Genome-wide DNA Copy-number Analysis in ACTS-CC Trial of Adjuvant Chemotherapy for Stage III Colonic Cancer

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Abstract. *Background: The adjuvant chemotherapy trial of TS-1 for colon cancer phase III trial was designed to validate the non-inferiority of the oral fluoropyrimidine S-1 to uracil and tegafur/leucovorin as adjuvant chemotherapy for stage III colonic cancer. As a prospective biomarker study of this trial, DNA copy number was studied using formalin-fixed, paraffin-embedded specimens. Materials and Methods: FFPE blocks were obtained from 795 patients of the 1,535 patients enrolled in the study. The quality of extracted DNA was assessed using arbitrarily primed polymerase chain reaction and microfluidic analysis. Genomic copy-number alterations in cancer were analyzed by high-density single-nucleotide polymorphism arrays. Copy-number changes in Japanese patients with colonic cancer were compared with those in Western countries using data from a previously reported meta-analysis. We then compared genome-wide segment copy number and clinicopathological features of colorectal cancer. Results: Genome-wide copy number was analyzed in 161 samples and DNA copy-number alteration profiles showed frequent DNA copy-number gains at chromosome 7, 8q and 13, and losses at 4, 5q, 8p, 17p and 18q. The weighted kappa statistic from comparing copy-number alteration status with data from Western countries was 0.828 (95% confidence interval=0.786 -0.871). DNA copy-number alterations of 8,684 segments were compared with clinicopathological features in 161 patients. Location of the tumor correlated with genomic segments of chromosome 4, 5, 7, 8, 13, 14, 18 and 20. Differentiation of the tumor correlated with segments in chromosome 4, 6, 8, 11, 13, 14,15, 16, 17 and 20. Conclusion: Somatic copy-number alteration profiles of stage III colonic cancer in the Japanese ACTS-CC trial closely agreed with the results of previous Western studies. Location and differentiation of the tumor correlated with DNA copy-number alterations. Our findings will facilitate understanding the characteristics of colonic cancer. Further investigation may contribute to the exploration of valid biomarkers.*

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Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide. Studies performed in the 1990s demonstrated that postoperative adjuvant chemotherapy using intravenous administration of fluorouracil (FU) plus leucovorin (LV) improved the survival of patients with stage III (T, any N1-2, M0) colonic cancer (1, 2). Postoperative intravenous therapy with 5-FU/LV has come to be a standard adjuvant chemotherapy for colonic cancer. Subsequent phase III trials have demonstrated the non-inferiority of the oral fluoropyrimidines capecitabine and uracil plus tegafur (UFT)/LV owing to their easy administration (3, 4). Oral fluoropyrimidines have been widely employed as adjuvant therapy for colorectal cancer in Japan (5, 6). The randomized controlled phase III, the adjuvant chemotherapy trial of TS-1 for colon cancer (ACTS-CC) (TRICC0706), was designed to evaluate the non-inferiority of S-1 (an oral fluoropyrimidine consisting of tegafur, 5-chloro-2,4-dihydropyrimidine, and potassium oxonate) to UFT/LV in stage III colonic cancer and rectosigmoidal cancer (7). Screening of suitable patients for adjuvant chemotherapy is advisable, as response to treatment is diverse and increasingly appears to be dependent on aberrations in individual tumors.

Somatic DNA copy-number alterations (SCNAs; distinguished from germline copy-number variations, CNVs) are common in cancer (8, 9). Two major types of genomic variability, chromosomal instability (CIN) and microsatellite instability (MSI), have been recognized as alternative mechanisms of colorectal carcinogenesis with the more common, CIN, being present in about 65-70% of CRC cases (10, 11). Severity of loss of heterozygosity (LOH) also differs between tumors: some have LOH at many loci in different chromosomes, whereas others have less frequent LOH. These differences might reflect biological behavior of CRC (12).

Recently, the prognostic relevance of SCNAs in CRC has been suggested (13-16). However, in order to identify the major changes influencing clinical outcome, it is necessary to carry out detailed assessments such as array comparative genomic hybridization (CGH) based on large-scale prospective studies. Moreover, for the development of a biomarker study, it is important to include prospective translational studies with stringent quality control for reliable analysis (17). As the clinical outcome of CRC has been shown to differ between countries and regions (18), identification of biological differences between carcinomas is useful.

The present study used genome-wide copy-number analysis to identify candidate loci influencing the prognosis of patients with CRC or the efficacy of oral fluoropyrimidines as a biomarker study of the ACTS-CC trial. This report outlines the biomarker study of the ACTS-CC trial and the analysis of SCNAs in colonic cancer tissues from Japanese patients.

