



Distribution of Metastatic Cancer Cells in Colorectal Mesentery

Chen-Sen Ma¹ · Yi-Xin Tong¹ · Jian-Ping Gong¹

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Abstract

Background To investigate the distribution of metastatic cancer cells in the mesentery (referred to as metastasis V) and enrich the understanding of the metastasis of colorectal cancer.

Methods A total of two hundred ninety-nine patients who received colorectal operations at the Department of Gastrointestinal Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology between April 2018 and December 2018 were included. Samples were acquired from the dissected mesentery after the operation, and hematoxylin–eosin staining or immunohistochemistry was used to detect metastatic cancer cells. Pathological factors, including tumor position, tumor size, invasion depth, tumor differentiation, lymph node involvement, local vessel invasion, and perineural invasion, were recorded.

Results Metastatic cancer cells in the colorectal mesentery (metastasis V) were detected in 62 of 299 patients. Metastasis V was closely correlated with tumor invasion depth, lymph node metastasis, tumor differentiation, and perineural and vessel invasion by cancer cells. Metastasis V occurred more frequently in patients with T3 stage (26.27%) and T4 stage (40.00%) than in patients with T1 and T2 stages (0% and 2%, respectively). Metastasis V was frequently detected in patients with N2a and N2b stage tumors (51.72% and 61.54%, respectively). Metastasis V was more frequently detected in patients with perineural metastasis and local vessel invasion. In addition, metastasis V incidences in colon and rectal cancer were similar.

Conclusion The incidence rate of metastasis V is correlated with tumor staging factors and occurs more frequently in advanced-stage patients.

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✉ Jian-Ping Gong
jpgong@tjh.tjmu.edu.cn

¹ Department of GI Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Ave., Wuhan 430030, Hubei, People's Republic of China

Introduction

Colorectal cancer (CRC) is a high-mortality disease worldwide [1]. A high local regional recurrence rate is one of the reasons for the high mortality rate [2, 3]. Over time, surgeons have paid attention to reducing recurrence. RJ Heald introduced the total mesorectum excision (TME) for rectal cancer 30 years ago [4]. Recently, Hohenberger proposed complete mesocolic excision (CME) for colon cancer [5]. Their work had increased the lymph node harvest number and reduced local regional recurrence of CRC. However, lymph node dissection protocols cannot thoroughly explain the promotion of prognosis.

A previous study reported the existence of metastatic cancer cells in adipose tissue, and metastatic cancer cells have been closely related to poor prognosis [6]. In addition, we have also discovered metastatic cancer cells and nodules in mesentery of gastric cancer [7] and colorectal cancer [8]. The metastatic cancer cells and nodules in the mesentery were not continuous with primary tumor, metastatic lymph node, or hematogenous metastasis. We assume that this metastatic pathway was different from the conventional routes (direct invasion, peritoneum dissemination, lymphatic metastasis, and hematogenous metastasis); thus, we named it “metastasis V” [7–9]. Metastasis V left behind after an operation is termed “cancer leak” and leads to poor prognosis [10]. We aimed in the current study to investigate the distribution of metastasis V in the colorectal mesentery and provide information for clinical treatment.

Materials and methods

Patients, samples, and sectional tissue analysis

A total of 299 patients who underwent colorectal surgery for CRC at the Department of Gastrointestinal Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology between April 2018 and December 2018 were included in this study. All participants signed written informed consent forms for this study. This study was approved by the Ethics Committee of the first author’s institute. Clinical samples of CRC were obtained from resected colorectal tissue specimens. The demographic characteristics of the 299 patients are described in Tables 1 and 2. The clinicopathological stages were determined according to the 8th edition of the AJCC Cancer Staging Manual.

