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Predictive and prognostic impact of the different features of tumor budding in stage II colorectal cancer

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Tumor budding is a significant independent prognostic factor in colorectal cancer. Routine reporting of tumor budding is now advocated for in the colorectal cancer standard approach recommended by the International Tumor Budding Consensus Conference guidelines. However, the current tumor budding assessment system only emphasizes tumor budding quantity and ignores other features. Therefore, this study aimed to further determine the prognostic value of tumor budding based on a more comprehensive feature analysis. To this end, we conducted a retrospective pathology review of the different characteristics of tumor budding (that is quantity, structure, cell atypia, location, stromal reaction, and immunohistochemical phenotype) in 224 specimens of stage II colorectal cancer at our institution between 2009 and 2015. The mean age of the patients was 60.3±9.2 years (range, 39–84 years). Among various features of tumor budding, single-cell budding, anaplasia-like cell atypia, myxoid stroma, high tumor budding quantity, and loss of CDX2 expression were independent predictors of recurrence and mortality in patients with stage II colorectal cancer. Based on these results, we suggest that in addition to tumor-budding quantity, other tumor budding features play important biological roles in the development of colorectal cancer. Our findings provide prognostic information that could help with guiding clinical management and oncology care models for patients with stage II colorectal cancer.

Key words: colorectal cancer, recurrence, prognostic factor, tumor budding, predictive biomarker, prognostic biomarker, adjunct chemotherapy

The tumor-node-metastasis (TNM) classification system remains the gold standard for risk stratification in patients with colorectal cancer (CRC). However, heterogeneity in survival within each TNM stage indicates the need for additional prognostic factors. Tumor budding (TB) is defined as the phenomenological occurrence of a single tumor cell or small clusters of up to four tumor cells at the invasive tumor front or in the intratumoral stroma [1]. TB is a significant independent adverse prognostic factor in CRC [2] and is associated with a higher tumor grade, a higher TNM stage, lymphovascular invasion (LVI), and lymph node and distant metastases [3–5].

In CRC, TB is advocated as a quantitative prognostic factor for the optimal management of patients in three different clinical settings. First, in endoscopically resected pT1 CRC, TB indicates a high risk of lymph node metastasis. Therefore, patients with pT1 CRC and TB may require a second surgical resection [6, 7]. Second, stage II CRC with high-grade TB is associated with reduced recurrence-free survival when compared with stage II CRC with low-grade or no TB. Therefore, patients with stage II CRC and high-grade TB may be considered candidates for adjuvant therapy [8]. Lastly, intratumoral TB in preoperative biopsy specimens could help identify patients who require neoadjuvant therapy and potentially predict their therapeutic response [9–11].

TB is considered a morphological hallmark of epithelial-mesenchymal transition (EMT) [12–14], wherein transformed tumor cells acquire the ability to resist apoptosis, invade, and disseminate [15]. Several studies [16–18] have shown that TB was associated with the presence of immature tumor stroma. Therefore, a potential hypothesis for the molecular events in TB is that tumor stroma affects the local tumor microenvironment via the release of various cytokines, growth factors, and chemokines, which enhance EMT, thereby driving TB and metastasis.

Previous studies have confirmed that the quantity of TB played a significant role in the clinical management of stage II CRC, and the International Tumor Budding Consensus Conference (ITBCC) guidelines [19] provided a standardized counting system for routine reporting. However, not all TB-positive patients have a poor outcome. The current risk assessment system based on TB only focuses on TB quantity while ignoring other features of TB. Several other histologic features have been proposed. In many malignancies, the presence of tumor anaplasia is a major adverse prognostic indicator [20]. Carcinomas with overt anaplasia are classified as being high-grade or poorly differentiated. Furthermore, the tumor microenvironment is reflected by the type of tumor stroma [21]. An inflammatory stroma, which is rich in tumor-infiltrating lymphocytes, is a good prognostic factor [22]. An immature myxoid stroma at the invasion front is a reliable poor prognostic factor for stage II CRC [18]. Immunohistochemical studies of TB indicate that TB can be expressed by epithelial and mesenchymal markers in some cases [12], and the immunophenotype of TB may be different from that of the main tumor. Therefore, abnormal immunohistochemical expression, abnormal features, or density of vessels in different layers of TB may have prognostic significance. In daily clinical practice, varying degrees of TB are observed in most CRCs. Therefore, this retrospective study aimed to analyze different characteristics of TB (including TB quantity, structure, cell atypia, location, stromal reaction, and immunohistochemical phenotype) to further evaluate the risk stratification utility of TB features in CRC.

Patients and methods

Ethics approval and informed consent. The study design was approved by the Ethics Committee of Jilin Central Hospital. The requirement for informed consent was waived owing to the retrospective nature of the study.

Patients and sample collection. This study included patients with CRC who were admitted to Jilin Central Hospital (Jilin City, Jilin Province, China) between January 2009 and December 2015. Patients who had received preoperative neoadjuvant therapy were excluded. Among 1,755 patients with CRC, 224 with stage II (pT3/4 and pN0) CRC with follow-up data were selected for the analysis. All 224 CRC cases were positive for TB (TB counting ≥1). We included cases of well-differentiated to moderately differentiated adenocarcinoma. We also excluded those of specific histological subtypes of adenocarcinoma such as poorly cohesive carcinoma, micropapillary adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, and medullary adenocarcinoma. The median follow-up duration was 42.0 months. Supplementary Figure S1 shows the inclusion and exclusion criteria for participation in this study.

