ANTICANCER RESEARCH *26*: 2909-2914 (2006)

C-MYC Locus Amplification as Metastasis Predictor in Intestinal-type Gastric Adenocarcinomas: CGH Study in Brazil

ROMMEL RODRÍGUEZ BURBANO[1,2], PAULO PIMENTEL ASSUMPÇÃO[3], MARIANA FERREIRA LEAL[2], DANIELLE QUEIROZ CALCAGNO[1], ADRIANA COSTA GUIMARÃES[1], ANDRÉ SALIM KHAYAT[1], SYLVIA SATOMI TAKENO[2], ELIZABETH SUCHI CHEN[2] and MARÍLIA DE ARRUDA CARDOSO SMITH[2]

[1]*Human Cytogenetics and Toxicological Genetics Laboratory, Department of Biology, Center of Biological Sciences, Federal University of Pará, Belém, PA;*
[2]*Genetics Division, Department of Morphology, Federal University of São Paulo, São Paulo, SP;*
[3]*Department of Pathology and Surgery Service 4, João de Barros Barreto University Hospital, Federal University of Pará, Belém, PA, Brazil*

Abstract. *Background: The genetic events involved in gastric cancer, the third most frequent cancer in the world with a high incidence in Pará State, Brazil, remain largely unknown. Materials and Methods: Twenty-one primary gastric adenocarcinomas were investigated by comparative genomic hybridization (CGH) and the relationships between genomic abnormalities and histopathological features were evaluated. Results: Eighty-one percent of cases presented DNA copy-number changes. Chromosomal gains were the most frequent finding, losses occurring only in the diffuse type. The main copy-number gains were on chromosome 8, principally on 8q24.1 (8/21 cases), 8p21 (3/21) and 8p23.2-8p12 (2/21). Gain of region 8q24.1, where C-MYC is located, was the main finding, exclusively in the intestinal type with metastasis. Conclusion: C-MYC locus amplification may be predictor of aggressiveness in intestinal-type gastric cancer, playing an important role in its development and progression. Moreover, other genes on 8q24 should be investigated. Gastric adenocarcinomas of differing histopathological features were associated with distinct genetic alterations, supporting the hypothesis that they evolve through distinct genetic pathways.*

Correspondence to: Rommel Rodríguez Burbano, Laboratório de Citogenética Humana e Genética Toxicológica, Departamento de Biologia, Centro de Ciências Biológicas, Universidade Federal do Pará, Campus Universitário do Guamá, Av. Augusto Correa, 01, CEP 66075-900, Belém, Pará, Brazil. Tel: (091)211 17 27 / (091) 242 60 26, Fax: (091) 211 16 01, e-mail: rommel@ufpa.br

Key Words: Gastric cancer, comparative genomic hybridization, 8q24.

Gastric cancer is the third most frequent cancer type (1) and the second most important cause of cancer deaths in the world (2). In northern Brazil, Pará State presents a high incidence of this neoplasia and its capital, Belém, was ranked eleventh in number of gastric cancers per inhabitant among all cities in the world with cancer records (2). Food factors may be related to the high incidence of this neoplasia in Pará, especially the considerable consumption of salt-conserved food, reduced use of refrigerators and the low consumption of fresh fruit and vegetables (3).

The molecular events in gastric carcinogenesis remain largely unknown. Carcinogenesis is widely regarded as a multistep process involving the accumulation of genetic alterations in cellular oncogenes, tumor-suppressor genes, cell cycle regulators and DNA repair genes (4).

Comparative genomic hybridization (CGH) enables the detection of genetic imbalances and for chromosomal map determination of chromosome or chromosomal subregion gains and losses in relation to the normal reference metaphase (5). Importantly, CGH has facilitated the identification of consistent sites of chromosomal imbalance in a wide variety of solid tumors (6).

Many chromosomal alterations have been identified by CGH in gastric cancer, but there are significant discrepancies among the reported results. In several studies, gains on 1p, 3p, 3q, 5p, 6p, 7p, 7q, 8p, 8q, 11q, 12q, 13q, 16p, 17q, 20q and 22q and deletions on 1p, 3p, 4p, 4q, 5q, 6p, 9p, 15q, 16q, 17p, 18q, 19p, 20q and 21q were observed in gastric cancer (7-23).

