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ORIGINAL ARTICLE – COLORECTAL CANCER

Tumor Budding as a Prognostic Marker in Rectal Cancer Patients on Propensity Score Analysis

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ABSTRACT

Background. Tumor budding is associated with adverse histology. It is a predictor of poor oncologic outcomes in colorectal cancer. However, it remains unclear whether tumor budding is a predictor of poor prognosis for rectal cancer patients regardless of neoadjuvant chemoradiotherapy (nCRT).

Patients and Methods. This study analyzed 2888 rectal cancer patients who underwent radical surgery from 2007 to 2014. Among these patients, 939 underwent nCRT while 1949 did not receive nCRT. Tumor budding was defined as positive if the number of isolated tumor cells or small clusters of up to five tumor cells at the invasive front of the tumor was five or more. If the number was less than five, it was defined as negative. Patients were categorized according to tumor budding status. We used 1:1 propensity score matching to adjust for potential baseline confounders between the two groups.

Results. Among 2888 patients, 939 received nCRT while 1949 did not receive nCRT. A total of 418 patients who received nCRT were matched (209 in each group). A total of 1024 patients without nCRT were also matched (512 in each group). In matched patients, 5-year overall survival (OS) and 5-year disease-free survival (DFS) rates for the positive budding group were significantly lower than those in the negative budding group regardless of nCRT. On multivariate analysis of prognostic factors, positive budding was associated with poorer disease-free survival independent of nCRT.

Conclusion. Tumor budding positivity is a prognostic indicator of poor outcomes in rectal cancer patients regardless of neoadjuvant chemoradiotherapy.

Tumor budding is a histologic feature in which tumor cells will detach from the invasive margin of the tumor and migrate into the stroma surrounding the tumor.[1] As one of potential prognostic biological variables, tumor budding has been demonstrated as a histological phenomenon in several studies.[2–4] It is a characteristic microscopic feature representing tumor dedifferentiation, the first and paramount sign of tumor invasion.[2,3]

Many studies have suggested that tumor budding is an independent poor prognostic factor because it is related to high tumor grade, infiltrating tumor border, lymphovascular invasion, and perineural invasion.[5–8] A few studies have also reported that tumor budding is a prognostic factor in rectal cancer.[4,9,10] However, these studies enrolled small numbers of patients. In addition, potential bias was a concern in many studies because of lack of equal distribution between groups. Thus, whether poor oncologic outcomes observed for rectal cancer patients with tumor budding are due to aggressive features of the tumor or tumor budding itself remains unclear. Thus, the objective of this study was to investigate the prognostic significance

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of tumor budding using propensity score matching for a large number of rectal cancer patients who underwent radical surgery with or without neoadjuvant treatment.

PATIENTS AND METHODS

Between January 2007 and December 2014, a total of 3113 patients with primary rectal adenocarcinoma underwent radical resection at one single center. Patients were excluded from this study if they had no record of tumor budding staining or if they had a recording of "not identified," recurrent or metastatic cancer, or hereditary rectal cancer. Among them, 223 patients had no record of tumor budding. After excluding them, an analysis of 2900 patients was performed, and records of "not identified" were found for two patients during the analysis. After excluding these two patients, the analysis was finally conducted for 2888 patients.

All patients underwent preoperative staging with rectum magnetic resonance imaging (MRI) or computed tomography (CT) scans of abdominopelvic cavity and thorax. Neoadjuvant treatment was offered to patients with clinical T3 or higher or with clinical nodal involvement. Chemoradiation regimen consisted of a long course of radiation with 4500–5400 cGy in 5–6 weeks with synchronous 5-fluorouracil based chemotherapy. Surgery was performed at 6 and 8 weeks after completion of chemoradiation.

Tumor node metastasis stage was defined according to the Cancer Staging Manual, Seventh Edition, by the American Joint Committee on Cancer.[11] Tumor budding was also evaluated for surgical specimen using hematoxylin-and-eosin-stained slides by two pathologists at our institution specialized in colorectal cancer. Tumor budding was evaluated in the original pathologic assessment. All data for this study were retrieved from the original pathology report (review of slides was not conducted in this study). Tumor budding was defined as positive if the number of isolated tumor cells or small clusters of up to five tumor cells at the invasive front of the tumor was five or more, as described by Ueno et al.[12] Otherwise, it was defined as negative. Perineural invasion was defined as the presence of tumor cells found within the perineural space.

Patients were categorized according to tumor budding status: negative tumor budding (< 5 buds) or positive tumor budding (≥ 5 buds). For rectal cancer patients, the effect of neoadjuvant treatment on tumor budding is an important issue. This would have been a significant confounder for results. Thus, this study also divided patients according to whether they underwent neoadjuvant treatment (Fig. 1).

