The association of p53 expression levels with clinicopathological features and prognosis of patients with colon cancer following surgery

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Abstract. The present study aimed to examine the association of p53 expression levels with clinicopathological features and prognosis of patients with colon cancer following surgery. The present study included 484 patients with colon cancer that underwent colon resection between December 2003 and December 2011. All follow-ups were censored in December 2013 with a median follow-up time of 43 months. Kaplan-Meier survival curves and Cox regression analysis were used to determine predictors for overall survival rate. p53 expression status (positive or negative) was significantly different between patient groups when categorized by age distribution, disease course, tumor location, maximum tumor diameter, depth of tumor invasion, Dukes' stage, distant metastasis and lymph node (LN) metastasis ($P<0.05$). Cox regression analysis revealed that age, surgery type, histological subtypes, tumor size, tumor location, LN metastasis, distant metastases, Dukes' stage and p53 expression status are independent factors influencing the survival rate of patients with colon cancer following surgery ($P<0.05$). Therefore, the present study revealed that the loss of p53 expression levels in tumors was associated with aggressive clinicopathological characteristics in patients with colon cancer.

Introduction

Colorectal cancer (CRC) or colon cancer, is the third most frequently diagnosed type of cancer in males, the second in females and the fourth most frequent cause of cancer mortality (1,2). In 2012, it was estimated that 1,360,602 million new cases (women, 614,304; men, 746,298) of CRC were diagnosed (3). The burden of CRC worldwide is expected to increase by 60% to >2.2 million new cases and 1.1 million CRC-associated mortalities by 2030 (4). The curative or palliative treatment for colon cancer is dependent on various factors, including the patient's health and tumor stage, and curative surgery is typically recommended if colon cancer is diagnosed early and palliative care is provided for advanced colon cancer (5,6). The U.S. National Comprehensive Cancer Network and American Society of Clinical Oncology have provided guidelines for the recommended follow-up of colon cancer (7,8) and have identified that surveillance and detailed follow-up following curative resection of colon cancer may reduce the 5-year mortality rate and improve overall survival (OS) and the re-resection rate for recurrent disease (9-11). However, complications from surgery still occur frequently, resulting in an average length of hospital stay of 8-12 days subsequent to a standard colon resection and, therefore, the risk factors following colectomy for colon cancer require further investigation (12,13).

The prognosis of patients with colon cancer is dependent on clinical, pathological and biological factors, including age (14), gender, Dukes' stage (15), quantity of blood loss during surgery (16), lymph node (LN) invasion, bowel wall penetration, invasive margin character and type of tumor, and these may be effective prognostic indicators for predicting the survival rate of patients with colon cancer following surgery (17-20). In addition, patients with tumor perforation or incomplete resection have a poor prognosis following curative resection of Dukes' B colon cancer (21). In previous studies, p53 protein expression levels have been established to predict the outcome of Dukes' stage B CRC (22) and the p53 status may provide an effective prognostic factor for patients with Dukes' B and C colon cancer (23). The p53 tumor suppressor protein (encoded by *TP53*) is involved in DNA damage repair, cell cycle regulation, apoptosis, aging and cellular senescence and is mutated in ~50% of types of cancer (24). Additionally, p53 overexpression is associated with a reduced survival rate in patients with stage III tumors and p53 expression levels are able to predict CRC biology and clinical behavior (25,26). Using multivariate analysis, a p53 codon 72 mutation, a

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mutation hotspot identified in Egyptian populations, has been suggested to be an independent prognostic factor for disease-free survival (DFS) in patients with colon cancer from this geographical region (27). In addition, p53 overexpression was an independent predictor of tumor recurrence in patients with colon cancer (28) and increased nuclear p53 expression levels were associated with the presence of distal metastasis in stage II colon cancer (29). p53 immunoreactivity was implicated to be closely associated with clinicopathological variables, including pathological type, lymphocytic infiltration, tumor grade and the tumor site in CRC (30). The p53 functional status was demonstrated to provide a prognostic marker for patients with colon cancer (31); however, a previous study did not identify a significant association between p53 and DFS or OS in patients with locally advanced colon cancer (32). The prognosis of patients with colon cancer is primarily dependent on standard clinicopathological factors. In the present study, the association of p53 expression levels with clinicopathological features and prognosis was investigated to evaluate their prognostic value in patients with colon cancer following surgery.

