Chromosomal aberrations detected by comparative genomic hybridization predict outcome in patients with colorectal carcinoma

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Abstract. To obtain comprehensive information regarding the correlation between genomic changes and clinicopathological parameters such as disease stage, metastases, and survival, we investigated genomic changes by comparative genomic hybridization (CGH) in 73 patients with colorectal cancer (CRC), and assessed the associations of such charges with clinicopathological parameters. Gains of 8q21-22, 13q21-31 and 20q12-qter and loss of 17p12-pter were detected in >50% of stage I tumors. Gain of 8q23-qter and losses of 8p12-pter and 18q12-qter were observed more frequently in stage III/IV tumors than in stage I tumors (all P<0.05). Loss of 8p12-pter and gain of 8q23-qter were linked to nodal metastasis (all P<0.05). Loss of 18q12-qter and gain of 8q23-qter were associated with distant organ metastasis at diagnosis and/or recurrence after surgery (all P<0.05). Moreover, losses of 8p12-pter and 18q12-qter and gains of 8q23 and 8q24-qter were associated significantly with unfavorable prognosis (all P<0.05). Furthermore, combined examination of the above four changes can provide a more accurate assessment for patient's prognosis. Specifically, 11 of 19 patients with these four changes died, but only 1 of 21 cases without these four changes died during the follow-up period (P<0.0001). Multivariate analysis revealed that loss of 18q12-qter is an independent prognostic marker (P=0.031). Our findings indicate that genetic aberrations detected by CGH may predict outcome in patients with CRC.

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Abbreviations: CGH, comparative genomic hybridization; DSCNAs, DNA sequence copy number aberrations; CRC, colorectal cancer; LOH, loss of heterozygosity

Key words: comparative genomic hybridization, colorectal cancer, prognosis, DNA sequence copy number aberrations

Introduction

The prognoses of cancer patients are determined largely by tumor stage. However, patients with tumor of identical grade and stage often have significantly different clinical outcome or responses to therapy. Although influenced by numerous factors, the biological behavior of a tumor is affected primarily by genomic alterations of tumor cells. In general, the number of genetic aberrations parallels the clinical progression of tumors (1-3).

In CRC, a concept is widely accepted that accumulation of genetic aberrations is necessary for carcinogenesis and that the multistep progression of genetic alterations reflect clinical tumor progression (4,5). Many detailed reports have been published that several genomic aberrations are required for initiation and progression of CRC (6). These aberrations include activation of the *SRC* and *RAS* oncogenes (7,8) along with inactivation of the *FAP* and *DCC* tumor suppressors (9,10) and loss of *TP53* function (11). Although the correlation between genomic changes and clinicopathological parameters for CRC has beem extensively studied, data showed different (12-23) information on this relationship, and scarce data exist including patient's prognosis. A clear understanding of the correlation between genomic changes and clinicopathological parameters could provide specific information for tumor therapies. Therefore, we investigated genomic changes by CGH in 73 patients with CRC, and assessed the associations of such charges to clinicopathological parameters.

Materials and methods

Patients. Tumorous colorectal tissues were collected from 73 patients who underwent curative surgery for CRC from 1990 to 1993. Of the 73 patients, 52 were men, and 21 women (mean 64 years, range, 54-91 years). Tumors were classified according to the UICC system and the clinicopathological data

Table I. Characteristics of the patients.

Characteristics	No. of patients
Mean age (years)	64 (range: 54-91)
Sex	
Female	21
Male	52
Differentiation	
Well	38
Moderate	16
Poor	19
UICC classification	
Stage I	11
Stage II	31
Stage III	18
Stage IV	13
Localization	
Cecum	5
Colon	39
Rectum	29
Lymph node metastasis	
Positive	27
Negative	46
Distant organ metastasis	
Positive	13
Negative	60
Recurrence	
Positive	21
Negative	49
Not clear	3
Outcome	
Death	23
Alive	50

are summarized in Table I. None of the patients had received chemotherapy or radiation therapy before surgery. All patients were followed until December 30, 2004. We excluded patients who died of unrelated causes. After surgical resection, tissues were frozen and stored at -80˚C until used.

