Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Prognostic Value of Tumour Size in Colon Cancer – Smaller is Better?

Siyi Lu
Peking University Third Hospital
Bingyan Wang
Peking University Third Hospital
Zhenzhen Liu
Peking University Third Hospital
Fei Li
Peking University Third Hospital
Yongqu Lu
Peking University Third Hospital
Yan Meng
Peking University Third Hospital
Junwei Wang
Peking University Third Hospital
Hao Wang
Peking University Third Hospital
Limei Guo
Peking University Third Hospital
Xin Zhou
Peking University Third Hospital
Wei Fu (fuwei@bjmu.edu.cn)
Peking University Third Hospital

Research Article

Keywords: colon cancer, tumour size, prognosis, tumour microenvironment, microsatellite status

Posted Date: June 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-566870/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

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

Methods: A total of 498 patients with colon cancer were included in this study. The correlation of tumour size with prognosis, mismatch repair status and other clinicopathological characteristics as well as tumour microenvironment was analysed.

Results: For stage IIA microsatellite stable (MSS) colon cancer, tumours sized <3.5 cm and ≥5 cm were associated with a poorer disease free survival (DFS) compared with tumours sized between 3.5 and 5 cm (p=0.002). Small tumour size (HR=5.098, p=0.001) and large tumour size (HR=2.749, p=0.029) 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=0.009), which was also associated with smaller tumour size (p=0.027).

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

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) tumour status, and mutation status of *BRAF* and *KRAS*. Traditionally, a smaller tumour size is deemed to be associated with better prognosis in patients with cancer because as the tumour grows, tumour cells would acquire additional mutations, and when they reach a certain point, these cells would acquire the ability to metastasise 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 tumour size in pancreatic cancer,[7] breast cancer,[8] and certain T stage colon cancer [9–16] have a poorer prognosis, which is contrary to common knowledge; further, several studies have revealed an early spreading phenomenon of cancer cells.[15, 17–20]

Most previous studies on tumour size were based on data extracted from open databases,[6, 9–11, 13, 15, 16, 21] which cannot be analysed in depth owing to loss of information for several essential high-risk factors, such as lymphovascular invasion (LVI), perineural invasion (PNI), tumour deposit, *BRAF* or *KRAS* mutation, positive circumferential resection margin (CRM), and MMR status. The correlation between tumour 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,[22]. Moreover, the MSI status is a marker of favourable outcomes in patients with stage II cancer,[23, 24] which might correlate with a larger tumour size and may be a confounding factor for prognosis prediction according to tumour size.

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

Methods

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 analysed. 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 centre, 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 tumour and adenoma; and 3) neoadjuvant therapy or distant metastasis at initial diagnosis. Ethical approval was obtained from 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.

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, tumour size, tumour location, tumour differentiation, and status of PNI, LVI, tumour deposit, MMR, and *BRAF* or *KRAS* mutation. The tumour 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 tumour recurrence. DFS was defined as the period from surgical treatment to tumour 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).

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 transgelin (TAGLN) (Abcam, ab155272, 1:200) were used to investigate cancer-associated fibroblasts (CAFs).[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 three washes with phosphate-buffered saline, the slides were co-incubated with horseradish peroxidase-labelled goat anti-rabbit or anti-mouse secondary antibodies. The slides were counter-stained with haematoxylin. The infiltration density of the stained cells per field was evaluated by two independent pathologists who were blinded to the patients' clinical data. For each stained slide, three randomised fields of tumour centre were selected under a high-power field of 200 (NanoZoomer-XR, Hamamastu, Japan), and $CD3^+$-, $CD8^+$-, $CD68^+$-, $RCN3^+$-, and $TAGLN^+$-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 analysed using independent sample t-test and one-way ANOVA. Categorical variables were analysed 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 ($p<0.1$) 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 tumour size and TME for the DFS was determined using the X-tile programme. The y-axis of the X-tile plot represented all possible cut-off values for the large tumour 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 tumour 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 colouration indicated a direct association between a decreasing tumour size and poorer DFS. All statistical analyses were conducted using SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA). A p value of <0.05 was recognized as statistically significant.

Results

Patient Characteristics

According to the inclusion and exclusion criteria, 498 patients were eventually enrolled in this study. The detailed flow chart of the patient selection process is shown in Figure 1. The baseline clinicopathological characteristics of the patients are described in Supplementary Table 1. Right-sided tumours were found in 241 (48.4%) patients, while left-sided tumours were found in 257 (51.6%) patients. LVI, PNI, tumour deposits, MSI, and CRM positivity were found in 98 (19.7%), 84 (16.9%), 70 (14.1%), 77 (15.5%), and 8 (1.6%) patients, respectively. The median tumour size was 4.5 cm. The follow-up period ranged from 1 to 108 months, and the median follow-up time was 31 months. The cut-off value of the tumour size for the DFS was determined using the X-tile software, as shown in Figure 2; the patients were divided into three groups: <3.5 cm (n=97, 19.5%), 3.5–5 cm (n=157, 31.5%), and ≥5 cm (n=244, 49%).

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

To study the relationship between tumour size and microsatellite status, we compared the clinicopathological characteristics of MSS and MSI tumours. In total, 421 (84.5%) patients belonged to the MSS group and 77 (15.5%) patients to the MSI group. The patient characteristics according to the microsatellite status are shown in Table 1. MSI was significantly associated with open surgery (p=0.043), right-sided tumour (p<0.001), larger tumour size (p<0.001), and poorer differentiation (p<0.001). 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 3A, p=0.2236). Similarly, there was no correlation found between the microsatellite status and DFS among those with T3N+M0 colon cancer (Figure 3C, p=0.7128). Conversely, MSI was observed to be associated with a better DFS tendency among those with stage IIA cancer (Figure 3B, p=0.0677). 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) tumour size, the MSS group had a poor DFS tendency compared with the MSI group (Figure 3D, p=0.0672). Interestingly, based on the results of the log-rank test, tumour size could not differentiate the prognosis among the patients including the MSI group (Figure 3E, p=0.0689), while tumour size could differentiate the prognosis among the patients excluding the MSI group (Figure 3F, p=0.0484). A survival analysis based on tumour size for the DFS was also performed among the MSI group, which showed that tumour size was not correlated with prognosis (Figure 3G, p=0.3603).

