

Original Article

Tumor budding and size as risk factors of lymph node metastasis in early colorectal cancer

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Abstract: This study was designed to investigate risk factors for lymph node metastasis of the Chinese people with early stage colorectal cancer, which was confirmed to a carcinoma that invaded the submucosa after radical resection. In total, 68 patients revealing submucosal invasive colorectal carcinoma on pathology who underwent curative radical resection from October 2007 to September 2011 were evaluated retrospectively. Tumor size, depth of submucosal invasion, histological grade, lymph-vascular invasion, tumor budding, and microacinar structure were reviewed independently by two pathologists. Student t-test for continuous variables and Chi-square test for categorical variables were used for comparing the clinic-pathological features between two groups (whether lymph node involvement existed or not). Continuous variables are expressed as the mean \pm standard error while statistical significance is accepted at $P < 0.05$. As results, the mean age of the patients was 65.7 ± 9.6 years (range from 47 to 84). The mean tumor size (the largest diameter) was 21.5 ± 1.2 mm (range from 5 to 84 mm). Histologically, 11 patients (16.2%) had metastatic lymph node. The lymph node metastasis was significantly associated with the tumor budding ($P = 0.038$) and the tumor size ($P = 0.031$) while other factors were not statistically significant. Tumor budding seems to have a high sensitivity (80.0%) for lymph node metastasis, acceptable specificity (55.6%). Therefore, tumor budding should be performed in order to clarify early colorectal cancer with lymph node metastasis through pathologic analysis.

Keywords: Tumor budding, tumor size, risk factors, lymph node metastasis, colorectal cancer

Introduction

Early colorectal cancer (ECC) is defined as adenocarcinoma restricted to the mucosa or invading the submucosa, which includes ECC classified as tumor in situ (Tis) and T1 according to the Tumor-Lymph Node (LN)-Metastasis (TNM) classification. An increase in colorectal cancer (CRC) screening and progress in techniques has resulted greater frequency of detection of ECCs. With recent advances in endoscopic techniques and improved endoscopes, ECCs are often resected by endoscopes, regardless of their size or location [1, 2].

To our knowledge, patients who have ECC confined to the mucosa do not usually show either lymph node (LN) metastasis or distant metastasis. Local treatments such as endoscopic resection and local excision are considered

adequate management. A complete local treatment of intramucosal carcinoma is accepted as a curative treatment because there is little risk of lymph node metastasis. However, 10% of patients who have ECC with submucosal invasion (T1) exhibit LN metastasis [3, 4]. This suggests that an additional radical resection of the affected colon or rectum is necessary for patients who have specific risk factors for LN metastasis after histopathologic examination. Therefore, accurate identification of the risk factors for LN metastasis and careful examination of resected colorectal tissues can play crucial roles in reducing cancer recurrence.

The previous reports on the risk factors after endoscopic polypectomy included carcinoma at the surgical margin, lymphovascular invasion, poorly differentiated adenocarcinoma, and level of invasion [5, 6]. Recently, some investiga-

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Table 1. Characteristics of patients observed in the current study