Evaluation of histological features. The initial clinical and pathological stages of the disease in all patients were revised according to the American Joint Committee on Cancer staging system (8th edition). Hematoxylin and eosin-stained slides for each CRC case were reviewed independently by two pathologists. The histological type and grade were defined according to the latest World Health Organization classification system. In all specimens, the following histological features were evaluated: tumor size, LVI, perineural invasion (PNI), TB construction (single-cell or cluster), TB location (submucosa, muscularis propria, or subserosa), TB cell atypia (nonspecific or anaplasia-like), TB stromal reaction (inflammatory, fibrotic, or myxoid), and TB quantity. TB was defined as the dissociation of small tumor complexes containing < 5 cells that "budded" into the peritumoral stroma. TB was scored by two observers according to the ITBCC guidelines [19]. Hematoxylin and eosin-stained sections were evaluated at medium power magnification (×20) to determine the densest area of TB at the invasive tumor front ("hotspot"). Tumor bud cell anaplasia was defined as any ×400 magnification field with ≥3 nuclei with a diameter of ≥5 lymphocyte nuclei) [20]. An inflammatory stroma is characterized by the presence of lymphocytes infiltrating the surrounding tumor microenvironment. A fibrotic stroma has neither a myxoid nor inflammatory, but it typically consists of only fine mature collagen fibers stratified into multiple layers in all reactive fibrous zones. A myxoid stroma is characterized by an amorphous stromal substance comprising an amphophilic or a slightly basophilic extracellular matrix When a mixed pattern was present, the predominant type was considered. TB features were independently evaluated by two single-blinded pathologists to reduce interobserver variability. The final classifications of TB features were determined based on the agreement between at least two pathologists.

Immunohistochemistry. Immunohistochemical analysis was performed as described previously [23]. Tissue sections were stained using the following primary antibodies: rabbit monoclonal CDX2 (EP25; Zhongshan Golden Bridge Biotechnology LLC, Beijing, China; ready-to-use), Ki-67 (30-9; Ventana, Tucson, AZ, USA; ready-to-use), epidermal growth factor receptor (EGFR; EP22; Zhongshan Golden Bridge Biotechnology LLC; ready-to-use), p53 (4A4+UMAB4; Zhongshan Golden Bridge Biotechnology LLC; ready-to-use), BRAF V600E (VE1; Ventana; ready-to-use), and microsatellite instability (MSI) proteins, including MLH1 (ES05), PMS2 (EP51), MSH2 (RED2), and MSH6 (EP49) (Zhongshan Golden Bridge Biotechnology LLC; ready-to-use).

CDX2 and EGFR immunohistochemical staining was performed as described previously [24]. The extent to which TB cells were stained (0–100%) and the staining intensity (0, negative; 1, light brown; 2, brown; 3, dark brown) were evaluated. The final scores were defined as the products of the extent and intensity scores. Next, each case was scored as high or low using the median final score as the cut-off point for the log-rank test. P53 immunohistochemical staining patterns were classified into two subgroups: (a) wild-type pattern,

indicated by scattered strong or moderate nuclear staining in tumor cells; (b) mutant pattern, in which the majority of tumor cells (>60% of tumor cells and virtually 100% in most cases) showed diffuse strong nuclear positivity, or the tumor cells were completely devoid of any staining [25]. Only staining for cytoplasmic BRAF V600E was considered positive [26]. MSI status was classified into two subgroups: a) MSI-high (MSI-H), if any one of the four mismatch repair proteins (MLH1, PMS2, MSH2, and MSH6) was nuclear-negative in all tumor cells, but positive in internal controls; b) MSI-stable (MSS) or MSI-low (MSI-L), if all four mismatch repair proteins were positive in cancer cells [27]. The p53- and BRAF-staining patterns and MSI status were reported by two single-blinded observers.

Statistical analyses. R software (version 3.6.1, www.r-project.org) was used for all statistical analyses. The R statistical packages "rms," "barplot," "survival," "Hmisc," "MASS," and "pROC" were used to plot the distribution of risk scores and recurrence or distant metastasis (RDM), and plot calibration, ROC curves, build a nomogram, and draw Kaplan-Meier curves, while "rmda" was used to draw the DCA curves and "forestplot" was used to draw the forest plot.

The clinicopathological findings and TB features of the CRC specimens were compared using the chi-square or Fisher's exact test for categorical variables. The nonparametric Mann-Whitney *U* test was used to analyze age, tumor size, Ki-67 labeling index, and TB quantity because these data were not normally distributed.

Multivariate logistic regression analyses were performed to identify significant independent TB features for predicting recurrence. Variables with $p<0.1$ in the univariate analysis were included in the multivariate analyses.

Patient survival rates were analyzed using the Kaplan-Meier method and log-rank test. p-values were obtained based on two-tailed statistical analysis, and the significance level was set at 5% ($p<0.05$). A multivariate Cox proportional hazards model with the stepwise Wald method was used to obtain the hazard ratio (HR) of TB features for recurrence by adjusting for covariates.

