—**Original**—

Genome-Wide Screening of Laser Capture Microdissected Gastric Signet-Ring Cell Carcinomas

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Abstract

Gastric signet-ring cell carcinoma comprises a distinct category of gastric cancers and has been reported to have poor prognosis. In an attempt to define genetic changes involved in the pathogenesis of this lesion in an *in vivo* state, we isolated signet-ring cell carcinoma cells from freshly fixed smears of tumor tissues of 7 primary gastric signet-ring cell carcinomas by laser capture microdissection and applied comparative genomic hybridization (CGH) to screen for DNA sequence copy number changes. Frequent chromosomal gains were detected on 2q, 5p, 7q, 14q and 20q, each in 6/7 cases, on 9q, 12q, 17q, and 19q, each in 5/7 cases, and on 18p in 4/7 cases. Frequent losses were observed on 6p and 17p, each in 5/7 cases, on 6q, and 21p, each in 4/7 cases, and on 3p, 8p and 8q, each in 3/7 cases. Losses on 6p have rarely been observed in conventional types of gastric carcinomas reported in the literature. These data provide the first evidence for the occurrence of specific genomic aberrations in gastric signet-ring cell carcinomas. Our observation of frequent losses on 6p chromosomal arm may provide novel abnormalities of potential significance in gastric signet-ring cell carcinomas, suggesting the involvement of genes residing in this region in the genesis of the disease.
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Key words: comparative genomic hybridization, laser capture microdissection, gastric adenocarcinoma, signet-ring cell, DNA copy number, genetic changes, chromosome

Introduction

Gastric adenocarcinoma accounts for nearly one-tenth of all malignancies and is the second most frequent cause of cancer mortality worldwide[1]. Primary gastric adenocarcinoma with signet-ring cell histology comprises 3 to 39% of gastric carcinomas[2]. The diagnosis of signet-ring cell carcinoma is usually considered if an adenocarcinoma contains a predominant component (>50%) of tumor cells with signet-ring cell morphology. In Japan, gastric signet-ring cell carcinoma has a lower incidence (3.4%) than in Western countries[3].

The genetic events underlying the neoplastic process in gastric adenocarcinomas remain largely unknown. Efforts to understand this complex mechanism have led to a number of molecular and cytogenetic studies. Frequent allelic losses on chromosomes 1q, 5q, 7q, 12q, 17q, 18q, and 21q have been detected

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Table 1 Clinicopathological characteristics of the gastric signet-ring cell carcinomas

Patient	Sex	Age	Size	Site*	Depth[†]	TNM stage[‡]
1	F	73	80 × 60mm	F	ss	T2N2M0
2	F	63	18 × 22mm	M	m	T1N0M0
3	F	31	6 × 6mm	M	m	T1N0M0
4	F	65	4 × 4mm	M	mp	T2N0M0
5	M	54	90 × 55mm	M	m	T1N0M0
6	M	50	13 × 11mm	M	ss	T2N0M0
7	M	47	13 × 14mm	M	m	T1N0M0

＊F, fundus; M, mid-body. † ss, subserosa; m, mucosa; mp, muscularis propria.
‡ T1N0M0＝Ia, T2N0M0＝Ib, T2N2M0＝IIIa.

in gastric adenocarcinomas[4,5]. In addition, homozygous deletions of 3p were shown in several gastric cancer cell lines[6]. In a study by Hollstein et al[7], inactivation of *p53* tumor suppressor gene on chromosome 17p was demonstrated in the majority of gastric cancers. In contrast to the losses of genetic materials on chromosomal arms, amplifications of several chromosomal regions have also been reported. Amplification of 7q 31–32 region that harbors *MET* oncogene, encoding the hepatocyte growth factor receptor[8,9], and amplification of 17q 11-21 region that harbors *ERBB2* oncogene, encoding a growth factor receptor-like protein with high homology to the epidermal growth factor receptor[10], have been demonstrated in gastric cancers.

Comparative genomic hybridization (CGH) analyses of gastric adenocarcinomas, excluding those of cell lines, have so far been performed using DNA extracted from whole frozen or paraffin-embedded tumor tissues or tissue sections which contain various types of cells including vascular, lymphatic, and stromal cells, and thus heterogenous in nature. Consequently, genetic alterations obtained from these samples might be underrepresented or overrepresented. With the advent of laser capture microdissection (LCM) technology[11], pure population of specific cells under morphologic confirmation can be isolated from tissue sections or tumor cell smears on glass slides[12].