Materials and Methods

Sample collection. The biomarker study was predefined in the ACTS-CC protocol. Formalin-fixed paraffin-embedded (FFPE) cancer specimens were obtained from patients with stage III colonic cancer who underwent curative surgery, and were randomly assigned to receive five courses of UFT/LV [300 600 mg/day according to body surface area (BSA)] and LV (75 mg/day) on days 1 28 followed by 7 days of rest, or four courses of S-1 (80, 100, or 120 mg/day according to BSA) on days 1 and 28 followed by 14 days of rest. This study was conducted in accordance with the 'Declaration of Helsinki' and the 'Ethical Guidelines for Clinical Research'. The protocol was approved at each participating site by the relevant independent Ethics Committee or Institutional Review Board. All patients provided written informed consent before participating. The study protocol of ACTS-CC trial was registered at ClinicalTrials.gov (NCT00660894).

DNA preparation. Manual microdissection was performed to extract genomic DNA (gDNA) from 10-μm-thick FFPE specimens. After a review by a pathologist of representative hematoxylin and eosin (H&E)-stained slides to estimate the tumor load per sample, samples were stained with nuclear fast red (Sigma-Aldrich, St Louis, MO, USA) and prepared for manual microdissection. Malignant cells were selected under magnification (×5 to ×10), and dissected using a scalpel as described previously (17). After proteinase K treatment, phenol- chloroform extraction and ethanol precipitation, DNA concentrations were measured by spectrophotometry (NanoDrop1000: Thermo Fisher Scientific, Waltham, MA, USA).

Arbitrarily primed polymerase chain reaction (AP-PCR) amplification was performed in a total volume of 20 μl containing 3 μl DNA (30, 3, or 0.3 ng), 10 μl of 2 × HotStarTaq DNA polymerase (Qiagen, Hilden, Germany), and the primer 5'-AGGTCACTGA-3' [19]. PCR cycling conditions were 95˚C for 5 min followed by 40 cycles at 94˚C for 1 min, 36˚C for 1 min, and 72˚C for 2 min. DNA quality was then assessed and classified into four grades according to PCR-amplified DNA fragment length in at least two of three input templates for each sample by microfluidic analysis (Bioanalyzer; Agilent Technology, Santa Clara, CA, USA): grade A, ≥700 bp; grade B, <700 bp and ≥400 bp; grade C, <400 bp and ≥200 bp; and grade D, <200 bp. Samples classified as grade A or grade B were used for DNA microarray, and the remainder were stored for future validation steps.

Microarray hybridization. gDNA copy-number alterations were analyzed by high-density single-nucleotide polymorphism (SNP) arrays (Human 250K StyI array; Affymetrix, Santa Clara, CA, USA) using samples of grade A or grade B quality. For GeneChip arrays (Affymetrix), 250 ng DNA was used according to the manufacturer's protocols. Briefly, gDNA was digested with StyI restriction enzyme, and adaptors were added to restriction fragment ends using T4 DNA ligase. Adaptor-modified samples were PCR-amplified using Clontech® Titanium Taq (Takara Bio Inc., Tokyo, Japan), and amplification products were purified using a Clontech® DNA-amplification cleanup kit (Clontech Laboratories, Inc., Mountain View, CA, USA). Purified DNA (90 μg) was fragmented using Affymetrix® Fragmentation Reagent.

We excluded from further analysis any samples that contained <90 μg DNA after PCR amplification, and stored them for future validation steps. Biotin-labeling of fragmented samples was accomplished using the GeneChip DNA-labeling reagent (Affymetrix). Biotin-labeled DNA was hybridized on StyI Affymetrix microarrays at 49˚C for 16 18 h in an Affymetrix® rotation oven. After hybridization, a probe array wash and stain procedures were carried out on the automatic Affymetrix® Fluidics Stations according to the manufacturer's instructions. Microarrays were then scanned, and raw data were collected by the Affymetrix® GeneChip Scanner 3000.

Data analysis and software. To detect SCNAs, the scanned image CEL files were imported into Partek Genomics Suite v6.6 (Partek Inc., St. Louis, MO, USA). We filtered out probes that detect SNPs on Sty I fragments >700 bp, then normalized individual probe signals from HapMap project (http://hapmap.ncbi.nlm.nih.gov/) base-line data to 1.0 (haploid genome). The genomic segmentation algorithm used three criteria to find a segment: firstly, breakpoints (region boundaries) were chosen to give optimal statistical significance ($p<0.001$); secondly, detected regions contained a minimum of 10 probes; and thirdly, segments had a copy number >2.3 or <1.7, as previously described (20). Depending on the differences in sample frequency (delta; % of gain – % of loss), we classified 293 cytogenetic bands as "gain" (Delta >2%), "loss" (Delta <2%), or "unchanged" (2% > Delta >2%). Concordance was calculated by weighted kappa statistics using the R-software package (https://www.r-project.org/). Detected DNA segments,

Table I. *Patient characteristics*.