Results

Demographic and clinical findings. The baseline clinicopathological characteristics of the participants are summarized in Table 1. 37% (83/224) of CRC patients had distant metastasis during postoperative follow-up, and 17 of them had local recurrence. All the patients had tumors with negative surgical margins. The number of harvested lymph nodes was at least 12, and there was no case of perforation or intestinal obstruction. The mean age was 60.3±9.2 years (range, 39–84 years). RDM was correlated with advanced age ($p=0.009$) and lymphovascular invasion (LVI) ($p=0.004$). There were no statistically significant differences between RDM and sex, tumor site, T stage, tumor size, perineural invasion, *TP53* status, *BRAF* mutation, or MSI status.

Table 1 summarizes the association between RDM and the studied TB features. Single-cell construction, anaplasia-like cell atypia, myxoid stroma, and loss of CDX2 expression were observed in 34 (41%), 12 (14.5%), 31 (36.1%), and 24 (28.9%) patients, respectively, in the recurrence group (Figures 1A–1D). These differences were statistically significant ($p=0.001$, $p=0.014$, $p<0.001$, and $p<0.001$, respectively). TB quantity was significantly higher in the RDM group than in the non-RDM group (9.5±4.2 vs. 14.0±3.6; $p<0.001$). Differences in TB location, EGFR status, and Ki-67 labeling index did not reach statistical significance (Figures 1E–1H).

TB features and predictors of recurrence or distant metastasis. Finally, 224 patients were included and randomly allocated to a training cohort (n=162) and an internal validation cohort (n=62) using a ratio of 3 to 1 based on the data splitting approach [28]. Based on the univariate logistic regression analysis results in the training cohort, five factors, including TB construction, TB cell atypia, TB stromal reaction, TB quantity, and TB CDX2 expression were linked to RDM status (Figure 2A). TB construction (single-cell vs. cluster; odds ratio [OR], 7.483; 95% confidence interval [CI]: 2.040–27.445), TB cell atypia (nonspecific vs. anaplasia-like; OR, 2.532; 95% CI: 0.168–2.793), TB stromal reaction (myxoid vs. fibrotic; OR, 21.051; 95% CI: 6.4357–68.857), TB quantity (high vs. low; OR, 3.782; 95% CI: 1.781–8.029), and TB CDX2 expression (low vs. high; OR, 5.993; 95% CI: 1.3911–25.822) remained independent predictors of RDM in the multivariate analyses (Supplementary Table S1).

The calibration curve of the nomogram was highly consistent with the standard curve, indicating high reliability of the nomogram's prediction ability (Figure 2B). The decision curve analysis (DCA) curves for the developed nomogram and TB quantity in the training and internal validation cohorts are shown in Figures 2C and 2D. Compared with TB quantity, the DCA of the nomogram showed higher net benefits, indicating that it had better clinical outcome values than TB quantity (Figures 2E, 2F).

Survival analyses. To identify the variables for building the OS predictive nomogram, Cox univariate and multivariate regression analyses were performed in the training cohort. Overall survival was significantly associated with TB construction, TB cell atypia, TB stromal reaction, TB quantity, TB CDX2 expression, and EGFR expression (Figure 3A). In multivariate Cox proportional hazards model, TB construction (single-cell vs. cluster; HR, 2.983; $p=0.014$), TB cell atypia (anaplasia-like vs. nonspecific; HR, 8.065; $p=0.003$), TB stromal reaction (myxoid vs. fibrotic; HR, 5.464; $p<0.001$), TB quantity (high vs. low; HR, 1.542; $p<0.001$), TB EGFR expression (low vs. high; HR, 0.185; $p=0.039$), and TB CDX2 expression (low vs. high; HR, 10.619; $p=0.003$) were independent predictors of mortality (Supplementary Table S2). We used these seven variables to build a predictive OS nomogram (Figure 3B). C-indices of the OS nomogram and TB quantity were 0.799 and 0.605, respectively. Calibration curves based on the seven variables are shown in Figures 3C and 3D.

Table 1. Demographic and clinicopathological features of 224 patients with colorectal cancer.

Variables		All patients	Recurrence or distant metastasis		p-value
			Absent No. (%)	Present No. (%)	
Age (years)[a]		60.3±9.2 (39–84)	57.1±9.7 (39–84)	62.3±7.9 (43–80)	0.009
Sex	Male	106	68 (48.2)	38 (45.8)	0.723
	Female	118	73 (51.8)	45 (54.2)	
Tumor site	Proximal colon	164	100 (70.9)	64 (77.1)	0.313
	Distal colon or rectum	60	41 (29.1)	19 (22.9)	
T stage	T3	192	122 (86.5)	70 (84.3)	0.651
	T4	32	19 (13.5)	13 (15.7)	
Tumor size (mm)[a]		53.9±15.3 (23.1–73.4)	53.9±16.3 (23.1–73.4)	53.8±13.2 (27.8–68.2)	0.11
Lymphovascular invasion	Absent	112	81 (57.4)	31 (37.3)	0.004
	Present	112	60 (42.6)	52 (62.7)	
Perineural invasion	Absent	135	88 (62.4)	47 (56.6)	0.393
	Present	89	53 (37.6)	36 (43.4)	
TP53	Wild-type	86	49 (34.8)	37 (44.6)	0.144
	Mutant	138	92 (65.2)	46 (55.4)	
BRAF	Low	203	128 (90.8)	75 (90.4)	0.917
	High	21	13 (9.2)	8 (9.6)	
	Not available	8	–		
MSI	Stable	198	127 (94.1)	71 (87.7)	0.098
	Unstable	18	8 (5.9)	10 (12.3)	
Ki-67 status (%)[a]		73.5±14.5 (33.5–97.5)	73.5±11.5 (33.5–93.5)	77.5±12.5 (38.5–97.5)	0.732
TB construction	Single-cell	63	29 (20.6)	34 (41.0)	0.001
	Cluster	161	112 (79.4)	49 (59.0)	
TB location	Submucosa	20	11 (7.8)	9 (10.8)	0.318
	Muscularis propria	154	102 (72.3)	52 (62.7)	
	Subserosa	50	28 (19.9)	22 (26.5)	
TB cell atypia	Nonspecific	205	134 (95.0)	71 (85.5)	0.014
	Anaplasia-like	19	7 (5.0)	12 (14.5)	
	Inflammatory	46	30 (21.3)	16 (19.3)	
TB stromal reaction	Fibrotic	132	96 (68.1)	36 (43.4)	<0.001
	Myxoid	46	15 (10.6)	31 (37.3)	
TB quantity[a]		11.2±4.5 (2–23)	9.5±4.2 (2–18)	14.0±3.6 (7–23)	<0.001
EGFR	Negative	93	65 (46.1)	28 (33.7)	0.070
	Positive	131	76 (53.9)	55 (66.3)	
CDX2	Negative	36	12 (8.5)	24 (28.9)	<0.001
	Positive	188	129 (91.5)	59 (71.1)	