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

To assess the capability of tumour size to predict the DFS in patients with MSS colon cancer, we conducted a survival analysis on T3N0-2M0 MSS colon cancer cases. Tumours with a small (<3.5 cm) and large (≥5 cm) size showed a poorer DFS than did tumours with a median tumour size among the patients with T3N0M0 MSS colon cancer (Figure 4A, p=0.0021), while no significant difference in the DFS

was found among the patients with T3N+M0 MSS colon cancer with different tumour sizes (Figure 4B, p=0.8155). To further investigate the association between tumour 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 4C and 4D, p=0.2329 and p=0.1739, respectively). As tumour size could distinguish patients with stage IIA colon cancer with a poor DFS, we analysed the correlation between tumour size and clinicopathological parameters among those with stage IIA colon cancer. A total of 50 (21.1%) patients belonged to the <3.5 cm group, 80 (33.8%) to the 3.5–5 cm group, and 107 (45.1%) to the ≥5 cm group. The patient characteristics according to tumour size are shown in Table 2. Tumour site was related to tumour size, and right-sided tumours were relatively larger than those left-sided (p=0.046). The number of harvested lymph nodes was also related to tumour size; the larger the tumour, the more the retrieved lymph node (p<0.001). The remaining characteristics, including sex, age, CRM, LVI, PNI, histopathology, KRAS mutation, BRAF mutation, and recurrence site, did not show any significant association with tumour size (p>0.05).

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 <0.1 to indicate a significant difference. The univariate analysis showed that the DFS was only associated with a small tumour size (HR=4.883, p=0.001), a large tumour size (HR=2.903, p=0.021), and LVI status (HR=2.739, p=0.015). All of the abovementioned parameters were evaluated in the multivariate analysis for the DFS. A small tumour size (HR=5.098, p=0.001), a large tumour size (HR=2.749, p=0.029), and LVI status (HR=2.889, p=0.012) were still associated with the DFS, as shown in Table 3. Thus, the results suggested that among the patients with stage IIA MSS colon cancer who underwent radical surgery, tumour size could be an independent prognostic factor for the DFS.

Relationship of TME with Tumour 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. Representative immunoreactivity of the positive cells are shown in Figure 5. According to the X-tile programme, high densities of CD3+ T cells, CD8+ T cells, CD68+ macrophages, RCN3+ CAFs, and TAGLN+ CAFs were defined as >263/mm2, >97/mm2, >142/mm2, ≥271/mm2, and >40%/mm2, respectively. TAGLN+ CAFs were associated with a small tumour size in both T3N0-2M0 and stage IIA MSS CRC cases (p=0.01 and p=0.027, respectively), as shown in Table 4, 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=0.0447, p=0.0043, and p=0.0023, respectively), while the densities of CD3 and CD68 were not correlated with the DFS, as shown in Figure 6. The subgroup analysis showed that only a high TAGLN+ density among those with stage IIA CRC was associated with a poorer DFS (p=0.0013, Figure 6F). The multivariate analysis showed that RCN3 (HR=4.629, p=0.011) and TAGLN (HR=5.014, p=0.007) were

independent prognostic factors of the DFS in patients with T3N0-2M0 MSS CRC (Table 5). A subgroup analysis was also performed among the patients with stage IIA MSS CRC, and TAGLN (HR=9.651, p=0.009) remained an independent prognostic factor of the DFS. The details are shown in Table 6.

Discussion

Tumour size was defined as the maximal tumour diameter obtained from pathology reports on resected cancer specimens, which is an important prognostic factor in solid tumours, such as breast cancer,[29] lung cancer,[30] and prostate cancer.[31] However, the prognostic value of tumour size in colon cancer remains unclear. Some studies have shown that tumour size is not related to the prognosis of colon cancer,[32, 33] while other studies showed that the prognosis of a larger tumour size is worse,[5, 6] which is consistent with our traditional view. Recently, studies have found that the prognosis of small tumours is worse under certain infiltration depths through large-data analysis in open databases.[9-16] However, there is an inevitable lack of data in open databases, which makes it impossible to conduct in-depth analyses between tumour size and pathological features and to examine the actual impact of tumour size on prognosis. Patients with stage II colon cancer with MSI have been reported to have a better prognosis, and this type of tumour is more common in larger tumours,[23, 24] which is likely to be a confounding factor leading to a good prognosis for large tumours. 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 tumours (≥5 cm) (p<0.001). The survival analysis showed that there was no significant difference in the prediction of tumour size for the DFS when only those with MSI were included (p=0.0689). Interestingly, when we excluded these patients, we obtained a different finding: Both a small tumour size (p=0.0205) and large tumour size (p=0.0484) 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 tumour size and prognosis.

In our study, except for the microsatellite status, no correlation was found between tumour size and other widely accepted high-risk factors, including mutations in BRAF or KRAS, poorly differentiated tumours, LVI, PNI, and positive CRM, indicating that the prognostic value of tumour size was not affected by these factors among the patients with stage IIA colon cancer. The unfavourable impact of a small and large tumour size on the DFS might be attributed to two metastasis theories of cancer. The first theory is that cancer cell metastasis begins at early stages of tumour development, and distant metastasis is considered to occur a few years ago before the primary lesion is diagnosed.[15, 17-20] Hu et al.[18] developed a novel calculation method (named SCIMET) that utilised 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 cm^3). They further analysed 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 ago 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 tumourigenesis, tumours can be divided into those that are prone to metastasis and those that are not, which explains our findings that a small tumour 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 tumour volume increases.[4] After the tumour grows to a certain extent, selection pressure including immunity, ischaemia, and hypoxia will force tumours to gain heterogeneity, and the larger the tumour, the greater the selection pressure; eventually, some tumour cells evolve into clones that are prone to distant metastatic seeding, leading to a poor prognosis of patients.[34, 35] Furthermore, in our study, tumour 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, tumour 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=0.0447). Our previous studies have also shown that $CD8^+$ T cells play an important role in tumour prognosis.[39] A number of studies have revealed that CAF, a major component of tumour 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 tumours, 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=0.011) and $TAGLN^+$ CAF (HR=5.014, p=0.007) were independent prognostic factors, which is consistent with the findings of previous studies on CAFs, including one of our recent study.[25, 37, 38, 40, 41] Interestingly, a multivariate analysis of stage IIA MSS CRC showed that $TAGLN^+$ CAF density remained an independent prognostic factor. By stabilising the cytoskeleton through actin cross-linking, TAGLN protein favour tumour cell invasion and migration, and remodelling of extracellular matrix.[43, 44] Yu et al.[25] found that when *TAGLN* coding gene in human CAFs was silenced, the ability of CAFs to promote tumour metastasis and invasion became attenuated, suggesting that TAGLN might be responsible for tumour metastasis via the action of CAFs. Our results suggest that tumour size is independent of the $CD3^+$, $CD8^+$, $CD68^+$, and $RCN3^+$ density but that a small tumour size is associated with an increased $TAGLN^+$ 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 tumour size.

To our knowledge, this study is the first to reveal the prognostic value of tumour size in colon cancer with different microsatellite statuses and to compare the relationship between tumour size and pathological features. However, some limitations exist in this study. Firstly, this was a single-centre retrospective study, which inevitably faces the problem of a small sample size. Secondly, 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 tumour size and overall survival. Thirdly, the sample size of the IHC staining group was relatively small, and additional samples were needed to further verify our results. In general, tumour 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 tumours are associated with a decreased DFS in patients with stage IIA MSS colon cancer who underwent surgical treatment. Further, a high TAGLN^{+} CAF density is associated with a decreased DFS and small tumour size.

