risk: the Fukuoka colorectal cancer study. *Scand J Gastroenterol* 2011;46:165–72.
23. Geng L, Yao Z, Yang H, et al. Association of CA repeat polymorphism in estrogen receptor beta gene with postmenopausal osteoporosis in Chinese. *J Genet Genomics* 2007;34:868–76.
24. Westberg L, Baghaei F, Rosmond R, et al. Polymorphisms of the androgen receptor gene and the estrogen receptor beta gene are associated with androgen levels in women. *J Clin Endocrinol Metab* 2001;86:2562–8.
25. Moore JT, McKee DD, Slentz-Kesler K, et al. Cloning and characterization of human estrogen receptor beta isoforms. *Biochem Biophys Res Commun* 1998;247:75–8.
26. Jassam N, Bell SM, Speirs V, et al. Loss of expression of oestrogen receptor beta in colon cancer and its association with Dukes' staging. *Oncol Rep* 2005;14:17–21.
27. Arai T, Esaki Y, Sawabe M, et al. Hypermethylation of the hMLH1 promoter with absent hMLH1 expression in medullary-type poorly differentiated colorectal adenocarcinoma in the elderly. *Mod Pathol* 2004;17:172–9.
28. Wong NA, Malcomson RD, Jodrell DI, et al. ERbeta isoform expression in colorectal carcinoma: an in vivo and in vitro study of clinicopathological and molecular correlates. *J Pathol* 2005;207:53–60.
29. Campbell PT, Cotterchio M, Dicks E, et al. Excess body weight and colorectal cancer risk in Canada: associations in subgroups of clinically defined familial risk of cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:1735–44.
30. Laake I, Thune I, Selmer R, et al. A prospective study of body mass index, weight change, and risk of cancer in the proximal and distal colon. *Cancer Epidemiol Biomarkers Prev* 2010;19:1511–22.
31. Lin J, Zhang SM, Cook NR, et al. Body mass index and risk of colorectal cancer in women (United States). *Cancer Causes Control* 2004;15:581–9.
32. Slattery ML, Ballard-Barbash R, Edwards S, et al. Body mass index and colon cancer: an evaluation of the modifying effects of estrogen (United States). *Cancer Causes Control* 2003;14:75–84.
33. Reeves GK, Pirie K, Beral V, et al. Cancer incidence and mortality in relation to body mass index in the million women study: cohort study. *Br Med J* 2007;335:1134.
34. Terry PD, Miller AB, Rohan TE. Obesity and colorectal cancer risk in women. *Gut* 2002;51:191–4.
35. Giovannucci E, Michaud D. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 2007;132:2208–25.
36. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 1998;139:4252–63.
37. Zhu BT, Han GZ, Shim JY, et al. Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: insights into the structural determinants favoring a differential subtype binding. *Endocrinology* 2006;147:4132–50.

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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

FANCJ Expression Predicts the Response to 5-Fluorouracil-Based Chemotherapy in MLH1-Proficient Colorectal Cancer

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ABSTRACT

Purpose. Fanconi anemia protein, FANCJ, directly interacts with MLH1, a key protein involved in DNA mismatch repair. Deficient mismatch repair, or microsatellite instability, is a potent marker for the ineffectiveness of 5-fluorouracil (5-FU) in colorectal cancer (CRC). We investigated the significance of FANCJ expression in CRC, focusing on the effects of 5-FU-based adjuvant chemotherapy.

Methods. Clinicopathologic features and immunohistochemical expression of FANCJ and MLH1 were studied in 219 patients with CRC. We also analyzed 5-FU sensitivity in CRC cell lines with varying levels of FANCJ expression.

Results. FANCJ expression was elevated in tumor tissues compared with normal epithelial tissue. High expression of FANCJ was significantly associated with 5-FU resistance measured by the SDI test ($P < 0.05$) and poor recurrence-free survival (RFS) ($P < 0.05$). Among patients with stage II/III tumors who received 5-FU, patients with tumors exhibiting high FANCJ expression had significantly worse RFS than did patients with tumors exhibiting low FANCJ expression ($P < 0.01$). Among patients who did not receive adjuvant chemotherapy, FANCJ expression was not correlated with RFS ($P = 0.76$). High FANCJ expression was correlated with 5-FU resistance in tumors with normal MLH1 expression ($P < 0.05$) but not in tumors not expressing MLH1 ($P = 0.9$). In vitro, FANCJ overexpression was correlated with 5-FU resistance in MLH1-proficient HCT116 3-6 cells but not in MLH1-deficient HCT116 cells.

Conclusions. FANCJ could be a useful biomarker to predict the response to 5-FU and prognosis of CRC, particularly in tumors with normal MLH1 expression.

Colorectal cancer (CRC) is the second leading cause of cancer-related death in developed countries. Although surgical resection alone is potentially curative in early stages of the disease, patients at high risk for recurrence usually receive adjuvant chemotherapy.[1–3] 5-Fluorouracil (5-FU) is one of the most widely used antimetabolite drugs against CRC. Several markers have been proposed to predict the effects of 5-FU-based chemotherapy.[4–7] Microsatellite instability (MSI) is one such candidate marker.[8,9] A high frequency of MSI (MSI-H) is correlated with ineffectiveness of 5-FU-based adjuvant chemotherapy in stage II and III CRC.[8,10]

Because the DNA mismatch repair (MMR) system repairs repeat stretches looping out of the DNA strand, some MMR deficiencies lead to alterations in repeat sequences in the genome. Such alterations in microsatellite sequences are referred to as MSI.[11,12] In eukaryotic cells, MSH2/MSH6 (MutS-α complex) and MLH1/PMS2 (MutL-α complex) play critical roles in MMR-dependent DNA repair and in the damage-signaling process.[13] In terms of the mechanisms underlying MMR deficiency, the loss of MLH1 is the most frequent event and accounts for approximately 10–20 % of all cases of sporadic CRC.[14,15]

Fanconi anemia protein FANCJ is one of the proteins that directly interact with MLH1.[16] *FANCJ* is one of 14 Fanconi anemia (FA) genes in which mutations have been described

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in FA patients.[17,18] FA is a rare chromosomal instability disorder characterized by congenital abnormalities, bone marrow failure, predisposition to cancer, and cellular hypersensitivity to DNA interstrand cross-links. In the repair of cross-link-induced damage, cells lacking the FANCJ/MLH1 interaction are highly sensitive to mitomycin C.[16,19] FANCJ and MLH1 were recently reported to interact in the response to MMR-related DNA damage[20] and 5-FU causes DNA damage that is recognized by MMR proteins.[21,22]

The goal of our present study was to examine the clinical significance of FANCJ expression in CRC and to evaluate the influence of FANCJ/MLH1 expression on the outcome of 5-FU-based chemotherapy. Therefore, we conducted retrospective studies to investigate the relationship between FANCJ/MLH1 expression and clinicopathological features, the response to 5-FU, and survival rates. We also investigated the relationship between FANCJ/MLH1 status and 5-FU sensitivity in vitro.

PATIENTS AND METHODS

Patients and Specimens

We retrospectively analyzed 219 consecutive patients with any stage of CRC who underwent surgical resection at the Department of Surgery and Science, Kyushu University Hospital, between 1999 and 2008. The background and follow-up duration of the patients are summarized in Table 1. Histological diagnosis was based on the World Health Organization criteria.[23] Pathologic staging was performed according to the tumor-node-metastasis classification system revised in 2002.[24] We trialed 5-FU-based adjuvant chemotherapy in patients with stage II/III disease, such as oral tegafur-uracil (UFT), UFT/leucovorin, 5-FU/leucovorin, S-1 (tegafur, gimeracil, and oteracil), and 5′-deoxy-5-fluorouridine, according to the individual patient's general condition. Written, informed consent was obtained from all patients. The institutional review board of our university approved this study.

Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

We obtained cancer and normal epithelial tissue specimens from each patient. We extracted total RNA from each surgical specimen using ISOGEN (Nippongene, Tokyo, Japan) and cDNAs were synthesized from RNAs using SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA). Quantitative PCR amplification was performed by using a LightCycler® 480 System II (Roche Diagnostics, Basel, Switzerland) and a QuantiFast™ SYBR® Green PCR kit (QIAGEN, Hilden, Germany). The *FANCJ* transcript level was determined using β-actin as an endogenous control and human reference RNA (Promega, Madison, WI) as a standard for quantitation. The oligodeoxynucleotide primers were as follows: *FANCJ*, forward 5′-GACGCAATC AAATACAAAGGAGAGAA-3′ and reverse 5′-GCACGG GCATTGTCATCTGAG-3′; and β-actin, forward 5′-CTG GCACCACACCTTCTACAATG-3′ and reverse 5′-GGCG TACAGGGATAGCACAGC-3′.

Immunohistochemistry

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections. Four-micrometer thick sections were deparaffinized and heat-induced epitope retrieval was performed at 121 °C for 15 min. The slides were incubated with rabbit polyclonal antibodies against FANCJ [Novus Biologicals, Littleton, CO (#100–416; at 1:200 dilution) and Sigma, St. Louis, MO (B1310, 1:200 dilution)], and a mouse monoclonal antibody against MLH1 (BD Bioscience, San Jose, CA; 1:100 dilution) overnight at 4 °C. The sections were then treated with Envision Plus (Dako, Glostrup, Denmark), anti-rabbit secondary antibodies for FANCJ, and anti-mouse secondary antibodies for MLH1. Staining for FANCJ and MLH1 was completed using the streptavidin–biotin–peroxidase complex method with diaminobenzidine as a chromogen. We evaluated the nuclear staining of FANCJ and MLH1. High FANCJ expression has been reported in invasive breast cancers.[25] We used an invasive breast cancer specimen showing strong nuclear staining of FANCJ as a positive control (Fig. S1a). The immunoreactivity score (IRS) for FANCJ was determined as the multiplication of the values for the grade of intensity (0: no staining; 1: weak; 2: moderate; and 3: strong) and the number of positive cells (1: 0–10 %; 2: 11–50 %; 3: 51–80 %; and 4: >80 %). FANCJ expression was classified as high when the IRS was ≥6 (median value) or low when the IRS was ≤4. FANCJ staining was considered to be specific because FANCJ was detected in HCT116 and HCT116 3-6 whole-cell extracts by western blotting (Fig. S1e), similar staining patterns were achieved using antibodies from two manufacturers (Novus Biologicals; Fig. S1b, Sigma; Fig. S1c),[26,27] and the western blot signal decreased in cells treated with two siRNAs targeting the *FANCJ* gene (Fig. S1f). MLH1 staining was evaluated as previously described.[14] FANCJ and MLH1 expression was determined by four researchers, including two clinicopathologists, all of whom were blinded to the patients' clinical characteristics.