In the present investigation, a CGH study was carried out on 21 samples of primary gastric adenocarcinoma to define the pattern of chromosomal imbalance and to compare the results with clinical and histopathological data.

Materials and Methods

Tissues specimens and DNA extraction. Surgical samples of primary tumors were obtained from 21 patients at Pará State João de Barros Barreto University Hospital (HUJBB), Brazil. Patient ethnicity, age and tumor anatomical sites were obtained from the tumor registries. The study cohort comprised males in the age range 42 to 71 years (mean age, 56.5 years). These patients had never undergone chemotherapy or radiotherapy prior to surgery, nor had they any other diagnosed cancer. The Ethics Committee of HUJBB approved this genetic study.

The tumor samples were collected immediately after surgical resection, frozen in liquid nitrogen and stored at –80˚C prior to DNA extraction. The DNA was isolated by digestion with proteinase K (2 μg/ml), followed by phenol-chloroform extraction. The tumors were classified according to the TNM classification of the International Union Against Cancer (UICC) criteria (24) and the Lauren classification (25).

Comparative genomic hybridization. Metaphase chromosomes were prepared using phytohemaglutinin-stimulated peripheral blood lymphocytes from a subject with a normal karyotype (26). The slides were 3 to 5 days old prior to treatment with pepsin (20 μg/ml) in 0.01M HCl at 37˚C. Before hybridization, normal lymphocyte metaphases were denatured at 72˚C in 70% formamide/2xSSC and dehydrated in ethanol series. Tumor and reference DNA were labeled by nick translation using biotin-14-dATP (GIBCO BRL, USA) and digoxigenin-11-dUTP (Boehringer, Mannheim, Germany), respectively. The nick-translation reaction was stopped by heating at 70˚C for 15 min. The CGH procedure was similar to published standard protocols (5). Only strong uniform hybridization metaphases were included in the analysis. A minimum of ten metaphases per specimen were analyzed using a Zeiss Axiophot fluorescence microscope (Zeiss, Oberkochen, Germany) with single band pass excited filters for UV/FITC (490 nm), DAPI (360 nm) and rhodamine (570 nm), and were analyzed using the ISIS digital image analysis system (MetaSystems, Altlussheim, Germany). At least ten representative metaphases were fully analyzed and combined to produce an amplification-deletion map for each chromosome.

An internal control with differentially labeled normal DNA was performed and 15 successfully hybridized metaphases were analyzed. In cases of no gains or losses, the green-to-red fluorescence ratio (FR) was 1. Gains in a tumor were detected as an increased FR, whereas losses were observed as a decreased ratio. Chromosomal regions were interpreted as over-represented (gains) when the ratio exceeded 1.20 and under-represented (losses) when the ratio was less than 0.80. When the ratio exceeded 1.5 it was considered to be a highly amplified region. All findings were confirmed with a confidence interval of 99%. Repetitive heterocromatic, telomeric and subtelomeric regions were excluded from the analysis.

Results

Copy number changes in the DNA sequences were detected in 81% of the 21 primary gastric adenocarcinomas. The CGH analysis demonstrated that chromosomal gains were more common than losses (Table I). A schematic summary of the CGH imbalances detected is shown in Figure 1. Normal profiles were seen in only four gastric cancer (cases 10, 12, 20 and 21). Copy number gains were frequently detected along chromosome 8, mainly on 8q24.1 (by just the position of different segments in 8/21 cases), 8p21 (3/21) and 8p23.2-8p12 (2/21). A gain on 14q31 was observed in two cases. Losses on 2q12-q14.3, 2q32.2-q33, 5q35.2, 7p21 9p23-p12, 9q13-q34.1, 16p13.2, 17q21.3-q24, 18q11.2, 20p11.2 and 20q13.2 were found in two patients for each region. In one case, copy number changes varied across the genome. The most frequently observed chromosomal aberration involved chromosome 8 (Figure 1). Over-representation of 8q24.1-q24.2 was found in three tumors (cases 11, 15, 18) and five other cases (1, 2, 4, 5 and 7) showed an increased copy number in the same region. The relationships between the CGH results and clinical and histological parameters were also investigated (Table I).