Declarations

Ethics approval and consent to participate

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 for publication

All authors consent for publication.

Availability of data and materials

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.

Competing interest

The authors declare no competing interests.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 91959110 and 81972702) and the Natural Science Foundation of Beijing (Grant No.7204324) and Clinical Medicine Plus X - Young Scholars Project, Peking University(PKU2020LCXQ002), the Fundamental Research Funds for the Central Universities.

Authors’ contributions

S.L and B.W collected and analyzed data, and wrote the manuscript. F.L, Z.L, Y.M and J.W contributed to data collection. Y.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.

Acknowledgements

Not applicable.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
2. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-64.
3. Gill S, Loprinzi CL, Sargent DJ, Thome SD, Alberts SR, Haller DG, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? J Clin Oncol. 2004;22(10):1797-806.
4. Schmidt-Kittler O, Ragg T, Daskalakis A, Granzow M, Ahr A, Blankenstein TJ, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. Proc Natl Acad Sci U S A. 2003;100(13):7737-42.
5. Kornprat P, Pollheimer MJ, Lindtner RA, Schlemmer A, Rehak P, Langner C. Value of tumor size as a prognostic variable in colorectal cancer: a critical reappraisal. Am J Clin Oncol. 2011;34(1):43-9.
6. Saha S, Shaik M, Johnston G, Saha SK, Berbiglia L, Hicks M, et al. Tumor size predicts long-term survival in colon cancer: an analysis of the National Cancer Data Base. Am J Surg. 2015;209(3):570-4.
7. Muralidhar V, Nipp RD, Mamon HJ, Punglia RS, Hong TS, Ferrone C, et al. Association Between Very Small Tumor Size and Decreased Overall Survival in Node-Positive Pancreatic Cancer. Ann Surg Oncol. 2018;25(13):4027-34.
8. Wo JY, Chen K, Neville BA, Lin NU, Punglia RS. Effect of very small tumor size on cancer-specific mortality in node-positive breast cancer. J Clin Oncol. 2011;29(19):2619-27.
9. Huang B, Feng Y, Mo SB, Cai SJ, Huang LY. Smaller tumor size is associated with poor survival in T4b colon cancer. World J Gastroenterol. 2016;22(29):6726-35.
10. Dai W, Mo S, Xiang W, Han L, Li Q, Wang R, et al. The Critical Role of Tumor Size in Predicting Prognosis for T1 Colon Cancer. The Oncologist. 2019;25(3):244-51.
11. Huang B, Feng Y, Zhu L, Xu T, Huang L, Cai G. Smaller tumor size is associated with poor survival in stage II colon cancer: An analysis of 7,719 patients in the SEER database. Int J Surg. 2016;33 Pt A:157-63.
12. Lee SY, Kim CH, Kim YJ, Kim HR. Macroscopic serosal invasion and small tumor size as independent prognostic factors in stage IIA colon cancer. Int J Colorectal Dis. 2018;33(8):1139-42.

13. Muralidhar V, Nipp RD, Ryan DP, Hong TS, Nguyen PL, Wo JY. Association Between Very Small Tumor Size and Increased Cancer-Specific Mortality in Node-Positive Colon Cancer. Dis Colon Rectum. 2016;59(3):187-93.
14. Santullo F, Biondi A, Cananzi FCM, Fico V, Tirelli F, Ricci R, et al. Tumor size as a prognostic factor in patients with stage IIa colon cancer. Am J Surg. 2018;215(1):71-7.
15. Wang Y, Zhuo C, Shi D, Zheng H, Xu Y, Gu W, et al. Unfavorable effect of small tumor size on cause-specific survival in stage IIA colon cancer, a SEER-based study. Int J Colorectal Dis. 2015;30(1):131-7.
16. Huang B, Chen C, Ni M, Xue X, Cai G, Cai S. The association between small tumor size and poor survival in T4 mucinous adenocarcinoma of colon without distant metastasis. J BUON. 2017;22(1):170-7.
17. Hu Z, Li Z, Ma Z, Curtis C. Multi-cancer analysis of clonality and the timing of systemic spread in paired primary tumors and metastases. Nat Genet. 2020;52(7):701-8.
18. Hu Z, Ding J, Ma Z, Sun R, Seoane JA, Scott Shaffer J, et al. Quantitative evidence for early metastatic seeding in colorectal cancer. Nat Genet. 2019;51(7):1113-22.
19. Klein CA. Parallel progression of primary tumours and metastases. Nat Rev Cancer. 2009;9(4):302-12.
20. Hosseini H, Obradovic MMS, Hoffmann M, Harper KL, Sosa MS, Werner-Klein M, et al. Early dissemination seeds metastasis in breast cancer. Nature. 2016;540(7634):552-8.
21. Pan H, Cui J, Cai K, Zhou Y. Increased cancer-specific mortality of very small size in carcinoembryonic antigen-elevated rectal cancer. Annals of Translational Medicine. 2019;7(18):447-.
22. Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010;28(3):466-74.
23. Klingbiel D, Saridaki Z, Roth AD, Bosman FT, Delorenzi M, Tejpar S. Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial. Ann Oncol. 2015;26(1):126-32.
24. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010;28(20):3219-26.
25. Yu B, Chen X, Li J, Qu Y, Su L, Peng Y, et al. Stromal fibroblasts in the microenvironment of gastric carcinomas promote tumor metastasis via upregulating TAGLN expression. BMC Cell Biol. 2013;14:17.
26. Balanescu P, Ladaru A, Balanescu E, Pompilian V, Gologanu D, Caraiola S, et al. Circulating Reticulocalbin 1 and Reticulocalbin 3 in Systemic Sclerosis Patients: Results of a Case Control Study. Clin Lab. 2016;62(6):1109-16.
27. Lee HO, Hong Y, Etlioglu HE, Cho YB, Pomella V, Van den Bosch B, et al. Lineage-dependent gene expression programs influence the immune landscape of colorectal cancer. Nat Genet. 2020;52(6):594-603.

