Association between Ki67 expression and therapeutic outcome in colon cancer

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Abstract. Ki67 is a commonly used proliferation marker in pathological diagnosis of tumors; however, its prognostic value in colon cancer is controversial. A total of 312 consecutive patients with stage I-III colon cancer who underwent radical surgery with or without adjuvant chemotherapy were included in the present study. Ki67 expression was assessed using immunohistochemistry and was classified according to 25% intervals. The association between Ki67 expression and clinicopathological features was analyzed. Long-term postoperative survival, including disease-free survival (DFS) and overall survival, was calculated, and its association with Ki67 was analyzed. High Ki67 expression (>50%) was associated with improved DFS in patients treated with adjuvant chemotherapy postoperatively, but not in patients who received surgery alone (P=0.138). Ki67 expression was significantly associated with histological differentiation of the tumor (P=0.01), while it was not associated with other clinicopathological factors. Multivariate analysis demonstrated that pathological T and N stage were independent prognostic factors. In conclusion, high Ki67 expression was associated with a good therapeutic outcome in patients receiving adjuvant chemotherapy in colon cancer.

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Introduction

Colon cancer is the third most common cancer type globally and the third-leading cause of cancer death (1). The traditional prognostic factors based on morphological features or blood markers are not sufficient to stratify the risk of post-operative tumor progression (2). Although various genomic or molecular biomarkers, ranging from tissue markers to serum-derived markers, have been developed for more exact prediction of tumor recurrence (3), novel biomarkers are still needed to screen out the patients with poor therapeutic response, as well as those at high risk of tumor progression.

Ki67 is broadly used to evaluate cell proliferation and aggressiveness in various malignant tumors (4,5). Although the expression levels of Ki67 are higher in malignant tumors compared with normal tissues in the majority of solid tumor types, the prognostic value of Ki67 is still controversial (6). In colon cancer, some reports have mentioned that high Ki67 expression was associated with poor prognosis (7,8), while others reported the opposite conclusion, showing that high Ki67 expression reflected improved clinical outcome (9,10). Even high-quality meta-analyses have reported contradicting conclusions (11,12), depending on the evidence they relied on.

To investigate the prognostic significance of Ki67 in colon cancer, the associations of Ki67 expression levels with clinicopathological variables and survival data from 312 patients with colon cancer were analyzed. All cases were divided into four grades based on 25% intervals of the Ki67-positive cell percentage in immunohistochemical staining to identify an optimal cut-off value for prognostic evaluation.

Patients and methods

Patient recruitment. A total of 312 consecutive patients with stage I-III colon cancer treated at Peking University

Cancer Hospital (Beijing, China) between January 2004 and December 2010 were retrospectively included. Radical surgery was performed in all patients with or without adjuvant chemotherapy. Adjuvant chemotherapy with the regimens of FOLFOX, XELOX or capecitabine alone was performed for patients with lymph node metastasis and those with high-risk microsatellite stable stage II tumors, including patients with perforated tumors, pT4N0 lesions, vascular invasion and/or bowel obstruction (13), following the NCCN guidelines of colon cancer (Version 3.2022). Within the 195 patients who underwent chemotherapy, 35 patients underwent capecitabine alone, while 160 patients accepted combined chemotherapy (FOLFOX or XELOX). All patients were given adjuvant chemotherapy for 6 months after surgery, equally 8-12 cycles. Patients were followed up regularly every 6 months post-surgery, with physical examination, serum CEA testing, chest radiography, computed tomography and colonoscopy once per year. The follow-up lasted 5 years, and patients missing in follow-up were excluded.

Detection of tissue Ki67. Sections (5-μm-thick) were cut from paraffin-embedded blocks of tumor tissue. Immunohistochemistry staining was performed as previously reported (14). Repaired tissue sections were incubated with the Ki67 primary monoclonal antibody (dilution, 1:200; cat. no. ZM-0166; OriGene Technologies, Inc.). The Dako REAL EnVision Detection System (cat. no. K5007; Agilent Technologies, Inc.) was used for staining and detection. Ki67 expression was defined as follows: +, >0 and ≤25%; ++, >25 and ≤50%; +++, >50 and ≤75%; and ++++, >75%; among which, + and ++ were defined as low expression, while +++ and ++++ were defined as high expression (Fig. 1), as reported previously (15).

Statistical analysis. Categorical variables such as clinicopathological characteristics are presented as patient numbers and percentages. The association between Ki67 expression and clinicopathological variables was analyzed using the χ^2 test. The 5-year disease-free survival (DFS) rate and overall survival (OS) rate were analyzed using Kaplan-Meier survival curves with log-rank tests based on different Ki67 expression level. Multivariate analysis was performed using a Cox proportional hazard model with the Enter-method to detect which factors independently affected DFS or OS. SPSS software (version 21; IBM Corp.) was used for statistical analysis. $P<0.05$ (two-tailed test) was considered to indicate a statistically significant difference.

Results

Clinicopathological characteristics. A total of 312 patients of the 409 CRC cases were enrolled, including 178 men (57.1%) and 134 women (42.9%), with a median age of 67 years (range, 28-83 years). The number of patients with a Ki67 expression level of +, ++, +++ and ++++ was 53 (17%), 91 (29.2%), 90 (28.8%) and 78 (25%), respectively. All clinicopathological parameters are listed in Table I. High Ki67 expression was significantly associated with poor histological differentiation of the tumor ($P<0.05$), while no other associations were observed (Table II).

Prognosis analysis for Ki67 expression. The 5-year DFS and OS rates for all included patients were 70.2% (219/312) and 76.9% (240/312), respectively. The expression level of Ki67 was associated with DFS and OS. Patients in the ++++ group had higher DFS and OS rates than those in the + or ++ group. The same trend was also observed based on the low- or high-expression classification. In patients treated with surgery and adjuvant chemotherapy, high Ki67 expression was associated with improved DFS but not OS, whereas in patients treated with surgery alone, there was no statistical association between Ki67 expression and DFS or OS (Fig. 2). In relation to the administration of chemotherapy, there was no statistical association between chemotherapy protocols and DFS or OS (Fig. S1). These results exclude the affection of chemotherapy protocols to survival of patients treated with surgery and adjuvant chemotherapy.

Table I. Clinicopathological characteristics of included patients.

Clinicopathological parameters	No. (%)
Median age (range)	67 (28-83)
Sex (M:F)	178:134
Location	
Right	122 (39.1)
Left	163 (52.2)
Middle	27 (8.7)
Histological differentiation	
Well	24 (7.7)
Moderate	262 (84)
Poor	13 (4.2)
Mucinous and signet	13 (4.2)
TNM stage	
I	27 (8.7)
II	140 (44.9)
III	145 (46.5)
Vascular invasion	68 (21.9)
Ki67 expression	
+	53 (17)
++	91 (29.2)
+++	90 (28.8)
++++	78 (25)

Multivariate analysis of DFS. To identify independent prognostic factors for tumor progression, multivariate analysis using a Cox proportional hazards regression model (Enter method) was performed. Ki67 expression level and other variables, including tumor location, histological differentiation, pathological T and N stage, vascular invasion, and adjuvant chemotherapy, were analyzed. The results suggested that pathological T stage and N stage were independent prognostic factors, whereas the Ki67 did not pass multivariate analysis to be an independent prognostic factor (Table III).

Discussion

Ki67 has been well established as a pathologic proliferation marker in cancer, which was first identified in Hodgkin

Table II. Association between Ki67 expression and clinicopathological parameters.

Clinicopathological parameters	Ki67 expression		P value
	Low (%)	High (%)	
Sex			0.541
Male	85 (59)	93 (55.4)	
Female	59 (41)	75 (44.6)	
Age			0.351
<60	32 (22.2)	45 (26.8)	
≥60	112 (77.8)	123 (73.2)	
Tumor location			0.641
Right	60 (41.7)	62 (36.9)	
Left	73 (50.7)	90 (53.6)	
Middle	11 (7.6)	16 (9.5)	
Histological differentiation			0.01
Well	13 (9)	11 (6.5)	
Moderate	117 (81.3)	145 (86.3)	
Poor	3 (2.1)	10 (6)	
Mucinous and signet-ring	11 (7.6)	2 (1.2)	
T stage			0.157
T1-2	12 (8.3)	22 (13.1)	
T3	109 (75.7)	129 (76.8)	
T4	23 (16)	17 (10.1)	
N stage			0.388
N0	72 (50)	95 (56.5)	
N1	35 (24.3)	40 (23.8)	
N2	37 (25.7)	33 (19.6)	
Vascular invasion			0.752
Yes	30 (21.1)	38 (22.6)	
No	112 (78.9)	130 (77.4)	

Table III. Multivariate analysis of DFS by Cox proportional hazards regression (Enter method).

Variable	HR	95% CI	P-value
T stage			0.008
T1	1		
T2	1.012	0.953-1.632	
T3	3.876	1.845-6.723	
T4	6.343	3.002-9.194	
N stage			0.002
N0	1		
N1	1.528	0.733-3.187	
N2	3.266	1.59-6.71	
Ki67			0.341
+	1		
++	1.057	0.581-1.925	
+++	0.881	0.464-1.647	
++++	0.597	0.289-1.232	
Vascular invasion			0.329
No	1		
Yes	1.298	0.769-2.191	
Histological differentiation			0.42
Well	1		
Moderate	1.529	0.641-3.649	
Poor	0.844	0.223-3.191	
Mucinous and Signet	2.092	0.697-6.276	
Tumor location			0.398
Right	1		
Left	0.762	0.478-1.213	
Middle	1.118	0.545-2.295	
Adjuvant chemotherapy			0.651
Yes	1		
No	1.199	0.546-2.632	

lymphoma cell nuclei 40 years ago (16). The function of Ki67 is complicated and has not yet been completely revealed. Based on current knowledge, Ki67 is a key protein for the formation of the perichromosomal layer, which is a ribonucleoprotein sheath coating the condensed chromosomes to prevent aggregation of mitotic chromosomes, during mitosis (5). During interphase, Ki67 maintains the normal distribution of heterochromatin antigens (17). The role of Ki67 in carcinogenesis has been well established that it promotes cell proliferation and tumor growth (18); and high Ki67 expression is associated with poor prognosis in numerous types of malignant tumors (19,20).

The prognostic value of Ki67 in colorectal cancer is still controversial. A meta-analysis including 34 studies and 6,180 patients with colorectal cancer by Luo *et al* (11) suggested that high Ki67 expression was associated with decreased DFS and OS, especially in patients with colon cancer who underwent surgery alone, but was not associated with prognosis in patients treated with surgery and adjuvant chemotherapy. Interestingly, another meta-analysis including 8,293 patients based on 30 studies by Xiong *et al* (12) reported that high Ki67 expression was associated with improved prognosis in patients treated with surgery and adjuvant therapy but worse prognosis in patients treated with surgery alone. The differences in conclusions between the two meta-analyses may have been due to the different clinical evidence selected for analysis. There are increasing reports demonstrating that high Ki67 expression is associated with improved response to adjuvant chemotherapy (10,12). For example, Fluge *et al* (21) investigated Ki67 expression in 409 patients with CRC, reporting that high Ki67 expression was associated with improved relapse-free survival not in all patients but only in the patients who received chemotherapy. Similarly, other studies have also provided evidence suggesting that high expression levels of Ki67 are associated with improved response to adjuvant chemoradiotherapy in CRC (22), although contradictory evidence also exists (15,23). Due to the inconsistencies among reports, more well-designed studies are required to clarify the prognostic value of Ki67 expression in colon cancer. The present study demonstrated that high Ki67