		Efficacy analysis set		DNA analysis set		SNP array analysis set	
		N=1518	(%)	N=777	(%)	N=161	(%)
Age	Median (range), years	66.0 (23-80)		66.0 (23-80)		66.0 (34-80)	
	≥70 Years	536	(35.3)	276	(35.5)	58	(36.0)
Gender	Male	814	(53.6)	442	(56.9)	96	(59.6)
	Female	704	(46.4)	335	(43.1)	65	(40.4)
Tumor location	Right colon (C, A, T)	592	(39.0)	320	(41.2)	73	(45.3)
	Left colon (D, S)	592	(39.0)	303	(39.0)	60	(37.2)
	Rectosigmoid	334	(22.0)	154	(19.8)	28	(17.4)
Depth of tumor invasion*	T1	88	(5.8)	41	(5.3)	8	(5.0)
	T2	153	(10.1)	73	(9.4)	14	(8.7)
	T3	862	(56.8)	440	(56.6)	87	(54.0)
	T4a	353	(23.3)	186	(23.9)	42	(26.1)
	T4b	62	(4.1)	37	(4.8)	10	(6.2)
Histology of tumor	Well diff. adenoca.	509	(33.6)	271	(33.8)	53	(33.0)
	Mod. diff. adenoca.	905	(59.6)	441	(56.8)	97	(60.2)
	Poorly diff. adenoca.	52	(3.4)	33	(4.3)	10	(6.2)
	Mucinous/other	52	(3.5)	32	(4.1)	1	(0.6)
Number of LN metastases	Median (range)	2 (1-26)		2 (1-25)		2 (1-25)	
LN metastasis*	N1a	662	(43.6)	326	(42.0)	66	(41.0)
	N1b	531	(35.0)	275	(35.4)	56	(34.8)
	N2a	231	(15.2)	116	(14.9)	27	(16.8)
	N2b	94	(6.2)	60	(7.7)	12	(7.5)
Stage*	IIIA	225	(14.8)	107	(13.8)	21	(13.0)
	IIIB	1076	(70.9)	494	(63.6)	101	(62.7)
	IIIC	217	(14.3)	176	(22.7)	39	(24.2)

SNP: Single nucleotide polymorphism; C: cecum; A: ascending colon, T: transverse colon; D: descending colon, S: sigmoid colon, Well-diff.: well-differentiated adenocarcinoma, Mod. diff.: moderately differentiated adenocarcinoma, Poorly diff.: poorly differentiated adenocarcinoma. *TNM Classification of Malignant Tumours (35).

Table II. *Comparison with meta-analysis data*.

		Meta-analysis					
		Primary carcinoma			Liver metastases		
		Gain	Unchanged	Loss	Gain	Unchanged	Loss
ACTS-CC study (7)	Gain	124	21	3	124	13	11
	Unchanged	10	17	10	22	7	8
	Loss	0	16	92	9	3	96
Meta-analysis (liver metastases) (21)	Gain	116	13	5			
	Unchanged	34	4	16			
	Loss	5	6	94			

representing potential deletions or highly polymorphic regions, were plotted along chromosomes to produce copy-number genome view. Either Pearson's correlation test or the Wilcoxon test was used to assess correlations between segmental copy number and clinicopathological parameters. All statistical analysis used the SAS software package ver.9.3, JMP software version 8.11 (SAS Institutes Inc., Cary, NC, USA) and R software package.

Results

Patients and sample preparation. In total, 1,535 patients from 358 hospitals were enrolled in the ACTS-CC trial between April 2008 and June 2009. Among the 1,518 patients included in the efficacy analysis, FFPE samples

were obtained from 795 patients (52.4%). Genomic DNAs were successfully extracted from 777 samples. The characteristics of the patients are shown in Table I. There were no significant differences between the characteristics of the patients in the efficacy analysis set, the DNA analysis set, and the SNP array analysis set.

Quality assessment of samples and SNP array analysis. Quality assessments were performed for 777 samples (Figure 1). Of these, 482 passed the AP-PCR assessment, and 161 were successfully amplified. These pre-determined quality control processes allowed 616 samples to be prepared for future validation steps. The success rate for SNP array detection was affected by the quality of DNA (data not shown): gDNA samples of grade A quality had a success rate of 45.1%, which was significantly higher than that of grade B samples (28.6%). The average SNP call rate was 86.2% [95% confidence interval (CI)=85.4-87.0%].

Copy-number alterations. Figure 2 shows the frequency of copy-number alterations across all samples. The fraction of samples with copy-number gain (red) or loss (blue) was plotted along each chromosome. The most common changes were losses of chromosomes 4, 5q, 8p, 15q, 17p, and 18q, and gains of chromosomes 7, 8q, 13, and 20. Concordance of the copy-number gain or loss of each cytogenetic band with meta-analysis data reported by Diep *et al*. (21) was assessed by weighted kappa statistics (Table II, Figure 3). A contingency table is shown for both the present study data (Japan, primary carcinomas, n=161) and meta-analysis data (Western countries, primary carcinomas, n=720; liver metastases, n=139). The weighted kappa statistic was 0.828 (95% CI=0.786-0.871) for primary colonic cancer and 0.735 (95% CI=0.668-0.802) for liver metastases, whereas that between primary carcinomas and liver metastases in the meta-analysis was 0.748 (95% CI=0.692-0.804).

