とができる．現在までに4例のISR症例に付加手術をおこなった（表1）．いずれも縫合不全，吻合部狭窄，腸管脱を認めていない．4例中3例は人工肛門を閉鎖し，最長6ヵ月の観察期間でKirwan score[4]は2が2例，3が1例であった．この手技は単純，簡単で，要する時間も10分から20分程度である．ISR後の排便機能低下を抑制する有効な手段のひとつになると考えている．

文　献

1) Denost Q, Laurent C, Capdepont M, et al: Risk factors for fecal incontinence after intersphincteric resection for rectal cancer. Dis Colon Rectum 54：963-968, 2011
2) Lunniss PJ, Phillips RK: Anatomy and function of the anal canal longitudinal muscle. Br J Surg 79：882-884, 1992
3) Macchi V, Porzionato A, Stecco C, et al: Histo-topographic study of the longitudinal anal muscle. Clin Anast 21：447-452, 2008
4) Kirwan WO, O'Riordain MG, Waldron R: Declining indications for abdominoperineal resection. Br J Surg 76：1061-1063, 1989

(2011年8月30日受付)
(2012年2月14日受理)

World J Surg (2012) 36:424–430
DOI 10.1007/s00268-011-1378-y

World Journal of Surgery

Risk Factors for Postoperative Recurrence in Patients with Pathologically T1 Colorectal Cancer

Shuji Iida · Hirotoshi Hasegawa · Koji Okabayashi · Konosuke Moritani · Makio Mukai · Yuko Kitagawa

Published online: 21 December 2011

Abstract

Background The evolution of diagnostic procedures has resulted in an increase in early detection of pathologically T1 (pT1) colorectal cancer (CRC). However, the risk factors affecting long-term outcomes of patients with pT1 CRCs have been unclear. The aim of the present study was to identify risk factors for postoperative recurrence and overall survival in patients with pT1 CRC.

Methods Between January 1990 and January 2003, a total of 284 patients with pT1 CRC underwent radical surgery in the authors' institution. The impact of clinicopathological factors on postoperative recurrence and overall survival was estimated by univariate and multivariate analysis.

Results The median follow-up period was 55 months (interquartile range: 47.1 months). Postoperative recurrence occurred in 8 (2.8%) patients. The overall 5-year and 10-year disease-free survival rates were 98.4 and 92.7%. Multivariate analysis showed the presence of lymphatic invasion only was an independent risk factor for postoperative recurrence in pT1 CRC patients (hazard ratio: 11.622; $P = 0.003$). The 5-year and 10-year disease-free survival rates of the patients in N-ly− group, the N-ly + group, and the N+ group were 99.5%/98.2% and 96.3%/75.2%, and 93.3%/93.3%, respectively. Additionally, 4 of the 8 recurrences were found more than 5 years after the operation.

Conclusions Lymphatic invasion was an independent risk factor for recurrence in pT1 CRC patients.

Presented at the American Society of Colon and Rectal Surgeons, 2009 Annual Meeting, Hollywood, Florida, 2–6 May 2009.

S. Iida · H. Hasegawa (✉) · K. Okabayashi · K. Moritani · Y. Kitagawa
Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi Shinjuku-ku, Tokyo, Japan
e-mail: hasegawa@z8.keio.jp

M. Mukai
Department of Pathology, School of Medicine, Keio University, Tokyo, Japan

Introduction

Colorectal cancer (CRC) is one of the most common malignancies and a leading cause of cancer-related deaths in Europe and the United States [1]. Likewise, it is also the third leading cause of cancer deaths in Japan, and 41,000 of the deaths in Japan were attributable to CRC in 2006 [2].

Recent advancements in the diagnosis of CRC have led to an increase in the detection of pathologically T1 (pT1) CRCs, resulting in less invasive treatment including local resections, such as endoscopic submucosal dissection (ESD) and transanal endoscopic microsurgery (TEM) in selected cases [3–5]. However, these patients treated with local excision have a relatively high risk of recurrence because these treatments leave metastatic lymph nodes in 8–15% of pT1 CRC patients without definitively retrieving regional lymph nodes [6], resulting in 4.1–39% of local recurrence in patients with pT1 CRC treated by TEM [7]. To minimize local recurrence due to incomplete lymph node dissection, radical surgery has been recommended in Japanese guideline for treatment of CRC. Consequently, patients with pT1 CRC have a good prognosis, and the recurrence rate has been reported to be 1.3% after radical surgery [8].

To allow these local excisions and define the indication, it is necessary to clarify the risk factors that affect lymph node metastasis and prognosis by conducting a cohort study focusing on patients with curative surgical resection.

Most previous studies evaluated risk factors for lymph node metastasis, such as tumor budding and lymphatic invasion [9–11], and there is little evidence with regard to risk factors of postoperative recurrence in patients with pT1 CRC. The aim of the present study was to identify the predictive risk factors that may affect postoperative relapse in patients with pT1 CRC.

Patients and methods

Patients

Between January 1990 and January 2003, 2,151 patients with CRC underwent radical resection in the authors' institution, and, of these, 284 patients with pT1 CRC formed the basis of the present study. Of those 284 patients, 107 patients had undergone endoscopic mucosal resection prior to eventual radical surgery. The pathological findings of these patients were confirmed by the specimens retrieved by surgical excision or endoscopic mucosal resection. The authors' treatment strategy for pT1 CRC was based on the Japanese treatment guideline for CRC [12]. The Japanese guideline recommends that pT1 CRC other than pSM1 CRC without any lymphatic and vascular invasion, which is confirmed by local excision including endoscopic mucosal resection, should be removed together with regional lymph nodes by definitive surgical resection, whereas pSM1 CRC without any lymphatic and vascular invasion can be observed only with scheduled follow-up. All the clinical data, including age, sex, site distribution, tumor differentiation, depth of tumor invasion, tumor size, lymph node metastasis, and lymphatic and vascular invasion, were collected from prospectively maintained database.

Surveillance system

Before 2000, all patients underwent postoperative surveillance that consisted of measurement of the serum tumor markers carcinoembryonic antigen (CEA) and CA19-9, abdominal computed tomography (CT), and a chest X-ray every 6 months. After 2000 the chest X-ray was replaced by chest CT. Colonoscopy was performed one year after the operation and every 3 years thereafter. Postoperative surveillance was usually discontinued in patients without any relapse by 6 years after surgery, but it was continued if the patient wished.

Pathological findings

The histopathological diagnosis was made by independent, experienced pathologists. The depth of submucocal invasion was measured as vertical distance from the muscularis mucosae to the deepest point of tumor invasion in the submucosa. According to previous reports, the level of submucosal invasion was defined as follows: SM1 if the deepest point of invasion was in the upper third of the submucosa; SM2 if the deepest point of invasion was in the middle third of the submucosa; and SM3 if the deepest point of invasion was in the lower third of the submucosa [13]. Lymphatic invasion and venous invasion were defined as positive when tumor cells were seen in the lumen of a lymphatic vessel and in the lumen of a vein, respectively. The standard staining methods in the authors' institution (e.g., with hematoxylin and eosin was used, and Elastica van Gieson (EVG) staining was used to identify venous walls.

Statistical analysis

Continuous variables were compared by the Mann–Whitney *U*-test. Categorical variables were compared with Fisher's exact test. Overall survival was analyzed by Kaplan–Meier survival curves, and comparisons were made by the log-rank test. The hazard ratio for postoperative recurrence and survival was calculated by Cox's proportional hazard model. All the reported P values were two-tailed, and $P < 0.05$ was considered statistically significant. All analyses were performed with SPSS version 17 statistical software package (SPSS, Chicago, IL).

Results

The patients' background is shown in Table 1. Data from the records of 284 patients were analyzed in the present study. The median follow-up period was 55 months (interquartile range: 47.1 months).

Eleven (65%) of 17 patients who had lymph node metastases were positive for lymphatic invasion. In univariate analysis, the presence of lymphatic invasion and the depth of invasion (pSM3) were associated with an increased incidence of lymph node metastasis (Table 2). Other factors had no significant correlation with the presence of lymph node metastasis. In multivariate analysis, the presence of lymphatic invasion (HR: 5.916, CI: 2.027–17.265, $P = 0.001$), and the depth of invasion (pSM3) (HR: 6.579, CI: 1.704–25.396, $P = 0.006$) were significant risk factors for lymph node metastasis (Table 2).

The 5-year and 10-year disease-free survival (DFS) rates were 98.4 and 92.7% (Fig. 1a) and the overall 5-year and 10-year survival rates were 97.0% and 91.4% (Fig. 1b). In univariate analysis for risk factors that affect overall survival, only lymphatic invasion was significantly associated

Table 1 Patient background

	Number (%)
Age, years[a]	62 ± 10
Sex	
Male	200 (70)
Female	84 (30)
Site distribution	
Right colon	67 (24)
Left colon	163 (57)
Rectum	54 (19)
Lymph node metastasis	
(−)	267 (94)
(+)	17 (6)
Number of harvested lymph nodes[a]	9.60 ± 8.50
Differentiation	
Well	233 (82)
Moderate	48 (17)
Other	3 (1)
Lymphatic invasion	
(−)	204 (72)
(+)	80 (28)
Vascular invasion	
(−)	251 (88)
(+)	33 (12)
Depth of invasion	
pSM1	67 (24)
pSM2	93 (33)
pSM3	124 (44)

95% CI 95% confidence interval

[a] Mean ± SD

with poor prognosis (HR: 8.98, CI: 1.8–44.7, $P = 0.007$) (Table 3).

Based on previous reports [14, 15], the authors selected tumor differentiation, depth of invasion, lymph node metastasis, lymphatic invasion, and vascular invasion as potential risk factors (Table 3). Multivariate analysis showed lymphatic invasion was the only independent risk factor for postoperative recurrence in pT1 CRC patients (HR: 11.622, CI: 2.272–59.465, $P = 0.003$).