Materials and methods

Study design and subjects. Between December 2003 and December 2011, 484 patients with primary colon cancer were enrolled onto the present study at the Department of Gastroenterology, The Affiliated ZhongDa Hospital of Southeast University (Nanjing, China). The inclusion criteria were as follows: i) Patients that underwent radical and palliative surgery for colon cancer treatment; ii) patients with a pathologically confirmed colon cancer diagnosis; and iii) patients with complete follow-up data. The exclusion criteria were as follows: i) Patients who had preoperative chemotherapy or radiotherapy; and ii) patients with pathologically positive margins. The patients were composed of 296 males and 188 females with an average age of 61.94 years (range, 25-88). The age distribution was categorized as <40 (n=63), 40-60 (n=226) and >60 years (n=195). There were 58 cases with Dukes' A colon cancer, 189 cases with Dukes' B colon cancer, 201 cases with Dukes' C colon cancer and 36 cases with Dukes' D colon cancer according to the revised Dukes' staging (33). The types of tissue samples included 210 mass samples, 183 ulceration samples and 91 invasion samples.

Following approval from the Affiliated ZhongDa Hospital of Southeast University, written informed consent was obtained from all patients. Protocols of the present study were based on the ethical principles of The Declaration of Helsinki for medical research that includes human patients (34).

Immunohistochemistry. Surgically resected tumor specimens from enrolled patients were obtained and were formalin-fixed and paraffin-embedded to create tissue blocks from which 4 μm tissue sections were cut. Conventional dewaxing and hydration with a series of graded alcohol was performed. Briefly, the tissue sections were rinsed with dimethylbenzene twice (10 min/wash), followed by rinsing with a descending series of graded alcohol (100, 95, 90, 80 and 70%; 3-5 min/wash) and final 3 min rinse in distilled water. Subsequent antigen retrieval was performed using a microwave, followed by the addition of 3% hydrogen peroxide to block endogenous peroxidase activity. Primary mouse anti-human p53 antibody (DO-7; 1:100; cat. no. MAB-0674; Fuzhou Maixin Biotech Co., Ltd., Fuzhou, China) was added, and the tissue sections were incubated at 4˚C overnight. Following incubation for 20 min, the sections were treated with horseradish peroxidase-labeled goat anti-mouse secondary antibody (1:1,000; cat. no. ab6785; Abcam, Cambridge, MA, USA) for 30 min at room temperature. The immunohistochemistry analysis was performed using an SP ultrasensitive immunostaining kit according to the manufacturer's protocol, followed by DAB for visualization (both Fuzhou Maixin Biotech Co., Ltd.). The replacement of the primary antibody with PBS was used as a negative control. As a positive control, tissue sections that were positively stained for p53 were included in each staining run (Fuzhou Maixin Biotech Co., Ltd.). If ≥10% of the malignant nuclei were stained, the slide was scored as positive based on the standard scoring for p53 expression status as described in a previous study (23).

Follow-up of patients with colon cancer following surgery. All patients were followed up via outpatient review, telephone calls, letters and other forms of communication. The follow-up time was measured from the date of surgery and patients were followed up semiannually during the first 2 to 5 years and annually thereafter. All follow-ups were completed by December 2013, there was a median follow-up duration of 43 months and the longest follow-up was 120 months. The survival rate was calculated from the time of surgery to the date of mortality, or date of final follow-up.

