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Yeun-Jun Chung · Jung-Ran Choi · Sang-Won Park
Kyoung-Mee Kim · Mun-Gan Rhyu

Evidence for two modes of allelic loss: multifocal analysis on both early and advanced gastric carcinomas

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Abstract To assess the extent and the timing of allelic loss required for the progression of gastric carcinoma, the intratumoral distribution of loss of heterozygosity (LOH) was compared in early and advanced tumors: early loss is uniformly observed in all tumor areas and late loss is localized in parts of tumor tissue. Tumor sites (167 sites) obtained from 42 gastric carcinoma tissues (26 advanced cancers and 16 early cancers) were examined for LOH on chromosomes 5q, 9p, 13q, 17p, and 18q. By using two or three microsatellite markers for each chromosome arm, it was shown that of 29 tumors showing LOH in at least one tumor site, 15 (51.7%, 12 advanced and three early cancers) harbored multiple losses on three or more chromosome arms, and 89.4% (84 of 94) of these losses was uniformly found in all tumor sites tested. In the remaining 14 tumors (48.3%, eight advanced and six early tumors) with sporadic losses on one or two chromosome arms, 44% (11 of 25) of the losses were commonly shared among the sites tested. Such marked difference (P<0.001, Fisher's exact test) in the intratumoral distribution of multiple and sporadic LOH patterns proposes two distinct LOH subtypes: multiple losses (high LOH), occurring at an early stage with a few additional losses, and sporadic losses (low LOH), taking place relatively late during tumor progression. The multifocal LOH findings imply that, rather than being gradual, the allelic losses take place in two manners that are already determined at an early stage.

Keywords Gastric carcinoma · Loss of heterozygosity · Heterogeneity · Multifocal analysis

Yeun-Jun Chung · Jung-Ran Choi · Sang-Won Park
Mun-Gan Rhyu (✉)
Department of Microbiology, College of Medicine,
The Catholic University of Korea, 505 Banpo-dong, Socho-gu,
Seoul 137-701, Korea
e-mail: rhyumung@cmc.cuk.ac.kr
Tel.: +82-2-5901215, Fax: +82-2-9602534

Kyoung-Mee Kim
Department of Clinical Pathology, College of Medicine,
The Catholic University of Korea, Seoul, Korea

Introduction

Tumor suppressor genes that are inactivated by two rate-limiting genetic events, a germ-line mutation in one allele and a chromosomal loss involving the other allele, have been identified in various hereditary cancers [13]. Functional loss of tumor suppressor by biallelic alterations indicates that the chromosomal loss around the tumor suppressor gene locus is a hallmark of somatic gene inactivation in cancer development. Studies on loss of heterozygosity (LOH) using polymorphic DNA markers have indeed supplied an insight into the loss of genetic material prevailing in specific chromosomal regions; the mapping of minimally deleted regions has allowed the identification of the genome segments suspected of containing tumor suppressor genes [9]. LOH studies on gastric carcinoma, using polymorphic markers that are intragenic to tumor suppressor genes, have also provided a strong evidence for the involvement of well-known tumor suppressor genes, such as APC (5q), p15 and p16 (9p), Rb (13q), p53 (17p), and DCC (18q) [1, 18, 19, 26].

Nonetheless, there have been major limitations regarding the fact that gastric carcinomas are heterogeneous entities based on the instability of the cancer genome. The genetic instability gives rise to marked intra- and inter-tumoral variations in terms of both histological pattern [14, 15] and genetic alterations [3, 4, 6, 16]. A microdissected tumor piece used for a genetic study represents only a portion of intratumoral genetic heterogeneity. Additional genetic alterations, localized in parts of a tumor lesion that occur in the late stage during tumorigenesis, are unlikely to be observed using the tumor DNA, which has been microdissected from a tumor area. Instead, the signal of localized alteration would be compromised in pools of tumor DNA from multiple sites. Thus, controversies still exist over the LOH incidence on a chromosome arm in gastric cancers [2, 8, 17, 20, 23, 26, 27]. In addition, the genome-wide allelotype studies demonstrated that the extent of chromosomal loss estimated by means of fractional allelic loss (FAL, the ratio of LOH-positive markers to total informative markers)

varied among individual patients, ranging from those harboring no loss to those losing up to 60% of chromosomal loci tested [2, 12]. The inherent instability of the cancer genome seems to cause non-specific LOH and non-random and specific LOH, contributing to the genetic diversity that is too extensive to predict the concurrent patterns of obligatory frequent and optional infrequent LOH, especially when using a number of genetic markers for allelotyping.

