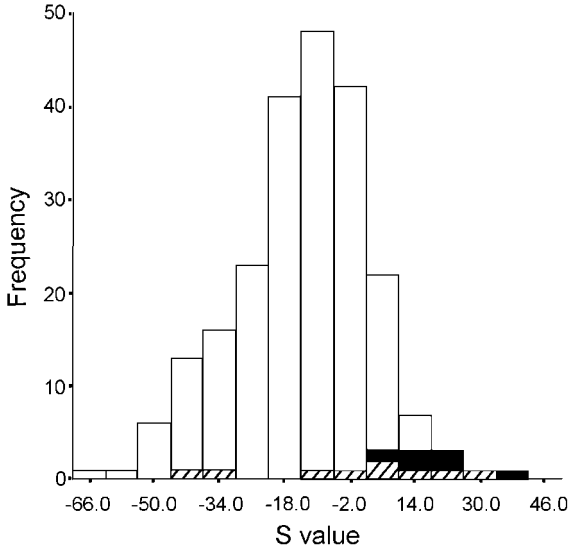

Figure 5. Distribution of summary statistic for adenoma-carcinoma pairs. □, pairs from the different patient; ■, pairs from the same patient within the same lesion (adjacent pair); ▨, pairs from same patient in the different lesion (non-adjacent pair). *S* value was used to evaluate the similarity between the adjacent and non-adjacent pairs of adenomas and carcinomas. Briefly, *S* value was defined as positive when the same chromosomal changes were present in the paired adenomas and carcinomas. In contrast, it was defined as negative when different chromosomal changes were present in the paired lesion. The *S* value was scored higher when the chromosomal changes of low frequency was observed simultaneously in the paired lesion. In contrast, the chromosomal changes of high frequency were given the lower score. Higher *S* values (similarities) were observed within case pairs. Closer similarities were observed in the adjacent pairs.

Discussion

In this study, pairs of gastric adenoma and carcinoma were investigated for chromosomal abnormality. We demonstrated more frequent chromosomal losses and gains in gastric carcinomas than the adenomas. We also demonstrated the pattern of chromosomal alterations in paired adenomas and carcinomas showed great similarity than the non-case pairs, and this similarity was more prominent in the adjacent pairs than the non-adjacent pairs.

