Prognostic Value of Tumor Size in Colon Cancer—Smaller is Better?

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Abstract

Background: The prognostic value of tumor size in colon cancer remains controversial. This study aimed to reveal the correlation between tumor size and prognosis of colon cancer.

Methods: A total of 491 patients with colon cancer were included in this study. The correlation of tumor size with prognosis, mismatch repair status, and other clinicopathological characteristics as well as tumor microenvironment was analyzed.

Results: For stage IIA microsatellite stable (MSS) colon cancer, tumors sized <3.5 cm and ≥5 cm were associated with a poorer disease free survival (DFS) compared with tumors sized between 3.5 and 5 cm (P = .002). Small tumor size (HR = 5.098, P = .001) and large tumor size (HR = 2.749, P = .029) were found to be independent prognostic factors for stage IIA MSS colon cancer. Moreover, high expression of transgelin (TAGLN), a marker of cancer-associated fibroblasts (CAFs), was found to be an independent prognostic factor for poorer DFS (HR = 9.651, P = .009), which was also associated with smaller tumor size (P = .027).

Conclusion: Small (<3.5 cm) and large (≥5 cm) tumor sizes are associated with decreased DFS in stage IIA MSS colon cancer. Enrichment of TAGLN⁺ CAFs is associated with decreased DFS and small tumor size.

Keywords
colon cancer, tumor size, prognosis, tumor microenvironment, microsatellite status

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related deaths.¹ Approximately 70% of all CRC cases are colon cancer.² Local recurrence and distant metastasis remain the main causes of poor prognosis in patients with stage II-III colon cancer.³ Multiple factors are correlated with the prognosis of patients with T3N0-2M0 colon cancer, such as N stage, mismatch repair (MMR) tumor status, and mutation status of BRAF and KRAS. Traditionally, a smaller tumor size is deemed to be associated with better prognosis in patients with cancer because as the tumor grows, tumor cells would acquire additional mutations, and when they reach a certain point, these cells would acquire the ability to metastasize and survive in distance.⁴ A number of previous studies have shown the same phenomenon in stage T3 CRC.⁵,⁶ Interestingly, several studies have also shown that patients with a smaller tumor size in pancreatic cancer,⁷ breast cancer,⁸ and certain T stage colon cancer⁹-¹⁶ have a poorer prognosis, which is contrary to common knowledge; further, several studies

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have revealed an early spreading phenomenon of cancer cells.\textsuperscript{15,17-20}

Most previous studies on tumor size were based on data extracted from open databases,\textsuperscript{6,9,11,13,15,16,21} which cannot be analyzed in depth owing to loss of information for several essential high-risk factors, such as lymphovascular invasion (LVI), perineural invasion (PNI), tumor deposit, \textit{BRAF} or \textit{KRAS} mutation, positive circumferential resection margin (CRM), and MMR status. The correlation between tumor size and these pathological features is still unclear. For example, microsatellite instability (MSI) accounts for 22\% and 12\% of stage II and III colon cancers, respectively.\textsuperscript{22} Moreover, the MSI status is a marker of favorable outcomes in patients with stage II cancer,\textsuperscript{23,24} which might correlate with a larger tumor size and may be a confounding factor for prognosis prediction according to tumor size.

Hence, to comprehensively study the prognostic value of tumor size in colon cancer, we analyzed the relationship between tumor size and other clinicopathological characteristics emphasizing the tumor microenvironment (TME) in this study.

\textbf{Methods}

\textit{Study Population}

A total of 1321 patients with CRC with prospective follow-up data treated in the Department of General Surgery at Peking University Third Hospital between January 2010 and December 2018 were retrospectively analyzed. The inclusion criteria were as follows: (1) postoperative pathology confirming the diagnosis of stage T3 colon cancer (as most stage T3 rectal cancer are subjected to preoperative chemoradiation in our center, the rectal cancer group were not included in prognosis analysis); (2) radical surgery; and (3) complete inpatient data, including clinical, pathological, and follow-up data. Conversely, the exclusion criteria were as follows: (1) congenital megacolon, colonic torsion, colorectal trauma, and other benign diseases; (2) colorectal neuroendocrine tumor and adenoma; (3) neoadjuvant therapy or distant metastasis at initial diagnosis; and (4) patients with less than 3 months of follow-up. Ethical approval was obtained from Peking University Third Hospital (IRB000006761-M2020046), and this study adhered to the tenets of the Declaration of Helsinki. The need of informed consent was waived by Institutional Review Boards of Peking University Third Hospital.