In this study, in an attempt to define genetic changes involved in the pathogenesis of gastric signet ring cell carcinoma in an *in vivo* state, we isolated pure carcinoma cells from freshly fixed tumor cell smears of primary gastric signet-ring cell carcinomas by laser capture microdissection and applied CGH to screen for DNA sequence copy number changes.

Materials and Methods

Specimens

Tumor tissue and blood samples were obtained from 7 consecutive gastric signet-ring cell carcinoma patients. Informed consents from the patients were obtained by the Center for Digestive Diseases, Second Hospital, Nippon Medical School before the study was done. We used an epithelial aggregate separation and isolation method for preparation of tumor cell smears[12]. Immediately after surgery, the surface of the sectioned tumor was gently scraped with the edge of a plain, uncharged, RNase-free microscopic glass slide. The materials obtained were spread evenly onto the surface of a second uncharged slide and immediately fixed in 95% ethanol for approximately 2 min. Multiple slides (20 to 30) were prepared and stored at−20℃ until use. Routine hematoxylin-eosin stained sections from the orignial tumor specimens were reviewed to confirm the histopathologic type and diagnosis. Signet-ring cell carcinoma cells could be detected easily because of their typical enriched intracytoplasmic mucin and peripheral compressed nuclei.

Patients

Clinicopathological characteristics of the patients are summarized in **Table 1**. There were 4 females and 3 males. The mean age of the patients was 54.7 years (range, 31–73 years). Pathologic diagnosis and classification of the tumors were based on the Japanese Classification of Gastric Cancer[13]. The diagnosis of signet-ring cell carcinoma was considered if an adenocarcinoma contained a predominant
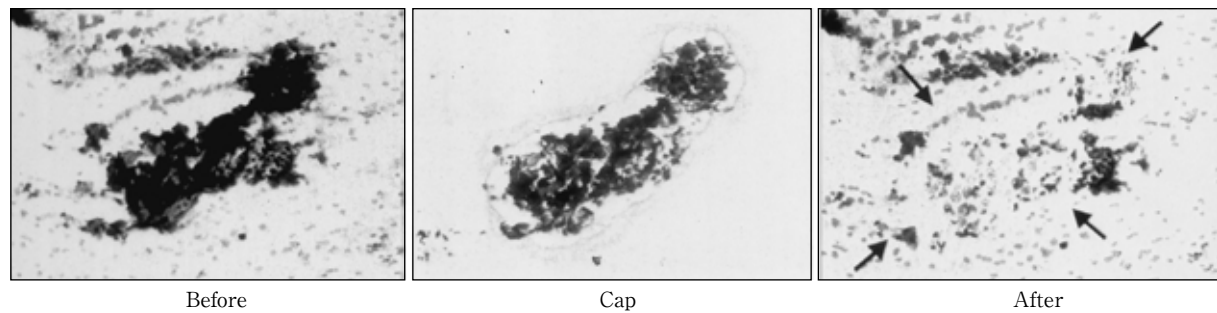
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Fig. 1　Epithelial aggregate preparation and subsequent LCM of a signet-ring cell carcinoma lesion. Gastric signet-ring cell carcinoma cells could be microdissected after a rapid cytological staining.

component (>50%) of tumor cells with signet-ring cell morphology. The clinical stages of the tumors were distributed as stage Ia, 4 cases; stage Ib, 2 cases; and stage IIIa, 1 case.

Laser Capture Microdissection

LCM was done using the PixCell LM 100 system (Arcturus Engineering, Mountain View, CA). The tumor smears were stained using a rapid cytological staining method without the use of coverslips according to the standard LCM method available at the NIH LCM web page (http://dir.nichd.nih.gov/l cm/lcm. htm). By LCM, between 800 to 1500 signet-ring cell carcinoma cells could be microdissected from one slide.

Comparative Genomic Hybridization

Extraction of DNA from microdissected tumor cells was performed using QIAamp DNA Mini Kit(QIAGEN, GmbH, Germany) according to standard protocols, and concentration, purity, and molecular weight of the DNA were examined. CGH was performed essentially as described previously[14]. Briefly, genomic tumor cell DNA was labeled with fluorescein isothiocyanate-deoxyuridine triphsphate (FITC-dUTP) and normal reference DNA with Texas Red isothiocyanate (Texas Red-dUTP) using a standard nick translation reaction. Equal amounts of labeled tumor DNA and labeled normal reference DNA (200 ng) and 10 μg of Cot-1 blocking DNA were mixed, co-precipitated, and resuspended in 10 μl of hybridization mixture (50% formamide, 0.1% Tween-20, and 10% dextran sulfate in 2× standard saline citrate at pH 7.0). The probe mixture was denatured and hybridized to normal metaphase preparations.