Microdissection and DNA extraction. A microdissection technique was used as described previously to reduce contamination of normal tissue (25). Test DNA was extracted from tumor tissues, while normal DNA for reference DNA was extracted from peripheral blood lymphocytes of a cytogenetically normal male. High molecular weight DNA was isolated using a DNA extraction kit (SepaGene, Sanko Jyunyaku, Tokyo, Japan) according to the manufacturer's instructions.

Comparative genomic hybridization. The CGH analysis, including digital image analysis, was performed as described previously (1,2). Briefly, reference DNA from lymphocytes of healthy blood donors and tumor DNA were labeled with SpectrumRed-dUTP and SpectrumGreen-dUTP (Vysis Inc., Downers Grove, IL) by nick translation, respectively. Each labeled DNA sample (200 ng) and Cot-1 DNA (10 μg) were dissolved in 10 μl of hybridization buffer and co-hybridized on normal denatured metaphase chromosomes for 72 h at 37˚C. Specimens were mounted in antifade solution containing 0.15 ng/ml 4',6'-diamino-2-phenylindole as a counterstain. Images were captured with an Olympus BX50 fluorescence microscope equipped with a 100X UplanApo objective and a CCD camera (Sensys1400, Photometrics Ltd., Tucson, AZ). A digital image analysis system (QUIPS XL, Vysis) was used for image analysis. At least fire and usually 10, representative images were analyzed for each specimen, and the results were combined to produce an average fluorescence ratio for each chromosome. Gains of DNA sequences were defined as chromosomal regions with a fluorescence ratio above 1.25 and losses as regions with a ratio below 0.75. A positive control with known aberrations and a negative control were included in each CGH experiment as quality controls. Over-representations were considered to be high-level gains when the fluorescence ratio exceeded 1.5.

Statistical analysis. Statistical analysis was performed with commercially available software (SAS Institute Inc., Cary, NC). Correlations between DNA sequence copy number aberrations (DSCNAs) and clinicopathological features were analyzed with Fisher's exact test or Chi-square test. For survival analysis, Kaplan-Meier survival curves were plotted, and the statistical significance of the survival curves was tested by log-rank test. Multivariate analysis was carried out with the Cox proportial-hazards regression model (SAS System). Probability (P) values <0.05 were considered significant.

Results

Chromosomal aberrations detected by CGH in CRC. Although DNA sequence copy number aberrations (DSCNAs) were found in various chromosomal regions, they were detected preferentially in certain regions. The CGH results of 73 CRCs are summarized in Fig. 1. Three tumors had no detectable chromosomal aberrations. Gains were observed for 13q21-q31 in 69.9% (51/73) of tumors, 8q23 in 68.5% (50/73), 8q21-q22 in 67.1% (49/73), 8q24-qter in 53.4% (39/73), 20q12-qter in 49.3% (36/73), 7p14-p21 in 38.4% (28/73), and 7q21-q31 in 30.1% (22/73). Losses were detected for 17p12-pter (51/73, 69.9%), 22q13 (34/73, 46.6%), 8p12-pter (29/73, 39.7%), and 18q12-qter (27/73, 37%). High-level gains (amplifications) were observed in 5p, 6q, 7, 8q, 9p, 12, 13q, 18, 20 and X.