Statistical analysis. Clinical and pathological data obtained from the follow-up of patients were coded into a database using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Statistical analysis was performed using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using the χ^2 test. Survival distributions were estimated using Kaplan-Meier survival analysis. Estimated probabilities of survival rate were compared with the log-rank method. To identify independent prognostic factors, variables that were significantly associated with the prognostic information from the univariate analysis were applied in the Cox regression model. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. The clinical and pathological characteristics of 484 patients with colon cancer are presented in Table I. There were 96 patients (19.38%) with a disease course length of <1 month, 152 cases (31.40%) with 1-3 months, 65 cases (13.43%) with 3-6 months, 75 cases (15.50%) with 6-12 months and 96 cases (19.84%) with ≥12 months. The median disease course length was 3-6 months. The tumor location was categorized as right (cecum, ascending colon and hepatic flexure), transverse and left (splenic flexure, descending colon and sigmoid colon), and these accounted for 245 cases (50.62%), 50 cases (10.33%) and 189 cases (39.05%), respectively. With regard to the type of surgery

Table I. Clinical and pathological characteristics from patients with colon cancer.

Characteristics	Number of patients (%)
Gender	
Male	296 (61.16)
Female	188 (38.84)
Age, years	
<40	63 (13.02)
40-60	226 (46.69)
>60	195 (40.29)
Time course of disease, months	
<1	96 (19.83)
1-3	152 (31.40)
3-6	65 (13.43)
6-12	75 (15.50)
≥12	96 (19.84)
Tumor location	
Left hemicolon	245 (50.62)
Colon transversum	50 (10.33)
Right hemicolon	189 (39.05)
Surgery	
Radical surgery	403 (83.26)
Palliative surgery	81 (16.74)
Perioperative blood transfusion, ml	
0	295 (60.95)
≤400	100 (20.66)
400-800	89 (18.39)
Tumor size, cm	
≤4	212 (43.80)
4-8	255 (52.69)
>8	17 (3.51)
Borrmann type	
Fungating	210 (43.39)
Infiltrative	91 (18.80)
Ulcerative	183 (37.81)
Histological subtypes	
Moderately/well-differentiated adenocarcinomas	383 (79.13)
Poorly differentiated adenocarcinoma	43 (8.89)
Mucinous adenocarcinoma	49 (10.12)
Signet-ring cell carcinoma	9 (1.86)
Depth of invasion	
Mucosa and submucosa	24 (4.96)
Muscular coat	33 (6.82)
Full-thickness	243 (50.21)
Beyond serosa	184 (38.02)
Dukes' stage	
Dukes' A	58 (11.98)
Dukes' B	189 (39.05)
Dukes' C	201 (41.53)
Dukes' D	36 (7.44)
Distant metastasis	
Positive	40 (8.26)
Negative	444 (91.74)
Lymph node metastasis	
Positive	228 (47.11)
Negative	256 (52.89)
p53 status	
Positive	253 (52.27)
Negative	231 (47.73)

that was selected, 403 cases (83.26%) had radical surgery and 81 cases (16.74%) had palliative surgery for the treatment of colon cancer. The quantities of perioperative blood transfusion that were administered to patients were 0 ml (did not have a blood transfusion) in 295 cases (60.95%), ≤400 ml in 100 cases (20.66%) and 400-800 ml in 89 cases (18.39%). A maximum tumor diameter of ≤4 cm was observed in 212 cases (43.80%), 4-8 cm in 255 cases (52.69%) and >8 cm in 17 cases (3.51%). Gross types of colon cancer tissue samples were categorized as fungating type (210 cases, 43.39%), infiltrative type (91 cases, 18.80%) and ulcerative type (183 cases, 37.81%), respectively. The histological subtypes were divided into moderately/well-differentiated adenocarcinomas (383 cases, 79.13%), poorly differentiated adenocarcinoma (43 cases, 8.89%), mucinous adenocarcinoma (49 cases, 10.12%) and signet-ring cell carcinoma (9 cases, 1.86%).

Association of p53 expression with clinical characteristics. As summarized in Table II, positive nuclear p53 immunoreactivity was identified in 253 patients (52.27%) and tumors from 231 patients (47.73%) were negative for p53 expression. The p53 expression status (positive or negative) was significantly different between patient subgroups when categorized according to age distribution, disease course, tumor location, maximum tumor diameter, depth of tumor invasion, Dukes' stage, distant metastasis and LN metastasis ($P<0.05$). However, p53 expression levels were not statistically different between the two genders or the gross tumor type.

Univariate survival rate analysis. The mean OS was 79.78±3.85 months for patients with colon cancer at the time of the final follow-up. The 3-, 5- and 10-year survival rates were 68.02, 59.70 and 52.27%, respectively. The univariate analysis (Table III) revealed that age, course of disease, tumor location, surgery, perioperative blood transfusion, tumor size, histological subtypes, LN metastasis, depth of invasion, distant metastasis, Dukes' stage and p53 protein expression status were significantly associated with postoperative survival rate in patients with colon cancer ($P<0.05$). Statistical associations were not detected between gender and gross tumor type.

Multivariate survival rate analysis. In the multivariate analysis, age, surgery, histological subtypes, tumor size, tumor

Table II. The association of p53 expression levels with clinicopathological characteristics in patients with colon cancer.

Clinical pathology index	p53 expression status		Positive rate, %	χ^2	P-value
	Positive	Negative			
Gender				0.056	0.812
Male	156	140	52.70		
Female	97	91	51.59		
Age, years				27.241	<0.001
<40	28	35	44.44		
40-60	95	131	42.04		
>60	130	65	66.51		
Time course of disease, months				11.644	0.020
<1	50	46	52.08		
1-3	92	60	59.21		
3-6	23	42	35.38		
6-12	39	36	52.00		
≥12	49	47	51.04		
Tumor site				12.352	0.002
Left hemicolon	147	98	60.00		
Colon transversum	20	30	40.00		
Right hemicolon	86	103	45.5		
Tumor size, cm				29.932	<0.001
≤4	81	131	38.21		
4-8	161	94	63.14		
>8	11	6	64.71		
Borrmann type				4.294	0.117
Fungating	102	108	48.57		
Infiltrative	56	35	61.54		
Ulcerative	95	88	51.91		
Histological subtypes				9.421	0.024
Moderately/well-differentiated adenocarcinomas	187	196	48.83		
Poorly differentiated adenocarcinoma	30	13	69.77		
Mucinous adenocarcinoma	30	19	61.22		
Signet-ring cell carcinoma	6	3	66.67		
Distant metastasis				4.053	0.044
Positive	27	13	67.50		
Negative	226	218	50.90		
Lymph node metastasis				35.801	<0.001
Positive	152	76	66.67		
Negative	101	155	39.45		
Dukes' stage				41.643	<0.001
Dukes' A	17	41	29.31		

location, LN metastasis, distant metastasis, Dukes' stage and p53 expression levels were identified as independent factors that may influence the survival rate of patients with colon cancer following surgery (P<0.05; Table IV; Fig. 1).

Discussion

The present study enrolled 484 patients with primary colon cancer that had radical and palliative surgery and complete follow-up data was available. The clinicopathological features and colon cancer-associated prognostic factors were investigated to provide a reliable theoretical understanding of influencing factors in the prognosis of colon cancer, based on long-term follow-up data that was applicable to the patients in the geographical location of the present study. There were significant differences in p53 expression levels in various categories, including age distribution, disease course, tumor location, tumor size, depth of invasion, Dukes' stage, distant

Table III. Univariate analysis of associations between clinicopathological characteristics and follow-up data in patients with colon cancer.

Characteristics	Number of patients (%)	Average length of survival, months	10-year survival rate	P-value
Gender				0.162
Male	296 (61.16)	79.47±3.37	49.66	
Female	188 (38.84)	79.87±3.51	56.38	
Age, years				<0.001
<40	63 (13.02)	80.96±4.68	50.79	
40-60	226 (46.69)	79.39±5.97	63.27	
>60	195 (40.29)	66.41±4.02	40.00	
Time course of disease, month				0.039
<1	96 (19.83)	80.16±2.85	48.96	
1-3	152 (31.40)	77.88±3.53	45.39	
3-6	65 (13.43)	80.56±3.42	56.92	
6-12	75 (15.50)	78.47±3.43	66.67	
≥12	96 (19.83)	80.12±4.01	52.08	
Tumor location				0.006
Left hemicolon	245 (50.62)	74.96±5.21	46.12	
Colon transversum	50 (10.33)	85.37±5.67	64.00	
Right hemicolon	189 (39.05)	84.69±3.68	59.26	
Surgery				<0.001
Radical surgery	403 (83.26)	94.18±3.68	60.54	
Palliative surgery	81 (16.74)	26.08±2.82	11.11	
Perioperative blood transfusion, ml				0.042
0	295 (60.95)	81.23±4.66	53.89	
≤400	100 (20.66)	78.50±6.72	55.00	
400-800	89 (18.39)	66.18±8.06	40.44	
Tumor size, cm				0.024
≤4	212 (43.80)	98.05±7.09	70.75	
4-8	255 (52.69)	78.50±4.90	38.04	
>8	17 (3.51)	66.18±7.07	35.29	
Borrmann type				0.516
Fungating	210 (43.39)	84.59±5.51	60.23	
Infiltrative	91 (18.80)	67.15±3.65	49.45	
Ulcerative	183 (37.81)	79.36±3.05	50.27	
Histological subtypes				<0.001
Moderately/well-differentiated adenocarcinomas	383 (79.13)	97.15±6.98	56.4	
Poorly differentiated adenocarcinoma	43 (8.89)	66.70±5.70	30.77	
Mucinous adenocarcinoma	49 (10.12)	86.74±5.98	48.82	
Signet-ring cell carcinoma	9 (1.86)	22.61±5.22	0.00	
Depth of invasion				<0.001
Mucosa and submucosa	24 (4.96)	116.72±5.82	91.67	
Muscular coat/muscularis	33 (6.82)	106.21±4.65	84.85	
Full-thickness	243 (50.21)	80.33±4.51	60.90	
Beyond serosa	184 (38.02)	39.59±3.62	29.89	
Dukes' stage				<0.001
Dukes' A	58 (11.98)	116.44±2.84	93.1	
Dukes' B	189 (39.05)	101.86±3.04	64.55	
Dukes' C	201 (41.53)	66.83±7.13	36.82	
Dukes' D	36 (7.44)	29.13±3.98	8.33	
Distant metastasis				0.004
Positive	40 (8.26)	39.59±3.29	12.50	

Table III. Continued.

Characteristics	Number of patients (%)	Average length of survival, months	10-year survival rate	P-value
Negative	444 (91.74)	87.58±4.96	55.85	
Lymph node metastasis				<0.001
Positive	228 (47.11)	44.59±4.74	33.77	
Negative	256 (52.89)	86.74±5.06	68.75	
p53 status				<0.001
Positive	253 (52.27)	37.61±2.61	30.83	
Negative	231 (47.73)	85.18±3.68	75.76	

Table IV. Multivariate analysis for overall survival rate in patients with different prognostic variations of colon cancer.

Characteristic	B	SE	Wald χ^2	P-value	EXP(B)	95% CI
Age	0.022	0.055	0.158	0.011	1.522	1.018-1.838
Course of disease	-0.320	0.286	1.444	0.230	0.726	0.431-1.224
Surgery	0.789	0.257	9.424	0.002	2.200	1.330-3.640
Blood transfusion	-0.028	0.091	0.082	0.775	0.974	0.818-1.164
Pathological type	0.157	0.052	9.026	0.003	1.170	1.056-1.297
Tumor diameter	0.149	0.063	5.487	0.019	1.160	1.025-1.314
Tumor site	0.087	0.035	6.022	0.014	1.091	1.018-1.169
Lymph node metastasis	0.406	0.072	31.607	<0.001	1.501	1.303-1.729
Distant metastasis	0.286	0.087	10.891	0.001	1.332	1.123-1.579
Dukes' stage	0.830	0.159	27.393	<0.001	2.293	1.680-3.128
p53 expression	0.864	0.400	27.939	0.031	2.372	1.680-3.128

B, coefficient for the constant; SE, standard error; EXP(B), exponentiation of the coefficient constant; CI, confidence interval.

metastasis and LN metastasis, which suggests that the loss of p53 expression is associated with aggressive clinicopathological features in patients with colon cancer. In addition, the age, surgery type, histological subtypes, tumor size, tumor location, LN metastasis, distant metastasis, Dukes' stage and p53 expression status were independent factors that influenced the survival rate of patients with colon cancer following surgery.

A previous retrospective tumor microarray study also established that p53 may be a potential biological diagnostic marker (29) and p53 status has been demonstrated to be associated with colon cancer prognosis (15). However, a previous study was not able to demonstrate a significant association between p53 expression levels and clinical outcomes in patients with colon cancer (35). Notably, the association between p53 expression levels and clinicopathological data in patients with colon cancer was evaluated in a previous study, and the results suggested that right-sided colon tumors may develop in a p53-independent manner and, therefore, p53 status in cancer cells has prognostic value only for left-sided colorectal tumors (36) However, the association between p53 expression levels and clinicopathological characteristics in CRC has yet to be elucidated. A previous study demonstrated that p53 nuclear expression levels were not associated with a patient gender, age, tumor location, differentiation, T stage, N stage or status of lymphatic and vascular vessel invasion (37). The presence of p53 protein in tumor cells was not associated with age, gender, nodal status and tumor stage, but it was previously identified that well to moderately differentiated tumors exhibited significantly higher expression levels of p53 compared with poorly differentiated tumors (38). However, the present study indicated that the loss of p53 expression in colon cancer may be a predictor of a more aggressive tumor phenotype and other clinicopathological characteristics may also require consideration in conjunction with p53 status to design an effective follow-up strategy to improve patient survival rate.

Clinicopathological characteristics have been used as reliable prognostic factors for the clinical outcome of colon cancer, including age and tumor location, and may predict the development of metastases, which cause mortality in patients with colon cancer (39-41). When the length of survival was stratified by tumor location and analyzed at the end of the follow-up period, the survival rate in patients with right and left colon tumors were 53.9 and 51.4 months, respectively (42). With respect to surgery type, advances in surgical procedures are associate with improved outcomes and previous studies have identified that surgical treatment