Metastasis V detection

All the mesentery from the colonic wall to the ligation clip of the vessel root was obtained after the local lymph nodes were retrieved. The specimen was divided into 1 cm × 1 cm pieces and numbered (see Supplementary Figure S1) and fixed in 4% formaldehyde solution for at least 24 h. After fixation, HE slices were generated for each piece of mesentery for the first screening. The number of slices for each specimen varied with the area of the entire obtained mesentery. If a potential or suspect metastasis V was found in a specimen, IHC (pan-cytokeratin, PCK) staining slices were generated and investigated for confirmation. The slices were checked by two senior pathologists.

Statistical analysis

Chi-square and ANOVA analyses were used to inspect the inter-group differences in incidence and metastasis V numbers, respectively. We considered a *P* value less than 0.05 to be statistically significant. Standard statistical analyses were performed with SPSS version 22.0.

Results

Patients and baseline analysis

Two hundred ninety-nine patients were enrolled in the study. As shown in Table 1, the participants were 117 females (39.13%) and 182 males (60.87%). The average patient age was 56.69 ± 11.74 years (range 25–86). Among the patients, 289 (96.66%) received radical operations, and 10 (3.34%) received palliative operations because of distant metastasis or peritoneal metastasis with complications, such as obstruction and perforation. There were 48, 131, 110, and 10 patients with stages I, II, III, and IV disease, respectively. The average number of retrieved lymph nodes was 16.16 ± 9.16 (2–75). Metastasis V was detected in 62 of the 299 (20.74%) patients.

Metastasis V occurred more frequently in patients with advanced colorectal cancer

Tumor stage is closely related to poor prognosis in CRC [11]. We compared the incidence and average metastasis V numbers among patients with each stage of cancer. The Chi-square analysis showed significant differences in the incidence rates between each stage ($P < 0.05$), and the incidence of metastasis V in patients with advanced stage (stage III 45/110; 40.91%; stage IV 7/10; 70.00%) was higher than that in patients with stage II (9/131; 6.87%) and stage I (1/52; 1.92%). The ANOVA results showed that there was no significant difference in the average metastasis V number between those with stage I and stage II disease, and the average metastasis V number in stage III and stage IV patients was much higher than that in stage I and stage II patients (Fig. 1a). Therefore, metastasis V occurs more frequently in patients with advanced colorectal cancer.

Metastasis V occurred more frequently in patients with colorectal cancer with invasion depths greater than T3

We compared the incidence and the metastasis V number at different tumor invasion depths (T stage) (Table 1, Fig. 1b). Most metastasis V occurred in stage T3 and T4

Table 1 Data in 299 colorectal cancer patients

Parameters	Results ^a
<i>Gender</i>	
Female	27/117 (23.08%)
Male	35/182 (19.23%)
Age (year, mean, range)	56.69 ± 11.74 (25–86)
Lymph nodes retrieved (mean ± SD, range)	16.16 ± 9.12 (2–75)
<i>Location of tumor</i>	
Colon	24/138 (17.39%)
Rectum	38/161 (23.60%)
<i>Histologic grade (G)^b</i>	
G1	2/17 (11.76%)
G2	32/195 (15.42%)
G3	28/87 (32.18%)
<i>Invasive depth</i>	
T1	0/7 (0.00%)
T2	1/50 (2.00%)
T3	51/217 (26.27%)
T4	10/25 (40.00%)
<i>Lymph node metastasis</i>	
N0	9/181 (4.97%)
N1	24/68 (35.29%)
N2a	18/34 (52.94%)
N2b	11/16 (68.75%)
<i>Distant metastasis</i>	
M0	55/289 (19.03%)
M1	7/10 (70.00%)
<i>LVI</i>	
Negative	39/243 (16.05%)
Positive	23/46 (50.00%)
<i>Perineural invasion</i>	
Negative	28/225 (12.44%)
Positive	34/74 (45.94%)

LVI local vessel invasion of cancer cells

^aThe data were showing as (number of patients with metastasis V)/(number of total patients in the category) (%)

^bHistologic Grade classification according to AJCC 8th, G1: well differentiated; G2: moderately differentiated; G3: poorly differentiated

patients. Chi-square analysis showed significant differences in incidence rates between different T stages. Metastasis V was more likely to occur in patients with stages T3 (51/217; 23.50%) and T4 (10/25; 40.00%) than in those with stages T1 (0/7; 0%) and T2 (1/50; 2.00%) (Table 1). Moreover, ANOVA showed that the average metastasis number was also much higher in T3 and T4 patients than in T2 and T1 patients (Fig. 1b). The above results support that metastasis V occurs more frequently in patients with colorectal cancer with deeper invasion.