Surgical Specimen Chemosensitivity Test

The succinate dehydrogenase inhibition (SDI) test was conducted as previously described.[28,29] Tumors were

TABLE 1 Relationship among clinico-pathologic factors and FANCJ protein expression

Characteristic	All patients ($N = 219$)	Patients with tumors exhibiting low expression of FANCJ ($N = 109$)	Patients with tumors exhibiting high expression of FANCJ ($N = 110$)	P	Stage II/III Patients ($N = 136$)	Patients with tumors exhibiting low expression of FANCJ ($N = 63$)	Patients with tumors exhibiting high expression of FANCJ ($N = 73$)	P
Age (year)	65.7 ± 12.1	64.6 ± 12.4	66.8 ± 11.7	0.22	65.4 ± 12.7	63.7 ± 13	66.9 ± 12.4	0.17
Sex								
Male	142 (65)	73 (67)	69 (63)	0.51	91 (67)	43 (68)	48 (66)	0.76
Female	77 (35)	36 (33)	41 (37)		45 (33)	20 (32)	25 (34)	
Depth of invasion								
T1, T2	36 (17)	24 (22)	12 (11)	0.025	5 (4)	3 (5)	2 (3)	0.53
T3, T4	183 (83)	85 (78)	98 (89)		131 (96)	60 (95)	71 (97)	
Tumor grade								
Well differentiated	66 (30)	35 (32)	31 (28)	0.33	35 (26)	15 (24)	20 (27)	0.62
Other	153 (70)	74 (68)	79 (72)		101 (74)	48 (76)	53 (73)	
Lymph node metastasis								
–	115 (53)	61 (56)	54 (49)	0.31	70 (51)	31 (49)	39 (53)	0.62
+	104 (47)	48 (44)	56 (51)		66 (49)	32 (51)	34 (47)	
MSI								
MSI-S or L	160 (84)	75 (83)	85 (85)	0.75	98 (81)	42 (78)	54 (84)	0.42
MSI-H	30 (16)	15 (17)	15 (15)		23 (19)	12 (22)	11 (16)	
Stage of tumor								
I	26 (12)	19 (17)	7 (6)	0.11	–	–	–	0.98
II	83 (38)	40 (37)	43 (39)		68 (50)	31 (49)	37 (51)	
III	78 (36)	37 (34)	41 (37)		68 (50)	32 (51)	36 (49)	
IV	32 (15)	13 (12)	19 (17)		–	–	–	
CDDP sensitivity[a]								
High	125 (57)	27 (42)	32 (48)	0.47	33 (44)	15 (43)	18 (45)	0.85
Low	98 (43)	37 (58)	34 (52)		42 (56)	20 (57)	22 (55)	
5-FU sensitivity[a]								
High	80 (37)	48 (45)	32 (30)	0.021	50 (38)	31 (50)	19 (27)	0.005
Low	135 (63)	59 (55)	76 (70)		83 (62)	31 (50)	52 (73)	

MSI-S microsatellite stable; *MSI-L* low frequency of microsatellite instability; *MSI-H* high frequency of microsatellite instability; *CDDP* cis-dichloro-diamine-platinum; *5-FU* 5-fluorouracil

Data are numbers with percentages in parentheses unless otherwise indicated

Stage IV CRC were excluded

[a] Chemosensitivity was determined based on the succinate dehydrogenase inhibition test

classified as highly sensitive when cell viability in response to 5-FU was below the 75th percentile (median value) and the response to cis-dichloro-diamine-platinum (CDDP) was below the median value. Other tumors were classified as showing low sensitivity.

Microsatellite Instability Analysis

Microsatellite analysis using fluorescence-labeled primers and an automated DNA sequencer has already been described in detail.[30,31] Five human dinucleotide microsatellites (D2S123, D5S107, D10S197, D11S904, and D13S175) were used as markers. MSI status was classified as showing high frequency (MSI-H) when MSI was detected in at least two markers, as low frequency (MSI-L) when MSI was detected in one marker, and as microsatellite stable when no positive MSIs were detected.[32–34]

Cell Lines, Culture Conditions, and Reagents

HCT116 and HCT116 3-6 cells were kindly provided by Prof. Richard Boland (Baylor University Medical Center). Parental HCT116 cells have a hemizygous nonsense mutation in the hMLH1 gene located on chromosome 3. HCT116 3-6 cells were generated by microcell transfer of a single normal human chromosome 3 into HCT116 cells.[35] HCT116 and HCT116 3-6 cells were maintained in Dulbecco's modified Eagle's medium (Mediatech, Manassas, VA) containing 10 % fetal bovine serum (Invitrogen) in 5 % CO_2 at 37 °C.

FANCJ Overexpression in CRC Cell Lines

We generated adenoviruses expressing human *FANCJ* or luciferase genes with the ViraPower™ Adenoviral Expression System, according to the manufacturer's instructions (Invitrogen).[36] We used HCT116 or HCT116 3-6 cells after adenovirus infection (multiplicity of infection, 100) for 48 h. Transient transfections with FANCJ (2 μg DNA/dish) were performed using an X-treme GENE transfection kit, according to the manufacturer's instructions (Roche, Mannheim, Germany). The transfected cells were incubated for an additional 24 h. Cells transfected with empty vector were used as negative controls.

Western Blotting

Cells in the logarithmic growth phase were lysed in lysis buffer (1.0 % NP40; 50 mM Tris, pH 8.0; 150 mM NaCl; 0.5 % deoxycholate; 0.1 % SDS; 2 mM PMSF; 2 mM NaF, and 2 mM Na_3VO_4). The protein content of the supernatants was determined using a RC DC protein assay kit (Bio-Rad, Hercules, CA). Equal amounts of protein (10 μg/lane) were resolved. The membranes were blotted with two rabbit polyclonal antibodies for FANCJ (Novus Biologicals and Sigma), MLH1 (Santa Cruz, Santa Cruz, CA) and mouse monoclonal antibodies for β-actin (Sigma). The bound antibodies were detected using horseradish peroxidase-conjugated anti-rabbit (FANCJ and MLH1) or anti-mouse (β-actin) immunoglobulin (Amersham Pharmacia Biotech, Schenectady, NY, USA).

Cell Growth Analysis

Growth-suppressive effects were measured after treating cells for 96 h. Cell viability was assayed using cell counting kit-8 (Dojindo Laboratories, Tokyo, Japan) according to the manufacturer's instructions on a 96-well plate reader (iMark; Bio-Rad). The relative cell growth compared with control cells treated with dimethyl sulfoxide alone was calculated and plotted, and the mean growth-inhibitory concentration (IC_{50}) was determined. The experiment was repeated three times, each time in triplicate.

Statistical Analysis

Relationships among the clinicopathologic factors and FANCJ/MLH1 expression were analyzed by using χ^2 tests and logistic regression analysis. Survival curves were plotted using the Kaplan–Meier method, and significant differences among subgroups were compared using the log-rank test. Cox proportional hazards multivariate regression analysis with the forward stepwise procedure was performed to identify independent significant prognostic factors. Differences were considered significant at $P < 0.05$.

RESULTS

Elevated FANCJ Expression in CRC

First, we measured *FANCJ* transcript levels by quantitative RT-PCR in a set of 95 CRC specimens. *FANCJ* expression was significantly elevated in tumor tissues compared with that in normal epithelium ($P < 0.001$; Fig. 1a, b). Next, we examined FANCJ protein expression by immunohistochemistry (Fig. 1c–f). FANCJ protein expression also was apparent in tumor tissues but not in normal epithelium (Fig. 1d, e). On the other hand, there were some patients with tumors that exhibited low FANCJ expression (Fig. 1f). We classified all patients into two groups according to FANCJ expression. High FANCJ expression was significantly associated with 5-FU resistance measured by the SDI test ($P = 0.021$; Table 1). FANCJ expression also was associated with tumor depth but not with MSI status or CDDP sensitivity.

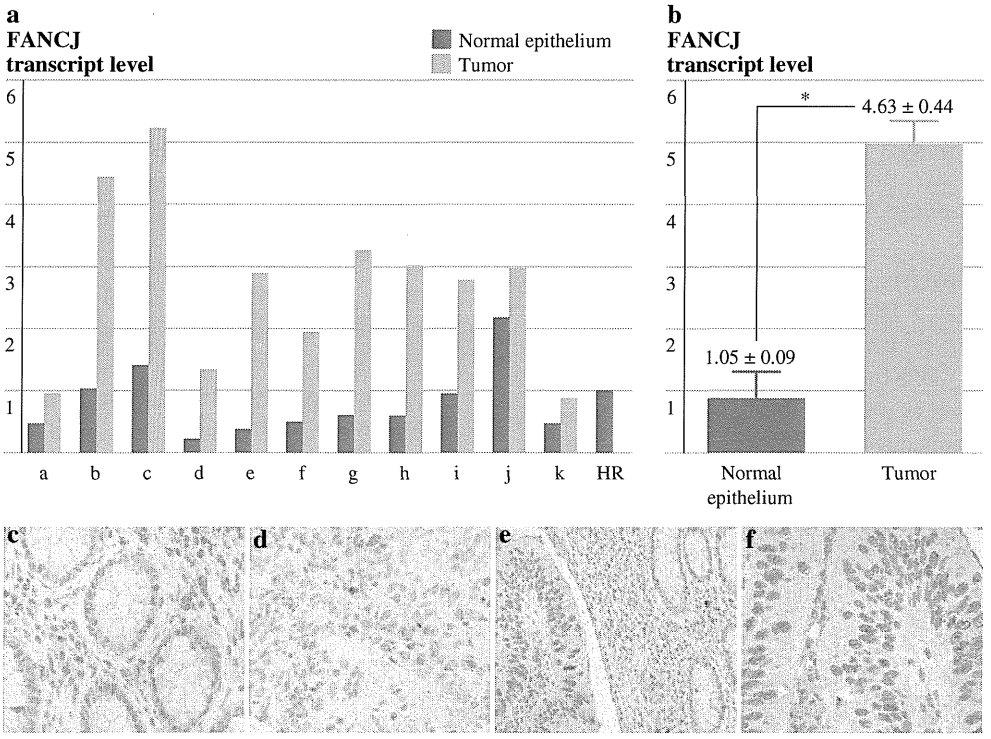

FIG. 1 FANCJ expression. FANCJ transcript levels in 11 specimens (**a:** *a–k*) and a summary in 95 (**b**). $*P < 0.0001$. Data are means ± standard error. **c** A tumor showing high FANCJ expression (the tumor (**d**) and normal (**e**) tissue). **f** A tumor showing low FANCJ expression. Magnification, ×100 (**c**), × 400 (**d–f**). *HR* human reference

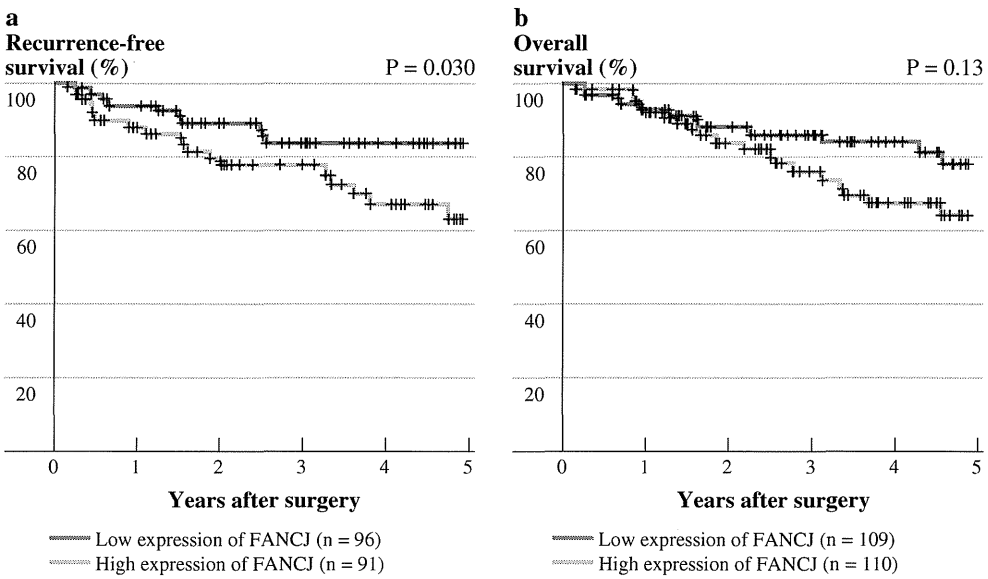

FIG. 2 Kaplan–Meier estimates of 5 year overall survival (OS) and 5 year recurrence-free survival (RFS) according to FANCJ expression. High expression of FANCJ was significantly associated with poor RFS (**a**) and nonsignificantly with poor OS (**b**)

Prognostic Significance of FANCJ Protein Expression

The recurrence-free survival (RFS) of patients with tumors displaying high FANCJ expression was significantly worse than that in patients with tumors displaying low FANCJ expression ($P = 0.03$; Fig. 2a). Similarly, the overall survival (OS) of patients with tumors displaying high FANCJ expression was worse, although the difference was not statistically significant ($P = 0.13$; Fig. 2b). These data suggest that high FANCJ expression is associated with poor prognosis.

In multivariate models adjusted for lymph node metastasis (yes or no), histological differentiation (well or others) and tumor depth (T1, T2 or T3, T4), high FANCJ

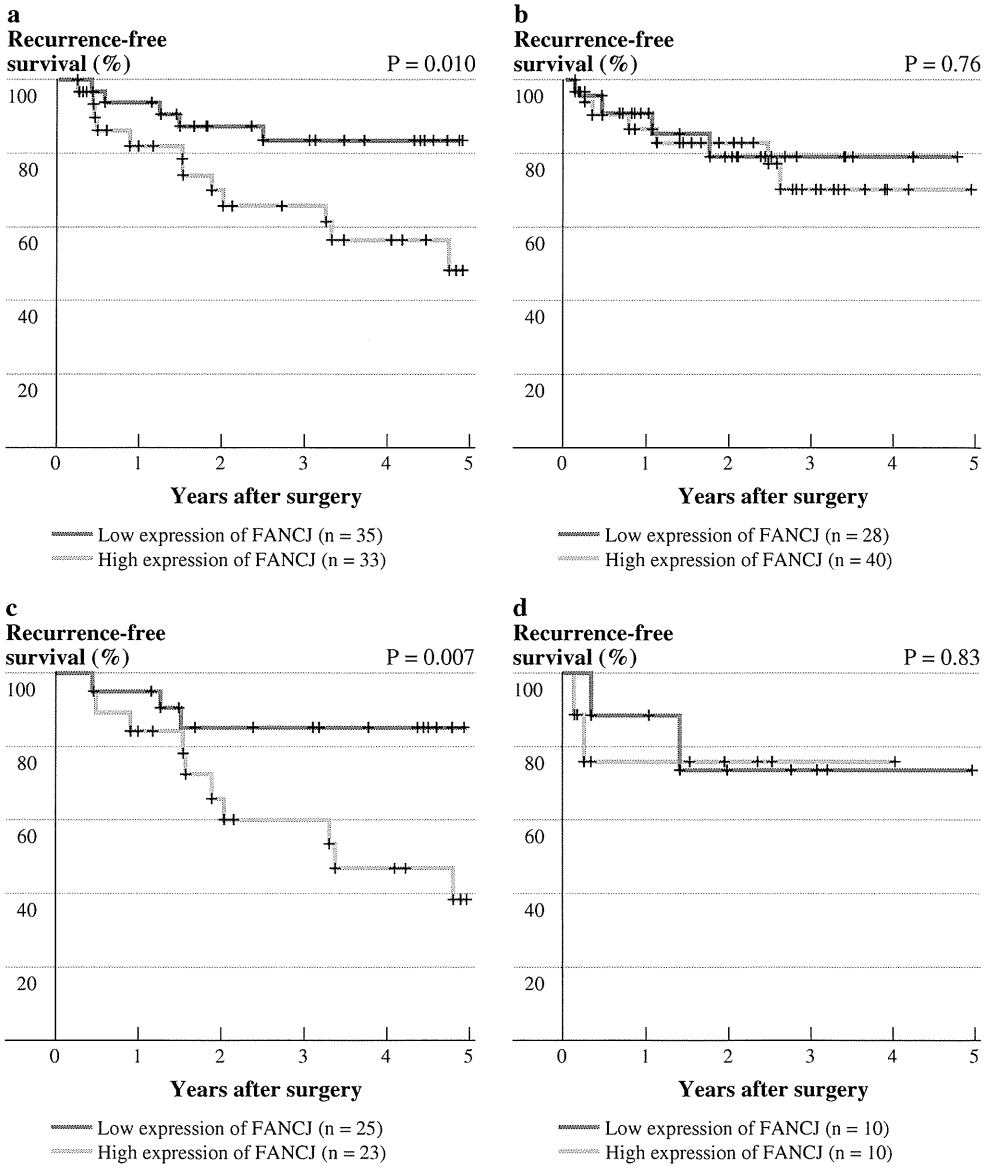

FIG. 3 Recurrence-free survival (RFS) in patients with stage II or III CRC according to adjuvant chemotherapy status. **a** Surgery plus 5-FU-based adjuvant chemotherapy. **b** Surgery alone. Patients with stage III (**c**) or stage II disease (**d**) who were treated by surgery plus 5-FU-based adjuvant chemotherapy

expression was associated with RFS (hazard ratio, 2.06; 95 % confidence interval (CI), 0.98–4.61; $P = 0.056$), although it did not reach statistical significance.

Association Between FANCJ Expression and Survival of Patients with Stage II/III CRC Treated with 5-FU-Based Adjuvant Chemotherapy

Because FANCJ expression was significantly associated with 5-FU sensitivity, as measured by the SDI assay (Table 1), we focused on the 136 patients with stage II/III disease stratified by chemotherapy treatment status to evaluate the correlation between FANCJ expression and the survival benefit of 5-FU. There were no differences in clinicopathological factors, including tumor stage, between the two groups classified by the level of FANCJ expression (Table 1). Among patients who received 5-FU-based adjuvant chemotherapy, high FANCJ expression was significantly correlated with worse RFS ($P = 0.01$; Fig. 3a). On the other hand, among patients who did not receive adjuvant chemotherapy, FANCJ expression was not correlated with RFS ($P = 0.76$; Fig. 3b). In terms of pathological stage, high FANCJ expression was correlated with poor prognosis in patients with stage III tumors ($P = 0.007$; Fig. 3c) but not in patients with stage II tumors (Fig. 3d).

Next, we divided the patients into two groups according to FANCJ expression to evaluate the effects of 5-FU-based chemotherapy (Fig. S2a). Among patients with tumors displaying high FANCJ expression, RFS was worse in patients who received adjuvant chemotherapy than in patients who did not ($P = 0.049$; Fig. S2b). Among

patients with tumors displaying low FANCJ expression, there was no difference in RFS without relation to adjuvant chemotherapy status (Fig S2c). Although not statistically significant, the RFS in patients given 5-FU-based chemotherapy was shorter than that of patients not given adjuvant thermotherapy (Fig S2a). This difference may arise from their different tumor stages. Approximately three-quarters of patients given adjuvant chemotherapy had stage III disease, and approximately three-quarters of patients who were not given adjuvant chemotherapy had stage II disease. The RFS of patients with stage III disease was worse than that of patients with stage II disease (Fig. S2d). Taking these results into consideration, patients with tumors exhibiting low FANCJ expression tended to have a greater survival benefit from 5-FU-based adjuvant chemotherapy than did patients with tumors exhibiting high FANCJ expression.

Association Between FANCJ/MLH1 Expression and 5-FU Resistance

We next examined MLH1 expression by immunohistochemistry in the same clinical samples (Fig. S3). The complete absence of nuclear staining for MLH1 was considered as abnormal MLH1 expression and was observed in 40 patients (18 %; Fig. S3b). Although MLH1 expression was significantly associated with MSI-H ($P < 0.001$, data not shown), it did not correlate with FANCJ expression ($P = 0.25$; Table 1) or OS ($P = 0.86$, data not shown).

We also analyzed the association between FANCJ expression and 5-FU sensitivity measured by the SDI method according to MLH1 status. High FANCJ expression was correlated with low sensitivity to 5-FU, particularly in tumors showing normal MLH1 expression ($P = 0.016$), but not in MLH1-deficient tumors ($P = 0.90$; Table 2). Indeed, in patients with normal MLH1 expression given 5-FU-based adjuvant chemotherapy, high FANCJ expression was correlated with worse RFS ($P = 0.026$; data not shown). On the other hand, there was no significant correlation between FANCJ expression and RFS in patients with abnormal MLH1 expression ($P = 0.12$; data not shown).

TABLE 2 Relationship between FANCJ expression and 5-FU sensitivity according to MLH1 status

	FANCJ expression		*P*
	Low ($N = 107$)[a]	High ($N = 108$)[b]	
Abnormal staining of MLH1			
High sensitivity to 5-FU ($N = 18$)	11 (46)	7 (44)	0.9
Low sensitivity to 5-FU ($N = 22$)	13 (54)	9(56)	
Normal staining of MLH1			
High sensitivity to 5-FU ($N = 62$)	37 (45)	25 (27)	0.016
Low sensitivity to 5-FU ($N = 113$)	46 (55)	67 (73)	

5-FU 5-fluorouracil

[a] Two missing data

[b] Two missing data

5-FU Resistance in MMR-Proficient CRC Cells Induced by FANCJ Overexpression

To confirm the effects of FANCJ/MLH1 expression on 5-FU sensitivity, we performed in vitro analysis. We developed adenoviruses expressing FANCJ or luciferase genes and infected these into MLH1-deficient HCT116 and MLH1-proficient HCT116 3-6 cells. We then examined the 5-FU sensitivity of these infectants. The increased expression of FANCJ was confirmed by western blotting (Fig. 4a). In cell growth analysis, HCT116 3-6 cells overexpressing FANCJ showed significantly increased resistance to 5-FU (Fig. 4b). In contrast, FANCJ

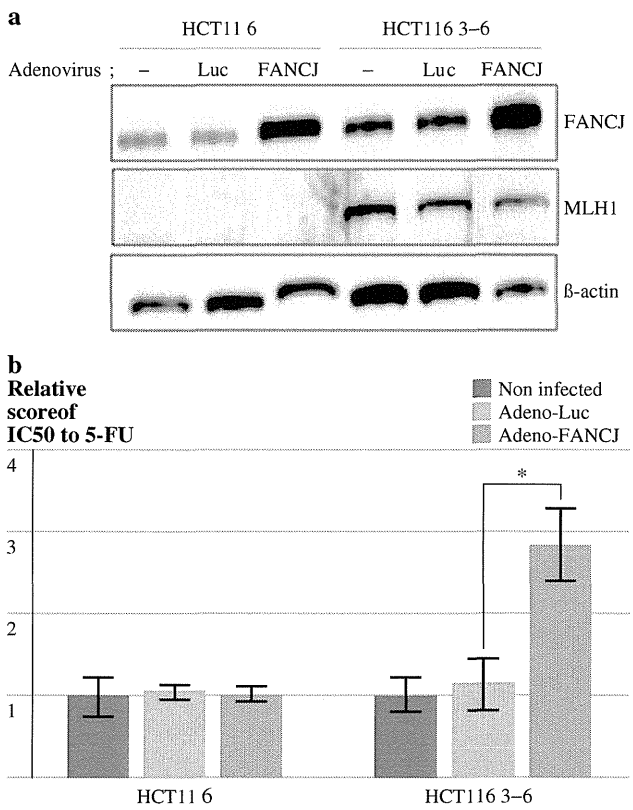

FIG. 4 FANCJ expression and 5-FU resistance in CRC cell lines. **a** Western blotting of extracts from noninfected (−) and adenovirus-infected HCT116 and HCT116 3-6 cells. **b** Relative score of IC_{50} to 5-FU of adenovirus-infected HCT116 and HCT116 3-6 cells. $^*P < 0.05$. **b** Data are shown as relative scores of IC_{50} to 5-FU, which was normalized by that of non-infected cells of each cell line as 1.00. *Error bars* represent standard errors. *Luc* luciferase

overexpression had little effect on 5-FU sensitivity in HCT116 cells (Fig. 4b). Induction of ectopic FANCJ expression using other methods yielded similar results (Fig. S4), confirming that high expression of FANCJ in MLH1-proficient cells confers 5-FU resistance.

DISCUSSION

5-FU-based adjuvant chemotherapy is a standard treatment for CRC. Therefore, it is particularly important for us to be able to predict the therapeutic effect of 5-FU. We set out to determine whether FANCJ expression could be a useful biomarker to predict sensitivity to 5-FU and prognosis in patients with CRC. Our study revealed that FANCJ was overexpressed in CRC tissues and that its overexpression was significantly correlated with 5-FU resistance. Interestingly, this association was only observed in patients with tumors showing normal MLH1 expression.

FA proteins are known to participate in the DNA repair pathway, but few studies have examined their clinical significance.[37,38] As for FANCJ, an earlier study showed that FANCJ expression was associated with the malignancy of breast cancer.[25] In our current study, high FANCJ expression was significantly associated with tumor depth and worse prognosis. These results suggest that FANCJ expression also is correlated with the malignancy of CRC. FANCJ expression was also correlated with low sensitivity to 5-FU as measured by the SDI method ($P = 0.028$). Significant correlations between SDI-determined sensitivity and the response to antitumor drugs have already been reported.[28,39] Furthermore, high FANCJ expression was correlated with worse prognosis in an analysis of stage II/III tumors treated by 5-FU-based adjuvant chemotherapy. These results provide strong support for the association between high FANCJ expression and 5-FU resistance.

We also found that high FANCJ expression was correlated with low sensitivity to 5-FU, as measured by the SDI method, in tumors with normal MLH1 expression, but not in MLH1-deficient tumors. We reproduced similar correlations in vitro using isogenic CRC cell lines; i.e., increased FANCJ expression conferred 5-FU resistance in MLH1-proficient HCT116 3-6 cells, but not in MLH1-deficient HCT116 cells. This implies a causal relationship between FANCJ overexpression and 5-FU resistance when MLH1 is normally expressed. FANCJ-MLH1 interaction is essential for the establishment of normal cellular response to DNA interstrand cross-link,[16,19] and for the proper MMR signaling and apoptotic responses to agents that induce O^6-methylguanine lesions.[20] Because 5-FU also causes DNA damage that is recognized by MMR factors,[22] overexpressed FANCJ may compromise cellular response to 5-FU in an MLH1-dependent manner. As an alternative possibility, unregulated FANCJ function is supposed to be associated with chemoresistance, which requires interaction with MLH1.[40] In our case, unleashed FANCJ protein produced by overexpression may confer 5-FU resistance in MLH1-proficient CRC.

There are some limitations to this study. First, this was a retrospective study and had limited ability to adjust for propensity, which can confound the association with survival. Second, we followed the patients for a median of 3–4 years, because some patients were treated by surgery within 5 years. A more homogeneous study population and a longer follow-up period may be necessary to better understand the long-term influence of FANCJ expression on survival.

CONCLUSIONS

Our study revealed that FANCJ expression may be a useful biomarker to predict sensitivity to 5-FU and prognosis in CRC. Patients with MLH1-proficient tumors displaying high FANCJ expression might receive little survival benefit from 5-FU-based adjuvant chemotherapy. However, selection of patients for 5-FU treatment should be based on the results of detailed, well-designed experiments, and randomized, double-blind, clinical trials. Further studies are expected to evaluate the significance of FANCJ as a predictive factor for 5-FU sensitivity and prognosis in CRC.

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REFERENCES

1. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. *Lancet*. 1995;345:939–44.
2. O'Connell MJ, Laurie JA, Kahn M, et al. Prospectively randomized trial of postoperative adjuvant chemotherapy in patients with high-risk colon cancer. *J Clin Oncol*. 1998;16:295–300.
3. Wilkinson NW, Yothers G, Lopa S, et al. Long-term survival results of surgery alone versus surgery plus 5-fluorouracil and leucovorin for stage II and stage III colon cancer: pooled analysis of NSABP C-01 through C-05. A baseline from which to compare modern adjuvant trials. *Ann Surg Oncol*. 2010;17:959–66.
4. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon

cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol.* 2009;27:1814–21.
5. Morris M, Platell C, Iacopetta B. Tumor-infiltrating lymphocytes and perforation in colon cancer predict positive response to 5-fluorouracil chemotherapy. *Clin Cancer Res.* 2008;14:1413–7.
6. Lecomte T, Ferraz JM, Zinzindohoue F, et al. Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. *Clin Cancer Res.* 2004;10:5880–8.
7. Nobili S, Napoli C, Landini I, et al. Identification of potential pharmacogenomic markers of clinical efficacy of 5-fluorouracil in colorectal cancer. *Int J Cancer.* 2011;128:1935–45.
8. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med.* 2003;349:247–57.
9. Jover R, Zapater P, Castells A, et al. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. *Eur J Cancer.* 2009;45:365–73.
10. Elsaleh H, Powell B, McCaul K, et al. P53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. *Clin Cancer Res.* 2001;7:1343–9.
11. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell.* 1996;87:159–70.
12. Tokunaga E, Oki E, Oda S, et al. Frequency of microsatellite instability in breast cancer determined by high-resolution fluorescent microsatellite analysis. *Oncology.* 2000;59:44–9.
13. Zavodna K, Bujalkova M, Krivulcik T, et al. Novel and recurrent germline alterations in the MLH1 and MSH2 genes identified in hereditary nonpolyposis colorectal cancer patients in Slovakia. *Neoplasma.* 2006;53:269–76.
14. Barrow E, Jagger E, Brierley J, et al. Semiquantitative assessment of immunohistochemistry for mismatch repair proteins in Lynch syndrome. *Histopathology.* 2010;56:331–44.
15. Park JW, Chang HJ, Park S, et al. Absence of hMLH1 or hMSH2 expression as a stage-dependent prognostic factor in sporadic colorectal cancers. *Ann Surg Oncol.* 2010;17:2839–46.
16. Peng M, Litman R, Xie J, et al. The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. *EMBO J.* 2007;26:3238–49.
17. Levran O, Attwooll C, Henry RT, et al. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat Genet.* 2005;37:931–3.
18. D'Andrea AD, Grompe M. The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer.* 2003;3:23–34.
19. Cantor SB, Xie J. Assessing the link between BACH1/FANCJ and MLH1 in DNA crosslink repair. *Environ Mol Mutagen.* 2010;51:500–7.
20. Xie J, Guillemette S, Peng M, et al. An MLH1 mutation links BACH1/FANCJ to colon cancer, signaling, and insight toward directed therapy. *Cancer Prev Res (Phila).* 2010;3:1409–16.
21. Liu A, Yoshioka K, Salerno V, Hsieh P. The mismatch repair-mediated cell cycle checkpoint response to fluorodeoxyuridine. *J Cell Biochem.* 2008;105:245–54.
22. Fischer F, Baerenfaller K, Jiricny J. 5-Fluorouracil is efficiently removed from DNA by the base excision and mismatch repair systems. *Gastroenterology.* 2007;133:1858–68.
23. Jass JR, Sobin LH. Histological typing of intestinal tumours. WHO International Histological Classification of Tumours No. 15. 2nd ed. Berlin: Springer; 1989.
24. Sobin LH Wittekind Ch, eds. TNM classification of malignant tumours. 6th ed. New York: Wiley; 2002.
25. Eelen G, Vanden Bempt I, Verlinden L, et al. Expression of the BRCA1-interacting protein Brip1/BACH1/FANCJ is driven by E2F and correlates with human breast cancer malignancy. *Oncogene.* 2008;27:4233–41.
26. Cantor SB, Bell DW, Ganesan S, et al. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell.* 2001;105:149–60.
27. Yu X, Chini CC, He M, et al. The BRCT domain is a phosphoprotein binding domain. *Science.* 2003;302:639–42.
28. Takeuchi H, Baba H, Inutsuka S, et al. Antitumor chemosensitivity differs between clinical sarcoma and adenocarcinoma tissues. *Anticancer Res.* 1994;14:169–71.
29. Oki E, Baba H, Tokunaga E, et al. Akt phosphorylation associates with LOH of PTEN and leads to chemoresistance for gastric cancer. *Int J Cancer.* 2005;117:376–80.
30. Oda S, Oki E, Maehara Y, Sugimachi K. Precise assessment of microsatellite instability using high resolution fluorescent microsatellite analysis. *Nucleic Acids Res.* 1997;25:3415–20.
31. Oki E, Oda S, Maehara Y, Sugimachi K. Mutated gene-specific phenotypes of dinucleotide repeat instability in human colorectal carcinoma cell lines deficient in DNA mismatch repair. *Oncogene.* 1999;18:2143–7.
32. Oki E, Kakeji Y, Zhao Y, et al. Chemosensitivity and survival in gastric cancer patients with microsatellite instability. *Ann Surg Oncol.* 2009;16:2510–5.
33. Oda S, Maehara Y, Ikeda Y, et al. Two modes of microsatellite instability in human cancer: differential connection of defective DNA mismatch repair to dinucleotide repeat instability. *Nucleic Acids Res.* 2005;33:1628–36.
34. Tajiri T, Suita S, Shono K, et al. A microsatellite instability analysis in neuroblastoma based on a high resolution fluorescent microsatellite analysis. *Cancer Lett.* 1998;124:59–63.
35. Koi M, Umar A, Chauhan DP, et al. Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. *Cancer Res.* 1994;54:4308–12.
36. Russell WC. Update on adenovirus and its vectors. *J Gen Virol.* 2000;81:2573–604.
37. Rudland PS, Platt-Higgins AM, Davies LM, et al. Significance of the Fanconi anemia FANCD2 protein in sporadic and metastatic human breast cancer. *Am J Pathol.* 2010;176:2935–47.
38. Ozawa H, Iwatsuki M, Mimori K, et al. FANCD2 mRNA overexpression is a bona fide indicator of lymph node metastasis in human colorectal cancer. *Ann Surg Oncol.* 2010;17:2341–8.
39. Furukawa T, Kubota T, Hoffman RM. Clinical applications of the histoculture drug response assay. *Clin Cancer Res.* 1995;1:305–11.
40. Xie J, Litman R, Wang S, et al. Targeting the FANCJ-BRCA1 interaction promotes a switch from recombination to poleta-dependent bypass. *Oncogene.* 2010;29:2499–508.

RESEARCH COMMUNICATION

Constipation and Colorectal Cancer Risk: The Fukuoka Colorectal Cancer Study

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Abstract

Constipation has been suspected to be linked to colorectal cancer risk, but epidemiological evidence is inconclusive. We described the prevalence of constipation and related lifestyle factors in a community and examined the relation of constipation and other bowel habits to colorectal cancer risk. The prevalence study was based on 833 community controls in the Fukuoka Colorectal Cancer Study, and 212 cases of Dukes' stage A were used in a study on bowel habits and colorectal cancer risk. Bowel habits were assessed by in-person interview. Odds ratio (OR) and 95% confidence interval (CI) of colorectal cancer were estimated with adjustment for dietary and nondietary factors. Constipation was reported by 10.3% of men and 27.7% of women. Individuals with less frequent bowel movements had a lower intake of total energy and were physically less active. The multivariate-adjusted OR (95% CI) of colorectal cancer were 1.51 (1.02-2.25) for self-reported constipation, 1.60 (1.05-2.44) for functional constipation, and 1.24 (0.81-1.90) for infrequent bowel movements (<1 stool/day). Self-reported constipation was fairly common in Japanese adults. Constipation was associated with a moderately increased risk of colorectal cancer.

Keywords: Bowel habits - constipation - colorectal cancer - Japanese

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Introduction

Constipation is a fairly common symptom among patients in routine clinical practice and also in free-living populations. Chronic constipation is perceived by approximately 10-30% of adults in Western countries (Higgins et al., 2004; Peppas et al., 2008). A Japanese study reported that the prevalence of self-perceived constipation was 14% among men and 36% among women in a rural middle-aged and elderly population (Nakaji et al., 2004), while another study in Korea showed that 10% among men and 23% among women felt constipation (Jun et al., 2006).

Constipation has long been suspected to be linked to colorectal cancer risk, but epidemiological evidence is limited and inconclusive. Previous studies have been based on self-reported constipation, frequency of bowel movements, or use of laxatives. Several case-control studies have reported an increased risk of colorectal cancer associated with constipation (Wynder et al., 1967; Haenszel et al., 1973) or infrequent bowel movement (Vobecky et al., 1983; Roberts et al., 2003), whereas others have not shown an association with constipation (Higginson, 1966; Wynder et al., 1969; Jain et al., 1980; Nakamura et al., 1984). One case-control study showed an increased risk of colorectal cancer associated with constipation, but not with infrequent bowel movement (Kune et al., 1988). A meta-analysis of 9 case-control studies reported an odds ratio (OR) of 1.48 (95% confidence interval [CI] 1.32-1.66) for constipation or infrequent bowel movement (Sonnenberg and Müller, 1993). Use of laxatives has also been a matter of interest, because it is more objective than self-reported constipation. Laxative use was positively associated with colorectal cancer, the OR being 1.46 (95% CI 1.33-1.61) in a meta-analysis of 11 case-control studies (Sonnenberg and Müller, 1993). At least 6 prospective

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studies have addressed the association of bowel movement or laxative use with colorectal cancer risk (Watanabe et al., 2004; Kojima et al., 2004; Simons et al., 2010; Otani et al., 2006; Park et al., 2009; Dukas et al., 2000). A non-significant increase in the risk of colorectal cancer was observed among men and women combined with less than a bowel movement per day compared to those who had daily or more bowel movement in one study (Watanabe et al., 2004), and very infrequent bowel movement (every 6 days or less) was associated with a more than 2-fold increase in the risk of colorectal cancer in women but not in men in another study (Kojima et al., 2004). On the contrary, frequent bowel movement was associated with an increased risk of colorectal cancer in a recent prospective study (Simons et al., 2010). In that study, constipation was related to a decreased risk (Simons et al., 2010). One of these studies showed a marked increase in the risk associated with use of laxatives (Watanabe et al., 2004). Neither bowel movement nor laxative use was measurably associated with colorectal cancer risk in three prospective studies in Japan (Otani et al., 2006), Europe (Park et al., 2009), and USA (Dukas et al., 2000). None of these previous prospective studies, except for one (Simons et al., 2010), examined the relationship between self-reported constipation and colorectal cancer risk.

In the study reported here, we examined the relation of constipation and bowel movement to colorectal cancer risk in a community-based case-control study in Japan (Kono et al., 2004). We also described the prevalence of constipation in the community and lifestyle factors related to constipation

Materials and Methods

The Fukuoka Colorectal Cancer Study is a community-based case-control study to investigate etiological factors of colorectal cancer among residents in Fukuoka City and three adjacent areas. The study has been approved by the ethics committee of Kyushu University and collaborating hospitals. Details of the methods have been described elsewhere (Kono et al., 2004).

Study subjects

Cases comprised a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas who were admitted to one of the eight participating hospitals for the first surgical treatment. Eligible cases were aged 20–74 years at time of diagnosis, lived in the study area, had no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, and were mentally competent to give informed consent and to complete the interview. During the period from September 2000 to December 2003, 840 (80%) of 1,053 eligible cases participated in the interview. The number of cases according to the Dukes' classification was as follows: stage A 215, stage B 226, stage C 281, stage D 116, and unrecorded 2. In the analysis on the relation between bowel habits and colorectal cancer risk, we used only cases with Dukes' stage A.

Controls were randomly selected in the study area by frequency-matching with respect to gender and 10-year age class. Eligible criteria were the same as described for the cases except that controls had no history of colorectal cancer. A total of 1,500 persons living in 15 geographical areas were selected as control candidates by two-stage random sampling, and were invited to participate in the study by mail. There were 113 persons who were found to be ineligible, and 5 persons were diagnosed as having colorectal cancer after the interview survey. After exclusion of these 118 persons, 833 (60%) of the 1,382 eligible candidates participated in the study.

We described bowel habits in the 833 community-based control subjects. The association between bowel habits and colorectal cancer risk was examined in the 212 cases of Dukes' stage A and 791 controls aged 40 years or older. When dietary factors were assessed, we excluded subjects who were in the top or bottom 1% of energy intake within each stratum of sex and age categories (<55, 55–64 and ≥65 years).

Bowel habits and lifestyle features

Research nurses interviewed cases and controls in person regarding bowel habits as well as lifestyle factors, using a uniform questionnaire. The index dates were the date of onset of symptom or screening for cases and the time of interview for controls. Bowel habits in the past year were ascertained by closed-ended questions with respect to 10 items: self-reported constipation, frequency of bowel movements, consistency of stool, abdominal bloating, feeling of incomplete evacuation, time required for defecation, regularity of bowel movements, use of laxatives, and 2 items about incontinence of feces (Table 1). Eight options were prepared for the question on the frequency of bowel movements, and 5 pre-coded answers were given to the question on the time for defecation. Regularity of the bowel habit and feeling of incomplete evacuation were answered dichotomously. Three to four options were prepared for the remaining 7 questions. Answers to most of the questions, except for regularity of bowel movements and feeling of incomplete evacuation with two precoded answers, were collapsed into 2 or 3 categories in the analysis.

Approximately one year after the survey, 29

Table 1. Questions on Bowel Habits and Reproducibility of Each Response*

Question	Options	κ†
1. How often have you felt constipation?	4	0.68
2. How often have you had a bowel movement?	8	0.68
3. What has the consistency of your stool been?	4	0.42
4. How often have you had abdominal distension?	3	0.61
5. Have you had a feeling of incomplete evacuation?	2	0.49
6. How long has it taken to pass a bowel movement?	5	0.61
7. Have your bowel movements been regular?	2	0.58
8. How often have you used laxatives?	4	0.67
9. How often have you had fecal incontinence?	3	--‡
10. How often have you had your underwear stained with feces?	3	0.65

*Agreement between the first and the second survey with a one-year interval among 29 subjects was assessed; κ, eighted kappa; †Used Fleiss-Cohen weights. Unweighted kappa statistics were used for questions with two options; ‡All of the subjects were in a single cell of cross tabulation

control subjects were re-interviewed by using the same questionnaire. Reproducibility was assessed by kappa statistics ranged 0.42 to 0.68 (see Table 1). Fecal incontinence was not included in the present analysis because those who reported fecal incontinence were very few. With reference to Rome Criteria III (Longstreth et al., 2006), those who reported 2 or more of the following 5 symptoms were classified as having functional constipation: less than one bowel movement per 3 days, hard stools, feeling of incomplete evacuation, taking more than 5 minutes to have a bowel movement, and use of laxatives more than once per week.

Details of the methods on lifestyle factors have been described elsewhere (Kono et al., 2004). Smoking habit was categorized into lifelong non-smoker, former smoker, and current smoker. Alcohol consumption 5 years prior to the index date was ascertained. Individuals reported height (cm), current body weight (kg), and body weight (kg) 10 years earlier. Current body mass index (kg/m^2) and body mass index (kg/m^2) 10 years earlier were obtained. Questions on physical activities elicited type of job, activities in commuting and housework and leisure-time activities 5 years before. As described in detail previously (Isomura et al., 2006), leisure-time physical activity (including activities in commuting and housework) was expressed as a sum of metabolic equivalents (MET) multiplied by hours of weekly participation in each activity, i.e., MET-hours per week. Parental colorectal cancer was also elicited.

Dietary assessment

The method of dietary assessment was described previously (Uchida et al., 2007). Consumption frequencies and portion sizes of 148 food/beverage items over one year prior to the index date were ascertained by a computer-assisted interview. Typical dishes for each item were shown on the display window of the personal computer. Individuals were asked to report their usual consumption over one year prior to the index date. Intakes of nutrients and alcohol were calculated based on the food composition tables in Japan (Science and Technology Agency, Japan: Standard Tables of Food Composition, 2000). Estimated intakes of nutrients and foods generally showed a fairly high validity in comparison with those based on the 28-day diet record over one year (Uchida et al., 2007). Pearson correlation coefficients of log-transformed values of total energy intake, energy-adjusted dietary fiber, and alcohol between the first interview (and the second interview in parentheses) and diet record were as follows: total energy intake 0.56 (0.34), energy-adjusted dietary fiber 0.48 (0.44), and alcohol 0.65 (0.58).

Statistical analysis

Between-group comparisons of proportions were assessed by χ^2-test. Age-adjusted means and proportions were used in examining the characteristics of the controls according to the frequency of bowel movements; age-adjusted means were obtained by analysis of covariance using a continuous variable for age, and age-adjusted proportions were calculated by the direct method of standardization with men and women each in 3 age classes (<55, 55–64 and ≥65 years) as standard population. Trend was assessed by Mantel-Haenszel method for proportions and linear regression analysis for means. Current alcohol intake and current BMI were used in the analysis on lifestyle factors in relation to constipation and bowel movement.

Logistic regression analysis was used to estimate OR and 95% CI of colorectal cancer for each bowel habit category. Statistical adjustment was made for sex, age (year), residential area (Fukuoka City or others), parental history of colorectal cancer, past and current smoking status, alcohol drinking 5 years before (0, 0.1–0.9, 1.0–1.9 or ≥ 2 units per day), body mass index 10 years earlier (<22.5, 22.5–24.9, 25.0–27.4 or ≥27.5 kg/m^2), type of job (sedentary or non-sedentary), leisure-time physical activity (0, 0.1–15.9 or ≥ 16 MET-hours per week) and natural logarithm of total calorie intake. Body weight 10 years before was not ascertained for 2 cases and 10 controls, and was replaced with the current body weight. Statistical significance was declared if two-sided p was <0.05. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary: NC, USA).

Results

Table 2 summarizes bowel habits in the control subjects by sex. Constipation was reported by 10.3% of men and 27.7% of women. Individuals with less than one bowel movement per day were more frequent among women compared with men. Laxative use and irregular bowel movement were almost three-fold more frequent in women than in men. Hard stools and abdominal distension were also more common in women. On the other hand, defecation time was shorter in women than in men, and there was no difference in the feeling of incomplete evacuation between men and women.

Table 3 shows characteristics of bowel habits according to self-reported constipation by sex. In both

Table 2. Bowel Habits in the Controls by Sex

Bowel habits		Men	Women	P*
Self-reported constipation	(–)	461 (89.7)	230 (72.3)	<0.01
	(+)	53 (10.3)	88 (27.7)	
Frequency	>1 stool/day	86 (16.8)	38 (12.0)	<0.01
	1 stool/day	369 (71.9)	198 (62.5)	
	<1 stool/day	58 (11.3)	81 (25.6)	
Consistency of stool	Hard	37 (7.2)	39 (12.4)	<0.01
	Normal	371 (72.5)	248 (79.0)	
	Loose	104 (20.3)	27 (8.6)	
Abdominal distention	Never	361 (70.4)	204 (64.2)	0.06
	Ever	152 (29.6)	114 (35.8)	
Incomplete evacuation	(–)	408 (81.0)	245 (79.0)	0.50
	(+)	96 (19.0)	65 (21.0)	
Defecation) time (min	1-2	147 (28.6)	148 (46.5)	<0.01
	3-5	262 (51.0)	137 (43.1)	
	≥6	105 (20.4)	33 (10.4)	
Regularity	Regular	463 (90.1)	227 (71.4)	<0.01
	Irregular	51 (9.9)	91 (28.6)	
Laxative use†	(–)	491 (95.5)	278 (87.4)	<0.01
	(+)	23 (4.5)	40 (12.6)	

Number (%), total numbers differ due to missing values; *P value was calculated by χ^2-test; † (+), once per week or more frequent use

Table 3. Bowel Habit Characteristics According to Self-reported Constipation by Sex*

Bowel habits	Men		Women	
	(–)	(+)	(–)	(+)
Number	461	53	230	88
<1 stool/day	29 (6.3)	29 (54.7)	29 (12.7)	52 (59.1)
Hard stool	16 (3.5)	21 (40.4)	12 (5.3)	27 (30.7)
Abdominal dist	120 (26.1)	32 (60.4)	57 (24.8)	57 (64.8)
Incomplete evac	76 (16.8)	20 (39.2)	27 (12.1)	38 (44.2)
Long defecation	83 (18.0)	22 (41.5)	15 (6.5)	18 (20.5)
Irreg movement	22 (4.8)	29 (54.7)	31 (13.5)	60 (68.2)
Laxative use	11 (2.4)	12 (22.6)	9 (3.9)	31 (35.2)

*All p<0.01 between (-) and (+) based on χ^2-test.

Table 4. Lifestyle Characteristics According to Bowel Movements

Characteristics*	Bowel movement (per day)			P for trend†
	>1	1	<1	
Men				
Number	86	369	58	
Age, mean±SD	60±9	59±10	60±12	0.62
Parental CRC (%)	8.3	5.4	1.4	0.10
Current smoking (%)	37.3	48.7	49.8	0.06
Alcohol ≥20 g/day (%)	45.8	52.9	34.6	0.34
Sedentary job (%)	62.7	63.0	56.6	0.55
High physical act (%)‡	40.8	32.7	26.3	0.07
Height, mean±SD (cm)	164±6	166±6	166±7	0.04
BMI, mean±SD (kg/m^2)	24±3	23±2	23±3	0.10
Dietary intake, geometric mean				
Total energy (kcal/day)	2377	2311	2151	0.02
Carbohydrate (g/day)§	267	264	272	0.60
Protein (g/day)§	70.7	69.7	67.7	0.09
Fat (g/day)§	52.0	51.5	54.1	0.38
Fiber (g/day)§	12.7	12.4	13.4	0.36
Women				
Number	38	198	81	
Age, mean±SD	63±8	59±11	56±13	0.001
Parental CRC (%)	2.1	6.2	5.3	0.86
Current smoking (%)	22.7	16.3	11.0	0.26
Alcohol ≥20 g/day (%)	6.2	5.6	2.6	0.53
Sedentary job (%)	76.9	83.7	82.2	0.56
High physical act (%)‡	43.7	39.6	34.5	0.33
Height, mean±SD (cm)	153±5	153±6	154±5	0.65
BMI, mean±SD (kg/m^2)	23±3	23±4	22±3	0.36
Dietary intake, geometric mean				
Total energy (kcal/day)	2087	1972	1846	0.01
Carbohydrate (g/day)§	271	266	272	0.63
Protein (g/day)§	79.8	76.9	75.3	0.03
Fat (g/day)§	59.9	61.0	60.5	0.91
Fiber (g/day)§	16.5	15.6	15.2	0.09

*Proportions were age-standardized by the direct method, and means were age-adjusted by analysis of covariance; †Mantel-Haenszel method for proportions and linear regression analysis for means; ‡Those who have ≥16 MET-hours/week; §Energy-adjusted per 2000 kcal/day

men and women, those reporting constipation were more likely to have infrequent bowel movements, hard stools, abdominal distention, feeling of incomplete evacuation, long time for defecation (≥6 min), and irregular bowel movements. Functional constipation was defined for 41% of those who reported constipation (58/141), and for 6% of subjects who did not perceive constipation (44/691). The concordance between self-reported constipation and functional constipation was moderate, kappa statistics being 0.39.

Table 5. Bowel Habits and Colorectal Cancer Risk*

Habits	Cases	Controls	OR†(95% CI)	OR‡(95% CI)
Self-reported constipation				
(–)	166	660	1.00 (reference)	1.00 (reference)
(+)	46	130	1.43 (0.97-2.10)	1.51 (1.02-2.25)
Functional constipation§				
(–)	172	694	1.00 (reference)	1.00 (reference)
(+)	40	97	1.66 (1.11-2.50)	1.60 (1.05-2.44)
Stool frequency				
>1/day	42	123	1.41 (0.94-2.10)	1.35 (0.89-2.05)
1/day	131	537	1.00 (reference)	1.00 (reference)
<1/day	39	128	1.25 (0.83-1.90)	1.24 (0.81-1.90)
Stool consistency				
Hard	28	72	1.71 (1.06-2.77)	1.72 (1.04-2.82)
Normal	129	590	1.00 (reference)	1.00 (reference)
Loose	54	122	2.10 (1.44-3.08)	2.02 (1.36-3.00)

*Total numbers differed due to missing values; †Adjusted for sex, age, and resident area; ‡Adjusted for sex, age, resident area, cigarette smoking, alcohol consumption, body mass index 10 years earlier, type of job, leisure-time physical activity, parental colorectal cancer, total calorie intake; §Functional constipation was defined if two or more of the following five symptoms were reported; less than one bowel movement/3days, having hard stools, feeling of incomplete evacuation, taking more than 5 minutes to have a bowel movement, and laxative use more than once per week

Lifestyle characteristics according to bowel movement frequency are shown in Table 4. Individuals with less frequent bowel movements had lower intake of total energy intake in both men and women. Physical activity tended to be lower in both men and women with less than daily bowel movements. Men with less frequent bowel movement were taller, and such women consumed less protein. Smokers were more frequent in men with infrequent bowel movements and in women with frequent bowel movements. Fiber intake tended to be lower in women with infrequent bowel movements.

Table 5 shows adjusted OR of colorectal cancer risk according to bowel habits. In the multivariate model, both self-reported constipation and functional constipation were statistically significantly related to a moderately increased risk of colorectal cancer. Loose or hard stool was also associated with an increased risk of colorectal cancer.

Discussion

The present study showed that self-reported constipation was fairly common in Japanese adults, the prevalence being 10% in men and 28% in women. It was also found that individuals with a lower intake of total energy had fewer frequencies of bowel movement. While bowel movement was unrelated to colorectal cancer risk, self-reported constipation, functional constipation, and loose or hard stool were associated with a moderately increased risk of colorectal cancer.

The prevalence rates of self-reported constipation observed here were comparable to the rates reported in Japan (Nakaji et al., 2004) and Korea (Jun et al., 2006). In these studies, 10-14% of men and 23-36% of women reported constipation. Infrequent bowel movements (less than once per day) was reported among 11% of men and 26% of women in the present study. These figures are also

in agreement with those reported elsewhere in Japan, i.e., 8-12% in men and 23-32% in women (Watanabe et al., 2004; Kojima et al., 2004; Otani et al., 2006).

Functional constipation defined on the basis of specific items of bowel habits was found to be less concordant with self-perceived constipation in the present study. The definition of functional constipation used in the present study did not necessarily accord with the Rome Criteria III (Longstreth et al., 2006), but a large discordance between self-reported and functional constipation has been observed in different populations. For instance, only 14% of women who reported constipation were diagnosed with functional constipation according to the Rome Criteria III in a study of outpatients (Digesu et al., 2010).

A positive association between total energy intake and bowel movements in this study corroborated previous community-based studies (Otani et al., 2006; Dukas et al., 2000). The present study also added a supportive evidence for a protective association between physical activity and bowel movement (Müller-Lissner et al., 2005). There was no evident association between dietary fiber and bowel movement. Although wheat bran increases stool weight and shortens oroanal transit time (Müller-Lissner, 1988), dietary fiber intake does not seem to differ between individuals reporting constipation and those not (Müller-Lissner et al., 2005).

While fewer bowel movements (<3 per week) was related to an increased risk of colorectal cancer in some case-control studies (Vobecky et al., 1983; Roberts et al., 2003), no such association has been observed in prospective studies in Western countries (Park et al., 2009; Dukas et al., 2000) as well as in Japan (Otani et al., 2006). The present study did not provide evidence that fewer bowel movements were related to an increased risk of colorectal cancer. On the other hand, the present study suggested an increased risk of colorectal cancer associated with self-reported constipation and functional constipation. It is notable that both self-reported and functional constipation were associated with colorectal cancer risk to almost the same extent. Having loose or hard stool was associated with an increased risk of colorectal cancer in the present study. Self-reported diarrheal stool was associated with a 11-fold increase in the risk of rectal cancer, not of colon cancer, only in women in a Japanese prospective study, in which the usual state of stool was classified into hard, normal, soft, diarrheal, and diarrhea alternated with constipation (Otani et al., 2006). In the EPIC-Norfolk study in which stool consistency was classified into hard, soft, and loose stool, loose stool was associated with 3-fold increased risk of colorectal cancer in men and women combined (Park et al., 2009). Hard stool was not associated with the risk of colorectal cancer in either of these two studies (Otani et al., 2006; Park et al., 2009). The present findings on loose stool are consistent with the previous observations, and loose stool may be a reflection of bowel inflammation, and thereby being associated with an increased risk of colorectal cancer. We have no proper explanation to the present finding on hard stools.

A strength of the study was that bowel habits were evaluated in a random sample in the community, although the participation rate did not exceed 60%. Another advantage was that multi-facets of bowel habits were evaluated in relation to self-reported constipation. However, the retrospective assessment of bowel habits in relation to colorectal cancer risk was a limitation.

In conclusion, using data from a community-based case-control study in Japan, the present study reported the prevalence of self-reported constipation as well as of functional constipation in the general population. Both self-reported and functional constipation were related to an increased risk of colorectal cancer

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References

Digesu GA, Panayi D, Kundi N, et al (2010). Validity of the Rome III Criteria in assessing constipation in women. *Int Urogynecol J Pelvic Floor Dysfunct*, **21**, 1185-93.

Dukas L, Willett WC, Colditz GA, et al (2000). Prospective study of bowel movement, laxative use, and risk of colorectal cancer among women. *Am J Epidemiol*, **151**, 958-64.

Haenszel W, Berg JW, Segi M, Kurihara M, Locke FB (1973). Large-bowel cancer in Hawaiian Japanese. *J Natl Cancer Inst*, **51**, 1765-79.

Higgins PD, Johanson JF (2004). Epidemiology of constipation in North America: a systematic review. *Am J Gastroenterol*, **99**, 750-9.

Higginson J (1966). Etiological factors in gastrointestinal cancer in man. *J Natl Cancer Inst*, **37**, 527-45.

Isomura K, Kono S, Moore MA, et al (2006). Physical activity and colorectal cancer: The Fukuoka Colorectal Cancer Study. *Cancer Sci*, **97**, 1099-1104.

Jain M, Cook GM, Davis FG, et al (1980). A case-control study of diet and colo-rectal cancer. Int J Cancer, 26, 757-68.

Jun DW, Park HY, Lee OY, et al (2006). A population-based study on bowel habits in a Korean community: prevalence of functional constipation and self-reported constipation. *Dig Dis Sci*, **51**, 1471-7.

Kojima M, Wakai K, Tokudome S, et al (2004). Bowel movement frequency and risk of colorectal cancer in a large cohort study of Japanese men and women. *Br J Cancer*, **90**, 1397-1401.

Kono S, Toyomura K, Yin G, Nagano J, Mizoue T (2004). A case-control study of colorectal cancer in relation to lifestyle factors and genetic polymorphisms: design and conduct of the Fukuoka colorectal cancer study. *Asian Pac J Cancer Prev*, **5**, 393-400.

Kune GA, Kune S, Field B, Watson LF (1988). The role of chronic constipation, diarrhea, and laxative use in the etiology of large-bowel cancer. *Dis Colon Rectum*, **31**, 507-12.

Longstreth GF, Thompson WG, Chey WD, et al (2006). Functional bowel disorders. Gastroenterology, 130, 1480-91.

Müller-Lissner SA (1988). Effect of wheat bran on weight of stool and gastrointestinal transit time: a meta analysis. *Br Med J*, **296**, 615-7.

Müller-Lissner SA, Kamm MA, Scarpignato C, Wald A (2005). Myths and misconceptions about chronic constipation. *Am J Gastroenterol*, **100**, 232-42.

Nakaji S, Matsuzaka M, Umeda T, et al (2004). A population-based study on defecatory conditions in Japanese subjects: methods for self-evaluation. *Tohoku J Exp Med*, **203**, 97-104.

Nakamura GJ, Schneiderman LJ, Klauber MR (1984). Colorectal cancer and bowel habits. *Cancer*, **54**, 1475-7.

Otani T, Iwasaki M, Inoue M, Sasazuki S, Tsugane S (2006). Bowel movement, state of stool, and subsequent risk for colorectal cancer: The Japan Public Health Center-Based Prospective Study. *Ann Epidemiol*, **16**, 888-94.

Park JY, Mitrou PN, Luben R, Khaw KT, Bingham SA (2009). Is bowel habit linked to colorectal cancer? – Results from the EPIC-Norfolk study. *Eur J Cancer*, **45**, 139-45.

Peppas G, Alexiou VG, Mourtzoukou E, Falagas ME (2008). Epidemiology of constipation in Europe and Oceania: a systematic review. *BMC Gastroenterol*, **8**, 5.

Roberts MC, Millikan RC, Galanko JA, Martin C, Sandler RS (2003). Constipation, laxative use, and colon cancer in a North Carolina population. *Am J Gastroenterol*, **98**, 857-64.

Science and Technology Agency, Japan (2000): *Standard Tables of Food Composition in Japan, Fifth Revised Edition*. Tokyo: Ministry of Finance Printing Bureau.

Simons CC, Schouten LJ, Weijenberg MP, Goldbohm RA, van den Brandt PA (2010). Bowel movement and constipation frequencies and the risk of colorectal cancer among men in the Netherlands Cohort Study on Diet and Cancer. *Am J Epidemiol*, **172**, 1404-14.

Sonnenberg A, Müller AD (1993). Constipation and cathartics as risk factors of colorectal cancer: a meta-analysis. *Pharmacology*, **47**, 224-33.

Uchida K, Kimura Y, Shirota T, Kono S (2007). Validity and reproducibility of the PC-assisted dietary interview used in the Fukuoka Colorectal Cancer Study. *Asian Pac J Cancer Prev*, **8**, 583-90.

Vobecky J, Caro J, Devroede G (1983). A case-control study of risk factors for large bowel carcinoma. *Cancer*, **51**, 1958-63.

Watanabe T, Nakaya N, Kurashima K, et al (2004). Constipation, laxative use and risk of colorectal cancer: The Miyagi Cohort Study. *Eur J Cancer*, **40**, 2109-15.

Wynder EL, Shigematsu T (1967). Environmental factors of cancer of the colon and rectum. *Cancer*, **20**, 1520-61.

Wynder EL, Kajitani T, Ishikawa S, Dodo H, Takano A (1969). Environmental factors of cancer of the colon and rectum. *Cancer*, **23**, 1210-20.

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ORIGINAL ARTICLE

Initial report of KSCC0803: feasibility study of capecitabine as adjuvant chemotherapy for stage III colon cancer in Japanese patients

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Abstract
Background A prospective feasibility study was planned to clarify the proportion of compliance and adverse events in the administration of capecitabine as adjuvant chemotherapy for colon cancer in Japanese patients.
Methods We aimed initially to register 92 cases of R0 stage III colon cancer. Capecitabine (2,500 mg/m^2/day) was given orally on days 1–14 every 3 weeks for 8 cycles. The proportion of treatments completed as planned was selected as the primary endpoint.
Results Ninety-seven cases were registered and treated between September 2008 and August 2009. The proportion of treatments completed in the full analysis set was 64/97 [66.0%; 95% confidence interval (CI), 55.7–75.3%] and in the per protocol set was 64/91 (70.3%; 95% CI, 59.8–79.5%). Adverse events which led to treatment discontinuation included hand–foot syndrome (HFS) (7), haematotoxicity (5) and increased hepatic damage (4). The proportions of patients with major grade 3/4 adverse events were HFS 22.7%, neutropenia 7.2%, diarrhoea 2.1%, and increased bilirubin 0.0%.
Conclusions This collaborative multi-facility study, the first of its kind in Japan, presented results of a safety confirmation experiment on capecitabine as adjuvant chemotherapy for stage III colon cancer. The results suggest that capecitabine may be administered safely to Japanese patients.

Keywords Capecitabine · Adjuvant chemotherapy · Colon cancer · Japanese · Feasibility study · X-ACT trial

YM was the principal investigator. YE, YK, YA, and ST were responsible for the study concept and design. YK, YS, HS, YO, SN, KA, MM, and KS provided patients. YK, HS and YO did the review. YE and YK collected and collated data. ST and YE analyzed the data. ST, YO, YK and YM interpreted the data. YE wrote the manuscript, which was approved by all authors.

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Introduction

Japanese Guidelines for the Treatment of Colorectal Cancer (2010) state that 5-fluorouracil (5FU)/leucovorin (LV) therapy, capecitabine, UFT/LV and FOLFOX4 and mFOLFOX6 are the standard treatments for postoperative adjuvant chemotherapy in stage III colon cancer in Japan [1]. The intravenous medications recommended in the guidelines cannot feasibly be used in the elderly and in patients for whom intensive therapy is not appropriate [2–4]. More effective, better tolerated and more convenient chemotherapy is required for these patients [5]. Moreover, a reported 84–89% of cancer patients would prefer oral to injected medications, given equivalent efficacy [6, 7].

Capecitabine is a novel oral fluorocytidine derivative discovered at the Nippon Roche Kamakura Laboratory (currently Chugai Pharmaceutical Co., Ltd. Kamakura Laboratory) that is designed to be converted to 5FU in steps to allow for selective delivery of high-dose 5FU to the target tumour while minimizing systemic exposure. After oral administration, capecitabine is absorbed unchanged from the gastrointestinal tract and metabolized in the liver to 5′-DFCR by carboxylesterase. The 5′-DFCR is subsequently converted to 5′-DFUR by cytidine deaminase, which is highly active primarily in the liver and tumour tissue. The 5′-DFUR is then selectively converted in the tumour tissue to 5FU by thymidine phosphorylase, which is highly active in tumour tissue [8, 9].

Based on the findings of capecitabine monotherapy in metastatic colon and rectal cancer, a phase III clinical trial (X-ACT trial) was conducted to compare capecitabine to bolus 5FU/LV therapy (Mayo Clinic regimen) as adjuvant chemotherapy in resected stage III colon cancer. Twelves et al. [10] reported that the capecitabine therapy in the study was at least equivalent to 5FU/LV therapy (Mayo Clinic regimen) in terms of the primary endpoint of 3-year disease-free survival (DFS) as well as overall survival (OS). In addition, capecitabine was associated with fewer gastrointestinal disturbances such as diarrhoea and stomatitis, indicating a superior safety profile [10]. Capecitabine was approved in the US and Europe in 2005 based on these results. Currently, the NCCN Guidelines include capecitabine monotherapy among the standard treatments for postoperative adjuvant chemotherapy in stage III colon cancer, along with 5FU/LV therapy and 5FU/LV + oxaliplatin therapy [11].

Occasionally, the treatment must be suspended due to the development of the characteristic adverse reaction of hand–foot syndrome (HFS). The treatment completion rate was 83.0% in the X-ACT trial, in which capecitabine was recognized as a standard postoperative adjuvant chemotherapy for colorectal cancer. Although capecitabine was approved in Japan based on the results of this trial, it has not yet been established to be a safe postoperative adjuvant therapy for colon cancer in Japanese patients. Accordingly, a feasibility study with an endpoint of the treatment completion rate of capecitabine in Japanese patients was planned.

Patients and methods

Inclusion criteria

Patients who met all of the following criteria were eligible for the study regardless of sex. (1) Histologically-confirmed colorectal cancer (adenocarcinoma). (2) Histological stage III [12] colon cancer or rectosigmoid cancer. (3) Curative colorectal cancer resection with D2 or more lymph node dissection. (4) Surgical procedure classified as histological curability A (cur A) [12] was performed. (5) Age 20–80 years old. (6) Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1. (7) No prior chemotherapy or radiotherapy for target disease. (8) Oral intake is possible. (9) Preservation of primary organ function [white blood cell (WBC) count $\geq$3,000/mm^3 and $<$12,000/mm^3, neutrophil count $\geq$1,500/mm^3, haemoglobin $\geq$9.0 g/dL, platelet count $\geq$100,000/mm^3, serum creatinine $\leq$1.5 times upper laboratory reference, serum total bilirubin $\leq$1.5 times upper laboratory reference, aspartate aminotransferase (AST), alanine aminotransferase (ALT) $\leq$2.5 times upper laboratory reference, alkaline phosphatase (ALP) $\leq$2.5 times upper laboratory reference]. (10) Able to start protocol treatment within 8 weeks of surgical procedure. (11) After basic screening, informed consent to participate in the study was obtained from patients. This study was approved by the Ethics Committees of the participating institutions and registered in the UMIN clinical trials registry (UMIN000001444).

Exclusion criteria

Patients who met any of the following criteria were excluded from participation in the study. (1) Pregnant or lactating, or planning to become pregnant. (2) History of hypersensitivity or severe adverse reaction to fluoropyrimidines. (3) Past organ transplant. (4) Serious concurrent disease [including interstitial pneumonia, pulmonary fibrosis, intestinal paralysis, ileus, poorly controlled diabetes, liver cirrhosis or hepatitis (type B or C), poorly controlled hypertension, history of myocardial infarction or unstable angina within past 6 months]. (5) Active multiple primary cancer (disease-free less than 5 years). (6) Concurrent infectious disease. (7) Judged to be unsuitable for participation in the clinical study by the investigator for any other reason.

Staging criteria

Japanese Classification of Colorectal Carcinoma, 7th edition (Second English edition) [12] was used for staging.

Treatment and assessment

Patients were enrolled and started on the protocol treatment within 8 weeks postoperatively. The protocol treatment consisted of eight 3-week cycles of 2,500 mg/m^2 of capecitabine per day in two divided doses for 14 days, followed by a 7-day rest period. To assess the protocol treatment, baseline tumour markers, peripheral blood counts and blood chemistry values were measured within 14 days of the start of administration.

General findings, peripheral blood counts, blood chemistry values and clinical findings (subjective symptoms and objective signs) were generally recorded at each visit during treatment. To assess HFS, photos of both the palms and the soles of the feet of patients were taken at baseline, at the onset of each grade of HFS and at recovery, with consent of the patient.

Protocol discontinuation criteria

Patients who met any of the following criteria were discontinued from the protocol treatment. (1) Onset of adverse event meeting the criteria for discontinuation of capecitabine in "dose modification". (2) Did not recover sufficiently to meet the start criteria for the next cycle of capecitabine even though 3 weeks had passed from the scheduled date of the start of the next cycle due to onset of adverse event in previous cycle. (3) 3 weeks had passed from the scheduled start date of the next cycle for reasons other than an adverse event (such as patient circumstances) (discontinuation of protocol treatment not required if treatment could be resumed without compromising efficacy). (4) Attending physician determines discontinuation of protocol treatment is necessary for reasons related to adverse event that does not meet the discontinuation criteria. (5) Confirmed recurrence. (6) Death. (7) Patient requests discontinuation from the protocol treatment for adverse event-related reason (relation to an adverse event cannot be ruled out). (8) Patient requests discontinuation from the protocol treatment for reason unrelated to adverse event [relation to an adverse event can be ruled out (e.g., relocation of patient or patient's family)]. (9) Patient found to have been ineligible for participation in the study after enrolment. (10) Pregnancy. (11) Confirmed secondary cancer or multiple cancers. (12) Investigator or primary or attending physician judges continuation of treatment to be inappropriate for any other reason.

Statistical analysis

The objective of the current study was to assess the feasibility of postoperative adjuvant chemotherapy with capecitabine for patients with stage III colon cancer. The primary endpoint was the treatment completion rate. The secondary endpoints were safety profile (rate and severity of adverse events), cumulative incidence of HFS and hepatic dysfunction (secondary analyses), 3- and 5-year DFS and OS.

Protocol completion was defined as the completion of eight 3-week cycles of 14 days of capecitabine followed by a 7-day rest period according to protocol in patients who were enrolled and started on treatment within 8 weeks postoperatively. If the eighth cycle was suspended, a ninth cycle was planned for patients meeting the treatment resumption criteria. These patients were considered to have completed eight cycles. Completion was judged by a central assessment committee in cases of protocol deviations or violations.

The objective of the current study was to find the estimated treatment completion rate in the patient population meeting the enrolment criteria for treatment with capecitabine as postoperative adjuvant therapy for colon cancer. The treatment completion rate was 83% in the X-ACT trial conducted outside of Japan using the same regimen [10]. Although the exact number of completing patients is unknown, the estimated 95% confidence interval (CI) was 80–86%. The study was estimated to require a sample size of at least 87 patients to ensure that the 95% confidence limit of the estimated treatment completion rate was within 10% of either side of the estimated value (if 70 of the 87 patients completed treatment, the estimated observation rate, or the estimated treatment completion rate, would be 80.5% with a 95% CI of 70.6–88.2%, resulting in a 9.9% difference between the estimated value and the lower confidence limit). A target enrolment size of 92 was selected, to account for patient attrition of approximately 5% due to ineligibility or other reasons.

Results

Patient baseline characteristics

From August 2008 to August 2009, 97 patients were enrolled at 42 centres belonging to the Kyushu Study Group of Clinical Cancer (KSCC). The per protocol set (PPS) excluded six of these patients due to incorrect administration. The patients had a median age of 65 years (range, 32–80 years). Males accounted for 60 of the patients, and females for 37. Surgical history included D2 lymph node dissection in 25 patients and D3 lymph node

Table 1 Patient characteristics

Characteristics	No. of patients
Age, years	
Median (range)	65 (32–80)
Gender	
Male/female	60/37
PS	
0/1	93/4
Lymph node dissection	
D0/D1/D2/D3	0/0/25/72
Primary tumor	
C/A[a]/T/D/S/RS	8/11/9/15/32/22
Stage	
IIIa/IIIb	74/23
Invasion	
SM/MP/SS/A[b]/SE/SI/AI	6/6/58/3/21/2/1
Nodal status	
N1/N2/N3	74/19/4
Regional lymph nodes	
ly0/ly1/ly2/ly3	21/47/21/8
Venous invasion	
v0/v1/v2/v3	27/45/18/7
Histological classification	
pap/tub1/tub2/por/muc/sig	2/31/59/3/2/0

C cecum, A^a ascending colon, *T* transverse colon, *D* descending colon, *S* sigmoid colon, *RS* rectosigmoid, *SM* carcinoma is limited to within the mucosa and submucosa, *MP* carcinoma is limited to within the mucosa submucosa, and proper muscle layer, *SS* carcinoma extends from the mucosa beyond the proper muscle but is not exposed on the serosal surface, A^b carcinoma extends beyond the proper muscle, *SE* carcinoma is exposed on the serosal surface, *SI* carcinoma definitely infiltrates other organs, *AI* carcinoma definitely infiltrates other organs, *pap* papillary adenocarcinoma, *tub1* well differentiated type (tubular adenocarcinoma), *tub2* moderately differentiated type (tubular adenocarcinoma), *por* poorly differentiated adenocarcinoma, *muc* mucinous adenocarcinoma, *sig* signet-ring cell carcinoma

dissection in 72 patients. Nodal status was N1 in 74 patients, N2 in 19 patients and N3 in four patients (Table 1).

Treatment completion rate

The treatment completion rate in the full analysis set (FAS), which was the primary endpoint, was 66.0% (64/97). The treatment completion rate in the PPS was 70.3% (64/91). Treatment was discontinued in 33 patients. Treatment was discontinued for an adverse event in 24 of these patients. The most common adverse event resulting in treatment discontinuation was HFS (seven patients), followed by haematological toxicities and liver dysfunction (five patients each). The reasons for treatment discontinuation in the remaining nine patients were incorrect

Table 2 Reasons for protocol discontinuation

Reasons	*N*
Adverse events	
Hand–foot syndrome	7
Haematological toxicities	5
Liver dysfunction	5
Fatigue	4
Diarrhoea	2
Rash	1
Extrapyramidal symptom	1
Skin ulcer	1
Ileus	1
Nausea/vomiting	1
Others	
Protocol violations/administrative reasons	6
Protocol violation/regimen changed	1
Relapse	3

Table 3 Most common treatment-related adverse events

Events	All grades (%)	Grade 3/4 (%)
Diarrhoea	23 (23.7)	2 (2.1)
Stomatitis	30 (30.9)	0 (0)
Hand–foot syndrome	64 (66.0)	22 (22.7)
Nausea	26 (26.8)	1 (1.0)
Vomiting	7 (7.2)	1 (1.0)
Neutropenia	56 (57.7)	7 (7.2)
Hyperbilirubinaemia	44 (45.4)	0 (0)
ALT	34 (35.1)	0 (0)
AST	33 (34.0)	0 (0)

ALT alanine aminotransferase, *AST* aspartate aminotransferase

administration in six patients and recurrence in three patients (Table 2).

Safety

The most common treatment-related adverse events were diarrhoea 23 (23.7%), stomatitis 30 (30.9%), HFS 64 (66.0%), neutropenia 56 (57.7%), and nausea/vomiting 33 (34.0%) (Table 3). The major grade 3/4 adverse events were HFS 22 (22.7%), neutropenia 7 (7.2%) and diarrhoea 2 (2.1%). Extrapyramidal symptoms and skin ulcer also occurred in one patient each.

Dose modification

Three dose levels were used for dose modification (levels 0, 1, 2). Patients were discontinued if adverse events could not be controlled at −2 levels. An analysis of dose