Correlation between copy-number alterations and clinicopathological parameters. DNA copy-number alterations of 8,684 segments were compared with clinicopathological features in 161 patients. Location and differentiation of the tumor correlated with the frequency of DNA copy-number alterations (Figure 4). Namely, the frequency of DNA copy-number alterations of tumors in left-sided colon was significantly higher than that of right-sided colon, while copy-number alterations frequency of well or moderately differentiated tumors was significantly higher than that of poorly differentiated or mucinous tumors. Median copy numbers and *p*-values of each DNA segment were plotted along genomic coordinates according to the tumor location (Figure 5) and differentiation (Figure 6). Location of the tumor correlated with genomic segments of chromosome 4, 5, 7, 8, 13, 14, 18 and 20.

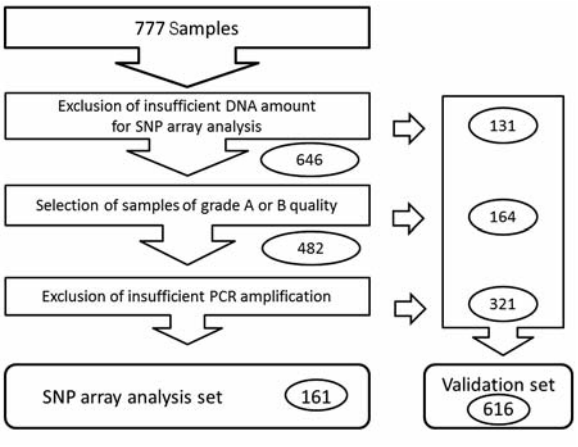

Figure 1. *Sample process chart*.

Differentiation of the tumor correlated with segments in chromosome 4, 6, 8, 11, 13, 14,15, 16, 17 and 20. Age and gender had no correlation with copy-number alterations (data not shown).

Discussion

Herein we report on an outline of the biomarker study in the ACTS-CC trial. Although strict quality control processes limited the number of samples subjected to array-CGH measurements, we obtained precise data from 161 out of 777 (20.7%) patients with CRC regarding cancer chromosomal alteration, and 616 gDNA samples that will be used for validation steps in PCR-based measurements. SCNA profiles of stage III colonic cancer in Japan were found to agree closely with the results of previous studies in Western countries. SCNAs were significantly frequent in left-sided tumors and well-differentiated tumors.

Previous articles have reported the usefulness of genomic analysis of FFPE cancer tissue samples using SNP arrays (22-24). FFPE samples could be advantageous for biomarker studies in clinical trials as they are readily available and are routinely examined in general municipal hospitals. Because the molecular profiles of FFPE samples might be affected by fixation and preservation methods, stringent quality control is important for reliable analysis. Our microarray data were obtained from approximately 20% of the total number of samples. The rate-limiting process was the PCR amplification step during target preparation prior to microarray hybridization. Although the successful execution rate rises if the number of PCR cycles or number of independent PCR reactions increases, as described by Thompson *et al*. (25), we carried out this process according to standard protocols so that PCR bias would not affect copy-number data.

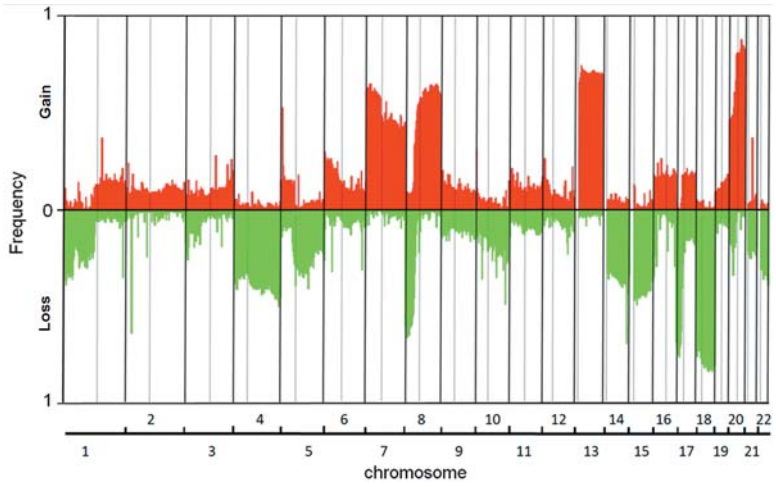

Figure 2. *Chromosome copy-number alterations in colorectal cancer cells. Frequency of copy-number alterations of genomic segments elucidated by circular segmentation analysis. Alterations are ordered by physical position for chromosomes 1 to 22. Copy-number gain, red bar; copy-number loss, green bar.*

