DNA copy number aberrations associated with the clinicopathological features of colorectal cancers: Identification of genomic biomarkers by array-based comparative genomic hybridization

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Received December 28, 2010; Accepted February 24, 2011

DOI: 10.3892/or.2011.1246

Abstract. The aim of the present study was to investigate the chromosomal aberrations that are linked with the crucial clinicopathological features of colorectal cancer (CRC) and its prognosis by array-based comparative genomic hybridization (CGH). Fresh-frozen tumor tissues of 94 cases of CRC were analyzed by using bacterial artificial chromosome (BAC) CGH slides spotted with 4030 human BAC clones, which covered the whole range of the human genome at an average interval of 0.83 mega base pairs. DNA copy number aberrations (DCNAs) were identified in association with clinicopathological features: a gain of 8q24.3 and losses of 9q33.1 and 20p12.2 were associated with lymph node metastasis, gain of 8q24.3 and loss of 9q33.1 with disease stage, gain of 8q21.11 and loss of 10q21.3 with lymphovascular invasion and losses of 3p25.1, 10p15.3, 12q15 and 17p13.1 for venous invasion. These aberrations can be regarded as genomic biomarkers to predict the clinical outcome of patients with CRC, and are expected to serve to individualize the treatment of CRC patients.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies in humans worldwide, and apart from some familial types, usually arising sporadically (1). Cytogenetically, CRC can be classified into two types based on the types of genetic abnormalities present (2,3). One is the major type, which is characterized by frequent chromosomal imbalances, that is, the chromosomal instability phenotype comprising more than 85% of all CRCs. The other is the minor type that frequently exhibits microsatellite instability originating from DNA replication errors. The microsatellite instability phenotype comprises 10-15% of all CRCs. In both types, the genomic instability leads to a degree of gains and losses of genomic DNA, which can be classified as DNA copy number aberrations (DCNAs).

Recently, microarray technology has been applied to the comparative genomic hybridization (CGH) methodology, thus leading to array-based comparative genomic hybridization (a-CGH) (4). The a-CGH method allows high-resolution and high-throughput screening of DCNAs across the whole genome. The DCNAs detected by the a-CGH method can be directly related to the DNA sequence information of a cancer to aid in the localization, identification and the validation of cancer-causing genes (4,5).

In general, cancers (including CRC) occur as a result of the accumulation of a number of genomic aberrations that are linked with carcinogenesis and cancer progression (2,3,6). Thus, the a-CGH analysis is suitable for studying the carcinogenic pathway of CRC with high-resolution and high-throughput results. Furthermore, the analysis of the cancer genome by a-CGH is expected to serve not only to clarify the relationship between the clinicopathological features and genomic abnormalities, but also to optimize the medical treatment of patients with CRC by making use of their cancer genome information. However, there has been little information available regarding the relationship between the tumor genome and its characteristics and the associated patient prognosis (7,8).

The cytogenetic, as well as biological, properties of CRCs differ from each other. Thus, treatments based on the differences would be of benefit for the patients with CRC. Clinical information, such as the presence of lymph node metastasis and lymphovascular and blood vessel invasion, are predictors of prognosis and indicators of the optimal medical treatment approach (i.e. what kinds of adjuvant chemotherapy should be administered). The aim of the present study was to identify the

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Key words: colorectal cancer, array-based comparative genome hybridization, DNA copy number aberration, genomic biomarker

Table I. Clinicopathological data.

Parameters	No.
Gender	
Male	51
Female	43
UICC stage	
I + II	38
III + IV	54
Lymph node metastasis	
Negative	41
Positive	43
Lymphovascular invasion	
Negative	54
Positive	31
Venous invasion	
Negative	36
Positive	50

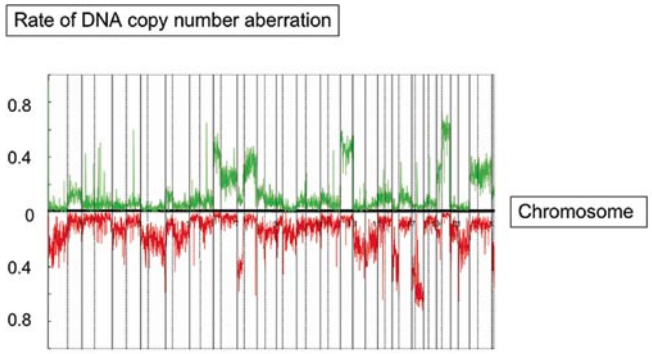

Figure 1. Overall frequency of DNA copy number aberrations detected by a-CGH for each BAC clone in 94 colorectal adenocarcinomas. The frequency of aberrations is depicted as the fraction of cases with DNA copy number gain or loss for the 4030 BAC clones (the entire genome). The green dots in the upper part of the profile indicate the frequency of tumors with DNA copy number gains, and the red dots in the lower part of the profile indicate the frequency of tumors with DNA copy number losses.