Discussion

Both environmental and genetic factors contribute to the occurrence of gastric cancer (27). CGH analysis of solid tumors revealed a number of recurrent copy number aberrations that had not been detected previously by any other technique (28). Cytogenetic changes are found in the majority of gastric carcinomas and may lead to increased or decreased gene expression. However, it is still unclear whether these abnormalities are the cause or consequence of the malignant phenotype of gastric carcinoma (29).

It is likely that these chromosomal aberrations reflect selective retention of genomic fragments housing "driver" genes, the products of which functionally contribute to gastric carcinogenesis (30). Many groups have tried to identify cytogenetic alterations that could be related to clinical and histological parameters, aiming to use the data with diagnostic, prognostic or even therapeutic intent. Our team had previously found chromosomal aberrations in gastric cancer by conventional cytogenetics and the FISH technique, with the goal of establishing a possible clinical application based on differences in aggressiveness, histological data or patient outcome (31, 32).

From the CGH analysis of 21 primary gastric adenocarcinomas, originating from patients living in a high incidence area in Brazil, some important differences between intestinal and diffuse types (according to Lauren's classification), as well as some cytogenetic alterations related to specific stages and patterns of numerical aberrations, were revealed. A very interesting finding in our analysis was the exclusive pattern of gains and losses. Each sample presented either one or the other, suggesting that tumors evolve in two different ways by either losing or gaining DNA (33).

We also identified many differences among the histological types and staging groups. Numerical aberrations

Table I. *Histological diagnosis and DNA copy number changes in 21 cases of gastric adenocarcinomas.*

Case	Age	Location	Lauren[a]	UICC[b]/AJCC[c]	Gains	Losses
1	47	Antrum	Diffuse	T3N0M0	–	2q12-q14.3, 2q32.2-q33, 9p22-p12, 9q13-q34.1, 14q11.2-q24.2, 5q31.1-q31.3, 12p13.2-p11.2, 12q14, 17p12-p11.2, 17q21.3-q24, 18q11.2-q12.1
2	59	Antrum	Intestinal	T4N1M0	11p15.4-p15.3	–
3	57	Antrum	Diffuse	T3N2M0	–	5q35.2
4	71	Antrum/body	Intestinal	T3N2M1	2p23.1-p22.2, 6q16.2-qter, 8q13-qter	–
5	47	Antrum	Intestinal	T3N2M0	16p12, 19p13.1	–
6	51	Body	Intestinal	T3N2M0	6p21.1, 10q23.1-q26.2, 14q31-q32.2, 16p13	–
7	55	Antrum/body	Diffuse	T3N2M1	–	1p22.3, 1q43, 2p25.2-p12, 2q12-q36, 3p25-p12, 3q25.1-q28, 5p15.2-p12, 5q11-q35.2, 7p21-p12, 7q11.22-q31.2, 9p23-p12, 9q13-q34.2, 11p14-p11.12, 11q13.2-q14.1, 13q12.1-q33, 16p13.2-p11.2, 16q12.1-q23, 17q21.1-q24, 18q12.1-q21.2, 20p12-p11.2, 20q11.2-q13.2
8	50	Cardia	Diffuse	T3N1M0	7q21.3	–
9	41	Antrum	Intestinal	T3N2M1	2q31, 8q24.1, 19q13.1-q13.2	–
10	62	Antrum	Diffuse	T3N1M0	–	–
11	61	Antrum	Intestinal	T3N2M1	8p21, 8q23-q24.2	–
12	54	Antrum	Diffuse	T3N0M0	–	–
13	42	Antrum/body	Intestinal	T3N2M1	7p21, 8p23.2-p11.2, 8q12-q24.2	–
14	49	Antrum	Intestinal	T3N2M1	8q24.1	–
15	45	Antrum	Intestinal	T3N2M1	8q24	–
16	45	Antrum	Diffuse	T3N2M0	–	16p13.2, 20p11.2, 20q13.2
17	54	Antrum/body	Intestinal	T3N2M1	8q21.1-8q24.1	–
18	46	Body	Intestinal	T3N2M1	8p23.2-8p12, 8q24, 14q23-q31, 20p13-p12	–
19	50	Antrum/body/fund	Diffuse	T3N2M1	–	7p21
20	46	Antrum	Diffuse	T3N1M0	–	–
21	60	Antrum/body	Diffuse	T3N2M0	–	–
Control	37	Lymphocytes			–	–

[a]Lauren classification; [b]International Union Against Cancer classification; [c]American Joint Committee on Cancer.

were detected in every intestinal-type tumor. Diffuse tumors, which nonetheless presented fewer numerical aberrations, were the only histological type where losses were detected (five cases). Four patients with diffuse tumors without systemic metastasis (M0) had no detected chromosomal imbalances. Each case of intestinal-type adenocarcinoma had chromosomal gains, but none showed DNA losses. The DNA gain patterns in intestinal tumors varied according to the TNM/UICC classification (Table I).