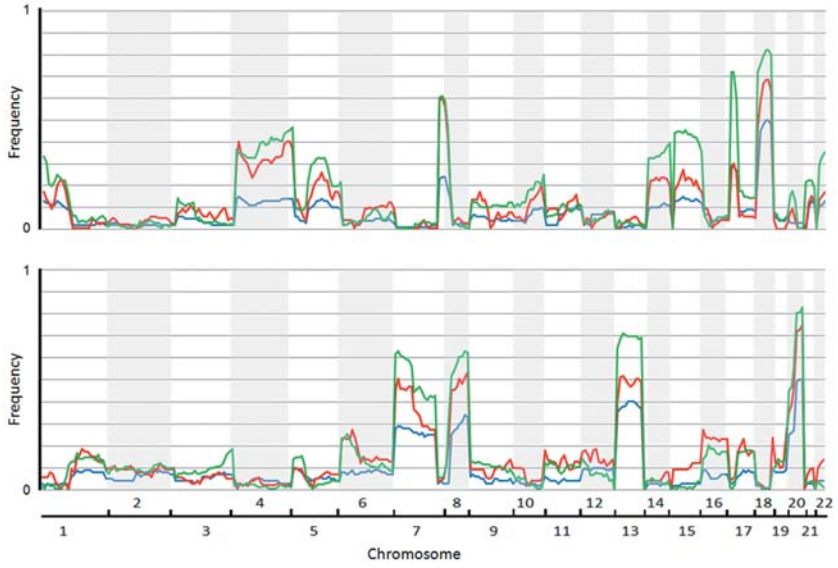

Figure 3. *Comparison of copy-number profiles with meta-analysis data. Frequencies of copy-number alterations in each cytogenetic band are ordered by physical position for chromosomes 1 to 22. Upper panel shows the frequency of loss; lower panel shows the frequency of gain. Green represents data from the present study, blue represents primary colonic cancer data, and red represents liver metastases data, from meta-analysis by Diep et al. (21).*

Many analyses have attempted to demonstrate new factors that are independently prognostic or predictive, but they have often failed to establish a role in therapeutic decision making. The five levels of evidence recommended by The American Society of Clinical Oncology Tumor Markers Guidelines Committee and the REMARK criteria have been used to establish the clinical utility of new biomarkers (26, 27). However, the methodological difficulties involved in implementing these guidelines led Simon *et al*. to suggest a refined grading system to assist in establishing the clinical utility of biomarkers (28). Although the ACTS-CC trial was not designed to evaluate the medical utility of biomarkers, the present study might determine those with high levels of evidence according to the grading system proposed by Simon *et al*. based on the following considerations: firstly, biomarker utility was accommodated in the design of the ACTS-CC trial; secondly, patients were prospectively enrolled, treated, and followed-up in a prospective controlled trial; thirdly, specimens were collected prospectively; fourthly, analysis focusing on markers was applied before the

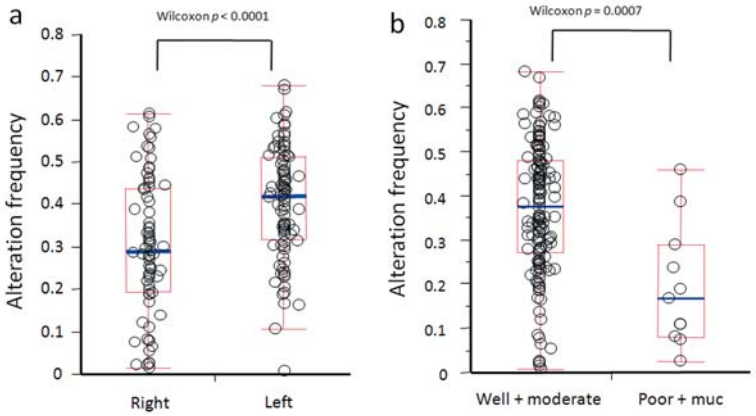

Figure 4. *Comparison of frequency of copy-number alterations by tumor location in the colon (a) and differentiation (b). Median and quartile box plots are shown. Muc: Mucinous.*

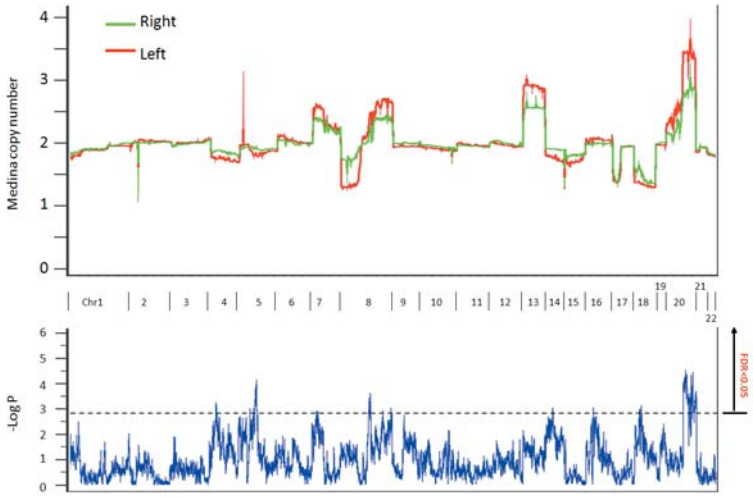

Figure 5. *Trend lines of median copy number (upper) and a Manhattan plot of their associations (lower) with tumor location in the colon, displayed along genomic coordinates. Dotted line: threshold line for false-discovery rate (FDR) (p=0.05).*