Notes: [a]data are expressed as mean ± standard deviation; all cases were stage II and had no lymph node metastasis
Abbreviations: MSI-microsatellite instability; TB-tumor budding

There was good agreement between actual and nomogram-predicted probabilities for 5-year OS, in the training and validation cohort, respectively. We compared the predictive powers of the OS nomogram with that of the conventional system based on TB quantity using ROC curve analysis. Our nomograms displayed better discriminatory powers in predicting postoperative OS in the derivation cohort than those competing models did. For the OS nomogram, the C-index was 0.799 (95% CI, 0.77–0.86), substantially higher than the TB quantity alone (Figures 3E, 3F).

Based on the OS nomogram's score, patients could be divided into low risk (score ≤120) and high risk (>120) of recurrence. We used Kaplan-Meier curves and the log-rank test to analyze OS in patients with CRC after stratification by OS nomogram (low-risk vs. high-risk) and TB quantity (low TB quantity was defined as ≤8 vs. high TB quantity was defined as >8) (Supplementary Figure S2).

Discussion

Our study showed that the histological and immunohistochemical features of TB in CRC specimens were predictive of tumor behavior. Among various features of TB, single-cell construction, anaplasia-like cell atypia, myxoid stroma, high TB quantity, and loss of CDX2 expression were independent predictors of recurrence and mortality in patients