It is particularly noteworthy that the gains at region 8q24.1, where the *C-MYC* oncogene is located, were identified exclusively and in every case of intestinal-type adenocarcinoma with systemic metastasis (M1). Reports from CGH studies included gains on 8q in approximately 18-56% of cases (4, 13, 34, 35). In a CGH-array study, Takada *et al.* (23) reported a high level of amplification on 8q24.1 in six poorly-differentiated gastric adenocarcinoma cell lines.

We detected a high amplification of 8q24.1-q24.2 in three tumors (cases 11, 15, 18), all of them intestinal-type with M1 metastasis. The overexpression of MYC is associated with over 50% of human cancers and is frequently related to a worse prognosis and a more invasive phenotype. MYC has been shown to contribute to tumorigenesis by inducing unrestrained cellular growth, proliferation, angiogenesis and genomic instability (36, 37).

The constitutive expression of *C-MYC* due to chromosomal translocation, mutation or amplification contributes to the development and progression of many cancers (12). Additionally, *C-MYC* induces incorrect replication initiation, DNA breakage, alterations of DNA repair, point mutations and causes remodeling of the 3D nuclear organization of telomeres and chromosomes, thus creating topological conditions that initiate genomic instability (38).

The enhanced expression of the MYC protein contributes to almost every aspect of tumor cell biology. Whereas the ability of MYC to drive unrestricted cell proliferation and to inhibit cell differentiation has long been recognized, a

