may be attractive therapeutic target against GC. The success of molecular-targeted therapy depends on the identification of a landmark to select patients with more benefit from the therapy, such as activating mutation or gene amplification of EGFR in non-small cell lung cancer [33], and overexpression or gene amplification of HER2 in breast cancer [34]. Thus, genetic alteration or expression status is possible to be a landmark for molecular-targeted therapy, and it is indispensable to evaluate the anticancer activity of PRL-3 inhibitor treatment against cancer cells with different genetic and expression status. Although neither *PRL-3* genomic amplification nor expression level was responsible for the sensitivity to PRL-3 inhibitor treatment, the inhibitor exhibited dose-dependent efficacy on all the tested cell lines with PRL-3 expression, and remarkably induced apoptosis in line with a previous report [35]. PRL-3 is not expressed in human adult stomach, and its expression is cancer-specific event [6,7]. Collectively, the presence of PRL-3 expression, but not expression level, may be sufficient to promote metastatic properties through activation of downstream signaling pathways, and the effective inhibition seems to have important implication for the success of this treatment. Combined with our previous findings demonstrating the high frequency of PRL-3 expression (55%, 95/173) [6], PRL-3-targeted therapy may be applicable for most patients with GC. The different sensitivity against PRL-3 targeting as shown in the present study may imply the additional alterations attenuating the dependence of PRL-3 signaling networks on cancer cells. Therefore, identification of molecules leading to the different sensitivity would shed light on the development of more sophisticated strategy.

Normal tissues with PRL-3 expression may be susceptible to adverse effects from the targeted therapy, especially in normal skeletal muscle and heart [28]. Interestingly, PRL-3 inhibitor treatment with the concentration of 10 μmol/L significantly repressed proliferation through apoptosis induction on all the tested GC cell lines, whereas did not on normal skeletal muscle C2C12 cells, implying that this concentration may act as an optimal dose of anticancer activity without severe effects against muscle cells, and normal cells may have a better apoptotic protective mechanism, even though PRL-3 is constitutively expressed [35]. As C2C12 cells might not be the best control because of relatively weak expression, further research will be necessary to validate our findings.

Conclusions

We have for the first time demonstrated that *PRL-3* genomic amplification is one of the predominant mechanisms inducing its expression, especially in more advanced stage, and that PRL-3-targeted therapy may have a great potential against gastric cancer with its expression.

List of Abbreviations used

PRL-3: Phosphatase of regenerating liver-3; GC: gastric cancer; PTP: protein tyrosine phosphatase; JCGC: Japanese Classification of Gastric Carcinoma; UICC: the Union Internationale Contre Le Cancer; FISH: fluorescence in situ hybridization; Q-PCR: quantitative-genomic polymerase chain reaction; EC_{50}: 50% effective concentration; IC_{50}: 50% inhibitory concentration; ANOVA: analysis of variance; DSS: disease specific survival.

Acknowledgements

This work was supported in part by the Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan and by the Japanese Foundation for Multidisciplinary Treatment of Cancer. The funding agencies had no role in the design of the study, data collection, or analysis; in the interpretation of the results; in the preparation of the manuscript; or in the decision to submit the manuscript for publication.

Authors' contributions

AO conceived of the study, performed the study, drafted the manuscript and participated in coordination. KY participated in coordination and assisted in editing of manuscript. SK, SS, NK, MW, HK, and KN helped in the collection and analysis of clinical data. MW participated in coordination. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 26 September 2010 Accepted: 6 April 2011
Published: 6 April 2011

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Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2407/11/122/prepub

doi:10.1186/1471-2407-11-122
Cite this article as: Ooki *et al.*: Therapeutic potential of PRL-3 targeting and clinical significance of *PRL-3* genomic amplification in gastric cancer. *BMC Cancer* 2011 11:122.

Ann Surg Oncol (2011) 18:2432–2441
DOI 10.1245/s10434-011-1625-3

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – COLORECTAL CANCER

Prognostic Significance of Preoperative Bowel Obstruction in Stage III Colorectal Cancer

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ABSTRACT

Background. Previous studies have suggested a detrimental prognostic effect of preoperative obstruction proximal to colorectal cancer (CRC). If such a detrimental effect is preserved in each stage of advanced (stage II or III) CRC, we can identify high-risk patients.

Methods. We enrolled 641 patients with pathologically confirmed advanced CRC (stage II, $n = 207$; stage III, $n = 434$) who had undergone curative resection of the primary lesion. The association of preoperative obstruction with clinicopathologic parameters was evaluated. Kaplan–Meier analysis and Cox proportional hazard models were used to estimate the effect of preoperative obstruction on disease-free survival in each stage.

Results. Preoperative obstruction was seen in 63 patients (9.8%) (stage II, $n = 16$; stage III, $n = 47$). Multivariable analysis showed that preoperative obstruction was significantly associated with preoperative elevation of carcinoembryonic antigen level in patients with colon cancer (odds ratio = 3.59; $P < 0.001$), while it was correlated with poor differentiation in patients with rectal cancer (odds ratio = 3.99; $P = 0.016$). Preoperative obstruction was a significant prognostic factor in stage III CRC ($P < 0.001$), but not in stage II disease. Multivariable prognostic analysis showed that preoperative obstruction was a remnant independent prognostic factor in stage III CRC. This finding was confirmed by separate analyses of colon and rectal cancer. Preoperative obstruction was associated with systemic recurrence ($P = 0.003$) rather than peritoneal or local recurrence.

Conclusions. These findings suggest that preoperative obstruction may predict worse long-term prognosis in patients with stage III CRC and may be a potential clinical marker to identify patients with high-risk stage III CRC.

Preoperative obstruction has been reported to occur in 7% to 47% of patients with colorectal cancer (CRC).[1–8] However, whether preoperative obstruction is prognostic is controversial.[1,5–11] This controversy may arise as a result of variations in study designs, including different stages and/or mixed curability.

Recently, increasing application of adjuvant chemotherapy and advances in chemotherapy regimens have improved the prognoses in stage III CRC.[12,13] Moreover, stage II patients may now be candidates for adjuvant chemotherapy. However, not all patients who undergo curative surgery receive optimal adjuvant chemotherapy because of cost issues and the capacity of medical facilities. Additionally, prognostic heterogeneity occurs in each cancer stage. Therefore, understanding important differences in prognostic clinical parameters other than staging is important when considering individual treatment strategies after curative surgery.

Because few reports have analyzed the role of preoperative obstruction in each stage, the objectives of this study were: (1) to evaluate the relationship between preoperative obstruction and clinicopathologic parameters of CRC; (2) to evaluate the prognostic impact of obstruction on advanced CRC patients with stage II and stage III

Electronic supplementary material The online version of this article (doi:10.1245/s10434-011-1625-3) contains supplementary material, which is available to authorized users.

First Received: 5 April 2010;
Published Online: 3 March 2011

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disease separately; and (3) if a prognostic significance of preoperative obstruction was confirmed, to determine its impact on patients' prognoses in different tumor location (colon vs. rectum) and consider the potential suitability for identifying high-risk patients.

PATIENTS AND METHODS

Between 1991 and 2005, a total of 641 sporadic CRC patients who had been clinically diagnosed with advanced disease (tumor, node, metastasis system [TNM] stage II and III) and underwent curative resection of the colon or the rectum at Kitasato University Hospital were analyzed. Through the observation period, our treatment policy was curative resection of the primary lesion with sufficient margin and appropriate lymph node dissection even for patients with obstruction. Except for unavoidable cases, such as perforation or suspicion of bowel necrosis, we first performed emergency ileostomy/colostomy, or insertion of a decompression tube if bowel obstruction required emergency decompression. After a thorough examination and nutritional improvement, we performed curative resection. We excluded the patients with prior chemo-immunotherapy or radiotherapy, and with severe systemic complications in other organs (heart, kidney, and liver). Patient demographics, tumor characteristics, and the postoperative course were assessed. Thirty-five surgeons performed curative resections, and all had at least 6 years of experience. Perioperative transfusion was defined as allogeneic blood transfusion during surgery or in the first 2 postoperative days and was performed at the discretion of the treating surgeons and anesthesiologists.[14] Pathological TNM classification was made according to International Union Against Cancer staging criteria.

Preoperative intestinal obstruction was defined as total absence of flatus or bowel movements for at least 24 h, accompanied by clinical signs of obstruction (abdominal distension, peristaltic abdominal pain, nausea, or vomiting) and by radiographic evidence of obstruction (dilated intestinal loops).

When adjuvant chemotherapy was used, it was started within 7 weeks after surgery. Adjuvant chemotherapy regimens consisted of 5-fluorouracil (5-FU) alone (infusion or oral), 5-FU/PSK (protein-bound polysaccharide K), 5-FU/leucovorin (LV), 5-FU/LV/CPT-11 (irinotecan), 5-FU/CPT-11, and others for at least 3 months or 3 cycles. Patients were followed up until cancer-related death, the recurrence of cancer, or the study end-point (March 31, 2007) if they survived. All patients were followed up at least every 3 months for the first year and every 6 months thereafter. Follow-up assessment involved a medical history, physical examination, biologic tests, measurement of serum carcinoembryonic antigen (CEA) and CA19-9 levels, colonoscopy, chest radiography, abdominal ultrasound, and chest/abdominal computed tomography. Serum CEA and CA19-9 levels were usually evaluated at every visit, and abdominal ultrasound and computed tomography were performed every 6 months. Chest computed tomography and colonoscopy were performed every year. Recurrence was diagnosed on the basis of imaging and, if necessary, either cytologic analysis or biopsy was performed. Postoperative therapy for recurrence or metastasis included surgical resection, 5-FU-based chemotherapy, or radiotherapy.

The relationship between preoperative obstruction and clinicopathologic features was assessed by chi-square test or Fisher's exact test, and multivariable logistic regression analysis was performed to obtain an adjusted effect. The follow-up time was calculated from the surgical date for the primary lesion to the date of recurrence or cancer-related death. Cumulative disease-free survival (DFS) was estimated by the Kaplan–Meier method, and statistical significance was tested by the log rank test. For the Kaplan–Meier estimate, we truncated the data at a follow-up period of 5 years to avoid having the number at risk be too small. Those with a survival time of more than 5 years were reported 5 years, and events after the end of 5-year follow-up period were computed as censored data. Multivariable analysis was performed by the Cox proportional hazard model to examine the interaction between obstruction and other variables and estimate the independent prognostic effect of preoperative obstruction by adjusting for confounding factors. Within the present study population, there were 184 recurrences of CRC (27 for stage II, 157 for stage III) which allowed up to 18, 3, or 15 variables to be included in a multivariable regression model for estimating in all stages, stage II, or stage III, respectively. When data were separated by the colon and rectal cancer, the numbers of events were 76 and 81, respectively. To avoid overfitting, all potential confounding factors of preoperative obstruction were reduced to a single composite characteristic by applying a propensity score.[15] A P value (2-sided) of <0.05 indicated statistical significance. Analyses were performed independently at our clinical research center by SPSS, version 17.0 (SPSS, Chicago, IL).

RESULTS

Patients' Characteristics and Association with Preoperative Obstruction

Clinicopathologic features are shown in Table 1. A total of 380 men and 261 women were analyzed (mean age, 61.3 ± 11.1 years). Of these patients, 207 had stage II and

TABLE 1 Patient characteristics and correlation with preoperative obstruction

Variable	No. of patients	%	Preoperative obstruction											
			Stage II and III disease (n = 641)				Colon cancer (n = 384)				Rectal cancer (n = 257)			
			Positive	Negative	Positive rate (%)	P[a]	Positive	Negative	Positive rate (%)	P[a]	Positive	Negative	Positive rate (%)	P[a]
Gender														
Male	380	59.3	39	341	10.3	0.688	30	193	13.5	0.261	9	148	5.7	0.327
Female	261	40.7	24	237	9.2		15	146	9.3		9	91	9.0	
Age (y)														
<60	274	42.7	29	245	10.6	0.594	18	137	11.6	>0.999	11	108	9.2	0.225
≥60	367	57.3	34	333	9.3		27	202	11.8		7	131	5.1	
Tumor location														
Right	169	26.4	24	145	14.2	0.051	24	145	14.2	0.203	–	–	–	–
Left	215	33.5	21	194	9.8		21	194	9.8		–	–	–	
Rectum	257	40.1	18	239	7.0		–	–	–		–	–	–	
Differentiation														
Nonpoor	583	91.0	51	532	8.7	0.009	38	314	10.8	0.080	13	218	5.6	0.025
Poor[b]	58	9.0	12	46	20.7		7	25	21.9		5	21	19.2	
T factor[c]														
T1	14	2.2	0	14	0.0	0.007	0	6	0.0	0.265	0	8	0.0	0.007
T2	40	6.2	0	40	0.0		0	16	0.0		0	24	0.0	
T3	563	87.8	57	506	10.1		42	305	12.1		15	201	6.9	
T4	24	3.7	6	18	25.0		3	12	20.0		3	6	50.0	
N factor[c]														
N0	207	32.3	16	191	7.7	0.454	13	118	9.9	0.517	3	73	3.9	0.089
N1	311	48.5	33	278	10.6		26	165	13.6		7	113	5.8	
N2	123	19.2	14	109	11.4		6	56	9.6		8	53	13.1	
ND (%)														
≥20	138	21.5	16	122	11.6	0.423	8	74	9.8	0.699	8	48	14.3	0.032
<20	503	78.5	47	456	9.3		37	265	12.3		10	191	5.0	
Intramural lymphatic invasion														
Negative	21	3.3	1	20	4.8	0.711	1	12	7.7	>0.999	0	9	0.0	>0.999
Positive	620	96.7	62	558	10.0		44	327	11.9		18	230	7.3	
Intramural vascular invasion														
Negative	62	9.7	7	55	11.3	0.654	7	34	17.1	0.300	0	21	0.0	0.376
Positive	579	90.3	56	523	9.7		38	305	11.1		18	218	7.6	

TABLE 1 continued

Variable	No. of patients	%	Preoperative obstruction											
			Stage II and III disease (n = 641)				Colon cancer (n = 384)				Rectal cancer (n = 257)			
			Positive	Negative	Positive rate (%)	P[a]	Positive	Negative	Positive rate (%)	P[a]	Positive	Negative	Positive rate (%)	P[a]
Preoperative CEA														
Normal (<2.5 ng/ml)	418	65.2	29	389	6.9	0.001	18	239	7.0	<0.001	11	150	6.8	>0.999
Elevated (>2.5 ng/ml)	223	34.8	34	189	15.2		27	100	21.3		7	89	7.3	
Preoperative CA19-9														
Normal (<37 ng/ml)	574	89.5	52	522	9.1	0.079	37	306	10.8	0.120	15	216	6.5	0.406
Elevated (>37 ng/ml)	67	10.5	11	56	16.4		8	33	19.5		3	23	11.5	
Laparoscopic operation														
Yes	83	12.9	5	78	6.0	0.321	2	64	3.0	0.041	3	14	17.6	0.105
No	558	87.1	58	500	11.6		36	282	11.3		15	225	6.3	
No. of total dissected lymph nodes														
<6	28	4.4	2	26	7.1	0.175	2	15	11.8	0.205	0	11	0.0	0.337
6–10	84	13.1	4	80	4.8		4	53	7.0		0	27	0.0	
11–15	129	20.1	10	119	7.8		6	77	7.2		4	42	8.7	
>15	400	62.4	47	353	11.8		33	194	14.5		14	159	8.1	
Anastomotic leakage														
Present	28	4.4	1	27	3.6	0.510	1	6	14.3	0.585	0	21	0.0	0.376
Absent	613	95.6	62	551	10.1		44	333	11.7		18	218	7.6	
Adjuvant chemotherapy														
Yes	527	82.2	50	477	9.5	0.493	7	34	17.1	0.300	13	191	6.4	0.544
No	114	17.8	13	101	11.4		38	305	11.1		5	48	9.4	
Perioperative transfusion														
Yes	122	19.0	16	106	13.1	0.178	10	33	23.3	0.021	6	73	7.6	0.795
No	519	81.0	47	472	9.1		35	306	10.3		12	166	6.7	

ND node density (metastatic lymph node ratio)

[a] Compared by Pearson's chi-square test or Fisher's exact test

[b] Poor includes poorly differentiated, mucinous, and undifferentiated types

[c] T and N factors indicate pathological T and N factor

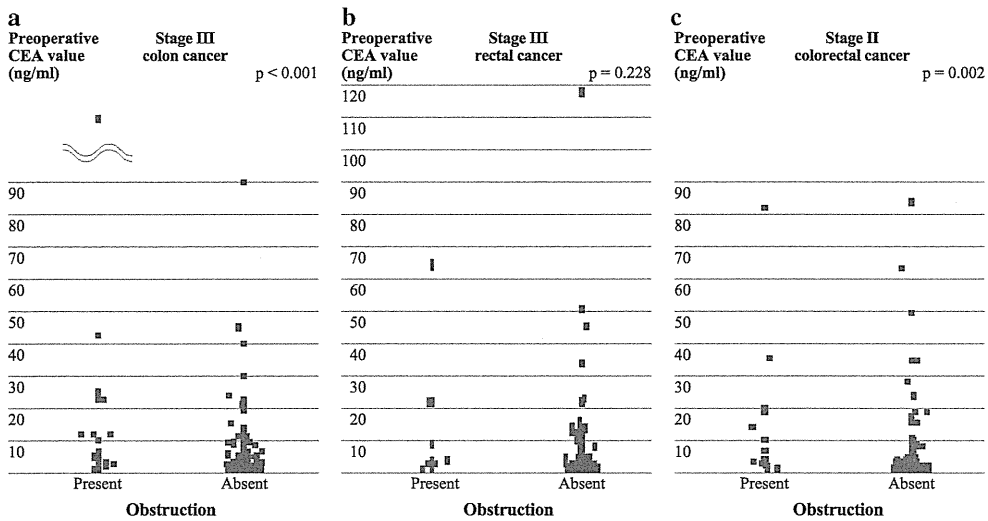

FIG. 1 Preoperative CEA values were compared between obstructed cases and nonobstructed cases, respectively, in (**a**) colon cancer ($n = 253$), (**b**) rectal cancer ($n = 181$), and stage II CRC ($n = 207$)

434 had stage III CRC. Preoperative obstruction occurred in 16 (7.7%) and 47 (10.8%) of patients with stage II and III CRC, respectively. Among these 63 patients, only 1 patient underwent emergency primary resection with curative intent. All other patients underwent curative resection electively after decompression of the bowel obstruction. Emergency ileostomy or colostomy was initially performed in 15 patients; the remaining 47 patients were initially treated by nonoral nutrition with/without a nasal or anal decompression tube. During the study period, 2 patients with bowel obstruction were resected primary cancer without radicality (not included in the study). Therefore, 63 patients (96.9%) among the 65 obstructed patients had radical resection regardless of 1- or 2-stage surgery.