\textit{Clinical Data and Follow-Up}

The medical records of all patients were reviewed. The AJCC eighth edition classification standard recommended by the NCCN guidelines was adopted for the pathological staging of the patients. The data included the TNM staging, tumor size, tumor location, tumor differentiation, and status of PNI, LVI, tumor deposit, MMR, and \textit{BRAF} or \textit{KRAS} mutation. The tumor size was determined as the longest diameter of the fixed specimen at the time of pathological examination. The diameter included the entire lesion containing both non-invasive and invasive tissue. The patients were followed up at 1 and 3 months after surgery and every 6 months thereafter. Abdominal and pelvic contrast-enhanced CT or MRI scans were routinely performed, and the CEA levels were assessed every 6 months for 2 years and then once every year for a total of 3 years at each follow-up. Colonoscopy was conducted within 1 year after surgery and then repeated every 2-3 years. The presence of new lesions revealed by biopsy or imaging was deemed as tumor recurrence. DFS was defined as the period from surgical treatment to tumor recurrence. Local recurrence was defined as the recurrence of local and regional lymph nodes in the original lesion area (any detectable local disease at follow-up, occurring either alone or in conjunction with distant recurrence). Distant metastasis was defined as systemic recurrence (any detectable disease at follow-up, except for local disease).

\textit{Immunohistochemical (IHC) Assessment}

IHC analysis was conducted on 50 formalin-fixed paraffin-embedded surgical specimens of T3N0-2M0 MSS CRC, including 35 stage IIA MSS CRC specimens. Primary antibodies against human CD3 (ZSGB-BIO, ZM-0417, 1:1), CD8 (ZSGB-BIO, ZA-0508, 1:1), and CD68 (ZSGB-BIO, ZM-0060,1:1) were used to observe CD3+, CD8+ T cells, and macrophages, respectively, while reticulocalbin 3 (RCN3) (Atlas antibodies, HPA043134, 1:150), and transgalmin (TAGLN) (Abcam, ab155272, 1:200) were used to investigate cancer-associated fibroblasts (CAFs).\textsuperscript{25,28} Tissue sections 4-μm-thick were deparaffinised and dehydrated. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 10 min at room temperature. Antigen retrieval was performed in ethylene diamine tetraacetic acid for 2 min at 100°C. The slides were incubated with the primary antibody at 37°C for 2 h. After 3 washes with phosphate-buffered saline, the slides were co-incubated with horseradish peroxidase-labeled goat anti-rabbit or anti-mouse secondary antibodies. The slides were counterstained with haematoxylin. The infiltration density of the stained cells per field was evaluated by 2 independent pathologists who were blinded to the patients’ clinical data. For each stained slide, 3 randomized fields of tumor center were selected under a high-power field of 200 (NanoZoomer-XR, Hamamastu, Japan), and CD3\textsuperscript{+}, CD8\textsuperscript{+}, CD68\textsuperscript{+}, RCN3\textsuperscript{+}, and TAGLN\textsuperscript{+}-stained cells were quantified using an image analysis software (Image-Pro Plus 6.0, Media Cybernetics, USA).
Statistical Analysis

The Kolmogorov-Smirnov method was used to determine the normality of the data. Normally distributed data were expressed as means ± standard deviations and analyzed using independent sample t test and one-way ANOVA. Categorical variables were analyzed using the chi-square test or Fisher’s exact test. Factors that influenced the DFS were assessed using the Cox proportional hazards model, which was established via univariate and multivariate analyses. Potential risk factors (P < .1) were adopted for the multivariate analysis with the backward stepwise method, following the results of the univariate analysis. The survival curve was drawn using the Kaplan-Meier method owing to the significant difference observed in the follow-up time of the patients; thus, all survival analyses were targeted at the cumulative survival rate of the patients. The optimal cut-off value of the tumor size and TME for the DFS was determined using the X-tile program. The y-axis of the X-tile plot represented all possible cut-off values for the large tumor size, with the size of the cut-off values increasing from top to bottom. Similarly, the x-axis of the X-tile plot represented all possible cut-off values for the small tumor size, with the size of the cut-off values increasing from left to right. The highest value (marked by the black circle) in the Kaplan-Meier log-rank chi-square test was generated by the optimal cut-off value. Green coloration indicated a direct association between a decreasing tumor size and poorer DFS. All statistical analyses were conducted using SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA). A P value of <.05 was recognized as statistically significant.