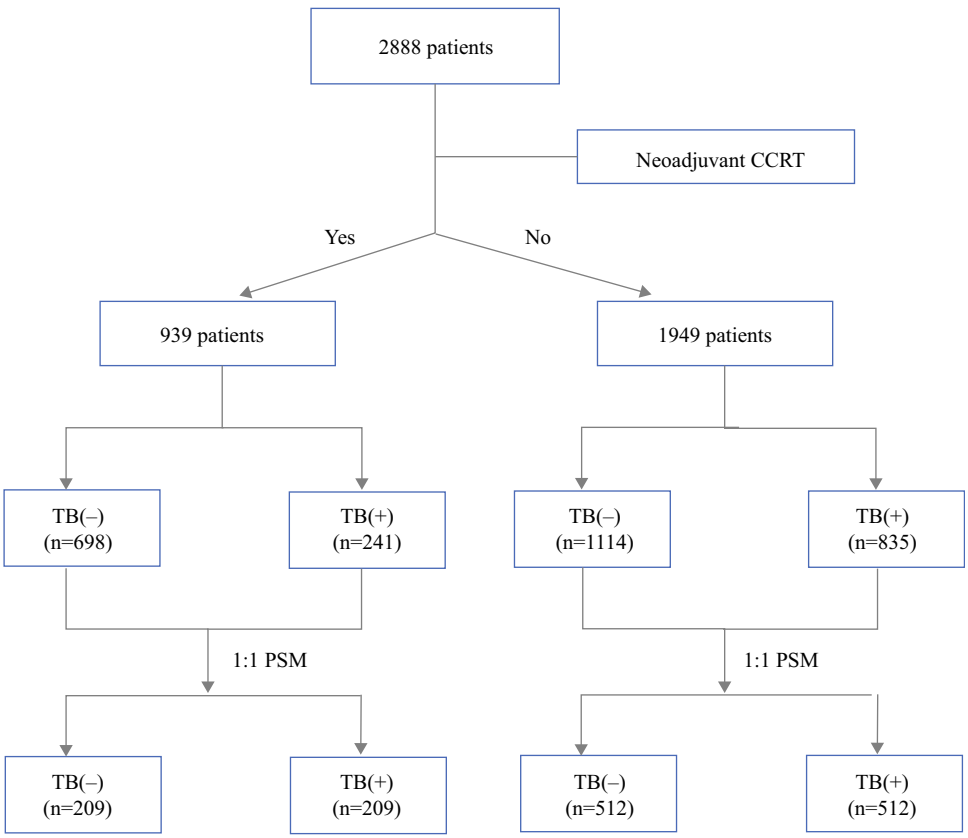

FIG. 1 Flowchart of this study

Postoperative surveillance was performed every 3 months for the first 2 years after surgery. It was then performed every 6 months for up to 5 years. Most patients were evaluated for serum carcinoembryonic antigen (CEA) levels. Most patients underwent CT scanning of the chest and the abdominopelvic cavity to evaluate disease status. Primary end points were disease-free survival (DFS) and overall survival (OS) according to tumor budding status.

TABLE 1 Characteristics of patients with neoadjuvant treatment according to tumor budding status