Relation of DSCNAs to clinicopathological parameters. Frequent DSCNAs with a difference of >30% in occurrence rates were analyzed among tumor stages (Table II). Gains of 8q21-22, 13q21-q31, 20q12-qter, 7p14-p21 and 7q21-q31 and losses of 17p12-pter and 22q13 were detected frequently in both stage I and stage III and IV tumors. In addition to these changes, gains of 8q23 and 8q24-qter and losses of 8p12-pter

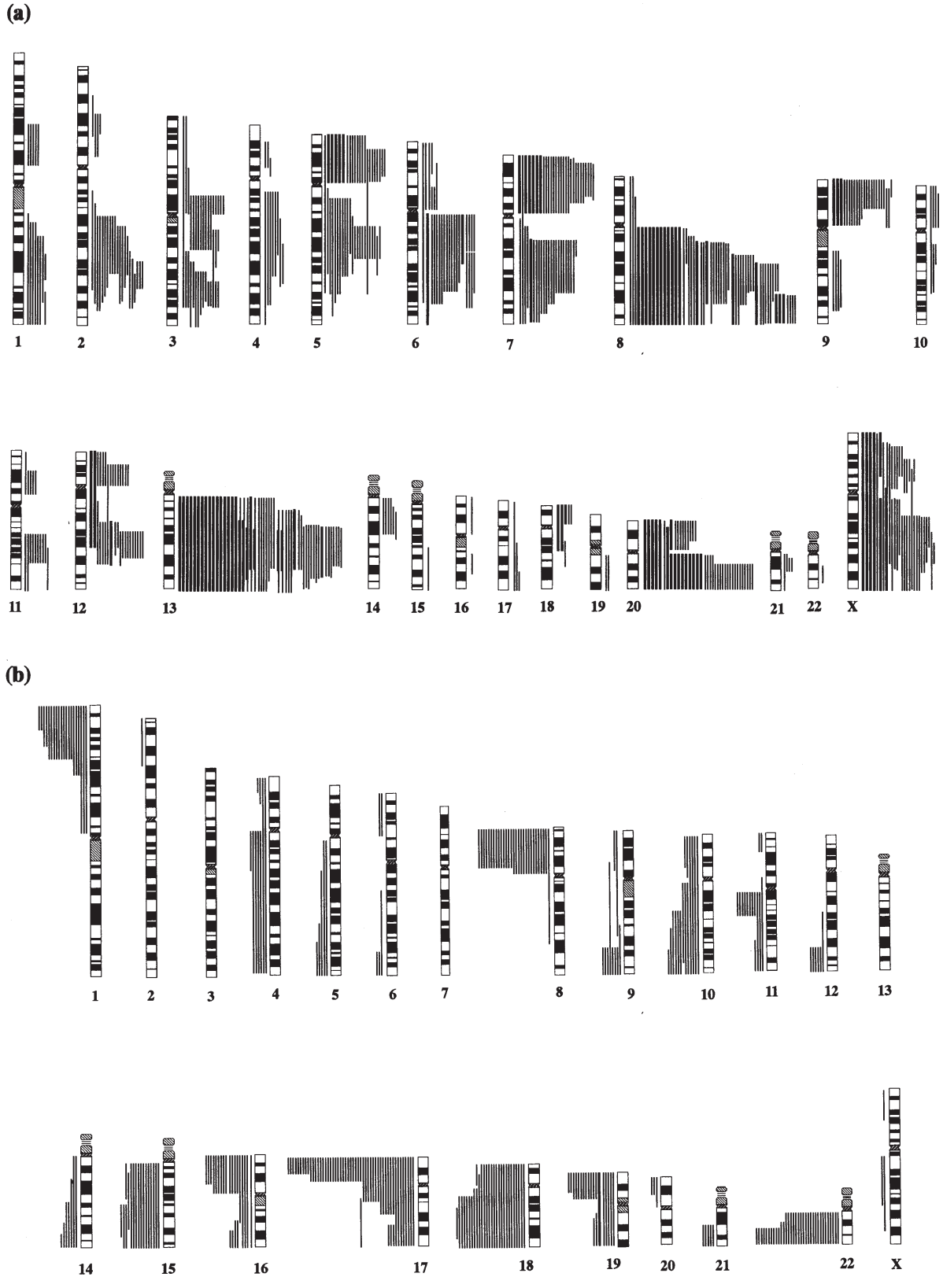

Figure 1. DNA copy number gains and losses detected by CGH in colorectal carcinomas. (a) Gains are shown on right of the chromosome ideograms. High-level amplifications are shown as thick lines. (b) Losses are shown on the left of the chromosome ideograms.