Characteristics		Lymph node positive group (n = 11)	Lymph node negative group (n = 57)	P-value
Age	Mean ± SD	65.6 ± 9.8	66.7 ± 9.5	0.832*
Sex	men/women	7 (63.6%)/4 (36.4%)	39 (68.4%)/18 (31.6%)	0.489#
Greatest tumor dimension (mm)	Mean ± SD	23.3 ± 17.9	17.8 ± 6.5	0.031*
Tumor location	Ascending colon	1 (9.1%)	5 (8.8%)	0.327 [§]
	Transverse colon	1 (9.1%)	4 (7%)	
	Descending colon	2 (18.2%)	10 (17.5%)	
	Sigmoid colon	4 (36.4%)	16 (28.1%)	
	Rectum	3 (27.2%)	22 (38.6%)	
Macroscopic shape of tumor	Ip	4 (36.4%)	28 (49.1%)	0.462 [§]
	Isp	2 (18.2%)	10 (17.5%)	
	Is	5 (45.4%)	19 (33.3%)	
Depth of invasion (μm)	< 1000	0	1 (1.8%)	0.398*
	1000 ≤ or < 2000	3 (27.3%)	8 (14%)	
	2000 ≤ or < 3000	1 (9.1%)	12 (21.1%)	
	3000 ≤	5 (45.5%)	28 (49.1%)	
Depth of invasion (by Kudo's classification)	Sm1	3 (27.3%)	14 (24.6%)	0.371*
	Sm2	1 (9.1%)	21 (36.8%)	
	Sm3	6 (54.5%)	12 (21.1%)	
Differentiation	Well	2 (18.2%)	26 (45.6%)	0.128*
	Moderately	8 (72.7%)	25 (43.9%)	
	Poorly	0	0	
Lymphovascular invasion	Absent	7 (63.6%)	45 (78.9%)	0.193#
	Present	3 (27.3%)	6 (10.5%)	
Tumor budding	Absent	2 (18.2%)	29 (50.9%)	0.038#
	Present	8 (72.7%)	18 (31.6%)	
Microacinar structure	Absent	4 (36.4%)	36 (63.2%)	0.184#
	Present	5 (45.6%)	11 (19.3%)	

Note: SD indicates standard deviation; *Student t test; #Chi-square test; [§]Mann-Whitney U test.

tors suggested that a tumor budding is another risk factor for lymph node metastasis of ECC including occult metastasis [7, 8]. Kye et al. showed that the tumor budding had a high sensitivity and a high negative predictive value, suggesting that a close examination of pathologic finding including tumor budding should be performed in order to manage ECC properly [9].

However, relevant studies on tumor budding in China are lacking. In the current study, we investigate risk factors for lymph node metastasis of ECC, which had been confirmed to be carcinoma that invaded the submucosa after radical resection.

Materials and methods

Design and patients

A total of 68 patients revealing submucosal invasive colorectal carcinoma on pathology

who underwent curative radical resection at the Department of Gastrointestinal Surgery, affiliated Hospital of Guangdong Medical College from October 2007 to September 2011 were evaluated retrospectively. The study protocol was reviewed and approved by the Committee on Human Investigations at affiliated Hospital of Guangdong Medical College of China. All patients have been informed and have consented to the clinical practice, and written documents have been signed by all patients.

All tumors were macroscopically polypoid type which protruded above the surrounding surface at endoscopic finding. These tumors were classified into three different kinds: Ip, Isp and Is. Ip is an abbreviation for pedunculated polyp which the base is narrow. Isp is an intermediate and broad-based forms. Finally, Is is a sessile polyp in which the base and the top of the lesion have the same diameter. We defined the right sided

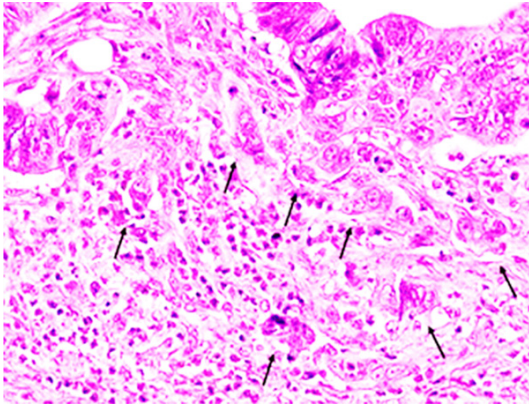


Figure 1. Characteristic of tumor budding in colon cancer.