28. Zhou Y, Bian S, Zhou X, Cui Y, Wang W, Wen L, et al. Single-Cell Multiomics Sequencing Reveals Prevalent Genomic Alterations in Tumor Stromal Cells of Human Colorectal Cancer. Cancer Cell. 2020.
29. Foulkes WD, Reis-Filho JS, Narod SA. Tumor size and survival in breast cancer–a reappraisal. Nat Rev Clin Oncol. 2010;7(6):348-53.
30. Ettinger DS, Wood DE, Aggarwal C, Aisner DL, Akerley W, Bauman JR, et al. NCCN Guidelines Insights: Non-Small Cell Lung Cancer, Version 1.2020. J Natl Compr Canc Netw. 2019;17(12):1464-72.
31. Mohler JL, Antonarakis ES, Armstrong AJ, D'Amico AV, Davis BJ, Dorff T, et al. Prostate Cancer, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2019;17(5):479-505.
32. Crozier JE, McMillan DC, McArdle CS, Angerson WJ, Anderson JH, Horgan PG, et al. Tumor size is associated with the systemic inflammatory response but not survival in patients with primary operable colorectal cancer. J Gastroenterol Hepatol. 2007;22(12):2288-91.
33. Miller W, Ota D, Giacco G, Guinee V, Irimura T, Nicolson G, et al. Absence of a relationship of size of primary colon carcinoma with metastasis and survival. Clin Exp Metastasis. 1985;3(3):189-96.
34. Makohon-Moore A, Iacobuzio-Donahue CA. Pancreatic cancer biology and genetics from an evolutionary perspective. Nat Rev Cancer. 2016;16(9):553-65.
35. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
36. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer. 2002;2(8):563-72.
37. Gout S, Huot J. Role of cancer microenvironment in metastasis: focus on colon cancer. Cancer Microenviron. 2008;1(1):69-83.
38. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423-37.
39. Wang B, Li F, Guo L, Lu S, Ma J, Ma Y, et al. Loss of survival advantage for deficient mismatch repair in patients with advanced colorectal cancer may be caused by changes in prognostic value of CD8+T cell. World J Surg Oncol. 2020;18(1):196.
40. Kiaris H, Chatzistamou I, Kalofoutis C, Koutselini H, Piperi C, Kalofoutis A. Tumour-stroma interactions in carcinogenesis: basic aspects and perspectives. Mol Cell Biochem. 2004;261(1-2):117-22.
41. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121(3):335-48.
42. Fukumura D, Xavier R, Sugiura T, Chen Y, Park EC, Lu N, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell. 1998;94(6):715-25.
43. Thompson O, Moghraby JS, Ayscough KR, Winder SJ. Depletion of the actin bundling protein SM22/transgelin increases actin dynamics and enhances the tumourigenic phenotypes of cells.

BMC Cell Biol. 2012;13:1.

44. Lee EK, Han GY, Park HW, Song YJ, Kim CW. Transgelin promotes migration and invasion of cancer stem cells. J Proteome Res. 2010;9(10):5108-17.

Tables

Table 1. Characteristics of patients according to microsatellite status

Variables	MSI	MSS	p *value*
Gender			0.064
Male	37 (48.1)	250 (59.4)	
Female	40 (51.9)	171 (40.6)	
Age, years ± SD	65 ± 14	67 ± 12	0.282
Surgery			0.043
Open	25 (32.5)	92 (21.9)	
Laparoscopy	52 (67.5)	329 (78.1)	
Site			<0.001
Right	52 (67.5)	189 (44.9)	
Left	25 (32.5)	232 (55.1)	
Tumor size			<0.001
>3.5 cm	6 (7.8)	91 (21.6)	
3.5-5 cm	13 (16.8)	144 (34.2)	
≥5 cm	58 (75.3)	6 (44.2)	
Lymph node status			0.324
Negative	48 (62.3)	237 (56.3)	
Positive	29 (37.7)	184 (43.7)	
Histopathology			<0.001
Well differentiation	0 (0.0)	54 (12.8)	
Moderate differentiation	43 (55.8)	277 (65.8)	
Poor differentiation	34 (44.2)	90 (21.4)	
LVI			0.131
Positive	20 (26.0)	78 (18.5)	
Negative	57 (74.0)	343 (81.5)	
PNI			0.099
Positve	8 (10.4)	76 (18.1)	
Negative	69 (89.6)	345 (81.9)	
Tumor deposits			0.769

Positive	**10 (13.0)**	**60 (14.3)**	
Negative	**67 (87.0)**	**361 (85.7)**	
LNH ± SD	**18.2 ± 6.2**	**17.1 ± 6.8**	**0.187**

MSI: microsatellite instability, MSS: microsatellite stable, SD: standard deviation, LVI: lymphovascular invasion, PNI: perineural invasion, LNH: lymph node harvest.

Table 2. Characteristics of stage IIA MSS colon cancer patients according to tumor size

Variables	Tumor size (%)			P value
	<3.5 cm	3.5-5 cm	≥5 cm	
Gender				0.316
Male	34 (68.0)	47 (58.8)	74 (69.2)	
Female	16 (32.0)	33 (41.2)	33 (30.8)	
Age, years ± SD	68.8 ± 11.8	67.7 ± 12.2	64.9 ± 12.7	0.123
LNH ± SD	13.8 ± 5.5	17.3 ± 8.2	19.3 ± 6.5	<0.001
CRM				0.211
Negative	49 (98.0)	80 (100.0)	107 (100.0)	
Positive	1 (2.0)	0 (0.0)	0 (0.0)	
LVI				0.351
Negative	46 (92.0)	76 (95.0)	95 (88.8)	
Positive	4 (8.0)	4 (5.0)	12 (11.2)	
PNI				0.960
Negative	45 (90.0)	71 (88.7)	97 (90.7)	
Positive	5 (10.0)	9 (11.3)	10 (9.3)	
Site				0.046
Right	20 (40.0)	33 (41.3)	61 (57.0)	
Left	30 (60.0)	47 (58.7)	46 (43.0)	
Histology				0.235
Well differentiation	10 (20.0)	11 (8.3)	18 (16.8)	
Moderate differentiation	30 (60.0)	62 (77.5)	78 (72.9)	
Poor differentiation	10 (20.0)	7 (8.8)	11 (10.3)	
KRAS mutation (N=215)				0.261
Wild	30 (62.5)	33 (47.1)	54 (55.7)	
Mutant	18 (37.5)	37 (52.9)	43 (44.3)	
BRAF mutation (N=215)				0.352
Wild	47 (97.9)	70 (100.0)	94 (96.9)	
Mutant	1 (2.1)	0 (0.0)	3 (3.1)	

Recurrence (N=42)				0.661
Local	2 (13.3)	2 (33.3)	4 (19.0)	
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.