In our previous genome-wide LOH study on gastric cancer [2], wide range of allelic loss was categorized into background nonspecific occurrence on arbitrary chromosomes when below mean FAL and a causative event having an influence on malignant phenotype when above mean FAL. The extent of loss above mean FAL mainly involved multiple chromosomal arms, including 5q, 9p, 13q, 17p, and 18q, all of which have consistently proven to be closely associated with gastric cancers [2, 18, 23, 26]. These findings suggest that the limited number of genetic markers on cancer type-specific chromosomes, rather than the extensive number of markers covering all autosomes, can be advantageous in representing LOH genotypes, while avoiding non-specific events resulting from the inherent instability of the cancer genome.

Therefore, in the present study, a set of genetic markers on gastric cancer-associated chromosome arms was expected to specifically represent cancer-causing LOH events. A polymerase chain reaction (PCR)-based LOH assay, using microsatellite makers on the five chromosomal arms, was performed on multiple pairs of tumor and normal DNA that were selected from histotopographically dissimilar areas in a given tumor lesion. The intratumoral distribution of chromosomal loss was evaluated to define the primary loss commonly shared in multiple tumor sites and the secondary loss localized in parts of tumors. In addition, to gain an insight into the order and the amount of chromosomal loss required for the progression of gastric cancer from early to advanced gastric carcinomas, both lesions were subjected to the multifocal analysis. The present multifocal study on both early and advanced cancers provided interesting findings concerning the multiple chromosomal losses underlying the progression of gastric cancer.

Materials and methods

Specimens

Archival paraffin-embedded gastric tumor samples were randomly collected from 51 patients admitted to St. Paul's and St. Mary's hospital, Seoul, Korea. None of the cases chosen for this study had family histories of gastric carcinoma. Based on the criteria of the Japanese Research Society for Gastric Cancer [11], 33 advanced tumors were found to have invaded beyond the muscularis propria, and 18 early tumors were identified within the mucosa or submucosa. For each case, tumor cells were precisely scrapped off from 5-μm thick sections under the microscope by using a 21 gage needle. A more than 60% purity of tumor cell population was selected with this microdissection procedure. The dissected cells (50–80 cells/μl) were placed in digestion buffer containing 50 mmol/l Tris-HCl (pH 8.5), 0.5% Tween 20, and 0.2 mg/ml proteinase K. The buffer was incubated at 50°C for 24 h and was used directly as template DNA after inactivating proteinase K by heating for 5 min at 95°C.

Analysis of genetic alterations with microsatellite markers

Microsatellite loci (13 loci) were examined for LOH using the following microsatellite markers: D5S299, D5S409, D9S165, D9S171, IFNA, D13S120, D13S133, D13S135, TP53, D17S786, D17S796, D18S70, and D18S386. Genomic DNA, extracted from the microdissected tissue, was amplified with 1 μCi of [^{32}P]dCTP in 10 μl of the PCR mixture. Cycles of amplification (32 cycles) were performed at 95°C for 50 s, 52–60°C for 50 s, and 72°C for 50 s. The amplified product was diluted with the gel-loading buffer containing 95% formamide and denatured completely by heating for 5 min, followed by ice cooling. Samples were electrophoresed on a 6% denaturing gel and visualized overnight by means of autoradiography.