Metastasis V occurred more frequently in patients with local regional lymph node metastasis

Local regional lymph node metastasis (N stage) can predict poor prognosis [6]. We analyzed the incidences and average metastasis V number in each N stage. Chi-square analysis showed significant differences in incidence rates between different N stages, and metastasis V appeared to occur more frequently in the N-positive groups (N1: 35.29%; N2a: 52.94%; N2b: 68.75%) than in the N-negative group (4.97%) (Table 1). ANOVA showed that the average metastasis V number in the N2b group was significantly higher than that in the N2a, N1, and N0 groups combined ($P < 0.05$); the average metastasis V number in the N2a group was significantly higher than that in the N1 and N0 groups combined ($P < 0.05$); and the average metastasis V number in the N1 group was significantly higher than that in the N0 group ($P < 0.05$) (Fig. 1c). Therefore, metastasis V occurs more frequently in patients with regional lymph node metastasis.

Metastasis V occurred more frequently in colorectal cancer with low histologic grade

We compared the incidence and metastasis V number in each histologic grade. Chi-square analysis showed significant differences in incidence rates between different grades. Metastasis V was more likely to occur in patients with G3 (28/87; 32.18%) than in those with G1 (2/17; 11.76%) and G2 (32/195; 15.42%) (Table 1). Moreover, ANOVA showed that the average metastasis V number was also much higher in G3 patients than in G2 patients, but it was not higher in G3 patients than in G1 patients, possibly because the G1 sample size was small (Fig. 1d). The above results support that metastasis V occurs more frequently in colorectal cancer patients with low histologic grades.

Metastasis V occurred more frequently in patients with local vessel invasion

A previous study showed that metastasis V is the appearance of cancer cells in the mesogastrium, mesocolon, or mesorectum independent of lymph node or vessel invasion [7, 8]. We explored whether metastasis V is related to local vessel invasion by cancer cells. The results showed that there was a significant difference between the groups with local vessel invasion (LVI-positive group) and without local vessel invasion (LVI-negative group), and specifically, the metastasis V incidence in the LVI-negative group (39/243, 16.05%) was significantly lower than the incidence in the LVI-positive group (23/46, 50.00%) ($P < 0.05$); the patients without LVI had a lower metastasis V number than the patients with LVI ($P < 0.05$).

Table 2 Cases in TNM classification

	TNM stage	Total ^a
I	T1N0M0	0/7 (0)
I	T2N0M0	1/41 (2.44%)
IIa	T3N0M0	8/123 (6.50%)
IIb	T4N0M0	1/8 (12.50%)
IIIa	T2N1M0	0/8 (0)
IIIb	T3N1M0	15/46 (32.61%)
IIIb	T3N2aM0	12/25 (48.00%)
IIIc	T3N2bM0	10/15 (66.67%)
IIIb	T4N1M0	5/12 (41.67%)
IIIc	T4N2aM0	1/1 (100.00%)
IIIc	T4N2bM0	2/3 (66.67%)
IV	T3N0M1	0/1 (0)
IV	T3N1M1	2/2 (100.00%)
IV	T3N2M1	3/4 (75.00%)
IV	T4N2M1	1/2 (50.00%)

^aThe data were showing as (number of patients with metastasis V)/(number of total patients in the category) (%)

(Fig. 1e). Thus, metastasis V is associated with LVI by tumor cells.

