and 1.3%, respectively ($p = 0.19$). These rates in patients with cancer of the rectum were 25.0 and 1.1% ($p < 0.0001$). Among various parameters, histological grade ($p < 0.0001$), location ($p = 0.025$), LNM ($p < 0.0001$), and venous invasion ($p = 0.0013$) were risk factors for recurrence. Among them, LNM ($p = 0.0008$) and histological grade ($p = 0.041$) were independent risk factors for recurrence after curative resection for T1 colorectal cancer. Time to recurrence was more likely to be shorter for patients with, than without nodal involvement. In patients with an unfavorable histological grade, all recurrences developed within 1 year.

Conclusions The recurrence rate after curative resection for node-negative T1 colorectal cancer was very low. The effectiveness of surveillance to detect recurrence after curative resection for T1 colorectal cancer should be validated in further studies.

Keywords Lymph node metastasis · Lymph node ratio · Venous invasion · Histological grade · Relapse

Introduction

Colorectal cancer is the second leading cause of cancer death in Japan, as well as in the United States [1], and its frequency is rapidly increasing in Japan [2, 3]. The most promising treatment for colorectal cancer is curative resection, and colorectal cancer is detected earlier thanks to advances in screening technology. Some T1 colorectal cancers can be treated by endoscopic mucosal resection or endoscopic submucosal dissection [4–7]. In Japan, endoscopic resection is indicated for patients with T1 colorectal cancer, of which the depth of submucosal invasion is <1000 μm [8]. Lymph node metastasis (LNM) is an important risk factor for recurrence in colorectal cancer [9], the rate of which is around 10% in T1 colorectal cancer [10, 11]. Therefore, radical resection is necessary for patients with a risk of LNM, even if invasion is limited to the submucosal layer. Currently, unfavorable histological grade, depth of submucosal invasion ≥1000 μm, lymphovascular invasion, and tumor budding are parameters for radical resection in patients with T1 colorectal carcinoma in Japan [8, 12]. However, the rate of recurrence after radical resection for T1 colorectal cancer is low. Therefore, the characteristics of recurrence in these patients remain obscure. The standard surveillance schedule after curative resection for colorectal cancer in Japan comprises serum tumor marker measurements every 3 months for the first 3 years and every 6 months for the next 2 years, computed tomography (CT) for chest and abdomen every 6 months for the first 3 years and every 12 months for the next 2 years, and colonoscopy every 1–2 years. This schedule seems to be rather intensive for T1 cancer. This multicenter study examined the characteristics of recurrence after curative resection for T1 colorectal cancer.

Patients and methods

Patients

We enrolled 798 patients with T1 colorectal cancer who had undergone curative resection with lymph node dissection between January 1991 and December 1996 at 14 hospitals that are members of the Study Group of the Japanese Society for Cancer of the Colon and Rectum (JSCCR) on Postsurgical Surveillance of Colorectal Cancer. All patients were followed up until December 2003. Although this study was a retrospective one, these data were prospectively collected in each institution. The local ethics committees approved this study. None of these patients had received preoperative radiotherapy or neoadjuvant chemotherapy. Patients with T1 cancer who were treated by endoscopic mucosal resection (EMR) or transanal resection (TAR) were excluded. Other exclusion criteria were cancers associated with inflammatory bowel disease or familial adenomatous polyposis.

Specimens were examined by pathologists of each institution. Sections were made every 3 mm.

The anatomical definition of the rectum was as follows: the upper rectum is located between the lower border of the second sacral vertebra and the peritoneal reflection, which is equivalent to 8 cm from the anal verge; and the lower rectum is between the peritoneal reflection and the upper border of the anal canal.

Resection for recurrence was considered in the absence of a medical contraindication to surgery, when technically feasible.

Most of the patients with LNM were administered with oral 5-fluorouracil or uracil-tegafur (UFT) as adjuvant chemotherapy for 1 year during the period of this study.

Preoperative investigations included barium enemas, colonoscopy, chest X-rays, ultrasonography (US) and/or CT of the liver, and blood tests for carcinoembryonic antigen (CEA). Most institutions established a 5- to 10-year follow-up period comprising serum tumor marker measurements every 3–6 months for the first 3 years and every 6 months for the next 2 years, hepatic imaging (US and/or CT) and chest X-rays every 6 months, annual pelvic CT for rectal cancer, and colonoscopy every 1–2 years.

We investigated risk factors for LNM, recurrence, and survival after radical resection for T1 colorectal cancer and evaluated recurrence sites and treatment.

Statistical analysis

Data were statistically analyzed using the StatView statistical package (StatView 5.0;Abacus Concepts,, Berkeley, CA, USA). All data are expressed as means ± standard deviation. Lymph node metastasis and recurrence rates were investigated using the χ^2 method for independence according to each parameter. Independent risk factors were determined by logistic regression analysis. Actuarial patient survival was calculated using the Kaplan–Meier method. Overall survival rates in all groups were compared using the log-rank test. Independent prognostic factors were determined using the Cox proportional hazard model. Statistical significance was established at $p < 0.05$ for all results.

Results

Lymph node metastasis

Table 1 shows the clinicopathological features of the patients. T1 rectal cancer was more likely to have a histological type other than well-differentiated adenocarcinoma, and was more likely to have lymphatic invasion and venous invasion than T1 colon cancer. The rates of LNM in T1 colorectal cancer, T1 cancer of the colon, and T1 cancer of the rectum were 10.5, 9.6, and 13.1%, respectively (Table 2). There was no difference in the rate of LNM between upper and lower rectal tumors (12.0 and 13.9%, respectively) in this study. Of the 798 patients, 714 (89%) were N0, 77 (10%) were N1, and 7 (1%) were N2. On univariate analysis, risk factors for LNM in T1 colorectal cancer were histological grade of poorly differentiated adenocarcinoma or mucinous carcinoma ($p < 0.0001$), lymphatic invasion ($p < 0.0001$), and venous invasion ($p < 0.0001$). Multiple logistic regression analysis revealed that unfavorable histological grade ($p = 0.0053$) and lymphatic invasion ($p < 0.0001$) were independent risk factors for LNM in T1 colorectal cancer.

The numbers of harvested lymph nodes in patients with node-positive and node-negative T1 colorectal cancer were 17 ± 14 and 13 ± 10, respectively ($p = 0.013$). The number of LNMs in patients with T1 colorectal cancer was associated with the lymph node ratio; namely, the ratio of metastatic to examined lymph nodes ($p < 0.0001$, Table 3). There was no N2 in patients with a lymph node ratio of less than 0.05.

Table 1 Clinicopathological characteristics of 798 T1 colorectal cancer patients who underwent curative surgery

Clinicopathological features	Colon (%)	Upper rectum (%)	Lower rectum (%)	*p* Value
Age (years)	60 ± 10	61 ± 10	60 ± 10	0.67
Gender				
Male	379 (64.9)	48 (52.2)	76 (62.3)	
Female	205 (35.1)	44 (47.8)	46 (37.7)	0.067
Primary site				
Cecum	17 (2.1)	92 (11.5)	122 (15.3)	
Ascending colon	71 (8.9)			
Transverse colon	58 (7.3)			
Descending colon	41 (5.1)			
Sigmoid colon	306 (38.3)			
Rectosigmoid	91 (11.4)			
Pathology				
Well-differentiated	422 (72.6)	57 (62.0)	75 (61.5)	
Moderately differentiated	153 (26.3)	35 (38.0)	42 (34.4)	
Poorly differentiated	4 (0.7)	0	3 (2.5)	
Mucinous	2 (0.3)	0	2 (1.6)	0.012
Unknown	3			
Lymph node metastasis				
Absent	528 (90.4)	81 (88.0)	105 (86.1)	
Present	56 (9.6)	11 (12.0)	17 (13.9)	0.32
Lymphatic invasion				
Absent	340 (61.9)	43 (48.9)	64 (53.3)	
Present	209 (38.1)	45 (51.1)	56 (46.7)	0.026
Unknown	35	4	2	
Venous invasion				
Absent	434 (79.9)	63 (70.8)	77 (64.2)	
Present	109 (20.1)	26 (29.2)	43 (35.8)	0.0005
Unknown	41	3	2	

Table 2 Risk factors for lymph node metastasis in T1 colorectal cancer

	Univariate analysis			Multivariate analysis		
	LNM (+) (%)	LNM (−) (%)	*p* Value	Odds ratio	95% CI	*p* Value
Gender						
Male	45/503 (8.9)	458/503 (91.1)	0.058			
Female	39/295 (13.2)	256/295 (86.8)				
Location						
Colon	56/584 (9.6)	528/584 (90.4)	0.15			
Rectum	28/214 (13.1)	186/214 (86.9)				
Histological grade						
Well or Mod	78/784 (9.9)	706/784 (90.1)	<0.0001	1		
Poorly or Muc	5/11 (45.5)	6/11 (54.5)		7.58	1.82–31.25	0.0053
Lymphatic invasion						
Absent	8/437 (1.8)	429/437 (98.2)	<0.0001	1		
Present	35/285 (12.3)	250/285 (87.7)		5.13	2.87–9.09	<0.0001
Venous invasion						
Absent	21/549 (3.8)	528/549 (96.2)	<0.0001	1		
Present	22/167 (13.2)	145/167 (86.8)		1.46	0.86–2.48	0.16