Results

Patient Characteristics

According to the inclusion and exclusion criteria, 491 patients were eventually enrolled in this study. The detailed flowchart of the patient selection process is shown in Supplementary Figure 1. The baseline clinicopathological characteristics of the patients are described in Supplementary Table 1. Right-sided tumors were found in 237 (48.3%) patients, while left-sided tumors were found in 254 (51.7%) patients. LVI, PNI, tumor deposits, MSI, and CRM positivity were found in 96 (19.6%), 83 (16.9%), 69 (14.1%), 74 (15.1%), and 8 (1.6%) patients, respectively. The median tumor size was 4.5 cm. The follow-up period ranged from 3 to 108 months, and the median follow-up time was 31 months. The cut-off value of the tumor size for the DFS was determined using the X-tile software; the patients were divided into 3 groups: <3.5 cm (n = 94, 19.1%), 3.5-5 cm (n = 155, 31.6%), and ≥5 cm (n = 242, 49.3%).

Comparison Between MSS and MSI Among the Patients With T3N0-2M0 Colon Cancer

To study the relationship between tumor size and microsatellite status, we compared the clinicopathological characteristics of MSS and MSI tumors. In total, 417 (84.9%) patients belonged to the MSS group and 74 (15.1%) patients to the MSI group. The patient characteristics according to the microsatellite status are shown in Supplementary Table 2. MSI was significantly associated with open surgery (P = .022), right-sided tumor (P < .001), larger tumor size (P < .001), and poorer differentiation (P < .001). To further investigate the relationship between MSI and prognosis, we conducted a survival analysis based on the microsatellite status among the patients with T3N0-2M0 colon cancer, and no significant difference was found (Figure 1(a), P = .2248). Similarly, there was no correlation found between the microsatellite status and DFS among those with T3N + M0 colon cancer (Figure 1(c), P = .7123). Conversely, MSI was observed to be associated with a better DFS tendency among those with stage IIA cancer (Figure 1(b), P = .068). Although there was no significant difference in the DFS between the MSI group and the MSS group in terms of having a large (≥5 cm) tumor size, the MSS group had a poor DFS tendency compared with the MSI group (Figure 1(d), P = .0675). Interestingly, based on the results of the log-rank test, tumor size could not differentiate the prognosis among the patients including the MSI group (Figure 1(e), P = .6085), while tumor size could differentiate the prognosis among the patients excluding the MSI group (Figure 1(f), P = .0482). A survival analysis based on tumor size for the DFS was also performed among the MSI group, which showed that tumor size was not correlated with prognosis (Figure 1(g), P = .3552).

Relationship of Tumor Size with Clinicopathological Parameters and Prognosis Among the Patients with Stage T3 MSS Colon Cancer

To assess the capability of tumor size to predict the DFS in patients with MSS colon cancer, we conducted a survival analysis on T3N0-2M0 MSS colon cancer cases. Tumors with a small (<3.5 cm) and large (≥5 cm) size showed a poorer DFS than did tumors with a median tumor size among the patients with T3N0M0 MSS colon cancer (Figure 2(a), P = .0021), while no significant difference in the DFS was found among the patients with T3N + M0 MSS colon cancer with different tumor sizes (Figure 2(b), P = .8158). To further investigate the association between tumor size and T3N + M0 MSS colon cancer, we performed a subgroup analysis among different N stage groups, and similar results were seen in the patients with T3N1M0 and T3N2M0...
MSS colon cancer (Figure 2(c) and 2(d), $P = .2327$ and $P = .1739$, respectively). As tumor size could distinguish patients with stage IIA colon cancer with a poor DFS, we analyzed the correlation between tumor size and clinicopathological parameters among those with stage IIA colon cancer. A total of 50 (21.2%) patients belonged to the <3.5 cm group, 80 (33.9%) to the 3.5-5 cm group, and 106 (44.9%) to the $\geq 5$ cm group. The patient characteristics according to tumor size are shown in Table 1.