Postoperative recurrence occurred in 8 patients (lung: 3, liver: 2, distant lymph node: 2, local: 1). The clinicopathological details of the 8 patients are shown in Table 4. Five of the 8 patients were negative for lymph node metastasis and positive for lymphatic invasion (N-ly+), 2 were negative for both lymph node metastasis and lymphatic invasion (N-ly−), and one was positive for lymph node metastasis (N+) (Table 4). In each group, based on the status of lymph node metastasis and lymphatic invasion, the 5-year and 10-year disease-free survival (DFS) rates of the patients in the N-ly− group, the N-ly+ group, and the N+ group were 99.5%/98.2%, 96.3%/75.2%, and 93.3%/93.3%, respectively (Fig. 2a). The N-ly+ group had a shorter 10-year DFS than the other two groups, but the difference was not statistically significant. The 5- and 10-year overall survival rates of the patients in the N-ly− group, the N-ly+ group, and the N+ group were 98.1%/93.7%, 94.7%/83.0%, and 93.3%/93.3%, respectively (Fig. 2b). There was no significant difference among the three groups. In 4 of the 8 patients with recurrence, the recurrences were found more than 5 years after the operation. Three of the four patients died due to the recurrent disease, and the other patient is still alive with liver metastasis.

Discussion

Patients with pT1 CRC have a favorable prognosis, but approximately 1.3% of them are reported to develop a recurrence after radical surgery [12]. Previous studies have shown that age, TNM stage, T-status, nodal status, distant metastasis, lymphatic and vascular invasion, and preoperative CEA level were prognostic factors of CRC patients [15–17], but most of these included pT2–T4 patients as well as pT1 patients, and there has been a paucity of evidence of a risk factor for recurrence in pT1 CRCs. The present study showed that lymphatic invasion was the only significant risk factor for recurrence in pT1 CRC patients treated by radical surgery.

Lymph node metastasis was reported to have a poor prognosis in advanced CRC [8, 14–16]. In many studies, lymphatic invasion was significantly associated with incidence of lymph node metastasis, a finding consistent with the results of the present study. Despite theses correlations, lymph node metastasis was not significant risk factor affecting DFS, and the N-ly+ group had a shorter 10-year OS and DFS rate than the N+ group in this study. There were thought to be three reasons for these unique results. First, stage migration might have occurred as a result of insufficient lymph node dissection because prognosis depends on the number of lymph nodes examined [18]. In this study, the mean number of lymph nodes dissected was only 9.6. Fewer than 9 lymph nodes were dissected in 5 of the 8 patients with recurrence. Notably, a patient (Patient 4) from whom no lymph nodes were dissected had undergone laparoscopic resection for cancer of the descending colon early in the study period. It was assumed that mobilization of the colon was inadequate, leading to eventually development of recurrence in regional lymph nodes. In addition, a patient with cancer of the rectum (patient 5) developed a lateral pelvic lymph node recurrence. It is important to note that lateral lymph node dissection is not performed in pT1

Table 2 Univariate and multivariate analysis of risk factors affecting lymph node metastasis

Factors	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, years	1.001	0.953–1.050	0.977			
Sex						
Male	0.579	0.213–1.576	0.285			
Female	1	–	–			
Site distribution						
Right colon	1	–	–			
Left colon	2.817	0.618–12.838	0.181			
Rectum	0.745	0.150–3.700	0.719			
Differentiation						
Well differentiated	1	–	–			
Moderately differentiated	1.128	0.309–4.122	0.855			
Lymphatic invasion						
(–)	1	–	–	1	–	–
(+)	5.261	1.875–14.762	0.002	5.916	2.027–17.265	0.001
Vascular invasion						
(–)	1	–	–			
(+)	1.693	0.460–6.233	0.429			
Depth of invasion						
pSM1	1	–	–	1	–	–
pSM2	0.242	0.053–1.09	0.066	0.205	0.017–2.412	0.208
pSM3	0.085	0.11–0.662	0.019	6.579	1.704–25.396	0.006

95% CI 95% confidence interval, *well* well differentiated, *mod* moderately differentiated

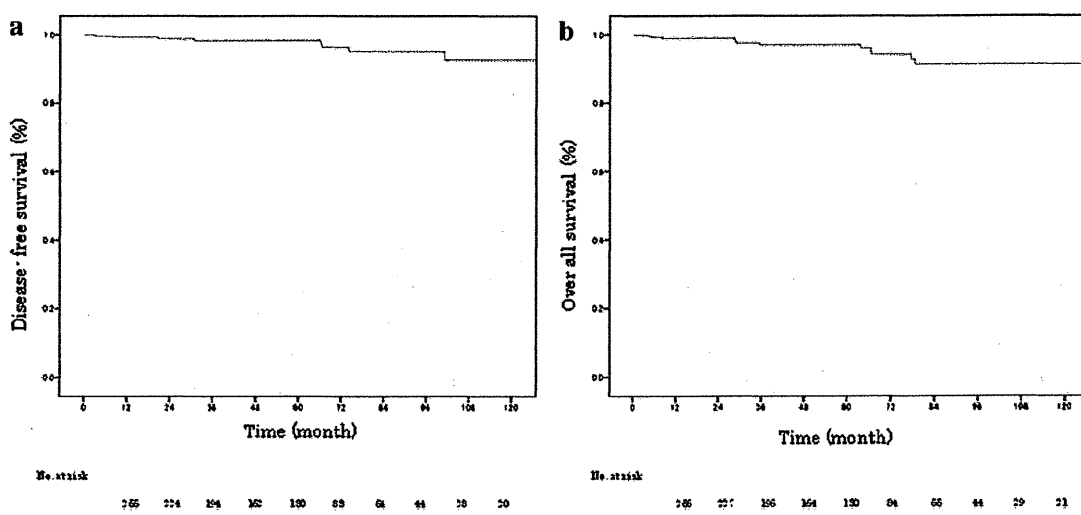

Fig. 1 Cumulative survival curves in 284 patients with pT1 colorectal cancer (CRC). **a** Disease-free survival, **b** Overall survival

CRC patients, even in Japan. Second, the use of adjuvant chemotherapy might have improved the prognosis of N+ patients. Referring to the pooled analysis of NSABP C-01 through C-05, the 10-year survival rate of the N-ly+ (stage I) group was better than in the stage II group treated by surgery alone (approximately 60%) and worse than in the stage II group treated by surgery plus 5-fluorouracil/leucovorin (FU/LV) (approximately 80%) [19]. In this study seven of the 17 N+ patients received adjuvant chemotherapy and had no recurrence. Considering the survival

Table 3 Univariate and multivariate analysis of risk factors affecting recurrence

Factors	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, years	1.001	0.931–1.076	0.980			
Sex						
Male	1.107	0.223–5.494	0.901			
Female	1	–	–			
Site distribution						
Right colon	1	–	–			
Left colon	1.447	0.161–12.979	0.742			
Rectum	3.810	0.780–18.621	0.098			
Lymph node metastasis						
(–)	1	–	–		–	–
(+)	2.581	0.317–21.040	0.376			
Differentiation						
Well-differentiated	1	–	–	1	–	–
Moderately differentiated	0.039	0.000–215.911	0.461	0.000	0.000	0.977
Lymphatic invasion						
(–)	1	–	–	1	–	–
(+)	8.984	1.808–44.652	0.007	11.622	2.272–59.465	0.003
Vascular invasion						
(–)	1	–	–	1	–	–
(+)	1.019	0.125–8.290	0.986	0.458	0.054–3.912	0.475
Depth of invasion						
pSM1	1	–	–	1	–	–
pSM2	0.939	0.058–15.106	0.965	0.719	0.042–12.311	0.820
pSM3	3.749	0.756–18.585	0.106	3.343	0.648–17.264	0.150

rt right; *lt* left; *95% CI* 95% confidence interval; *well* well differentiated; *mod* moderately defferentiated

Table 4 Details of recurrent patients

Site distribution	Depth	Lymphatic invasion	Vascular invasion	N	No. of harvested LN	DFS (month)	Prognosis	Recurrence site
Left colon	pSM 3	(+)	(–)	(+)	7	8	Dead	Lung
Left colon	pSM 3	(+)	(+)	(–)	13	31	Alive	Liver
Right colon	pSM 3	(+)	(–)	(–)	15	74	Alive	Lung
Left colon	pSM 3	(+)	(–)	(–)	0	101	Dead	Local
Rectum	pSM 3	(+)	(–)	(–)	20	21	Dead	Lateral pelvic LN
Left colon	pSM 2	(+)	(–)	(–)	8	66	Dead	Distant
Rectum	pSM 3	(–)	(–)	(–)	2	66	Dead	Lung
Rectum	pSM 1	(–)	(–)	(–)	3	3	Dead	Liver

N lymph node metastasis; *LN* lymph node; *DFS* disease-free survival

rate rose from 60 to 80% as a result of administering adjuvant chemotherapy to stage II patients, the administration of adjuvant chemotherapy for pT1 CRC patients with lymphatic invasion might have a potential benefit for improving overall survival. Finally, the expression of some novel molecular markers, such as oncogene mutations, changes in chromosomes, and vascular endothelial growth factors, might result in poor outcome in pT1 CRCs [20–22]. However, because these researches have just begun, the impact on survival has been unclear. Further analyses