colon as cecum, ascending, hepatic flexure and proximal transverse colon. And the left sided colon was defined as mid to distal transverse, descending, sigmoid and rectosigmoid colon. The circumferential ratio of the tumor represents the ratio of circumference of tumor to luminal circumference. All pathologic slides were re-examined in which tumor size, depth of submucosal invasion, histologic grade, lymphovascular invasion, tumor budding and microacinar structure by two pathologists independently. The depth of submucosal invasion was defined as the depth between muscularis mucosae and deep tumor margin measured in micrometers. Also, Kudo's classification was used with the relative invasion depth of the submucosal layer and they were as follow: sm1, infiltration into the upper third of the submucosal layer; sm2, middle third; or sm3, lower third [10]. Histologic grade was classified in the order of well, moderately, poorly differentiated and mucinous adenocarcinoma. Lymphovascular invasion was defined as the presence of tumor cells within small luminal structures lined by endothelial cells. An isolated cell or a small cluster of carcinoma cells in the invasive front was determined by looking at a budding focus, > 10 budding foci were considered as positive for tumor budding. Finally microacinar structure specifies small tubules that form cribriform structures within the medium or large glands, or small isolated round tubules within the stroma [11]. Detailed clinical data were recorded for each patient, with demographic data summarized in **Table 1**.

Statistical analysis

Student *t*-test for continuous variables and Chi-square test for categorical variables were used for comparing the clinic-pathological features between two groups whether lymph node involvement existed or not. Continuous variables are expressed as the mean \pm standard error whereas statistical significance is accepted at $P < 0.05$. The applied statistical software was stata 6.0.

Results

There were a total of 68 patients including 46 males and 22 females. The mean age of the patients was 65.7 ± 9.6 years range from 47 to 84. Histologically, 11 patients (16.2%) had metastatic lymph node. **Table 1** showed the clinic-pathological features in patients with ECC. The mean tumor size (the largest diameter) was 21.5 ± 1.2 mm (range, 5-84 mm). Tumor budding (**Figure 1**) were found in 38.24% (26/68) patients. The lymph node metastasis was significantly associated with the tumor budding ($P = 0.038$) and the tumor size ($P = 0.031$) while other factors were not statistically significant. In all patients, 27 patients underwent colonoscopic polypectomy before curative radical surgery. **Table 2** showed the causes of radical surgery after colonoscopic polypectomy. An incomplete polypectomy (44.4%) was the most frequent reason for a curative radical surgery.

In the study, **Table 3** showed the clinic-pathological features of 11 patients who had ECC with lymph node metastasis. Data of malignant polyps in one patient who underwent colonoscopic polypectomy at a local clinic before radical surgery could not be found. The tumor budding was found in eight (80%) of ten patients whose pathologic data of malignant polyps could be found. The microacinar structure was found in five patients. There was one patient having a recurrence after curative radical surgery. After the curative surgery, the patient was given an adjuvant chemotherapy using 5-FU and leucovorin (LF) regimen after curative surgery, but multiple unresectable liver metastases were found 2 months later. Although we gave FOLFOX chemotherapy, the liver metastases progressed and the patient passed away ten months after radical surgery.

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Table 2. Causes of radical resection after colonoscopic polypectomy

Causes	N (27)
Involved or closed resection margin	12 (44.4%)
Patient wanted (Sm1 or Sm2)	5 (18.5%)
No identification of tumor depth, lymphovascular invasion, or resection margin	4 (14.8%)
Lymphatic invasion (+)	3 (11.1%)
No lifting of tumor at trial for polypectomy	1 (3.7%)
Piecemeal resection	1 (3.7%)
Sessile polyp crectal invasive carcinoma	1 (3.7%)

Table 3. Clinicopathological features of 11 patients who had T1 colorectal cancer with lymph node metastases