LNM Lymph node metastasis, *CI* confidence interval, *Mod* moderately, *Muc* mucinous

Table 3 Recurrence and survival rates according to lymph node ratio

	Lymph node ratio (total positive/total examined)				*p* Value
	<0.05	0.05–0.19	0.2–0.39	0.4–1.0	
Lymph node metastasis					
N1	10	50	15	2	<0.0001
N2	0	1	3	3	
Recurrence rate (%)	0 (0/10)	11.8 (6/51)	11.1 (2/18)	20 (1/5)	0.11
5-Year overall survival (%)	100	90	83.3	80	–

Recurrence

Cancer recurred in 18 (2.3%) of the 798 patients during a median follow-up of 7.8 ± 3.5 years. The rates of recurrence of T1 cancer of the colon and rectum were 1.5 and 4.2%, respectively ($p = 0.02$). The recurrence rates among patients with T1 colon cancer with and without LNM were 3.6 and 1.3% ($p = 0.19$), and the recurrence rates in patients with T1 rectal cancer with and without LNM were 25 and 1.1%, respectively ($p < 0.0001$). The most frequent sites of recurrence were the liver and the lung in T1 colon and rectal cancers, respectively (Table 4). Most T1 colorectal cancers recurred at a single site during a median of 1.9 years (range, 0.3–4.4 years). T1 colorectal cancer recurred sooner in patients with than in those without nodal involvement, although the difference did not reach statistical significance ($p = 0.28$, Fig. 1). In patients with nodal involvement, cancer recurred in 78% of them within 1 year. On the other hand, most cancer did not recur within 1 year in patients with T1 colorectal cancer without LNM. The patients with unfavorable histological grade ($p = 0.033$) or LNM ($p = 0.0044$) were more likely to have a recurrence within 1 year (Table 5).

Table 4 Recurrence sites of T1 colorectal cancers

	Colon ($n = 9$)	Rectum ($n = 9$)	*p* Value
Number of recurrence sites			
Single	7	7	>0.99
Multiple	2	2	
Recurrence site			
Liver	6	1	0.17
Lung	1	3	
Local	1	2	
Other	3	4	

Other recurrence sites included bone and distant lymph nodes

The patients with a high lymph node ratio were more likely to develop recurrence, although there was no significant difference according to whether the ratio was high or low (Table 3). There was no recurrence in patients with a lymph node ratio of less than 0.05 in this study.

Risk factors for recurrence

Univariate analysis revealed that tumor location ($p = 0.025$), histological grade ($p < 0.0001$), LNM ($p < 0.0001$), and

venous invasion ($p = 0.0013$) were risk factors for recurrence (Table 6). Among them, multivariate analysis revealed that histological grade ($p = 0.041$) and LNM ($p = 0.0008$) were independent risk factors for recurrence after curative resection for T1 colorectal cancer.

Treatment for recurrence

Table 7 shows the details of the 18 patients with recurrence after curative resection for T1 colorectal cancer. Most of the liver metastases were detected by liver imaging such as CT or US. Pulmonary metastasis was detected by CEA or chest X-ray. Seven (39%) of the 18 patients underwent surgical resection for recurrence, and the procedure was curative in 6 of them (pulmonary metastasis, $n = 3$; liver metastasis, $n = 1$; supraclavicular LNM, $n = 1$; lateral pelvic LNM, $n = 1$). Three of the 6 patients who underwent curative resection for metastasis to the liver, lung, and lateral pelvic lymph nodes did not develop re-recurrence. Five patients received chemotherapy, and 6 did not receive any treatment for recurrence.

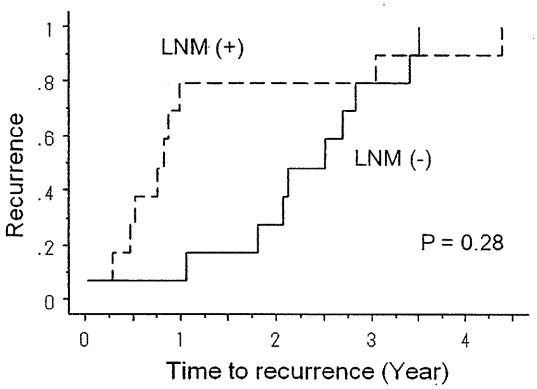

Fig. 1 Cumulative appearance rate of recurrence after curative resection for T1 colorectal cancer in patients with and without lymph node metastasis (*LNM*)

Prognosis after curative resection for T1 colorectal cancer

The 5-year overall survival rates after curative resection for T1 colon cancer in patients with and without LNM were 92.7 and 94.1%, respectively (Fig. 2a, $p = 0.49$). These rates in the patients with T1 rectal cancer with and without LNM were 82.1 and 93.8%, respectively (Fig. 2b, $p = 0.0018$). Table 8 shows the prognostic factors for patients after curative resection for T1 colorectal cancer. Univariate analysis showed that LNM ($p = 0.021$) and venous invasion ($p = 0.0099$) were factors indicating a poor prognosis, and the Cox proportional hazard model also revealed that these were independent factors indicating a poor prognosis ($p = 0.032$ and 0.030, respectively).

Discussion

The prognosis of T1 colorectal cancer is relatively good, and it is often treated by endoscopic resection or local excision.

Table 5 Time-dependent analysis of recurrence

	Univariate analysis			Multivariate analysis		
	Rec within 1 year	Rec 1 year or later	*p* Value	OR	95% CI	*p* Value
Gender						
Male (11)	4	7				
Female (7)	4	3	0.39			
Location						
Colon (9)	3	6				
Rectum (9)	5	4	0.34			
Histological grade						
Well or Mod (15)	5	10		1		
Poorly or Muc (3)	3	0	0.033	270964	0–	0.98
Lymph node metastasis						
Absent (9)	1	8		1		
Present (9)	7	2	0.0044	15.9	1.1–250	0.042
Lymph invasion						
Absent (8)	3	5				
Present (10)	5	5	0.60			
Venous invasion						
Absent (8)	2	6				
Present (10)	6	4	0.14			

The values given in parentheses are the total numbers

Rec Recurrence, *OR* odds ratio, *CI* confidence interval, *Mod* moderately, *Muc* mucinous

Table 6 Risk factors for recurrence in T1colorectal cancer

	Univariate analysis			Multivariate analysis		
	Rec (+) (%)	Rec (−) (%)	*p* Value	Odds ratio	95% CI	*p* Value
Gender						
Male	11/503 (2.2)	492/503 (97.8)	0.86			
Female	7/295 (2.4)	288/295 (97.6)				
Location						
Colon	9/584 (1.5)	575/584 (98.5)	0.025	1		
Rectum	9/214 (4.2)	205/214 (95.8)		1.74	0.63–4.85	0.29
Histological grade						
Well or Mod	15/784 (1.9)	769/784 (98.1)	<0.0001	1		
Poorly or Muc	3/11 (27.3)	8/11 (72.7)		6.21	1.08–35.6	0.041
Lymph node metastasis						
Absent	9/714 (1.3)	705/714 (98.7)	<0.0001	1		
Present	9/84 (10.7)	75/84 (89.3)		6.02	2.11–17.2	0.0008
Lymphatic invasion						
Absent	8/437 (1.8)	429/437 (98.2)	0.16			
Present	10/285 (3.5)	275/285 (96.5)				
Venous invasion						
Absent	8/549 (1.5)	541/549 (98.5)	0.0011	1		
Present	10/167 (6.0)	157/167 (94.0)		2.29	0.80–6.57	0.12

Rec Recurrence, *CI* confidence interval, *Mod* moderately, *Muc* mucinous

Table 7 Details of patients with recurrence

Case	Age	Gender	Tumor location	Histological type	Nodal involvement	Time (year) to recurrene	Recurrence site	First indicator
1	59	M	Ascending colon	Well	Absent	2.0	Others	Symptom
2	61	M	Lower rectum	Well	Present	4.4	Lung	Chest X-ray
3	74	F	Lower rectum	Muc	Present	0.5	Others	Symptom
4	62	F	Sigmoid colon	Well	Absent	2.8	Liver	Imaging
5	58	M	Rectosigmoid	Well	Absent	2.1	Liver, anastomosis	Imaging
6	66	M	Upper rectum	Well	Present	0.3	Liver	CEA, imaging
7	66	M	Lower rectum	Well	Absent	2.7	Others	CEA, imaging
8	67	M	Sigmoid colon	Mod	Absent	3.5	Others	Symptom, imaging
9	58	M	Rectosigmoid	Well	Present	0.7	Liver	Imaging
10	53	F	Rectosigmoid	Well	Absent	1.8	Others	Clinical examination
11	81	M	Ascending colon	Mod	Present	0.5	Liver, Lung	CEA, imaging
12	84	F	Lower rectum	Por	Present	0.9	Others	Clinical examination
13	60	M	Lower rectum	Mod	Present	3.0	Lung	Chest X-ray
14	81	F	Upper rectum	Mod	Present	1.0	Local, Others	CEA, imaging
15	71	M	Ascending colon	Well	Absent	2.5	Liver	CEA
16	51	M	Descending colon	Well	Absent	1.0	Liver	Imaging
17	48	F	Lower rectum	Por	Present	0.8	Others	Symptom
18	67	F	Lower rectum	Well	Absent	3.4	Lung	CEA, chest X-ray