After 72 h incubation, the slides were washed and mounted in anti-fade solution containing 4´, 6-diamidino-2-phenylindole (DAPI) as a counterstain. Normal male and female DNAs hybridized to normal metaphase preparations served as a negative control, and as a positive control, a previously characterized ovarian cancer cell line (A2780) was used[15].

For each tumor, at least 8 to 10 metaphases were analyzed including only metaphase spreads with high-intensity hybridization and low granularity. Image capturing and quantitative analysis of the ratio of green-to-red fluorescence intensities, representing tumor to normal DNA copy number along each chromosome were done using Leica digital image analysis system (Q-FISH/Q-CGH software package; Leica Microsystems Imaging Solutions Ltd, Cambridge, UK). A chromosomal region was considered to have increased DNA copy number if the average green-to-red fluorescence ratio exceeded the 1.15 threshold (a gain), amplified copy number if the ratio exceeded the 1.5 threshold, and decreased copy number if the ratio was below the 0.85 threshold (a loss). These threshold levels have been based on the average green-to-red fluorescence ratio levels and 95% confidence intervals derived from control experiments using normal-normal (microdissected normal lymphocytes) co-hybridizations. Telomeric and pericentromeric regions were excluded from the analysis. In addition, chromosomes 1p, 16p, 17p, 19, 22, were meticulously analyzed because these regions had been previously found to be prone to false-positive CGH results[16,17]. In each case, the averaged data from 3 repeated experiments was used for the analysis.
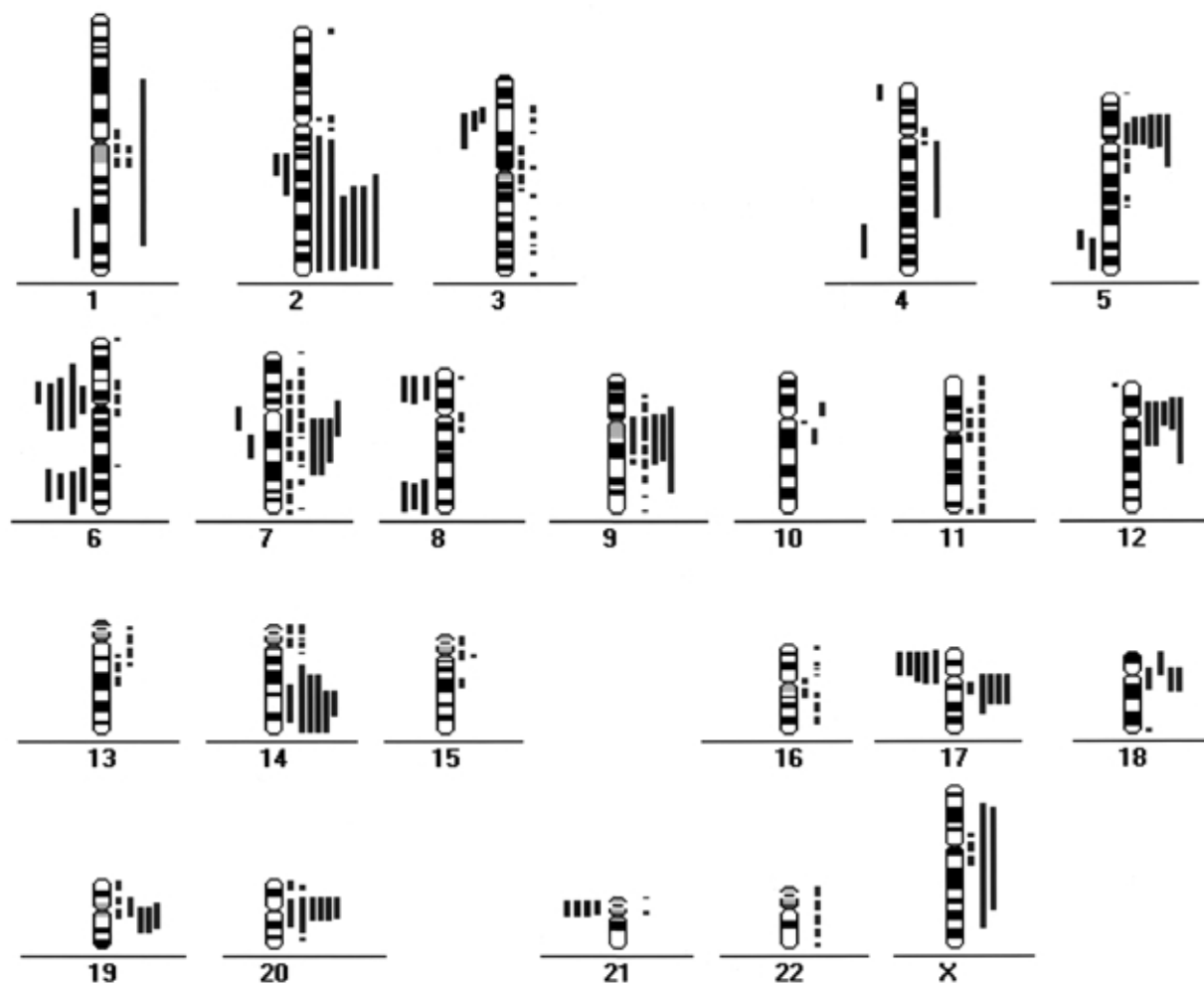
Fig. 2   Summary of the CGH data on the 7 signet-ring cell carcinomas. Gains of the DNA copy number are shown to the right of the chromosome ideograms, and losses are shown to the left. Each line represents a genetic aberration seen in one tumor.

