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Prognostic value of programmed death ligand 1, p53, and Ki-67 in patients with advanced stage colorectal cancer

Lisha Wang MD, PhD[1,2,3†], Zebing Liu MD, PhD[4†], Kurt W. Fisher MD[5], Fei Ren MD, PhD[1,2], Jiaojie Lv MD[1,2], Darrell D. Davidson MD[5], Lee A. Baldridge[5], Xiang Du MD, PhD[1,2*], Liang Cheng MD[5*]

[†]These authors contributed equally to this study

From the [1]Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai, China; [2]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China; [3]Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI, USA; [4] Department of Pathology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [5]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA.

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*Corresponding authors at : Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 350 West 11th Street, Indianapolis, IN 46202, USA (Email: liang_cheng@yahoo.com) or Department of Pathology, Fudan University Shanghai Cancer Center and Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China (Email: dx2008cn@163.com)

Abstract

Current prognostic indicators are ineffective for identifying advanced stage colorectal cancer (CRC) patients with high risk of recurrence after surgical resection. We investigated the prognostic value of p53, Ki-67, and programmed death ligand 1 (PD-L1) in 254 patients with stage II and III CRC. The expression of p53 was positive in 63% of cases. Up-regulation of p53 was associated with smaller tumor size (*P*=0.001) and higher Ki-67 labeling index (LI) (*P*=0.031). The tumor Ki-67 LI was high (≥ 20%) in 197 (78%) of the patients. High Ki-67 LI was associated with higher TNM stage (*P*=0.031), positive p53 expression (*P*=0.031), and negative PD-L1 expression (*P*=0.003). The five-year relapse-free survivals (RFS) were 53% and 89%, respectively, for the p53-positive and Ki-67 LI-high patients and the p53-negative and Ki-67 LI-low patients (*P*<0.001). In univariate analysis, negative p53 (*P*= 0.001), low Ki-67 LI (*P*=0.006), low PD-L1 expression (*P*=0.044), low TNM stage (*P*<0.001), recto-sigmoid location (*P*=0.026), and small size (*P*=0.013) were significantly related to RFS. In multivariate Cox regression analysis, positive p53 expression (hazard ratio [HR]: 2.48; 95% confidence interval: 1.34-4.59, *P*= 0.004), high Ki-67 LI (HR: 2.62; 95% CI: 1.12-6.14, *P*=0.027) and high TNM stage (HR: 2.598, 95% CI: 1.55-4.37, *P*<0.001,) were independent predictors of unfavorable prognosis. In summary, PD-L1, Ki-67, and p53 staining individually had significant prognostic value for patients with stage II and III CRC. Moreover, combining p53 H-score ≥ 35 and Ki-67 LI ≥ 20% identifies patients with poor clinical outcome.

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world, and its burden is expected to increase 60% by 2030 to more than 2.2 million new cases and 1.1 million deaths (1-3). Current prognostic factors are still insufficient for identifying patients at high risk of recurrence after surgery for stage II and III disease. These patients may benefit from aggressive adjuvant treatment. It would also be desirable to identify patients at low risk of recurrence who may safely be spared the morbidity and expense of adjuvant interventions after surgery (4-7).

TP53, a well-known tumor suppressor gene, encodes a nuclear protein that is a nexus for responses to various cellular stresses. Activation of the p53 pathway results in cell cycle arrest, apoptosis, or senescence (8). Inactivation of p53 is a key event in CRC progression and correlates with transitions from benign adenomas to malignant carcinomas (9). Although many investigations have addressed the prognostic value of p53 in CRC patients, no consistent conclusions have been drawn (10, 11). Cellular proliferation rate may be another tool to stratify CRC risk groups. The MIB-1 antibody recognizes an epitope on the Ki-67 cell cycle progression protein and is frequently used to measure proliferation or to predict behavior for many malignancies. In CRC patients, however, the Ki-67 index has not always had a significant association with clinical outcome (11). Finally, antitumor immune responses portend a favorable outcome for many tumors (12). Multiple cancer types implement an “immune shield” against inflammatory cell attack by expressing programmed death ligand 1 (PD-L1). This surface receptor on the tumor cell generates an immunosuppressive microenvironment and allows the tumor cell with foreign epitopes to escape immune-mediated destruction (13). PD-L1 ligand binding to programmed death-1 (PD-1) receptors on T-cells results in suppression of CD-8+ antigen-specific cytotoxic cell proliferation and causes apoptosis of PD-1 positive lymphocytes.

PD-L1 expression in tumor specimens is used as a predictive marker for tumor response to anti-PD-1 or anti-PD-L1 immunotherapy (14-16). The present investigation was designed to assess the value of using p53 and Ki-67 as prognostic markers either alone or in combination with PD-L1 in patients with stage II and III CRC.

Materials and Methods

Patients

Tumor samples were obtained from 254 consecutive patients who had undergone surgical resection for CRC without preoperative treatment at the Fudan University Shanghai Cancer Center (FUSCC) from 2007 through 2009. The inclusion criteria were as follows: primary sporadic colorectal adenocarcinoma (mucinous carcinoma and other less common variant types were excluded), 28-75 years of age, and no preoperative chemotherapy or radiotherapy (17). Patients were included if the final pathology report of the CRC included the tumor location, whether colo-cecal or recto-sigmoid, and tumor size by greatest dimension, whether lateral or radial. The patients were staged according to the American Joint Committee on Cancer/International Union against Cancer (AJCC/UICC) TNM staging system (2010, 7th edition) (18). All the patients had advanced-stage colorectal cancer (≥ T2). Histologic grading (differentiation) was based on the 2010 WHO classification of tumors of the digestive system (4th edition) (19). The study was carried out with the approval of the local Ethics Committee of FUSCC.