Well Well-differentiated adenocarcinoma, *Mod* moderately differentiated adenocarcinoma, *Por* poorly differentiated adenocarcinoma, *Muc* mucinous adenocarcinoma, *Others* bone and distant lymph node metastasis, *Imaging* computed tomography (CT) or ultrasonography (US), *CEA* carcinoembryonic antigen

Nevertheless, about 10% of T1 colorectal cancers metastasize to regional lymph nodes [10, 11]. Surgery with lymph node dissection is indicated for patients with T1 colorectal cancer with a risk of LNM. The features of recurrence after curative resection have remained obscure because recurrence after curative resection for T1 colorectal cancer is rare.

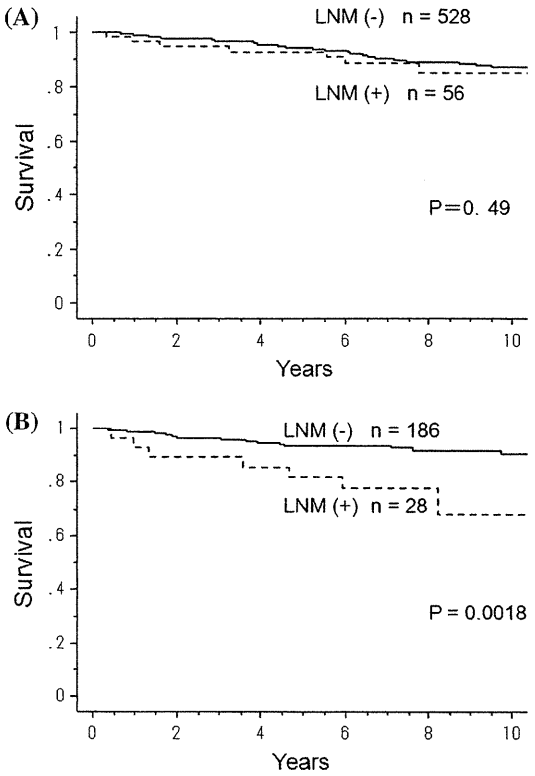

Fig. 2 Five-year overall survival curves for patients after curative resection for T1 cancer of the colon (**a**) and rectum (**b**). *Dotted* and *unbroken lines* indicate survival with and without lymph node metastasis (*LNM*), respectively

Table 8 Prognostic factors in patients with curative resection for T1 colorectal cancer

	n	Univariate analysis	Multivariate analysis		
		p Value	HR	95% CI	*p* Value
Gender					
Male	503				
Female	295	0.79			
Location					
Colon	584				
Rectum	214	0.81			
Histological grade					
Well or Mod	784				
Poorly or Muc	11	0.42			
Lymph node metastasis					
Absent	714		1		
Present	84	0.021	1.91	1.06–3.46	0.032
Lymphatic invasion					
Absent	447				
Present	310	0.34			
Venous invasion					
Absent	574		1		
Present	178	0.0099	1.70	1.05–2.76	0.030

HR Hazard ratio, *CI* confidence interval, *Mod* moderately, *Muc* mucinous

Histological grade was an independent risk factor for recurrence after curative resection for T1 colorectal cancer, as well as LNM. The recurrence rate among patients with poorly differentiated adenocarcinoma or mucinous carcinoma that invaded the submucosal layer was 27.3% in the present study. Blumberg et al. [13] have reported that a poorly differentiated tumor is a predictor of tumor-related mortality in patients with surgical resection for T1 rectal cancer. Cooper et al. [14] demonstrated that 37.5% of patients with grade III early cancer had adverse outcomes such as local and/or distant recurrence. Adjuvant chemotherapy or chemoradiotherapy might be necessary for such patients, even if they have T1 colorectal cancer. On the other hand, local resection for early distal rectal cancer is thought to be a reasonable alternative to abdomino-perineal resection or low anterior resection. However, the recurrence rate after local excision for rectal cancer was not necessarily low (6.6–18%) in previous studies [15–19]. The indication for local excision in patients with early distal rectal cancer should be determined carefully. A recent study demonstrated that the combination of gender and histological type was useful to determine the appropriate candidates for local excision for early distal rectal cancer [20].

The present study found that the most frequent sites of recurrence in T1 cancer of the colon and rectum were the liver and the lung, respectively. This is consistent with previous studies that enrolled mainly patients with advanced cancer [9, 21]. In the present study, all curative resections were performed in patients with metastasis in a single organ. The rate of curative resection for recurrence after radical resection for T1 colorectal cancer in our study was 33%, which is similar to previous findings, in which the rates of curative resection for liver and pulmonary metastasis were between 20 and 40% [9, 22, 23]. Curative resection for recurrence permanently cured 50% of the patients in our study; thus, we highly recommend curative resection for recurrence when possible.

Time to recurrence was shorter in T1 colorectal cancer patients with LNM than in those without LNM. Approximately 80% of recurrences in patients with LNM developed within 1 year, whereas most of the cancers did not recur within 1 year in those without LNM. If patients with T1 colorectal cancer do not have LNM, follow-up after initial surgery might be unnecessary, at least for the first year. In terms of the first indicator for recurrence, liver imaging such as CT or US was useful to detect liver metastasis, although a combination of CEA and chest X-ray detected all pulmonary metastases. From a comprehensive point of view, CEA

and CT from chest to abdomen seem to be useful to detect a recurrence after curative resection for T1 colorectal cancer at this moment.

The outcome after curative resection for T1 colorectal cancer in the present study was satisfactory. The 5-year overall survival rate of patients with T1 colon cancer was more than 90%, even if nodes were involved, and the prognosis after curative resection for T1 colon cancer did not differ between patients with and without LNM. This result may indicate that lymph node dissection for T1 colon cancer is more useful than that for colon cancers at other T-stages. At the same time, the good prognosis might be a result of the number of LNMs. In this study, most of the stage III patients with T1 colorectal cancer were N1. Furthermore, a recent study demonstrated that the lymph node ratio was associated with the survival rate [24]. The lymph node ratios in our study were less than those in a previous study which enrolled patients with stage II and III colon cancer. Especially, in our study, patients with a lymph node ratio of less than 0.05 did not have a recurrence, even if they had LNM. The lymph node ratio may be useful to predict the prognosis of patients with node-positive T1 colorectal cancer. On the other hand, the 5-year overall survival rate for patients with rectal cancer and LNM was 82.1%, even when tumor invasion was confined to the submucosal layer.

Lymph node metastasis (LNM) and venous invasion were independent factors for a poor prognosis in the present study. Others have reported that LNM is one of the strongest predictors of prognosis in patients with colorectal cancer [25, 26]. Venous invasion has also been reported as an independent adverse prognostic factor in T1, as well as in T2–4 tumors [14, 27–31].

The present study demonstrated that the rates of LNM in T1 cancer of the colon and rectum were 9.6 and 13.1%, respectively. These results are consistent with previous findings in which the rates of nodal involvement in T1 colorectal cancer were approximately 10% [11, 31–33 That is, surgery might have been unnecessary in almost 90% of patients who underwent radical resection for T1 colorectal cancer. Therefore, to identify a more accurate predictor of LNM in T1 colorectal cancer is important. Histological grade and lymphatic invasion were independent risk factors for LNM in T1 colorectal cancer in the present study.

Cooper et al. [14] demonstrated that unfavorable histology such as grade III, tumor margin <1.0 mm in endoscopically resected polyps, lymphatic and venous invasion, and unfavorable tumor grade were associated with LNM [12]. Our data support these results.

In the present study, tumor location in T1 colorectal cancer was not a risk factor for LNM. However, some studies have associated tumor location with LNM. Nascimbeni et al. [10] demonstrated that the lower third of the rectum was a risk factor for LNM in T1 colorectal cancer. Another study showed that the level of invasion, configuration, and location were risk factors for LNM in early invasive colorectal cancer [34] The frequencies of LNM in T1 colon and rectal cancers in these studies were 3.8 and 9.7%, respectively. The rate of LNM in our study was somewhat higher than in previous studies, because our patients were preoperatively diagnosed with a risk of LNM and were treated with surgical resection. Recent reports indicate that the depth of submucosal invasion and tumor budding are risk factors for LNM in T1 colorectal cancer [12, 35–37. One study has shown that 42.1% of patients with T1 colorectal cancer have LNM if tumor budding is present [12]. Kurokawa et al. [38] have reported that tumor matrilysin expression is a promising biomarker predicting nodal metastasis of colorectal cancer.

