Tumor Budding at the Invasive Margin Can Predict Patients at High Risk of Recurrence After Curative Surgery for Stage II, T3 Colon Cancer

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PURPOSE: The aim of this study was to identify indicators that can predict patients at high risk of tumor recurrence in Stage II, T3 colon cancer. METHODS: A total of 138 patients classified as Stage II, T3 underwent curative resection of colon cancer between 1981 and 1993. Clinical variables included age, gender, bowel obstruction, tumor location, and emergency presentation. For each colon tumor specimen, the following histopathological variables were assessed: maximum tumor diameter (<5 *vs.* ≥5 cm), depth, tumor grade (well and moderate *vs.* other), lymphatic and venous invasion (absent *vs.* present), perineural invasion, tumor necrosis, and tumor margin (expanding *vs.* infiltrating). We also categorized tumor budding, defined as a single cancer cell or small clusters of undifferentiated cancer cells in the invasive frontal lesion, into two categories: none or minimal (BD-1), and moderate or severe (BD-2). Univariate analysis for factors regarding recurrence and disease-specific survival were performed with the logistic regression model and the log-rank test. RESULTS: Among the factors analyzed, tumor budding was the only factor that was significantly associated with recurrence and survival. The numbers of patients with BD-1 and BD-2 tumors were 111 and 27, respectively. Forty-eight percent of BD-2 tumor patients developed recurrence, compared with 4.5 percent of BD-1 tumor patients ($P < 0.0001$). The cumulative disease-specific survival rates at five years for patients with BD-1 and BD-2 tumors were 98 and 74 percent, respectively ($P < 0.0001$). CONCLUSION: The presence of moderate or severe budding at the invasive margin in Stage II, T3 colon cancer indicated a high risk of tumor recurrence after curative surgery, providing useful information for the decision regarding postoperative adjuvant chemotherapy. [Key words: Colon cancer; T3; Stage II; Tumor budding; Chemotherapy; Recurrence; Survival]

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Within the last 15 years, postoperative adjuvant chemotherapy has reduced tumor recurrence and improved disease-free and overall survival in Stage III colon cancer.[1,2] Recent studies have demonstrated that a combination of 5-fluorouracil (5-FU), leucovorin, and irinotecan (CPT-11), or 5-FU, leucovorin, and oxaliplatin, is superior to 5-FU and leucovorin alone for patients with Stage III colon cancer.[3] However, the precise role for chemotherapy in Stage II colon cancer remains unclear. Several trials have failed to demonstrate a statistical advantage for adjuvant chemotherapy in patients with Stage II colon cancer, although slight improvements in the survival rates in treated groups have been reported.[4,5] These results may indicate that postoperative chemotherapy for patients with relatively low recurrence rates, such as those with Stage II, T3 colon cancer, should be limited to a subgroup of patients with reliable risk factors of recurrence.

The ability to distinguish patients with a poor prognosis would help modify selection procedures for future trials, targeting primarily those cases where tumor recurrence may be expected. Although numerous studies have sought to link recurrence and long-term survival to such factors as clinicopathologic features,[6–8] angiogenesis,[9,10] nodal micrometastases,[11,12] and immunohistochemical expression,[13–15] results are still inconclusive. The aim of this study was to identify indicators that can simply predict patients at high risk of tumor recurrence at Stage II, T3 colon cancer.

PATIENTS AND METHODS

Between 1981 and 1993, 138 patients with colon cancer underwent potentially curative surgery at the National Defense Medical College Hospital in Japan.

They were classified as T3N0M0 and Stage II, according to the International Union Against Cancer (UICC) TNM.[16] These 138 patients consisted of 80 males and 58 females, with an average age of 63 (range, 29–87) years. No patients received preoperative and postoperative adjuvant therapy. No patient had postoperative mortality because of complications in this study. All patients were observed for more than five years, with an average follow-up period of 93 (range, 68–214) months.