and 18q12-qter were observed frequently in stage III and IV tumors in comparison with stage I tumors (all $P<0.05$). Gains of 8q23 and 8q24-qter, and loss of 8p12-pter were associated with lymph node metastasis ($P=0.003$, $P=0.000025$, and $P=0.002$, respectively; Table III). Furthermore, combined examination of 8q24-qter gain and 8p12-pter loss can provide a more accurate assessment for lymph node metastasis. Specifically, 80% of patients with both gain of 8q24-qter and

Table II. Correlation between frequent DSCNAs and tumor stages.

	Stage I (n=11)	Stage II (n=31)	Stage III (n=18)	Stage IV (n=13)	P-value[a]	P-value[b] I vs. II	P-value[b] I vs. III and IV
Losses							
8p12-pter							
Positive	1 (9%)	9 (29%)	10 (55.6%)	9 (69.2%)			
Negative	10	22	8	4	0.006	NS[c]	0.003
17p12-pter							
Positive	8 (72.7%)	23 (74.2%)	11 (61.1%)	9 (69.2%)			
Negative	3	8	7	4	NS	NS	NS
18q12-qter							
Positive	0 (0%)	8 (25.8%)	8 (44.4%)	11 (84.6%)			
Negative	11	23	10	2	<0.0001	NS	0.0003
22q13							
Positive	3 (27.3%)	18 (58.1%)	10 (55.6%)	3 (23.1%)			
Negative	8	13	8	10	NS	NS	NS
Gains							
7p14-p21							
Positive	3 (27.3%)	12 (38.7%)	10 (55.6%)	3 (23.1%)			
Negative	8	19	8	10	NS	NS	NS
7q21-q31							
Positive	3 (27.3%)	10 (32.3%)	6 (33.3%)	3 (23.1%)			
Negative	8	21	12	10	NS	NS	NS
8q21-q22							
Positive	6 (54.5%)	20 (64.5%)	12 (66.7%)	11 (84.6%)			
Negative	5	11	6	2	NS	NS	NS
8q23							
Positive	5 (45.5%)	16 (51.6%)	16 (88.9%)	13 (100%)			
Negative	6	15	2	0	0.001	NS	0.002
8q24-qter							
Positive	4 (36.4%)	9 (29%)	15 (83.3%	11 (84.6%)			
Negative	7	22	3	2	0.0002	NS	0.006
13q21-q31							
Positive	7 (63.6%)	26 (83.9%)	9 (50%)	9 (69.2%)			
Negative	4	5	9	4	NS	NS	NS
20q12-qter							
Positive	6 (54.5%)	15 (48.4%)	7 (38.9%)	8 (61.5%)			
Negative	5	16	11	5	NS	NS	NS

[a]With Chi-square test. [b]With Fisher's exact test. [c]NS, not significant.

loss of 8p12-pter had lymph node metastasis (P<0.000001; Table III). Gain of 8q23 and loss of 18q12-qter were associated with distant organ metastasis (P=0.004 and P=0.002, respectively; Table IV). Moreover, recurrence after surgery was also correlated significantly not only with gain of 8q23 and loss of 18q12-qter but also with gain of 8q24-qter (P=0.005, P=0.004 and P=0.00073, respectively; Table IV).

Relation of clinicopathological parameters with patient outcome. Survival was examined with respect to clinicopathological parameters including patient age, sex, histological differentiation, depth of tumor invasion, the absence or presence of lymph node metastasis, distant organ metastasis, and recurrence after surgery. Univariate Kaplan-Meier analysis revealed that prognosis was significantly poorer for female patients (P=0.0086) and those with tumors having one of the following characteristics: recurrence after surgery (P<0.0001), distant organ metastasis (P<0.0001), lymph node metastasis (P<0.0001), and moderate or poor differentiation (P=0.0026).