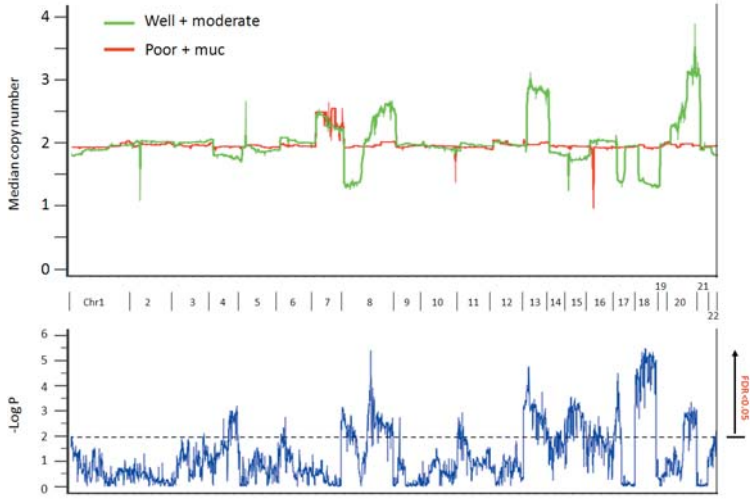

Figure 6. *Trend lines of median copy number (upper) and a Manhattan plot of their associations (lower) with differentiation of the tumor, displayed along genomic coordinates. Dotted line: Threshold line for false-discovery rate (FDR) (p=0.05). Muc: Mucinous.*

assays; and fifthly, an independent validation step was used to confirm the candidates identified by the microarray screening step. The current Japanese biomarker study of the ACTS-CC phase III trial could provide insights into the biology and can be used for the development of new drugs.

CRC has been shown to have molecular heterogeneity that results from different combinations of genetic and epigenetic alterations. Recently, molecularly defined subtypes of stage III colonic cancer were shown to have associations in clinical and pathological features and survival outcomes (29). These subtypes had significant relationship with patient characteristics, such as age, gender and race, and the pathological features, such as tumor location, histological grade, N stage and T stage. In the present study, location and differentiation of the tumor correlated with DNA copy-number alterations. Our study might provide new molecular markers to establish a new classifier.

Data from the Organization for Economic Co-operation and Development Health Care Quality Indicators showed that Japan has the highest 5-year relative survival rate for CRC (18), and a favorable 5-year overall survival (87.5%) for stage III colonic cancer treated with adjuvant UFT/LV was reported in a Japanese randomized phase III study (30). This result is an improvement on the findings of the NSABP-C06 trial, with a 5-year overall survival of 69.6%. Improved outcomes in Japanese populations are speculated to be caused by differences in colonic cancer surgery (D3 resection) (31), stage migration in pathological examination, or racial/ethnic bias (32). For the progress of treatment of CRC, investigations of these differences are important. The present study, suggesting differences in tumor biology between Japan and Western countries, could contribute to those investigations as well as to the surgical treatment and pathological management of colonic cancer.

Although the efficacy of oral fluoropyrimidines for the adjuvant treatment of colonic cancer has been demonstrated (33), most related phase III trials are being carried out in Japan (5). Our findings could be useful when these phase III findings are introduced to Western countries and will help explore the basic rationale of adjuvant chemotherapy for colonic cancer. Survival data of ATCS-CC trail has been opened and adjuvant chemotherapy using S-1 for stage III colonic cancer was confirmed to be non-inferior in disease-free survival compared to UFT/LV (34). Our future study will provide important prognostic information in stage III colonic cancer with implications for patient management.

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References

1 Wolmark N, Rockette H, Fisher B, Wickerham DL, Redmond C, Fisher ER, Jones J, Mamounas EP, Ore L and Petrelli NJ: The benefit of leucovorin-modulated fluorouracil as postoperative adjuvant therapy for primary colonic cancer: results from NSABP protocol C-03. J Clin Oncol *11*: 1879-1887, 1993.

2 Haller DG, Catalano PJ, Macdonald JS, O'Rourke MA, Frontiera MS, Jackson DV, and Mayer RJ: Phase III study of fluorouracil, leucovorin, and levamisole in high-risk stage II and III colonic cancer: final report of Intergroup 0089. J Clin Oncol *23*: 8671-8678, 2005.