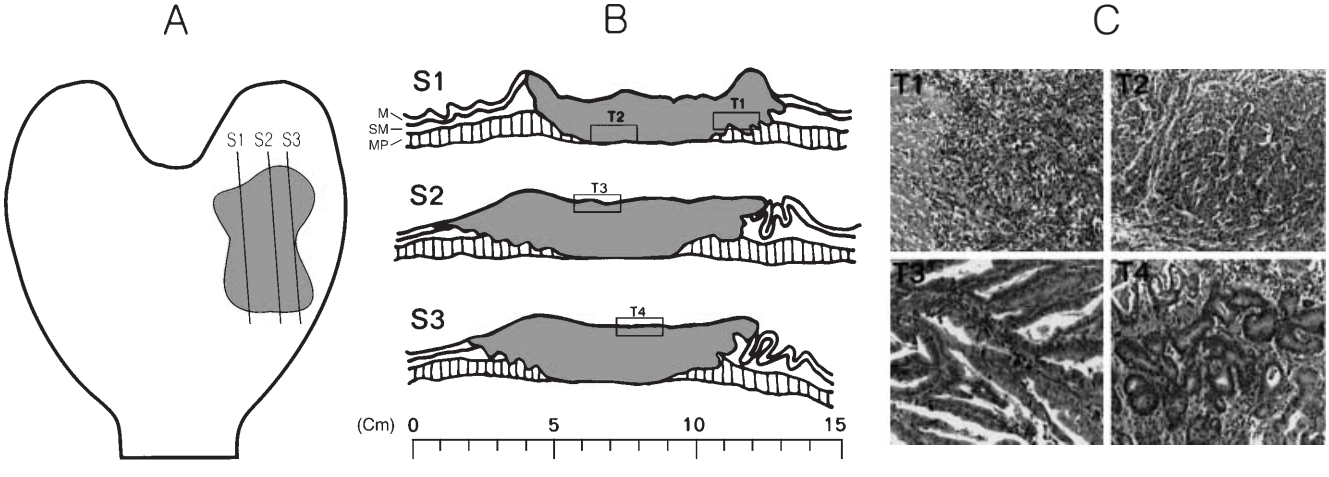

Fig. 1 Representative case (GC23) of multifocal selection of histotopographically distinct tumor sites. **A** Schematic illustration of multiple section lines (S1, S2, and S3) from a resected gastric tumor specimen. Tumor area is indicated by the *gray color*. **B** Four topographically separated tumor sites are illustrated at cut view of the corresponding section lines. Two deep areas (T1 and T2) and two surface areas (T3 and T4) were selected in a given tumor. **C** Microscopic appearances of the four tumor areas (hematoxylin and eosin staining; original magnification ×100). *M* mucosa, *SM* submucosa, *MP* muscularis propria