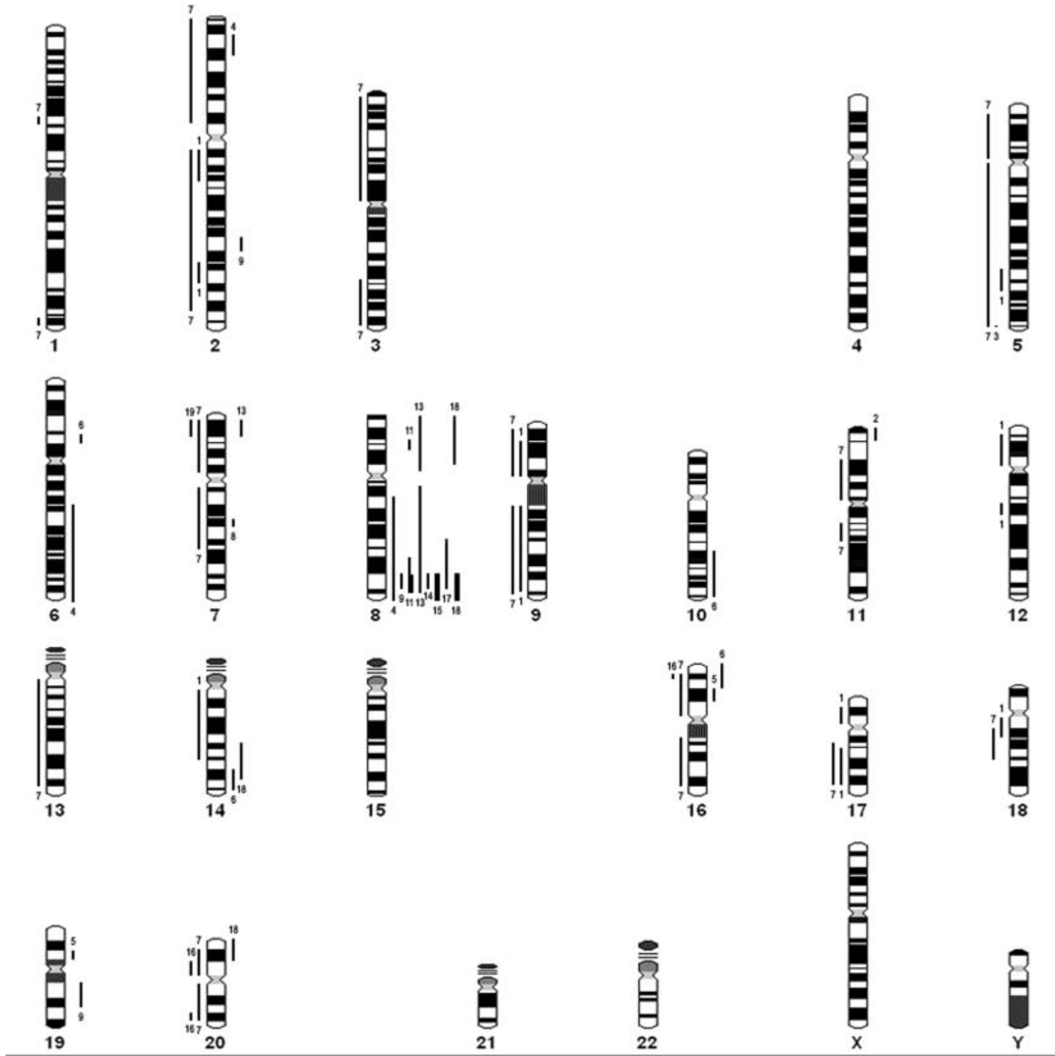

Figure 1. *Summary of the chromosomal alterations determined. Bars to the right of the chromosomes indicate gains. Bars to the left indicate losses. High levels of amplification are shown with bold lines. The numbers on the bars indicate the case study.*

recent work indicated that deregulated expression of MYC can drive cell growth and angiogenesis, reduce cell adhesion and promote metastasis and genomic instability. Conversely, the loss of the MYC protein not only inhibits cell proliferation and cell growth, but can also accelerate differentiation, increase cell adhesion and lead to an excessive response to DNA damage. Results in animal models suggest that MYC may be a target for the treatment of human cancer, but it is still unknown whether such drugs will be useful (39).

The *C-MYC* oncogene seems to be fundamental in the carcinogenesis process. *C-MYC* locus amplification may be a predictor of aggressiveness and poor outcome in intestinal-type gastric cancer and might play an important role in the development and progression of this neoplasia.

We previously demonstrated *C-MYC* amplification in intestinal-type adenocarcinoma by the FISH technique, as well as translocation of this gene in the diffuse-type. The highest amplification level, including the occurrence of double minutes, were also shown in intestinal-type gastric cancer with systemic metastasis (32), supporting our CGH. These results should provide useful information for the development of more accurate strategies in the management of gastric cancer. Moreover, some genes, other than *C-MYC*, on 8q24 should be investigated.

Other regions on chromosome 8 were also amplified, such as 8p21 (3/21) and 8p23.2-8p12 (2/21). These alterations suggest that the presence of trisomy 8 is a common biological phenomenon in adenocarcinoma of the stomach (32, 40, 41).

Several CGH studies identified the 20q region as the most frequent site of DNA gain in gastric cancer (10, 13, 14, 17, 28, 42-48). Among our samples, this or other cytogenetic abnormalities referred to could not be found, perhaps due to regional differences or to the limited sample number.

A gain on 7q31 was observed in the single case of cardiac cancer that we analyzed. The expression of the *C-MET* gene, located on 7q31, was associated with clinical stage and/or prognosis in gastric cancer (4). Other genetic aberrations occurred only in a few cases and could not be related to any specific clinical or pathological pattern, thus requiring further investigation in additional tumors.

Based on our results, we affirm that gastric adenocarcinomas of differing histopathological features are associated with distinct patterns of genetic alterations, suggesting their evolution through different genetic pathways.

Acknowledgements

This work was supported by Financiadora de Estudos e Projetos (FINEP CT-INFRA/FADESP) grant No. 0927-03 and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grant 2003/06540-5; R.R.B. had a post-doctorate fellowship (number 151127/2002-6) granted by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

References

1 Instituto Nacional do Câncer (INCA): Estimativa 2005: incidência de câncer no Brasil. Rio de Janeiro, INCA, pp. 33, 2004.