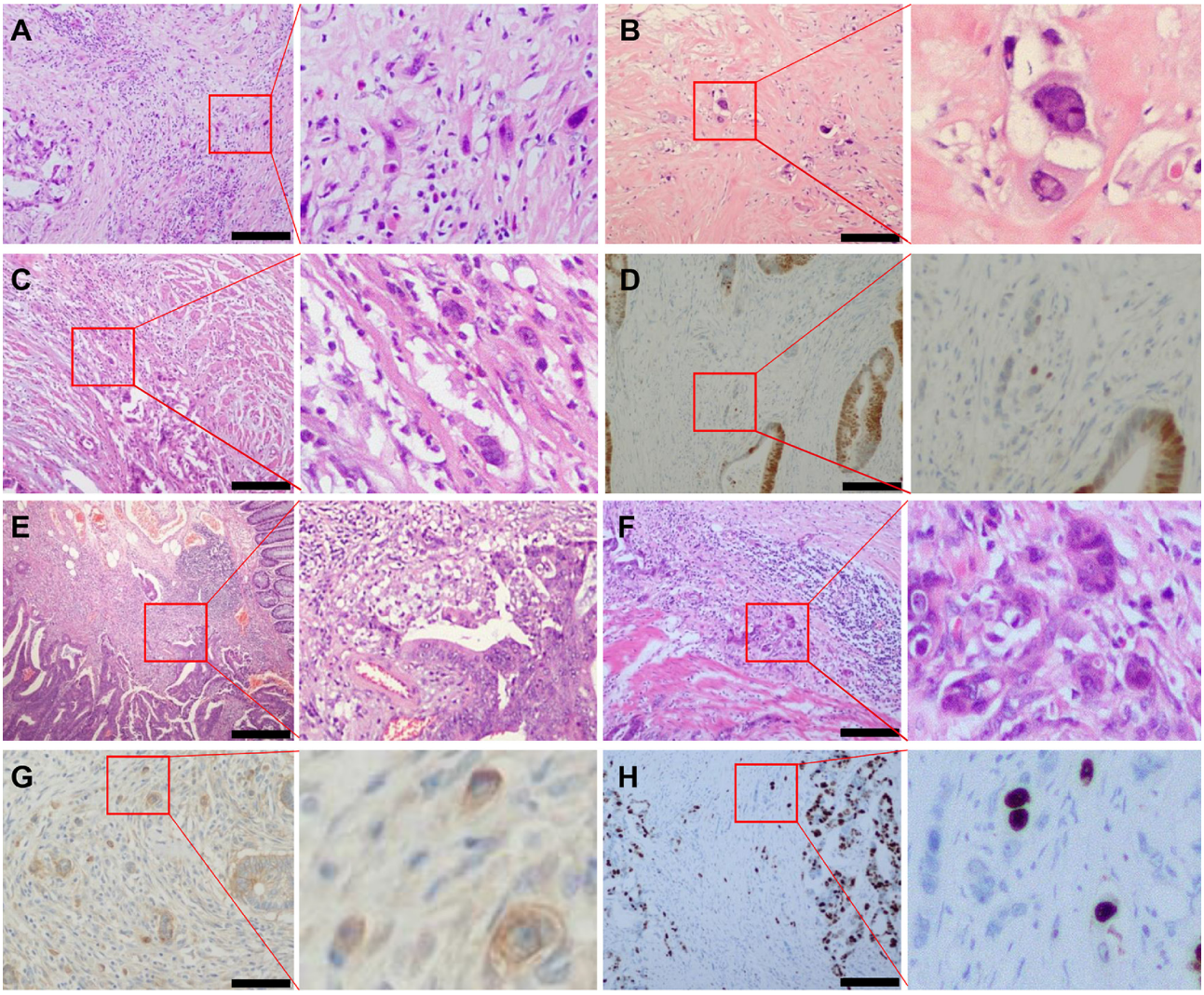

Figure 1. Histological and immunohistochemical features of tumor budding in colorectal cancer specimens. A) Representative photomicrograph of high-grade tumor budding showing a primary single-cell pattern. B) Photomicrograph of tumor budding characterized by high-grade anaplasia-like cell atypia. C) Myxoid stroma indicated by an amorphous stromal substance constituting a slightly basophilic extracellular matrix. D) Tumor budding with loss of CDX2 expression, as shown by a negative result for CDX2 immunohistochemical staining, relative to the primary tumor with weak staining. E) Tumor budding primarily present in the submucosa of colorectal cancer. F) Tumor budding primarily present in the muscularis propria and subserosa of colorectal cancer. G) Tumor budding showing positive epidermal growth factor receptor expression in the cytoplasm and membrane. H) Immunohistochemical staining for Ki-67 in tumor budding (scale bar = 50 µm).

with CRC. TB in CRC, especially stage II CRC, is a significant prognostic factor that is simple to use and can be easily assessed using routine light microscopy on hematoxylin and eosin-stained slides. A standardized consensus approach has been recommended by the ITBCC guidelines [19] for TB counting in routine diagnostics. However, the current TB scoring system focuses only on its quantity and ignores other features of TB. In addition, few studies have focused on the morphological heterogeneity among TB patterns in the field of CRC histopathology. To the best of our knowledge, this is the first study to investigate the different features of TB to develop a more precise risk stratification system than that using TB quantity alone. We showed that the histological and immunohistochemical features of TB, including its quantity, were independently associated with a significantly worse prognosis. Moreover, our results show that TB construction, cell atypia, stromal reaction, and CDX2 expression status could help further stratify patients with TB-positive CRC.

Morphological grading has remained an important indicator of prognostic stratification in patients with cancer. However, compared with the number of studies on the use of histological grade for risk stratification, few studies have examined the role of the histological grade of TB in CRC. The data analysis in this study revealed that high-grade

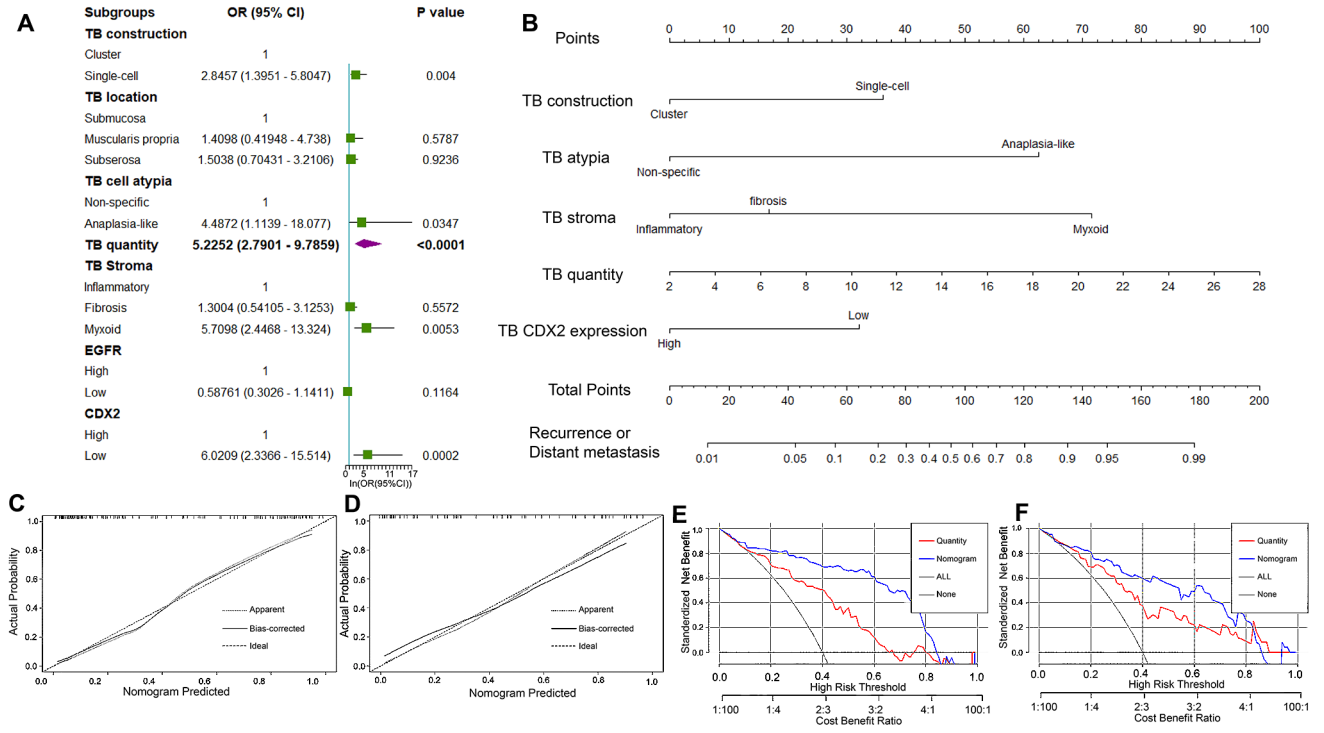

Figure 2. Predicted model of recurrence or distant metastasis. A) Forest plots to decipher the risk factors associated with recurrence or distant metastasis identified in multivariate logistic regression analysis. B) Newly developed nomogram for predicting recurrence or distant metastasis in stage II CRC patients. The calibration curve for predicting recurrence or distant metastasis of stage II CRC patients in the C) training and D) internal validation cohorts. Decision curve analysis of the nomogram and TB quantity alone for predicting recurrence or distant metastasis in stage II CRC patients in the E) training cohort and F) internal validation cohort. The gray line and black line represent the assumption regarding all patients with and without RDM, respectively.

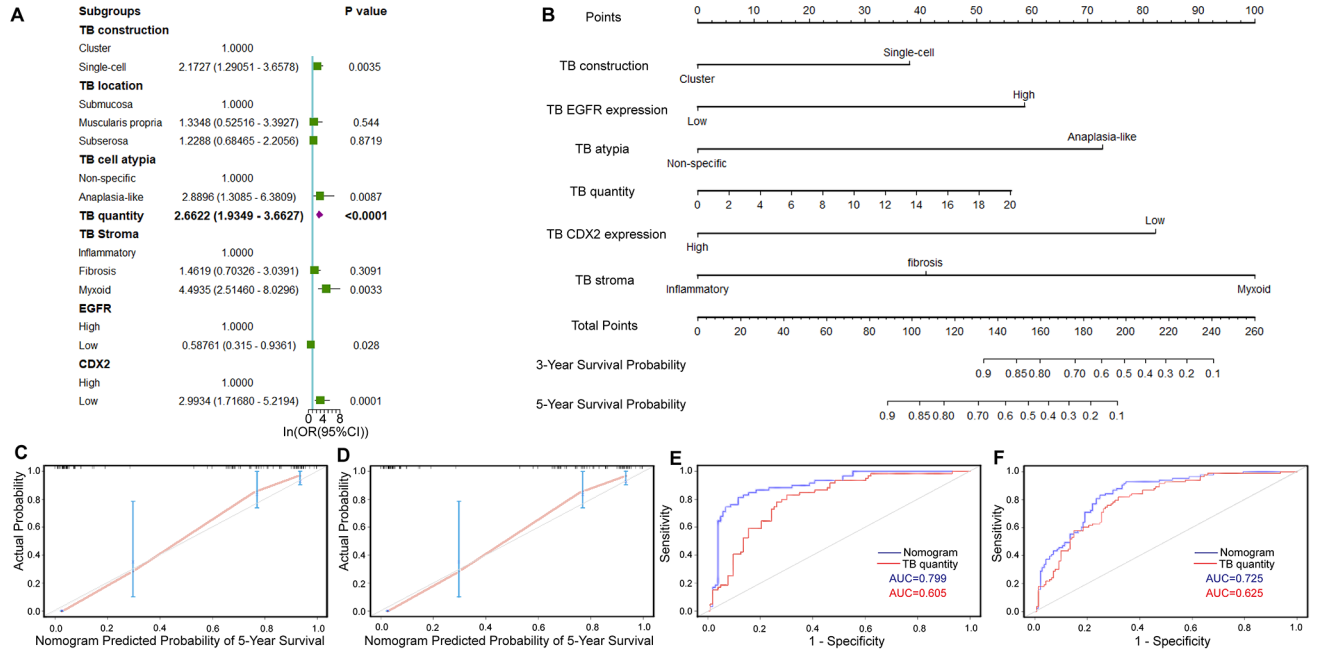

Figure 3. Predicted model of overall survival (OS). A) Forest plots to decipher the risk factors associated with OS identified in multivariate Cox regression analysis. B) OS predictive nomogram. The calibration curve of postoperative OS in stage II CRC patients in the C) training cohort and D) internal validation cohort. Comparison of predictive accuracy between OS-nomogram and TB quantity alone in the E) training cohort and F) internal validation cohort.

anaplasia-like cell atypia in TB was a significant prognostic factor. The environment at the tumor invasive front is not static, unlike that in the tumor core. Thus, the tumor cells at the invasive edge are more reflective of the biological behavior of the tumor [29, 30]. There has been an increasing interest in the role of the tumor microenvironment in cancer prognosis, particularly in the roles of lymphocytic infiltrates and stromal reactions. Tumor cells cannot act alone; the cross-talk between tumor cells and the surrounding stroma creates a dynamic tumor microenvironment that influences tumor progression [21, 31]. EMT is an underlying molecular mechanism of TB. In this study, fibrotic stroma was the most common finding in TB (58.9%), whereas inflammatory and myxoid stromal reactions were less common. Although the prognosis was worse in patients with TB-positive CRC, a myxoid stroma was an independent predictor of recurrence and mortality in patients with TB-positive CRC. A myxoid stroma is considered an immature stroma [16], which is a histological feature that predicts aggressive tumor behavior with a high potential to disseminate and metastasize [18]. Consistent with our findings, several studies [18, 32] have determined that a histological stroma categorization system could serve as a prognostic factor in stage II CRC. The composition of tumor-infiltrating lymphocytes within an inflammatory stroma has proven to be a useful biomarker for predicting therapeutic response and OS [33, 34]. Furthermore, in this study, the presence of an inflammatory stroma was associated with a favorable prognosis, although this association was not statistically significant. Therefore, our findings indicate that both TB counts and stromal features surrounding TB should be evaluated in CRC.

CDX2 has been reported to be a tumor suppressor and prognostic factor in CRC [35]. Although some meta-analyses [36–38] have reported that immunohistochemical staining for CDX2 may be a potential prognostic factor for CRC, the application of CDX2 as a biomarker remains controversial [39]. The reasons for that controversy arise from the low rate of CDX2 silencing (2–10%) in CRC [40]. Previous studies have shown that patients with a high TB count have more lymph node metastases and poorer prognoses [19]. These results suggest that TB is a concomitant finding that appears in association with tumor progression. However, their causal relation remains unclear, and the biological nature of TB has not been well established. Our data showed that CDX2 expression status differed between TB and the primary tumor. Loss of CDX2 expression in TB was observed in 16.1% (36/224) of CRC cases; furthermore, the rate of CDX2 expression loss was higher in TB than in the primary tumor, and it was associated with a poor prognosis. CDX2 is an intestine-specific transcription factor implicated in the proliferation, adhesion, differentiation, and migration of tumor cells [41]. A recent study [42] determined that CDX2 inhibited EMT and metastasis of CRC. Moreover, a previous study showed that CDX2 loss in poorly differentiated clusters is associated with poor prognosis in CRC [43]. Thus, loss of CDX2 expression in TB may reflect the dynamic EMT process. This may explain why TB has a significant adverse impact on patient outcomes. It is recommended that patients with the TB features of single-cell construction, anaplasia-like cell atypia, myxoid stroma, high TB quantity, and loss of CDX2 expression should receive more aggressive therapy, such as adjuvant systemic radiotherapy, to improve prognosis.

This study has several limitations. First, the statistical power was limited because this was a retrospective single-center study. Second, due to the retrospective study design, potential selection biases could not be ruled out. Third, the follow-up period was relatively short (median, 3.5 years). Finally, although the study focused on identifying the most significant predictor of recurrence, it is unclear whether the findings can be generalized. Due to the limited number of cases in the cohort, our study did not evaluate other clinicopathological features. However, our study focused on the prognostic significance of the novel TB assessment system in patients with the same stage, which is also conducive to individualized clinical management of the patients.

In addition to TB quantity, the histological and immunohistochemical features of TB were shown to be risk factors for recurrence and mortality in patients with CRC, and therefore, should be incorporated into the routine reporting of CRC. Our results may be meaningful in guiding the clinical management of CRC. Further studies with larger sample sizes and a multicenter design are needed to confirm these initial findings.

Supplementary information is available in the online version of the paper.

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Predictive and prognostic impact of the different features of tumor budding in stage II colorectal cancer