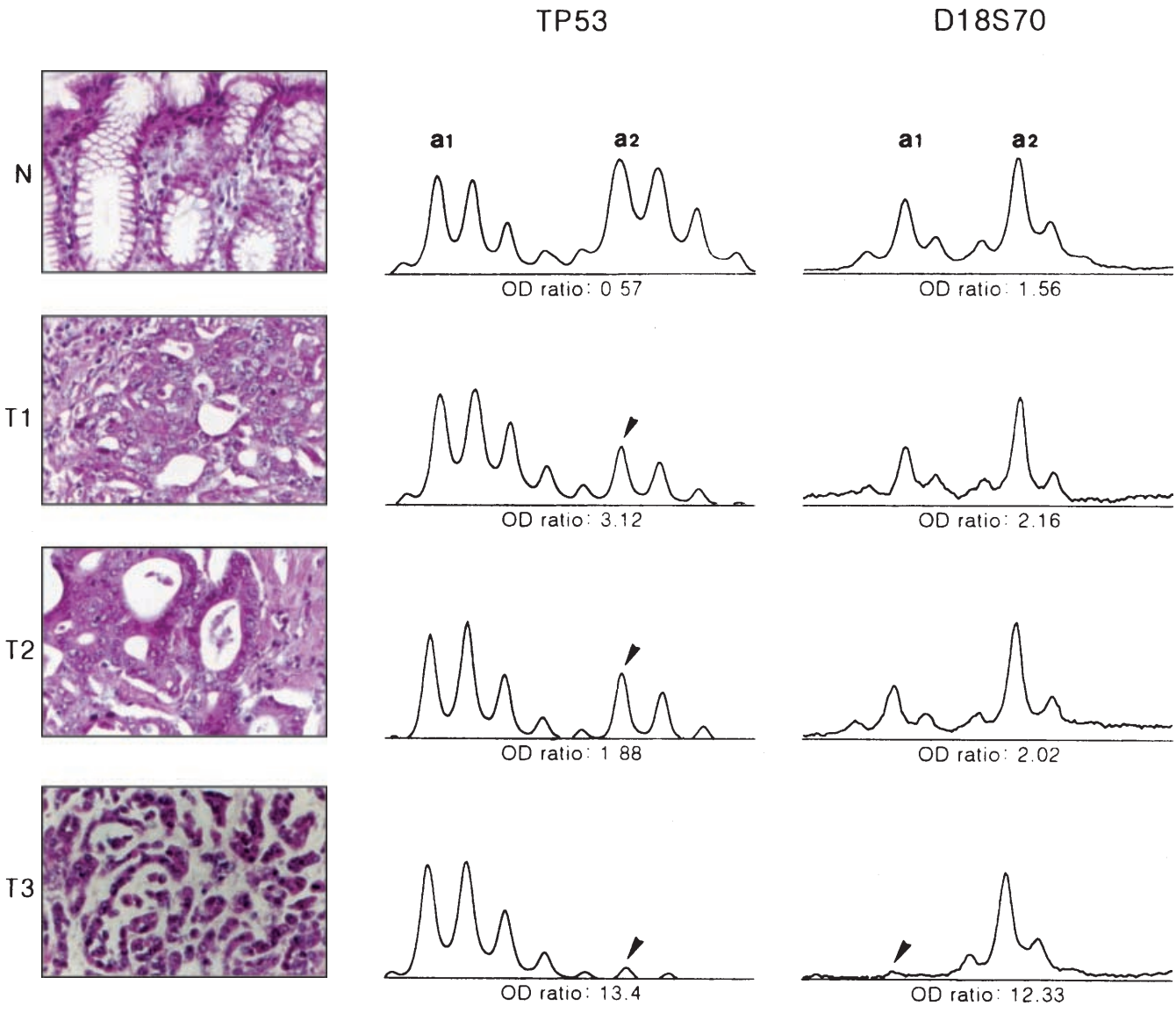

Fig. 2 Varying patterns of allelic imbalance observed in multiple tumor sites (T1, T2, and T3), microdissected from a gastric cancer patient, GC10. The degree of signal reduction in tumor DNA differs among distinct tumor areas and between two microsatellite markers, TP53 and D18S70, tested in the same tumor areas. The intensities of the electrophoretic bands of the microsatellite markers were measured with a densitometer. When the signal intensity ratio of the two alleles (a1 and a2) is compared between normal and tumor DNA, the a2 allele (*arrowhead*) at the TP53 locus is completely lost in the T3 site and incompletely lost in the T1 and T2 sites. The T3 tumor site also demonstrates complete loss of signal in the a1 allele (*arrowhead*) of the D18S70 marker. In addition, T1 and T2 sites demonstrate allelic imbalance of the borderline level, not loss of heterozygosity, at the D18S70 locus. *N* normal; *T* tumor tissues (hematoxylin and eosin staining; original magnification ×200)

Determination of LOH

The band intensity of the two separated alleles was measured by using the densitometer (Imagemaster, Pharmacia Biotech, San Francisco, Calif.). Because gastric carcinoma usually accompanies inflammatory cell infiltration, a complete allelic loss was not commonly observed even in microdissected tumor DNA. Thus, LOH at each tumor area was indicated if the intensity ratio of the two alleles from tumor DNA differed by more than twofold relative to the ratio from normal DNA. If novel bands not observed in normal DNA were found, they were interpreted as microsatellite instability. Of 51 gastric cancers tested, 9 cases showing widespread microsatellite instability were excluded in this LOH study because the presence of allelic imbalance cannot be determined with band shift arising from the instability. All of the experiment was repeated at least twice to ensure the reproducibility of the result.

Statistical analysis

Comparison of the proportion of intratumoral uniformity between multiple and sporadic LOH events and that of the incidence of LOH between early and advanced gastric cancers were made usingeither χ^2 or the Fisher's exact test. A P value of less than 0.05 was interpreted as significant.

Results

Multifocal analysis of allelic loss

Separated tumor areas (2–8 areas) that were histotopographically dissimilar were selected microscopically on

hematoxylin and eosin-stained sections (Fig. 1). The degree of reduction in signal intensity of tumor lesion was not always comparable to the presence of allelic loss because of the intratumoral genetic heterogeneity and the tumor cell contents. For example (Fig. 2), the three tumor sites of the patient GC10 were seen as LOH positive at the TP53 locus, showing different intensities of electrophoretic bands in the lanes of the lost alleles (a2). A relatively incomplete loss in the T1 and T2 sites and a clear absence of the same allele in the T3 tumor site were observed.

Microscopic examination showed more lymphocytes infiltrating into the tumor lesions of the T1 and T2 sites than into the tumor lesions of the T3 sites, indicating that incomplete loss at the TP53 locus in the T1 and T2 sites might be due to normal cell contamination. At the D18S70 locus, the T1 and T2 sites exhibited allelic imbalance (a1) of the borderline level, which did not meet the criteria of LOH, and the T3 sites had complete loss comparable to that of TP53. Considering the content of normal cells in the T1 and T2 sites that showed incomplete loss at TP53, the borderline degree of imbalance at D18S70 in the T1 and T2 sites was likely to reflect the coexistence of tumor cells with or without the loss of D18S70 and normal cells. The intratumoral heterogeneous distribution of chromosomal loss observed in this case suggested that the multifocal assay is capable of distinguishing a signal of genetic alteration localized in a part of tumor tissue.