Results

Fig. 1 illustrates an example of epithelial aggregate preparation and subsequent LCM of a signet-ring cell carcinoma lesion. Gastric signet-ring cell carcinoma cells could be microdissected after a rapid cytological staining. A summary of the CGH data on the 7 signet-ring cell carcinomas is depicted in **Fig. 2** Subsequently, **Table 2** shows the DNA copy number alterations. Common chromosomal gains were detected on 20q, 7q, 2q, 5p, 14q, each in 6/7 cases, 9q, 17q, 12q, 19q, each in 5/7 cases, and 18p in 4/7 cases. Frequent losses were observed on 6p, 17p, each in 5/7 cases, 6q, 21p, each in 4/7 cases, and 3p, 8p, and 8q, each in 3/7 cases. Minimal

overlapping regions for gain were assigned to 2q37, 5p13, 7q11.2, 9q12–21, 12q12, 14q24, 17q21, 18p11, 19q13 and 20q11.2, whereas minimal overlapping regions for loss were assigned to 3p21–26, 6p12, 6q25–26, 8p21–23, 8q24.2, 17p13, and 21p13. The common regions of chromosome 7 gain were 7q11.2 (5/7 cases) and 7q35–36 (4/7 cases).

Discussion

In the present CGH study, microdissected primary gastric signet-ring cell carcinoma cells were screened for DNA sequence copy number changes in an **in vivo** state. Since signet-ring cell carcinoma is attributed to adenocarcinomas containing a predominant component with signet-ring cell morphology, LCM