Table II. Top 10 loci associated with DNA copy number aberrations in 94 colorectal cancers.

Locus	Frequency (%)
DNA copy number gain	
20q11	76
20q13	71
20q12	68
13q12	61
13q34	58
7p14	58
13q22	56
13q13	56
7p21	55
13q33	54
DNA copy number loss	
18q23	74
18q21	71
18q12	70
22q11	67
18q22	67
18q23	66
17p11	63
18q22	63
4q35	61
17p13	60

DCNAs that are linked with these crucial clinicopathological features of CRCs and to provide the genomic information that can be valuable for the treatment of patients with CRC.

Materials and methods

Materials. The present study followed the ethical guidelines of the Institutional Review Board of the Yamaguchi University School of Medicine. Ninety-four surgically resected, fresh-frozen CRC sample tissues were available for the present study. The patients consisted of 51 males and 43 females, ranging from 39 to 87 years in age (Table I). The clinical staging of the tumors was according to the UICC TNM Classification, 2002 (9). Histopathologically, 63 of the tumors were diagnosed as well differentiated, 25 as moderately differentiated, and 6 as poorly differentiated adenocarcinomas.

Array-based comparative genomic hybridization. Tumor tissue sections (10-mm thick) were cut from each fresh-frozen cancer tissue specimen using a cryostat (Bright Instrument, Hunchington, UK). The sections were immediately fixed in 90% ethanol solution and stained with methylgreen (Sigma, Tokyo, Japan). Tumor tissues were microdissected using a 28-gauge needle. High molecular weight genomic DNA was extracted from the cancer tissue sections using a DNeasy Tissue kit (Qiagen Sciences, CA, USA).

Each 500 ng tumor DNA sample and reference DNA sample were labeled with FluoroLink Cy5-dCTP (Perkin-Elmer, MA, USA) and FluoroLink Cy3-dCTP (Perkin-Elmer) using a BioPrime DNA Labeling System (Promega, WI, USA), respectively. The fluorescence-labeled DNAs were applied to a MacArray Karyo4000 CGH array slide (Macrogen, Seoul, Korea). The array slide was spotted with 4030 human bacterial artificial chromosome (BAC) clones, which covered the whole range of the human genome at an average interval of 0.83 mega base pairs (Mb). Images of the 16-bit fluorescence intensity for spots were captured using a GenePix 4000A scanner (Axon Instruments, CA, USA), and the Cy5/Cy3 ratio values were calculated using the MAC Viewer software program (Macrogen). All fluorescence intensity ratios were converted to log base 2. Any inadequate spots were flagged by manual inspection.

Data analysis. The χ^2 test was used to identify the DCNAs by analyzing a-CGH data of CRC with and without nodal metastasis, lymphatic invasion, venous invasion, and high- and low-clinical stage. Figures were generated to determine

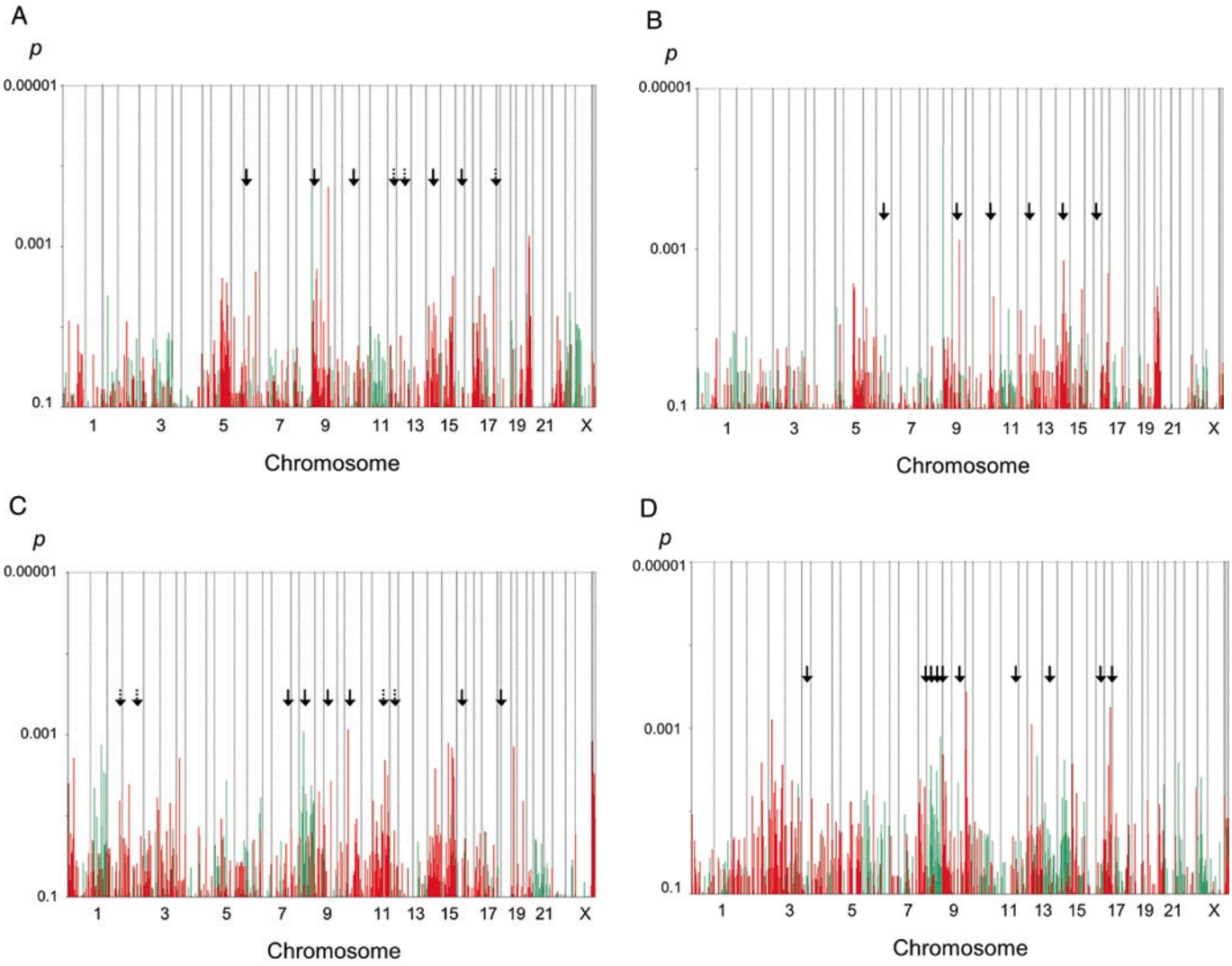

Figure 2. The results of the χ^2 test for the 4030 BAC clones and the differences in the p-values between lymph node metastasis negative and positive (A), low stage (stages I and II) and advanced stage (stages III and IV) (B), lymphovascular invasion negative and positive (C), and venous invasion negative and positive (D) colorectal cancers (CRCs). The green columns of each profile indicate an increase in the frequency of CRCs carrying DNA copy number aberrations (DCNAs) at the corresponding chromosome locus in the former categories, ie, lymph node metastasis negative (A), low stage (B), lymphovascular invasion negative (C), or venous invasion negative (D) categories. The red columns of each profile indicate the increase in frequency of CRCs carrying DCNAs at the corresponding chromosomal locus in the same categories (lymph node metastasis positive (A), high stage (B), lymphovascular invasion positive (C), or venous invasion positive (D) categories).

the difference by plotting the BAC DNA clones with p<0.1 based on the analyses (Figs. 1 and 2).

Results

The BAC clones indicated that the DCNAs differed for every case. Although there were differences in the DCNAs between each of the 94 cases of CRC, the most frequent DCN gain was of 20q11, which was observed in 76% of the cases. The frequency of DNA copy number gain at 13q and 7p (58%) was also high (Table II). On the other hand, the most frequent DNA copy number loss was at 18q23, and was detected in 74% of the cases (Table II).

Lymph node metastasis and DCNA. Fig. 2A shows the results of the χ^2 test between CRCs with lymph node metastasis and those without metastasis with regard to all 4030 of the BAC clones mounted on a-CGH slide, as well as the corresponding p-values. Fig. 2A shows that the CRCs with lymph node metastasis were associated with a gain of 11q and losses of 5q, 9, 17p and 20p (arrows, p<0.01). The figure also demonstrates that CRCs without lymph node metastasis were associated with a gain of X, and losses of chromosomes 14 and 15 (dotted arrows, p<0.01).

The specific BAC clones with p<0.01 are listed in Table III. CRCs with lymph node metastasis were significantly associated with a gain of clone 2748 (8q24.3) and losses of clone 4289 (9q33.1), clone 5951 (20p12.2) and clone 466 (20p12.2) (p<0.001).

Clinical stage and DCNA. CRCs in stages I and II were defined as low stage tumors, and those in stages III and IV as advanced stage tumors. Fig. 2B shows the results of the χ^2 test between low stage CRCs and advanced stage CRCs

Table III. BAC clones associated with lymph node metastasis in colorectal cancer.

Chromosome	BAC-start	Locus	ID	Locating genes	P-value
DNA copy number gain					
2	20378550	2p24.1	1357	PUM2	0.004108
8	1.46E+08	8q24.3	2748	ZNF16, TMED10P, C8orf77	0.000203
10	25715182	Xp21.3	4329		0.003777
10	29992424	Xp21.2	2150	MAGEB2, MAGEB3, MAGEB4, MAGEB1, NR0B1	0.008468
10	70247389	Xq13.1	2922	ZMYM3, NONO, ITGB1BP2	0.009193
10	74789201	Xq13.3	5171		0.009406
11	60762733	11q12.2	2373	PGA5, VWCE, DDB1	0.009995
15	84084157	15q25.3	2914	AKAP13, KLHL25	0.00766
19	858881	19p13.3	5529	C19orf22, KISS1R, ARID3A, WDR18, GRIN3B, C19orf6, ABCA7	0.008422
20	705254	20p13	5734	C20orf55, RPS10L, ANGPT4	0.003907
20	14725959	20p12.1	1089	C20orf133	0.007449
DNA copy number loss					
1	28059305	1p35.3	922	EYA3	0.008444
1	76485020	1p31.1	4085	ST6GALNAC3	0.009498
2	1.62E+08	2q24.2	5373	PSMD14, TBR1	0.008638
5	1.32E+08	5q23.3	5300	P4HA2, PDLIM4, SLC22A4	0.002469
5	1.52E+08	5q33.1	4740		0.002835
5	1.25E+08	5q23.2	4761		0.004705
5	1.51E+08	5q33.1	5345	SPARC, ATOX1	0.005411
5	1.59E+08	5q33.3	2853		0.005413
5	1.26E+08	5q23.2	4736	LMNB1, MARCH3	0.006226
5	1.37E+08	5q31.1	4668	SPOCK1	0.008393
5	1.35E+08	5q31.1	1539	TGFBI, SMAD5	0.009836
6	1.52E+08	6q25.1	173	ESR1, SYNE1	0.002059
6	98369873	6q16.1	4205		0.007345
6	14035344	6p23	814	RNF182	0.007675
9	1.17E+08	9q33.1	4289		0.000183
9	23737636	9p21.3	1099	ELAVL2	0.001923
9	21726045	9p21.3	4214	MTAP	0.002536
9	1.17E+08	9q33.1	4221	ASTN2, TRIM32	0.003014
9	42141245	9p12	5118		0.004607
9	919127	9p24.3	5884	DMRT1, DMRT3	0.004775
9	414664	9p24.3	5566	DOCK8, ANKRD15	0.008638
9	79353236	9q21.31	1119	TLE4	0.008638
14	71175222	14q24.2	4675	SIPA1L1	0.005054
14	34875953	14q13.2	2531	NFKBIA	0.005595
14	80102060	14q31.1	1280	C14orf145	0.007222
14	73441348	14q24.3	4034	ZNF410, C14orf44, COQ6, ENTPD5	0.008437
15	89157227	15q26.1	2434	BLM, FURIN, FES, MAN2A2	0.002363
15	89185022	15q26.1	2971	FURIN, FES, MAN2A2, HDDC3	0.003354
15	88125742	15q26.1	707	ANPEP, AP3S2	0.007458
15	71713010	15q24.1	4618	CD276	0.007576
17	72955036	17q25.3	2602	SEPT9	0.001816
17	11944544	17p12	2859	MAP2K4	0.004177
17	35871401	17q21.2	2983	TNS4	0.006952
17	2510194	17p13.3	5917	PAFAH1B1, KIAA0664	0.009045
17	410041	17p13.3	158	VPS53	0.009053
20	10407110	20p12.2	5951	C20orf94	0.000748
20	9192646	20p12.2	466	PLCB4	0.000894
20	10566034	20p12.2	768	JAG1	0.001033
20	10633685	20p12.2	5954		0.005326
20	10266917	20p12.2	5952	RPL23AP6, MKKS, C20orf94	0.00536
20	10740672	20p12.2	5957	FAT1P1	0.007335
20	16603508	20p12.1	1509	RPL7AL3, SNRPB2, OTOR	0.007452
20	12524678	20p12.1	1345		0.008638
20	168339	20p13	5621	C20orf96, ZCCHC3, SOX12, C20orf98, TRIB3	0.008801
22	27639138	22q12.1	460	ZNRF3	0.007449

Table IV. BAC clones associated with clinical stage in colorectal cancer.

Chromosome	BAC-start	Locus	ID	Locating genes	P-value
DNA copy number gain					
5	886825	5p15.33	5598	ZDHHC11, BRD9, TRIP13	0.005423
8	1.46E+08	8q24.3	2748	ZNF16, TMED10P, C8orf77	5.14E-05
11	94501857	11q21	1302	ENDOD1, SESN3	0.00567
15	19958051	15q11.2	2266	VSIG6	0.009342
DNA copy number loss					
5	1.25E+08	5q23.2	4761		0.00273
5	1.32E+08	5q23.3	5300	P4HA2, PDLIM4, SLC22A4	0.003043
5	1.26E+08	5q23.2	4736	LMNB1, MARCH3	0.003359
5	18102403	5p15.1	4466		0.00887
6	14035344	6p23	814	RNF182	0.005457
9	1.17E+08	9q33.1	4289		0.00078
9	1.17E+08	9q33.1	4221	ASTN2, TRIM32	0.008174
11	8182603	11p15.4	2704	LMO1	0.003937
12	6175040	12p13.31	591	CD9	0.005891
12	1.32E+08	12q24.33	5605	ZNF26, ZNF84, ZNF140	0.008955
12	96497364	12q23.1	4796		0.009111
14	73441348	14q24.3	4034	ZNF410, C14orf44, COQ6, ENTPD5	0.001408
14	71175222	14q24.2	4675	SIPA1L1	0.004751
14	80102060	14q31.1	1280	C14orf145	0.006465
14	63739020	14q23.2	2040	SYNE2, ESR2	0.006543
15	89157227	15q26.1	2434	BLM, FURIN, FES, MAN2A2	0.003217
17	11944544	17p12	2859	MAP2K4	0.002045
20	10407110	20p12.2	5951	C20orf94	0.002978
20	10566034	20p12.2	768	JAG1	0.003914
20	513026	20p13	5646	TCF15, SRXN1, SCRT2	0.005423
20	168339	20p13	5621	C20orf96, ZCCHC3, SOX12, C20orf98, TRIB3	0.006151
20	15275392	20p12.1	269	C20orf133	0.006172
20	16603508	20p12.1	1509	RPL7AL3, SNRPB2, OTOR	0.007495

for all 4030 BAC clones mounted on the a-CGH slides with the corresponding p-values. The figure also shows that the advanced stage CRCs were associated with a gain of 11q and losses of 5q, 9, 14, 17p and 20p (arrows, p<0.01).

The specific clones with p<0.01 are listed in Table IV. Advanced stage CRCs were significantly associated with a gain of clone 2748 (8q24.3) and a loss of clone 4289 (9q33.1) (p<0.001). These two BAC clones that were associated with advanced stage CRCs were also included in those that were linked with CRCs with lymph node metastasis.

Lymphovascular invasion and DCNA. Fig. 2C shows the p-values corresponding to the 4030 BAC clones that were estimated by the χ^2 test between CRCs with lymphovascular invasion and those without lymphovascular invasion. The CRCs with lymphovascular invasion were associated with an increased frequency of gains of 8q and 21, and losses of 10, 11 and Y (arrows, p<0.01). Further, Fig. 2C shows that the CRCs without lymphovascular invasion were associated with a gain of 1q, and losses of 1p, 14 and 15p (dotted arrows, p<0.01).

The specific BAC clones with p<0.01 are listed in Table V. CRCs with lymphovascular invasion were significantly associated with a gain of clone 4208 (8q21.11) and a loss of clone 4467 (10q21.3) (p<0.001).

Venous invasion and DCNA. Fig. 2D shows the results of the χ^2 test between CRCs with venous invasion and those without venous invasion concerning all 4030 of the BAC clones mounted on a-CGH slides with their corresponding p-values. The CRCs free of venous invasion were associated with gains of 8q, 14, 22 and Xp, and losses of 3p, 8p, 9p, 10p and 17p (arrows, p<0.01).

The specific BAC clones with p<0.01 are listed in Table VI. CRCs with venous invasion were significantly associated with losses of clone 230 (10p15.3), 1536 (17p13.1), 2562 (3p25.1) and clone 2084 (12q15) (p<0.001).

Discussion

The biological properties of cancers differ by patient and by cancer subtype. Thus, treatments based on an individual cancer would be of benefit to patients. Clinically significant

Table V. BAC clones with lymphovascular invasion in colorectal cancer.

Chromosome	BAC-start	Locus	ID	Locating genes	P-value
DNA copy number gain					
1	208000000	1q32.3	1078	SLC30A1, NEK2	0.001329
1	229000000	1q42.2	5630	SIPA1L2	0.002892
1	242000000	1q44	303		0.003034
1	157000000	1q23.2	1412	PIGM, KCNJ10	0.004509
5	135000000	5q31.1	2688	IL9, FBXL21, LECT2	0.003663
6	168000000	6q27	2706	MLLT4	0.005968
6	160000000	6q25.3	2872	SOD2, WTAP	0.009212
8	75824814	8q21.11	4208	PI15	0.00092
8	134000000	8q24.22	359	WISP1, NDRG1	0.004182
8	72080962	8q13.3	5059		0.004275
8	91413409	8q21.3	1131		0.005384
8	145000000	8q24.3	5267	HSF1, DGAT1, SCRT1, FBXL6, GPR172A, ADCK5, CPSF1	0.00651
8	145000000	8q24.3	1284	ZC3H3, GSDMDC1, C8orf73, NAPRT1, EEF1D	0.007132
8	48721528	8q11.21	2413	CEBPD	0.008533
8	99509595	8q22.2	4470	KCNS2, STK3	0.008827
8	66247296	8q13.1	4923		0.009245
8	143000000	8q24.3	4223		0.009392
8	128000000	8q24.21	939	SRRM1L	0.009718
15	70609648	15q24.1	334	ARIH1	0.009665
DNA copy number loss					
1	28068722	1p35.3	388	EYA3	0.001934
1	1463003	1p36.33	705	SLC35E2, CDC2L2, CDC2L1	0.00402
2	140000000	2q22.1	4557		0.004082
2	68918173	2p13.3	5007	ARHGAP25	0.00654
3	71826780	3p13	900	EIF4E3, GPR27, PROK2	0.005996
3	166000000	3q26.1	4469	SLITRK3	0.007001
3	199000000	3q29	2321	KIAA0226	0.00714
3	80273927	3p12.2	4988		0.00844
3	167000000	3q26.1	4805		0.009354
4	3180037	4p16.3	2855	HD	0.001934
9	117000000	9q33.1	4289		0.003724
9	21726045	9p21.3	4214	MTAP	0.004983
9	42141245	9p12	5118		0.00783
10	67611287	10q21.3	4467	CTNNA3	0.000866
11	128000000	11q24.3	1184	FLI1	0.003167
11	61848708	11q12.3	2681	ASRGL1, SCGB1A1, AHNAK	0.00654
11	101000000	11q22.1	35		0.007308
11	131000000	11q25	4032	C11orf39	0.009046
14	71175222	14q24.2	4675	SIPA1L1	0.002616
15	48316625	15q21.2	985	HDC, GABPB2	0.001262
15	76950406	15q25.1	4450	MORF4L1, CTSH, RASGRF1	0.001444
15	88125742	15q26.1	707	ANPEP, AP3S2	0.001934
15	89185022	15q26.1	2971	FURIN, FES, MAN2A2, HDDC3	0.00332
15	49208466	15q21.2	590	CYP19A1	0.007401
19	10175587	19p13.2	753	EDG5, ICAM1, ICAM4	0.001418
19	54042891	19q13.33	1194	PLEKHA4, PPP1R15A, TULP2, NUCB1, DHDH, BAX, FTL, GYS1	0.006621
Y	6062912	Yp11.2	4648	TSPY2, TSPYP1	0.001209
Y	10145611	Yp11.2	24	RBMY2GP, TTTY7	0.002883
Y	21750616	Yq11.223	899	RBMY2SP	0.00305
Y	21831283	Yq11.223	485	RBMY2EP, RBMY2TP, TSPYP4	0.003814
Y	19261015	Yq11.222	1442	USP9YP1, HSFY1, TTTY9B, OFDYP5	0.005537
Y	18634292	Yq11.221	412	CDY5P, ACTGP2, XKRY, SEDLP3, OFDYP1	0.005716
Y	6245999	Yp11.2	5143	RBMY2GP, TTTY7	0.00592
Y	6123032	Yp11.2	4330	TSPY2, TSPYP1, RBMY2GP	0.006672
Y	22359301	Yq11.223	429	RBMY1B, RBMY1A1	0.007153
Y	13424058	Yq11.21	4549	DDX3Y, CASKP	0.008572

Table VI. BAC clones associated with venous invasion in colorectal cancer.

Chromosome	BAC-start	Locus	ID	Locating genes	P-value
DNA copy number gain					
3	1.85E+08	3q27.1	1267	AP2M1, ABCF3, ALG3, CAMK2N2	0.004777
6	31614370	6p21.33	2279	BAT1, ATP6V1G2, NFKBIL1, LTA, TNF, LTB, LST1, NCR3, AIF1, BAT2, BAT3	0.005702
6	19479260	6p22.3	4509		0.007369
6	1.48E+08	6q24.3	838		0.007798
6	32917633	6p21.32	2086	PSMB8, TAP1, PSMB9, PPP1R2P1, HLA-DMB	0.008609
8	1.44E+08	8q24.3	2978	GML, CYP11B1, CYP11B2	0.001282
8	75824814	8q21.11	4208	PI15	0.002815
8	1.45E+08	8q24.3	1284	ZC3H3, GSDMDC1, C8orf73, NAPRT1, EEF1D	0.003108
8	1.18E+08	8q24.11	4296		0.003307
8	72080962	8q13.3	5059		0.00559
8	76752360	8q21.11	4850		0.00832
8	88062342	8q21.3	5174	CNBD1	0.008437
8	90966600	8q21.3	2711	C8orf1, NBN	0.008991
9	1.08E+08	9q31.2	4892		0.004541
12	1.03E+08	12q23.3	2790	NT5DC3	0.00217
13	31754912	13q13.1	148	FRY, BRCA2, IFIT1P	0.007934
14	63953132	14q23.2-	1066	MTHFD1, AKAP5, ZBTB25	0.002416
14	52954585	14q22.1	4506		0.00685
14	68799746	14q24.1	4082	GALNTL1	0.007369
14	76963463	14q24.3	1546	C14orf133, AHSA1, THSD3, SPTLC2	0.007369
15	89157227	15q26.1	2434	BLM, FURIN, FES, MAN2A2	0.007798
20	22946651	20p11.21	2292	SSTR4, THBD, CD93	0.00474
21	35144508	21q22.12	2331	RUNX1	0.002614
X	22016295	Xp22.11	4307	PHEX, ZNF645	0.003979
DNA copy number loss					
2	2.06E+08	2q33.3	825	ALS2CR19	0.002614
2	1.32E+08	2q21.1	2745	ARHGEF4, PLEKHB2	0.006666
2	2.10E+08	2q34	1526	MAP2	0.007483
3	14148375	3p25.1	2562	TMEM43, XPC, LSM3	0.000792
3	71826780	3p13	900	EIF4E3, GPR27, PROK2	0.002771
3	74941458	3p12.3	4309		0.002771
3	1.39E+08	3q22.3	418	NPM1P17	0.004333
3	36923131	3p22.3	3024	EPM2AIP1, MLH1	0.004417
3	63273432	3p14.2	1005	SYNPR	0.005318
3	26969537	3p24.2	4929		0.005992
3	64300928	3p14.1	348		0.006545
3	1.87E+08	3q27.2	2819	ETV5	0.00685
3	25613645	3p24.2	2268	RARB, TOP2B	0.00936
3	44861037	3p21.31	5340	KIF15, TMEM42, TGM4, ZDHHC3	0.009527
3	61112371	3p14.2	2781	FHIT	0.009527
3	833434	3p26.3	5701		0.009665
4	57604869	4q12	656	REST, C4orf14, POLR2B, IGFBP7	0.007076
4	1.66E+08	4q32.3	4287		0.008078
5	1.26E+08	5q23.2	4736	LMNB1, MARCH3	0.00767
5	1.25E+08	5q23.2	4761		0.007819
6	58452627	6p11.2	998		0.006476
8	6562724	8p23.1	4738	AGPAT5	0.004137
8	36251863	8p12	4919		0.005148
8	649574	8p23.3	5579	ERICH1, C8orf68	0.007967
8	250609	8p23.3	670	FBXO25	0.009945
9	414664	9p24.3	5566	DOCK8, ANKRD15	0.002083
9	14314735	9p22.3	5623		0.004436

Table VI. Continued.

Chromosome	BAC-start	Locus	ID	Locating genes	P-value
DNA copy number loss					
9	15666106	9p22.3	4639	C9orf93	0.006505
9	288712	9p24.3	2768	DOCK8	0.007162
9	502548	9p24.3	5569	ANKRD15	0.007419
10	543426	10p15.3	230	DIP2C	0.000366
10	183360	10p15.3	2532	ZMYND11	0.001822
10	185862	10p15.3	2166	ZMYND11	0.005187
10	854943	10p15.3	5553	LARP5	0.006853
12	66758603	12q15	2084	IFNG	0.000903
12	38170799	12q12	4224	ABCD2	0.006666
15	19051540	15q11.2	5162		0.002692
15	37896537	15q14-15q15.1	1056	GPR176	0.006096
17	10455531	17p13.1	1536	MYH3, SCO1, C17orf48	0.000563
17	3811567	17p13.2	1210	ATP2A3, ZZEF1	0.00282
19	21158366	19p12	2301	ZNF431	0.007369
20	3477726	20p13	1566	ATRN	0.008263
20	4970392	20p13-20p12.3	2878	C20orf30, PCNA, CDS2	0.009205
22	48278710	22q13.33	930		0.005187
Y	1805965	Yp11.31	5180		0.006476

information for patients with CRC, such as the presence of lymph node metastasis, the clinical stage, and lymphovascular and venous invasion, are predictors of prognosis and indicators of the likelihood of a response to treatment. In the present study, a-CGH yielded a more statistically meticulous analysis than previous chromosomal CGH.

Our previous CGH analysis showed that a gain of 8q24 was the most significant factor related to lymph node metastasis and advanced tumor stage in CRCs (7,8). Since advanced stage tumors are usually accompanied by lymph node metastasis, there were many overlapping DCNAs that were associated with lymph node metastasis and an advanced tumor stage in CRCs. However, elevated tumor stage alone was significantly associated with an increased frequency of a gain of X and chromosome 15 in the present study. Other factors that were determinants of an advanced tumor stage that were separate from lymph node metastasis were liver metastasis and peritoneal metastasis. However, since the number of these cases was limited in comparison to those with lymph node metastasis, the details could not be determined.

The 8q24 locus is frequently amplified not only in colorectal cancer but also in other types of cancer, e.g., breast cancer (10) and prostate cancer (11). A number of reports have speculated that aberrations of 8q24 are associated with malignant transformation of a cell, and the CMYC gene, which is located at 8q24, has been suggested as a dominant candidate gene for carcinogenesis (7,8,10,11). However, since numerous genes other than CMYC are included in this region, multiple genes located at 8q24 may take part in the carcinogenesis.

In the present study, the CRC patients with loss of 20p12.2 demonstrated a high frequency of lymph node metastasis. The JAG1 gene is located at 20p12.2, and the NOTCH1 gene is at 9q34.4. Although these loci are associated with ARAJIRU syndrome, elevated expression of JAG1 and NOTCH1 was also reported as a marker of poor prognosis in breast cancer (12,13). The same association has been reported in CRCs (14). Furthermore, JAG1 mRNA has been shown significantly increased in most familial adenomatous polyposis (FAP) adenomas in comparison to that of normal intestinal tissue (15). Table IV demonstrates that a loss of 20p12.2 (clone 768), on which JAG1 is located, has a significant correlation with the lymph node metastasis of CRC ($p<0.01$). Gain of 8q24.3 and loss of 20p12.2 were independent factors for lymph node metastasis. All 7 CRCs that had either 8q24.3 gain or 20p12.2 loss had lymph node metastasis. Among 32 CRCs with either aberration, 26 tumors demonstrated lymph node metastasis. Furthermore, CRCs with lymphovascular invasion showed an increase of 8q2 gain in addition to 10q21 loss, which is reported to be associated with Alzheimer's disease (16).

CRCs with venous invasion were associated with an increased frequency of losses of various BAC clones. For example, losses of 12q15 and 17p13.1 encoding IFNG and p53, respectively, were both associated with venous invasion. Furthermore, the results showed a high frequency of losses of the cadherin-related genes, for example, the β-catenin domain was associated with lymph node metastasis, and the α-3 catenin domain with lymphovascular invasion (14,16). Therefore, metastatic colorectal cancer might not express either cadherin or catenin proteins, which may result in exfoliated cancer cell metastases.

In conclusion, the present study examined the relationship between the clinicopathological features of CRC and DCNAs: a gain of 8q24.3 and losses of 9q33.1 and 20p12.2 were associated with lymph node metastasis, gain of 8q24.3 and loss of 9q33.1 with disease stage, gain of 8q21.11 and loss of 10q21.3 with lymphovascular invasion and losses of 3p25.1,

10p15.3, 12q15, and 17p13.1 for venous invasion. These aberrations can be regarded as genomic biomarkers to predict the clinical outcome of the patients with CRC, and are expected to serve to individualize the treatment of CRC patients. Further clarification of the genomic and clinical correlations is expected to provide more useful genomic biomarkers for clinical management of the patients with CRC.

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