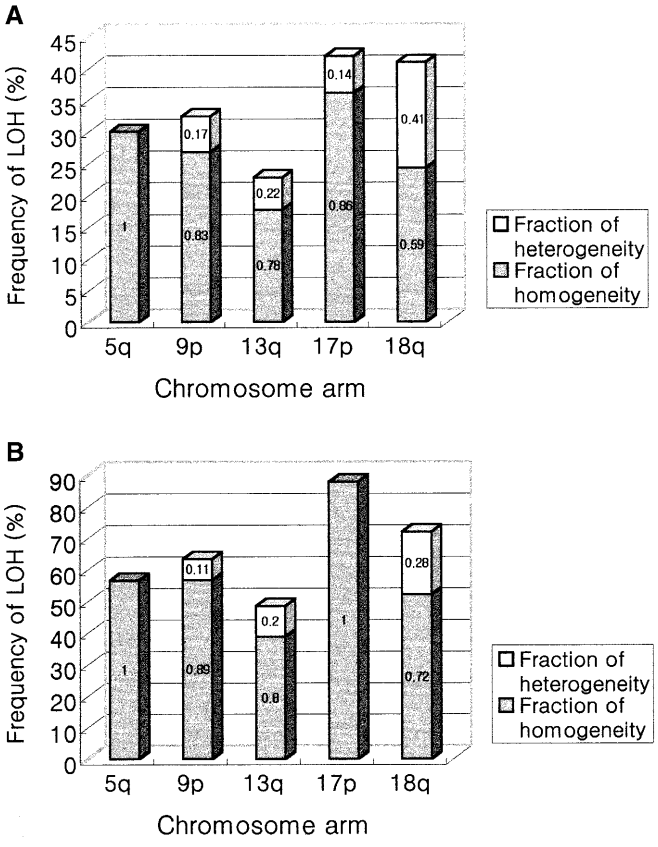

Fig. 3 Incidence and intratumoral distribution of loss of heterozygosity (LOH) on individual chromosome arms were evaluated in a total of 42 gastric cancers (**A**) and in 15 gastric cancers demonstrating high LOH (**B**). The fraction of homogeneity and heterogeneity are shown as the ratio of the number of uniform and heterogeneous events to LOH positive cases, respectively

The incidence and intratumor topography of LOH events

Using microsatellite markers on five gastric cancer-associated chromosome arms, a PCR-based multifocal LOH assay was performed on a total of 167 tumor sites microdissected from 42 gastric cancers (26 advanced cancers and 16 early cancers) and on the corresponding normal stomach tissues. LOH was observed in 33.8% (119 of 352) of the informative markers on the five chromosome arms, including 17p (41.9%), 18q (40.9%), 9p (32.4%), 5q (30%), and 13q (22.8%). Multiple foci from a given lesion were compared to determine whether the LOH events were shared in all tumor sites as an early event derived from a progenitor cell or localized in parts of tumor tissue as an additional event directing toward subclonal expansion. Losses on 5q were detected uniformly in all tumor sites tested, while homogeneous distribution of LOH was observed to be relatively low on 17p (86%), 9p (83%), 13q (78%), and 18q (59%; Fig. 3A). The results are summarized in Table 1.

Of 42 patients with gastric carcinoma, 29 (69%) harbored one or more LOH on the five chromosome arms, but no loss was found in the remaining 13 cases (31%). The mean FAL value of the LOH-positive tumors was calculated as 0.48. Of the 29 LOH-positive tumors, 15 cases above mean FAL (51.7%, 12 advanced and three early cancers) harbored multiple LOH events on three or more chromosome arms and were thus designated as high LOH. The remaining 14 cases below mean FAL (48.3%, eight advanced and six early tumors) demonstrated sporadic LOH on one or two chromosome arms, therefore indicating low LOH. There was marked difference in the intratumoral distribution of chromosomal loss between high- and low-LOH subtypes (Table 1). In cases with high LOH, 89.4% (84 of 94) of the allelic losses were uniformly observed in all tumor sites tested, whereas in low LOH cases, 44% (11 of 25) of the losses were found to share the common loss in the sites tested ($P<0.001$, Fisher's exact test; Fig. 4). The intratumoral distribution of LOH events on each chromosome in high-LOH cases was further analyzed to delineate the order of allelic loss during tumor progression. As shown in Fig. 3B, 17p loss was observed most frequently (87.9%) and most uniformly (100%) in the multiple sites, thus being interpreted as an obligatory early event in the progression of high-LOH gastric cancer. The loss of 18q was the second most frequent (72%) and the most heterogeneous (28%) and, therefore, indicative of late alterations required for malignant progression.

Table 1 Multifocal loss of heterozygosity (LOH) assay of 42 gastric carcinomas (+ uniform LOH detected in all tumor sites, ± hetergeneous LOH detected in restricted tumor sites, – LOH negative)