Clinical variables studied were age, gender, presence of bowel obstruction, tumor location, and emergency presentation, which was defined as the need for urgent surgery within 48 hours of admission.[17] With regard to tumor location, patients were divided into two groups: those with tumors in the left (sigmoid and descending colon) and those with tumors in the right colon (other parts of the colon).

For each colon tumor specimen, the following histopathologic variables were retrieved from histopathologic records according to the Japanese Classification of Colorectal Carcinoma[18] and then categorized as follows: maximum tumor diameter was divided into two categories (<5 or ≥ 5 cm). The tumor differentiations were classified as well/moderate and other.[19] Venous invasion and lymphatic invasion were classified as present or absent.

Subdivision of the pathologic tumor category pT3 (depth) was done based on histologic measurements of maximum tumor invasion beyond the outer border of the muscularis propria.[20] When the outer aspect of the muscular layer could not be estimated because of its destruction by the tumor, an estimate was made by drawing a straight line between both break points of the muscular layer. We divided patients into two groups according to the depth of tumor invasion: ≤ 15 and >15 mm.

Four other discrete histologic variables were also reviewed pathologically by one of the authors (MT). These included tumor budding, perineural invasion,[21] tumor necrosis,[22] and type of invasive front.[23] Tumor budding was defined as a single cancer cell or small clusters of undifferentiated cancer cells in the invasive frontal lesion, as shown in Figure 1. Using one hematoxylin and eosin slide containing the deepest portion of tumor penetration, tumor budding was classified into four grades: none, minimal, moderate, and severe, according to Hase *et al.*[24] Then, tumors were divided into two categories: none or minimal (BD-1) and moderate or severe (BD-2). Extramural perineural invasion[21] and tumor necrosis[22] were graded as absent or present. A positive judgment of perineural invasion was made when cancer cells were found to exist inside the perineurium. Type of invasive front[23] was defined as expanding or infiltrating following the morphologic guidelines previously defined by Jass and colleagues.[23]

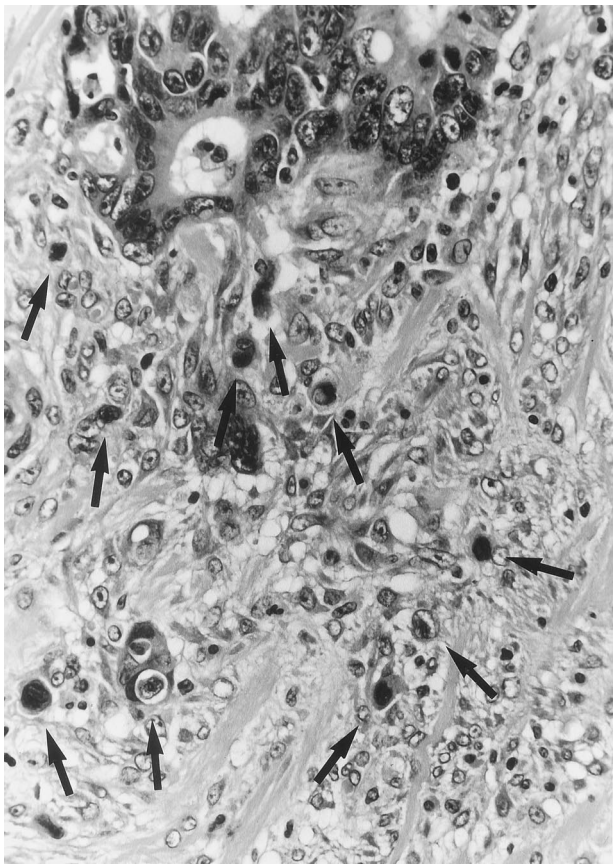

Figure 1. Histologic finding of tumor budding (arrow; original magnification, ×100).

Statistical analyses were performed by StatView® 4.11 software (Abacus Concepts, Berkeley, CA). Patient survival differences were examined by the Kaplan-Meier method with the log-rank test. Disease-specific survival was used for the survival analysis. Univariate analyses were performed using recurrence as the outcome variables with the logistic regression model. Chi-squared tests were performed when appropriate.

RESULTS

Clinical and pathologic features were analyzed by univariate analysis as risk factors for recurrence and are shown in Table 1. Based on the univariate analyses using the logistic regression model, tumor bud-

Table 1.
Risk Factors for Recurrence Analyzed by Univariate Analyses

	No. of Cases	Recurrence Rate (%)	*P* Value
Age			
<70 years	97	13.4	
≧70 years	41	12.2	0.8474
Gender			
Male	80	12.5	
Female	58	13.8	0.8238
Bowel obstruction			
Absent	80	15.5	
Present	58	11.3	0.1572
Tumor site			
Left colon	79	15.2	
Right colon	59	10.2	0.3863
Emergency presentation			
No	136	13.2	
Yes	2	0	>0.9999
Tumor size			
<5 cm	72	12.5	
≧5 cm	66	13.6	0.843
Depth*			
≦5 mm	103	10.6	
>5 mm	35	20	0.1572
Tumor budding			
BD-1	111	4.5	
BD-2	27	48	<0.0001
Grade			
Well and mod	131	17	
Others	7	14.3	0.92
Venous invasion			
Absent	14	21.4	
Present	124	12.1	0.3257
Lymphatic invasion			
Absent	72	9.7	
Present	66	16.7	0.2263
Perineural invasion			
Absent	125	12.8	
Present	13	15.4	0.7923
Necrosis			
Absent	121	12.4	
Present	17	17.6	0.5472
Tumor margin			
Expanding	111	11.7	
Infiltrating	27	18.5	0.3462

BD-1 = no or minimal tumor budding; BD-2 = moderate or severe tumor budding.

* Depth of penetration beyond the muscularis propria.

ding was the only factor that was significantly associated with recurrence.

There were 111 patients (80 percent) with colon cancer classified as BD-1 and 27 patients (20 percent) as BD-2. Forty-eight percent of patients with BD-2 cancer developed recurrence, compared with 4.5 percent with BD-1 ($P < 0.0001$).

As shown in Table 2, among the 138 patients, locoregional recurrence was observed in 2 (1.4 percent), liver metastases in 10 (7.2 percent), lung metastases in 2 (1.4 percent), and peritoneal dissemination in 4 (2.9 percent). BD-2 cancer patients had a significantly higher incidence of liver, peritoneal, and locoregional recurrence than those with the BD-1 tumor.

Risk factors for disease-specific survival after surgery were univariately analyzed by the log-rank test. Maximum tumor diameter, histological type, lymphatic invasion, and venous invasion had no significant impact on disease-specific survival. Tumor budding was the only factor that significantly affected the prognostic outcome. The cumulative disease-specific survival at five years for patients with BD-1 and BD-2 tumors was 98 and 74 percent, respectively ($P < 0.0001$), as shown in Figure 2.

DISCUSSION

Recent findings from randomized trials suggest that the survival benefit of chemotherapy in Stage II patients ranges from −2 to 8 percent.[5,25] There are concerns that any marginal difference in survival between treatment and control groups may not justify the discomfort, cost, and risk involved in administering adjuvant therapy. Accordingly, the need for adjuvant therapy in all Stage II patients is debatable, inasmuch as the event-free and overall five-year survival rates are reported to be 73 and 80 percent, respectively, with surgery alone.[6] To circumvent this problem, it is necessary to establish other prognostic factors that may characterize subgroups of Stage II patients and help distinguish those with a good prognosis from those with a poor prognosis.

In patients with Stage II colon cancer, pT4 tumors were identified as the major risk factor and had a significantly higher risk of distant metastases and a significantly lower disease-related survival rate compared with patients with pT3 tumors.[8,20] Based on these studies, we analyzed the risk factors of patents with UICC Stage II (T3N0M0) to identify high-risk subgroups that may benefit from adjuvant chemotherapy.

Numerous authors have reported new risk factors obtained by immunohistochemistry and molecular biology for recurrence in Stage II colon cancer patients, such as angiogenesis (vessel count,[9] vascular endo-

Table 2.
Grade of Budding and Recurrence Sites

	None	Liver	Lung	Peritoneal	Locoregional
BD-1 (n = 111)	106 (95.5)	3 (2.7)	1 (0.9)	1 (0.9)	0
BD-2 (n = 27)	14 (51.9)	7 (25.9)	1 (3.7)	3 (11.1)	2 (7.4)
Total (n = 138)	120	10	2	4	2
P value		0.0004	0.354	0.024	0.037

BD-1 = no or minimal tumor budding; BD-2 = moderate or severe tumor budding.
Figures are number and (percentage) unless otherwise specified.

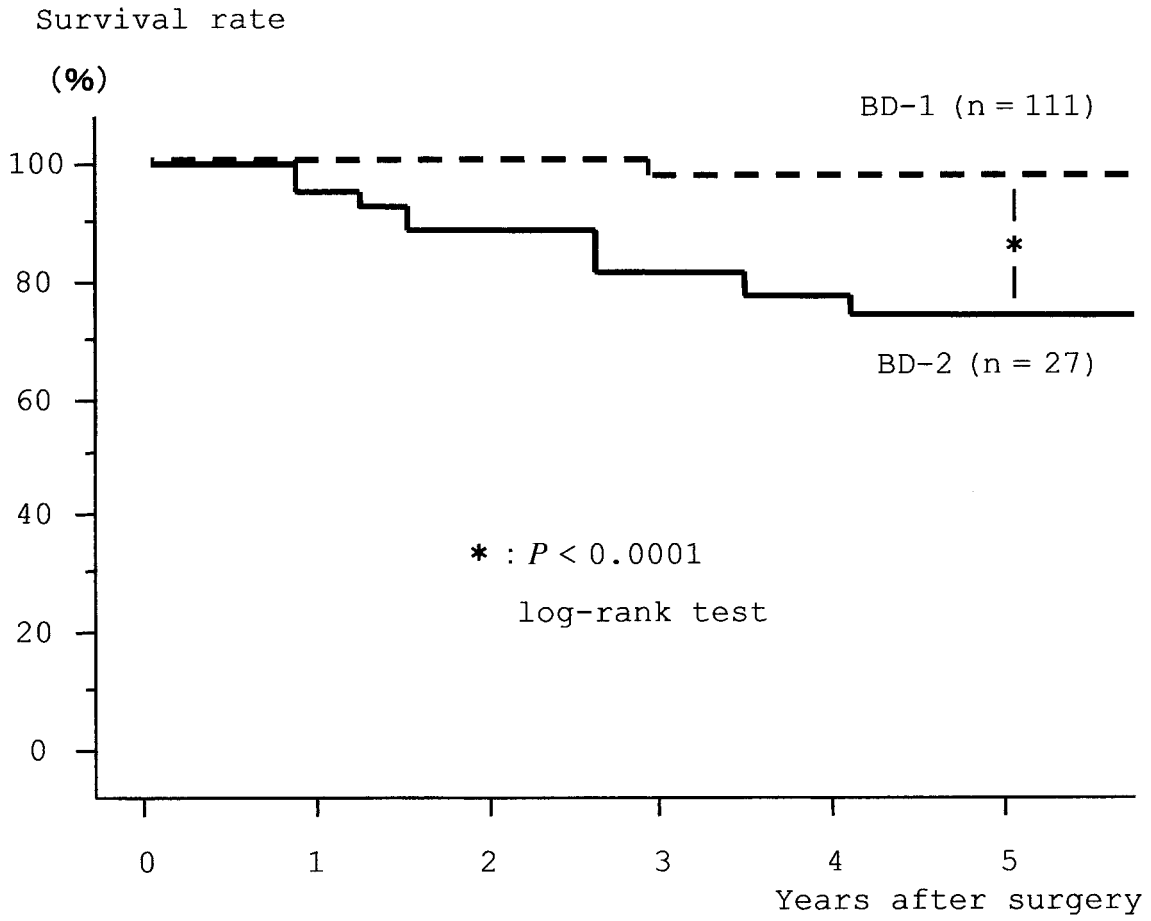

Figure 2. Disease-specific survival after surgery by budding grade.

thelial growth factor[10]), nodal micrometastases (p53,[11] cytokeratin[12]), immunohistochemical expression (p53,[13] c-erbB-2,[14] survivin[15]), S-phase fraction,[26] and aneuploidy.[27] However, these factors usually need additional and expensive examination and have not been used in further clinical studies. Although the literature provides some information about prognostic factors, such as pT3 classification,[20] perineural invasion,[21] tumor necrosis,[22] and type of invasive front,[23] which are also examined by the hematoxylin and eosin slides, they had no influence on prognosis in the present study.

Our results demonstrated that the presence of BD-2 indicated a high incidence of recurrence and poor prognosis when compared with that of BD-1 in Stage II, T3 colon cancer. The identification of a subset of node-negative patients with BD-2 could allow intensive postoperative follow-up and selective use of adjuvant chemotherapy.

Tumor budding can be examined by the hematoxylin and eosin technique in routine histopathological examinations for resected specimens without the burden of any additional procedures. Tumor budding differs from the classification of infiltrative growth

pattern of tumor margin expressed as infiltrative or pushing, which is usually examined by naked eye examination or low-power microscopy (×20). Tumor budding is examined by microscopic examination with special reference to the observation of microscopic clusters of undifferentiated tumor cells just ahead of the invasive front of the tumor.

In 1920, Broders[28] reported on the undifferentiated cancer cells that are seen in the invasive regions of the lip. He classified these cells into four grades, according to the proportion of undifferentiated cancer cells. He reported that there was a correlation between grade and prognosis. However, strictly speaking, Broders' classification differs from the definition of tumor budding in the size of clusters of cancer cells. Tumor budding has smaller clusters of fewer cancer cells than Broders' classification. The term *tumor budding* itself did not appear in the literature until Hase *et al.*[24] used it, and it was not reported in other organs. They reported that tumor budding reflects the biological activity of colorectal cancer, using clinicopathologic variables.[24,29] We also have reported the clinical significance of budding as a prognostic indicator to estimate the aggressiveness potential in rectal cancer[30] and as an indicator for treatment strategy for submucosal cancer[31] and colorectal liver metastases.[32]

For use in routine examination, the evaluation method should be simple, reproducible, and objective. Tumor budding can be identified and graded when performing a routine pathological examination. We observed one cut section containing the deepest portion of tumor penetration to evaluate the tumor budding grade, which is considered to be the simplest way to evaluate the biological attitude of cancer. However, the reproducibility and objectivity of the present study were not assessed. One of the authors[30] has reported that in the two-grade system based on the tumor budding intensity, the degree of grading agreement was nearly perfect based on the intraobserver study. Thus, it is meaningful for tumor budding to be entered into the clinical practice. The optimal evaluation system of budding as a routine examination has not been established and requires further investigation.

As we limited the subject of this study to patients with Stage II, T3 colon cancer, the number of patients may not be enough to draw a decisive conclusion. The further accumulation of patients with a prospective multi-institutional study may be required.

CONCLUSIONS

This study clarified that tumor budding correlated well with recurrence and survival in patients with Stage II, T3 colon cancer who underwent potentially curative surgery. We propose that tumor budding should be included in the pathological staging and used in the decision to use postoperative adjuvant chemotherapy for Stage II, T3 colon cancer patients.

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