	Before propensity score matching			After propensity score matching		
	TB(−) (n = 698) (%)	TB(+) (n = 241) (%)	p-Value	TB(−) (n = 209) (%)	TB(+) (n = 209) (%)	p-Value
Age (years)			0.738			0.447
< 65	499 (71.5)	175 (72.6)		146 (69.9)	154 (73.7)	
≥ 65	199 (28.5)	66 (27.4)		63 (30.1)	55 (26.3)	
Gender			0.093			0.915
Male	460 (65.9)	173 (71.8)		146 (69.9)	147 (70.3)	
Female	238 (34.1)	68 (28.2)		63 (30.1)	62 (29.7)	
CEA level (ng/ml)			0.014			0.869
< 5	656 (94.0)	215 (89.2)		188 (90.0)	189 (90.4)	
≥ 5	42 (6.0)	26 (10.8)		21 (10.0)	20 (9.6)	
ypTNM stage			< 0.001			0.955
I	374 (53.6)	26 (10.8)		24 (11.5)	26 (12.4)	
II	157 (22.5)	80 (33.2)		73 (34.9)	72 (34.4)	
III	167 (23.9)	135 (56.0)		112 (53.6)	111 (53.2)	
Cell types			0.002			0.875
WD/MD	657 (94.1)	212 (88.0)		187 (89.5)	186 (89.0)	
PD/MUC/SRC	41 (5.9)	29 (12.0)		22 (10.5)	23 (11.0)	
Neoadjuvant CTx regimen			0.069			0.095
5-FU	321 (46.0)	118 (49.0)		89 (42.6)	100 (47.8)	
FL	106 (15.2)	23 (9.6)		27 (12.9)	15 (7.2)	
Capecitabine	268 (38.4)	97 (40.2)		93 (44.5)	92 (44.0)	
Others	3 (0.4)	3 (1.2)		0 (0.0)	2 (1.0)	
Tumor regression grade			< 0.001			0.978
No	81 (11.6)	56 (23.2)		43 (20.5)	45 (21.5)	
Minimal	88 (12.6)	40 (16.6)		34 (16.3)	33 (15.8)	
Moderate	382 (54.7)	120 (49.8)		112 (53.6)	109 (52.2)	
Complete/near complete	147 (21.1)	25 (10.4)		20 (9.6)	22 (10.5)	
Lymphatic invasion						
Yes	67 (9.6)	81 (33.6)	< 0.001	55 (26.3)	59 (28.2)	0.742
No	631 (90.4)	160 (66.4)		154 (73.7)	150 (71.8)	
Vascular invasion						
Yes	52 (7.4)	47 (19.5)	< 0.001	36 (17.2)	41 (19.6)	0.614
No	646 (92.6)	194 (80.5)		173 (82.8)	168 (80.4)	
Perineural invasion						
Yes	47 (6.7)	62 (25.7)	< 0.001	39 (18.7)	50 (23.9)	0.232
No	651 (93.3)	179 (74.3)		170 (81.3)	159 (76.1)	
Adjuvant treatment						
(−)	115 (16.5)	28 (11.6)	0.07	25 (12.0)	27 (12.9)	0.882
(+)	583 (83.5)	213 (88.4)		184 (88.0)	182 (87.1)	

TB tumor budding, *WD* well differentiated, *MD* moderately differentiated, *PD* poorly differentiated, *MUC* mucinous carcinoma, *SRC* signet ring cell carcinoma, *CTx* chemotherapy, *5-FU* 5-fluorouracil, *FL* 5-FU/leucovorin.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 23.0 (SPSS, Chicago, IL, USA). Differences between the two groups were analyzed using χ^2 test, Fisher's exact test, or Mann–Whitney *U* test, as appropriate. To evaluate oncologic effects of tumor budding status, we used 1:1 propensity score matching to adjust for potential baseline confounders, including age, gender, preoperative CEA level, stage, lymphatic invasion, vascular invasion, perineural invasion, tumor regression grade, and adjuvant treatment. Before and after matching, survival rates were calculated by the Kaplan–Meier method and log-rank test. Multivariate analyses were performed using a Cox proportional hazard model. Results were considered statistically significant when *p* value was less than 0.05.

RESULTS

Patients with Neoadjuvant Chemoradiotherapy

Among 939 patients who underwent neoadjuvant treatment, 241 (25.7%) were positive for tumor budding. As presented in Table 1, many variables were differently distributed between patients with and without tumor budding before propensity score matching. Elevated preoperative CEA (≥ 5 ng/ml), advanced stage, poor histology, presence of lymphovascular and perineural invasion, and no response to neoadjuvant treatment were more common in patients with tumor budding than in those without tumor budding. Based on these findings, we performed propensity score matching at a 1:1 ratio. A total of 418 patients were matched. After matching, two groups (209 in each group) were well balanced for all variables (Table 1).

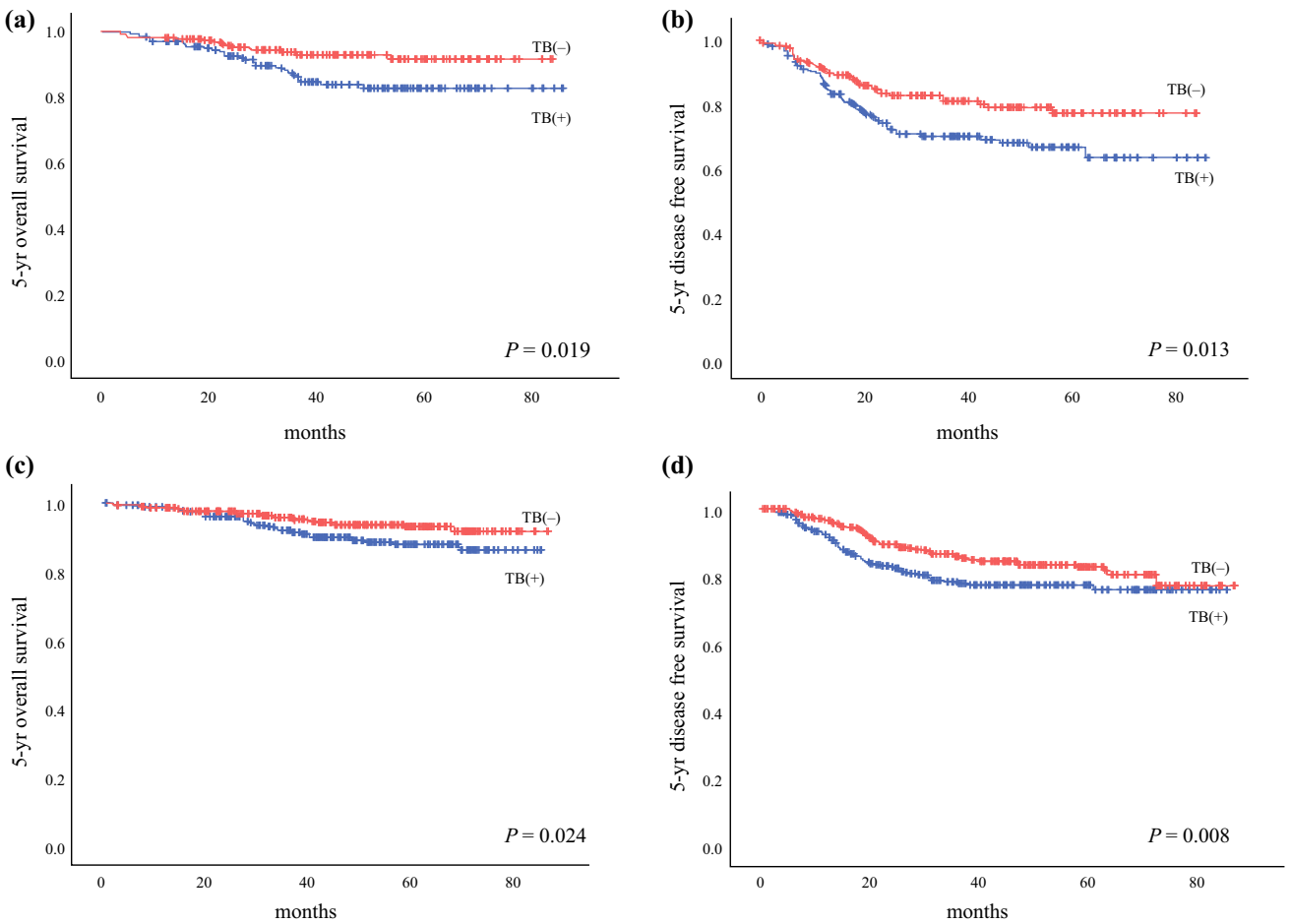

FIG. 2 Survival according to tumor budding in matched patients with neoadjuvant treatment: **a** 5-year overall survival; **b** 5-year disease-free survival. Survival according to tumor in matched patients without neoadjuvant treatment: **c** 5-year overall survival; **d** 5-year disease-free survival

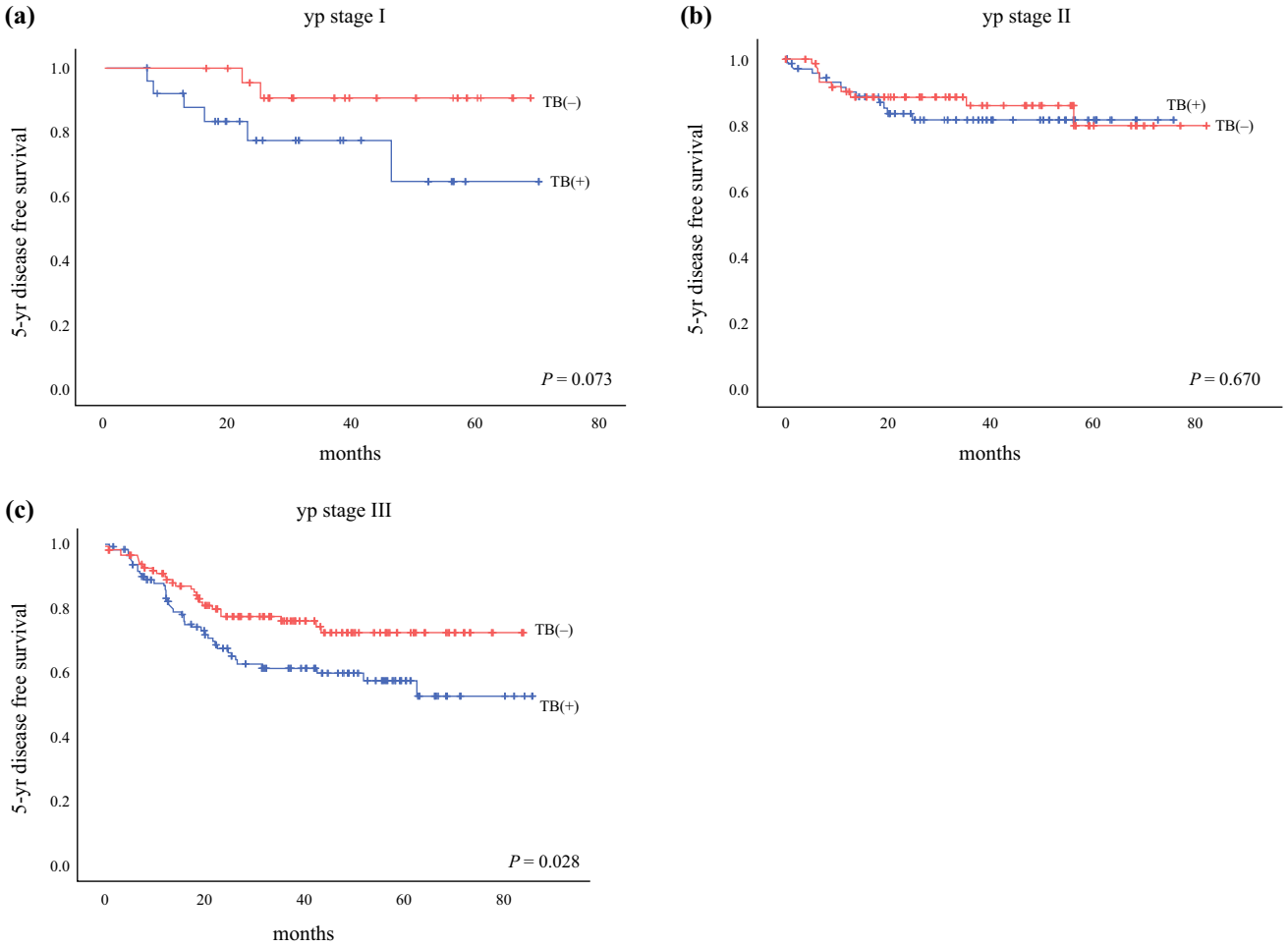

FIG. 3 Survival according to tumor budding in matched patients with neoadjuvant treatment: **a** yp stage I; **b** yp stage II; **c** yp stage III

To determine the impact of tumor budding on oncologic outcomes, we analyzed 5-year DFS and 5-year OS according to tumor budding. Before matching, patients with tumor budding showed significantly lower rates of 5-year DFS (65.4% versus 80.5%, $p < 0.001$) and 5-year OS (82.1% versus 94.7%, $p < 0.001$) than patients without tumor budding. Among matched patients, although such difference was decreased, it was still statistically significant. Patients with tumor budding showed significantly lower rates of 5-year OS (82.5% versus 91.4%, $p = 0.019$) and 5-year DFS (66.7% versus 77.2%, $p = 0.013$) than patients without tumor budding (Fig. 2a, b). When 5-year DFS and 5-year DRFS rates were analyzed according to tumor stage, they also showed significant difference for stage III. Tumor budding (TB)-positive patients at stage III showed a significantly lower 5-year DFS than TB-negative patients (57.3% versus 72.3%, $p = 0.028$; Fig 3). The 5-year distant recurrence-free survival (DRFS) rate was also lower in the TB-positive group with stage III than in TB-negative patients (57.0% versus 73.8%, $p = 0.026$).

To determine whether tumor budding positivity was an independent prognostic factor for survival in rectal cancer, the Cox proportional hazard model was used to analyze matched patients. On multivariate analysis, positive tumor budding was an independent poor prognostic factor for both 5-year DFS ($p = 0.014$) and OS ($p = 0.022$) (Table 2).

Patients without Neoadjuvant Treatment

Among 1949 patients who did not receive neoadjuvant treatment, 835 (42.8%) were positive for tumor budding. A total of 1024 patients were matched (512 in each group). After matching, the two groups were well balanced for all variables (Table 3).

In terms of oncologic outcomes, 5-year DFS (71.4% versus 89.5%, $p < 0.001$) and 5-year OS (86.9% vs. 95.0%, $p < 0.001$) were significantly lower in tumor-budding-positive patients than in tumor-budding-negative patients of the unmatched group. These rates were similar in matched patients. Patients with tumor budding showed significantly lower rates of 5-year OS (87.7% versus

TABLE 2 Prognostic factors of survival for matched patients with neoadjuvant treatment

	Before propensity score matching			After propensity score matching		
	TB(−) (n = 1114) (%)	TB(+) (n = 835) (%)	p-Value	TB(−) (n = 512) (%)	TB(+) (n = 512) (%)	p-Value
Age (years)			0.377			0.747
< 65	726 (65.2)	528 (63.2)		315 (61.5)	321 (62.7)	
≥ 65	388 (34.8)	307 (36.8)		197 (38.5)	191 (37.3)	
Gender			0.729			0.482
Male	685 (61.5)	507 (60.7)		304 (59.4)	316 (61.7)	
Female	429 (38.5)	328 (39.3)		208 (40.6)	196 (38.3)	
CEA level (ng/ml)			< 0.001			0.832
< 5	955 (85.7)	638 (76.4)		415 (81.1)	409 (79.9)	
≥ 5	137 (12.3)	174 (20.8)		87 (17.0)	94 (18.3)	
Unknown	22 (2.0)	23 (2.8)		10 (2.0)	9 (1.8)	
TNM stage			< 0.001			0.651
I	529 (47.5)	112 (13.4)		103 (20.1)	100 (19.5)	
II	295 (26.5)	184 (22.0)		127 (24.8)	140 (27.3)	
III	290 (26.0)	539 (64.6)		282 (55.1)	272 (53.2)	
Cell types			< 0.001			0.542
WD/MD	1089 (97.8)	754 (90.3)		487 (95.1)	492 (96.1)	
PD/MUC/SRC	25 (2.2)	81 (9.7)		25 (4.9)	20 (3.9)	
Lymphatic invasion			< 0.001			0.691
Yes	185 (16.6)	400 (47.9)		174 (34.0)	167 (32.6)	
No	929 (83.4)	435 (52.1)		338 (66.0)	345 (67.4)	
Vascular invasion			< 0.001			0.269
Yes	118 (10.6)	203 (24.3)		107 (20.9)	92 (18.0)	
No	996 (89.4)	632 (75.7)		405 (79.1)	420 (82.0)	
Perineural invasion			< 0.001			0.302
Yes	59 (5.3)	237 (28.4)		59 (11.5)	71 (13.9)	
No	1055 (94.7)	598 (71.6)		453 (88.5)	441 (86.1)	
Adjuvant treatment			< 0.001			0.741
(−)	673 (60.4)	587 (70.3)		336 (65.6)	342 (66.8)	
(+)	441 (39.6)	248 (29.7)		176 (34.4)	170 (33.2)	

TB tumor budding, *CEA* carcinoembryonic antigen, *WD* well differentiated, *MD* moderately differentiated, *PD* poorly differentiated, *MUC* mucinous carcinoma, *SRC* signet ring cell carcinoma.

92.8%, p = 0.024) and 5-year DFS (77.6% versus 82.8%, p = 0.008) than patients without tumor budding (Fig. 2c, d). We also analyzed 5-year DFS and 5-year DRFS rates according to pathological stage in matched patients. TB-positive patients at stage III showed a significantly lower 5-year DFS than TB-negative patients (68.2% versus 77.8%, p = 0.003; Fig. 4). The 5-year DRFS rate was also lower in the TB-positive group at stage III (72.0% versus 80.7%, p = 0.007).

To determine whether tumor budding positivity was an independent prognostic factor for survival in rectal cancer, the Cox proportional hazard model was used to analyze matched patients. On multivariate analysis, positive tumor budding was an independent poor prognostic factor for both 5-year DFS (p = 0.009) and OS (p = 0.026) (Table 4).

DISCUSSION

Nowadays, tumor budding has been widely accepted as an adverse histologic factor in colorectal cancer. A higher intensity of budding is associated with more aggressive tumor behavior and poorer oncologic outcome.[2,5,12–15]

TABLE 3 Characteristics of patients without neoadjuvant treatment according to tumor budding status

Factors	Overall survival			Disease-free survival		
	Univariate	Multivariate		Univariate	Multivariate	
	p	HR (95% CI)	*p*	*p*	HR (95% CI)	*p*
TB						
≥ 5 versus < 5	0.033	2.102 (1.111–9.979)	0.022	0.031	1.665 (1.108–2.504)	0.014
Age (years)						
≥ 65 versus < 65	0.007	1.939 (1.058–3.555)	0.032	0.177	1.109 (0.806–1.527)	
Gender						
Female versus male	0.044	1.947 (0.903–4.196)	0.089	0.02	1.487 (0.989–2.236)	0.056
CEA level (ng/l)						
≥ 5 versus < 5	0.192			0.778		
Stages						
2 versus 1	0.04	1.755 (0.952–3.235)	0.071	0.01	2.089 (1.003–4.348)	0.004
3 versus 1	0.024	4.952 (1.796–13.648)	0.002	0.005	2.662 (1.748–4.054)	<0.001
Cell type						
PD/MUC/SRC versus WD/MD	0.002	3.252 (1.638–6.456)	0.001	0.426	2.762 (1.691–4.509)	
Lymphatic invasion						
Yes versus no	0.082			0.132		
Vascular invasion						
Yes versus no	0.239	3.483 (2.218–5.469)		0.004	2.089 (1.003–4.348)	0.049
Perineural invasion						
Yes versus no	0.011	2.604 (1.383–4.903)	0.003	0.05	2.077 (1.345–3.208)	0.001

However, it has not been accepted as a definite high-risk factor in colorectal cancer yet. Accepted factors include poor differentiation, lymphatic/vascular/perineural invasion, and obstruction or perforation. Recently, the International Tumor Budding Consensus Conference (ITBCC) group has recommended that tumor budding is a high-risk factor in the management for colorectal cancer.[16]

In this study, we investigated the prognostic significance of tumor budding in rectal cancer patients regardless of neoadjuvant treatment. We observed associations between tumor budding positivity and other adverse histologic features such as elevated preoperative CEA level, advanced stage, poor differentiation, and presence of lymphatic/vascular/perineural invasion. This study adjusted these features to evaluate effects of tumor budding on oncologic outcomes. Results showed that tumor budding positivity was an independent prognostic factor in rectal cancer.

Many studies have investigated oncologic outcomes according to tumor budding in colorectal cancer. Those studies, including our previous study, have suggested that patients with tumor budding show worse oncologic outcomes and higher lymph node metastasis.[2,4,5,8,9,17–19] However, some studies have concluded that tumor budding is not an independent prognostic factor.[20] Several studies have reported scoring systems for the prognostic value of tumor budding including other factors.[6,13,21–23] However, these studies had confounding bias between groups. They also restricted subjects to certain stages. Furthermore, they had limited sample sizes. Few studies have included only rectal cancer patients. Studies that classify patients according to neoadjuvant treatment have not been reported yet.

In this study, the TB-positive group at stage III showed a lower 5-year DFS rate regardless of neoadjuvant treatment. The role of adjuvant chemotherapy for patients with rectal cancer after neoadjuvant treatment remains controversial. The present study revealed that 5-year DFS and 5-year DRFS of stage III rectal cancer patients with positive TB were significantly lower than those of patients with negative TB. Our findings suggest that stage III rectal cancer patients with positive TB with or without nCRT are good candidates for a clinical trial to test the effect of an adjuvant chemotherapy.

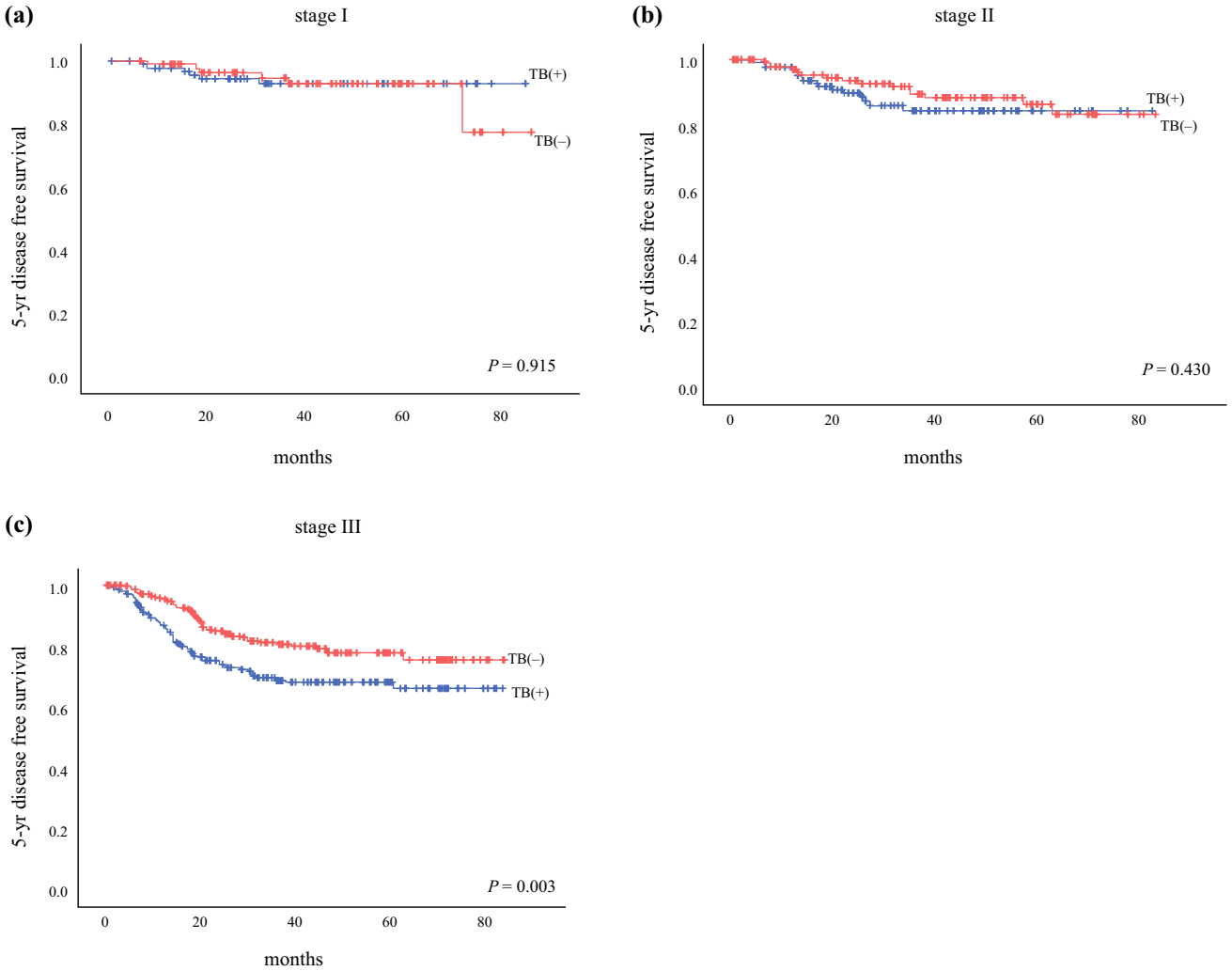

FIG. 4 Survival according to tumor in matched patients without neoadjuvant treatment; **a** stage I; **b** stage II; **c** stage III

One strength of this study was that we divided patients into those who received nCRT and those who did not receive nCRT. For rectal cancer patients, neoadjuvant treatment could affect tumor budding. It could be a significant confounder in our results. Only one study[14] has specifically investigated tumor budding in post-irradiation rectal cancer, although neoadjuvant radiotherapy has become the standard treatment for locally advanced rectal cancer.[24–27] The present study showed that tumor budding was a significant prognostic factor associated with poor prognosis in rectal cancer following curative resection with neoadjuvant treatment even after adjusting for confounding factors. Although tumor budding positivity alone is not enough for accurate calculation of expected survival, this study suggests that tumor budding is a key factor for predicting prognosis and establishing treatment strategies.

This study has some limitations. First, it was retrospective in nature. In addition, two patients with tumor budding staining reported as "not identified" were excluded because the number of these patients was small and classifying them into a TB-negative group could lead to bias. Despite these limitations, to the best of our knowledge, this is the first study to evaluate the prognostic significance of tumor budding using propensity score matching for rectal cancer patients with or without receiving neoadjuvant treatment.

In conclusion, tumor budding is an independent poor prognostic factor in rectal cancer patients. Because of its value as a prognostic indicator, tumor budding could be a good index to estimate the aggressiveness of rectal cancer. More data are needed through more extensive literature review. A multiinstitutional prospective study is also needed in the future to confirm our finding.

TABLE 4 Prognostic factors of survival for matched patients without neoadjuvant treatment

Factors	Overall survival			Disease-free survival		
	Univariate	Multivariate		Univariate	Multivariate	
	p	HR (95% CI)	*p*	*p*	HR (95% CI)	*p*
TB						
≥ 5 versus < 5	0.049	1.690 (1.066–2.678)	0.026	0.010	1.511 (1.109–2.057)	0.009
Age (years)						
≥ 65 versus < 65	< 0.001	2.668 (1.691–4.208)	< 0.001	0.046	1.109 (0.806–1.527)	0.525
Gender						
Female versus male	0.098			0.012	1.535 (1.102–2.140)	0.011
CEA level (ng/l)						
≥ 5 versus < 5	0.367			0.873		
Stages						
2 versus 1	0.019	2.559 (0.849–7.711)	0.095	0.003	1.887 (0.969–3.674)	0.062
3 versus 1	0.009	4.952 (1.796–13.648)	0.002	< 0.001	4.013 (2.218–7.261)	< 0.001
Cell type						
PD/MUC/SRC versus WD/MD	< 0.001	4.968 (2.821–8.751)	< 0.001	< 0.001	2.762 (1.691–4.509)	< 0.001
Lymphatic invasion						
Yes versus no	0.243			0.103		
Vascular invasion						
Yes versus no	< 0.001	3.483 (2.218–5.469)	< 0.001	< 0.001	3.022 (2.209–4.134)	< 0.001
Perineural invasion						
Yes versus no	0.061			< 0.001	2.923 (2.032–4.205)	< 0.001

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