	Samples	Tumor sites[a]	Histological type[b]	Microsatellite loci												
				D5S409	D5S299	D9S165	D9S171	IFNA	D13S133	D13S135	D13S120	TP53	D17S786	D17S796	D18S70	D17S386
Advanced	GC1	3	Intestinal		+	+	+		+		+		+	+	+	
	GC2	5	Intestinal	+	+		+	+	±	±		+		+	–	+
	GC3	3	Int/Dif	+	–		+			–	+	+	+	+	–	+
	GC4	6	Diffuse		+	+		+		–	–	+		+	+	+
	GC5	2	Intestinal	+	+	+	–	+	+	+		+	+	+	–	–
	GC6	7	Intestinal	–			±		+	–	–	+			+	+
	GC7	3	Intestinal	–	–	+	–	–	+	±		+	–	+	+	
	GC8	2	Intestinal	+	+	+		+		–			+	+		–
	GC9	3	Diffuse	–		–			+	+		+		+	+	
	GC10	3	Int/Dif				–	–	+	+		+	+	+	±	–
	Gc11	5	Intestinal	–	–	+		+	+	–	–	–			±	±
	GC12	8	Int/Dif		–	+		–	–			+	–	–	±	±
	GC13	3	Intestinal	–		–		–		–	–	±		±	±	±
	GC14	4	Int/Dif	–		±		±		–		–	–		–	+
	GC15	3	Intestinal			+	–			–	±		–		–	–
	GC16	3	Int/Dif		–				–	–			–	–	±	+
	GC17	3	Diffuse	–		–		–	–	–	–	±	–	±	–	
	GC18	5	Int/Dif	–		–		–	–				–	–		+
	GC19	3	Intestinal		–	–		–	–	–		–	–	–	±	
	GC20	3	Intestinal	–	–		–	–	–	–	–		–	–	±	–
	GC21	2	Diffuse			–					–		–	–	–	
	GC22	6	Diffuse	–		–	–	–	–			–	–	–	–	
	GC23	4	Int/Dif	–		–		–				–	–	–	–	
	GC24	8	Diffuse	–	–			–	–	–	–	–	–		–	–
	GC25	7	Intestinal		–		–	–		–	–	–		–	–	–
	GC26	6	Diffuse	–		–	–	–		–			–	–	–	–
Early	GC27	3	Intestinal	+	+	+	+		–	–	–		+	+		+
	GC28	4	Intestinal	+	+	–		–	–	–		+			+	+
	GC29	3	Intestinal	–	–	±	–	–	–	–		+	+	+	–	+
	GC30	4	Intestinal					+		–			+	+	–	
	GC31	3	Intestinal		–		–			+	–	±	–		–	
	GC32	3	Intestinal	+			–		–	–	–			–		–
	GC33	3	Intestinal		–					+					–	–
	GC34	4	Intestinal	+		–	–	–	–		–	–		–	–	–
	GC35	2	Intestinal		–	–				–	–	–	–			±
	GC36	3	Intestinal		–	–	–		–		–	–	–		–	–
	GC37	5	Int/Dif		–	–			–		–	–		–		–
	GC38	4	Intestinal	–	–	–				–	–		–	–	–	
	GC39	3	Diffuse	–		–		–	–	–		–	–		–	–
	GC40	3	Intestinal	–				–		–		–	–		–	–
	GC41	3	Intestinal						–	–	–	–	–			–
	GC42	3	Intestinal	–	–	–				–		–			–	–
Intratumoral heterogeneity (%)[c]				0 0/8	0 0/7	18.2 2/11	20 1/5	14.3 1/7	12.5 1/8	28.6 2/7	33.3 1/3	21.4 3/14	0 0/8	14.3 2/14	53.8 7/13	28.6 4/14

[a] Number of tumor sites studiesd in each gastric carcinoma.

[b] Lauren (1965) classification [14]: Int/Dif, hetergeneous histology composed of both intestinal and diffuse types

[c] Number of heterogeneous LOH cases/number of total LOH cases at each marker

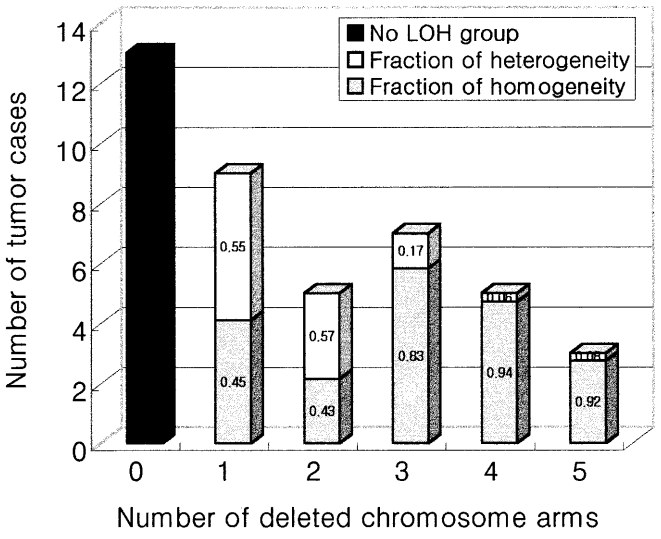

Fig. 4 Distribution of the 42 gastric cancers according to the number of chromosome arms involved in allelic loss. Intratumoral homogeneity of loss of heterozygosity (LOH) in each patient is indicated as the ratio in bars. The uniformity is significantly higher in the high-LOH group (involving three or more chromosome arms) than in the low-LOH group (involving one or two arms; $P<0.001$, Fisher's exact test)