3 Twelves C, Wong A, Nowacki MP, Abt M, Burris H 3rd, Carrato A, Cassidy J, Cervantes A, Fagerberg J, Georgoulias V, Husseini F, Jodrell D, Koralewski P, Kröning H, Maroun J, Marschner N, McKendrick J, Pawlicki M, Rosso R, Schüller J, Seitz JF, Stabuc B, Tujakowski J, Van Hazel G, Zaluski J and Scheithauer W: Capecitabine as adjuvant treatment for stage III colonic cancer. N Engl J Med *352*: 2696-2704, 2005.

4 Lembersky BC, Wieand HS, Petrelli NJ, O'Connell MJ, Colangelo LH, Smith RE, Seay TE, Giguere JK, Marshall ME, Jacobs AD, Colman LK, Soran A, Yothers G, and Wolmark N: Oral uracil and tegafur plus leucovorin compared with intravenous fluorouracil and leucovorin in stage II and III carcinoma of the colon: results from National Surgical Adjuvant Breast and Bowel Project Protocol C-06. J Clin Oncol *24*: 2059-2064, 2006.

5 Rousseau B, Chibaudel B, Bachet JB, Larsen AK, Tournigand C, Louvet C, André T, de Gramont A; GERCOR (French Oncology Research Group): Stage II and stage III colonic cancer treatment advances and future directions. Cancer J *16*: 202-209, 2010.

6 Watanabe T: Ongoing colorectal cancer adjuvant trials in Japan. Curr Colorectal Cancer Rep *6*: 168-174, 2010.

7 Mochizuki I, Takiuchi H, Ikejiri K, Nakamoto Y, Kinugasa Y, Takagane A, Endo T, Shinozaki H, Takii Y, Takahashi Y, Mochizuki H, Kotake K, Kameoka S, Takahashi K, Watanabe T, Watanabe M, Boku N, Tomita N, Matsubara Y and Sugihara K: Safety of UFT/LV and S-1 as adjuvant therapy for stage III colonic cancer in phase III trial: ACTS-CC trial. Br J Cancer *106*: 1268-1273, 2012.

8 Hanahan D and Weinberg RA: The hallmarks of cancer. Cell *100*: 57-70, 2000.

9 Knutsen T, Gobu V, Knaus R, Padilla-Nash H, Augustus M, Strausberg RL, Kirsch IR, Sirotkin K and Ried T: The interactive online SKY/M-FISH & CGH Database and the Entrez Cancer Chromosomes search database: Linkage of chromosomal aberrations with the genome sequence. Genes Chromosomes Cancer *44*: 52-64, 2005.

10 Lengauer C, Kinzler KW and Vogelstein B: Genetic instabilities in human cancers. Nature *396*: 643-649, 1998.

11 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava S: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res *58*: 5248-5257, 1998.

12 Watanabe T, Kobunai T, Yamamoto Y, Matsuda K, Ishihara S, Nozawa K, Yamada H, Hayama T, Inoue E, Tamura J, Iinuma H, Akiyoshi T and Muto T: Chromosomal instability (CIN) phenotype, CIN high or CIN low, predicts survival for colorectal cancer. J Clin Oncol *30*: 2256-2264, 2012.