We conclude that unfavorable histological grade and lymphatic invasion are risk factors for LNM in patients with T1 colorectal cancer. Thus, these patients should undergo curative resection with lymph node dissection, even if the depth of tumor invasion is limited to the submucosal layer. Lymph node metastasis (LMN) and unfavorable histological grade were independent risk factors for recurrence after curative resection for T1 colorectal cancer. The optimal surveillance schedule after curative resection for T1 colorectal cancer should be validated in further studies.

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Effect of classification based on combination of mutation and methylation in colorectal cancer prognosis

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Abstract. Colorectal cancer (CRC) is caused by an accumulation of genetic alterations and epigenetic alterations. The molecular classification of CRCs based on genetic alterations and epigenetic alterations is evolving. Here, we examined mutations and methylation status in CRCs to determine if the combination of genetic and epigenetic alterations predicts prognosis. We examined 134 sporadic CRCs. We used the direct sequencing method to identify mutations in BRAF and AKT1, which are downstream of KRAS and PIK3CA, respectively, in the EGFR pathway. We used the Methylight method to determine the methylation status of hMLH1, p16, MINT1, MINT2 and MINT31. Both BRAF and AKT1 mutations were found in only one case (0.75%). Aberrant methylation of hMLH1, p16, MINT1, MINT2 and MINT31 was detected in 22.4, 35.1, 32.8, 59.7 and 41.0% of cases, respectively. The clinicopathological factors were not significantly correlated to mutation or methylation. Among the patients who had no mutation in the EGFR pathway, the overall survival was significantly shorter in the patients with methylation compared to the patients with no methylation in hMLH1 and p16 (p=0.0318). Methylation could play a key role in the prognosis of patients without mutations in the EGFR pathway. The combination of genetic and epigenetic alterations may be a good marker for the prognosis of CRC patients.

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world and the second most common cause of cancer-related death (1). Of patients who undergo potentially curative surgery, 17% develop local recurrence or distant metastasis leading to a shorter survival time (2). Therefore, it is important to identify molecular markers of biological and prognostic significance and predictive value in patients with advanced CRC (3,4).

CRC develops as a result of progressive accumulation of genetic alterations and epigenetic alterations (3-5). The elucidation of the human genome sequence (6) has showed that about 50-70 gene mutations are detected in CRC. Many studies have reported the importance of the mutations in the EGFR pathway, including the RAS/RAF pathway and the PI3K/AKT pathway (7-10). Gene mutations in the EGFR pathway are related to the efficiency of cetuximab or panitumumab therapy in metastatic CRC (11-14). The RAS/RAF pathway mediates the cellular response to extracellular signals that regulate cell growth, differentiation, and apoptosis (15). The PI3K/AKT pathway plays a central role in carcinogenesis since it is frequently activated and deregulated in the carcinogenic process of various human cancers (16). We previously examined the mutation of KRAS and PIK3CA in CRC patients and found that PIK3CA mutation is predictive of poor survival (17).

Gene methylation has been recognized as a third mechanism of Knudson's two-hit theory, and it is clear that methylation is associated with not only carcinogenesis but also the evolution and metastatic processes of cancer (18,19). Epigenetic changes usually begin early in carcinogenesis, are potentially reversible, and can advance to gene alterations. Therefore, the detection of aberrant methylation is important for the early diagnosis, prognosis, and treatment of patients with CRC (20-22).

In the present study, we examined the mutation of BRAF and AKT1, which are downstream of KRAS and PIK3CA, respectively, and the methylation status of hMLH1, p16, MINT1, MINT2 and MINT31 to clarify whether the combination of genetic and epigenetic alterations might be used as parameters to predict prognosis in CRC.

Materials and methods

Patients and tissue samples. A total of 158 patients who had undergone surgical resection for primary sporadic colorectal

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cancer at the Department of Surgical Oncology, Tokyo Medical and Dental University (Tokyo, Japan), between March 2000 and April 2003 were targeted in our previous study. Of them, 134 patients, for whom genomic DNA was available were included in this study. This research was approved by the institutional review board of Tokyo Medical and Dental University, and written informed consent was obtained from all participants. The patients comprised 86 men and 48 women, ranging in age from 37 to 88 (mean, 64.5 years). Tumors were classified as proximal (proximal to the splenic flexure) or distal. There were 48 cancers in the proximal colon and 86 cancers in the distal colon, including the rectum. Histological classification and tumor staging were performed according to the International Union Against Cancer Tumor-Node-Metastasis (TNM) classification. No patient received preoperative chemotherapy or radiotherapy. After surgery, patients with stage III CRC received oral or intravenous 5-fluorouracil (5-FU)-based adjuvant chemotherapy, and patients with stage IV tumors received 5-FU-based systemic chemotherapy without any radiotherapy. Patients were prospectively followed-up after surgery for a median of 49 months. All resected specimens were fixed in 10% pH-neutral formalin and embedded in paraffin. In all cases, archival H&E slides of the primary tumors were retrieved and reviewed to confirm pathological features.

DNA extraction and mutation analysis. Tissue blocks were cut into 10-μm thick sections with a microtome. The blade was changed and the microtome was cleaned after each specimen. After the specimens were deparaffinized and washed, tumor tissue was manually dissected with a razor blade in comparison to H&E slide. Tumor tissues were incubated overnight with proteinase K in digestion buffer, and then genomic DNA was extracted by a standard phenol-chloroform method. Exon 1 of the KRAS gene, exons 9 and 20 of the PIK3CA gene, exon 15 of the BRAF gene, and exon 4 of the AKT1 gene were selected for mutation analysis, because mutations cluster in these regions. The exons were sequenced after PCR amplification. Primer sequences and PCR conditions are available upon request. PCR products were purified with Microcon YM-100 Centrifugal Filters (Millipore, MA) and Centri-Sep Columns (Princeton Separations, Adelphia, NJ) and then directly sequenced with a Big Dye Terminator Cycle Sequencing kit (3130 Genetic Analyser, Applied Biosystems, Foster City, CA).

Methylight analysis. Sodium bisulfite conversion and DNA recovery was performed using EpiTect Bisulfite (Qiagen). Following sodium bisulfite conversion, genomic DNA was analyzed by the Methylight technique, a fluorescence-based, real-time PCR (Q-PCR) assay (23) and the ABI Prism 7300 Real-Time PCR System (Taqman; Applied Biosystems). Six sets of primers and probes designed specifically for bisulfite-converted DNA were used. One set was used to detect methylation in the gene of interest and the other five sets served as reference sets for ß-actin (ACTB) to normalize for input DNA. The reference primers and probes were designed in a region of the ACTB gene that lacks CpG dinucleotides, thus allowing for equal amplification regardless of the methylation levels. Primer and probe sequences are available upon request. *Sss*I-treated HCT-15 DNA was used as a fully methylated positive control (100% methylation ratio). Parallel TaqMan PCR was performed with specific primers for the bisulfite-converted methylated sequence for a particular locus and with the ACTB reference primers. In each case, triplicate threshold cycle (Ct) values were obtained and averaged, and expression levels were then evaluated by the 2-ΔΔCt method (24). As an internal standard, each individual sample was normalized to its ß-actin (ACTB) content and compared to the gene expression level of *Sss*I-treated HCT-15 DNA (calibration sample) as follows: $2^{-\Delta\Delta Ct}$, where ΔΔCt = (Ct-target-Ct-reference) treated-sample - (Ct-target-Ct-reference) calibrator sample. We defined the percentage of fully methylated reference (PMR) to be $2^{-\Delta\Delta Ct}$ x 100%.

Statistical analysis. All statistical analyses were performed with StatView Software (version 5.0). To estimate differences between groups, the χ^2 test, Fisher's exact test, Student's t-test and log-rank test were used as appropriate. The Kaplan-Meier method was used to estimate survival. Survival was calculated from the date of surgery. P-values <0.05 were considered to be significant.

Results

Mutation and methylation status in relation to clinicopathological parameters of 134 CRCs. KRAS mutations in exon 1 were found in 30.6% (41/134) of the cases, and PIK3CA mutations in exons 9 and 20 were found in 13.4% (18/134) of the cases. Both BRAF and AKT1 mutations were found in only one case (0.75%). One case with an AKT1 mutation also had a PIK3CA mutation, while one case with a BRAF mutation had no other mutations. There were no correlations between mutations in KRAS, BRAF, PIK3CA, and AKT1. There were no correlations between the RAS/RAF and PIK/AKT pathways. There were also no statistically significant differences between patients with mutations and patients without mutations in these pathways. The frequency of mutations is summarized in Table I.