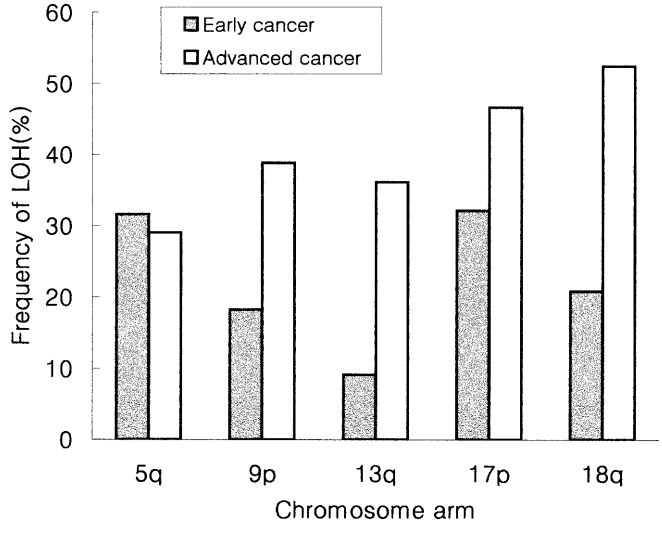

Fig. 5 Comparison of loss of heterozygosity (LOH) frequency on the five chromosome arms in early and advanced gastric cancers

Comparison of LOH between early and advanced gastric cancers

LOH events were detected more frequently (40.8% vs 21%; $P<0.001$, χ^2 test) and more heterogeneously (22.6% vs 11.5%; $P=0.215$ χ^2 test) in multiple sites from advanced cancers than they were from early cancers. The LOH frequencies on individual chromosome arms were compared between early and advanced gastric cancers (Fig. 5). LOHs on chromosomes 5q and 17p with intratumoral homogeneity demonstrated similar incidences in both early and advanced gastric cancers: 31.6% vs 29% and 32.1% vs 46.6%, respectively. LOHs on 9p, 13q, and 18q with intratumoral heterogeneity were detected less than half as frequently in early cancers than in advanced cancers: 18.2% vs 38.8%, 9.1% vs 36.1%, and 20.8% vs 52.4%, respectively. Interestingly, of nine LOH-positive early cancers, three (33%) and six (67%) were found to be of high- and low-LOH subtypes, respectively, supportive of the suggestion that the two distinct LOH types are determined at an early stage of tumor progression.

Discussion

The allelotype studies on gastric cancer [2, 8, 20, 23, 27] have faced two major limitations, inter-individual diversity and intra-individual heterogeneity, both of which are likely to be due to non-specific genetic alterations resulting from the genetic instability. The present multifocal LOH analysis examined five chromosome arms known to include candidate tumor suppressor genes that play a role in the development of gastric cancer in order to focus on non-random causative allelic loss. We envisaged multiple allelic losses (high LOH) as a genotype determined at an early stage in a dosage-dependent manner but also in a locus-specific manner.

When the amount of LOH events occurring on the entire autosomal chromosomes was measured using a FAL value, a bimodal distribution of FAL values showed two peaks, indicative of LOH-related and LOH-unrelated subgroups in gastric cancer [2], breast cancer [12], and rhabdomyosarcoma [25]. Most of LOH-related gastric carcinomas have been thought to have specific and non-specific allelic losses due to the genetic instability [2]. In this study, which focuses on the five gastric cancer-associated chromosome arms, the mean FAL value of LOH-positive tumors was 0.48, which was in accordance with the arbitrary cut off of 2–3 of five chromosome arms tested. There was also a significant difference ($P<0.001$, Fisher's exact test) in the topographical uniformity between the high- and low-LOH subtypes. Such a marked difference in the proportion of topographical uniformity led us to categorize into high- and low-LOH gastric cancers according to the amount of allelic loss. Additionally, the number of LOHs on five gastric cancer-associated chromosome arms was thought to correspond to the specific losses comprising an above mean FAL value calculated from the genome-wide allelotype study. Considering the intratumoral distribution of multiple allelic losses, gastric tumors with a high LOH were likely to progress with the concurrent early LOHs on multiple chromosome arms, accompanied by a few additional losses (LOH-driven tumors). Tumors with a low LOH suggested a trend in which incidental allelic loss occurs subsequently to other predominant type(s) of genetic alterations (non-LOH-driven tumors), for example, point mutation and epigenetic change.