Tumor site was related to tumor size, and right-sided tumors were relatively larger than those left-sided ($P = .047$). The number of harvested lymph nodes was also related to tumor size; the larger the tumor, the more the retrieved lymph node ($P < .001$). The remaining characteristics, including sex, age, CRM, LVI, PNI, histopathology, KRAS mutation, BRAF mutation, and recurrence site, did not show any significant association with tumor size ($P > .05$).

**Univariate and Multivariate Analyses for the DFS Among the Patients With Stage IIA MSS Colon Cancer**

According to the Cox proportional hazards models, we adopted a $P$ value of $<.1$ to indicate a significant difference. The univariate analysis showed that the DFS was only associated with a small tumor size ($P < .001$). The remaining characteristics, including sex, age, CRM, LVI, PNI, histopathology, KRAS mutation, BRAF mutation, and recurrence site, did not show any significant association with tumor size ($P > .05$).

**Figure 1.** Comparison of DFS in different MMR tumor status and different tumor size in T3N0-2M0 colon cancer (A) Kaplan-Meier analysis for DFS rate between different MMR tumor status in T3N0-2M0 colon cancer patients ($P = .2248$). (B) Kaplan-Meier analysis for DFS rate between different MMR tumor status in stage IIA colon cancer patients ($P = .068$). (C) Kaplan-Meier analysis for DFS rate between different MMR tumor status in T3N + M0 colon cancer patients ($P = .7123$). (D) Kaplan-Meier analysis for DFS rate between different MMR tumor status in stage IIA colon cancer patients with large tumor size ($P = .0675$). (E) Kaplan-Meier analysis for DFS rate between different tumor size in T3N0-2M0 colon cancer patients ($P = .0685$). (F) Kaplan-Meier analysis for DFS rate between different tumor size in T3N0-2M0 MSS colon cancer patients ($P = .0482$). (G) Kaplan-Meier analysis for DFS rate between different tumor size in T3N0-2M0 MSI colon cancer patients ($P = .3552$).
(HR = 4.883, \( P = .001 \)), a large tumor size (HR = 2.903, \( P = .021 \)), and LVI status (HR = 2.739, \( P = .015 \)). All of the abovementioned parameters were evaluated in the multivariate analysis for the DFS. A small tumor size (HR = 5.098, \( P = .001 \)), a large tumor size (HR = 2.749, \( P = .029 \)), and LVI status (HR = 2.889, \( P = .012 \)) were still associated with the DFS, as shown in Table 2. Thus, the results suggested that among the patients with stage IIA MSS colon cancer who underwent radical surgery, tumor size could be an independent prognostic factor for the DFS.

**Relationship of TME With Tumor Size and Prognosis**

The formalin-fixed paraffin-embedded surgical specimens of T3N0-2M0 MSS CRC were collected from a total of 50 patients, 35 of whom had stage IIA colon cancer. A variety of cell types in TME were studied via immunochemical staining. According to the X-tile program, high densities of CD3\(^+\) T cells, CD8\(^+\) T cells, CD68\(^+\) macrophages, RCN3 + CAFs, and TAGLN + CAFs were defined as >263/mm\(^2\), >97/mm\(^2\), >142/mm\(^2\), ≥271/mm\(^2\), and >40%/mm\(^2\), respectively. TAGLN + CAFs were associated with a small tumor size in both T3N0-2M0 and stage IIA MSS CRC cases (\( P = .01 \) and \( P = .027 \), respectively), as shown in Supplementary Table 3, whereas CD3\(^+\) T cells, CD8\(^+\) T cells, and CD68\(^+\) macrophages were not. We further investigated the relationship between TME and prognosis. The Kaplan-Meier survival analysis showed that low CD8\(^+\) density, high RCN3 + density, and high TAGLN + density were associated with a poor DFS in the patients with T3N0-2M0 MSS CRC (\( P = .0447 \), \( P = .0043 \), and \( P = .0023 \), respectively), while the densities of CD3 and CD68 were not correlated with the DFS, as shown in Supplementary Figure 4. The subgroup analysis showed that only a high TAGLN + density among those with stage IIA CRC was associated with a poorer DFS (\( P = .0013 \), Supplementary Figure 4(f)). The multivariate analysis showed that RCN3 (HR = 4.629, \( P = .011 \)) and TAGLN (HR = 5.014, \( P = .007 \)) were independent prognostic factors of the DFS in patients with T3N0-2M0 MSS CRC (Supplementary Table 4). A subgroup analysis was also performed among the patients with stage IIA colon cancer who underwent radical surgery, tumor size could be an independent prognostic factor for the DFS.
MSS CRC, and TAGLN (HR = 9.651, \(P = .009\)) remained an independent prognostic factor of the DFS. The details are shown in Supplementary Table 5.