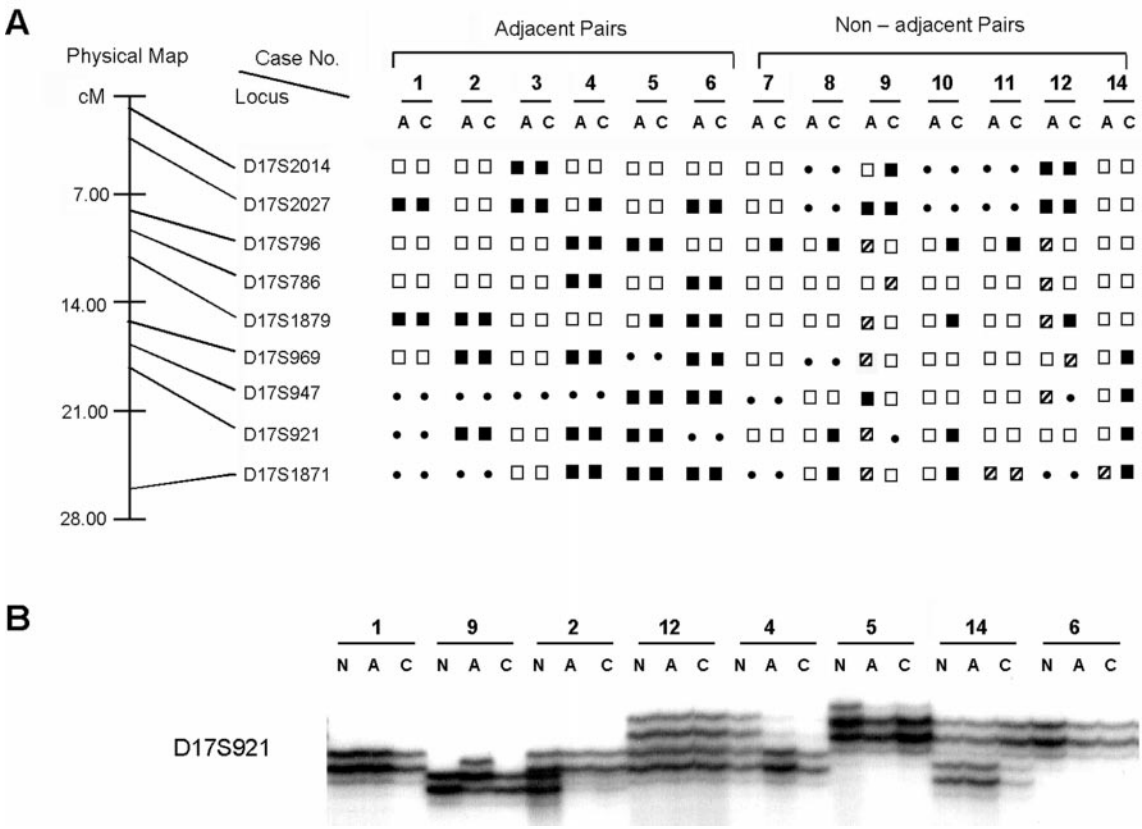

Figure 6. A: Schematic representation of LOH in paired gastric adenomas and carcinomas with 9 microsatellite markers mapped from D17S2014 to D17S1871. The markers are listed in relative positions from centromeric to the most telomeric locus at intervals of approximately 4cM. The conserved deletion area were frequent in the adjacent pairs of gastric adenomas and carcinomas (cases 1–6), whereas different deletion patterns were observed in the non-adjacent pairs of adenomas and carcinomas (cases 7–12 and 14). □, retention of heterozygosity; ■, allelic loss; ▨, microsatellite instability; ●, not informative. **B:** Representive autoradiographs of loss of heterozygosity (LOH) by D17S921 marker. The adenoma (A), carcinoma (C), and corresponding non-tumorous tissue (N) are shown with D17S921 marker indicated at the left. Cases 2, 4, and 5 showed losses in both adenoma and carcinoma, whereas case 14 showed loss only in carcinoma. Case 9 also showed microsatellite instability phenotype in adenoma.

The frequent LOH of the several chromosomal arms in gastric carcinomas, which have previously been reported imply the presence of tumor suppressor genes. Many segments of chromosomal arms had been reported to have frequent losses.[5,10,12–17,30,36] Among these chromosomal arms, losses on 17p, 3q, and 5q are known to be associated with specific target gene inactivation. Loss of 17p is known to be associated with *p53* inactivation by either mutation or deletion,[5,10,37] *FHIT* gene inactivation with 3p deletion,[38] and *APC* gene deletion on 5q[39] in gastric carcinomas have been reported. In this study we demonstrated deletion of chromosome 17p as a frequent and early genetic event in gastric carcinogenesis. In our cases, the common deletion area on chromosome 17p encompasses the *p53* locus, suggesting *p53* alteration as an early genetic event in gastric carcinogenesis. Several common deletion areas on chromosome 1p,[40] 6q,[41,42] 7q,[14,43] 11,[17] and 16q[16] have been identified in gastric carcinomas by fine deletion mapping analysis. However, no gastric carcinoma-related specific tumor suppressor gene has been identified so far in these chromosomal areas.

We also found frequent chromosomal gains on 8q, 13q, and 20p by CGH analysis. Recent chromosomal copy number analysis of gastric carcinoma by CGH has reported frequent gains on the several chromosomal arms,[18–20,44] which had not been reported in earlier studies on the changes of DNA copy number by PCR-LOH analysis. The PCR-LOH study is useful in identifying small interstitial deletions, because the microsatellite markers are highly polymorphic and evenly distributed on the chromosomes.[45] However, it is impossible to differentiate between chromosomal gains and losses in many cases. This problem can be resolved by CGH or arbitrarily primed PCR fingerprinting analysis, which can differentiate between chromosomal gains and losses.[46] Of the 3 chromosomal arms with frequent gains in this study, we detected the gains of 8q and 13q by arbitrarily primed PCR fingerprinting analysis (data not shown) by using two primers, BLUE and MCG1. We could not, however, confirm the chromosomal gains of 20p because no corresponding band for the chromosomal arm of 20p was present with these two primers. Our findings, together with those of previous studies, support the hypothesis that chromosomal gains associated with specific oncogene activation are also important in gastric carcinogenesis.

Our evaluation of chromosomal alterations in gastric carcinomas permitted the identification of the striking intertumoral heterogeneity of chromosomal losses and

gains. Gastric carcinomas are not a homogenous disease and different patterns of genetic alterations have been implicated in the development of diffuse- and intestinal-type carcinomas.[47] All of the gastric carcinomas in this study were the intestinal type, probably because all of the carcinomas were associated with gastric adenomas. Although the selected cases in our series were a relatively homogeneous subset of tumors pathologically, there were also remarkable intertumoral genetic heterogeneities. These findings indicate that many different etiological and genetic events can result in these phenotypically similar gastric carcinomas. This intertumoral genetic heterogeneity was also present between the gastric adenomas, although the chromosomal changes were not as frequent as the carcinomas. These heterogeneites of chromosomal changes were present in the non-adjacent pairs of gastric adenomas and carcinomas. In contrast to the remarkable intertumoral genetic heterogeneity in our cases, relatively clonal genetic changes were found in adjacent pairs of gastric adenomas and carcinomas. By CGH analysis, the clonal genetic changes were found in all of the adjacent pairs of adenomas and carcinomas while most of the non-adjacent pairs showed different clonal genetic changes. The fine deletion mapping analysis on the short arm of chromosome 17 also demonstrated the clonal genetic changes in adjacent pairs, whereas most of the non-adjacent pairs with 17p loss did not show the clonal changes. The common deletion areas were found in all of the the adjacent pairs of gastric adenomas and carcinomas, while many of the non-adjacent pairs showed different patterns of deletion. Additionally, different microsatellite mutator phenotypes were observed in some of the non-adjacent pairs of gastric adenomas and carcinomas. These findings suggest that different genetic changes were involved in the multiple tumor formation. Although the sample numbers are small, the common genetic changes within adjacent pairs of adenomas and carcinomas, and accumulated genetic changes in the carcinomas provide an evidence that gastric adenomas progress to carcinomas through the accumulation of a series of genetic alterations and suggest a stepwise mode of carcinogenesis.

Acknowledgments

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