In particular, the intratumoral homogeneity of multiple losses is likely to be implicated in predicting the genetic progression of early cancer. For example, early cancers in the present study, GC27, GC28, and GC29, showing multiple losses, are expected to be followed by few subsequent genetic changes and, therefore, retain the same or similar genotype until reaching the advanced stage. An increased number of lost alleles has been correlated with several phenotypic features of poor prognosis, such as advanced-grade and lymph-node metastasis [21, 22, 26]. Thus, the potential outcome of these three early cancers with multiple allelic losses would be more progressive toward advanced cancer. Although early gastric carcinomas are considered to relate to a good prognosis, the patterns of growth and dissemination have been too diverse to predict the duration required for proceeding to advanced tumors [7]. Our study, hereby, suggests that high-LOH early cancer is more likely to proceed toward an advanced stage.

In order to understand the genetic progression of gastric carcinoma, an analytical study has been done on the difference in the incidence of LOH events on given chromosomal regions between early and advanced lesions [17, 26]. In the multifocal analysis on both early and advanced cancers, the frequencies of LOHs on 5q and 17p that tended to be uniformly distributed throughout tumor lesions were similar between early and advanced gastric cancers, while LOHs on 9p, 13q, and 18q were more frequent in advanced lesions than in early lesions. Consequently, based on both frequency and intratumoral heterogeneity of chromosomal loss, it was likely that allelic losses on 5q and/or 17p at an early stage and allelic losses on 9p, 13p, and/or 18q at a late stage accumulated progressively during gastric carcinogenesis. The possibility of genetic heterogeneity due to lower tumor cell contents (i.e. contamination by normal cells) has been considered, because the histological heterogeneity of gastric cancer may lead to variable degrees of tumor cell contents that in turn effect the LOH determination. Based on the fact that the diffuse-type samples (GC4, GC9), which are the least distinguishable and the most difficult to isolate and exhibit much less heterogeneity of LOH than expected, we believe that the contamination by normal cells has not been serious. Thus, intratumoral heterogeneity shown in the present study reflects the heterogeneous tumor cell population rather than the differing levels of normal cell contamination among regions tested.

Allelic loss on 5q has been reported to be somewhat less frequent than that on other cancer-associated chromosome arms, such as 9p, 17p, and 18q [2, 8]. Low frequency of 5q loss was statistically interpreted as a borderline level between random and non-random events or as a secondary change added in late-stage cancer. Because 5q LOH was more common in intestinal-type gastric carcinoma than in the diffuse type [21, 26], it is likely that the relatively low incidence of 5q loss is due to the histological preference for the intestinal type. Also, in the present study, 5q loss was more frequently observed in the intestinal type (34.8%) than in the diffuse type (14.3%). Additionally, 5q loss that was spread uniformly on the entire tumor was found at the similar incidence rate in both early and advanced cancers. Therefore, 5q loss is thought to play a role in the early stage of an intestinal-type tumor.

The frequency of 18q loss has been reported to vary, ranging from 27% to 70%, even among advanced cancers [1, 5, 8, 10, 17, 23, 24]. Such varying frequencies of 18q loss appear to be attributable to the heterogeneous tumor cell populations. Since the loss occurring at the late stage of cancer might be localized in a subclonal population, tumor DNA obtained from a microdissected tumor area might represent only certain parts of tumor, thus resulting in the underestimation of localized genetic alterations. In this study, the high incidence (52.4%) of 18q LOH in advanced cancers is, therefore, thought to be due to the multifocal LOH assay that can distinguish the compromised signal of subclonal alterations in the heterogeneous tumor cell populations.

In previous reports, there have been controversial findings on the histological type associated with the allelic loss on18q [1, 8, 26]. In the present study, even though the incidence rate of LOH on 18q in intestinal type (39%) was somewhat higher than that of LOH on 18q in diffuse type (25%), this finding was statistically insignificant. There was also no significant correlation between the intratumoral heterogeneity of 18q loss and the heterogeneous histological phenotype. Our results suggest that 18q LOH may contribute to the progression of gastric cancer without cell-type specificity.

In summary, deletion on chromosome 5q was likely to occur at an early stage and favorably in intestinal-type gastric tumors, whereas 17p alterations were widely related to tumor progression mainly at early and partly at late stages. 18q deletions seemed to play a role in the progression toward advanced gastric cancer. In terms of the pattern of LOH occurrence, we propose two modes of allelic loss: high LOH, occurring multiply at an early stage with a few additional losses and low LOH, taking place subsequently to other predominant type(s) of genetic alterations. Multiple allelic losses were likely to be an early and consistent genotype, with few successive alterations, thus indicating that early gastric carcinoma harboring multiple losses tends to proceed toward advanced tumors with the same or similar genotype. The present results obtained from multifocal analysis have an important implication for the genetic progression of gastric cancers.

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