Table 2   DNA copy number changes in gastric signet-ring cell carcinomas

Patient		Copy number changes
1	Gain:	1p13-35, 2p15-25, **2q11.2-23**, 2q31-36, **5p13**, 6p12, 6q12, 7p22, **7q11.2, 7q21-22, 7q35, 9q12-31**, 11p12, 11q12-25, 12p13, **12q12-13, 14q24**, 15q21-26, 16q21, **17q21, 18p11.1-q11.3**, 18q12-23, 19p12, **20q11.2**, 21p13, 22p13-q11.2, 22q13, X
	Loss:	**3p21-26**, 3q13.1-22, 4p14-16, 4q21-35, **6p23, 6q24-27, 8p21-23, 8q11.2**, 13q14-34, 16q23, **17p12-25**, 20q13, **21q22**
2	Gain:	2p22-24, **2q14.1-37**, 4q13.5, **5p12**, 6p21.1-24, 7p22, **7q11.2-36**, 8p12, 12p12-13, **14q13-24**, 15q11.2-26, 16p13.2, 16q11.2-21, 17p11.2, **17q21-25, 18p11.1, 19q12**, 20p11.2, **20q12-13.3**, X
	Loss:	2q12-34, 4p15, 4q32, **6p12-25**, 7q12-15, 7q21-34, **8p22, 8q24**, 10p14, 10q21-26, 14q11.2-23, 16q22, **17p13**, 19q13, 21q21-22
3	Gain:	1p21-34.3, 1q22, 2p16, 3p12, 4p13, 4q21, **5p13**, 5q11.2-23, 6q14-22, **7q11.2-22**, 8q24, 10p12, 10q11.2-26, 11q22, **14q21-31**, 16p11.2, **17q24-25**, 18q22, 19p13.1-13, Xp22.2, Xq13-28
	Loss:	2p11.2, 2p22-25, **3p13, 6p12-25, 6q26**, 9q21-34, 11q13-24
4	Gain:	1p36.2-31, 1q33, 2p12-25, **2q12-37**, 4p16, **5p11**, 6p12, 7p11.2-13, **7q36**, 8q11.2, **9q12-34**, 10p12, 11p12, **12q12-24.3, 14q21-32**, 15p13, 17p12-13, **18p11.1-12**, 18q21, **19q12**, 20p13, **20q11.2-13, 21p13**, X
	Loss:	**3p12-26**, 3q13.3-29, 4q34, 5q15.2-34, **6q12.2**, 7p21, 7q21, **8q13-23**, 10p14, 13p13, 13q11-33, 15q24, **21p12**
5	Gain:	2p12-25, **2q22-37**, 2p12-26, 4q21-34, **6q14, 7q12-36**, 8q12-24.2, **9p12, 12q12**, 13q34, 14p11.2, **14q21**, 15q21, 16p22-24, 17p13, **17q21-25, 18q21, 19q13.4, 20q11.1-13.3**, 21p11.1-q22, Xp11.4
	Loss:	1p36.3, 1q32-44, 4p15.3, **17p13, 21p13**
6	Gain:	1p13-31, 1q21-25, 1q32-43, **2q31-37**, 3p21, 3q12-13.3, 4p12, 4q25, **5p13**, 6q12-27, 7p25, 8q11, 9p12-24, **9q12-34**, 10q23, 11q22-25, **12q12-22**, 13p13-q34, 14p13, **14q32**, 15q22, 16q11.2-12.1, 17p11.2, **18p11.2, 19q13, 20p-q11.2**
	Loss:	1p12, 1q12, 2p12-23, 2q13-37, 3q21-24, 4p14-16, 4q25.5, 5p15, **6p11.2**, 7p12-22, 7q31, **8p12-q24.3**, 9q22-34, 10p13-15, 10q24-26, 11q23-25, 12p12-23, 13p13-q14, 15p-q26, 16p13.3-24, **17p13**, 19p13.1, **21p13-q22**
7	Gain:	2p12-25, **2q12, 2q33-37**, 3p12, 4p15, 4q12-18, **5p13**, 5q11.2-35, 6p22, 6q14, **7q11.2-31**, 8q12, **9q21-34**, 11p14, **12q12.1**, 13q21, **17q11.2-31, 19q13.1-34, 20q11.2**, 21q11.1, 21q22
	Loss:	**3p21, 6p12, 6q25**, 14q21-32, 15p12, **17p13, 21p13**, 22q13

provided a highly effective approach for obtaining a pure cell population. We could identify a variety of chromosomal aberrations in gastric signet-ring cell carcinomas. Frequent chromosomal gain (≧40% of cases) was detected, in order of frequency, on 20q, 7q, 2q, 5p, 14q, 9q, 17q, 12q, 19q, and 18p, and frequent loss (≧40% of cases) was observed, in the same order, on 6p, 17p, 6q, 21p, 3p, 8p, and 8q.

Several studies have reported changes in DNA copy number in gastric carcinomas[18-21]. However, these studies were not specifically directed at the detection of genetic aberrations in signet-ring cell subtype of gastric carcinoma *per se*. This distinct category of gastric adenocarcinoma has some specific morphologic and clinical features[3-22-23].

CGH studies of cell lines and tumor tissues each carry some inherent problems. As a result of long-term culture, cell lines often undergo genomic alterations such as amplifications or develop subclones with

different genetic profiles. On the other hand, whole tumor tissue contains unwanted interfering cells. These factors may influence the CGH results. With the advent of laser capture microdissection, it is now feasible to isolate specific cell types of interest from tissue sections or cell smears under direct morphological confirmation[11]. We applied this method to obtain pure signet-ring cell carcinoma cells for our analyses. In general approximately 800 to 1,500 signet-ring cell carcinoma cells could be obtained from one preparation. As in our study, DNA copy number gains at 17q and 20q have been detected in previous CGH studies of gastric carcinomas of various histological types including intestinal, diffuse, or mixed types. On the other hand, the losses of chromosomal DNA copy number observed in our study is consistent with previous studies of gastric adenocarcinomas[24], and suggests the presence of candidate tumor suppressor genes involved in gastric

Table 3　Comparison of common DNA copy number aberrations reported in gastric cancers