Table 3. Cox proportional hazards model for DFS in stage IIA MSS colon cancer patients

Variables	DFS			
	Univariable		Multivariable	
	HR (95%CI)	P *value*	HR (95%CI)	P *value*
Gender(male vs female)	0.974 (0.518-1.831)	0.934	-	-
Age, years	1.014 (0.988-1.041)	0.305	-	-
Surgery(open vs laparoscopy)	0.951 (0.440-2.055)	0.898	-	-
Tumor location(right vs left)	1.108 (0.603-2.035)	0.741	-	-
Tumor size	-	0.005	-	0.003
(<3.5 vs 3.5-5)	4.883 (1.893-12.596)	0.001	5.098 (1.969-13.153)	0.001
(≥5 vs 3.5-5)	2.903 (1.171-7.193)	0.021	2.749 (1.107-6.828)	0.029
LVI (+ vs -)	2.739 (1.214-6.181)	0.015	2.889 (1.263-6.609)	0.012
PNI (+ vs -)	1.517 (0.594-3.869)	0.383	-	-
Histopathology	-	0.257	-	-
(poor vs well differentiation)	1.930 (0.614-6.066)	0.260	-	-
(medium vs well differentiation)	1.098 (0.384-3.138)	0.862	-	-

HR: hazard ratio, CI: cofidence interval, LVI: lymphovascular invasion, PNI: perineural invasion.

Table 4. The relationship between tumor size and TME in stage T3 MSS colorectal cancer patients

Variables	Tumor size		p *value*
	<3.5cm	≥3.5cm	
CD3			0.630
High	10 (55.6)	20 (62.5)	
Low	8 (44.4)	12 (37.5)	
CD8			0.750
High	11 (61.1)	21 (65.6)	
Low	7 (38.9)	11 (34.4)	
CD68			0.157
High	10 (55.6)	24 (75.0)	
Low	8 (44.4)	8 (25.0)	
RCN3			0.198
High	7 (38.9)	7 (21.9)	
Low	11 (61.1)	25 (78.1)	
TAGLN			0.010
High	9 (50.0)	4 (12.5)	
Low	9 (50.0)	28 (87.5)	
TAGLN*			0.027
High	4 (57.1)	3 (10.7)	
Low	3 (42.9)	25 (89.3)	

*TAGLN of Stage IIA MSS colorectal cancer patients

Table 5. Cox proportional hazards model for DFS in 50 stage T3 MSS colorectal cancer patients

Variables	DFS			
	Univariate		Multivariate	
	HR (95%CI)	P *value*	HR (95%CI)[a]	P *value*
CD3 (High vs Low)	0.503(0.161-1.573)	0.238	-	-
CD8 (High vs Low)	0.326(0.103-1.031)	0.056	-	-
CD68 (High vs Low)	0.377(0.121-1.172)	0.092	-	-
RCN3 (High vs Low)	4.612(1.457-14.601)	0.009	4.629(1.424-15.047)	0.011
TAGLN (High vs Low)	5.008(1.583-15.840)	0.006	5.014(1.550-16.222)	0.007

HR: hazard ratio, CI: confidence interval

[a] Adjusted by tumor size, CD3 and CD68

Table 6. Cox proportional hazards model for DFS in 35 stage IIA MSS colorectal cancer patients

Variables	Score	N	DFS			
			Univariate		Multivariate	
			HR (95%CI)	P *value*	HR (95%CI)[a]	P *value*
TAGLN	Low	28	1 (-)	-	1 (-)	-
	High	7	9.651(1.764-52.810)	0.009	9.651(1.764-52.810)	0.009