Relation of DSCNAs to patient prognosis. Univariate analysis revealed that survival of patients with CRC was affected negatively by losses of 18q12-qter and 8p12-pter and gains of 8q23 and 8q24-qter (P=0.0008, P=0.0014, P=0.0011, and P=0.0016, respectively; Fig. 2a-d). Furthermore, combined examination of these DSCNAs can provide a more accurate assessment for

Table III. Relation of DSCNAs to lymph node metastasis.

	pN_{1-2}[a]	pN_0[b]	P-value[c]
8p12-pter loss			
Positive	17	12	
Negative	10	34	0.002
8q23 gain			
Positive	24	26	
Negative	3	20	0.003
8q24-qter gain			
Positive	23	16	
Negative	4	30	0.000025
8p12-pter loss and 8q24-qter gain			
Positive	20	5	
Negative	7	41	<0.000001

[a]pN_{1-2}, regional lymph node metastasis by histopathological examination. [b]pN_0, no regional lymph node metastasis by histopathological examination. [c]With Fisher's exact test.

patient's prognosis. Specifically, 11 of 19 patients with these DSCNAs died, but only 1 of 21 cases without these four DSCNAs died during the follow-up period (P<0.0001; Fig. 2e).

Multivariate analysis revealed that lymph node metastasis, distant organ metastasis and loss of 18q12-qter are independent prognostic markers for CRC (P=0.014, P<0.0001, and P=0.031, respectively; Table V).

Discussion

CGH is a powerful method to evaluate biological characteristics of individual tumors by detecting DSCNAs. Analysis of correlation between DSCNAs and the clinicopathological parameters of tumors may clarify which genomic (or chromosomal region) aberrations are related to malignancies that lead to the premature death of cancer patients (1-2,16-27). Although many studies on CRC using CGH method have been published (16-23,26-27), little information considering survival analysis based on CGH data is available (22,23). In this study, we performed survival analysis of CGH data in 73 patients with CRC, and found that specific DSCNAs associated with the biological behavior of CRC. Survival of patients with CRC was negatively associated with gains of 8q23 and 8q24-qter, and losses of 18q12-qter and 8p12-pter. Furthermore, combined examination of the above four changes allows precise estimations of the prognoses of patients with CRC. However, only loss at 18q12-qter was determined to be an independent prognostic marker.

Our findings are in general agreement with previous CGH studies that whole or partial gains of chromosome/chromosome arms 6p, 7, 8q, 13q, and 20q, and whole or partial losses of chromosome/chromosome arms 5q, 8p, 17p, 18q, and 22q are frequent recurrent aberrations in CRCs (16-23). Interestingly, we found that loss at 17p12-pter and gains of 8q21-q22, 13q21-q31 and 20q12-qter were detected in >50% of stage I and stage III/IV tumors respectively, suggesting