Reference	No. of cases	Gains	Losses
Koizumi et al 97	33	2q, 3q, 7p, 13q,19q, Yp, Yq	1p, **6p**, 17p
Nessling et al 98	23	20q, 6p, 1q, 11q, 7q, 20p	4q, 5q, 9pter, 20q
Kokkola et al 98	35	20q, 17q, 8q	4q, 18q
Sakakura et al 99	58	1p, 8p, 8q, 11q, 16p, 20p, 20q, Xp, Xq	1p, 3p, 5q, 6q, 9p, 16q, 17p,18q, 19
Koo et al 00	37	2q, 7p, 7q, 8p, 8q, 13q, 17q, 18q, 20p, 20q	17p
Okada et al 00	5 (*cell line*)	7p, 20q, 22q	3p, 5q, 18q
Nakanishi et al 00	47	8q, 16p, 17q, 19q, 20q, 20p	4p, 4q, 5q, 17p,18q
Noguchi et al 00	38	20q, 17q, 15q, 12q, 11q	4q, 13q
Present study	7 (*signet-ring cell type*)	2q, 5p, 7q, 9q, 12q, 14q, 17q, 18p, 19q, 20q	3p, **6p**, 6q, 8p, 8q, 17p, 21p

signet-ring cell carcinogenesis on chromosomes 2q, 3p, 6p, 6q, 8p, 8q, 17p, and 21p. Deletions on 3p have been reported in a variety of malignancies including renal, breast, and gastric cancers[25-27]. In gastric cancers, 46% loss at 3p14 was found[27]. The *FHIT* gene is known to reside at 3p14 and its loss of expression has been observed in a majority of gastric carcinomas[28].

We found some losses at 3p26. As a representative gene, the *Von Hippel Landau* gene is an established tumor suppressor gene at 3p25-26. Chromosome 8p arm has been suggested to harbor genes suppressing tumor progression or metastasis, and has shown significant loss of heterozygosity in different cancers including gastric adenocarcinoma[24,29-31]. In addition, in a detailed analysis of allelic loss at 8p21-22 performed on gastric cancers, 44% of tumors showed allelic loss for at least one marker at 8p21-22[28,] indicating that 8p22 deletion is a frequent event in gastric cancer. Loss of 8p21, 8p22, and 8p23 observed in our study is in accord with these findings.

Frequent loss of DNA copy number at a chromosomal region has been interpreted as evidence that the region might harbor a tumor suppressor gene that has been inactivated during malignant transformation[32]. Some of the losses of DNA copy number observed in our series 2q, 3p, 6p, 8p, 8q, 17p and 21p corresponded to the results of previous CGH, LOH, and cytogenetic studies of gastric cancers[33] and others such as loss of 6p provided new abnormalities of potential relevance to gastric signet-ring cell carcinomas. We performed a review of the existing literatures on CGH analysis of gastric carcinomas and found that loss of 6p was rarely observed in these tumors (**Table 3**). In fact, in the only study in which loss of 6p was detected, nearly half of the cases were more or less associated with the signet-ring cell carcinoma component[34]. In addition the loss of 6p was predominantly observed in the undifferentiated tumor type. Chromosomal arm 6p contains several genes with known function such as endothelin-1, interleukin-17F, and the tumor necrosis factor receptor superfamily, member 21. Allelic deletions on the 6p are one of the common, possibly early, genetic changes that occur in the pathogenesis of cervical carcinoma[38]. Recent studies in cervical carcinoma have also indicated the presence of at least two putative tumor suppressor genes on 6p[39].

Deletion at 6q is known as a reletively frequent cytogenetic aberration in gastric cancers. However, no significant loss of heterozygosity on 6p in gastric cancers has so far been found; thus our finding of frequent losses on 6p chromosomal arm appears novel for gastric signet-ring cell carcinoma. Subsequently, this region may harbor genes involved in the multistep process of signet-ring cell carcinogenesis.

In conclusion, the results of our CGH analysis in gastric signet-ring cell carcinoma suggest that inactivation of putative genes located on 6p chromosomal arm may be an important event in the development of this tumor type.

References

1. Whelan SL, Parkin DM, Masuyer E:Trends in cancer incidence and mortality. Lyon: IARC Scientific, 1993.

2. Theuer CP, Nastanski F, Brewster WR, Butler JA, Anton-Culver H:Signet-ring cell histology is associated with unique clinical features but does not affect gastric cancer survival. Am surg 1999; 65: 915–921.

3. Maehara Y, Sakaguchi Y, Moriguchi S, et al: Signet ring cell carcinoma of the stomach. Cancer 1992; 69: 1645–1650.

4. Sano T, Tsujino T, Yoshida K, Nakayama K, Haruma K, Ito H, Nakamura Y, Kajiyama G, Tahara E: Frequent loss of heterozygosity on chromosomes 1q, 5q, and 17q human gastric adenocarcinomas. Cancer Res 1991; 51: 2926–2931.

5. Tamura G, Ogasawara S, Nishizuka S, Sakata K, Maesawa C, Suzuki Y, Terashima M, Saito K, Satodate R: Two distinct regions of deletion on the long arm of chromosome 5 in differentiated adenocarcinomas of the stomach. Cancer Res 1996; 56: 612–615.

6. Kastury K, Baffa R, Druck T, Cotticelli MG, Inoue H, Massimo N, Rugge M, Huang D, Croce CM, Palazzo J, Huebner K: Potential gasterointestinal tumor suppressor locus at the 3p14.2 FRA36b site identified by homozygous deletions in tumor cell lines. Cancer Res 1996; 56: 978–983.

7. Hollstein MC, Sidransky D, Vogelstein B, Harris CC: p53 mutations in human cancers. Science(Washington DC) 1991; 253: 49–50.

8. Kuniyasu H, Yasui W, Kitadai Y, Yokozaki H, Io H, Tahara E: Frequent amplification of the c-met gene in scirrhous type stomach cancer. Biochem Biophys Res Commun 1992; 189: 227–232.

9. Tahara E: Molecular mechanism of stomach carcinogenesis. J Cancer Res Clin Oncol 1993; 119: 265–272.

10. Tsujino T, Yoshida K, Nakayama H, Ito H, Shimosato T, Tahara E: Alterations of oncogenes in metastatic tumors of human gastric carcinomas. Brit J Cancer 1990; 62: 226–230.

11. Simone NL, Bonner RF, Gillespie JW, Emmert-Buck MR, Liotta LA: Laser-capture microdissection: Openning the microscopic frontier to molecular analysis. Trends Genet 1998; 14: 272–276.

12. Maitra A, Wistuba II, Virmani AK, Sakaguchi M, Park I, Stucky A, Milchgrub S, Gibbons D, Minna JD, Gazdar AF:Enrichment of epithelial cells for molecular studies. Nat Med 1999; 5 : 459–463.

13. Japanese Gastric Cancer Association: Japanese Classification of Gastric Carcinoma (2 nd English Edition). Gastric Cancer 1998; 1 : 10–24.

14. Kallioniemi A, Kallioniemi O-P, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992; 258: 818–821.

15. Arai R, Ghazizadeh M, Kawanami O : Comparative genomic hybridization analysis of cisplatin-resistant ovarian carcinoma cells. J Nippon Med Sch 2000; 67: 416–417.

16. Kallioniemi O-P, Kallioniemi A, Piper J, et al:Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 1994; 10: 231–243.

17. Isola JI, Kallioniemi O-P, Chu LW, et al: Genetic abberations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. Am J Pathol 1995; 147: 905–911.

18. Kokkola A, Monni O, Puolakkainen P, Larramendy ML, Victorzon M, Nordling S, Haapiainen R, Kivilaakso E, Knuutila S: 17q12–21 amplicon, a novel recurrent genetic change in intestinal type of carcinoma: a comparative genomic hybridization study. Gene Chromosome Cancer 1997; 20: 38–43.

19. Nessling M, Solinas-Toldo S, Wilgenbus KK, Barchard F, Lichter P: Mapping of chromosomal imbalances in gastric adenocarcinoma revealed amplified protooncogene MYCN, MET, WNT2 and erbB-2. Gene Chromosomes Cancer 1998; 23: 307—316.

20. Vidgren V, Varis A, Kokkola A, Monni, Puolakkainen P, Nordling S, Forozan F, Kallioniemi A, Vakkari ML, Kivilaakso E, Knuutila S O, et al: Concomitant gastrin and ERBB 2 gene amplification at 17q12-q21 in intestinal type gastric cancer. Gene Chromosomes Cancer 1999; 24: 24–29.

21. Noguchi T, Wirtz HC, Michaelis S, Gabbert HE, Muller W:Chromosomal imbalances in gastric cancer: correlation with histologic subtypes and tumor progression. Am J Clin Pathol 2000; 115: 828–834.

22. Kim JP, Kim SC, Yang HK: Prognostic significance of signet ring cell carcinoma of the stomach. Surg Oncol 1994; 3: 221–227.

23. Otsuji E, Yamaguchi t, Sawai K, Takahashi T : Characterization of signet ring cell carcinoma of the stomach. J Surg Oncol 1998 ; 67: 216–220.

24. Yustein AS, Harper JC, Petroni GR, Cummings OW, Moskaluk CA, Powell SM: Allelotype of gastric adenocarcinoma. Cancer Res 1999; 59: 1437–1441.

25. Chudek J, Wilhem M, Bugert P, Herbers J, Kovacs G: Detailed microsattelite analysis of chromosome 3p region in non-papillary renal cell carcinomas. Int J Cancer 1997; 73: 225–229.

26. Matsumoto S, Kasumi F, Sakamoto G, Ondo M, Nakamura Y, Emi M: Detailed deletion mapping of chromosome arm 3p in breast cancers: a 2cM region on 3p14.3–21.2 and a 5cM region on 3p24.3–25.1 commonly deleted in tumors. Genes Chromosomes Cancer 1997; 20: 268–274.

27. Kastury K, Baffa R, Druck T, Cotticelli MG, Inoue H, Massimo N, Rugge M, Huang D, Croce CM, Palazzo J, and Huebner K: Potential gasterointestinal tumor suppressor locus at the 3p14.2 FRA36b site identified by homozygous deletions in tumor cell lines. Cancer

Res 1996; 56: 978–983.

28. Baffa R, Veronese ML, Santoro R, Mandes B, Palazzo JP, Rugge M, Santoro E, Croce CM, Huebner K: Loss of FH1T expression in gastric carcinoma. Cancer Res 1998; 58: 4708–4714.

29. Takle L, Knowles M: Deletion mapping implicates two tumor suppressor genes on chromosome 8p in the development of bladder cancer. Oncogene 1996; 12: 1083–1087.

30. Vocke C, Pozzatti R, Bostwick D, Florence C, Jennings S, Strup S, Duray P, Liotta L, Emmert-Buck M, Mineham W: Analysis of 99 microdissected prostate carcinomas reveals a high frequency of allelic loss on chromosome 8p12–21. Cancer Res 1996; 56: 2411–2416.

31. Ichikawa T, Nihei N, Kuramochi H, Kawana Y, Killary A, Rinker-Schaeffer C, Barrett J, Isaacs J, Kugoh H, Oshimura M, Shimazaki J: Metastasis suppressor genes for prostate cancer. Prostate 1996; 6 (Suppl): 31–35.

32. Knudson AG: Hereditary cancer, oncogenes, and antioncogenes. Cancer Res 1985; 45: 1437–1443.

33. Tzeng CC, Meng CL, Jin L, Hsieh HF: Cytogenetic studies of gastric adenocarcinoma. Cancer Genet Cytogenet 1991; 55: 67–71.

34. Koizumi Y, Tanaka SI, Mou R, Koganei H, Kokawa A, Kitamura R, Yamauchi H, Ookubo K, Saito T, Tominaga S, Matsumura K, Shimada H, Tsuchida N, Sekihara H: Changes in DNA copy number in primary gastric carcinomas by comparative genomic hybridization. Clin Cancer Res 1997; 3 : 1067–1076.

35. Koo SH, Kwon KC, Shin AY, Jeon YM, Park JW, Kim SH, Noh SM: Genetic alterations of gastric cancer: comparative genomic hybridization and fluorescence in situ hybridization studies. Cancer Genet Cytogenet 1997; 117: 97–103.

36. Sakakura C, Mori T, Sakabe T, Ariyama Y, Shinomiya T, Date K, Hagiwara A, Yamaguchi Y, Takahashi T, Nakamura Y, Abe T, Inazawa J: Gains, losses, and amplifications of genomic materials in primary gastric cancers analyzed by comparative genomic hybridization. Genes. Cromosomes Cancer 1999; 24: 299–305.

37. Okada K, Sugihara H, Bamba M, Bamba T, Hattori T: Sequential numerical changes of chromosomes 7 and 18 in diffuse-type stomach cancer cell lines: combined comparative genomic hybridization, fluorescence in situ hybridization, and ploidy analyses. Cancer Genet Cytogenet 2000; 118: 99–107.

38. Karsemaekers AM, van de Vijver MJ, Kenter GG, Fleuren GJ: Genetic alterations during the progression of squamous cell carcinomas of the urterine cervix. Genes Chromosomes Cancer 1999; 26: 346–354.

39. Chatterjee A, Pulido HA, Koul S, Beleno N, Perilla A, Posso H, Manusukami M, Murty VVVS: Mapping the sites of putative tumor suppressor genes at 6p25 and 6p21. 3 in cervical carcinoma: occurrence of allelic deletions in precancerous lesions. Cancer Res 2001; 61: 2119–2123.