2 Cancer Databases and Other Resourcess: International Agency for Research on Cancer (IARC) page. [http://www.iarc.fr]. Accessed January 28, 2005.

3 Moutinho V: Epidemiologic aspects of gastric cancer in Belém. *ABCD*: Arquivos Brasileiros de Cirurgia Digestiva *2*: 204-213, 1986.

4 Kimura Y, Noguchi T, Kawahara K, Kashima K, Daa T and Yokoyama S: Genetic alterations in 102 primary gastric cancers by comparative genomic hybridization: gain of 20q and loss of 18q are associated with tumor progression. Mod Pathol *17(11)*: 1328-1337, 2004.

5 Kallioniemi A, Kallioniemi O-P, Sudar D, Rutovitz D, Gray JW, Waldman F and Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science *258(5083)*: 818-821, 1992.

6 Du Manoir S, Speicher MR, Joos S, Schroeck E, Popp S, Doehner H, Kovacs G, Robert-Nicoud M, Lichter P and Cremer T: Detection of complete and partial chromosome gains and losses by comparative genomic *in situ* hybridization. Hum Genet *90(6)*: 590-610, 1993.

7 Sakakura C, Mori T, Sakabe T, Ariyama Y, Shinomiya T, Date K, Hagiwara A, Yamaguchi T, Takahashi T, Nakamura Y, Abe T and Inazawa J: Gains, losses, and amplifications of genomic materials in primary gastric cancers analyzed by comparative genomic hybridization. Genes Chromosomes Cancer *24(4)*: 299-305, 1999.

8 Okada K, Sugihara H, Bamba M, Bamba T and 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 *15;118(2)*: 99-107, 2000.

9 Kim YH, Kim NG, Lim JG, Park C and Kim H: Chromosomal alterations in paired gastric adenomas and carcinomas. Am J Pathol *158(2)*: 655-662, 2001.

10 Noguchi T, Wirtz HC, Michaelis S, Gabbert HE and Mueller W: Chromosomal imbalances in gastric cancer. Correlation with histologic subtypes and tumor progression. Am J Clin Pathol *115(6)*: 828-834, 2001.

11 Varis A, Puolakkainen P, Savolainen H, Kokkola A, Salo J, Nieminen O, Nordling S and Knuutila S: DNA copy number profiling in esophageal Barrett adenocarcinoma: comparison with gastric adenocarcinoma and esophageal squamous cell carcinoma. Cancer Genet Cytogenet *127(1)*: 53-58, 2001.

12 van Dekken H, Alers JC, Riegman PH, Rosenberg C, Tilanus HW and Vissers K: Molecular cytogenetic evaluation of gastric cardia adenocarcinoma and precursor lesions. Am J Pathol *158(6)*: 1961-1967, 2001.

13 Wu MS, Chang MC, Huang, SP, Tseng CC, Sheu JC, Lin YW, Shun CT, Lin MT and Lin JT: Correlation of histologic subtypes and replication error phenotype with comparative genomic hybridization in gastric cancer. Genes Chromosomes Cancer *30(1)*: 80-86, 2001.

14 Hidaka S, Yasutake T, Kondo M, Takeshita H, Yano H, Haseba M, Tsuji T, Sawai T, Nakagoe T and Tagawa Y: Frequent gains of 20q and losses of 18q are associated with lymph node metastasis in intestinal-type gastric cancer. Anticancer Res *23(4)*: 3353-3357, 2003.

15 Peng DF, Sugihara H, Mukaisho K, Tsubosa Y and Hattori T: Alterations of chromosomal copy number during progression of diffuse-type gastric carcinomas: metaphase- and array-based comparative genomic hybridization analyses of multiple samples from individual tumours. J Pathol *201(3)*: 439-450, 2003.

16 Varis A, Van Rees B, Weterman M, Ristimaki A, Offerhaus J and Knuutila S: DNA copy number changes in young gastric cancer patients with special reference to chromosome 19. Br J Cancer *88(12)*: 1914-1919, 2003.

17 Wang Q, Wang B, Guan X, Gao H, Cheng H, Zhang Q, Huang C, Li P and Fu S: Analysis of comparative genomic hybridization and loss of heterozygosity in 43 primary gastric carcinomas. Chin Med J(Engl) *116(4)*: 517-523, 2003.

18 Weiss MM, Snijders AM, Kuipers EJ, Ylstra B, Pinkel D, Meuwissen SG, Van Diest PJ, Albertson DG and Meijer GA: Determination of amplicon boundaries at 20q13.2 in tissue samples of human gastric adenocarcinomas by high-resolution microarray comparative genomic hybridization. J Pathol *200(3)*: 320-326, 2003.

19 Kimura Y, Noguchi T, Kawahara K, Kashima K, Daa T and Yokoyama S: Genetic alterations in 102 primary gastric cancers by comparative genomic hybridization: gain of 20q and loss of 18q are associated with tumor progression. Mod Pathol *17(11)*: 1328-1337, 2004.

20 Peng DF, Sugihara H, Mukaisho K, Ling ZQ and Hattori T: Genetic lineage of poorly differentiated gastric carcinoma with a tubular component analysed by comparative genomic hybridization. J Pathol *203(4)*: 884-895, 2004.

21 Weiss MM, Kuipers EJ, Postma C, Snijders AM, Pinkel D, Meuwissen SG, Albertson D and Meijer GA: Genomic alterations in primary gastric adenocarcinomas correlate with clinicopathological characteristics and survival. Cell Oncol *26(5-6)*: 307-317, 2004.
22 Morohara K, Nakao K, Tajima Y, Nishino N, Yamazaki K, Kaetsu T, Suzuki S, Tsunoda A, Kawamura M, Aida T, Tachikawa T and Kusano M: Analysis by comparative genomic hybridization of gastric cancer with peritoneal dissemination and/or positive peritoneal cytology. Cancer Genet Cytogenet *161(1)*: 57-62, 2005.
23 Takada H, Imoto I, Tsuda H, Sonoda I, Ichikura T, Mochizuki H, Kanoue T and Inazawa J: Screening of DNA copy-number aberrations in gastric cancer cell lines by array-based comparative genomic hybridization. Cancer Sci *96(2)*: 100-110, 2005.
24 Union Internationale Contre Le Cancer: TNM Classification of Malignant Tumour. New Jersey, Wiley & Sons Inc., 1997.
25 Lauren P: The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Pathol Microbiol Scand *64*: 31-49, 1965.
26 Dracapoli NC: Current Protocols in Human Genetics. New York, Wiley & Sons, 1999.
27 Ponz de Leon M: Genetic predisposition and environmental factors in gastric carcinoma. Rec Results Cancer Res *136*: 179-202, 1994.
28 Kurihara Y, Ghazizadeh M, Bo H, Shimizu H, Kawanami O, Moriyama Y and Onda M: Genome-wide screening of laser capture microdissected gastric signet-ring cell carcinomas. Nippon Med Sch *69(3)*: 235-242, 2002.
29 Stock M and Otto F: Gene deregulation in gastric cancer. Gene *360(1)*: 1-19, 2005.
30 Tay ST, Leong SH, Yu K, Aggarwal A, Tan SY, Lee CH, Wong K, Visvanathan J, Lim D, Wong WK, Soo KC, Kon OL and Tan P: A combined comparative genomic hybridization and expression microarray analysis of gastric cancer reveals novel molecular subtypes. Cancer Res *63(12)*: 3309-3316, 2003.
31 Lima EM, Rissino JD, Harada ML, Assumpcao PP, Demachki S, Guimaraes AC, Casartelli C, Smith MA and Burbano RR: Conventional cytogenetic characterization of a new cell line, ACP01, established from a primary human gastric tumor. Braz J Med Biol Res *37(12)*: 1831-1838, 2004.
32 Calcagno DQ, Leal MF, Taken SS, Assumpcao PP, Demachki S, Smith M de A and Burbano RR: Aneuploidy of chromosome 8 and C-MYC amplification in individuals from northern Brazil with gastric adenocarcinoma. Anticancer Res *25(6B)*: 4069-4074, 2005.
33 Teixeira MR and Heim S: Multiple numerical chromosome aberrations in cancer: what are their causes and what are their consequences? Semin Cancer Biol *15(1)*: 3-12, 2005.
34 Wu CW, Chen GD, Fann CS, Lee AF, Chi CW, Liu JM, Weier U and Chen JY: Clinical implications of chromosomal abnormalities in gastric adenocarcinomas. Genes Chromosomes Cancer *35(3)*: 219-231, 2001.