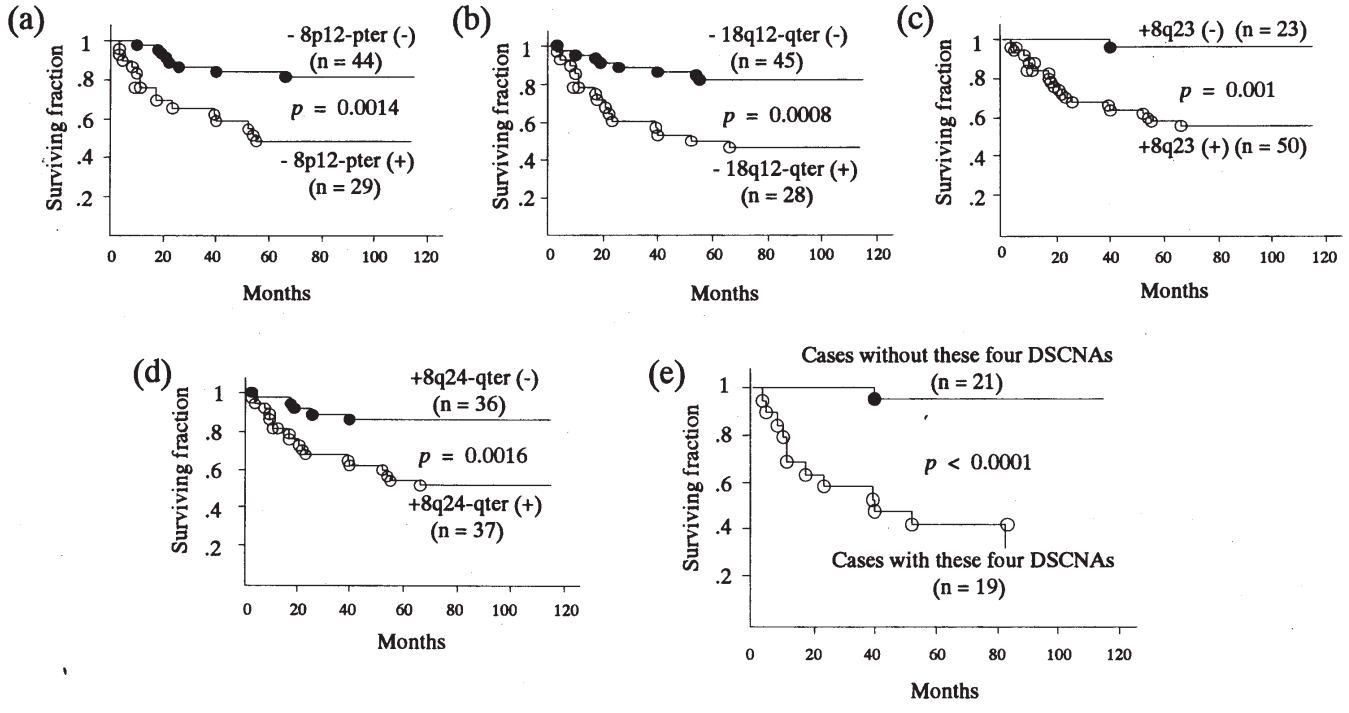

Figure 2. (a) Overall survival according to loss of 8p12-pter. (b) Overall survival according to loss of 18q12-qter. (c) Overall survival according to gain of 8q23. (d) Overall survival according to gain of 8q24-qter. (e) Overall survival according to these four changes.

Table IV. Relation of DSCNAs to distant organ metastasis at diagnosis and recurrence after surgery.

	18q12-qter loss			8q23 gain			8q24-qter gain		
	Positive	Negative	P-value[c]	Positive	Negative	P-value[c]	Positive	Negative	P-value[c]
DOM[a]									
Positive	10	3		13	0		10	3	
Negative	17	43	0.002	37	23	0.004	29	31	0.05
Recurrence[b]									
Positive	13	8		19	2		17	4	
Negative	12	37	0.004	28	21	0.005	18	31	0.0007

[a]DOM, distant organ metastasis at diagnosis. [b]Three cases of recurrence are not clear. [c]With Fisher's exact test.

Table V. Multivariate analysis of patients survival.

	Multivariate analysis	
Factors	P-value	Risk ratio
Sex (female vs. male)	0.835	1.11
Histological differentiation[a] mod. and por vs. wel.	0.913	1.06
pN (pN_0 vs. $pN_{1,2}$)[b]	0.014	4.35
pM (pM_0 vs. pM_1)[c]	<0.0001	25
8p12-pter loss	0.061	2.77
8q23 gain	0.231	4.11
8q24-qter gain	0.984	1.02
18q12-qter loss	0.031	5.26

[a]wel., mod., and por., well, moderately, poorly differentiated adenocarcinoma, respectively. [b]pN, histopathological classification of regional lymph node metastasis. [c]pM, histopathological classification of distant organ metastasis.