### Discussion

Tumor size was defined as the maximal tumor diameter obtained from pathology reports on resected cancer specimens, which is an important prognostic factor in solid tumors, such as breast cancer,\textsuperscript{29} lung cancer,\textsuperscript{30} and prostate cancer.\textsuperscript{31} However, the prognostic value of tumor size in colon cancer remains unclear. Some studies have shown that tumor size is not related to the prognosis of colon cancer,\textsuperscript{32,33} while other studies showed that the prognosis of a larger tumor size is worse,\textsuperscript{5,6} which is consistent with our traditional view. Recently, studies have found that the prognosis of small tumors is worse under certain infiltration depths through large-data analysis in open databases.\textsuperscript{9-16} However, there is an inevitable lack of data in open databases, which makes it impossible to conduct in-depth analyses between tumor size and pathological features and to examine the actual impact of tumor size on prognosis. Patients with stage II colon cancer with MSI have been reported to have a better prognosis, and this type of tumor is more common in larger tumors,\textsuperscript{23,24} which is likely to be a confounding factor leading to a good prognosis for large tumors. By comparing the clinicopathological characteristics of MSI and MSS among the patients with T3N0-2M0 cancer, we found that MSI was indeed significantly associated with large tumors (\(\geq 5\) cm) \((P < .001)\). The survival analysis showed that there was no significant difference in the prediction of tumor size for the DFS when only those with MSI were included \((P = .0685)\). Interestingly, when we excluded these patients, we obtained a different finding: Both a small tumor size and large tumor size \((P = .0482)\)

### Table 1. Characteristics of Stage IIA MSS Colon Cancer Patients According to Tumor Size.

| Variables               | Tumor Size (%) | \(P\) Value |
|------------------------|----------------|-------------|
|                       | \(< 3.5 \text{ cm}\) | \(3.5-5 \text{ cm}\) | \(\geq 5 \text{ cm}\) |
| Gender Male           | 34 (68.0) | 47 (58.8) | 73 (68.9) | .322 |
| Female                | 16 (32.0) | 33 (41.2) | 33 (31.1) | |
| Age, years (SD)       | 68.8±11.8 | 67.7±12.2 | 64.9±12.7 | .123 |
| LNH±SD                | 13.8±5.5 | 17.3±8.2 | 19.3±6.5 | <.001 |
| CRM Negative          | 49 (98.0) | 80 (100.0) | 106 (100.0) | .210 |
| Positive              | 1 (2.0) | 0 (0.0) | 0 (0.0) | |
| LVI Negative          | 46 (92.0) | 76 (95.0) | 94 (88.7) | .329 |
| Positive              | 4 (8.0) | 4 (5.0) | 12 (11.3) | |
| PNI Negative          | 45 (90.0) | 71 (88.7) | 96 (90.6) | .920 |
| Positive              | 5 (10.0) | 9 (11.3) | 10 (9.4) | |
| Site Right            | 20 (40.0) | 33 (41.3) | 60 (56.6) | .047 |
| Left                  | 30 (60.0) | 47 (58.7) | 46 (43.4) | |
| Histology Well        | 10 (20.0) | 11 (8.3) | 17 (16.0) | .218 |
| Differentiation       | 30 (60.0) | 62 (77.5) | 78 (73.6) | |
| Poor differentiation  | 10 (20.0) | 7 (8.8) | 11 (10.4) | |
| KRAS mutation (N = 215) Wild | 30 (62.5) | 33 (47.1) | 54 (55.7) | .250 |
| Mutant                | 18 (37.5) | 37 (52.9) | 43 (44.3) | |
| BRAF mutation (N = 215) Wild | 47 (97.9) | 70 (100.0) | 94 (96.9) | .352 |
| Mutant                | 1 (2.1) | 0 (0.0) | 3 (3.1) | |
| Recurrence Local      | 2 (13.3) | 2 (33.3) | 4 (19.0) | .661 |
| Distant               | 13 (86.7) | 4 (66.7) | 17 (81.0) | |

LNH: lymph node harvest, SD: standard deviation, CRM: circumferential resection margin, LVI: lymphovascular invasion, PNI: perineural invasion.
were significant in the prediction of the DFS, which confirms our previous hypothesis. In addition, in this study, we gained insight into the mechanisms related to tumor size and prognosis.

In our study, except for the microsatellite status, no correlation was found between tumor size and other widely accepted high-risk factors, including mutations in BRAF or KRAS, poorly differentiated tumors, LVI, PNI, and positive CRM, indicating that the prognostic value of tumor size was not affected by these factors among the patients with stage IIA colon cancer. The unfavorable impact of a small and large tumor size on the DFS might be attributed to 2 metastasis theories of cancer. The first theory is that cancer cell metastasis begins at early stages of tumor development, and distant metastasis is considered to occur a few years ago before the primary lesion is diagnosed.15,17-20 Hu et al18 developed a novel calculation method (named SCIMET) that utilized multi-region sequencing data to estimate the size of the primary lesion during metastasis and found that metastatic seeding generally occurred before clinical diagnosis (the primary lesion was sized less than 1 cm³). They further analyzed genome sequencing data (primary/metastatic paired samples) of 39 cases with metastatic CRC, revealing the dissemination time point during the metastasis process; their analysis showed that CRC metastasis occurred 4.1 (interquartile range = 3.2-4.6) years before primary diagnosis, which further revealed the prevalence of early metastasis.17 Similarly, early metastasis has also been confirmed in breast cancer.20 In the process of tumorogenesis, tumors can be divided into those that are prone to metastasis and those that are not, which explains our findings that a small tumor size represented an aggressive biological pattern and poor prognosis in stage IIA colon cancer. The second theory is that cancer cells acquire metastatic potential through an accumulation of mutations as the tumor volume increases.8 After the tumor grows to a certain extent, selection pressure including immunity, ischemia, and hypoxia will force tumors to gain heterogeneity, and the larger the tumor, the greater the selection pressure; eventually, some tumor cells evolve into clones that are prone to distant metastatic seeding, leading to a poor prognosis of patients.34,35 Furthermore, in our study, tumor size was no longer associated with prognosis once lymph node metastasis occurred (node positive cancer), which indicates that the prognosis of cancer is comparable after the human immune system fails to fight against dissemination of cancer cells.

Notably, tumor metastasis is a multi-phase process in which cancer cells spread from the primary lesion and invade surrounding tissue and distant organs.36 This process depends tightly on the surrounding TME, which includes CD8+ T cells and CAFs.37,38 In our study, the patients with a low CD8+ density had a poorer prognosis than those with a high CD8+ density (P = .0447). Our previous studies have also shown that CD8+ T cells play an important role in tumor prognosis.39 A number of studies have revealed that CAF, a major component of tumor stroma, contributes actively to the development and progression of cancer.40,41 It could increase the permeability of the vessels and capillary density, leading to distant metastasis by secreting a major source of growth factors that promote the development of tumors, including VEGF.42 A multivariate analysis of the DFS according to TME and clinical parameters in T3N0-2M0 MSS CRC showed that both RCN3+ CAF (HR = 4.629, P = .011) and TAGLN+ CAF (HR = 5.014, P = .007) were independent prognostic factors, which is consistent with the findings of