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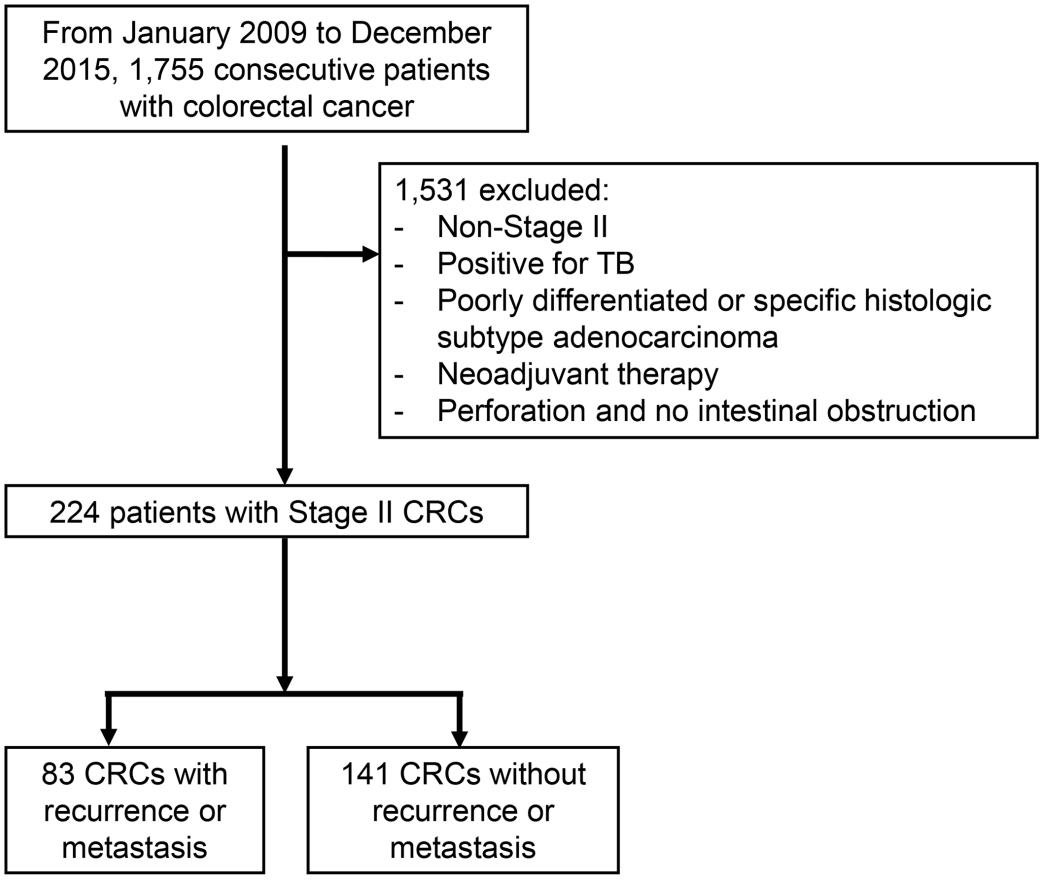
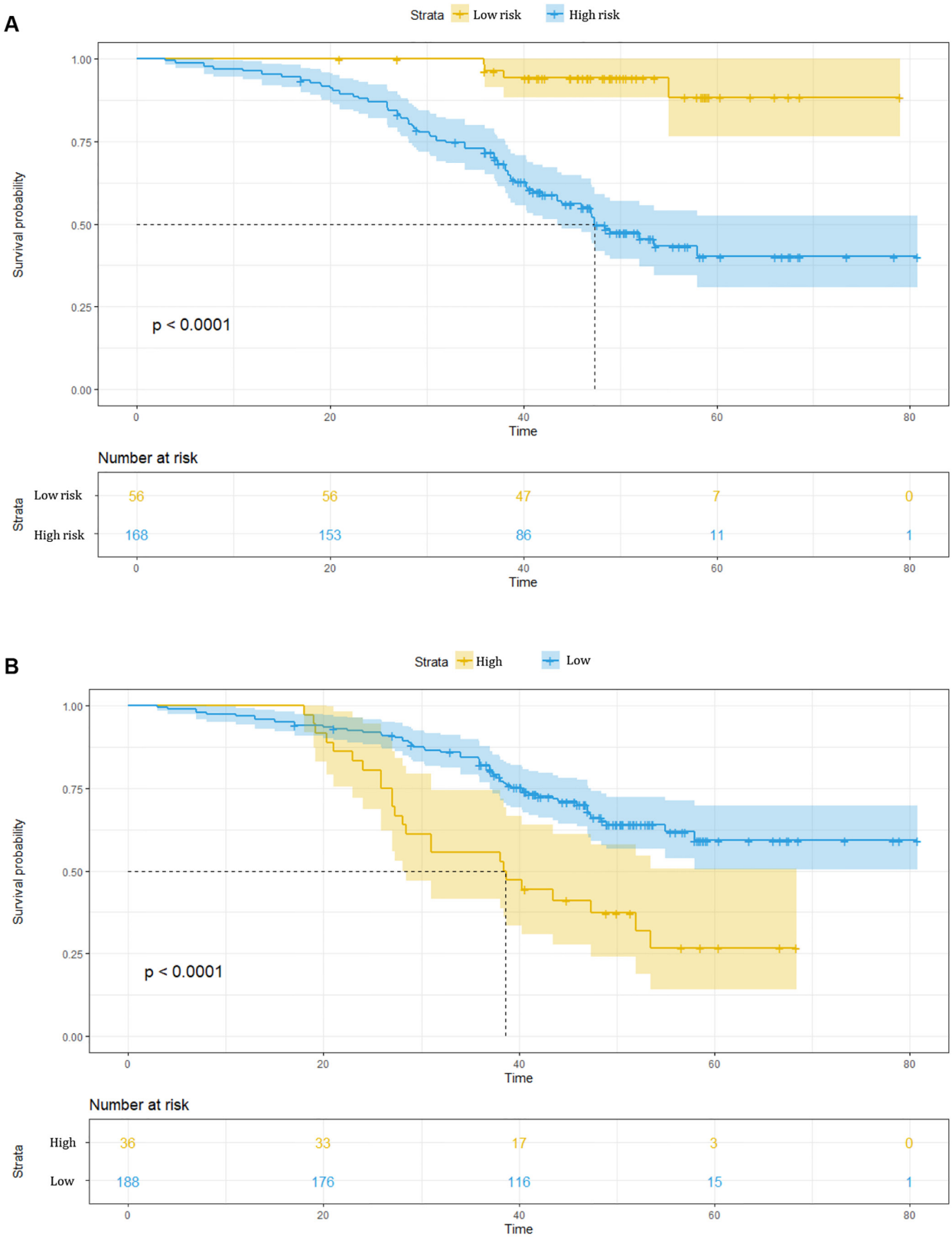
Supplementary Information



Supplementary Figure S1. Flowchart of patient inclusion and exclusion.



Supplementary Figure S2. Survival curves for subgroup analysis in patients with different risks of postsurgical mortality stratified by nomogram score and TB quantity score. A) OS according to OS nomogram. B) OS according to tumor budding quantity (<8 vs. ≥8). Abbreviation: OS-overall survival