Metastasis V occurred more frequently in patients with perineural invasion

The results showed that there was a significant difference between the groups with perineural invasion (neuro-positive group) and without perineural invasion (neuro-negative group), and specifically, the incidence of metastasis V in the neuro-negative group (28/225, 12.44%) was significantly lower than that in the neuro-positive group (34/74, 45.94%) ($P < 0.05$). The patients without perineural invasion had a lower metastasis V number than the patients with perineural invasion ($P < 0.05$) (Fig. 1f). Thus, metastasis V is associated with perineural invasion by tumor cells.

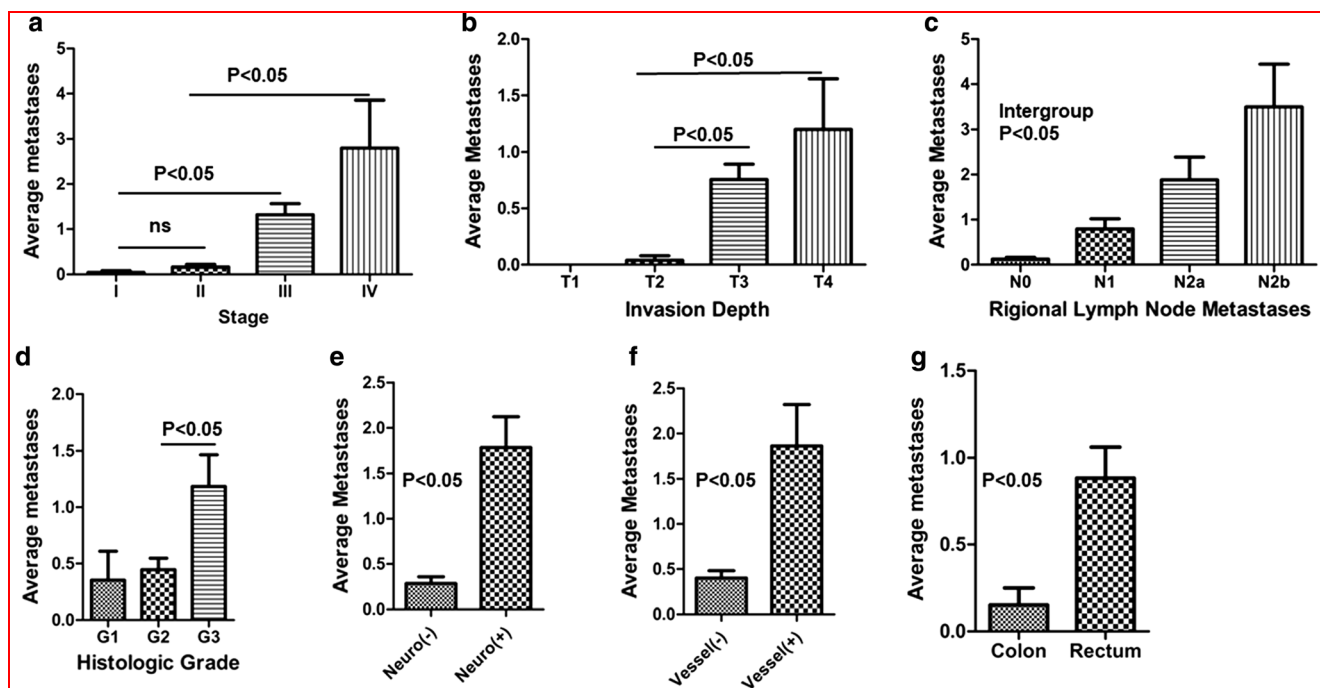


Fig. 1 Average metastases numbers in each category. **a** Average metastasis V number in stages I, II, III, IV, respectively. The stages were determined according to AJCC 8th. **b** Average metastasis V number in invasion depth T1, T2, T3, and T4, respectively. The invasion depth was determined according to AJCC 8th. **c** Average metastasis V number in local lymph node metastasis category N0, N1, N2a, N2b, respectively. The N stages were determined according to AJCC 8th. **d** Average metastasis V number in each histological differentiation category according to AJCC 8th. G1: well differentiated; G2: moderately differentiated; G3: poorly differentiated. **e** Average metastasis V number in subgroup without perineural metastasis [Neuro (-)] vs subgroup with perineural metastasis (Neuro (+))