HR: hazard ratio, CI: confidence interval

[a] Adjusted by age and tumor size

Figures

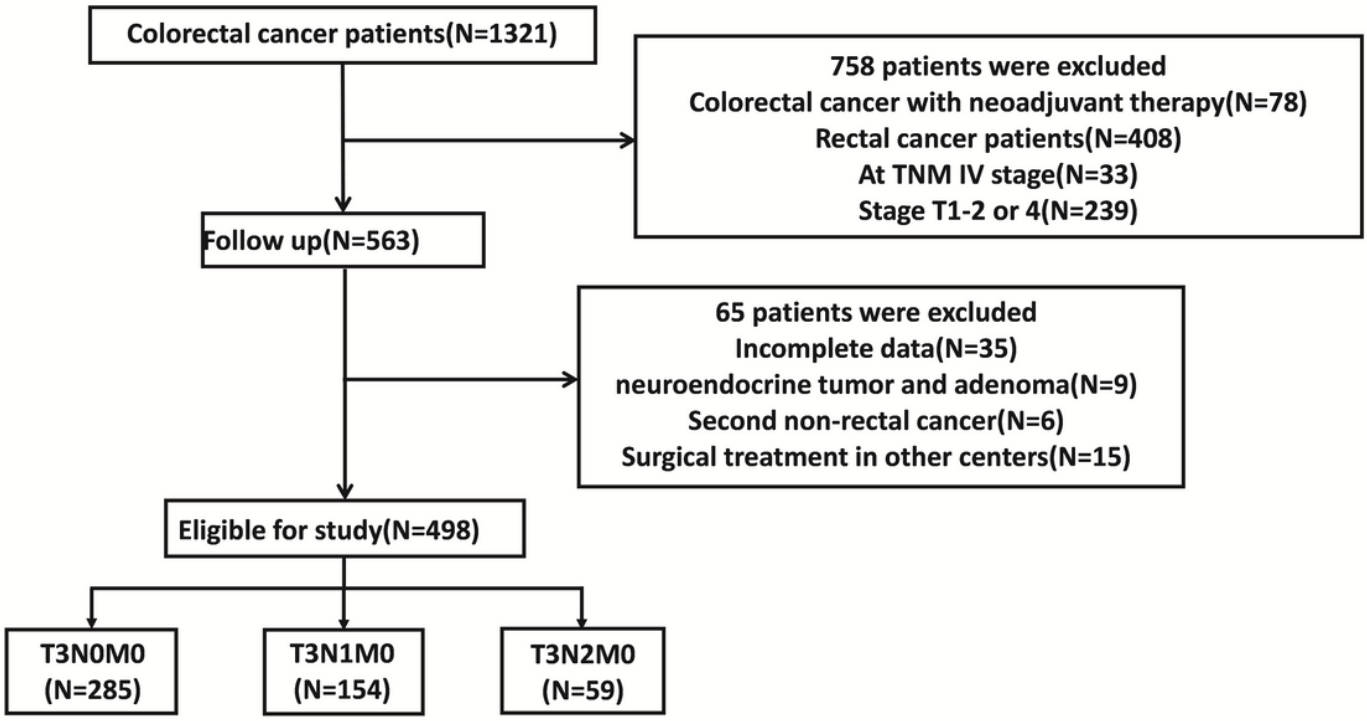

Figure 1

Flow chart of eligible cases selection

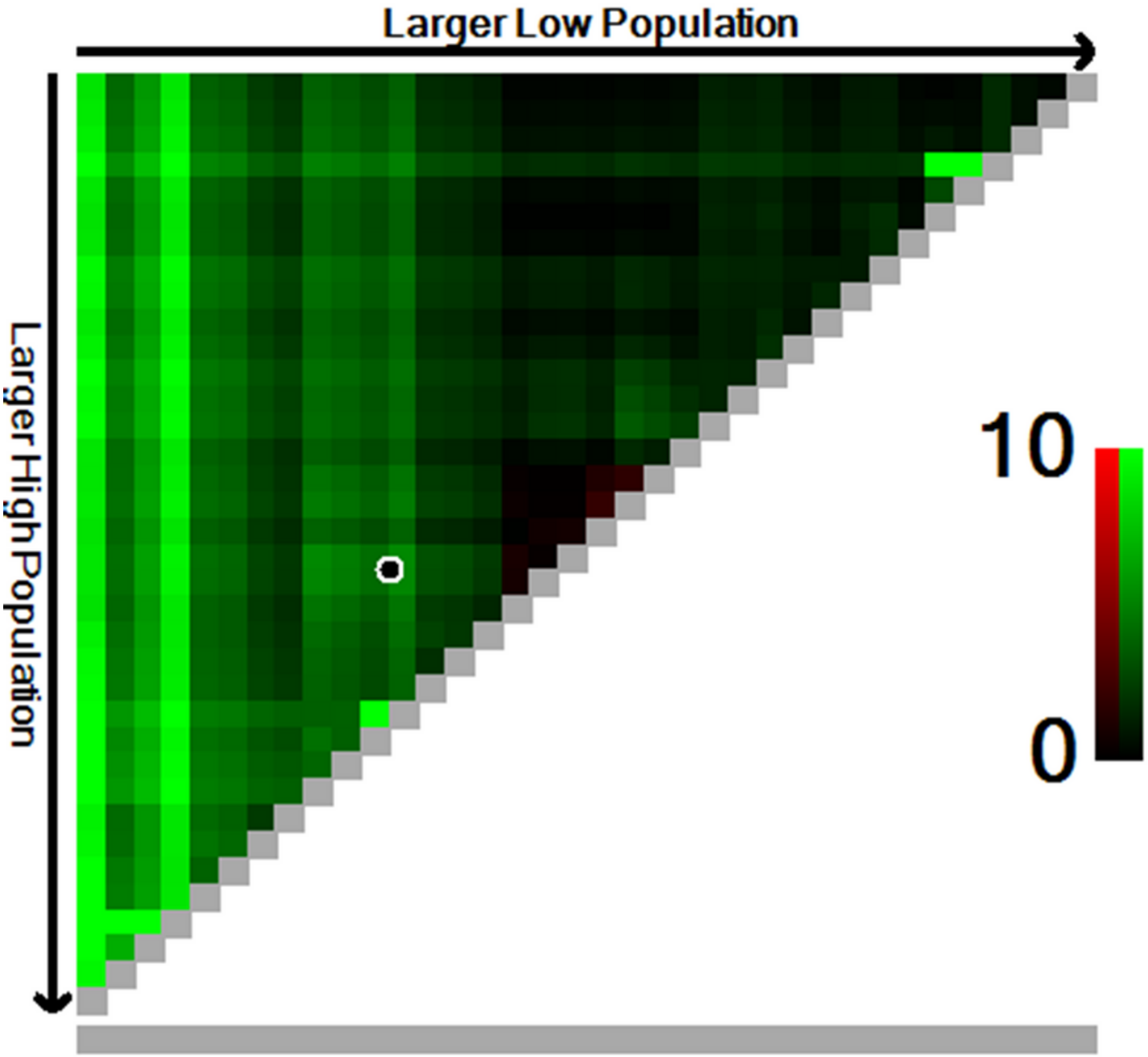

Figure 2

X-tile plot of tumor size in patients with stage T3N0-2M0 colon cancer. Brighter pixels indicate a stronger association between tumor size and DFS. When the study cohort was divided into large, medium and small tumor size subsets using a cut-off point of 3.5cm and 5.0 cm, Kaplan-Meier log-rank chi-square value of large/medium/small tumor size sub-setting reach highest (marked by the black circle).