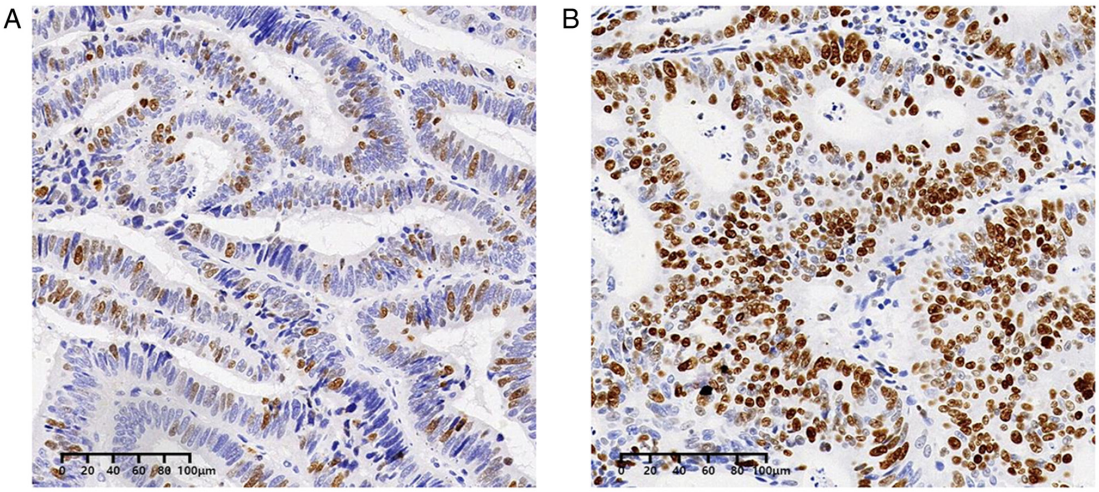

Figure 1. (A) Low and (B) high expression of Ki67 in colon cancer tissue (original magnification, x200).