### Table 2. Cox Proportional Hazards Model for DFS in Stage IIA MSS Colon Cancer Patients.

| Variables                        | DFS Univariable | DFS Multivariable |
|----------------------------------|-----------------|-------------------|
| Gender (male vs female)          | 1.027 (.546-1.931) | .934 | - |
| Age, years                       | 1.014 (.988-1.041) | .305 | - |
| Surgery (open vs laparoscopy)    | .951 (.440-2.055) | .898 | - |
| Tumor location (right vs left)   | 1.108 (.603-2.035) | .741 | - |
| Tumor size (<3.5 vs 3.5-5)       | 4.883 (1.893-12.596) | .001 | 5.098 (1.969-13.153) | .001 |
| (≥5 vs 3.5-5)                    | 2.903 (1.171-7.193) | .021 | 2.749 (1.107-6.828) | .029 |
| LVI (+ vs -)                     | 2.739 (1.214-6.181) | .015 | 2.889 (1.263-6.609) | .012 |
| PNI (+ vs -)                     | 1.517 (594.3-869) | .383 | - |
| (Poor vs well differentiation)   | 1.930 (614.6-606) | .260 | - |
| (Medium vs well differentiation) | 1.098 (384.3-138) | .862 | - |

HR: hazard ratio, CI: confidence interval, LVI: lymphovascular invasion, PNI: perineural invasion.
previous studies on CAFs, including one of our recent studies.\textsuperscript{25,37,38,40,41} Interestingly, a multivariate analysis of stage IIA MSS CRC showed that TAGLN\textsuperscript{+} CAF density remained an independent prognostic factor. By stabilizing the cytoskeleton through actin cross-linking, TAGLN protein favors tumor cell invasion and migration, and remodeling of extracellular matrix.\textsuperscript{33,44} Yu et al\textsuperscript{25} found that when TAGLN coding gene in human CAFs was silenced, the ability of CAFs to promote tumor metastasis and invasion became attenuated, suggesting that TAGLN might be responsible for tumor metastasis via the action of CAFs. Our results suggest that tumor size is independent of the CD3\textsuperscript{+}, CD8\textsuperscript{+}, CD68\textsuperscript{+}, and RCN3\textsuperscript{+} density but that a small tumor size is associated with an increased TAGLN\textsuperscript{+} density in both patients with T3N0-2M0 MSS and stage IIA MSS CRC. This indicates that a significant increase in CAF and the production of TAGLN protein may be a mechanism underlying the poor prognosis in patients with stage IIA MSS colon cancer with a small tumor size.

To our knowledge, this study is the first to reveal the prognostic value of tumor size in colon cancer with different microsatellite statuses and to compare the relationship between tumor size and pathological features. However, some limitations exist in this study. First, this was a single-center retrospective study, which inevitably faces the problem of a small sample size. Second, the follow-up time of this study was insufficient, and more meaningful results may be obtained by extending the follow-up time, such as the correlation between tumor size and overall survival. Third, the sample size of the IHC staining group was relatively small, leading to the absence of subdividing of patients in the N\textsuperscript{+} and N0 groups during statistical analysis in this study. However, the groups might very different in biologic phase and disease prognosis. Thus, additional samples were needed to further verify our results. In general, tumor size is routinely measured in clinical practise and is an available and promising marker for predicting prognosis in stage IIA colon cancer.

Conclusions

In summary, our research demonstrated that small and large tumors are associated with a decreased DFS in patients with stage IIA MSS colon cancer who underwent surgical treatment. Further, a high TAGLN\textsuperscript{+} CAF density is associated with a decreased DFS and small tumor size.

Authors’ Contributions

Y.M., B.W., and H.L. collected and analyzed data, and wrote the manuscript. F.L., Z.L., S.L., and J.W. contributed to data collection. Z.L and L.G. contributed to pathological analysis. X.Z. and H.W. provided intellectual contribution. L.G., X.Z., and W.F. supervised the project, discussed data analysis, and reviewed the manuscript.

Declaration of Conflicting Interests

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Ethics Approval

Ethical approval was obtained from the Institutional Review Boards of Peking University Third Hospital (IRB00006761-M2020046), and this study adhered to the tenets of the Declaration of Helsinki. The need of informed consent was waived by Institutional Review Boards of Peking University Third Hospital.

Consent

All authors consent for publication.

Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Supplemental Material

Supplemental material for this article is available online.

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