Tissue Microarray and Immunohistochemistry

Tissue microarrays (TMA) were constructed from paraffin-embedded blocks of the 254 CRC primary tumors as previously described(17). Each case in the TMA was represented in duplicate cores, 1 mm in size for each core. Each TMA block contained 118 individual tissue cores. Immunoreaction for p53 (DO-7 clone; Dako, Carpinteria, CA, USA; prediluted), Ki-67 (MIB-1 clone; Dako, Carpinteria, CA; prediluted) and PD-L1 (SP142, Spring Bioscience, Pleasanton, CA, USA; pre-diluted) were performed using the FLEX Detection System (Dako, Carpinteria, CA). Diaminobenzidine (3, 3-diaminobenzidine) was used as the chromogen. For antigen retrieval EnVisionTM FLEX Target Retrieval (Tris/EDTA) Solution, high pH (Dako, Carpinteria, CA) was used. Immunostaining was performed on the DAKO Autostainer Plus (EnVisionTM FLEX+). Positive and negative controls were stained concurrently and showed appropriate immunostaining. PD-L1 expression was assessed in tumor cells as well as tumor-infiltrating immune cells (i.e., immune cells present both within the tumor and around the tumor periphery) (17). IHC evaluation considered p53 and Ki-67 expression only in the nuclei of tumor cells (**Figure 1**). The interpretation of immunoreactivity for p53 was evaluated using the "hybrid scoring system" (H-score) criteria. An H-score was calculated as the sum of the product of the staining intensity in tumor cells (0, no staining; 1, weak staining; 2, moderate staining; 3, intense staining) and the proportion of cells showing that staining intensity (0–100%). If more than one staining intensity was present, the H-score was the sum of the h-scores for each intensity level. Thus, the possible H-score ranged from 0 to 300(20). Currently there is no consensus cutoff for p53 immunohistochemistry in CRC. In this study, cutoff score for p53 positivity was optimized using receiver operating characteristic (ROC) curve analysis (**Figure 2**). An H-score ≥ 35 defined a positive p53 expression; and an H-score <35 defined a negative p53 expression. For

Ki-67 labeling index (LI), each TMA core was scored based on the percentage of positively stained malignant nuclei regardless of stain intensity. Immunoreactivity to Ki-67 was "low" if nuclear staining of tumor cells was <20% (Ki-67 LI-low) and "high" if ≥20% (Ki-67 LI-high), as previously described (21). For each tissue sample, H-score for p53 and Ki-67 LI was averaged across evaluable TMA tissue cores.

Statistical Analysis

To determine the association of p53 expression with clinico-pathological features, cutoff scores were determined by ROC curve analysis. The ROC curve is a plot of the sensitivity and (1-specificity) for an outcome at each value of the protein expression score. An H-score can therefore be selected from the curve such that a cutoff at this value leads to the greatest number of patients correctly classified as with (maximizing sensitivity) and without (maximizing specificity) the clinical end point (22). ROC curve analysis was performed for stages and survival. The most frequently obtained H-score was selected as the final cutoff score above which tumors should be considered positive for the outcome (23). χ^2 tests were used to determine the association between p53 and Ki-67 expression with clinic-pathological characteristics, whereas the Kaplan-Meier method and log-rank test were used for univariate survival analysis. Cox proportional hazards regression was carried out for multivariable survival analysis. Hazard ratios (HR) and 95% confidence intervals (CI) were obtained. A two sided *P* value of ≤0.05 was considered statistically significant. All analyses were carried out using SPSS 22.0.

Results

Clinicopathologic Characteristics

A total of 254 CRC specimens were included in this study. The ages of the patients ranged from 28 to 75 years (median 56 years). The mean follow-up time was 42 months (range 21-68 months). Sixty-eight patients (28%) had relapsed at the time of last follow-up. The metastatic sites included lung (n = 23), liver (n = 22), bone (n = 8), abdomen (n = 7), brain (n = 3), and other organs (n=5).

Correlation of p53 and Ki-67 Expression with Other Clinicopathologic Variables

The association of p53 and Ki-67 expression with clinicopathologic parameters is summarized in **Table 1**. The prevalence of positive p53 expression was 63%. Notably, up-regulation of p53 was associated with greater tumor size (*P*=0.001) and high Ki-67 LI (*P*=0.031). There was no significant relationship between p53 expression and gender, age, location, differentiation, lymphovascular invasion (LVI), perineural invasion (PNI), TNM stage, pT stage, pN stage, or PD-L1 expression in tumor-infiltrating immune cells. One hundred ninety-seven patients (77%) had high Ki-67 LI. High Ki-67 LI was associated with TNM stage III (*P*=0.031), positive p53 expression (*P*=0.031), and negative PD-L1 expression (*P*=0.003). There was no significant association between Ki-67 LI and other clinicopathologic parameters. Although Ki-67 labeling correlated with PD-L1 expression (*P*=0.031), there was no association between p53 and PD-L1 expression (*P*=0.308). Furthermore, we correlated PD-L1 expression in tumor cells with p53 and Ki-67 status. No significant correlation was observed (data was not shown).

Prognostic Significance of p53, Ki-67 and PD-L1 Expression

To evaluate whether p53, Ki-67 and PD-L1 expression in CRC correlates with patients' prognosis, Kaplan-Meier survival curves were constructed using five-year recurrence free survival (RFS). The five-year RFS rate for patients with PD-L1 positive tumors was 60%, and the five-year RFS rate for the PD-L1 negative tumors was 70% (P=0.041, **Figure 3A**). Our data also revealed that both positive p53 expression and high Ki-67 labeling index (LI) in CRC was inversely correlated with the five-year RFS (P=0.001 and P=0.003, respectively, **Figure 3B, C**). The five-year RFS rate for the p53-positive/Ki-67 LI-high patients was 53% and for the p53-negative/Ki-67 LI-low patients was 89% (P<0.001, **Figure 3D**).

Furthermore, Kaplan-Meier analysis was performed in stage II and III CRC patients, respectively. The analysis of patients with stage II CRC showed that the five-year RFS rate for the p53 positive group was 76.7%, which was significantly lower than 91.5% observed for the p53 negative group (P=0.035, **Figure 4A**). No significant association between Ki-67 LI or combined p53-Ki-67 status and prognosis was observed in our analysis (P=0.371 and P=0.086, respectively, **Figure 4B, C**). The analysis of patients with stage III CRC showed that the five-year RFS rate for the p53 positive group was 55.4%, which was significantly lower than 77.8% observed for the p53 negative group (P=0.006, **Figure 4D**). The five-year RFS rate for the Ki-67 LI-low group was 59.1%, which was significantly lower than 90% observed for the Ki-67 LI-high group (P=0.013, **Figure 4E**). The five-year RFS rate for the p53-positive/Ki-67 LI-high patients was 53% and for the p53-negative/Ki-67 LI-low patients was 89% (P=0.002, **Figure 4F**). However, PD-L1 expression was not a strong prognostic marker in patients with CRC stratified by stages (P=0.494 and P=0.073, respectively).

Next, we examined whether p53, Ki-67 and PD-L1 expression were independent prognostic markers for CRC. In univariate Cox regression analysis, positive p53 (*P*=0.001) expression, high Ki-67 LI (*P*=0.006), increased PD-L1 (*P*=0.044) expression, higher pathologic TNM stage (*P*<0.001), recto-sigmoid location (*P*=0.026), LVI (*P*=0.007), and greater tumor size (*P*=0.013) were significantly related to shorter RFS (**Table 2**). In multivariate Cox regression analysis of these parameters, positive p53 expression (*P*=0.017, HR 2.370, 95 % CI 1.167-4.811), Ki-67 LI-high (*P*=0.025, HR 2.759, 95 % CI 1.136-6.704) and TNM stage (*P*=0.029, HR 1.978, 95 % CI 1.071-3.653) were independent prognostic factors (**Table 2**). Separate Cox regression analysis in stage II and III CRC patients is provided in **Table 3 and Table 4**. Our results indicated that high Ki-67 LI was an independent prognostic marker for RFS in stage III CRC (*P*=0.015, HR 6.919, 95 % CI 1.464-32.703); however, LVI was the only significant prognostic marker for RFS in stage II CRC (*P*=0.045, HR 4.160, 95 % CI 1.032-16.767).

Discussion

Current prognostic indicators are still ineffective for identifying stage II and III CRC patients with high risk of recurrence after resection with curative intent. Current parameters also cannot reliably identify patients who are unlikely to recur after surgery alone. In the present study, we evaluated the immunophenotypes of 254 CRCs, and correlated these findings with the patients' clinical outcomes. We found that PD-LI, Ki-67, and p53 staining each had individual significant prognostic value for patients with stage II and III CRC. However, the combination of p53 and Ki-67 labeling is superior to any individual markers for predicting the outcome for CRC patients after complete resection, especially for those patients who are positive or negative for both immunohistochemical stains.

This is the first study, to our knowledge, investigating these three markers in consecutive patients from a single, well-defined cohort of CRC radical resection patients with long-term follow-up. Mutations of the *TP53* tumor suppressor gene play a pivotal role in the progression of CRC. P53 alteration might, therefore, represent a clinically useful marker of prognosis. Many studies have investigated *TP53* gene mutations in CRC, but there is still controversy regarding the significance of mutation or the clinical validity of p53 expression for prognosis. In a pooled analysis of prognostic value studies from for p53 alteration 4416 CRC patients in 28 published studies, neither p53 overexpression nor *TP53* mutation emerged as a powerful indicator of treatment success(24). However, another analysis of *TP53* mutations from 3,583 CRC patients being followed by 25 different research groups in 17 countries identified tumor site, exon 5 mutation, and adjuvant treatment as important factors in determining the prognostic significance of *TP53* genetic alteration for any stage CRC (25). Available data about the prognostic value of p53 alteration between individual studies are inconclusive (26, 27), in large part due to the variability between institutions of systematic parameters such as patient selection, amount of tissue studied, and variation in immunostaining criteria.

In this study, all patients were consecutive CRC surgery patients from a single institution with a pathologic stage of II or III. We performed ROC analysis to determine the most discriminatory cutoff score for p53 expression. For the data in this study an H-score ≥ 35 defined positive p53 expression; and an H-score <35 defined negative p53 expression. In our patients, the prevalence of positive p53 expression was 63.0%. Positive p53 staining was associated with increased tumor size and high Ki-67 LI. In view of different patterns of immunoexpression associated with mutant *TP53*(28), further research is required to correlate p53 expression in CRC with mutational analysis in order to establish practical cutoff scores, which can be used to infer

the presence of a *TP53* mutation. Our study revealed that positive p53 expression in stage II and III CRC was inversely correlated with the five-year RFS. Multivariate analysis indicated that positive p53 expression independently predicted poor prognosis of CRC patients. Therefore, patients with high p53 expression need more aggressive treatment and close follow-up to reduce the risk of relapse or metastasis.

Many studies have shown a predictive role of Ki-67 in various human malignancies, including breast cancer, gastrointestinal stromal tumors, and gastrointestinal neuroendocrine tumors (29, 30). Earlier studies have emphasized predictive and prognostic roles for *BRAF* mutation in CRC in association with high Ki-67 proliferative index (31, 32). One early retrospective investigation of 465 patients from 5 collaborative adjuvant chemotherapy trials failed to find association between clinical outcome and Ki-67 staining. However, these investigators did find that positive p53 staining correlated with responsiveness to chemotherapy and increased survival in the adjuvant chemotherapy treatment group (11). High Ki-67 LI, low thymidylate synthase staining intensity and positive p53 staining were significantly correlated, but do not appear to be independent predictive variables (11). In our study, high Ki-67 LI was associated with advanced TNM stage, p53 overexpression, and PD-L1 expression. Kaplan-Meier analysis revealed that high Ki-67 LI in stage II and III CRC was inversely correlated with the five-year RFS. Multivariate analysis indicated that high Ki-67 LI independently predicted poor prognosis of CRC patients. Patients with high Ki-67 LI require more aggressive treatment and active surveillance.

When patients were further divided into stage II and III disease, Kaplan-Meier analysis revealed that the five-year RFS rate for the p53 positive group was significantly lower than the p53 negative group in stage II CRC. There was a trend toward a high Ki-67 LI correlation with

shorter RFS. The five-year RFS rates for the p53-positive/high Ki-67 LI group were significantly lower than the p53-negative/low Ki-67 LI group in stage III CRC. In addition, by multivariate analysis we found that LVI was the significant prognostic marker for RFS in stage II CRC and high Ki-67 LI was the independent prognostic marker in stage III CRC. Over the years, several high-risk clinical features, including T4 primary tumors, poorly differentiated tumors, perforation and/or obstruction, LVI, PNI, and less than 12 lymph nodes examined (33), have been identified to support the role of adjuvant chemotherapy for stage II CRC. Our study confirmed the independent prognostic value of LVI for the clinical outcome in stage II CRC. We also demonstrated the prognostic importance of Ki-67 LI in stage III CRC.

Discrepancies between our study and previous negative studies for association between Ki-67 or p53 and prognosis or survival may be attributed to the differences in study design, sample size, ethnic populations and stain scoring rules. One limitation of our study is the small size of arrayed samples. The main concern is that due to tumor heterogeneity, biomarker scores obtained from small TMA cores may not accurately reflect scores obtained from whole slide tissue sections. Given this limitation, further research is warranted to determine the efficacy of TMA in reflecting the prognostic value of different biomarkers. Studies have also shown that mismatch-repair deficient cancers are predicted to a very large number of mutation-associated neoantigens that might be recognized by the immune system (34, 35). PD-1 blockade was an effective treatment for CRC patients with mismatch-repair deficient tumors (36). Further studies are needed to assess the potential for combining microsatellite instability (MMI) status and other clinical features or biomarkers to predict prognosis in CRC.

PD-L1, Ki-67, and p53 staining individually had significant prognostic value for patients with stage II and III CRC. Moreover, combining p53 H-score $\geq$ 35 and Ki-67 LI $\geq$ 20% identifies

patients at high risk of recurrence. Such information may potentially be used clinically to guide patient management.

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Figure Legends

Figure 1: p53 and Ki-67 expression in colorectal cancer. (A) Photomicrograph showing high level of p53 expression (IHC, ×200). (B) Photomicrograph showing high Ki-67 labelling index (LI) (IHC, ×200). (C) Photomicrograph showing high level of p53 expression (IHC, ×200). (D) Photomicrograph showing low Ki67 LI (IHC, ×200).

Figure 2: Receiver operating characteristic curves for p53 with recurrence free survival (A) and stages (B). AUC, the area under the curve.

Figure 3: Kaplan-Meier survival analysis for all stages of colorectal cancer patients. Recurrence-free survival according to (A) PD-L1 expression (positive vs. negative), (B) Ki-67 labelling index (high vs. low), (C) p53 expression (positive vs. negative), and (D) combined p53-Ki-67 status.

Figure 4: Kaplan-Meier survival analysis for stage II and stage III colorectal cancer patients, respectively. Recurrence-free survival according to (A, D) p53 expression (positive vs. negative), (B, E) Ki-67 labelling index (high vs. low), and (C, F) combined p53-Ki-67 status.

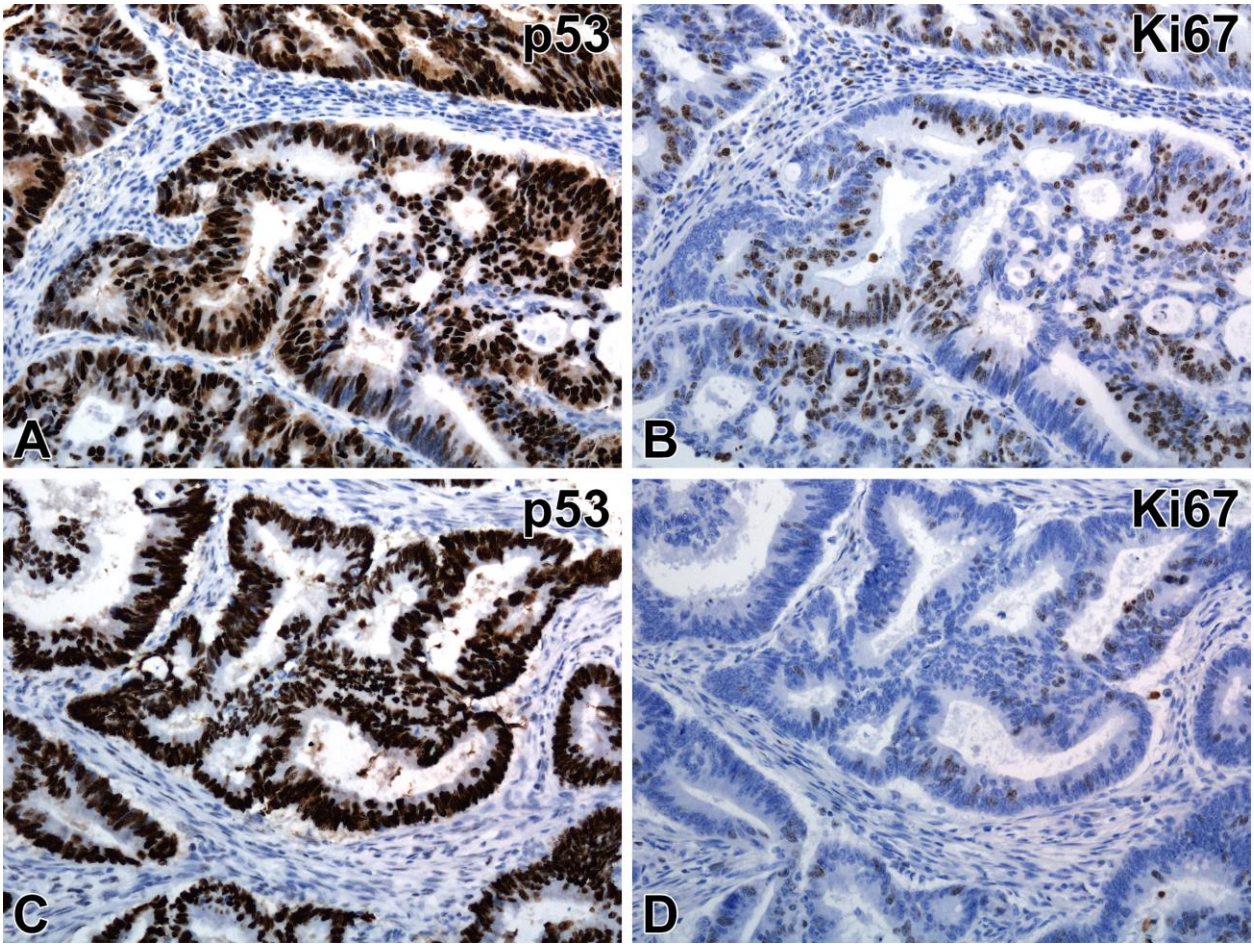

Figure 1

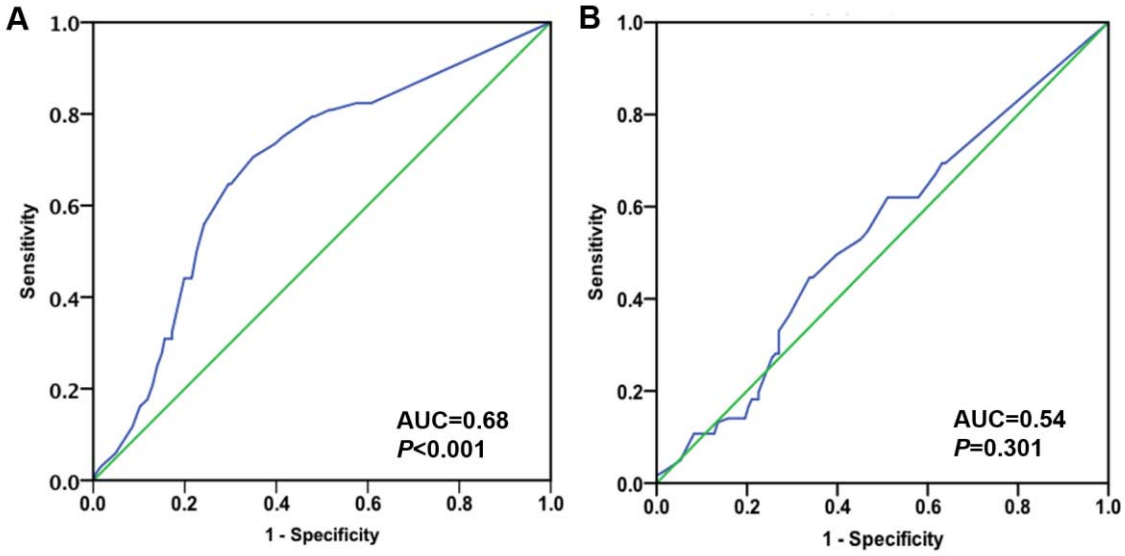

Figure 2

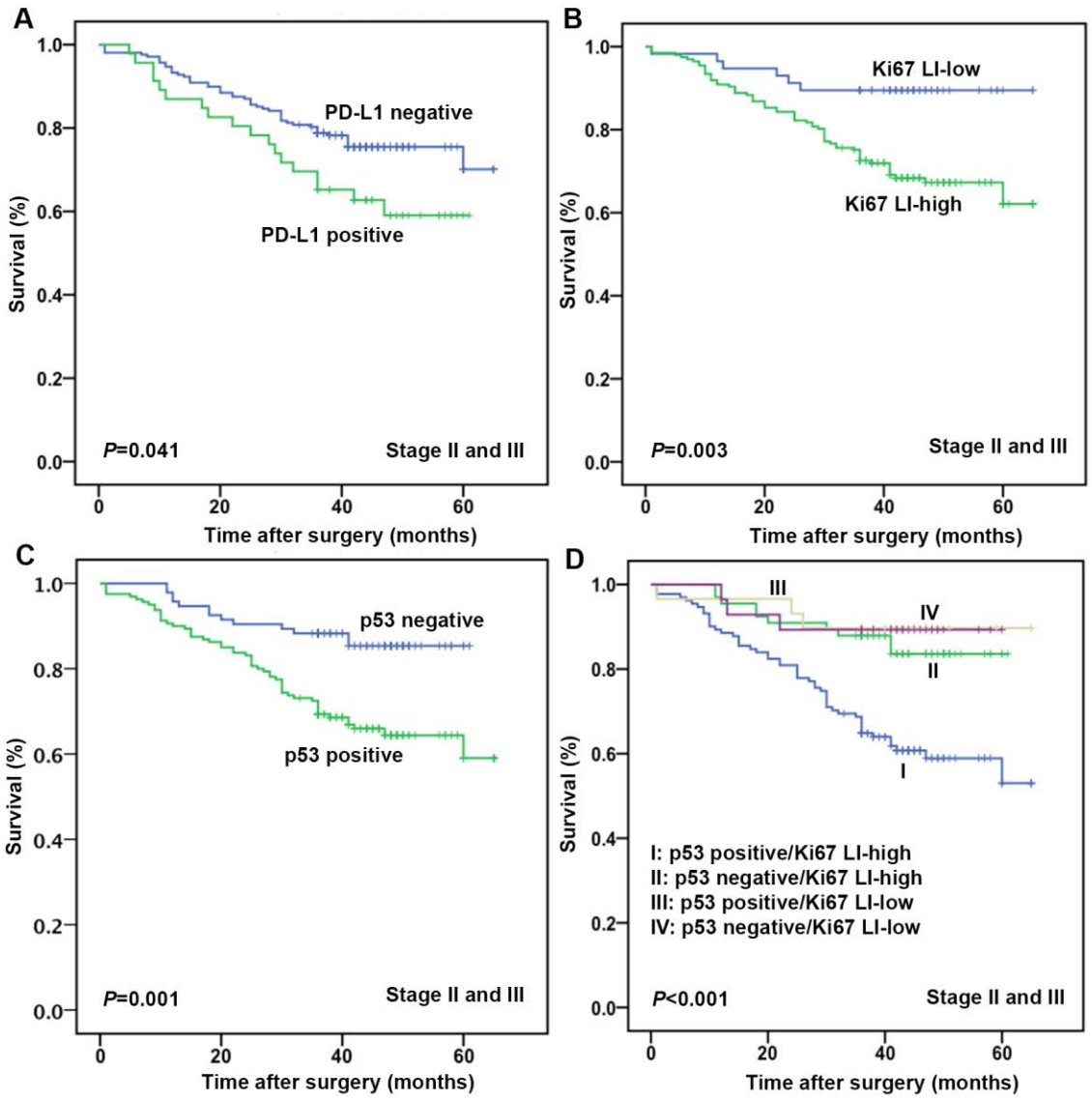

Figure 3

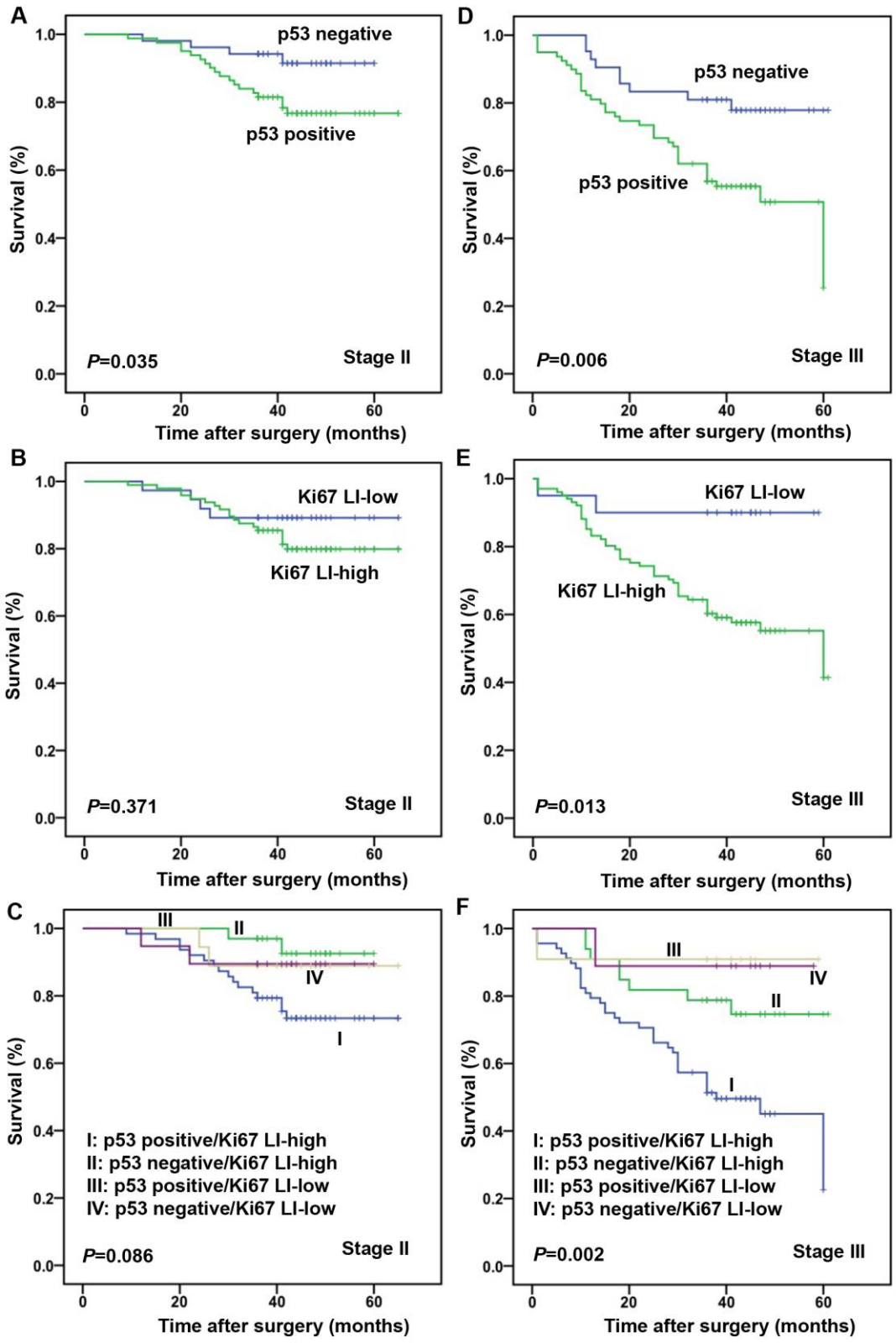

Figure 4

Table 1 Correlation of p53 and Ki67 expression with clinicopathologic features in 254 colorectal cancer (CRC) patients

		p53 expression (%)		χ^2 test	Ki-67 expression (%)		χ^2 test	p53+Ki-67 expression (%)		χ^2 test
Clinicopathologic features	n	Positive	Negative	*P* value	High LI	Low LI	*P* value	Positive	Negative	*P* value
Gender				0.630			0.202			0.567
Male	160	99 (61.9%)	61 (38.1%)		120 (75.0%)	40 (25.0%)		86 (53.8%)	74 (46.3%)	
Female	94	61 (64.9%)	33 (35.1%)		77 (81.9%)	17 (18.1%)		54 (57.4%)	40 (42.6%)	
Age（years）				0.357			0.185			0.492
<60	104	69 (66.3%)	35 (33.7%)		85 (81.7%)	19 (18.3%)		60 (57.7%)	44 (42.3%)	
≥60	150	91 (60.7%)	59 (39.3%)		112 (74.7%)	38 (25.3%)		80 (53.3%)	70 (46.7%)	
Tumor size (cm)				0.001*			0.211			0.008*
<5	169	118 (69.8%)	51 (30.2%)		135 (79.9%)	34 (20.1%)		103 (60.9%)	66 (39.1%)	
≥5	85	42 (49.4%)	43 (50.6%)		62 (72.9%)	23 (27.1%)		37 (43.5%)	48(56.5%)	
Location				0.615			0.770			0.987