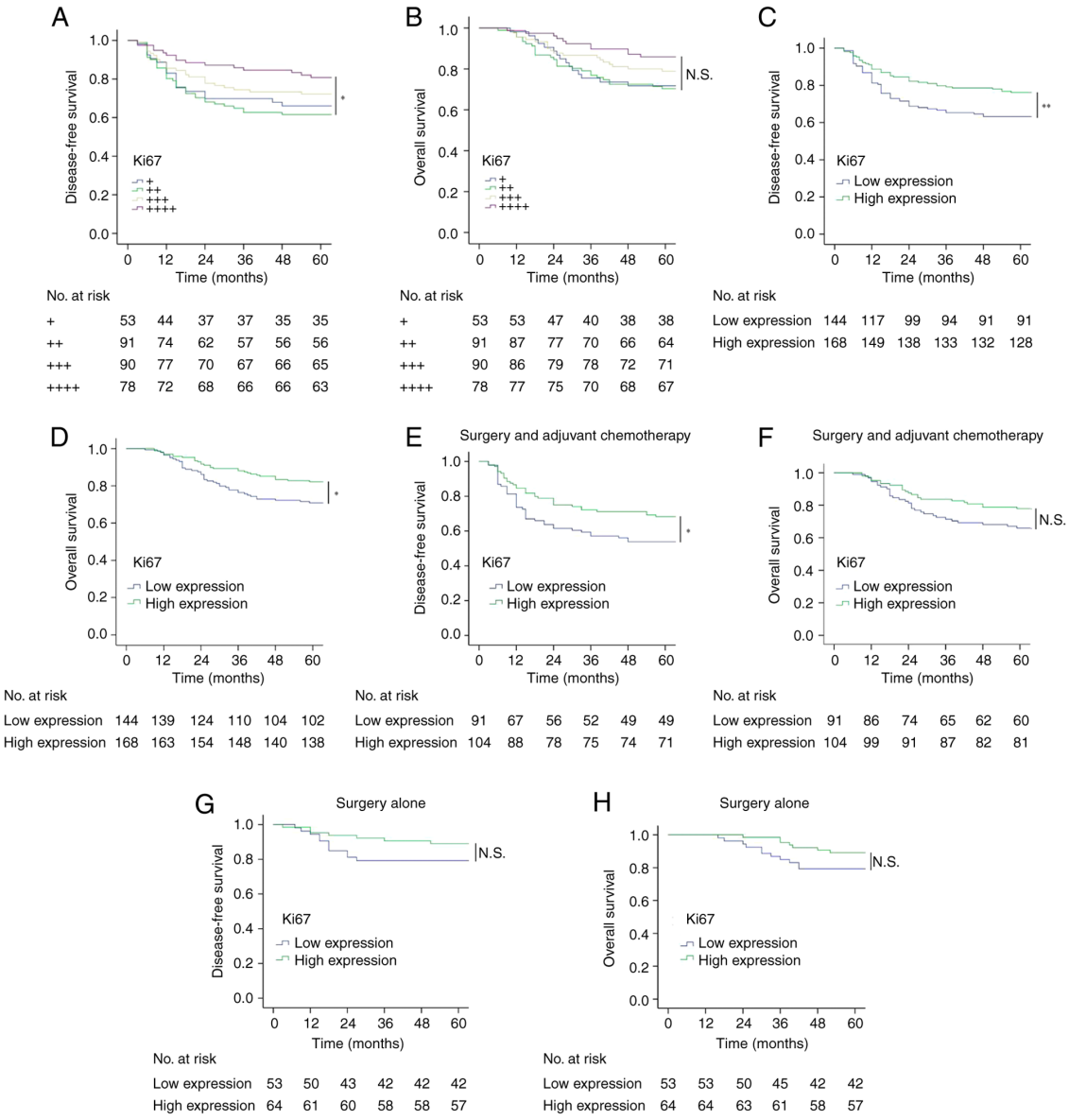

Figure 2. 5-year (A) DFS and (B) OS of patients with different expression levels of Ki67; 5-year (C) DFS and (D) OS in low and high Ki67 expression classification; 5-year (E) DFS and (F) OS in low and high Ki67 expression classification within the patients treated with surgery and adjuvant chemotherapy; 5-year (G) DFS and (H) OS in low and high Ki67 expression classification within the patients treated with surgery alone. *P<0.05, **P<0.01. N.S., no significance; DFS, disease-free survival; OS, overall survival.

expression was associated with improved DFS for patients who were treated with surgery and adjuvant chemotherapy but not for those treated with surgery alone, suggesting that Ki67 is a potential predictive marker for therapeutic outcome, which should be further investigated for the development of precision medicine. On the other hand, the evaluation criteria of Ki67 is crucial in the present study. The assessment of Ki67 varies among different studies. Some researchers used global positive percentage (10), while others used 'hot spot' field counting (24). A high-quality international study published in 2016 validated the analytical variability of different Ki67 evaluation criteria, finding that the global percentage method has the highest interlaboratory agreement, whereas the hot-spot methods is marginally acceptable (25). In the present study, all marked cells in the fields were evaluated including the moderate and strong positive cells. The present study also suggested that the 50% cutoff value was suitable to stratify patients with colon cancer into postoperative tumor progression risk groups, which is supported by other reports (8,26).

Unlike other studies, the present study divided patients into two groups depending on the therapy they received. The results demonstrated that the prognostic value of Ki67 was different for patients treated with surgery alone compared with those treated with surgery and chemotherapy. Although high Ki67 expression was not observed to be associated with poor survival in the surgery alone group in the present study, which was inconsistent with the results of a single study (27), the patients with high Ki67 expression in the surgery and chemotherapy group had improved therapeutic outcomes, suggesting that tumors with high proliferative activity exhibited increased sensitivity to chemotherapy (22,28). High-expression of Ki67 suggests active proliferation and mitosis of tumor, so this kind of tumor in inclined to be more sensitive to chemotherapy. However, the colon cancer is highly heterogeneous, patients of different race and countries often respond variously to the same treatment. On the other hand, acquired chemo-resistance after therapy is another factor affecting clinical outcome, which would 'dilute' the contribution of Ki67 to prognosis. Therefore, the conclusions from different studies are usually inconsistent, more clinical evidences are needed to demonstrate the prognostic value of Ki67 in colon cancer. Based on our findings, Ki67 is a potential prognostic marker for outcome prediction for patients with colon cancer receiving adjuvant chemotherapy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CD and YP designed the study and enrolled patients. QL, DR, LW and JF collected patients' information and analyzed data. WD and DM provided technological support for immunohistochemistry work. CD and YP confirmed the authenticity of the raw data. All authors participated in writing the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The research was approved by the Ethics Committee of Southern University of Science and Technology (Shenzhen, China) and Peking University Cancer Hospital (Beijing, China). All patients provided their signed informed consent for the use of their tissue samples and medical records for research.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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