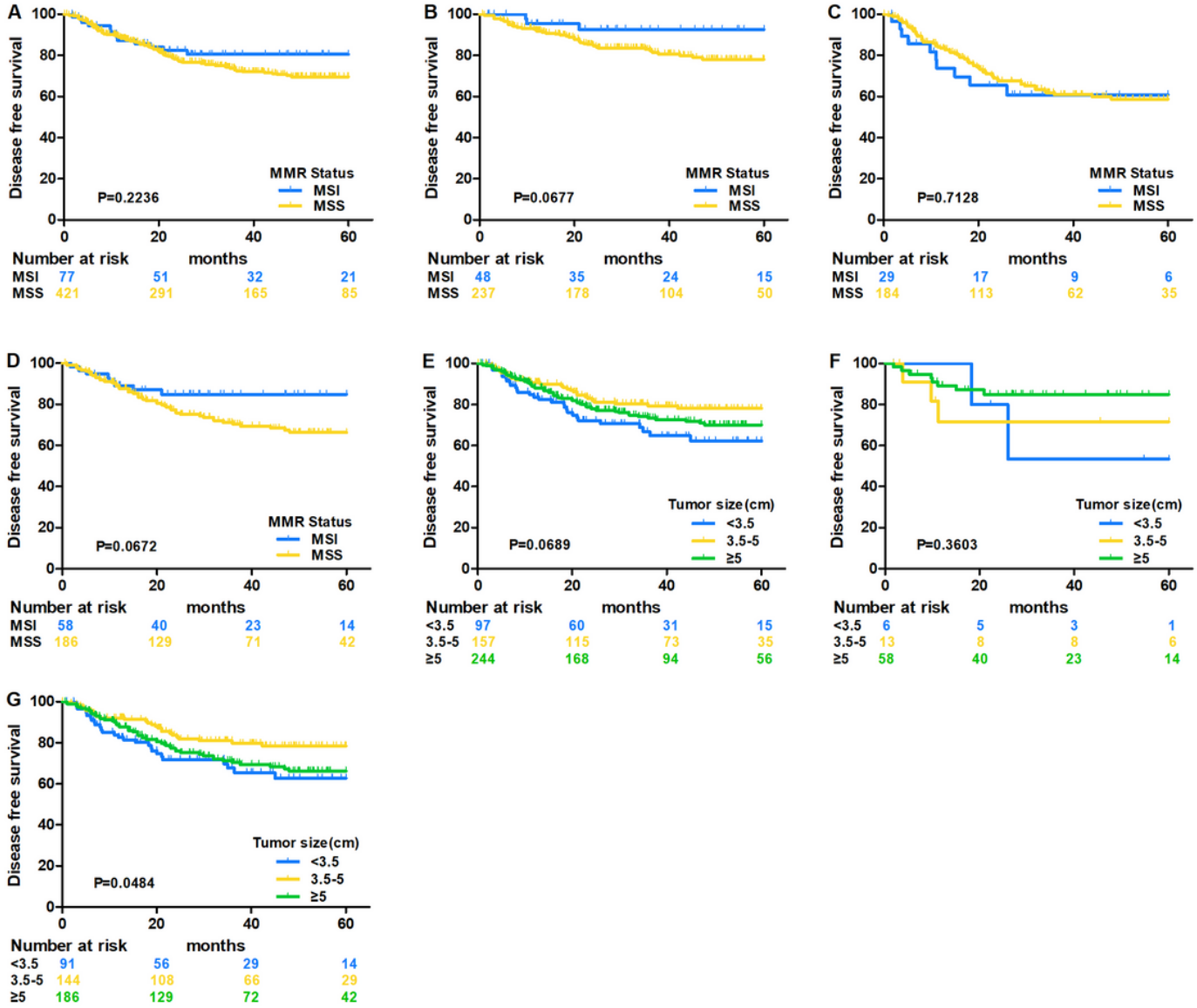

Figure 3

Comparison of DFS in different MMR tumor status and different tumor size in T3N0-2M0 colon caner (A) Kaplan–Meier analysis for DFS rate between different MMR tumor status in T3N0-2M0 colon cancer patients (p=0.2236). (B) Kaplan–Meier analysis for DFS rate between different MMR tumor status in stage IIA colon cancer patients (p=0.0677). (C) Kaplan–Meier analysis for DFS rate between different MMR tumor status in T3N+M0 colon cancer patients (p=0.7128). (D) Kaplan–Meier analysis for DFS rate between different MMR tumor status in stage IIA colon cancer patients with large tumor size (p=0.0672). (E) Kaplan–Meier analysis for DFS rate between different tumor size in T3N0-2M0 colon cancer patients (p=0.0689). (F) Kaplan–Meier analysis for DFS rate between different tumor size in T3N0-2M0 MSS colon cancer patients (p=0.0484). (G) Kaplan–Meier analysis for DFS rate between different tumor size in T3N0-2M0 MSI colon cancer patients (p=0.3603).

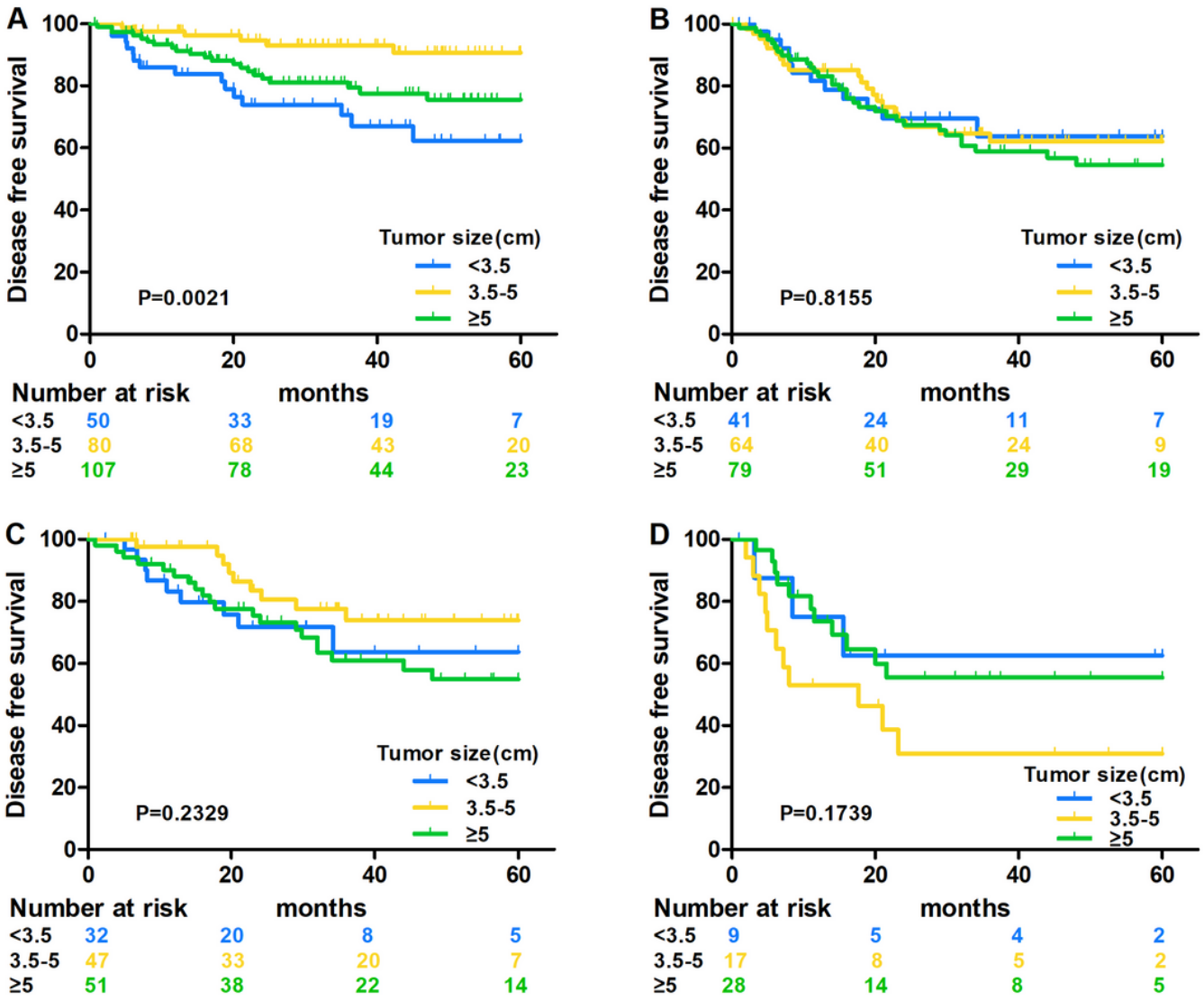

Figure 4

Survival curve based on tumor size in T3N0-2M0 MSS colon cancer (A) Kaplan–Meier analysis for DFS rate between different tumor size in Stage IIA MSS colon cancer patients (p=0.0021). (B) Kaplan–Meier analysis for DFS rate between different tumor size in T3N+M0 MSS colon cancer patients (p=0.8155). (C) Kaplan–Meier analysis for DFS rate between different tumor size in T3N1M0 MSS colon cancer patients (p=0.2329). (D) Kaplan–Meier analysis for DFS rate between different tumor size in T3N2M0 MSS colon cancer patients (p=0.1739).