that genes located at these chromosomal regions contribute to the development and progression of CRC. Gain of 20q was detected in 85% CRCs with liver metastasis (21), and a similar frequency (75%) was observed in primary CRCs (22) and colorectal adenomas (24). Gains at 8q, 13q, and 20q appear especially to be involved in progression from colorectal adenoma to carcinoma (26). Recently, Gaasenbeek *et al* reported also that loss at 17p and gains of 8q, 13q, and 20q occur early in the establishment of primary CRCs (27). In our study, gain at 8q21-q22 was detected in >50% of stage I CRCs, but gain at 8q23-qter was observed frequently in higher-stage tumors. With respect to the relation of 17p loss to tumor stages of CRC, our results are consistent with data of Ghadimi *et al* (28), but different from that of Al-Mulla *et al* (18). Consistent with the findings of other reports, we observed frequently gain of 8q23-qter and losses of 8p12-pter and 18q12-ter in higher-stage tumors (16,19,22,28). These results suggest that genetic aberrations in these regions play an important role in tumor progression and metastases.

Loss at 8p12-pter and gain at 8q23-qter are associated significantly with nodal metastasis, whereas loss of 18q12-qter and gain of 8q23-qter are significantly correlated with distant organ metastasis at diagnosis and/or recurrence after surgery. These results indicate that the different pathways of tumor dissemination are reflected by a non-random accumulation of chromosomal alterations with specific changes being responsible for the different characteristics of the metastatic phenotype. Gain at 8q23-qter is associated significantly with nodal metastasis in CRCs (28). In the present study, gain at 8q24-qter has a stronger association with nodal metastasis than 8q23 gain, while several candidate genes located at 8q24-qter associated to poor prognosis or aggressive behavior in several tumor types (29-31). Combined assessment of 8p12-pter loss and 8q24-qter gain can provide more accurate assessment for the status of nodal metastasis. Specifically, 80% of patients with both gain of 8q24-qter and loss of 8p12-pter had lymph node metastasis. Therefore, detection of specific DSCNAs may enable prediction at the time of diagnosis of the likelihood of nodal metastasis, distant organ metastasis, and recurrence after surgery and could provide specific information for tumor therapy.

Scarce CGH data on the establishment of correlation between specific chromosomal aberrations and outcomes of patients with CRC have been reported (22,23). We found that losses of 8p12-pter and 18q12-qter, and gains of 8q23 and 8q24-qter are significantly associated with unfavorable prognosis, and multivariate analysis revealed that loss of 18q12-qter is an independent prognostic marker in CRCs. Some researchers have reported that loss of 8p detected by LOH is related to poor prognoses of patients with CRC (12). Chromosome arm 8q23-qter contains the candidate genes *C-MYC* and *PRL-3*. A strong association between amplification of *C-MYC* and *PRL-3* and advanced clinical stage and/or metastases of CRC has been indicated (29,30). Furthermore, the relation of 8q23-qter gain detected by CGH to patient outcome has already been described in other tumors (32,33). Many reports mention allelic loss of 18q and lack of expression of *DCC* as an adverse prognostic indicator of CRC (34). Indeed, our analysis indicates that loss of 18q12-qter

related to an adverse prognosis and distant organ metastasis at diagnosis and/or recurrence after surgery. It is reported that three candidate tumor-suppressor genes, *DCC*, *DPC4*, and *JV181*, are located in this region (35,36). Loss of 18q is associated with poor clinical outcome or metastases in CRC (14,15,34), but it has not been reported yet that loss of 18q12-qter is an independent marker for CRCs, and further examinations for demonstrating this result are essential.

In conclusion, the prognoses of patients with CRC are largely influenced by genomic alteration of specific chromosomal regions in tumor cells. The findings of the present study elucidate that the CGH method can be used to predict the likelihood of outcomes of patients with CRC and provide information useful in treatment decisions.

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