13 Diep CB, Thorstensen L, Meling GI, Skovlund E, Rognum TO and Lothe RA: Genetic tumor markers with prognostic impact in Dukes' stages B and C colorectal cancer patients. J Clin Oncol *21*: 820-829, 2003.
14 Watanabe T, Wu TT, Catalano PJ, Ueki T, Satriano R, Haller DG, Benson AB 3rd and Hamilton SR: Molecular predictors of survival after adjuvant chemotherapy for colonic cancer. N Engl J Med *344*: 1196-1206, 2001.
15 Kim MY, Yim SH, Kwon MS, Kim TM, Shin SH, Kang HM, Lee C and Chung YJ: Recurrent genomic alterations with impact on survival in colorectal cancer identified by genome-wide array comparative genomic hybridization. Gastroenterology *131*: 1913-1924, 2006.
16 Postma C, Terwischa S, Hermsen MA, van der Sijp JR and Meijer GA: Gain of chromosome 20q is an indicator of poor prognosis in colorectal cancer. Cell Oncol *29*: 73-75, 2007.
17 Bosman F, Yan P, Tejpar S, Fiocca R, Cutsem E, Kennedy R, Dietrich D and Roth A: Tissue biomarker development in a multicentre trial context: a feasibility study on the PETACC3 stage II and III colonic cancer adjuvant treatment trial. Clin Cancer Res *15*: 5528-5533, 2009.
18 OECD: Survival and mortality for colorectal cancer. Health at a Glance 2009- OECD Indicators. OECD Publishing, Paris: Chapter-5.9, 2009.
19 Madico G, Akopyants NS and Berg DE: Arbitrarily primed PCR DNA fingerprinting of Escherichia coli O157: H7 strains by using templates from boiled cultures. J Clin Microbiol *33*: 1534-1536, 1995.
20 Olshen AB, Venkatraman ES, Lucito R and Wigler M: Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics *5*: 557-572, 2004.
21 Diep CB, Kleivi K, Ribeiro FR, Teixeira MR, Lindgjaerde OC and Lothe RA: The order of genetic events associated with colorectal cancer progression inferred from meta-analysis of copynumber changes. Genes Chromosomes Cancer *45*: 31-41, 2006.
22 Monzon FA, Hagenkord JM, Lyons-Weiler MA, Balani JP, Parwani AV, Sciulli CM, Li J, Chandran UR, Bastacky SI and Dhir R: Whole-genome SNP arrays as a potential diagnostic tool for the detection of characteristic chromosomal aberrations in renal epithelial tumors. Mod Pathol *21*: 599-608, 2008.
23 Jacobs S, Thompson ER, Nannya Y, Yamamoto G, Pillai R, Ogawa S, Bailey DK and Campbell IG: Genome-wide, high-resolution detection of copy number, loss of heterozygosity, and genotypes from formalin-fixed, paraffin-embedded tumor tissue using microarrays. Cancer Res *67*: 2544-2551, 2007.
24 Tuefferd M, De Bondt A, Van Den Wyngaert I, Talloen W, Verbeke T, Carvalho B, Clevert DA, Alifano M, Raghavan N, Amaratunga D, Göhlmann H, Broët P and Camilleri-Broët S: Genome-wide copy-number alterations detection in fresh frozen and matched FFPE samples using SNP 6.0 arrays. Genes Chromosomes Cancer *47*: 957-964, 2008.
25 Thompson ER, Herbert SC, Forrest SM and Campbell IG: Whole genome SNP arrays using DNA derived from formalin-fixed, paraffin-embedded ovarian tumor tissue. Hum Mutat *26*: 384-389, 2005.
26 Hayes DF, Bast RC, Desch CE, Fritsche H Jr, Kemeny NE, Jessup JM, Locker GY, Macdonald JS, Mennel RG, Norton L, Ravdin P, Taube S and Winn RJ: Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst *88*: 1456-1466, 1996.
27 McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M and Clark GM; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics: Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst *97*: 1180-1184, 2005.
28 Simon R, Paik S and Hayes DF: Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst *101*: 1446-1452, 2009.
29 Sinicrope FA, Shi Q, Smyrk T, Thibodeau SN, Dienstmann R, Guinney J, Bot BM, Tejpar S, Delorenzi M, Goldberg RM, Mahoney M, Sargent DJ and Alberts SR: Molecular markers identify subtypes of stage III colonic cancer associated with patient outcomes. Gastroenterology *148*: 88-99, 2015.
30 Shimada Y, Hamaguchi T, Mizusawa J, Saito N, Kanemitsu Y, Takiguchi N, Ohue M, Kato T, Takii Y, Sato T, Tomita N, Yamaguchi S, Akaike M, Mishima H, Kubo Y, Nakamura K, Fukuda H and Moriya Y: Randomized phase III study of adjuvant chemotherapy with oral uracil and tegafur plus leucovorin versus intravenous fluorouracil and levofolinate in patients with stage III colorectal cancer who have undergone Japanese D2/D3 lymph node dissection: Final results of JCOG0205. Eur J Cancer *50*: 2231-2240, 2014.
31 West NP, Kobayashi H, Takahashi K, Perrakis A, Weber K, Hohenberger W, Sugihara K and Quirke P: Understanding optimal colonic cancer surgery: comparison of Japanese D3 resection and European complete mesocolic excision with central vascular ligation. J Clin Oncol *30*: 1763-1769, 2012.
32 Hashiguchi Y, Hase K, Ueno H, Shinto E, Naito Y, Kajiwara Y, Kuroda T, Yamamoto J and Mochizuki H: Impact of race/ethnicity on prognosis in patients who underwent surgery for colonic cancer: analysis for white, African, and East Asian Americans. Ann Surg Oncol *19*: 1517-1528, 2012.
33 Andre T, Afchain P, Barrier A, Blanchard P, Larsen AK, Tournigand C, Louvet C and de Gramont A: Current status of adjuvant therapy for colonic cancer. Gastrointest Cancer Res *1*: 90-97, 2007.
34 Yoshida M, Ishiguro M, Ikejiri K, Mochizuki I, Nakamoto Y, Kinugasa Y, Takagane A, Endo T, Shinozaki H, Takii Y, Mochizuki H, Kotake K, Kameoka S, Takahashi K, Watanabe T, Watanabe M, Boku N, Tomita N, Nakatani E and Sugihara K on behalf of the ACTS-CC study group: S-1 as adjuvant chemotherapy for stage III colonic cancer: a randomized phase III study (ACTS-CC trial). Ann Oncol *25*: 1743-1749, 2014.
35 Sobin LH, Wittekind C and Gospodarowicz MK: TNM Classification of Malignant Tumours-Seventh Edition. Wiley-Blackwell, 2009.

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