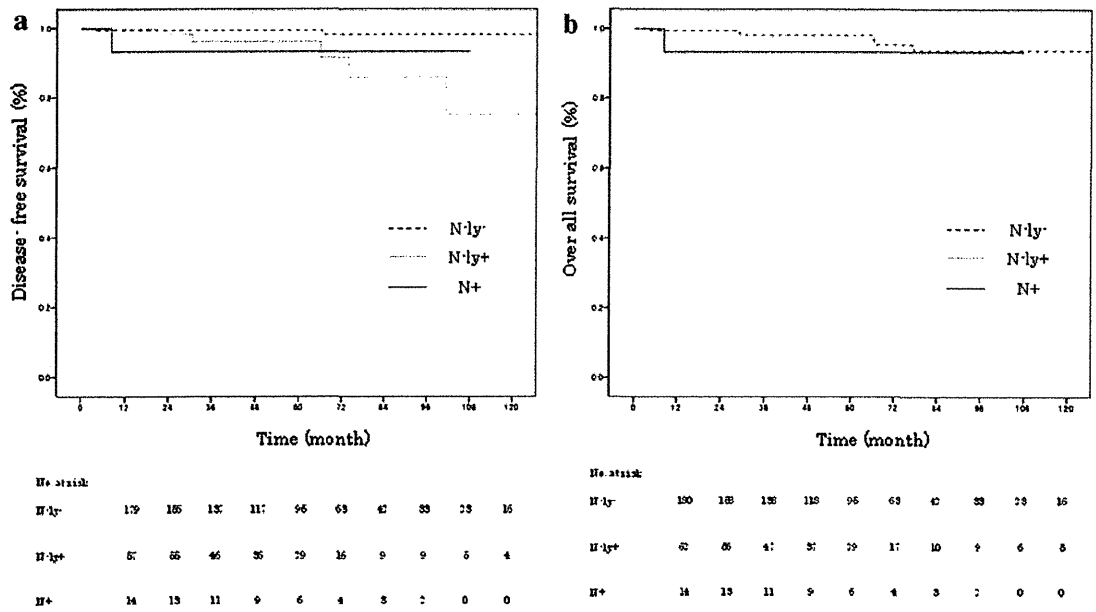

Fig. 2 Cumulative survival curves in N-ly−, N-ly+, N+ groups. **a** Disease-free survival, **b** Overall survival

using propensity score or case-matching and the research results for new biological markers should be carried out to clarify these issues.

Hematoxylin and eosin staining potentially caused inter-observer variability in the evaluation of lymphatic channel and venous involvement in this series. One feature of the present study was that EVG staining was used to identify venous invasion. This procedure was performed to distinguish clearly lymphatic channels from veins. It is effective because elastic fiber located in the venous wall stains violet with this procedure. Suzuki et al. demonstrated that EVG staining reduced about 40% the interobserver variability in evaluating the histology and significantly increased the positivity rate for venous invasion and the odds ratio [23].

In 4 of the 8 patients with recurrence in this study, the relapse was found more than 5 years after the operation, and only one patient was salvaged and alive without a re-relapse. In a previous study the cumulative recurrence rates in stage I, stage II, and stage III were 21.6%/31.0%/44.7% at 1 year, 68.6%/76.9%/87.0% at 3 years, and 96.1%/92.9%/97.8%at 5 years, respectively. Thus, the proportion of stage I patients who experienced a recurrence increased gradually and steadily over a 5-year period, whereas the proportions of stage II and III cancer patients with recurrence rapidly increased for the first 3 years [8]. After 5 years, the recurrence rates were extremely low in patients classified in all three stages: 0.14, 0.94, and 0.67%, respectively [8]. However, regarding the low rate of salvage and incidence of recurrence 5 years after operation, long-term postoperative follow-up of pT1 patients may be unnecessary.

The limitation of this study is the small number of cases because of the rarity of recurrence in early cancer. The recurrence rate after radical surgery in the present study was 2.8%, which is reported to be 1.3% [8] in another study. It is necessary to accumulate evidence of other studies because there is a limit to the number of cases seen in a single institution.

Another limitation of the present study was that tumor budding was not fully evaluated. Tumor budding has been reported to be associated with lymph node metastasis in pT1 CRC [11]. Although the role of budding in recurrence is unclear, given the strong correlation with lymph node metastasis, it is considered to be a promising candidate. Further investigation will be necessary to clarify the role of budding in recurrence in pT1 CRC.

Conclusions

Lymphatic invasion was found to be an independent risk factor for recurrence in pT1 CRC patients who had been treated by radical resection.

References

1. Landis SH, Murray T, Bolden S et al (1999) Cancer statistics, 1999. CA Cancer J Clin 49:8–31
2. Fujimori T, Satonaka K, Yamamura-Idei Y et al (1994) Non-involvement of ras mutations in flat colorectal adenomas and carcinomas. Int J Cancer 57:51–55

3. Stipa S, Lucandri G, Stipa F et al (1995) Local excision of rectal tumours with transanal endoscopic microsurgery. Tumori 81:50–56
4. Kobayashi N, Saito Y, Uraoka T et al (2009) Treatment strategy for laterally spreading tumors in Japan: before and after the introduction of endoscopic submucosal dissection. J Gastroenterol Hepatol 24:1387–1392
5. Burghardt J, Buess G (2005) Transanal endoscopic microsurgery (TEM): a new technique and development during a time period of 20 years. Surg Technol Int 14:131–137
6. Okabe S, Shia J, Nash G et al (2004) Lymph node metastasis in T1 adenocarcinoma of the colon and rectum. J Gastrointest Surg 8:1032–1039 discussion 1039–1040
7. Dias AR, Nahas CS, Marques CF et al (2009) Transanal endoscopic microsurgery: indications, results and controversies. Tech Coloproctol 13:105–111
8. Kobayashi H, Mochizuki H, Sugihara K et al (2007) Characteristics of recurrence and surveillance tools after curative resection for colorectal cancer: a multicenter study. Surgery 141:67–75
9. Fujimori T, Fujii S, Saito N et al (2009) Pathological diagnosis of early colorectal carcinoma and its clinical implications. Digestion 79(Suppl 1):40–51
10. Wang LM, Kevans D, Mulcahy H et al (2009) Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. Am J Surg Pathol 33:134–141
11. Yamauchi H, Togashi K, Kawamura YJ et al (2008) Pathological predictors for lymph node metastasis in T1 colorectal cancer. Surg Today 38:905–910
12. JSCCR (Japanese Society for Cancer of the Colon and Rectum) Guidelines 2009 for the treatment of colorectal cancer
13. Wirsing K, Lorenzo-Rivero S, Luchtefeld M et al (2006) Local excision of stratified T1 rectal cancer. Am J Surg 191:410–412
14. Chok KS, Law WL (2007) Prognostic factors affecting survival and recurrence of patients with pT1 and pT2 colorectal cancer. World J Surg 31:1485–1490. doi:10.1007/s00268-007-9089-0
15. Compton CC, Fielding LP, Burgart LJ et al (2000) Prognostic factors in colorectal cancer. College of American Pathologists consensus statement 1999. Arch Pathol Lab Med 124:979–994
16. Mehrkhani F, Nasiri S, Donboli K et al (2009) Prognostic factors in survival of colorectal cancer patients after surgery. Colorectal Dis 11:157–161
17. Bach SP, Hill J, Monson JR et al (2009) A predictive model for local recurrence after transanal endoscopic microsurgery for rectal cancer. Br J Surg 96:280–290
18. Wang HS, Liang WY, Lin TC et al (2005) Curative resection of T1 colorectal carcinoma: risk of lymph node metastasis and long-term prognosis. Dis Colon Rectum 48:1182–1192
19. Wilkinson NW, Yothers G, Lopa S et al. Long-term survival results of surgery alone versus surgery plus 5-fluorouracil and leucovorin for stage II and stage III colon cancer: pooled analysis of NSABP C-01 through C-05. A baseline from which to compare modern adjuvant trials. Ann Surg Oncol 17:959–966
20. Zhou W, Goodman SN, Galizia G et al (2002) Counting alleles to predict recurrence of early-stage colorectal cancers. Lancet 359:219–225
21. Zheng S, Han MY, Xiao ZX et al (2003) Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World J Gastroenterol 9:1227–1230
22. Lin LJ, Zheng CQ, Jin Y et al (2003) Expression of survivin protein in human colorectal carcinogenesis. World J Gastroenterol 9:974–977
23. Suzuki A, Togashi K, Nokubi M et al (2009) Evaluation of venous invasion by Elastica van Gieson stain and tumor budding predicts local and distant metastases in patients with T1 stage colorectal cancer. Am J Surg Pathol 33:1601–1607

Int J Colorectal Dis
DOI 10.1007/s00384-012-1471-1

ORIGINAL ARTICLE

Laparoscopic resection for sigmoid and rectosigmoid colon cancer performed by trainees: impact on short-term outcomes and selection of suitable patients

Satoshi Ogiso · Takashi Yamaguchi · Meiki Fukuda · Takahide Murakami · Yoshihisa Okuchi · Hiroaki Hata · Yoshiharu Sakai · Iwao Ikai

Accepted: 13 April 2012

Abstract
Purpose This study aimed (1) to evaluate the impact of clinical factors, particularly operation by trainees, on the short-term outcomes of laparoscopic resection for sigmoid and rectosigmoid cancer, and (2) to determine patients suitable for operation by trainees.
Methods From a prospectively maintained single-institution database, we identified 133 patients who underwent laparoscopic resection for sigmoid or rectosigmoid cancer between 2007 and 2010. Gender, age, body mass index (BMI), previous abdominal surgery, tumor location, tumor size, tumor stage, extent of lymph node dissection, and primary surgeon were evaluated using univariate and multivariate analyses to determine the predictive significance of these variables on surgical outcomes including operative time, blood loss, complication, postoperative stay, and retrieved lymph nodes.
Results Multivariate analysis showed that location of the tumor in the rectosigmoid ($p<0.001$), higher BMI ($p<0.001$), operation by trainees ($p<0.001$), male gender ($p=0.002$), and greater tumor depth ($p=0.011$) were independently predictive of longer operative time. Larger tumor size ($p=0.025$) and higher BMI ($p=0.040$) were independently predictive of greater blood loss. Larger tumor size was also related to longer postoperative stay ($p=0.001$) and a greater number of retrieved lymph nodes ($p=0.001$).
Conclusions This study identified operation by trainees as an independent risk factor for longer operative time but with no negative impact on any of the other outcomes. Female patients with a low BMI, sigmoid cancer, shallow tumor depth, and/or small tumor are suitable for operation by trainees.