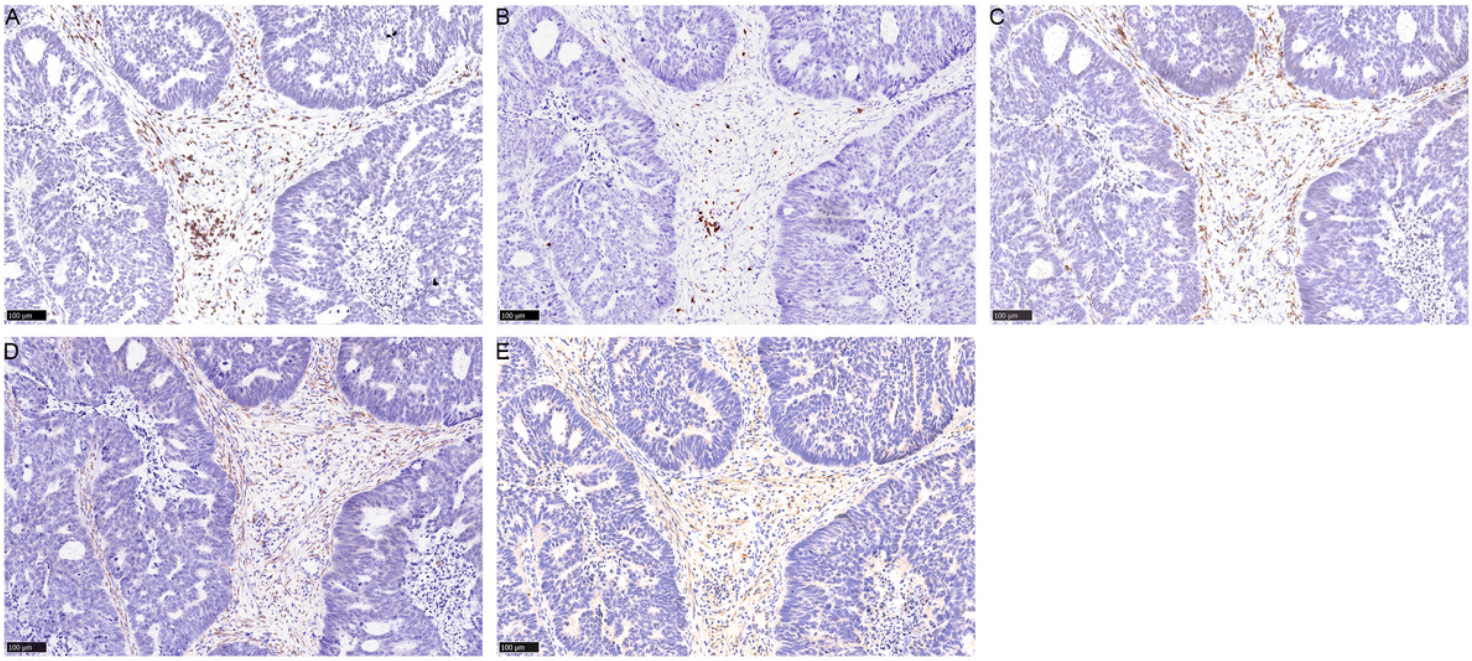

Figure 5

Major immune cells and CAFs in TME by IHC staining Characterizations of CD3+ (A), CD8+ (B), CD68+ (C), RCN3+ (D), and TAGLN+ cells (E).

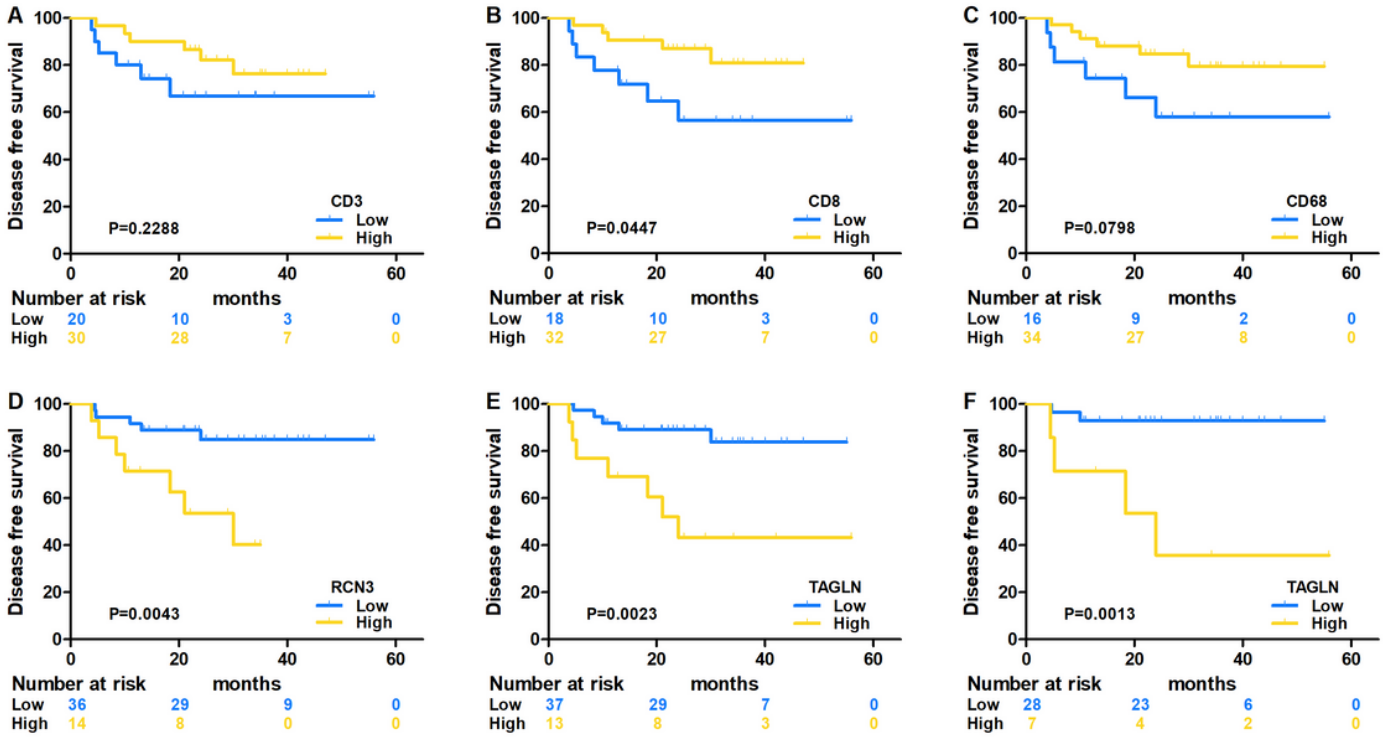

Figure 6

The correlation between TME and DFS Kaplan–Meier analysis for DFS between low and high densities of major immune cell groups and CAFs in T3N0-2M0 MSS colorectal cancer patients, namely, CD3+ group (p=0.2288) (A), CD8+ group (p=0.0447) (B), CD68+ group (p=0.0798) (C), RCN3+ group (p=0.0043) (D), TAGLN+ group (p=0.0023) (E), and (F) TAGLN+ group in stage IIA MSS colorectal cancer patients (p=0.0013).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx