Table 1. Topoisomerase I expression and clinicopathological findings

		Number of patients	Topoisomerase I Negative	Topoisomerase I Positive	*P*
Location of tumor	Colon	55	29	26	0.383
	Rectum	49	30	19	
Histopathology	Well-differentiated type	54	39	15	0.001
	Moderately or poorly differentiated type	50	20	30	
Dukes' classification	A or B	41	29	12	0.020
	C	63	30	33	

Correlation with Topo I protein expression and clinicopathological findings of patients

Topo I protein expression was detected in 45 of 104 patients (43.2%). Topo I protein expression was more frequently detected in moderately differentiated type or poorly differentiated type colorectal carcinoma than in well-differentiated carcinoma (Table 1). Moreover, Topo I was positive in only 12 of 41 patients (29.3%) in Dukes' A and B, while in 33 of 63 patients (52.4%) in Dukes' C (Table 1).

Topo I protein expression and prognosis of patients

The overall and disease-free 5-year survival rates of 104 patients with colorectal cancer were 64.9% and 75.5%, respectively. The disease-free 5-year survival rate of 50 patients with moderately differentiated or poorly differentiated carcinoma (63.6%) was lower than that of 54 patients with well differentiated carcinoma (86.4%, $P = 0.01$). And the disease-free 5-year survival rate of 63 patients in Dukes' C (64.4%) was lower than that of 41 patients in Dukes' A and B (94.3%, $P = 0.002$). Moreover, when the 104 patients were divided into two sub-groups according to their immunohistochemical findings, the disease-free 5-year survival rate of the 45 Topo I-positive patients (62.7%) was significantly lower than that of the 59 Topo I-negative patients (84.3%, $P = 0.005$, Fig. 3).

In order to understand whether Topo I protein expression is one of the prognostic factors of patients with colorectal cancer or not, variables (histological type, Dukes' classification, Topo I protein expression) were analyzed by Cox's proportional hazards regression model. Analysis determined that Topo I protein expression was not a prognostic factor independent from Dukes' classification (Table 2).

Topo I protein expression of tumors and effectiveness of adjuvant chemotherapy in patients with Dukes' C carcinoma

Out of the 63 Dukes' C patients, 47 were treated with 5-fluorouracil-based chemotherapy postoperatively. An oral dose of 600 mg/day of 1-(2-tetrahydrofuryl)-5-fluorouracil/uracil (1:4) (UFT; Taiho Pharmaceutical, Tokushima, Japan) was administered to these patients for at least 1 year. Postoperative chemotherapy was not performed on 13 patients because of advanced age

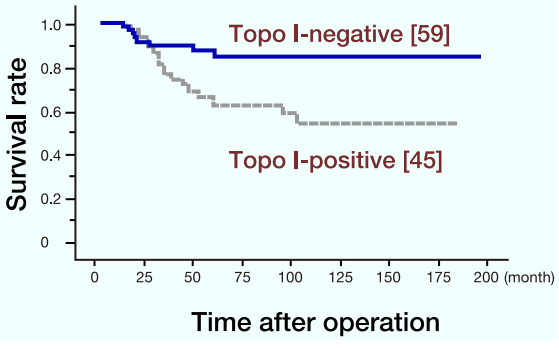

Fig. 3. The disease-free 5-year survival curve of 45 topoisomerase I (Topo I)-positive patients (dotted line) is significantly lower than that of 59 Topo I-negative patients (solid line) ($P = 0.005$). [ ], number of patients.

Table 2. Multivariate survival analysis in patients with colorectal cancer

Variable	Hazard ratio	95% Confidential interval	*P*
Histological type of tumors			
Moderately and poorly differentiated types versus well-differentiated types	1.379	0.548– 3.467	0.495
Dukes' classification			
C versus A and B	3.822	1.307–14.09	0.044
Topoisomerase I protein expression			
Positive versus negative	2.007	0.908– 4.937	0.083

(over 75) and 3 patients refused postoperative chemotherapy. At the end of 2006, 23 Dukes' C patients died from cancer recurrence. Fifteen were in the chemotherapy group (32%, 15/47) and 8 were in the non-chemotherapy group (50%, 8/16). Thus, 5-fluorouracil-based postoperative chemotherapy reduced the percentage of cancer recurrence from 50% to 32% in Dukes' C patients ($P = 0.2$).

Among the 23 Dukes' C patients who died from cancer recurrence, 16 were treated with Topo I inhibitor (CPT-11) just after detection of the recurrence. We observed that among the 16, 12 had Topo I-positive primary tumors, while 4 had Topo I-negative primary tumors. The survival periods just after the start of CPT-11 chemotherapy ranged from 2 to 43 months. Of these 16 patients, CPT-11 chemotherapy prolonged the survival of 12 patients who had Topo I-positive primary tumors over the 4 patients who had Topo I-negative primary tumors (Table 3).

Table 3. Survival time just after starting CPT-11 chemotherapy in 16 patients with recurrent colorectal cancer

	Number of patients	50% Survival period† (month)	*P*
Topoisomerase I-negative	4	4	0.041
Topoisomerase I-positive	12	12	

† After starting chemotherapy.

Discussion

It is known that Topo I expression is not observed in normal colon tissue, but we found Topo I-positive cells in basal cell layer of normal skin adjacent to rectal cancer. Bauman et al. (1997) and Hafian et al. (2004) reported that the expression of Topo I and Topo II protein were detected in normal tissue with proliferating cells including normal tonsil and normal skin. So, closed correlation between Topo I protein expression and cell proliferative activity is thought to be possible. In this study, we demonstrated the frequent occurrence of Topo I expression in surgically resected colorectal cancer (43.2%). We found that the percentage of patients who had Topo I-positive tumors was much higher in Dukes' C than in Dukes' A and B. Moreover, Topo I protein expression was more frequently detected in moderately or poorly differentiated adenocarcinomas than in well differentiated carcinomas. These findings indicate that Topo I expression closely correlated with tumor progression and histopathological differentiation in colorectal cancer. Also in human sarcomas, the incidence of detectable Topo I protein expression increased with tumor progression (Caleman et al., 2002). However, Staley et al. (1999) reported no correlation between Topo I expression and Dukes' classification in 29 patients with colorectal cancer. But the number of patients in their study was too small to elucidate a clear correlation between Topo I protein expression and tumor progression in colorectal cancer. Further investigation is needed.

We found that the 5-fluorouracil-based postoperative chemotherapy prolonged the survival of patients with Dukes' C colorectal cancer instead of Topo I protein expression of tumors. Recently, Topo I inhibitors have frequently been used in the treatment of advanced or recurrent colorectal cancers (Paradiso et al., 2004). But correlation between clinical effectiveness of Topo I inhibitors and tumor expression of Topo I protein has not been well studied in human colorectal cancer patients. In our study, we found that when CPT-11 chemotherapy had been used for patients with recurrent tumors, the survival periods of patients who had Topo I-positive primary tumors were significantly prolonged than those of patients who had Topo I-negative primary tumors. In vitro, it has been shown that tumors with a higher level of Topo I protein responded to Topo I inhibitors, but RNA expression was not predictive for the antiproliferative effect of Topo I inhibitors (Mcleod et al., 1996; Jansen et al., 1997). However, ATP-binding cassette transporters called ABCG2 or carboxilesterases have been reported to correlate with tumor sensitivity against Topo I inhibitors (Pavillard et al., 2002; Sanghani et al., 2003; Wierdl et al., 2003; Candeil et al., 2004). In order to prolong the survival of patients with advanced or recurrent colorectal cancer or to prevent ineffective chemotherapy for such patients, clinical importance of Topo I protein expression in colorectal cancer, especially sensitivity of tumors to Topo I inhibitors, should be investigated extensively.

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