Keywords Laparoscopic surgery · Colorectal resection · Sigmoid cancer · Laparoscopic training · Patient selection · Trainees

S. Ogiso (✉) · T. Yamaguchi · M. Fukuda · T. Murakami · Y. Okuchi · H. Hata · I. Ikai
Department of Surgery, Kyoto Medical Center,
1-1, Mukaihata-cho, Fukakusa, Fushimi-ku,
Kyoto 612-8555, Japan
e-mail: oggy_so@ybb.ne.jp

Y. Sakai
Department of Surgery, Graduate School of Medicine,
Kyoto University,
Kyoto, Japan

Published online: 28 April 2012

Introduction

Laparoscopic approaches are accepted for colorectal cancer surgery. Several randomized clinical trials of laparoscopic versus open colectomy for colorectal cancer suggest equivalent long-term outcomes with both techniques [1–7]. It is currently inevitable that laparoscopic colorectal procedures are included in general surgery training programs due to their increasing popularity. However, a suitable training program has not yet been developed for the clinical setting [8–11]. Laparoscopic training for surgical residents often begins with basic laparoscopic colorectal procedures such as laparoscopic sigmoid and rectosigmoid resection. During the training period, careful patient selection is essential for maintaining the quality and safety of the procedure performed by novice surgeons. This study aimed (1) to evaluate the impact of clinical factors, particularly operation by trainees, on the short-term outcomes of laparoscopic resection for sigmoid and rectosigmoid cancer, and (2) to select suitable patients for operation by trainees.

Methods

Patients

From a prospectively maintained single-institution database, we identified 156 consecutive patients who underwent laparoscopic resection for sigmoid or rectosigmoid cancer between 2007 and 2010. The indications for laparoscopic surgery were colon cancer tumors without forming a bulky mass, massive lymph node involvement, or invasion of the adjacent organs, as determined by using computed tomography during preoperative examinations. An additional indication was evidence of metastatic disease that could not be curatively resected using open surgery. The surgeries were performed by an experienced, board-certificated, expert surgeon (T.Y.) or by six trainees; of these trainees, four had no prior experience in open or any laparoscopic surgeries and two had experience (2 and 12 years) in open and only basic laparoscopic surgeries. In the first step, trainees with no experience were required to act as endoscopists for about 20 laparoscopic colorectal procedures and encouraged to view a collection of video recordings of laparoscopic colectomy in order to learn the essentials of the standardized techniques used in these operations. They experienced other basic laparoscopic procedures (e.g., cholecystectomy, stoma creation, omental patch repair for gastroduodenal perforation) in the same term. However, 4–6 months later, they proceeded to the next step, in which they started to act as primary surgeons during oncologic laparoscopic colorectal procedures such as laparoscopic sigmoid or ileocecal resection under the supervision of the expert surgeon, thereby acquiring basic laparoscopic skills (prior experience in open colon surgery was not required). In the present study, we excluded 23 patients who underwent Hartmann's procedure or simultaneous resection of other organs. The remaining 133 patients were evaluated. Data on gender, age, body mass index (BMI), previous abdominal surgery, tumor location, tumor size, tumor depth, tumor stage, extent of lymph node dissection, primary surgeon, operative time, blood loss, conversion to open surgery, pathology, 30-day morbidity, mortality, and postoperative stay were collected prospectively. Tumors were staged according to the sixth tumor–node–metastasis classification of the International Union Against Cancer on the basis of the histological findings of the surgical specimens. The protocol was approved by the local ethics committee, and informed consent was obtained from all participating patients.

Surgical procedures

Patients were placed in the Lloyd–Davis position with the head and right side of the bed lowered. First, a 12-mm camera port was inserted below the umbilicus with the open method. After creation of a pneumoperitoneum, four working ports were inserted: 5-mm ports in the right and left upper abdominal quadrants, and 12-mm ports in the right and left lower abdominal quadrants. The mesocolon was mobilized using the mediolateral approach, and no splenic flexure mobilization was needed. In accordance with the oncologic surgical principles recommended by the Japanese Society for Cancer of the Colon and Rectum [12, 13], a D3 lymph node dissection, including the pericolic nodes (local nodes), mesocolic nodes (intermediate nodes), and nodes at the origin of the inferior mesenteric artery (IMA) (main nodes), was performed with division of the IMA at its origin, except in the case of elderly patients or tumors confined to the submucosa, in which case a D2 lymph node dissection, which includes only the local and intermediate nodes, with division of the IMA distal to the first branch to the descending colon (the origin of the superior rectal artery [SRA] or sigmoid artery [SA]) was performed. The distal colon or rectum was transected intracorporeally using an Endopath Endo-Cutter or Echelon 60 (Ethicon Endo-Surgery, Cincinnati, OH, USA). Then, the specimen was extracted through the left lower quadrant port or the camera port, which was extended to about 4 cm. Anastomosis was performed intracorporeally by the double-stapling technique or extracorporeally by functional end-to-end anastomosis.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences™ (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA), and significance was defined as $p<0.05$. Where appropriate, we used Fisher exact tests, chi-square tests, Student's t tests, Welch's tests, or Pearson product–moment correlation coefficients to investigate the relationships between the patients' clinical characteristics and surgical outcomes. A multivariate analysis was performed using a multiple linear regression model with a stepwise method (significance level to enter=0.05; significance level to stay=0.1) or a multiple logistic regression model.

Results

The number of laparoscopic colorectal cancer resections performed by the expert and novice surgeons between 2007 and 2010 are shown in Table 1. Trainees performed a substantial portion of the laparoscopic resections for tumors in the ascending, sigmoid, and rectosigmoid colon and the cecum (209 [82.3 %] of 254 cases), which was significantly higher than that for tumors in other locations (75 [54.3 %] of 138; $p<0.001$).

The patients and tumor characteristics of the 133 patients included in the study are summarized in Table 2. Gender,

Table 1 The number of laparoscopic resections in each tumor location

Tumor location [*n* (%)]	Performed by expert	Performed by trainees	Total
Cecum	4 (13.3)	26 (86.7)	30
Ascending colon	15 (22.1)	53 (77.9)	68
Transverse colon	11 (27.5)	29 (72.5)	40
Descending colon	11 (42.3)	15 (57.7)	26
Sigmoid/rectosigmoid colon	26 (16.7)	130 (83.3)	156
Upper rectum	18 (48.6)	19 (51.4)	37
Lower rectum/anal canal	23 (65.7)	12 (34.3)	35
Total	108 (27.6)	284 (82.4)	392

mean age, mean BMI, and previous history of abdominal surgery were similar between the groups. Tumor size was significantly larger (5.1 cm versus 3.1 cm; $p<0.001$), and the incidence of T3/T4 tumors was significantly higher (81.8 % versus 49.5 %; $p=0.011$) in the patients operated on by the expert surgeon than in the patients operated on by the trainees. On the contrary, nodal involvement and tumor stage were not different. The incidence of D3 lymph node dissection was significantly higher for the expert surgeon than for the trainees (86.4 % versus 63.1 %; $p=0.046$).

Operative outcomes are summarized in Table 3. Operative time was significantly longer in the patients operated on by the trainees than in those operated on by the expert surgeon (244.2 min versus 214.7 min; $p=0.007$), but blood loss was not significantly different. The incidence of patients with less than 12 retrieved lymph nodes was larger in surgeries performed by the trainees than in surgeries performed by the expert (22.5 % versus 4.5 %), but the difference was not statistically significant ($p=0.074$). The overall morbidity rate was 9 (6.8 %) in 133 patients with a

Table 2 Patient clinical characteristics

	Performed by expert ($n=22$)	Performed by trainees ($n=111$)	*P* value
Gender (male/female) (range)	12 (54.5)/10 (45.5)	66 (59.5)/45 (40.5)	0.669
Age (years) (range)	68.5 (44–85)	67.7 (31–93)	0.766
Body mass index (kg/m^2) (range)	23.1 (18.4–28.0)	22.5 (14.8–28.8)	0.465
Previous abdominal surgery [n (%)]	5 (22.7)	37 (33.3)	0.467
Tumor location (sigmoid/rectosigmoid) [n (%)]	18 (81.8)/4 (18.2)	79 (71.2)/32 (28.8)	0.445
Tumor size (cm) (range)	5.1 (0–9)	3.1 (0–7)	<0.001
Pathologic T stage [*n* (%)]			(Tis/T1/T2 vs. T3/T4)
Tis	1 (4.5)	9 (8.1)	0.011
T1	3 (13.6)	32 (28.8)	
T2	0 (0)	15 (13.5)	
T3	7 (31.8)	16 (14.4)	
T4	11 (50)	39 (35.1)	
Pathologic N stage [*n* (%)]			(N0/N1 vs. N2)
N0	13 (59.1)	69 (62.2)	0.745
N1	6 (27.3)	27 (24.3)	
N2	3 (13.6)	15 (13.5)	
Tumor stage [*n* (%)]			(0/I/II vs. III/IV)
0	1 (4.5)	8 (7.2)	0.837
I	3 (13.6)	40 (36.0)	
II	9 (40.9)	18 (16.2)	
III	8 (36.4)	35 (31.5)	
IV	1 (4.5)	10 (9.0)	
Lymph node dissection (D3/D2) [*n* (%)]	19 (86.4)/3 (13.6)	70 (63.1)/41 (36.9)	0.046
Anastomosis (DST/FEEA) [*n* (%)]	19 (86.4)/3 (13.6)	96 (86.5)/15 (13.5)	1

All continuous variables are described as mean with range

DST double stapling technique, *FEEA* functional end-to-end anastomosis

Table 3 Surgical outcomes

	Performed by expert (n=22)	Performed by trainees (n=111)	P value
Operative time (min) (range)	214.7 (149–336)	244.2 (150–417)	0.007
Blood loss (ml) (range)	31.6 (0–340)	16.8 (0–590)	0.140
Transfusion	0	0	NA
Intraoperative complication [n (%)]	0	1 (0.9)	1
Tear in the rectal stump	0	1 (0.9)	1
Conversion [n (%)]	0	0	NA
Positive circumferential resection margin	0	0	NA
Number of retrieved lymph nodes <12 [n (%)]	1 (4.5)	25 (22.5)	0.074
Postoperative complication [n (%)]	2 (9.1)	7 (6.3)	1
Anastomotic leakage	0	0	
Wound infection	0	6 (5.4)	0.589
Enteritis	0	1 (0.9)	1
Prostatitis	1 (4.5)	0	0.165
Subcutaneous fluid collection	1 (4.5)	0	0.165
Postoperative stay (days) (range)	12.4 (7–34)	12.6 (3–50)	0.875

All continuous variables are described as mean with range

similar incidence for the expert and novice surgeons. There were no cases of conversion to open surgery, positive circumferential margin, anastomotic leakage, or 30-day mortality.

Correlations between operative outcomes and clinical factors, as determined by univariate analysis, are summarized in Table 4. Gender (p=0.004), BMI (p<0.001), tumor location (p<0.001), and experience of the primary surgeon (p=0.004) were significantly associated with operative time; BMI (p=0.030), tumor location (p=0.042), and tumor size (p=0.019) were significantly associated with blood loss; age (p=0.040), tumor size (p=0.001), tumor depth (p=0.001), and tumor stage (p=0.010) were significantly associated with postoperative hospital stay; and tumor size (p=0.001), tumor depth (p=0.001), tumor stage (p=0.013), and extent of lymph node dissection (p=0.012) were significantly associated with the number of retrieved lymph nodes. Multivariate analysis showed that male gender (p=0.002), higher BMI (p<0.001), location of the tumor in the rectosigmoid colon (p<0.001), greater tumor depth (p=0.011), and operation by trainees (p<0.001) were independently predictive of longer operative time; higher BMI (p=0.040) and larger tumor size (p=0.025) were independently predictive of greater blood loss; larger tumor size was also related to longer postoperative stay (p=0.001) and a greater number of retrieved lymph nodes (p=0.001) (Tables 5 and 6).

Discussion

Laparoscopic resection for colorectal cancer is one of the most advanced laparoscopic procedures. Yet, lymph node dissection along the surgical trunk (right-sided colon cancer)

Table 4 Correlations between surgical outcomes and clinical factors

Independent variable	P value for operative outcomes			
	Operative time	Blood loss	Postoperative stay	Retrieved LN (<12 vs. ≥12)
Gender	0.004	0.311	0.226	0.096
Age	0.472	0.428	0.040	0.350
Body mass index	<0.001	0.030	0.411	0.163
Previous abdominal surgery	0.495	0.176	0.451	0.569
Tumor location (sigmoid vs. rectosigmoid)	<0.001	0.042	0.141	0.985
Tumor size	0.489	0.019	0.001	0.001
Tumor depth (Tis/T1/T2 vs. T3/T4)	0.322	0.081	0.001	0.001
Tumor stage (0/I/II vs. III/IV)	0.489	0.155	0.010	0.013
Experience of the primary surgeon (expert vs. trainee)	0.004	0.264	0.437	0.052
Lymph node dissection (D3/D2)	0.461	0.293	0.279	0.012

LN lymph nodes

Table 5 Stepwise linear regression analysis

Dependent variable	Predictive factor	*P* value	*β*	*R*	Model utility test
Operative time	Intercept	0.001		0.613	<0.001
	Tumor location (rectosigmoid)	<0.001	0.348		
	Body mass index	<0.001	0.345		
	Experience of the primary surgeon (trainee)	<0.001	0.261		
	Gender (male)	0.002	0.222		
	Tumor depth	0.011	0.189		
Blood loss	Intercept	0.056		0.252	0.014
	Tumor size	0.025	0.193		
	Body mass index	0.040	0.176		
Postoperative hospital stay	Intercept	<0.001		0.276	0.001
	Tumor size	0.001	0.276		

and around the root of middle colic vessels (transverse colon cancer) is still technically challenging [14]. Splenic flexure mobilization—required for tumors located in the left transverse or descending colon—is also reported to be difficult and time-consuming [15]. Pelvic procedures during rectal cancer resection, including rectal mobilization, transection, and anastomosis, are quite demanding in narrow and deep surgical fields. Moreover, they are associated with some disadvantages such as long operative time [4, 16, 17] and increased rate of positive surgical margins [6]. Meanwhile, laparoscopic ileocecal resection, sigmoid resection, and rectosigmoid resection are considered comparatively basic among all laparoscopic colorectal procedures. Therefore, laparoscopic training for surgical residents often begins with these procedures. In our institution, 83.3 % of all laparoscopic colorectal resection cases for sigmoid colon and rectosigmoid cancer (130/156) are performed by trainees at their initial laparoscopic training. These operations provide the trainees with sufficient experiences in advanced laparoscopic procedures and enable the development of their surgical techniques. Herein, we evaluated the impact of the operation by trainees on the short-term outcomes of laparoscopic resection for sigmoid and rectosigmoid cancer and determined the characteristics of patients suitable for operation by novice surgeons. We excluded cases of Hartmann's procedure and simultaneous resection of other organs such as the liver. These cases tend to correlate with longer operative time, greater blood loss, and/or longer hospital stay. Therefore, these cases should be analyzed independently of sigmoid or rectosigmoid resection and anastomosis in order to clearly distinguish the clinical factors that influence the operative outcomes of the latter.

In this study, multivariate analyses showed that operation by trainees was significantly associated with longer operative time. However, it had no negative impact on any of the other short-term outcomes evaluated in the current study. Furthermore, male gender, higher BMI, location of the tumor in the rectosigmoid, and greater tumor depth were significantly associated with longer operative time. The present findings are valuable in supporting the early introduction of laparoscopic resection for sigmoid and rectosigmoid cancer during the initial training period of novice surgeons. In addition, our findings indicate that female patients with a low BMI, sigmoid cancer, and/or shallow tumor depth are suitable for operation by trainees. Additionally, multivariate analyses showed that higher BMI and larger tumor size were independently predictive of greater blood loss; larger tumor size was also related to longer postoperative stay, indicating that larger tumors should best be managed by expert surgeons.

In our surgical training program, we pay special attention to safety and quality. These parameters were reflected in the careful selection of patients for operation by trainees. As such, trainees operated on significantly smaller tumors with significantly higher incidence of shallow depth to avoid oncologic impairments during the resection of advanced cancers. We usually consider that small tumors with shallow depth do not require wide lymph node dissection (D3

Table 6 Stepwise logistic regression analysis

Dependent variable	Predictive factor	*P* value	Odds ratio	95 % CI
Retrieved lymph nodes (<12 vs. 12)	Tumor size	0.001	1.556	1.193–2.029
Postoperative complication	No parameter			

dissection) as compared to advanced tumors [12, 18]. In the case of the former, it is decided preoperatively that D2 lymph node dissection, including only local and intermediate nodes, will be performed with dividing the SRA or the SA at its origin. This may explain the significant differences in the extent of lymph node dissection between expert and novice surgeons. In the current study, we performed multivariate analyses to eliminate the influence of these differences in patients' characteristics between the expert and the trainees; thus, we assume that the impact of primary surgeons was evaluated successfully without bias.

In this study, we have focused on colorectal cancer regional nodal evaluation—reported as a staging measure, prognostic variable, and quality indicator. A minimum of 12 nodes is endorsed as a consensus standard for hospital-based performance by the American Society for Clinical Oncology, the National Comprehensive Cancer Network, and the American Joint Committee on Cancer [19]. Twelve or more lymph nodes (mean, 19.0) were retrieved from 86 of 111 patients (77.5 %) operated on by trainees, and D3 lymph node dissection was performed in 70 patients (63.1 %); deep tumors (T3/T4) were observed in 55 patients (49.5 %), and positive nodes in 42 patients (37.8 %). Lymph node removal achieves two objectives in oncologic procedures: therapeutic effect and adequate nodal staging. With regard to the therapeutic effect, we assume that the number of lymph nodes retrieved by trainees was enough considering the not so high incidence of advanced tumors. Furthermore, the expert surgeon ensured that the trainees removed all lymph nodes together with mesocolon included within the preoperatively planned area during the operation. Therefore, we assume that trainees satisfied the oncologic principles suggested by the Japanese Society for Cancer of the Colon and Rectum [13], which do not recommend a minimum number of retrieved lymph nodes but rather a wider area of dissection compared to the Western guidelines [22] for assuring complete lymph node removal irrespective of their number, although these principles have been criticized as excessive [21]. Aside from the therapeutic effect of lymph node removal, it is commonly assumed that stage migration is reflected in the positive association between survival and the number of lymph nodes retrieved. Recently, analysis of the pathological staging of 131,953 patients from the Surveillance, Epidemiology and End Results database by a beta-binomial model suggested that the minimum number of nodes required for adequate nodal staging depends on the T stage: to achieve a probability of correct staging of 90 %, a single node needs to be examined for T1, four nodes for T2, 13 nodes for T3, and 21 nodes for T4 disease [23]. Based on this staged classification, the quality of lymph node removal by trainees would be acceptable for nodal staging of patients with the highest incidence of shallow tumors compared to those performed by the expert surgeon. The incidence of patients from whom less than 12 lymph nodes were retrieved was higher in surgeries performed by trainees, yet the difference was not statistically significant. An important factor, which could account for the difference in the number of lymph nodes retrieved, is that trainees performed a significantly higher number of D2 lymph node dissection than the expert. In fact, D2 lymph nodes dissection has been reported to yield fewer lymph nodes for histopathological examination than D3 lymph node dissection [20, 21]. The higher incidence of D2 lymph node dissection was attributable to the significantly higher incidence of shallow tumor depth in cases of operation by trainees because of the aforementioned reason; therefore, the higher incidence of patients with less than 12 retrieved lymph nodes could be associated with our policy of careful patient selection for operation by trainees. Multivariate analysis did not identify operation performed by trainees, but tumor size as an independent predictor of the number of retrieved lymph nodes.

Tumor size was also an independent predictor of postoperative hospital stay and blood loss. The relation between longer postoperative stay and larger tumor size is not very clear. Some patients with large tumors had poor general condition and required postoperative rehabilitation or transfer to other hospitals, which may explain the longer postoperative stay. Greater blood loss in patients with larger tumors may result from the greater difficulty in intra-abdominal laparoscopic handling along with a more limited surgical field. Significant association between greater tumor depth and longer operative time may be explained similarly: deeply invasive tumors require careful intra-abdominal manipulation to prevent intra-operative oncologic impairment such as tumor cell spillage.

Significant association between rectosigmoid cancer (as opposed to sigmoid cancer) and longer operative time, as well as between male gender and longer operative time suggests the influence of pelvic diameters. Previous reports showed that resection of low rectal tumors in males is usually performed through a narrower pelvic space. This anatomical constraint limits the working space and directly increases the difficulty in safe and quick access while minimizing visibility and retraction, thereby resulting in a significantly longer operative time [24–26]. Similar relationships may influence the outcomes of sigmoid and rectosigmoid colon cancer. Another reason for longer operative time in male patients may be the presence of greater abdominal visceral fat deposits in men than in women [27]. Higher BMI was also significantly

associated with longer operative time and greater blood loss, yet the BMI of our patients were lower than that of Western populations. The latter observation is in agreement with our previous report on laparoscopic anterior resection for rectal cancer [24].

In this study, the overall morbidity rate was 6.8 % (9/133). Six of the patients had wound infections. Adverse events such as anastomotic leakages, conversions, positive circumferential margin, and 30-day mortality were not observed or occurred at a very low rate. This indicates that the procedure can be performed by the trainees in a safe way and in the absence of morbidity or violation of oncologic surgical principles. We believe that the assistance of the expert surgeon was essential to maintain the quality and safety of operations by trainees, providing good laparoscopic view with adequate retraction in the surgical field.

Nevertheless, our study has certain limitations. Operative outcomes, including complications, anastomotic leakage, conversion, mortality, and positive circumferential margin, should be examined to generalize the present findings. Moreover, this study only analyzed the short-term outcomes, and long-term follow-up is required to ensure that oncological procedures were not compromised.

In conclusion, this study identified operation by trainees as an independent risk factor for longer operative time but with no negative impact on any of the other short-term outcomes. Female patients with a low BMI, sigmoid cancer, shallow tumor depth, and/or small tumor are suitable for operation by trainees.

Competing interests The authors declare that they have no competing interests.

References

1. Milsom JW, Böhm B, Hammerhofer KA, Fazio V, Steiger E, Elson P (1998) A prospective, randomized trial comparing laparoscopic versus conventional techniques in colorectal cancer surgery: a preliminary report. J Am Coll Surg 187:46–55
2. Lacy AM, García-Valdecasas JC, Delgado S, Castells A, Taurá P, Piqué JM, Visa J (2002) Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. Lancet 359:2224–2229
3. Clinical Outcomes of Surgical Therapy Study Group (2004) A comparison of laparoscopically assisted and open colectomy for colon cancer. N Engl J Med 350:2050–2059
4. Leung KL, Kwok SP, Lam SC, Lee JF, Yiu RY, Ng SS, Lai PB, Lau WY (2004) Laparoscopic resection of rectosigmoid carcinoma: prospective randomised trial. Lancet 363:1187–1192
5. COLOR Study Group (2000) COLOR: a randomized clinical trial comparing laparoscopic and open resection for colon cancer. Dig Surg 17:617–622
6. Guillou PJ, Quirke P, Thorpe H, Walker J, Jayne DG, Smith AM, Heath RM, Brown JM, MRC CLASICC trial group (2005) Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. Lancet 365:1718–1726
7. Kitano S, Inomata M, Sato A, Yoshimura K, Moriya Y, Japan Clinical Oncology Group Study (2005) Randomized controlled trial to evaluate laparoscopic surgery for colorectal cancer: Japan Clinical Oncology Group Study JCOG 0404. Jpn J Clin Oncol 35:475–477
8. Ogiso S, Yamaguchi T, Hata H, Kuroyanagi H, Sakai Y (2010) Introduction of laparoscopic low anterior resection for rectal cancer early during residency: a single institutional study on short-term outcomes. Surg Endosc 24:2822–2829
9. Verheijen PM, vd Ven AW, Davids PH, Clark DA, Pronk A (2010) Teaching colorectal surgery in the laparoscopic era: is it safe? J Surg Educ 67:217–221
10. Maeda T, Tan KY, Konishi F, Tsujinaka S, Mizokami K, Sasaki J, Kawamura YJ (2010) Accelerated learning curve for colorectal resection, open versus laparoscopic approach, can be attained with expert supervision. Surg Endosc 24:2850–2854
11. Bosker R, Hoogenboom F, Groen H, Hoff C, Ploeg R, Pierie JP (2010) Elective laparoscopic recto-sigmoid resection for diverticular disease is suitable as a training operation. Int J Colorectal Dis 25:471–476
12. Japanese Society for Cancer of the Colon and Rectum (2012) Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer. Int J Clin Oncol 17:1–29
13. Okuno K (2007) Surgical treatment for digestive cancer. Current issues—colon cancer. Dig Surg 24:108–114
14. Hasegawa S, Kawamura J, Nagayama S, Nomura A, Kondo K, Sakai Y (2007) Medially approached radical lymph node dissection along the surgical trunk for advanced right-sided colon cancers. Surg Endosc 21:1657
15. Akiyoshi T, Kuroyanagi H, Oya M, Ueno M, Fujimoto Y, Konishi T, Yamaguchi T (2010) Factors affecting difficulty of laparoscopic surgery for left-sided colon cancer. Surg Endosc 24:2749–2754
16. Law WL, Lee YM, Choi HK, Seto CL, Ho JW (2006) Laparoscopic and open anterior resection for upper and mid rectal cancer: an evaluation of outcomes. Dis Colon Rectum 49:1108–1115
17. Braga M, Frasson M, Vignali A, Zuliani W, Civelli V, Di Carlo V (2005) Laparoscopic vs. open colectomy in cancer patients: long-term complications, quality of life, and survival. Dis Colon Rectum 48:2217–2223
18. Hida J, Okuno K, Yasutomi M, Yoshifuji T, Uchida T, Tokoro T, Shiozaki H (2005) Optimal ligation level of the primary feeding artery and bowel resection margin in colon cancer surgery: the influence of the site of the primary feeding artery. Dis Colon Rectum 48:2232–2237
19. Wong SL, Ji H, Hollenbeck BK, Morris AM, Baser O, Birkmeyer JD (2007) Hospital lymph node examination rates and survival after resection for colon cancer. JAMA 298:2149–2154
20. Liang JT, Huang KC, Lai HS, Lee PH, Sun CT (2007) Oncologic results of laparoscopic D3 lymphadenectomy for male sigmoid and upper rectal cancer with clinically positive lymph nodes. Ann Surg Oncol 14:1980–1990
21. Hashiguchi Y, Hase K, Ueno H, Mochizuki H, Shinto E, Yamamoto J (2011) Optimal margins and lymphadenectomy in colonic cancer surgery. Br J Surg 98:1171–1178
22. Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, Guillem J, Miedema B, Ota D, Sargent D, National Cancer Institute Expert Panel (2001) Guidelines 2000 for colon and rectal cancer surgery. J Natl Cancer Inst 93:583–596

23. Gönen M, Schrag D, Weiser MR (2009) Nodal staging score: a tool to assess adequate staging of node-negative colon cancer. J Clin Oncol 27:6166–6171
24. Ogiso S, Yamaguchi T, Hata H, Fukuda M, Ikai I, Yamato T, Sakai Y (2011) Evaluation of factors affecting the difficulty of laparoscopic anterior resection for rectal cancer: "narrow pelvis" is not a contraindication. Surg Endosc 25:1907–1912
25. Targarona EM, Balague C, Pernas JC, Martinez C, Berindoague R, Gich I, Trias M (2008) Can we predict immediate outcome after laparoscopic rectal surgery? Multivariate analysis of clinical, anatomic, and pathologic features after 3-dimensional reconstruction of the pelvic anatomy. Ann Surg 247:642–649
26. Akiyoshi T, Kuroyanagi H, Oya M, Konishi T, Fukuda M, Fujimoto Y, Ueno M, Miyata S, Yamaguchi T (2009) Factors affecting the difficulty of laparoscopic total mesorectal excision with double stapling technique anastomosis for low rectal cancer. Surg 146:483–489
27. Tsujinaka S, Konishi F, Kawamura YJ, Saito M, Tajima N, Tanaka O, Lefor AT (2008) Visceral obesity predicts surgical outcomes after laparoscopic colectomy for sigmoid colon cancer. Dis Colon Rectum 51:1757–1765

Ephrin-A1 mRNA is associated with poor prognosis of colorectal cancer

HIROFUMI YAMAMOTO[1], MITSUYOSHI TEI[1], MAMORU UEMURA[1], ICHIRO TAKEMASA[1], YOSHIO UEMURA[2], KOHEI MURATA[3], MUTSUMI FUKUNAGA[4], MASAYUKI OHUE[5], TADASHI OHNISHI[6], KIMIMASA IKEDA[7], TAKESHI KATO[8], SHU OKAMURA[3], MASAKAZU IKENAGA[9], NAOTSUGU HARAGUCHI[1], JUNICHI NISHIMURA[1], TSUNEKAZU MIZUSHIMA[1], KOSHI MIMORI[10], YUICHIRO DOKI[1] and MASAKI MORI[1]

[1]Department of Surgery, Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka; [2]Department of Surgery, Kinki Central Hospital, Hyogo; [3]Department of Surgery, Suita Municipal Hospital; [4]Department of Surgery, Sakai City Hospital; [5]Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases; [6]Department of Surgery, NTT West Osaka Hospital; [7]Department of Surgery, Minoh City Hospital, Osaka; [8]Department of Surgery, Kansai Rosai Hospital, Hyogo; [9]Department of Surgery, National Hospital Organization Osaka National Hospital, Osaka; [10]Department of Molecular and Cellular Biology, Division of Molecular and Surgical Oncology, Kyushu University, Medical Institute of Bioregulation, Ohita, Japan

Received September 13, 2012; Accepted November 2, 2012

DOI: 10.3892/ijo.2012.1750

Abstract. We previously studied hypoxic tumor cells from hepatic metastases of colorectal cancer (CRC) and determined several potential prognostic factors, including expression of ephrin-A1 (EFNA1), which was highly induced by hypoxia. Here, we further evaluated the prognostic impact of EFNA1 expression. Samples from a total of 366 CRC patients from 11 institutes were analyzed by either microarray (n=220) or quantitative reverse-transcriptase polymerase chain reaction (n=146). EFNA1 was an independent prognostic factor for CRC ($p<0.05$). *In vitro* assays revealed that loss of EFNA1 following siRNA treatment was associated with reduced proliferative activity and decreased invasion and migration of CRC cell lines. EFNA1 expression is a useful marker for predicting high risk of relapse and cancer-related death in patients who have undergone curative resection for CRC.

Introduction

Colorectal cancer (CRC) is one of the most common human malignancies worldwide. Despite recent advances in treatment with chemotherapy and with biologic agents such as bevacizumab or cetuximab (1), CRC is still a major cause of cancer death (2). Furthermore, the indications for these therapies have been limited due to side-effects and the small number of known target genes (3). Thus, there is a crucial need to explore novel cancer-related genes that may serve as diagnostic markers and molecular targets in CRC therapy.

Hypoxia is a main feature of cancer, with intratumoral hypoxia affecting every major aspect of cancer biology, including cell invasion, metastasis, and cell death (4). In tumor cells under hypoxia, hypoxia inducible factor 1 (HIF-1) plays a critical role in promoting the expression of hypoxia-response genes that are associated with an aggressive tumor phenotype (5-7). These hypoxia-related genes include several angiogenic factors, such as vascular endothelial growth factor (VEGF), that play important roles in cancer biology. The anti-VEGF antibody bevacizumab is used clinically for treatment of several human cancers (8), supporting the use of hypoxia-induced genes as clinically relevant therapeutic targets.

Ephrin-A1 (EFNA1) is known as an angiogenesis factor, and is induced through a HIF-dependent pathway (9,10). EFNA1 was originally isolated as a secreted protein in conditioned media from cultures of human umbilical vein endothelial cells treated with tumor necrosis factor-α (11), and the gene was found to be induced by tumor necrosis factor-α in these cells (12). EFNA1 expression has also been observed in tumor endothelial cells and tumor cells, and shown to induce endothelial cell migration (13), capillary assembly *in vitro*, and corneal angiogenesis *in vivo* (14). EFNA1 and its receptor, Eph receptor 2 (EphA2), are associated with carcinogenesis, angiogenesis (13,15-17), and tumorigenesis in various cancers, including urinary bladder carcinoma (18), breast cancer (19,20), gastric cancer (21), glioma (22), and malignant mesothelioma (23).

Previously, we detected several potential prognostic factors and therapeutic targets in hypoxic tumor cells from hepatic metastases of CRC *in vivo* (24). In these experiments, Ephrin-A1

Correspondence to: Dr Hirofumi Yamamoto, Department of Surgery, Gastroenterological Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita City, Osaka 565-0871, Japan
E-mail: hyamamoto@gesurg.med.osaka-u.ac.jp

Abbreviations: RT-PCR, reverse transcription-polymerase chain reaction

Key words: colorectal cancer, ephrin-A1, hypoxia, prognosis

gene (*EFNA1*) expression was highly induced in hypoxic regions of liver metastases (fold-change = 1.58, p=0.005). Thus, we hypothesized that *EFNA1* expression may be a novel prognostic factor in CRC patients. In the present study, we examined the correlation between *EFNA1* expression and prognosis in CRC patients, and we analyzed the biological significance of *EFNA1* expression in human CRC.

Materials and methods

Cell culture. The colon carcinoma cell lines DLD1, Lovo, HCT116, HT29, SW480, and CaCo2 were obtained from the American Type Culture Collection. KM12sm was a kind gift from Professor T. Minamoto (Cancer Research Institute, Kanazawa University, Japan). All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin, at 37°C in a humidified incubator with 5% CO_2. Human umbilical vein endothelial cells (HUVECs) were grown on MCDB131 culture medium (Chlorella Inc., Tokyo, Japan) supplemented with 10% fetal bovine serum, antibiotics, and 10 ng/ml fibroblast growth factor. For culture under hypoxic conditions, cells were grown for up to 72 h at 37°C in a continuously monitored atmosphere of 1% O_2, 5% CO_2, and 94% N_2 using a multigas incubator (Model 9200, Wakenyaku Co., Kyoto, Japan). Control cells were cultured under normoxic conditions (21% O_2).

Patients and clinical sample collection. For microarray analysis, we prospectively collected 220 primary CRC samples from consecutive patients who had curative operations between 2003 and 2006 at Osaka University Hospital and its nine associated hospitals. For quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), tumor samples were consecutively collected from 146 CRC patients who had curative surgery from 1993 to 2002 at the Department of Surgery, Medical Institute of Bioregulation, Kyushu University. None of the included patients had preoperative chemotherapy or irradiation. After surgery, patients with stage III/IV tumors were treated with 5-fluorouracil (5-FU)-based chemotherapy. The Human Ethics Review Committee of Osaka University and Kyushu University approved the use of the resected samples.

Immediately after surgical resection, a piece of each primary colorectal cancer tissue sample was collected from the fresh specimens, and stored in RNA Stabilization Reagent (RNA Later; Ambion, Inc., Austin, TX, USA) at -80°C until RNA extraction.

RNA extraction and real-time quantitative RT-PCR analysis. Total RNA was extracted with a single-step method, using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD, USA) at Osaka University and Isogen (Nippon Gene, Tokyo, Japan) at Kyushu University. Complementary DNA (cDNA) was generated using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI, USA). Real-time monitoring of PCR reactions was performed using the LightCycler™ system (Roche Applied Science, Indianapolis, IN, USA) for quantification of mRNA expression, as described previously (25). The housekeeping gene porphobilinogen deaminase (PBGD) (26,27) was used as an internal standard. The sequences of PBGD primers were as follows: sense primer, 5'-AACGGCGGAAGAAAACAG-3' and antisense primer, 5'-TCCAATCTTAGAGAGTGCA-3'. The *EFNA1* primer sets were designed to flank one intron, and were tested to ensure amplification of only cDNAs to avoid amplification of possibly contaminating genomic DNA. The sequences of these PCR primers were as follows: *EFNA1* sense primer, 5'-TGCCGTCCGGACGAGACAGGC-3' and *EFNA1* antisense primer, 5'-CTGGAGCCAGGACCGGGACTG-3'.

Immunohistochemistry. Immunohistochemical analysis was performed as described previously (28). Frozen sections (4 μm) were fixed in 4% paraformaldehyde for 5 min. The slides were incubated with anti-EFNA1 rabbit polyclonal antibody (1:200; Abcam, Cambridge, UK) overnight at 4°C. Negative control sections were incubated with normal rabbit serum instead of the primary antibody. All slides were evaluated in a blinded manner by a pathologist.

Western blot analysis. Western blot analysis was performed as we previously described (29). To detect EFNA1 protein expression, extracted protein was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by western blot analysis using the EFNA1-specific antibody (1:500; Abcam).

Transfection reagents. EFNA1 siRNA was purchased from Invitrogen (Carlsbad, CA, USA) and control negative siRNA was purchased from Qiagen Inc. (Valencia, CA, USA). siRNA sequences for *EFNA1* and for the irrelevant control were as follows: *EFNA1* siRNA #1, 5'-CCAUACAUGUGCAGCUGA AUGACUA-3'; *EFNA1* siRNA #2, 5'-CAGAGGUGCGGG UUCUACAUAGCAU-3'; and control negative siRNA, 5'-AAT TCTCCGAACGTGTCACGT-3'. CRC cell lines were transfected with siRNA using lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol.

Cell proliferation assay. Cell growth was measured by adding WST-1 reagent (Roche) and incubating at 37°C for 2 h. Absorbance was measured at 450 nm with background subtraction at 630 nm. Results were given as the mean ± SD of five separate experiments.

In vitro migration/invasion assay. The invasion assay was performed using transwell cell culture chambers (BD Biosciences, Bedford, MA, USA) as described previously (24). Briefly, colorectal cancer cells at a concentration of 20,000 cells/ml were placed in the top chamber of a two-chamber assay system and incubated for 24 h with and without 10% FBS placed in the lower chamber. After 48 h, cells that had invaded the undersurface of the membrane were fixed with 100% methanol and stained with 1% toluidine blue. Four microscopic fields were randomly selected for cell counting.

Cell migration assays were performed using BD Falcon cell culture inserts containing polyethylene terephthalate membranes (8-μm pore size) from BD Biosciences. Similar to the invasion assays, the cells were placed in the top of the chamber, while 10% FBS was added to the lower chamber. Results were given as the mean ± SD of four separate experiments.

Tumor cell and endothelial cell co-culture migration assays. The co-culture migration assay was performed using BD Falcon cell culture inserts containing collagen type IV (3-μm pore size) according to the manufacturer's instructions (BD Biosciences). Briefly, HUVECs at a concentration of 20,000 cells/ml were placed in the top chamber of a two-chamber assay system and incubated for 24 h, while HCT116 colorectal cancer cell lines transfected with control negative siRNA or *EFNA1* siRNA were placed in the lower chamber. After the incubation period, the cells on the upper side were removed using a cotton swab; then the coated filters were removed. The undersurface of the membrane was fixed with 100% methanol and stained with 1% toluidine blue. HUVEC migration was quantified microscopically by counting the cells that had migrated into the filters. Results were given as the mean ± SD of four separate experiments.

Statistical analysis. For clinicopathological analyses, study samples were divided into high- and low-expression groups based on the median EFNA1 mRNA expression levels in tumor tissue. All statistical analyses were carried out using the StatView J-5.0 program (Abacus Concepts, Inc., Berkeley, CA). The post-operative period was measured from the date of surgery to the date of the last follow-up or death. Differences were estimated using Fisher's exact probability test. Survival curves were calculated by the Kaplan-Meier method, and compared statistically using the log-rank test. To estimate relative risk (RR) and 95% confidence interval (95% CI), univariate and multivariate analysis were performed using the Cox proportional hazards regression model. Data are reported as mean ± SD. Mean values were compared using the Mann-Whitney test. A probability value of <0.05 was deemed to be statistically significant.

Results

Patient profiles. The patients selected for microarray analysis included 131 males (59.5%) and 89 females (40.5%). The primary tumor was in the colon in 141 patients and in the rectum in 79 patients; 98.2% of tumors were well or moderately-differentiated adenocarcinomas, and 4 patients (0.8%) had poorly-differentiated adenocarcinomas. In regards to TNM staging, 109 patients (49.5%) were stage I or II and 111 patients (50.5%) were stage III or IV. Detailed information is shown in Table I.

Table I. Clinicopathological factors of CRC patients analyzed by microarray.

	High EFNA1 expression	Low EFNA1 expression	p-value
Age at surgery (years)			
>67	60	62	0.787
≤67	50	48	
Gender			
Male	66	65	0.891
Female	44	45	
Tumor site			
Colon	66	75	0.212
Rectum	44	35	
Depth of tumor invasion			
T1, 2	12	18	0.246
T3, 4	98	92	
TNM stage			
I or II	52	57	0.498
III or IV	58	53	
Lymph node metastasis			
Present	57	52	0.589
Absent	53	58	
Venous invasion			
Present	70	61	0.272
Absent	40	49	
Histological type[a]			
Differentiated	106	110	
Undifferentiated	4	0	

[a]Differentiated type included well and moderately-differentiated adenocarcinoma. Undifferentiated type included poorly-differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma.

The CRC patients analyzed by qRT-PCR included 83 males (56.8%) and 63 females (43.2%). The primary tumor site was the colon in 92 patients (63.0%), and 8.9% of patients had poorly-differentiated adenocarcinoma or mucinous adenocarcinoma. Detailed information is shown in Table II. As shown in Tables I and II, high expression and low expression were divided based on the median value in each assay. No significant differences were observed in the clinicopathological factors between high and low *EFNA1* expression groups in the two data sets (Tables I and II).

Survival analysis stratified by EFNA1 mRNA expression. Kaplan-Meier survival curves demonstrated that patients with high *EFNA1* expression showed significantly shorter survival than those with low *EFNA1* expression, in terms of both disease-free survival (DFS) in microarray data (Fig. 1A) and cancer-related survival (CRS) in qRT-PCR data (Fig. 1B). Next, we performed univariate analysis of clinicopathological factors and found that lymph node metastasis, venous invasion, tumor differentiation, depth of tumor invasion, and *EFNA1* expression were significantly associated with DFS based on microarray data and CRS based on qRT-PCR data (Tables III and IV). Multivariate Cox regression analysis revealed that *EFNA1* expression and lymph node metastasis remained independent prognostic factors (Tables III and IV).

EFNA1 expression in CRC cell lines and colorectal tumor tissue. Immunohistochemistry of the CRC tissue samples showed that tumor cells expressed EFNA1 mainly at the plasma membrane (Fig. 2), while normal colonic epithelium scarcely expressed

Table II. Clinicopathological factors of CRC patients analyzed by qRT-PCR.

	High EFNA1 expression	Low EFNA1 expression	p-value
Age at surgery (years)			
>66	43	46	0.367
≤66	30	27	
Gender			
Male	43	40	0.371
Female	30	33	
Tumor site			
Colon	42	50	0.126
Rectum	31	23	
Depth of tumor invasion			
T1	19	25	0.185
T2, 3, 4	54	48	
TNM stage			
I or II	33	43	0.072
III or IV	40	30	
Lymph node metastasis			
Present	33	28	0.502
Absent	40	45	
Venous invasion			
Present	13	15	0.838
Absent	60	58	
Histological type[a]			
Differentiated	69	66	0.275
Undifferentiated	4	7	

[a]Differentiated type included well and moderately-differentiated adenocarcinoma. Undifferentiated type included poorly-differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma.

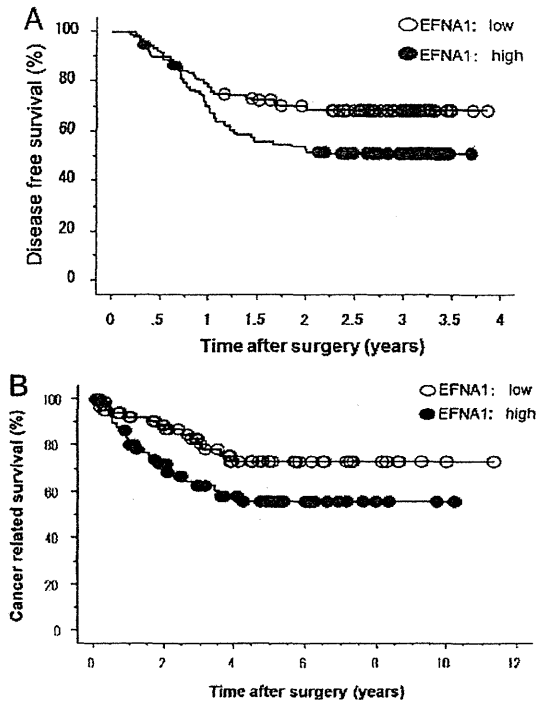

Figure 1. Kaplan-Meier survival curves of colorectal cancer patients according to *EFNA1* mRNA expression. Patients in the high *EFNA1* mRNA expression group had poorer survival than those in the low expression group, as shown by microarray analysis data (p=0.011) (A) and RT-PCR analysis (p=0.029) (B).

Table III. Univariate and multivariate analyses of the relationships between clinicopathological factors and disease-free survival in microarray data.

Factor	p-value	OR	95% CI	p-value
Lymph node metastasis	<0.001	2.393	1.489-3.848	<0.001
Venous invasion	<0.001	1.801	1.073-3.002	0.026
EFNA1 expression	0.011	1.586	1.026-2.452	0.038
Tumor differentiation	0.008	3.868	1.515-9.874	0.005
Depth of invasion	0.009	1.681	0.585-4.828	0.335
Tumor site	0.069			
Age	0.143			

EFNA1. Western blot analysis showed that the EFNA1 protein was expressed in the seven CRC cell lines tested (Fig. 3A).

EFNA1 mRNA overexpressed in CRC cell lines under hypoxia. Fig. 3B shows the EFNA1 mRNA expression in four CRC cell lines. EFNA1 mRNA was progressively induced with hypoxia in all CRC cell lines examined, and highly expressed after 48 h under hypoxia in HT29, DLD1, and Lovo. On the other hand, EFNA1 mRNA in HCT116 was expressed after 6 h of hypoxia.

Effects of EFNA1 on growth, invasion, and migration of CRC cells. To assess the potential relevance of EFNA1 as a therapeutic target, *in vitro* knockdown experiments were performed in HCT116. Western blot analysis showed moderate and strong reductions in EFNA1 after treatment with siRNA #1 and

Table IV. Univariate and multivariate analyses of the relationships between clinicopathological factors and cancer-related survival in qRT-PCR data.

Factor	p-value	OR	95% CI	p-value
Lymph node metastasis	<0.0001	3.344	1.707-7.769	0.0008
Venous invasion	0.0005	1.784	0.942-3.585	0.0744
EFNA1 expression	0.0288	2.037	1.026-3.889	0.0417
Tumor differentiation	0.0015	1.046	0.652-4.082	0.2954
Depth of invasion	0.0001	2.253	1.199-13.611	0.0242
Tumor site	0.6044			
Age	0.1434			