Metastasis V occurred more frequently in patients with rectal cancer

The prognosis of rectal cancer is much worse than that of colon cancer [12]. Thus, we compared the metastasis V incidence among tumors in different parts of the colorectum. Metastasis V occurred more frequently in rectal cancer patients (23.60%) than in patients with colon cancer (17.39%) (Table 1). The Chi-square analysis was not significant ($P > 0.05$). However, ANOVA showed that there was a significant difference between the average metastasis V numbers in rectal cancer patients and colonic cancer patients, and the average metastasis V number in rectal cancer patients was significantly higher than that in colon cancer patients (Fig. 1g). Therefore, the incidence of metastasis V was similar between colonic cancer and rectal cancer patients, but the metastasis V number was higher in rectal cancer patients than in colon cancer patients.

We concluded that metastasis V occurs more frequently in advanced stage patients (stage III and stage IV) and patients with deeper tumor invasion, local lymph node metastasis, low histologic grade, LVI, and perineural metastasis. We also analyzed the relationship between metastasis V and clinical factors involved above in colon cancer and rectal cancer separately. The results in colon cancer and rectal cancer showed similar trend except for the differentiation category (Figures S2, S3; Tables S1, S2). There was no significant difference between the metastasis V incidence rates in colon cancer and rectal cancer patients.

Discussion

Local recurrence is an important factor that affects CRC prognosis [13]. Lymph node dissection has been proven to be effective at improving the prognosis of colorectal cancer [14–16]. Further, metastatic cancer cells have been identified in the mesentery, and patients with metastatic cancer cells in adipose tissue around tumors have a relatively worse prognosis [17]. However, the distribution of metastatic cancer cells has rarely been studied.

Metastatic cancer cells in the adipose tissue were first called tumor deposits (TD) by Gabriel et al. [16] and were considered the result of vascular tumor dissemination. TD was further studied and defined as adenocarcinoma within adipose or fibrous tissue around that was not associated with a lymph node [17–19]. Later, isolated tumor cells (ITC) was also proposed and focused on single tumor cell or cell clusters in lymph node, hematologic system, bone marrow, etc., and has also proven associated to poor prognosis [20, 21].

In our previous studies, we discovered metastatic cancer cells in the mesentery of the stomach, colon, and rectum and proposed the definition of metastasis V [7–9]. Metastasis V defined a novel metastasis route of gastric cancer and colorectal cancer that occurred in the mesentery of stomach and colorectum. An important character of metastasis V is removable by complete mesentery excision operations and can “leak out” from ruptured mesentery. Complete mesentery excision in gastric cancer has been proven less “cancer leak” and with favorable prognosis [10]. We are also expanding the study on colorectal cancer. Thus, the distribution of metastasis V is meaningful for clinical strategy selection.

In this study, we have shown that metastasis V incidence is related to tumor stage. Metastasis V occurs more frequently in N-positive patients, and those with cancer in the N2b stage have a much higher metastasis V incidence than those with stage N2a, who in turn have a much higher incidence than those with N1. Consistent with our previous study, metastasis V occurs more in T3 or T4 stage disease than in T1 or T2 disease. Interestingly, we also noticed that metastasis V occurs more frequently in patients with local perineural or vessel invasion.

The limitation of this study is the lack of long-term survival data; we will continue to follow these patients and report the follow-up results, and an additional randomized clinical trial will be conducted to study the efficiency and reliability of CME/TME in preventing “cancer leak” and reduce tumor recurrence.

Authors' contribution JG conceived of and designed the study; CM contributed to data acquisition, analysis and interpretation and wrote the article; YT revised and reviewed the article; all authors have reviewed and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards All procedures performed in the study were done so in accordance with the ethical standards of Tongji Hospital Ethics Committee, Wuhan, China.

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