For all subjects, preoperative obstruction was more common in colon cancer ($P = 0.058$), related to preoperative CEA elevation ($P = 0.001$), T factor ($P = 0.007$), and poor differentiation ($P = 0.009$) (Table 1). Preoperative obstruction was significantly associated with preoperative CEA (odds ratio = 3.56; $P < 0.001$) for colon cancer, while for rectal cancer, preoperative obstruction was related with poor differentiation (odds ratio = 3.99; $P = 0.016$) and metastatic lymph node ratio (node density) $\geq$20% (odds ratio = 3.18, $P = 0.021$), by multivariable logistic regression analyses. Among patients with stage III colon cancer, preoperative CEA values were significantly higher in those with obstructive colon cancer (14.80 ± 7.55) than those with nonobstructive colon cancer (3.88 ± 0.54) ($P < 0.001$, Fig. 1a), contrarily, that was not true in stage III rectal cancer (Fig. 1b). The mean number of total dissected lymph nodes tended to be greater in obstructed cases than in nonobstructed cases (stage II: 33.25 ± 5.24 and 25.23 ± 1.15, respectively; stage III: 26.17 ± 2.45, 23.01 ± 0.87), although there was no difference in the distribution of patient numbers categorized by the recovered node number (Table 1).

Kaplan–Meier Estimate of DFS

The overall follow-up period ranged from 2 to 207 months (median, 77 months), and the mean DFS was 46.7 months during our 5-year follow-up. Because a cumulative DFS probability of 50% was not reached by the end of the 5-year follow-up, the overall median DFS time was not determined. Five-year cumulative DFS was 51.4% for the obstructed group and 72.9% for the nonobstructed group. The median survival time was 32 months for patients with obstruction; median survival time was not available for patients without obstruction at the end of the 5-year follow-up period, indicating a significantly poorer prognosis in the obstructed group ($P < 0.001$, Supplementary Table 1). Preoperative obstruction was significantly associated with poorer prognosis for stage III patients ($P < 0.001$, Fig. 2a), but not for stage II patients ($P = 0.478$, Supplementary Table 2). For stage III patients, DFS was 41.0% for obstructed vs. 65.6% for nonobstructed patients; for stage II patients, DFS was 81.3% vs. 87.4%, respectively. There was no statistical difference in prognosis between primary resection and delayed (2-stage) resection in stage III patients (data not shown).

When tumor location was analyzed separately, preoperative obstruction was significantly associated with a poor outcome in patients with stage III colon cancer or rectal cancer (Tables 2 and 3). Among patients with stage III colon cancer, the DFS was 48.1% in those with obstruction compared with 70.0% in those without obstruction ($P = 0.004$, Fig. 2b). Among patients with stage III rectal cancer, the DFS was 26.7% in those with obstruction

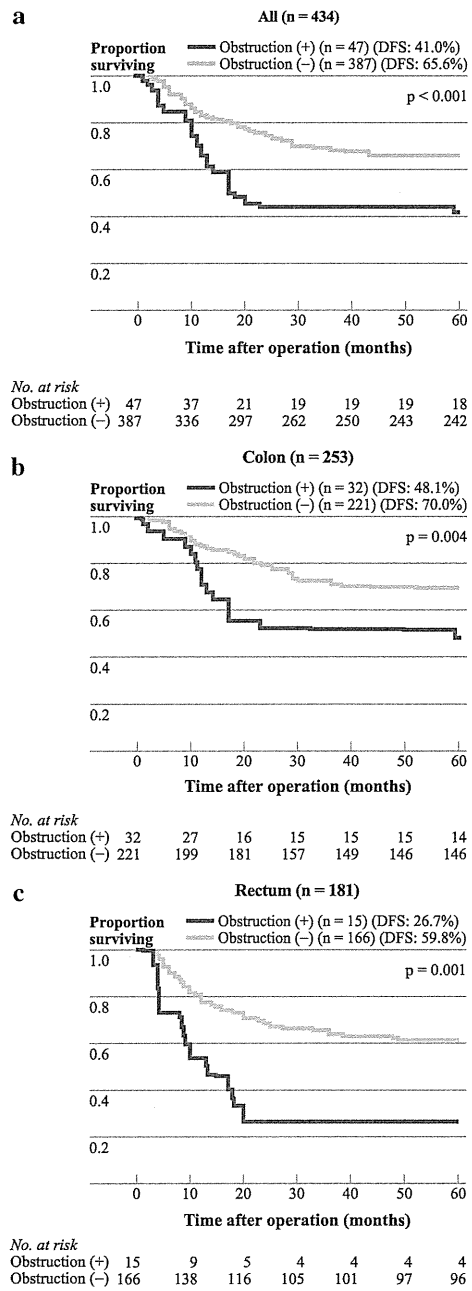

FIG. 2 Kaplan–Meier analysis of 5-year DFS according to preoperative obstruction in patients with stage III CRC. **a** All stage III CRC ($n = 434$). **b** Stage III colon cancer ($n = 253$). **c** Stage III rectal cancer ($n = 181$)

compared with 59.8% in those without obstruction ($P = 0.001$, Fig. 2c).

Multivariable Prognostic Analysis of Preoperative Obstruction

The Cox proportional hazard model was applied to estimate the effect of preoperative obstruction on survival. The crude hazard ratio (HR) of obstructed patients compared to nonobstructed patients was 2.21 (95% confidence interval [CI], 1.50–3.27; $P < 0.001$). After controlling for clinicopathologic factors, the adjusted HR of preoperative obstruction was 2.05 (95% CI, 1.35–3.11, $P = 0.001$). We also performed an analysis using a propensity score to adjust for the preoperative effect by transforming all other confounding variables into a single estimator. After the adjustment, the HR of preoperative obstruction became 1.92 (95% CI, 1.28–2.89, $P = 0.002$), suggesting that preoperative obstruction is an independent risk factor for prognosis (Supplementary Table 1).

The unadjusted effect of preoperative obstruction on DFS was 1.24 (95% CI, 0.60–2.10; $P = 0.506$) in stage II and 2.25 (95% CI, 1.49–3.41; $P < 0.001$) in stage III. Next, an evaluation of each stage was performed. The adjusted HR was not significant for stage II CRC (1.96; 95% CI, 0.48–7.97; $P = 0.347$), but was significant for stage III CRC (1.98; 95% CI, 1.26–3.09; $P = 0.003$). These respective values decreased to 1.62 (95% CI, 0.38–7.07; $P = 0.506$) and 1.88 (95% CI, 1.22–2.89; $P = 0.004$) after propensity score adjustment (Supplementary Table 3).

Prognostic Effect of Preoperative Obstruction in Stage III Colon and Rectal Cancer

Preoperative obstruction occurred in 32 cases (12.6%) of patients with stage III colon cancer ($n = 253$). Preoperative obstruction indicated significant poor outcome in stage III colon cancer (HR, 2.18; $P = 0.005$) (Table 2). Tumor location ($P = 0.014$), N factor ($P < 0.001$), and preoperative CEA ($P = 0.001$) were also significantly associated with patient prognosis in univariable analysis. The Cox proportional hazard model revealed that preoperative obstruction remained a potential independent prognostic factor in stage III colon cancer with an adjusted HR of 1.83 (95% CI, 1.02–3.25) ($P = 0.041$), after propensity score adjustment (Table 2).

We similarly analyzed stage III rectal cancer ($n = 181$). Preoperative obstruction occurred in 15 cases (8.3%) and showed a significant poor prognosis (HR, 2.78; $P = 0.002$) (Table 3). Differentiation ($P = 0.017$), T4 factor ($P = 0.019$), N factor ($P < 0.001$), node density ≥ 20 ($P = 0.004$), preoperative CEA level ($P = 0.046$), and preoperative CA19-9 ($P = 0.005$) were also significantly associated with patient outcome in univariable analysis. Subsequent analyses suggested that preoperative obstruction also had a tendency to have a detrimental effect on clinical outcome in stage III rectal cancer, with an adjusted HR of 2.17 (95% CI, 0.98–4.79) ($P = 0.056$) after propensity score adjustment (Table 3). Preoperative obstruction had a prognostic impact, especially on patients with poorly differentiated stage III rectal cancer (Supplementary Fig. 1).

TABLE 2 Prognostic analysis on 5-year DFS in patients with stage III colon cancer ($n = 253$)[a]

Variables	Univariable analysis		Multivariable analysis			
			Model 1		Model 2	
	HR (95% CI)	P[b]	HR (95% CI)	P[b]	HR (95% CI)	P[b]
Preoperative obstruction (present)	2.18 (1.26–3.77)	0.005	1.98 (1.09–3.58)	0.024	1.83 (1.02–3.25)	0.041
Gender (female)	0.89 (0.58–1.38)	0.606	0.85 (0.53–1.36)	0.495	n/d	n/d
Age (≥60 y)	0.73 (0.47–1.13)	0.160	0.76 (0.48–1.20)	0.236	n/d	n/d
Tumor location (left side)	0.58 (0.37–0.89)	0.014	0.49 (0.29–0.83)	0.008	n/d	n/d
Differentiation (poor[c])	1.38 (0.67–2.87)	0.386	1.15 (0.53–2.46)	0.726	n/d	n/d
T factor[d]						
T1	Ref.		Ref.		n/d	n/d
T2	n/d	n/d	n/d	n/d	n/d	n/d
T3	n/d	n/d	n/d	n/d	n/d	n/d
T4	n/d	n/d	n/d	n/d	n/d	n/d
N factor[d] (N2)	2.49 (1.59–3.90)	< 0.001	2.76 (1.54–4.93)	0.001	n/d	n/d
ND (≥20)	1.25 (0.79–1.96)	0.343	0.89 (0.46–1.73)	0.729	n/d	n/d
Intramural lymphatic invasion (positive)	20.86 (0.04–11498.43)	0.346	n/d	n/d	n/d	n/d
Intramural vascular invasion (positive)	1.53 (0.71–3.33)	0.281	1.45 (0.63–3.36)	0.385	n/d	n/d
Preoperative CEA (elevated)	1.77 (1.14–2.75)	0.01	1.92 (1.16–3.16)	0.011	n/d	n/d
Preoperative CA19-9 (elevated)	1.32 (0.66–2.64)	0.433	0.78 (0.36–1.68)	0.518	n/d	n/d
Laparoscopic operation (yes)	0.92 (0.55–1.56)	0.758	1.00 (0.55–1.79)	0.987	n/d	n/d
Number of total dissected lymph nodes						
<6	Ref.		Ref.		n/d	n/d
6–10	0.54 (0.18–1.61)	0.266	0.33 (0.10–1.09)	0.069	n/d	n/d
11–15	0.63 (0.23–1.73)	0.372	0.38 (0.12–1.16)	0.088	n/d	n/d
>15	0.90 (0.36–2.26)	0.823	0.35 (0.11–1.14)	0.081	n/d	n/d
Anastomotic leakage (present)	1.79 (0.25–12.88)	0.562	2.34 (0.28–19.32)	0.431	n/d	n/d
Adjuvant chemotherapy (yes)	2.35 (1.08–5.09)	0.031	2.32 (1.05–5.11)	0.037	n/d	n/d
Perioperative transfusion (yes)	0.88 (0.43–1.84)	0.741	0.60 (0.27–1.33)	0.206	n/d	n/d
Propensity score	n/d	n/d	n/d	n/d	5.53 (1.07–28.65)	0.042

ND node density (metastatic lymph node ratio)

[a] End point is date of death or March 31, 2007, if patient survived. There was no event in T1-, T2-, and intramural lymphatic invasion–negative cases, so that these variables were excluded from multivariable analysis. Multivariable model 2 indicates the adjusted effect of preoperative obstruction by applying propensity score, which is a conditional probability of presenting preoperative obstruction given by other clinicopathologic factors

[b] Cox proportional hazard model

[c] Poor includes poorly differentiated, mucinous, and undifferentiated types

[d] T and N factor indicate pathological factors

First Recurrence Site and Preoperative Obstruction in Stage III CRC Patients

First recurrence sites in stage III patients were analyzed with consideration of preoperative obstruction. Preoperative obstruction was significantly correlated with hepatic recurrence ($P = 0.019$ by Fisher's exact test) but not with other forms of recurrence (Supplementary Table 4).

DISCUSSION

In the present study, the prognostic value of preoperative obstruction was examined in curatively operated patients with advanced CRC (stage II and III). Multivariable analysis revealed that preoperative obstruction detrimentally affected on long-term prognosis in stage III colon and rectal cancer, but not in stage II CRC. Moreover, preoperative obstruction was associated with systemic

TABLE 3 Prognostic analysis on 5-year DFS in patients with stage III rectal cancer ($n = 181$)[a]

Variable	Univariable analysis		Multivariable analysis			
			Model 1		Model 2	
	HR (95% CI)	P^b	HR (95% CI)	P^b	HR (95% CI)	P^b
Preoperative obstruction (present)	2.78 (1.46–5.28)	0.002	2.34 (1.06–5.17)	0.035	2.17 (0.98–4.79)	0.056
Gender (female)	0.92 (0.58–1.45)	0.716	1.21 (0.72–2.01)	0.471	n/d	n/d
Age (≥60 y)	1.21 (0.77–1.90)	0.418	1.62 (0.98–2.69)	0.059	n/d	n/d
Differentiation (poor[c])	2.00 (1.13–3.51)	0.017	1.85 (0.95–3.57)	0.070	n/d	n/d
T factor[d]						
T1	Ref.		Ref.		n/d	n/d
T2	2.53 (0.31–20.54)	0.386	2.73 (0.31–24.28)	0.367	n/d	n/d
T3	4.35 (0.60–31.37)	0.145	3.77 (0.44–32.61)	0.229	n/d	n/d
T4	12.36 (1.52–100.75)	0.019	7.35 (0.67–81.01)	0.104	n/d	n/d
N factor[d] (N2)	2.42 (1.54–3.80)	< 0.001	1.55 (0.78–3.08)	0.209		
ND (≥20)	1.96 (1.24–3.11)	0.004	1.57 (0.77–3.18)	0.214	n/d	n/d
Intramural lymphatic invasion (positive)	20.32	0.617	n/d	n/d	n/d	n/d
Intramural vascular invasion (positive)	1.61 (0.59–4.41)	0.353	0.74 (0.22–2.48)	0.629	n/d	n/d
Preoperative CEA (elevated)	1.58 (1.01–2.49)	0.046	1.31 (0.78–2.18)	0.309	n/d	n/d
Preoperative CA19-9 (elevated)	2.42 (1.30–4.51)	0.005	2.24 (1.12–4.45)	0.022	n/d	n/d
Laparoscopic operation (yes)	0.70 (0.28–1.73)	0.434	0.86 (0.29–2.55)	0.781	n/d	n/d
Number of total dissected lymph nodes						
<6	Ref.		Ref.		n/d	n/d
6–10	0.93 (0.26–3.29)	0.908	1.40 (0.34–5.73)	0.643	n/d	n/d
11–15	1.23 (0.41–3.75)	0.711	1.15 (0.34–3.92)	0.822	n/d	n/d
>15	1.25 (0.45–3.47)	0.664	1.12 (0.32–3.99)	0.860	n/d	n/d
Anastomotic leakage (present)	0.79 (0.32–1.95)	0.601	1.08 (0.40–2.86)	0.884	n/d	n/d
Adjuvant chemotherapy (yes)	1.07 (0.61–1.88)	0.815	1.20 (0.62–2.33)	0.594	n/d	n/d
Perioperative transfusion (yes)	1.20 (0.74–1.96)	0.458	1.03 (0.57–1.85)	0.932	n/d	n/d
Propensity score	n/d	n/d	n/d	n/d	2.38 (0.55–10.23)	0.246

ND node density (metastatic lymph node ratio); *n/d* not determined

[a] End point is date of death or March 31, 2007, if patient survived. There was no event in intramural lymphatic invasion–negative cases, so that this variable was excluded from multivariable analysis. Multivariable model 2 indicates the adjusted effect of preoperative obstruction by applying propensity score, which is a conditional probability of presenting preoperative obstruction given by other clinicopathologic factors

[b] Cox proportional hazard model

[c] Poor includes poorly differentiated, mucinous, and undifferentiated types

[d] T and N factor indicate pathological factors

recurrence rather than local, lymph node, and peritoneal recurrence with regard to the first recurrent site in stage III CRC, suggesting that micrometastasis may occur in cases of preoperative obstruction. The tumor factors associated with preoperative obstruction differed between colon (preoperative CEA elevation) and rectal cancer (poor differentiation). Actually, the preoperative CEA level was significantly greater in obstructive colon cancer than in nonobstructive colon cancer, while this was not true for rectal cancer (Fig. 1a, b).

These findings suggest that obstructed CRC reflects a more advanced disease, possibly with radiologically undetectable occult metastases, which may be partly reflected by an elevated CEA level in colon cancer. This hypothesis could explain why bowel obstruction was associated with worse survival in stage III, but not in stage II disease. In addition, the preoperative CEA level was correlated with obstruction in stage II CRC (Fig. 1c), but neither obstruction nor preoperative CEA level was associated with a poor prognosis in stage II CRC. Collectively, factors other than the bowel obstruction itself (such as occult metastatic disease as reflected by elevated CEA level) may contribute to a worse outcome.

The prognostic effect of preoperative obstruction is controversial and may be due to the inclusion of several stages, and/or mixed curability.[1,5–11] In this study, each

stage and tumor location was analyzed separately in curatively operated CRC, and preoperative obstruction predicted a poor prognosis in stage III, but not in stage II. This result in stage II is different from a previous report.[16] In the present study, preoperative obstruction was particularly associated with a poor prognosis in right-sided stage III colon cancer ($P = 0.008$), corresponding to previous studies, although these reports included patients with both stage II and III.[1,8] Additionally, there is an argument in favor of 2-stage curative surgery.[3,17] In our study, there was no statistically significant difference in long-term prognosis between 1- and 2-stage curative surgery, but this comparison should be made with a large number of cases of obstructive CRC in the future.

The initiation of metastasis is suggested to begin earlier in tumorigenesis than previously thought.[18] Tumor cells are frequently present in the circulation or bone marrow of patients with cancer before clinical or histopathological metastasis.[19,20] Luminal distension or related inflammation was proved to increase the mucosal permeability of capillaries to macromolecules, and mucosal inflammation produces inflammatory cytokines, including interleukin-1, interleukin-6, receptor activator of nuclear factor κB, and tumor necrosis factor alpha, which are known to promote distant metastasis.[21–23] Thus, obstruction proximal to CRC may lead to a favorable environment for systemic metastasis.

Many parameters have been reported to be independent prognostic factors for stage III CRC, including the number of metastatic lymph nodes, negative lymph node count, metastatic lymph node ratio, the number of evaluated lymph nodes, and preoperative CEA.[24–33] Among these factors, the number of metastatic lymph nodes has only been available in clinical practice as TNM substaging and has been prospectively confirmed.[34] Interestingly, preoperative obstruction was the only prognostic discriminator in both colon cancer and rectal cancer in the present study. However, a negative lymph node count, metastatic lymph node ratio (which we called node density factor), and the number of evaluated lymph nodes did not have prognostic significance in colon cancer or rectal cancer (data not shown). Preoperative CEA are insufficient to use as a prognostic marker, and recently we noted a diminishing impact of preoperative CEA on prognosis in stage III disease with the advancement of adjuvant chemotherapy and diagnostic tools.[35,36] One study also suggested that intraperitoneal free cancer cells were an important prognostic marker in stage III disease.[14] In our current study, we present a novel prognostic marker in stage III disease.

In terms of molecular and genetic markers, DNA ploidy in patients with right-sided colon cancer alone has been prospectively confirmed in stage III CRC.[37] Ki-ras mutation on codon 12 was also reported a risk factor for relapse or cancer-related death in stage III.[38] TP53 mutation status is reported a prognostic predictor in stage III.[39] Other numerous markers have also been reported to indicate poor prognosis; however, all such genetic and molecular tools are unsuitable for routine application at present because they have not been validated. Additionally, testing for these markers is time consuming and expensive at present. In contrast, preoperative obstruction is easily identifiable for practical examinations and may have potential for patient selection after curative operation, in addition to intraperitoneal free cancer cells.

In conclusion, preoperative obstruction affected the long-term prognosis in patients with stage III CRC. To our knowledge, this study is the first to evaluate the prognostic impact of preoperative obstruction in each stage of advanced CRC among patients who underwent curative surgery. Preoperative obstruction may be a clinically applicable marker and a potential tool to identify patients with high-risk stage III CRC, at least in colon cancer.

ACKNOWLEDGMENT We thank Forte, Tokyo, Japan, for editorial assistance.

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Int. J. Radiation Oncology Biol. Phys., Vol. 79, No. 3, pp. 677–683, 2011

0360-3016/$–see front matter

doi:10.1016/j.ijrobp.2009.11.007

CLINICAL INVESTIGATION **Rectum**

A PHASE II TRIAL OF NEOADJUVANT PREOPERATIVE CHEMORADIOTHERAPY WITH S-1 PLUS IRINOTECAN AND RADIATION IN PATIENTS WITH LOCALLY ADVANCED RECTAL CANCER: CLINICAL FEASIBILITY AND RESPONSE RATE

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Purpose: We aimed to validate our hypothesis that a preoperative chemoradiotherapy regimen with S-1 plus irinotecan is feasible, safe, and active for the management of locally advanced rectal cancer in a single-arm Phase II setting.
Methods and Materials: Eligible patients had previously untreated, locally advanced rectal adenocarcinoma. Radiotherapy was administered in fractions of 1.8Gy/d for 25 days. S-1 was administered orally in a fixed daily dose of 80mg/m^2 on Days 1 to 5, 8 to 12, 22 to 26, and 29 to 33. Irinotecan (80mg/m^2) was infused on Days 1, 8, 22, and 29. Four or more weeks after the completion of the treatment, total mesorectal excision with lateral lymph node dissection was performed. The primary endpoint was the rate of completing treatment in terms of feasibility. The secondary endpoints were the response rate and safety.
Results: We enrolled 43 men and 24 women in the study. The number of patients who completed treatment was 58 (86.6%). Overall, 46 patients (68.7%) responded to treatment and 24 (34.7%) had a complete histopathologic response. Three patients had Grade 3 leukopenia, and another three patients had Grade 3 neutropenia. Diarrhea was the most common type of nonhematologic toxicity: 3 patients had Grade 3 diarrhea.
Conclusions: A preoperative regimen of S-1, irinotecan, and radiotherapy to the rectum was feasible, and it appeared safe and effective in this nonrandomized Phase II setting. It exhibited a low incidence of adverse events, a high rate of completion of treatment, and an extremely high rate of pathologic complete response. © **2011 Elsevier Inc.**

Chemoradiation, Rectal cancer, S-1, Irinotecan.

INTRODUCTION

In Japan the incidence of colorectal cancer (CRC) is increasing year by year. If this trend continues, forecasts estimate that about 170,000 people will have CRC in 2015. Colorectal cancer will become the most prevalent type of cancer in Japan, surpassing gastric cancer and lung cancer (1). In Europe and North America, CRC is the second leading cause of cancer-related death, behind lung cancer. Globally, the prevention, early diagnosis, and treatment of CRC are urgent tasks.

Advanced rectal cancer carries a poorer prognosis than advanced colon cancer. The control of local recurrence, a unique characteristic of rectal cancer, and improved overall survival are important goals of treatment. Total mesorectal excision (TME) has recently been shown to decrease the rate of local recurrence and is performed throughout the world as a standard procedure (2, 3). In the mid 1980s the Gastrointestinal Tumor Study Group showed that postoperative chemoradiotherapy improves the rate of recurrence-free survival (4). On the basis of these results, the National Institutes of Health in the United States has recommended resection plus postoperative chemoradiotherapy as standard therapy for pathologic Stage II and III rectal cancer since 1990 (5). Five controlled studies comparing preoperative radiotherapy followed by surgery with surgery alone subsequently showed that the rate of local recurrence is significantly lower in patients who receive preoperative radiotherapy than in those who receive surgery alone (6). Moreover, the Swedish Rectal Cancer Trial showed that preoperative radiotherapy significantly improves overall and disease-free survival (7). On the other hand, European Organisation for Research and

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Supplementary material for this article can be found at www.redjournal.org.

Conflict of interest: none.

Received April 1, 2009, and in revised form Nov 16, 2009. Accepted for publication Nov 18, 2009.

Treatment of Cancer Trial 22921, a large Phase III study, failed to prove that chemoradiotherapy improves survival rates, but the control of local recurrence at 5 years was significantly better in patients who received chemoradiotherapy than in those who received radiotherapy alone if chemotherapy was given at any time during the course of treatment (8). On the basis of these results, preoperative chemoradiotherapy was acknowledged to be standard treatment for locally advanced rectal cancer. However, the dose, duration, and radiation target volumes, as well as optimal concomitant agents, remain controversial. Recently, Guillem *et al.* (9) reported that patients with a complete response (CR) or nearly complete response to preoperative chemoradiotherapy have good long-term outcomes. Attention has thus focused on the relation between CR ratio (tumor downstaging) and survival outcome by preoperative chemoradiotherapy.

In Japan, however, few clinical trials of adjuvant radiotherapy have been conducted because the rate of local recurrence after the Japanese standard therapy (TME plus lateral lymph node dissection without neoadjuvant radiotherapy) is comparable to that including neoadjuvant chemoradiotherapy in Europe and North America. Because surgery alone has reached the most optimal outcome for decreasing local recurrence or improving survival of advanced rectal cancers in Japan at present, we wondered whether it is really necessary to evaluate chemotherapy combined with radiotherapy to improve clinical outcomes.

S-1 is an oral anticancer drug that combines tegafur, which is finally converted to the active agent of 5-fluorouracil (5-FU), with gimeracil and oteracil potassium. Gimeracil was added to increase the blood 5-FU concentration by inhibiting metabolism of 5-FU by dihydropyrimidine dehydrogenase mainly in the liver. On the other hand, oteracil potassium is widely distributed to gastrointestinal tissues and antagonizes orotate phosphoribosyl transferase, resulting in inhibition of 5-fluoronucleorides (active metabolites) generated from 5-FU, as well as reduced toxicity of 5-FU. Moreover, we also focused on the recently proven fact that components of S-1 markedly increase the radiosensitivity of cancer cells (even 5-FU–resistant cells) to radiotherapy in CRC (10). In addition, irinotecan hydrochloride decreases messenger ribonucleic acid levels of thymidylate synthase as a target enzyme of 5-FU (11), thereby augmenting its inhibition (12). Several studies have also shown that 5-FU induces topoisomerase I and that cancer cells overexpressing topoisomerase I increased chemosensitivity against irinotecan (13, 14). Such *in vitro* mechanisms provide a theoretic basis for combining S-1 and irinotecan plus radiation therapy (Fig. 1). At present, 5-FU–based chemoradiotherapy is used as a standard treatment for rectal cancer (4, 15); however, our 5-FU–based chemoradiotherapy was considered worthy of investigation.

A Phase I clinical study was performed to determine the maximum tolerated doses and recommended doses of S-1 and irinotecan. The pathologic response rate to the recommended dose, though not the primary endpoint in the Phase I study, however, was 94.7%, and the pathologic CR rate was surprisingly 31.6%, indicating that treatment with S-1 and irinotecan plus radiation was very active for locally advanced rectal cancer (16). In this Phase II clinical trial, we aimed to validate our hypothesis that a preoperative chemoradiotherapy regimen with S-1 plus irinotecan is feasible, safe, and effective for the management of locally advanced rectal cancer.

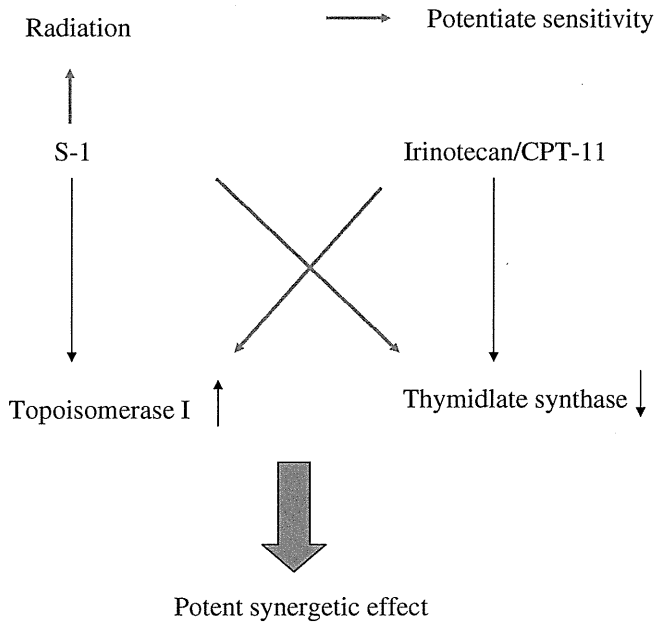

Fig. 1. Interaction of S-1 and irinotecan.

METHODS AND MATERIALS

This study was performed according to the guidelines of the Declaration of Helsinki, as amended in Edinburgh, Scotland, in October 2000. The protocol was approved by the Institutional Review Board of Kitasato University Hospital (Kanagawa, Japan). All patients gave written informed consent before study entry.

Eligibility criteria

Eligible patients had previously untreated clinical T3 or T4, N0 to N2, M0 locally advanced rectal cancer as confirmed histopathologically as adenocarcinoma in the rectum from August 2005 through December 2007, as well as an Eastern Cooperative Oncology Group performance status of 0 to 2. We used the International Union Against Cancer staging system. We described rectal cancer as involving the portion of the rectum above the peritoneal reflection and the portion of the rectum below the peritoneal reflection and ruled out other portions using the Japanese classification of CRC, and our definition of the rectum is thus the same as that of the International Union Against Cancer. Other eligibility criteria were as follows: age 20 to 80 years at enrollment; no severe disturbances of main organ functions (including bone marrow, heart, lung, liver, and kidney); no severe hematologic or blood chemical abnormalities such as leukocyte count of 4,000 to 12,000/mm^3, neutrophil count of 2,000/mm^3 or greater, platelet count of 100,000/μL or greater, hemoglobin concentration of 9.0 g/dL or greater, total bilirubin concentration of 1.5 mg/dL or less, serum aspartate aminotransferase and alanine aminotransferase levels less than twice the upper limit of normal, serum creatinine concentration less than the upper limit of the normal; normal electrocardiographic findings; and the ability

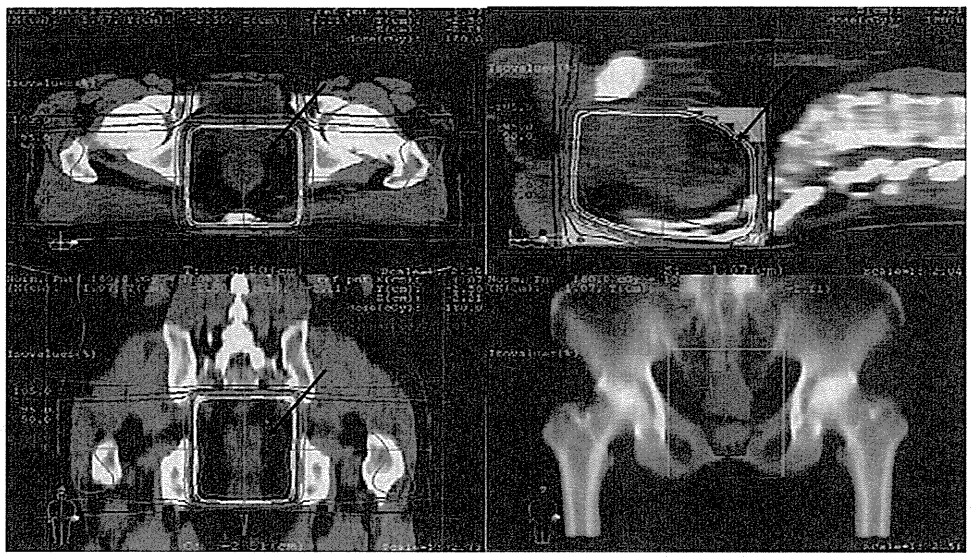

Fig. 2. Treatment field of radiation therapy.

to ingest solid foods and drugs orally. The eligible patients could not be transfused with red cells to meet these criteria.

Before enrollment in the study, we reviewed the histories of past and present disease and the general condition of all patients, assessed based on interview, physical examination, and blood tests. Locally advanced rectal cancer (clinical T3 or T4) without distant metastasis was confirmed by barium enema; colonoscopy including histopathologic evaluation; computed tomographic scans of the chest, abdomen, and pelvis; and magnetic resonance imaging (MRI) of the pelvis. Magnetic resonance imaging of the pelvis is useful to differentiate the clinical diagnosis of T3 and T4 and lymph node metastasis adjacent to the rectum. Differential diagnostic standards of MRI dictate that clinical T3 indicates a breach of the outer layer of the longitudinal muscle on T2 intensity imaging and T4 indicates irregular invasion to the extracorporeal region of the rectum on T1 intensity imaging.

Radiotherapy and chemotherapy

The treatment field of radiotherapy has been published previously (16). In brief, radiotherapy was administered in fractions of 1.8 Gy/d, given 5 days per week for 5 weeks. The total dose of radiation was 45 Gy. Patients were treated in the prone position, by use of a dedicated device (lead board) to minimize exposure of the small bowel. A computed tomography–based treatment planning system was mandatory to define the planned target volume (PTV), which allowed for setup error, organ movement, and a 1-cm circumference (clinical target volume) around both the primary tumors including regions invading surrounding organs or tissues and the adjacent swollen lymph nodes (gross tumor volume) (Fig. 2). The PTV was treated with radiation from a 10-MV linear accelerator, and we used a four-field box technique. The clinical target volume for the primary tumor used in this study typically included the perirectal lymph nodes. The target volumes used for radiotherapy in this study are far smaller in comparison to those usually described in North American and European practice, where the internal iliac nodes and often the external iliac nodes are electively irradiated. Thirty-eight patients had swollen lymph nodes included in the gross tumor volume preoperatively, and none was outside the PTV for radiotherapy. The response rate of the primary tumor was graded, but that of lymph nodes was not assessed.

S-1 (80 mg $\cdot$ m^{-2} $\cdot$ d^{-1}) was given orally after breakfast and dinner on Days 1 to 5, 8 to 12, 22 to 26, and 29 to 33. Irinotecan (80 mg $\cdot$ m^{-2} $\cdot$ d^{-1}) was given as an intravenous infusion over a period of 90 minutes on Days 1, 8, 22, and 29. The relative dose of irinotecan between the folinic acid, 5-FU, and irinotecan regimen and that used in this study is 180 mg/m^2 biweekly vs. 80 mg/m^2 weekly (180/160 = 1.125). The rationale for using a 1-week interval for chemoradiotherapy was to allow recovery of the patient's fatigue. It was our impression that a shorter interval duration would lead to several patients discontinuing the regimen before its completion.

Surgery

Total mesorectal excision with bilateral autonomic nerve preservation was performed, and lymph nodes were dissected from the middle rectal, internal iliac, and obturator lymph node regions. For sphincter-preserving surgery, the anorectal side of the rectum was divided, leaving a margin of at least 2 cm from the inferior border of the tumor. Abdominoperineal resection was done if the distal margin was insufficient.

Criteria for modification of treatment schedule and dosage

Our protocol specified that the regimens may be suspended for Grade 3 or worse diarrhea and nausea/vomiting, and we prospectively assessed hematologic, urinary, and dermatologic toxicities every 7 days by blood, urine, and dermatologic assessment. Toxicities were evaluated according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 2. If toxicity necessitated a dose reduction within a course of treatment, the dose could be decreased by one step (20%) of irinotecan and treatment resumed. If toxicity requiring a further dose reduction recurred after the dose was decreased by one step, the study was terminated in the patient, with no further decrease in dosage.

Method for calculating rate of completing treatment

The ratios of the total administered dose to the total scheduled dose up to the date of surgery were calculated for radiotherapy,

S-1, and irinotecan by the following formula: Administered dose/Scheduled dose × 100 (%). We defined completing treatment as administered dose equal to or over 75% of full dose, and such cases actually coincided with the patients who were given 100% of the dose of chemotherapy.

Method for calculating rate of response

After surgery, the responses of tumors to chemoradiotherapy were histopathologically evaluated by examining serial sections of the resected specimens. Responses were evaluated based on the degree of degeneration or necrosis and fusion of cancer cells. No response was assigned a grade of 0, and a CR was assigned a grade of 3. The criteria for histopathologically evaluating the response to preoperative chemoradiotherapy, according to the Histopathological Response Criteria of the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus edited by the Japanese Society for Cancer of the Colon and Rectum, have been previously described (16). In brief, complete, considerable, and slight responses coincide with Grade 3, Grade 2, and Grade 1, respectively.

Endpoints and statistical considerations

The primary endpoint was the rate of completing treatment in terms of feasibility. The secondary endpoints were the response rate, safety (incidences of adverse reactions and complications), local recurrence rate, and overall survival. The response rate is determined based on pathologic CR, as well as incidences of adverse reactions including hematologic, urologic, dermatologic, and symptomatic complications. Data on local recurrence and overall survival are not presented in this report, because follow-up is not sufficient to allow conclusions regarding survival outcome.

We calculated the required sample size for this study based on a target rate of treatment completion of 70% and a minimum completion rate of 50%, with an α error of 0.05 (1-sided) and a β error of 0.1. The required number of patients was estimated to be 50. In anticipation of 10% of patients being ineligible, we planned to enroll 55 patients. Ineligible patients were those who did not provide informed consent or who had rectal cancer located in portions other than those above the peritoneal reflection or below the peritoneal reflection. Patient enrollment was discontinued at the end of the month when the target number of 55 subjects had been reached. The final number of enrolled patients was 67. The final number was higher than the target number of 55 by 12, but less than 1 month had elapsed between the dates of enrollment of Patient 55 and Patient 67. Moreover, Patient 67 started treatment before the results for Patient 55 were analyzed. We therefore decided that the histopathologic findings from all enrolled patients should be included in this analysis and considered this a valid procedure. The final number of enrolled patients was therefore higher than the initially planned target number.

RESULTS

Table 1 shows the demographic characteristics of the 67 patients with locally advanced rectal cancer who were eligible for the study and received preoperative chemoradiotherapy at our hospital. Median follow-up was 26 months (range, 11 to 51 months).

Table 1. Clinical characteristics of patients with locally advanced rectal cancer who received preoperative chemoradiotherapy

Clinical characteristic	Data	%
Sex		
Male	43	64.2
Female	24	35.8
Age (y)		
Median	63	
Range	32–79	
ECOG performance status		
0	67	100
1	0	0
Tumor site		
Ra	23	34.3
Rab	7	10.5
Rb	37	55.2
Depth of invasion		
T3	56	83.6
T4	11	16.4
Preoperative chemoradiotherapy		
Lymph nodes		
N0	30	44.8
N1	36	53.7
N2	1	1.5

Abbreviations: ECOG = Eastern Cooperative Oncology Group; Ra = rectum above peritoneal reflection; Rab = rectum above and below peritoneal reflection; Rb = rectum below peritoneal reflection.
Data are presented as No. of patients, unless otherwise indicated.

Primary endpoint

Of the 67 patients, 66 (98.5%) completed treatment based on our definition of completing treatment. The dose of irinotecan was reduced by 20%, and the radiation and S-1 protocols were not changed (except in 1 patient, who forgot to take S-1 for several days and in whom final S-1 compliance was equal to or over 90% but less than 100%, as shown in Table 2). Eight patients exhibited irinotecan compliance equal to or over 70% but less than 80% (Table 2). Finally, 1 patient who did not complete treatment had Grade 3 anorexia, nausea, and vomiting, and these symptoms responded to treatment with fluid therapy; however, treatment was discontinued at the patient's request. On the other hand, the rate of completing treatment reached 86.6% (58 of 67) per the protocol according to the criteria of the Cancer and Leukemia Group B (CALGB) study (17), where rates of completing treatment were determined in two categories of patients—those having completed six cycles of oxaliplatin (56%) and those having completed at least four cycles of therapy (72%). In our study 58 patients completed treatment with 100% of the dosage (including four cycles of chemotherapy).

Secondary endpoints

The pathologic response was Grade 3 (pathologic CR in the primary cancer) in 25 (37.3%) of 67 patients (Table 3). Because we included 1 case with lymph node metastasis, the number of bona fide cases exhibiting pathologic CR was therefore 24 patients (34.7% [24 of 67]) (Table 4). The

Table 2. Treatment exposure

Relative dose intensity (%)	S-1* (median dose intensity, 80 mg · m^{-2} · d^{-1} × 25 days)		Irinotecan (median dose intensity, 80 mg · m^{-2} · d^{-1} × 4 days)	
	No. of patients	%	No. of patients	%
100	65	97	58	86.6
≥90 to <100	1	1.5	0	0
≥80 to <90	0	0	0	0
≥70 to <80	0	0	8	11.9
Missing	1	1.5	1	1.5

* The maximum dose of S-1 was 120 mg · m^{-2} · d^{-1}.

rate of pathologic CR was 31.6% in the Phase I setting (16), and our result was comparable in this Phase II setting. The total response rate involving both Grade 2 (considerable response) and Grade 3 (CR) was 68.7% (46 of 67 patients), whereas that including even Grade 1a/1b (slight response) reached 100%, if evaluated in the primary cancers (Table 3). Although no cancer cells were found in 54 patients (80.6%) on colonoscopy with biopsy after chemoradiotherapy, more than half of these patients were actually confirmed to have residual disease on histopathologic examination of the resected specimens.

Safety includes incidences of adverse reactions and complications, and adverse events as acute toxicities are summarized in Table 5. Adverse events are infrequent, and there was no Grade 4 hematologic or nonhematologic toxicity. Regarding hematologic toxicity, only 3 patients had Grade 3 leukopenia and 3 had Grade 3 neutropenia. One patient with Grade 3 leukopenia concurrently had Grade 3 thrombocytopenia. Regarding nonhematologic toxicity, only 3 patients had Grade 3 diarrhea, which promptly improved after treatment with a continuous intravenous infusion. One patient had Grade 3 anorexia and nausea; treatment was withdrawn before completion at the patient's request. Activity of either dihydropyrimidine dehydrogenase or orotate phosphoribosyl transferase enzyme was not assessed in this study, but such enzyme deficiency might have been involved in the patient with Grade 3 anorexia and nausea.

Surgical procedures and pathologic findings

Of the 67 patients, 50 (74.6%) underwent sphincter-preserving surgery and 17 (25.4%) underwent abdominoperineal resection. A diverting ileostomy was created in all patients who underwent sphincter-preserving surgery. We currently perform ileostomy for patients who had sphincter-preserving surgery in case of anastomotic leakage, because we are afraid that the low anterior resection was done after radiation therapy. Such ileostomy is a transient stoma and usually reversed 6 months to a 1 year later. For patients undergoing abdominoperineal resection, the sigmoid colon was diverted.

The median number of examined lymph nodes was 19 (range, 12 to 52). Among the 67 patients, 26 were found to have lymph node metastasis: 18 (26.9%) had pathologic N1 disease (1–3 metastatic regional lymph nodes) and 8 (11.9%) had pathologic N2 disease (≥4 metastatic regional lymph nodes). The relation between the response of the primary tumor and lymph node metastasis is shown in Table 4. Downstaging of the primary tumor according to clinical T stage was confirmed in 49 patients (73.1%). Of the 37 patients evaluated to have node-positive disease before treatment, 16 (43.2%) had no pathologic evidence of lymph node metastasis. In 1 patient with a Grade 3 response of the primary tumor, 2 metastatic lymph nodes were found in the field of the inferior mesenteric artery.

In 6 of the 26 patients with lymph node metastasis, metastatic lymph nodes along the internal iliac artery and obturator foramen were recognized but were dissected by surgery. These patients all had enlarged lymph nodes in these regions on computed tomography and/or MRI before treatment. Such patients with pathologic evidence of lymph node metastasis also received six courses of postoperative adjuvant chemotherapy with S-1 (80 mg/m^2), given for 14 days, followed by 14 days of rest, and irinotecan (125 mg/m^2), given on Days 1 and 15.

Table 3. Pathologic primary tumor response as secondary endpoint

Grade	Response to treatment	
	No. of patients	%
1a	5	7.5
1b	16	23.9
2	21	31.3
3	25	37.3

The response rate was good in 68.7% of patients, and the response rate was good or slight in 100%.

Table 4. Relation between response to treatment and lymph node metastasis

Grade	Response to treatment		
	No. of patients	No. of patients with lymph node metastasis	%
1a	5	1	20
1b	16	12	75
2	21	12	57.1
3	25	1	4

Table 5. Acute toxicity during treatment course

	Grade 1 [*n* (%)]	Grade 2 [*n* (%)]	Grade 3 [*n* (%)]	Grade 4 [*n* (%)]
Hematologic toxicity				
Leukopenia	0	10 (14.9)	3* (4.5)	0
Neutropenia	0	1 (1.5)	3 (4.5)	0
Thrombocytopenia	0	0	1* (1.5)	0
Nonhematologic toxicity				
Diarrhea	2 (3.0)	2 (3.0)	3 (4.5)	0
Anorexia/nausea	0	0	1 (1.5)	0

* One patient had leukopenia and thrombocytopenia.

Postoperative complications

Postoperative bleeding from a branch of the internal iliac vein required emergency surgery to achieve hemostasis. One patient with intestinal obstruction did not respond to conservative treatment and underwent reoperation (untethering). There were no perioperative or postoperative deaths or postoperative sequelae.

DISCUSSION

Our protocol is considered sufficiently safe, with high rates (86.6%) of completing treatment as compared with the previous studies. There was no Grade 4 toxicity, and all Grade 3 adverse events responded to conservative treatment. In the European Organisation for Research and Treatment of Cancer 22921 study, the rate of completing treatment was 82.0% in the two groups who received preoperative chemoradiotherapy (6). In the CALGB 89901 study, the incidence of Grade 3 or 4 diarrhea was 38% in patients who received preoperative chemotherapy with oxaliplatin plus 5-FU, and the percentage of patients who completed treatment was 72% (17), if we consider completing treatment to have been achieved with at least four cycles of therapy, similar to the definition we used. The recommended dose determined based on a Phase I clinical study of our regimen was thus deemed to be appropriate (16).

The low incidence of complications might be attributed primarily to the fact that the irradiated field was adequately reduced. The target volumes used for radiotherapy in this study are far smaller in comparison to those usually described in North American and European practice, where the internal iliac nodes and often the external iliac nodes are electively irradiated. We have to keep this difference in mind in determining whether we can safely use S-1 and irinotecan along with the more typical larger radiotherapy volumes used compared with the volumes used in this study. Reduced irradiated fields of our protocol can be reasoned for surgical procedures including lateral lymph node dissection, which is one of the standard surgical options in Japan.

The rate of pathologic CR in our study was 34.7%, which was clearly higher than the rates (11%–17%) in the previous studies (8, 18–21) (Table E1). In our study serial sections of tumor tissue were evaluated histopathologically. The reliability of the pathologic evaluation of CR is therefore considered higher than that in previous studies. The median number of dissected lymph nodes was 19 (range, 12–52), considered adequate for lymph node dissection. The addition of another anticancer agent to 5-FU–based chemotherapy plus radiotherapy at a dose of 45 Gy or higher was found to contribute to a higher rate of pathologic CR, consistent with the results of other studies (22, 23). The rate of pathologic CR to 5-FU/leucovorin regimens was 20% or less in most studies. In the CALGB 89901 study, in which patients also received oxaliplatin, the rate of pathologic CR improved to 25%, but serious diarrhea and a low rate of completing treatment were problems (17). With our regimen for chemoradiotherapy, the rates of completing treatment (86.6%) and of pathologic CR (34.7%) reached satisfactory levels. Such good outcomes might be attributed to increased radiosensitivity of tumor cells induced by components of S-1 or to synergism between irinotecan and tegafur (Fig. 1). UGT1A1 nucleotide polymorphisms, which are supposed to determine the sensitivity of irinotecan, were not assessed in our study. However, treatment could be completed safely, perhaps because the dose of irinotecan was lower than that used in folinic acid, 5-FU, and irinotecan regimens (88.9%).

Several retrospective studies have reported on the close association between the rate of pathologic CR and long-term outcomes (24, 25), but such a positive correlation between these factors has yet to be clearly shown in a prospective study. In our study overall survival is being followed up as a secondary endpoint. In addition to long-term outcomes, the relation between pathologic CR and the long-term outcome is an interesting issue. Some patients with a pathologic CR may have not required surgery, but postoperative histopathologic examinations are currently required to establish the occurrence of a pathologic CR. More than half of these patients with no cancer cells on colonoscopy with biopsy after chemoradiotherapy were actually confirmed to have residual disease on histopathologic examination of the resected specimens. It is therefore difficult to evaluate the bona fide response rate only on biopsy without surgery. New examination methods other than biopsy will hopefully be established to accurately evaluate pathologic CR before surgery.

Roels *et al.* (26) reported that the rates of recurrence in the pelvic cavity were 49% in the posterior region (presacral region), 21% in the lateral region (internal iliac lymph node region), and 12% in the inferior region (perineal region). Posterior and inferior lymph nodes can be adequately

removed by TME, whereas lateral lymph nodes were not included in the irradiated field in our study and were resected surgically. If these lateral lymph nodes had not been dissected, pelvic recurrence may have occurred. The irradiated field is thus expected to become an important issue in patients with enlarged lateral lymph nodes before treatment. The clinical significance of conventional lateral lymph node dissection has yet to be shown in clinical studies. To determine the optimal irradiated field for patients with lateral lymph node metastasis, we are now closely following local recurrence and outcomes, two other secondary endpoints of this study.

In conclusion, the regimen that we developed for preoperative treatment generated promising results. However, many issues remain unresolved, including the dose (including chemotherapy cycles), duration of chemoradiotherapy, radiation target volumes in patients with lateral lymph node metastasis, optimal concomitant agents, preoperative evaluation methods for response, role of adjuvant chemotherapy, and especially, survival benefit. To assess our regimen for locally advanced rectal adenocarcinoma, the durations of disease-/recurrence-free survival and overall survival should be carefully analyzed prospectively in Phase II trials, and then large Phase III trials might be anticipated.

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European Journal of Cardio-Thoracic Surgery Advance Access published January 4, 2012

European Journal of Cardio-Thoracic Surgery 0 (2012) 1–7
doi:10.1093/ejcts/ezr224

ORIGINAL ARTICLE

The value of pleural lavage cytology examined during surgery for primary lung cancer

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Received 24 August 2011; received in revised form 21 October 2011; accepted 3 November 2011

Abstract

OBJECTIVES: The pleural invasion (PL) score is a useful prognostic indicator in lung cancer. However, in many cases, the cancer may exfoliate itself into the pleural cavity and may progress to a malignant pleural effusion without invading the parietal pleura. This stage is not currently evaluated, but it is detectable by means of the pleural lavage cytology (PLC). However, PLC's contribution to TNM staging has not yet been clarified. The purpose of this investigation was to demonstrate the usefulness of PLC in the precise staging of patients with such an occult pleural dissemination.

METHODS: A total of 3231 patients who were included in a multi-institutional database were studied retrospectively. PLC was performed by washing the thoracic cavity with a small amount of physiological saline immediately after opening the thoracic cavity during lung cancer surgery.

RESULTS: The incidence of positive PLC findings was 4.58%. In comparison with the negative group, the survival curves were significantly worse ($P < 0.001$) and the incidence of recurrence with pleuritis carcinomatosa was significantly higher ($P < 0.001$). According to the subset analysis, the survival difference was prominent in earlier stage groups and lower PL score groups. The positive findings were confirmed to be a significantly poor prognostic indicator ($P = 0.016$) by multivariate analysis using the Cox proportional hazard model (Cox analysis). However, integration of the positive findings with the PL score was attempted for the convenience of TNM staging. To find the accurate PL score for positive PLC findings, the Cox analysis was re-estimated using the PL score upgraded stepwise. The most reliable model with the highest score for the likelihood ratio χ^2 statistic was obtained by scoring positive findings as PL3. So, it was considered to be the most reliable conversion.

CONCLUSIONS: Examining PLC in clinical practice is useful for detecting occult pleural dissemination before the appearance of a malignant pleural effusion. Evidence of positive findings should be treated as supplemental information to the precise diagnosis of TNM staging. Scoring positive PLC findings as PL3 (=T3) was appropriate.

Keywords: Pleural lavage cytology • Cytological examination of pleural lavage fluid • Occult pleural dissemination • TNM staging lung cancer • Non-small-cell lung cancer

INTRODUCTION

The peripheral lung cancer tends to grow by invading the visceral pleura and then progressing to the parietal pleura. This progression is expressed by a pleural invasion (PL) score from PL0 to PL3, which is considered to be useful for predicting prognosis by providing supplemental information to TNM staging [1]. However, it is evident that lung cancer may progress via another route. After reaching the surface of the visceral pleura, cancer cells may exfoliate themselves into the pleural cavity and potentially progress to a malignant pleural effusion. Although this type of progression is not currently considered for staging purposes, it is detectable by the cytological examination of the pleural cavity, such as via pleural lavage cytology (PLC). Several reports have suggested that PLC findings obtained during surgery are an important prognostic indicator [2–14]. However, PLC's contribution to TNM staging has not yet been clarified. The purpose of this investigation was to demonstrate the usefulness of PLC in the precise staging of patients with such an occult pleural dissemination.

MATERIALS AND METHODS

Patients

A multi-institutional retrospective database analysis was performed to identify patients with lung cancer who underwent