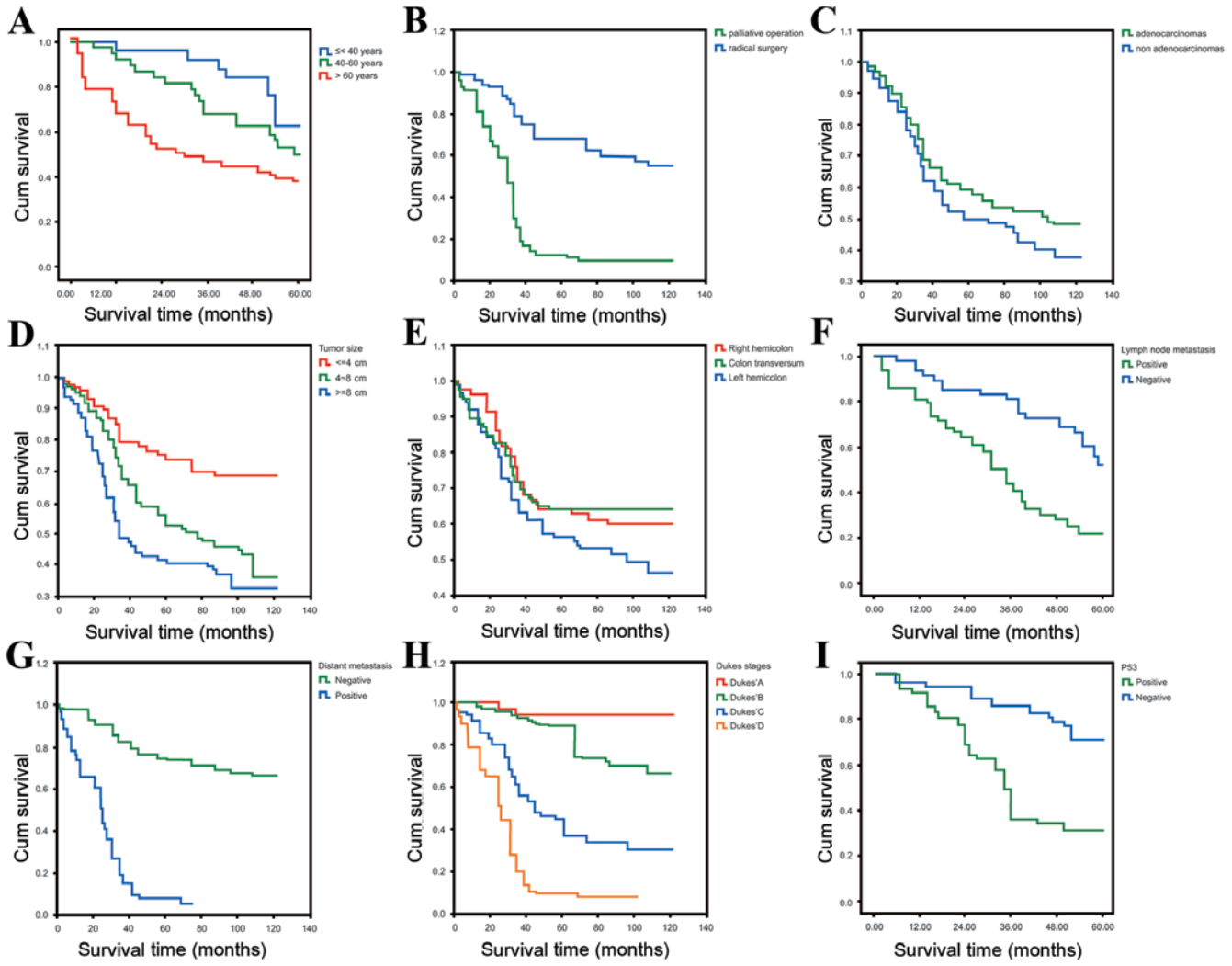

Figure 1. Kaplan-Meier curves estimating the 10-year survival rate following stratifying patients with colon cancer according to (A) age (<40, 40-60 and >60 years), (B) surgery (radical surgery or palliative surgery), (C) histological subtypes (adenocarcinomas or non-adenocarcinomas), (D) tumor size (≤4, 4-8 and >8 cm), (E) tumor location (left hemicolon, colon transversum or right hemicolon), (F) lymph node metastasis (positive or negative), (G) distant metastasis (positive or negative), (H) Dukes' stages (Dukes' A, Dukes' B, Dukes' C and Dukes' D) and (I) p53 status (positive or negative). Cum, cumulative.

of metastases is an independent prognostic factor for OS (41,43). Pulmonary metastasis has been demonstrated to occur in patients with low stage colon tumors and the survival rate following thoracotomy is dependent on the number of metastases (44). Patients with unilateral metastasis and Dukes' A primary tumor type exhibit the highest increase in survival rate following the resection of pulmonary metastasis from CRC (45). Furthermore, LN metastasis is a poor prognostic factor for colon cancer and the number of involved LNs has been implicated in the survival rate of patients with stage II and stage III colon cancer following surgical resection, which is consistent with the number of involved LNs providing an indicator of the care that the patient with colon cancer requires (17,46). Concordant with this, LN yield (LNY) is an independent prognostic factor in colon cancer and ≥12 LNs in the resected specimen obtained from surgery is the current recommended standard, regardless of age or disease site, and LNY is increased in right-sided colon cancer and reduces with age (47). It has been established using multivariate analysis that LN micrometastasis and lymphatic invasion are independent prognostic factors for CRC (48). The histological pattern may also be associated with an increased survival rate and patients with grade I moderately-differentiated tumors exhibit the highest rate of survival (41,42). Several clinicopathological factors were not previously identified to be effective independent predictors of survival rate in patients with colon cancer, including Dukes' stage, T stage, number of resected nodes and vascular or lymphatic invasion (49).

In conclusion, the results of the present study revealed that p53 expression levels are associated with the clinicopathological features of patients with colon cancer within the sampled geographical area. Additionally, the age, surgery type, histological patterns, tumor size, tumor location, LN metastasis, distant metastasis, Dukes' stage and p53 expression levels are independent factors that may influence the survival rate of patients with colon cancer following surgery.

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