Aberrant methylation of hMLH1, p16, MINT1, MINT2 and MINT31 was detected in 22.4% (30/134), 35.1% (47/134), 32.8% (44/134), 59.7% (80/134), and 41.0% (55/134), respectively. Aberrant methylation of p16 was significantly associated with tumor depth. The frequency of methylation is summarized in Table I.

Mutation or methylation status was not significantly correlated to the clinicopathological data (Tables I and II).

Relationship between the RAS/RAF and PIK/AKT pathways and methylation of hMLH1 or p16 in CRCs. The relationship between the RAS/RAF and PIK/AKT pathways and methylation of hMLH1 or p16 is summarized in Table III. Although not statistically significant, hMLH1 methylation tended to be associated with p16 methylation (p=0.06).

Prognostic value of mutations in the RAS/RAF and PIK/AKT pathways and methylation of hMLH1 and p16 in CRCs. There was no statistically significant difference in overall survival between patients with and without a mutation in the RAF/RAF and PIK/AKT pathways (p=0.2436; Fig. 1). Of

Table I. Mutation status in relation to clinicopathological parameters of 134 CRC.

		KRAS			PIK3CA			BRAF			AKT1		
		Wt	Mut	P-value	Wt	Mut	P-value	Wt	Mut	P-value	Wt	Mut	P-value
No. of cases	134	93	41		116	18		133	1		133	1	
Gender													
Male	86 (64.2)	63	23	0.1952	75	11	0.7705	85	1	0.4533	86	0	0.1791
Female	48 (35.8)	30	18		41	7		48	0		47	1	
Tumor site													
Proximal	48 (35.8)	29	19	0.0917	39	9	0.1775	47	1	0.1791	48	0	0.4533
Distal	86 (64.2)	64	22		77	9		86	0		85	1	
Histology													
Well	46 (34.3)	32	14	0.9765	38	8	0.3313	46	0	0.468	45	1	0.165
Others	88 (65.7)	61	27		78	10		87	1		88	0	
pT													
T1, T2	22 (16.4)	17	5	0.3809	18	4	0.4749	22	0	0.6564	22	0	0.6564
T3, T4	112 (83.6)	76	36		98	14		111	1		111	1	
pN													
Positive	59 (44.0)	39	20	0.3809	52	7	0.8144	59	0	0.3661	59	0	0.3661
Negative	75 (56.0)	54	21		64	11		74	1		74	1	
TNM stage													
I, II	65 (48.5)	45	20	0.7391	57	8	0.7109	64	1	0.3011	64	1	0.3011
III, IV	69 (51.5)	48	21		59	10		69	0		69	0	
Lymphatic invasion													
Positive	101 (75.4)	69	32	0.6331	91	10	0.036	100	1	0.5661	100	1	0.5661
Negative	33 (24.6)	24	9		25	8		33	0		33	0	
Venous invasion													
Positive	124 (92.5)	86	38	0.9666	111	13	0.0004	123	1	0.7756	123	1	0.7756
Negative	10 (7.5)	7	3		5	5		10	0		10	0	

Wt, wild-type; Mut, mutated type.

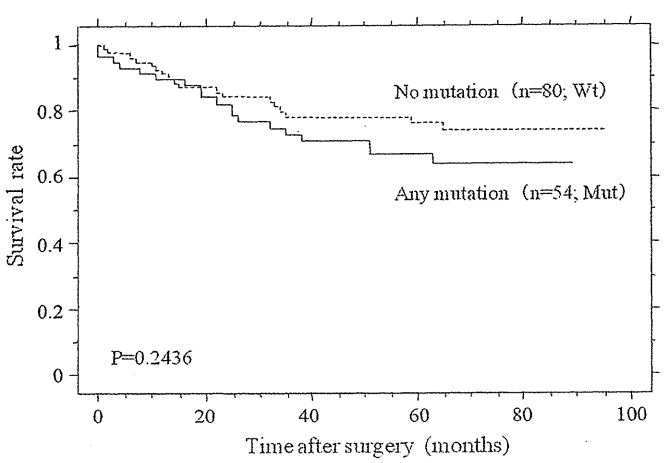

Figure 1. Overall survival in relation to mutation in the RAS/RAF and PIK/AKT pathways.

134 patients with CRC, 54 had a mutation in the RAS/RAF or PIK/AKT pathway. Among these 54 patients, there was no significant difference in overall survival based on methylation of hMLH1 or p16 (p=0.5463; Fig. 2). Of the 134 patients with CRC, 80 had no mutations in the RAS/RAF and PIK/AKT pathways. Among these wild-type patients, patients with methylated hMLH1 or p16 had a significantly shorter overall survival than those without methylation (p=0.0318; Fig. 3).

Discussion

In the present study, we examined mutations in the RAS/RAF and PIK/AKT pathways and the methylation status of hMLH1, p16, MINT1, MINT2 and MINT31. We analyzed their correlations with clinicopathological factors and prognosis to determine whether these factors are novel prognostic markers

Table II. Methylation status in relation to clinicopathological parameters of 134 CRC.

		hMLH1			p16			MINT1			MINT2			MINT31		
		Unm	Met	P-value	Unm	Met	P-value	Unm	Met	P-value	Unm	Met	P-value	Unm	Met	P-value
No. of cases	134	104	30		87	47		90	44		54	80		79	55	
Gender																
Male	86 (64.2)	69	17	0.33	55	31	0.9887	59	27	0.6436	34	52	0.8094	50	36	0.7972
Female	48 (35.8)	35	13		32	16		31	17		20	28		29	19	
Tumor site																
Proximal	48 (35.8)	35	13	0.33	29	19	0.4415	30	18	0.3904	16	32	0.2194	25	23	0.227
Distal	86 (64.2)	69	17		58	28		60	26		38	48		54	32	
Histology																
Well	46 (34.3)	35	11	0.7595	27	19	0.2935	31	15	0.9677	21	25	0.361	29	17	0.4867
Others	88 (65.7)	69	19		60	28		59	29		33	55		50	38	
pT																
T1, T2	22 (16.4)	18	4	0.6047	10	12	0.0391	11	11	0.0608	9	13	0.9491	10	12	0.1591
T3, T4	112 (83.6)	86	26		77	35		79	33		45	67		69	43	
pN																
Positive	59 (44.0)	45	14	0.8792	41	18	0.2563	44	15	0.1709	27	32	0.3177	36	23	0.8248
Negative	75 (56.0)	59	16		46	29		46	29		27	48		43	32	
TNM stage																
I, II	65 (48.5)	55	10	0.0591	40	25	0.4613	42	23	0.542	24	41	0.6739	39	26	0.8114
III, IV	69 (51.5)	49	20		47	22		48	21		30	39		40	29	
Lymphatic invasion																
Positive	101 (75.4)	82	19	0.0823	70	31	0.0685	72	29	0.0754	42	59	0.5955	60	41	0.8528
Negative	33 (24.6)	22	11		17	16		18	15		12	21		19	14	
Venous invasion																
Positive	124 (92.5)	95	29	0.3286	81	43	0.7485	85	39	0.2296	51	73	0.4901	73	51	0.9493
Negative	10 (7.5)	9	1		6	4		5	5		3	7		6	4	

Unm, unmethylated; Met, methylated.

Table III. Relationship between mutation in EGFR pathway and methylation of hMLH1 and p16 in CRC.

	PIK3CA			p16			hMLH1		
	Mut	Wt	P-value	High	Low	P-value	High	Low	P-value
KRAS									
Mut	6	35		20	21		8	33	
Wt	12	81	0.787	27	65	0.03	22	71	0.596
PIK3CA									
Mut	-	-	-	6	12		5	13	
Wt	-	-	-	41	74	0.848	25	91	0.556
p16									
High	-	-	-	-	-	-	15	32	
Low	-	-	-	-	-	-	15	71	0.06

Wt, wild-type; Mut, mutated type.

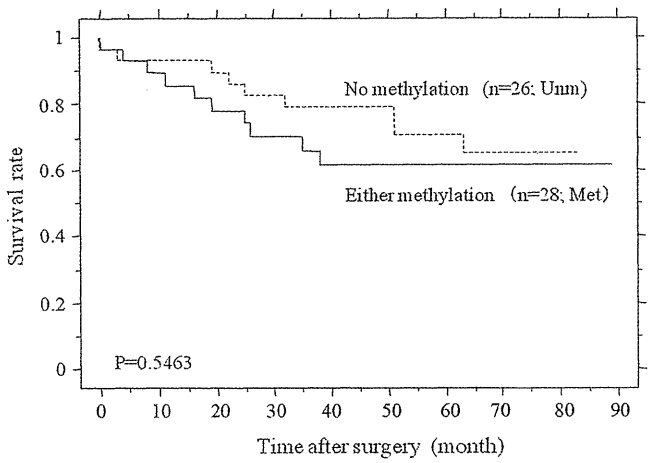

Figure 2. Overall survival in relation to hMLH1 and p16 methylation in the mutation group. Unm, unmethylated group; Met, methylated group.

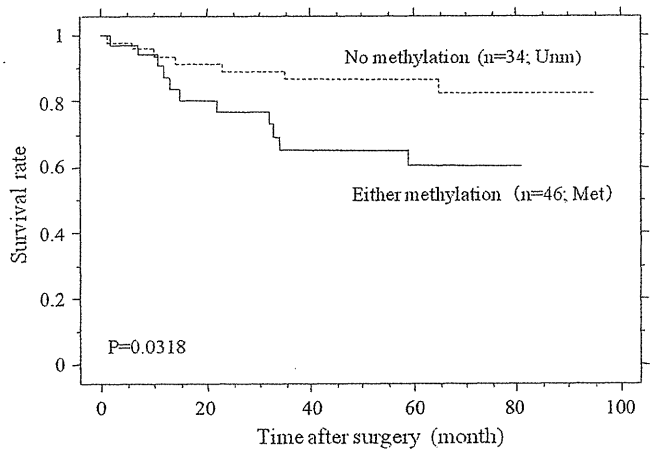

Figure 3. Overall survival in relation to hMLH1 and p16 methylation in the wild-type group. Unm, unmethylated group; Met, methylated group.

of CRC. We found that the combination of mutation and methylation may be a good prognostic marker for CRC.

Mutations in the RAS/RAF and PIK/AKT pathways are present in CRC (7-10,25). We previously examined the mutation of KRAS and PIK3CA in CRC patients, and found that PIK3CA mutation is predictive of poor survival in these patients (17). In the present study, we examined the mutation status of BRAF and AKT1, which are downstream of KRAS and PIK3CA, respectively. BRAF and AKT1 mutations were detected in one case each. BRAF mutation has been reported to occur in about 15% of CRC cases, while V600E accounts for approximately 90% of the mutations (26,27). The frequency of BRAF mutation in CRC patients differs among ethnic groups. Brim *et al* (28) analyzed BRAF mutation in CRC patients of different ethnic groups, African American, Omani and Iranian. Among these CRC patients, BRAF mutation was detected in 10% of the African Americans, 19% of the Omanis, and 2% of the Iranians. The frequency of BRAF mutation in Asia tends to be low, reported at approximately 5% (29-32). These differences among ethnic groups may be due to different lifestyle factors such as diet, alcohol and smoking (33,34). Our study indicates that the frequency of BRAF mutations in Asian patients with CRC is lower than in other ethnic groups. BRAF mutation in CRC is associated with microsatellite instability-high colorectal cancer (MSI-H CRC) (9,10,15,27). MSI-H CRC is often detected in the early stage of cancers. Most samples in the present study were from CRC patients in an advanced stage, so it is possible that the frequency of BRAF mutation was low. Carpen *et al* (35) found AKT1 mutation in 6% of CRC patients, but other studies have reported smaller frequencies of AKT1 mutation. Kim *et al* (36) found no AKT1 mutations in 104 CRC patients. In a study of 88 CRC patients, Bleeker *et al* (37) found only one case with an AKT1 mutation. Therefore, it is possible that the frequency of AKT1 mutation in CRC cases is lower than the 6% reported by Carpen *et al*. In the present study of CRC, the frequency of BRAF and AKT1 mutations was less than the frequency of KRAS and PIK3CA mutations; therefore, it is possible that the mutation of KRAS and PIK3CA is more important than the mutation of BRAF and AKT1 in carcinogenesis of CRC.

We examined the methylation status of five genes, but found no significant correlation between methylation status and clinicopathological factors. Many reports have found a relationship between methylation of these genes and CRC (38-40). The hMLH1 gene is methylated in MSI-H CRC, and the relationship between methylated hMLH1 and CRC prognosis has been discussed in many studies (41-43). Wettergren *et al* (44) reported that p16 hypermethylation may be a prognostic marker in CRC patients. Therefore, we focused on methylation of these two genes, hMLH1 and p16.

The combination of genetic alterations and epigenetic alterations may provide a good marker for the prognosis of CRC patients. Shen *et al* (45) analyzed both mutation and methylation in primary CRC and found that CRC consists of three distinct subclasses, each of which is fairly homogeneous. Lee *et al* (29) divided CRC patients into four groups based on classification of the RAS/RAF mutation and CIMP, and showed that this classification may be a very effective prognostic marker. Similarly, Ogino *et al* (46) showed that patients with CIMP-low and mutated BRAF have a shorter survival than those with other CIMP/BRAF types.

In the present study, overall survival was not associated with mutations in the RAS/RAF and PIK/AKT pathways. Thus, genetic classification was not useful as a prognostic marker among these patients. Overall survival of patients with mutations was not associated with the methylation status of hMLH1 and p16. However, among the patients without mutations, overall survival was significantly shorter in patients with any methylation than in those without methylation (p=0.0318). Thus, the combination of genetic and epigenetic classification has potential as a good prognostic marker among CRC patients. One possible reason for the lack of prognostic significance of epigenetic and genetic parameters among patients with mutations is that the genetic alterations may predominate in carcinogenesis of CRC; therefore, it is reasonable that the overall survival of wild-type patients is significantly shorter in when methylation occurs compared to no methylation; that is, methylation may play a central role in carcinogenesis of wild-type CRC. Thus, the combination

of genetic and epigenetic alterations may be used as a good marker for prognosis in CRC patients.

In conclusion, we found that genetic alteration by itself was not significantly associated with prognosis; however, the combination of genetic alteration and epigenetic alteration may be a good marker for the prognosis of CRC.

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Review

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Clinical Significance of Lymph Node Ratio and Location of Nodal Involvement in Patients with Right Colon Cancer

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Key Words

Colorectal carcinoma · Lymph node metastasis · Lymph node ratio · Complete mesocolic excision

Abstract

Background/Aims: Increasing negative lymph node count has been reported to be associated with better outcomes in patients with colon cancer. The present study aimed to clarify the clinical significance of the lymph node ratio (LNR) and location of lymph node metastasis (LNM) in patients with stage III right colon cancer. ***Methods:*** We enrolled 820 patients who had undergone curative resection due to colon cancer at a single institution between 1991 and 2005. Among them, 197 underwent curative resection for T2–T4 right colon cancer. We evaluated the oncological outcomes according to LNR (quartiles) and distribution of LNM (n1 = LNM adjacent to the colon or along the vascular arcades of the marginal arteries; n2 = LNM along the major vessels; n3 = LNM near the roots of the major vessels). ***Results:*** The rates of LNM in T2, T3 and T4 right colon cancer were 11.1, 38.6 and 58.0%, respectively ($p < 0.0001$). Recurrence rates were 27.3, 37.5 and 57.1% in patients with n1, n2 and n3 LNM, respectively ($p < 0.0001$). LNR ($p < 0.0001$) and distribution of LNM ($p = 0.046$) were independent risk factors for recurrence in patients with stage III right colon cancer. ***Conclusions:*** Some patients with extensive LNM benefited from lymph node dissection with high ligation. Those with T3–T4 right colon cancer are suitable candidates for lymph node dissection with high ligation. Adding the concept of LNR and location of LNM to conventional TNM staging could improve the accuracy of evaluating nodal status.

Introduction

Colorectal cancer is the second most common cause of cancer death in the USA and Japan, and the occurrence of this disease is rapidly increasing in Japan [1, 2]. Although there has been remarkable progress in chemotherapy for colorectal cancer, the standard therapy for resectable colorectal cancer is still curative resection with lymph node dissection. The introduction of total mesorectal excision led to a decrease in the local recurrence of rectal cancer and improved survival [3–5]. Complete me-

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socolic excision (CME) for colon cancer has recently been proposed as well as total mesorectal excision for rectal cancer [6]. Mesocolic excision with high ligation of the main feeding artery has been a standard technique for advanced colon cancer in Japan [7, 8]. However, high ligation of the supplying arteries and the draining veins for right colon cancer is sometimes complicated because of variations in vascular branches [9, 10]. The precise indication for CME in patients with stage III right colon cancer has not been clarified.

Recent studies have reported that the concept of the lymph node ratio (LNR), which is the proportion of metastatic to examined lymph nodes, is a prognostic factor in patients with stage III colorectal cancer [11–16]. On the other hand, it has been reported that the distribution of lymph node metastasis (LNM) is a prognostic factor in patients with stage III colon cancer [7]. However, LNR and the location of LNM have not been investigated simultaneously as prognostic factors in patients with stage III colon cancer.

The present study aimed to clarify the value of lymph node dissection with high ligation of the primary feeding artery for right colon cancer. We also evaluated the clinical significance of the LNR and the distribution of LNM in patients with stage III right colon cancer.

Patients and Methods

Patients

We reviewed the medical charts of 820 patients who underwent curative surgery for colon carcinoma at a single institution (the Department of Surgical Oncology, Tokyo Medical and Dental University, Tokyo, Japan) between January 1991 and December 2005. Among them, 197 underwent curative resection with high ligation of the main feeding arteries and CME for T2–T4 right colon cancer. The standard practice of nodal dissection for advanced colon cancer at our institution involves ligating the major artery to the region of resection at its origin. Resection of the right colon involves resection of the ileocolic artery and right colonic artery at their origin from the superior mesenteric artery. We usually ligate only the right branch of the middle colic artery, although the lymph nodes around the root of the middle colic artery are dissected. The resected specimen with the mesentery was stretched and pinned to a cork board. The surgeon identified the lymph nodes, isolated them and recorded both their number and distribution. In this study, the surgeons harvested the lymph nodes from the specimen to evaluate the precise location of nodal metastases; we acknowledge that this technique is not the custom in the vast majority of centers in North America or Europe. After formalin fixation, the specimens and lymph nodes were examined by the pathologist. Patients with familial adenomatous polyposis, kindred with cancer family syndrome or ulcerative colitis were excluded from this study. We evaluated the oncological outcomes according to the distribution of LNM.

Location of LNM

The distribution of LNM was classified as n0 (no LNM), n1 (LNM adjacent to the colon or along the vascular arcades of the marginal arteries), n2 (LNM along the major vessels) or n3 (LNM near the roots of the major vessels).

Lymph Node Ratio

We calculated the LNR as the proportion of metastatic to examined lymph nodes and then classified the LNRs into subgroups according to the following quartiles: >0 to <0.07, ≥ 0.07 to <0.15, ≥ 0.15 to <0.30 and ≥ 0.30.

Follow-Up Program

All patients were strictly followed up. During the first 3 years, patients were followed up every 3 months with clinical assessment and measurement of serum carcinoembryonic antigen, and every 6 months with chest X-rays and abdominal ultrasonography or computed tomography. For the next 2 years, all tests were performed every 6 months. Colonoscopy was performed 1 year after surgery and every 2 years for the next 4 years.

Statistical Analysis

Data were statistically analyzed using JMP 8 (SAS Institute Inc., Cary, N.C., USA). All data are expressed as means ± standard deviation. Data concerning age and number of lymph nodes were analyzed using the Mann-Whitney U test and Kruskal-Wallis test for 2 and 3 or more groups, respectively. Associations between each parameter and LNM or recurrence were analyzed using the χ^2 test. Independent risk factors for LNM and recurrence were determined using logistic regression analysis after forward stepwise selection. The actuarial survival of patients was calculated using the Kaplan-Meier method. Overall survival rates in all groups were compared using the log-rank test. Cox's proportional hazard regression analysis was applied to determine which factors independently affected postoperative survival. Forward selection was adopted in a stepwise regression procedure. Statistical significance was established at $p < 0.05$ for all results.

Results

Patient Characteristics

The characteristics of the patients, most of whom had lymphovascular invasion, are listed in table 1. The numbers of patients with stage I (T2), II and III cancers were 24, 91 and 82, respectively.

Lymph Node Metastasis

The mean number of lymph nodes harvested from 82 of the 197 patients was 20 ± 12. The median number of positive nodes was 3 (range 1–23). The rates of LNM in patients with T2, T3 and T4 right colon cancer were 11.1, 38.6 and 58.0%, respectively. The numbers of patients with n1, n2 and n3 LNM were 44 (53.6%), 24 (29.3%) and 14 (17.1%), respectively. The numbers of patients with TNM N1 and N2 were 54 (65.9%) and 28 (34.1%), respec-

Table 1. Risk factors for LNM

		Univariate analysis			Multivariate analysis		
		LNM (–)	LNM (+)	p value	odds ratio	95% CI	p value
Gender	male	61 (61)	39 (39)				
	female	54 (56)	43 (44)	0.45			
Location of colon cancer	ascending colon	60 (62)	37 (38)				
	cecum	34 (52)	31 (48)				
	transverse colon	21 (60)	14 (40)	0.47			
Depth of tumor invasion	T2	24 (89)	3 (11)		1		
	T3	62 (61)	39 (39)		3.1	0.79–12.1	0.10
	T4	29 (42)	40 (58)	0.0001	5.4	1.36–21.9	0.017
Histological type	well-differentiated adenocarcinoma	63 (68)	29 (32)		1		
	other	50 (49)	53 (51)	0.0049	1.8	0.91–3.40	0.093
	unknown	2	0				
Lymphatic invasion	absent	36 (97)	1 (3)		1		
	present	79 (49)	9 (51)	<0.0001	25.0	3.25–200	0.0020
Venous invasion	absent	17 (89)	2 (11)		1		
	present	98 (55)	80 (45)	0.0038	2.7	0.50–14.3	0.25

Values represent numbers of patients (percentage), unless indicated otherwise. CI = Confidence interval.

Table 2. Association between clinicopathological features and LNR and distribution of LNM

		LNR					Distribution of LNM			
		>0 to <0.07	≥0.07 to <0.15	≥0.15 to <0.30	≥0.30	p value	n1	n2	n3	p value
Number of lymph nodes retrieved		30 ± 11	21 ± 9	18 ± 7	16 ± 8	<0.0001	23 ± 11	18 ± 6	23 ± 11	0.16
Number of positive nodes		1 ± 0.4	2 ± 1	4 ± 1	7 ± 5	<0.0001	3 ± 2	4 ± 3	6 ± 6	0.10
Gender	male	6 (15)	13 (33)	10 (26)	10 (26)		22 (56)	9 (23)	8 (21)	
	female	14 (33)	9 (21)	12 (28)	8 (19)	0.25	22 (51)	15 (35)	6 (14)	0.45
Location of colon cancer	ascending colon	8 (22)	11 (30)	12 (32)	6 (16)		21 (57)	10 (27)	6 (16)	
	cecum	7 (23)	10 (32)	8 (26)	6 (19)		17 (55)	11 (35)	3 (10)	
	transverse colon	5 (36)	1 (7)	2 (14)	6 (43)	0.23	6 (43)	3 (21)	5 (36)	0.29
Depth of tumor invasion	T2	1 (33)	0	2 (67)	0		3 (100)	0	0	
	T3	11 (28)	17 (44)	7 (18)	4 (10)		20 (51)	9 (23)	10 (26)	
	T4	8 (20)	5 (13)	13 (33)	14 (35)	0.0058	21 (53)	15 (38)	4 (10)	0.14
Histological type	well-differentiated adenocarcinoma	9 (31)	11 (38)	6 (21)	3 (10)		14 (48)	10 (34)	5 (17)	
	other	11 (21)	11 (21)	16 (30)	15 (28)	0.10	30 (57)	14 (26)	9 (17)	0.72
Lymphatic invasion	absent	0	1 (100)	0	0		0	0	1 (100)	
	present	20 (25)	21 (26)	22 (27)	18 (22)	0.43	44 (54)	24 (30)	13 (16)	0.086
Venous invasion	absent	0	0	2 (100)	0		1 (50)	1 (50)	0	
	present	20 (25)	22 (28)	20 (25)	18 (23)	0.13	43 (54)	23 (29)	14 (18)	0.72

Values represent numbers of patients (percentage), unless indicated otherwise.

Table 3. Risk factors for recurrence

		Univariate analysis			Multivariate analysis after stepwise regression		
		recurrence (–)	recurrence (+)	p value	odds ratio	95% CI	p value
Gender	male	80 (80)	20 (20)				
	female	81 (84)	16 (16)	0.52			
Location of colon cancer	ascending colon	83 (86)	14 (14)				
	cecum	51 (78)	14 (22)				
	transverse colon	27 (77)	8 (23)	0.38			
Depth of tumor invasion	T2	26 (96)	1 (4)		1		
	T3	87 (86)	14 (14)				
	T4	48 (70)	21 (30)	0.0025	2.03	0.82–5.05	0.13
Histological type	well-differentiated adenocarcinoma	80 (87)	12 (13)				
	other	79 (77)	24 (23)	0.065			
	unknown	2	0				
Lymphatic invasion	absent	36 (97)	1 (3)				
	present	125 (78)	35 (22)	0.0065			
Venous invasion	absent	19 (100)	0		1		
	present	142 (80)	36 (20)	0.030	4792738	4.8×10^9–	0.022
Number of LNM	N0	108 (94)	7 (6)				
	N1	40 (74)	14 (26)				
	N2	13 (46)	15 (54)	<0.0001			
Location of LNM	n0	108 (94)	7 (6)		1		
	n1	32 (74)	12 (26)				
	n2	15 (63)	9 (38)				
	n3	6 (43)	8 (57)	<0.0001	3.91	1.03–14.93	0.046
LNR	0	108 (94)	7 (6)		1		
	<0.15	34 (81)	8 (19)				
	≥0.15 to <0.30	12 (55)	10 (45)		6.45	2.56–16.13	<0.0001
	≥0.30	7 (39)	11 (61)	0.0099			

Values represent numbers of patients (percentage), unless indicated otherwise. CI = Confidence interval.

tively. Among the various parameters studied, depth of tumor invasion ($p = 0.0001$), histological type ($p = 0.0049$), lymphatic invasion ($p < 0.0001$) and venous invasion ($p = 0.0038$) were risk factors for LNM in the univariate analysis (table 1). Multivariate analysis revealed that the depth of tumor invasion (T4; $p = 0.017$) and lymphatic invasion ($p = 0.0020$) were independent risk factors. None of the patients with T2 right colon cancer had n2 or n3 LNM (table 2). The distribution of LNM was not associated with any clinicopathological parameters (table 2).

Lymph Node Ratio

The LNR was associated with the depth of tumor invasion ($p = 0.0058$) and the number of positive lymph nodes ($p < 0.0001$; table 2). There was a negative correlation between the number of retrieved lymph nodes and the LNR ($p < 0.0001$).

Recurrence

Among 197 patients, 36 (18.3%) developed recurrence over a median follow-up period of 4.1 years. Recurrence rates were 6.1, 27.3, 37.5 and 57.1% in patients with n0, n1, n2 and n3 LNM, respectively ($p < 0.0001$). We found 3 locoregional recurrences in the present study (3/197, 1.5%). The locoregional recurrence rates in T2, T3 and T4 tumors were 0% (0/27), 0.99% (1/101) and 2.9% (2/69), respectively. Two of the 3 patients with locoregional recurrence also had distant metastasis. The depth of tumor invasion ($p =$

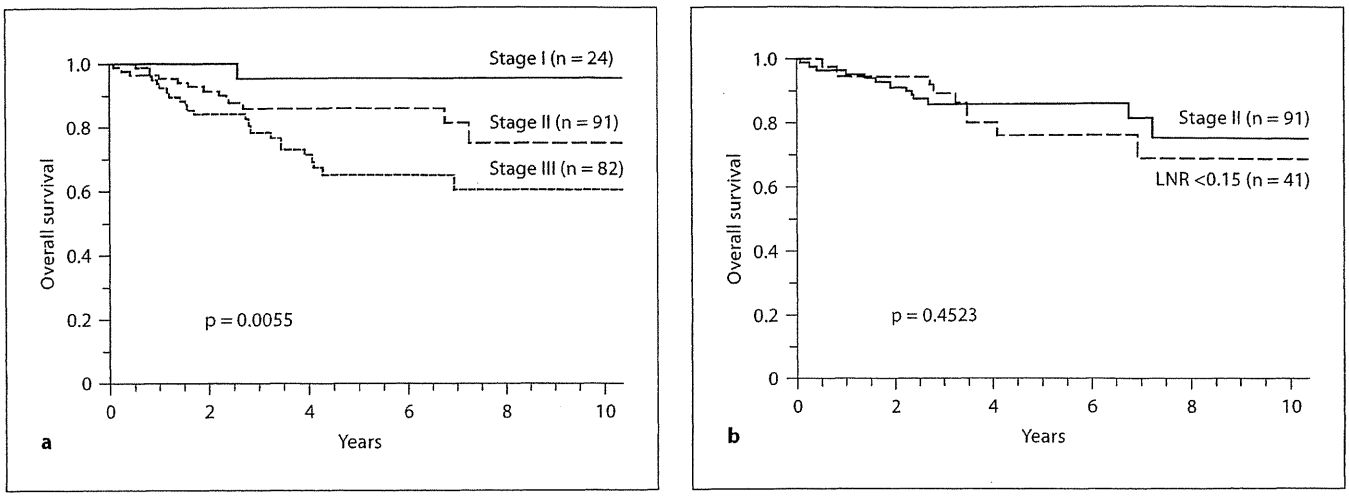

Fig. 1. a Overall survival curves according to the TNM stage. **b** Overall survival curves for patients with stage II cancer and stage III cancer (LNR <0.15).

0.0025), lymphatic invasion (p = 0.0065), venous invasion (p = 0.030), number of LNM (p < 0.0001), location of LNM (p < 0.0001) and LNR (p = 0.0099) were risk factors for recurrence in the univariate analysis. Among these, stepwise regression analysis selected depth of tumor invasion, venous invasion, location of LNM and LNR as risk factors for recurrence. Logistic regression analysis revealed that venous invasion (p = 0.022), the location of LNM (n0–n2 vs. n3, p = 0.046) and LNR (0–0.14 vs. 0.15–1.0, p < 0.0001) were independent risk factors for recurrence (table 3).

Survival

The 5-year overall survival rates in patients with stages I (n = 24), II (n = 91) and III (n = 82) right colon cancer were 95.2, 86.3 and 65.0%, respectively (p = 0.0055; fig 1a). In terms of LNM distribution, the 5-year overall survival rates in patients with n1, n2 and n3 LNM were 76.4, 60.0 and 37.5%, respectively (p = 0.0002). There was no difference in overall survival between patients with LNR <0.15 and those with stage II cancer (p = 0.45; fig. 1b). Depth of tumor invasion (p = 0.017) and lymphatic invasion (p = 0.025), number (p = 0.0006) and distribution (p = 0.0002) of LNM, and LNR (p < 0.0001) were prognostic factors according to the log-rank test (table 4). Among these, stepwise regression analysis selected depth of tumor invasion and location of LNM as variables for further analysis. Depth of tumor invasion (T2 vs. T3–T4, p = 0.030) and location of LNM (n0–n1 vs. n2–n3, p = 0.0050) were independent risk factors in the Cox proportional hazards model.

Discussion

Lymph flow in colorectal cancer was described a century ago [17]. Today, the standard treatment for resectable colorectal cancer is radical resection accompanied by systematic lymph node dissection. However, the optimal extent of lymph node dissection remains controversial. Some studies have shown an advantage of high ligation in patients with colorectal cancer [18–20], whereas others could not demonstrate such an advantage [21–24]. High ligation of major vessels is a standard procedure for advanced colorectal cancer in Japan, although few reports have addressed indications for this technique.

Vessels of the right colon vary anatomically. Garcia-Ruiz et al. [9] reported that it was unusual for the right colic artery to arise directly from the superior mesenteric artery (10.7%). Yamaguchi et al. [10] demonstrated variations in the anatomy of the veins of the right colon. Therefore, high ligation for right colon cancer is sometimes complicated, and thus indications for high ligation for right colon cancer should be clarified.

The present study demonstrated that among patients with LNM, 48.7% with T3 and 47.5% with T4 right colon cancer had more distant LNM than those with pericolic cancer. On the other hand, none of the patients with T2 right colon cancer had n2 or n3 LNM. This study also showed that the depth of tumor invasion was an independent risk factor for LNM. The extent of lymph node dissection should be determined according to the depth of tumor invasion. A suitable candidate for CME with high

Table 4. Prognostic factors in patients with T2–T4 right colon cancer

		Univariate analysis			Cox hazard model after stepwise regression		
		number	%	p value	HR	95% CI	p value
Gender	male	100	51				
	female	97	49	0.48			
Location of colon cancer	ascending colon	97	49				
	cecum	65	33				
	transverse colon	35	18	0.31			
Depth of tumor invasion	T2	27	14		1		
	T3	101	51		2.33	1.07–9.87	0.030
	T4	69	35	0.017			
Histological type	well-differentiated adenocarcinoma	92	47				
	other	103	52	0.093			
	unknown	2	1				
Lymphatic invasion	absent	37	19				
	present	160	81	0.025			
Venous invasion	absent	19	10				
	present	178	90	0.13			
Number of LNM	N0	115	58				
	N1	54	27				
	N2	28	14	0.0006			
Location of LNM	n0	115	58		1		
	n1	44	22				
	n2	24	12		1.64	1.17–2.28	0.0050
	n3	14	7	0.0002			
LNR	0	115	58				
	<0.15	42	21				
	≥0.15 to <0.30	22	11				
	≥0.30	18	9	<0.0001			

HR = Hazard ratio; CI = confidence interval.

ligation is a patient with T3–T4 right colon cancer, whereas high ligation might be unnecessary for T2 cancer.

Hohenberger et al. [6] recently described improved oncological outcomes after adopting CME for colon cancer. They reduced 5-year local recurrence rates from 6.5 to 3.6% and increased cancer-related 5-year survival rates from 82.1 to 89.1%. All of our patients underwent CME with high ligation for right colon cancer. In the present study, the locoregional recurrence rates in T2, T3 and T4 tumors were 0, 0.99 and 2.9%, respectively. The recurrence rates in patients with n2 and n3 LNM were 37.5 and 57.1%, respectively. This means that >40% of patients with LNM around the roots of major vessels did not develop recurrence after curative resection for right colon cancer, unless they had distant metastasis. Thus, CME with high ligation should be a standard treatment for advanced colon cancer, although the population of patients with n3 LNM who do not have distant metastasis may be small.

CME is not new [25], and surgical techniques have been applied in embryological tissue planes for colon cancer resection. However, this technique differs according to the surgeon. In fact, West et al. [26] reported that specimens resected at two different hospitals differed in terms of the area of the mesentery and the distance between the tumor and the high vascular tie.