Sex	Age (year)	Shape	No of (+) LN [#]	Size (cm)	Grade	Depth m Kudo	LVI [§]	Budding	MA ^x	Recurrence Site	DFS ^q (month)	OS ^w (month)
F	61	lsp	1	1.6	MD [§]	4000 Sm3	(-)	(+)	(+)	No	55	55
M	65	ls	2	1.4	MD [§]	1000 Sm1	(-)	(+)	(+)	No	48	48
M	62	ls	4	3.4	MD [§]	3600 Sm3	(+)	(+)	(+)	liver	2	10
F	61	ls	1	1.5	MD [§]	1500 Sm1	(-)	(+)	(-)	No	39	39
F	76	ls	2	1.6	MD [§]	3200 Sm3	(-)	(-)	(-)	No	16	16
M	77	lp	2	3.1	MD [§]	3000 Sm2	(-)	(+)	(+)	No	15	15
M	56	lp	2	2.8	WD [§]	2800 Sm2	(-)	(+)	(+)	No	15	15
F	49	lp	4	1.8	MD [§]	1500 Sm1	(-)	(-)	(-)	No	12	12
F	61	lsp	2	1.6	MD [§]	3000 Sm2	(+)	(+)	(-)	No	12	12
M	79	lp	1	2.1	MD [§]	1500 Sm1	(-)	(+)	(-)	No	14	14
F	57	/	/	/	/	/	/	/	/	No	18	18

Note: [#]Lymph node; [§]Moderately differentiated; [¶]Well differentiated; [§]Lymphovascular invasion; ^xMicroacinar structure; ^qDisease free survival; ^wOverall survival.

Discussion

Colorectal cancer is one of the most common causes of cancer death in developed countries [12]. Treatment methods based on colonoscopy and surgery have advanced rapidly, and a large number of patients with colorectal cancer achieve improvements after therapy. However, advanced stage colorectal cancer reduces the quality of life of patients receiving operative treatment or chemotherapy. Therefore, approaches allowing the early detection and diagnosis of colorectal cancer before lymph node metastasis are urgently being sought [13]. In the current study, 6 variables (tumor size, depth of invasion, histologic type in the mucosa, histologic type at the invasive front, budding and lymphatic invasion) are examined as predictive factors of LN metastasis in ECC. The analysis demonstrates that the tumor budding ($P = 0.038$) and the tumor size ($P = 0.031$) are independent predictors of LN metastasis. Other

factors are not related directly to LN status. These results demonstrate that the existence of tumor size and budding is the most important variables for predicting LN metastasis in ECC.

Various predictive factors for LN metastasis and outcome in patients with ECC have been reported previously, including tumor size [14], type of tumor growth [15], tumor differentiation [16], depth of invasion [16], lymphatic invasion [17], venous invasion [18] and budding formation. In the present study, the location of the primary tumor is not a significant predictor of LN status; however, tumor size is significantly greater in the LN-positive group than in the LN-negative group. A previous investigation also demonstrates that tumor size is an independent predictive factor of LN metastasis in early gastric cancer. In the stomach and large intestine, the submucosa, just under the muscularis mucosa has a relatively abundant, va-

lved plexus of lymphatics [8]. Considering this lymphatic distribution, tumor cells that invade the submucosa from a mucosal origin in the stomach and large intestine may migrate readily through the lymphatic route in this region, and larger mucosal tumors tend to have more frequent contact with the submucosal lymphatics. In fact, the most lymphatic invasion is observed in the submucosa in this series; in contrast, the type of tumor growth is not a predictive factor for LN metastasis. Previous investigators have reported that nonpolypoid-growth ECC is not associated with an adenoma-carcinoma sequence and exhibits rapid growth, aggressive behavior and a high frequency of LN metastasis compared with polypoid-growth carcinoma [15]. This discrepancy between the current results and previous reports may be because of a difference in the tumors examined or because of a recent increase in the detection rate of ECC at an earlier stage, before metastasis.

In conclusion, we examined pathologic findings including quantitative and qualitative factors for proper management of submucosal invasive ECC among Chinese population. We found that the tumor budding and tumor size seem to have a high sensitivity and acceptable specificity for lymph node metastasis of ECC. Therefore, we think that the examination for tumor budding after colonoscopic or local excision for submucosal invasive ECC should be performed routinely. Taken together, our findings will hopefully lead to an improved quality of life via the early detection of colorectal cancer. If any risk factor is detected, additional oncologic surgery would be necessary.

Disclosure of conflict of interest

None.

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