Colon	138	85 (61.6%)	53 (38.4%)		108 (78.3%)	30 (21.7%)		76 (55.1%)	62 (44.9%)	
Rectum	116	75 (64.7%)	41 (35.3%)		89 (76.7%)	27 (23.3%)		64 (55.2%)	52(44.8%)	
Differentiation [a]				0.308			0.900			0.386
High grade	208	128 (61.5%)	80 (38.5%)		161 (77.4%)	47 (22.6%)		112 (53.8%)	96 (46.2%)	
Low grade	46	32 (69.6%)	14 (30.4%)		36 (78.3%)	10 (21.7%)		28 (60.9%)	18 (39.1%)	
TNM stage				0.470			0.031*			0.741
II	133	81 (60.9%)	52 (39.1%)		96 (72.2%)	37 (27.8%)		72 (54.1%)	61 (45.9%)	
III	121	79 (65.3)	42 (34.7%)		101 (83.5%)	20 (16.5%)		68 (56.2%)	53 (43.8%)	
LVI				0.473			0.131			0.548
Positive	33	19 (55.6%)	14 (42.4%)		27 (81.8%)	6 (18.2%)		17 (51.5%)	16 (48.5%)	
Negative	140	90 (64.3%)	50 (35.7)		96 (68.6%)	44 (31.4%)		64 (45.7%)	76 (54.3%)	
PNI				0.259			0.186			0.089
Positive	28	15 (53.6%)	13 (46.4%)		17 (60.7%)	11 (39.3%)		9 (32.1%)	19 (67.9%)	
Negative	145	94 (64.8%)	51 (35.2%)		106 (73.1%)	39 (26.9%)		72 (49.7%)	73 (50.3%)	
pT stage				0.946			0.069			0.596
T2+T3	129	81 (62.8%)	48 (37.2%)		94 (72.9%)	35 (27.1%)		69 (53.5%)	60 (46.5%)	

		p53 expression (%)		χ^2 test	Ki-67 expression (%)		χ^2 test	p53+Ki-67 expression (%)		χ^2 test
Clinicopathologic features	n	Positive	Negative	*P* value	High LI	Low LI	*P* value	Positive	Negative	*P* value
T4	125	79 (63.2%)	46 (36.8%)		103 (82.4%)	22 (17.6%)		71 (56.8%)	54 (43.2%)	
pN stage				0.278			0.061			0.119
N0	134	82 (61.2%)	52 (38.8%)		97 (72.4%)	37 (27.6%)		73 (54.5%)	61 (45.5%)	
N1	69	41 (59.4%)	28 (40.6%)		55 (79.7%)	14 (20.3%)		33 (47.8%)	36 (52.2%)	
N2	51	37 (72.5%)	14 (27.5%)		45 (88.2%)	6 (11.8%)		34 (66.7%)	17 (33.3%)	
Ki-67				0.031*						
Low	57	29 (50.9%)	28 (49.1%)							
High	197	131 (66.5%)	66 (33.5%)							
p53							0.031*			
Negative	94				66 (70.2%)	28 (29.8%)				
Positive	160				131 (81.9%)	29 (18.1%)				
PD-L1[b]				0.308			0.003*			0.029*
Positive	46	32 (69.6%)	14 (30.4%)		43 (93.5%)	3 (6.5%)		32 (69.6%)	14 (30.4%)	
Negative	208	128 (61.5%)	80 (38.5%)		154 (74.0%)	54 (26.0%)		108 (51.9%)	100 (48.1%)	

Note. *P* value was obtained from χ^2 test. Abbreviations: LI, labeling index; LVI, lymphovascular invasion; PNI, perineural invasion. [a]Differentiation: well and moderately differentiated tumors were designated as low grade, and poorly differentiated tumors were designated as high grade. [b]PD-L1: PD-L1 expression in tumor-infiltrating immune cells. *Statistically significant.

Table 2 Univariate and multivariate analysis of variables on recurrence free survival in 254 colorectal cancer patients

	Univariate			Multivariate		
Parameters	HR	95% CI	*P* value	HR	95% CI	*P* value
Gender	0.827	0.500 to 1.367	0.459	0.740	0.391 to 1.400	0.355
Age	0.835	0.517 to 1.348	0.461	0.555	0.304 to 1.011	0.054
Location[a]	1.730	1.069 to 2.798	0.026*	1.480	0.812 to 2.699	0.200
Size	0.474	0.263 to 0.853	0.013*	0.937	0.463 to 1.899	0.857
LVI	2.279	1.247 to 4.166	0.007*	1.926	0.960 to 3.866	0.065
PNI	1.444	0.723 to 2.883	0.298	1.876	0.840 to 4.188	0.125
Differentiation	0.848	0.444 to 1.617	0.616	0.571	0.241 to 1.352	0.203
TNM	2.817	1.693 to 4.689	<0.001*	1.978	1.071 to 3.653	0.029*
PD-L1[b]	1.739	1.015 to 2.982	0.044*	1.415	0.732 to 2.735	0.302
p53	2.771	1.513 to 5.074	0.001*	2.370	1.167 to 4.811	0.017*
Ki-67	3.274	1.416 to 7.569	0.006*	2.759	1.136 to 6.704	0.025*

Note. Abbreviations: CI, confidence interval; HR, hazard ratio, determined by Cox proportional hazard models; LVI, lymphovascular invasion; PNI, perineural invasion. [a] Location: colon versus rectum (colon cancer patients had better survival). [b]PD-L1: PD-L1 expression in tumor-infiltrating immune cells. * Statistically significant.

Table 3 Univariate and multivariate analysis of variables on recurrence free survival in 133 stage II colorectal cancer patients.

	Univariate			Multivariate		
Parameters	HR	95% CI	*P* value	HR	95% CI	*P* value
Gender	1.763	0.761 to 4.082	0.186	1.717	0.640 to 4.605	0.283
Age	1.377	0.578 to 3.283	0.470	1.312	0.478 to 3.603	0.054
Location	1.502	0.651 to 3.465	0.340	1.672	0.590 to 4.737	0.334
Size	0.220	0.065 to 0.745	0.015*	0.420	0.105 to 1.675	0.219
LVI	3.714	1.226 to 11.256	0.020*	4.160	1.032 to 16.767	0.045*
PNI	1.486	0.493 to 4.484	0.482	0.719	0.146 to 3.545	0.685
Differentiation	1.241	0.458 to 3.364	0.672	1.325	0.301 to 5.837	0.710
PD-L1[a]	1.413	0.521 to 3.831	0.497	0.863	0.229 to 3.254	0.828
p53	3.027	1.024 to 8.945	0.045*	2.630	0.710 to 9.743	0.148
Ki-67	1.725	0.584 to 5.098	0.324	2.226	0.670 to 7.400	0.192

Note. Abbreviations: CI, confidence interval; HR, hazard ratio, determined by Cox proportional hazard models; LVI, lymphovascular invasion; PNI, perineural invasion. [a]PD-L1: PD-L1 expression in tumor-infiltrating immune cells.

*Statistically significant.

Table 4 Univariate and multivariate analysis of variables on recurrence free survival in 121 stage II colorectal cancer patients

	Univariate			Multivariate		
Parameters	HR	95% CI	*P* value	HR	95% CI	*P* value
Gender	0.583	0.296 to 1.150	0.120	0.360	0.132 to 0.979	0.045*
Age	0.559	0.313 to 0.998	0.049*	0.280	0.125 to 0.629	0.002*
Location	1.681	0.930 to 3.041	0.086	1.785	0.791 to 4.028	0.163
Size	0.829	0.421 to 1.632	0.587	1.744	0.726 to 4.191	0.213
LVI	1.444	0.696 toc2.996	0.324	1.406	0.608 to 3.252	0.426
PNI	1.471	0.605 to 3.579	0.395	3.028	0.983 to 9.326	0.054
Differentiation	0.731	0.310 to 1.728	0.476	0.318	0.099 to 1.024	0.055
PD-L1[a]	1.784	0.935 to 3.407	0.079	1.547	0.689 to 3.473	0.291
P53	2.658	1.280 to 5.518	0.009*	1.993	0.843 to 4.712	0.116
Ki-67	4.975	1.204 to 20.552	0.027*	6.919	1.464 to 32.703	0.015*

Note. Abbreviations: CI, confidence interval; HR, hazard ratio, determined by Cox proportional hazard models; LVI, lymphovascular invasion; PNI, perineural invasion. [a]PD-L1: PD-L1 expression in tumor-infiltrating immune cells.

*Statistically significant.

Highlights

- PD-L1, p53, and Ki-67 staining individually had prognostic value for CRC patients.
- p53, Ki-67 and TNM stage were independent prognostic factors in CRC.
- High expression of both p53 and Ki-67 predicted poor prognosis for CRC patients.