35 Stocks SC, Pratt N, Sales M, Johnston DA, Thompson AM, Carey FA and Kernohan NM: Chromosomal imbalances in gastric and esophageal adenocarcinoma: specific comparative genomic hybridization-detected abnormalities segregate with junctional adenocarcinomas. Genes Chromosomes Cancer *32(1)*: 50-58, 2001.
36 Ingvarsson S: The myc gene family proteins and their role in transformation and differentiation. Semin Cancer Biol *1(6)*: 359-369, 1990.
37 Arvanitis C and Felsher DW: Conditionally MYC: insights from novel transgenic models. Cancer Lett *226(2)*: 95-99, 2005.
38 Louis SF, Vermolen BJ, Garini Y, Young IT, Guffei A, Lichtensztejn Z, Kuttler F, Chuang TC, Moshir S, Mougey V, Chuang AY, Kerr PD, Fest T, Boukamp P and Mai S: C-Myc induces chromosomal rearrangements through telomere and chromosome remodeling in the interphase nucleus. Proc Natl Acad Sci USA *102(27)*: 9613-9618, 2005.
39 Adhikary S and Eilers M: Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol *6(8)*: 635-645, 2005.
40 Han K, Oh EJ, Kim YS, Kim YG, Lee KY, Kang CS, Kim BK, Kim WI, Shim SI and Kim SM: Chromosomal numerical aberrations in gastric carcinoma: analysis of eighteen cases using *in situ* hybridization. Cancer Genet Cytogenet *92(2)*: 122-129, 1996.
41 Panani AD, Ferti AD, Avgerinos A and Raptis SA: Numerical aberrations of chromosome 8 in gastric cancer detected by fluorescence *in situ* hybridization. Anticancer Res *24(1)*: 155-159, 2004.
42 Kokkola A, Monni O, Puolakkainen P, Nordling S, Haapiainen R, Kivilaakso E and Knuutila S: Presence of high-level DNA copy number gains in gastric carcinoma and severely dysplastic adenomas but not in moderately dysplastic adenomas. Cancer Genet Cytogenet *107(1)*: 32-36, 1998.
43 Nessling M, Solinas-Toldo S, Wilgenbus Kk, Borchard F and Lichter P: Mapping of chromosomal imbalances in gastric adenocarcinoma revealed amplified protooncogenes MYCN, MET, WNT2, and ERBB2. Genes Chromosomes Cancer *23(4)*: 307-316, 1998.
44 Fu S, Wang B, Guan X, Xia J, Lu S, Zhang G and Li P: [Comparative genomic hybridization analysis of primary gastric carcinomas]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi *16(6)*: 353-355, 1999.
45 Koo SH, Kwon KC, Shin SY, Jeon YM, Park JW, Kim SH and Noh SM: Genetic alterations of gastric cancer: comparative genomic hybridization and fluorescence *in situ* hybridization studies. Cancer Genet Cytogenet *117(2)*: 97-103, 2000.
46 Nakanishi M, Sakakura C, Fujita Y, Yasuoka R, Aragane H, Koide K, Hagiwara A, Yamaguchi T, Nakamura Y, Abe T, Inazawa J and Yamagishi H: Genomic alterations in primary gastric cancers analyzed by comparative genomic hybridization and clinicopathological factors. Hepatogastroenterology *47(33)*: 658-662, 2000.
47 Guan XY, Fu SB, Xia JC, Fang Y, Sham JS, Du BD, Zhou H, Lu S, Wang, BQ, Lin YZ, Liang Q, Li XM, Du B, Ning XM, Du JR, Li P and Trent JM: Recurrent chromosome changes in 62 primary gastric carcinomas detected by comparative genomic hybridization. Cancer Genet Cytogenet *123(1)*: 27-34, 2000.
48 Sakakura C, Hagiwara A, Yasuoka R, Fujita Y, Nakanishi M, Masuda K, Shimomura K, Nakamura Y, Inazawa J, Abe T and Yamagishi H: Tumour-amplified kinase BTAK is amplified and overexpressed in gastric cancers with possible involvement in aneuploid formation. Br J Cancer *84(6)*: 824-831, 2001.

Received March 16, 2006
Accepted April 11, 2006