Predictors of Lymph Node Metastasis and Prognosis in pT1 Colorectal Cancer Patients with Signet-Ring Cell and Mucinous Adenocarcinomas

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Key Words
Colorectal cancer • Signet-ring cell carcinoma • Mucinous carcinoma • Treatment • Prognosis

Abstract
Background/Aims: The local excision of early colorectal cancer is limited by the presence of lymph node metastasis (LNM). Signet-ring cell carcinomas (SRC) and mucinous adenocarcinomas (MAC) are two relatively infrequent histological subtypes. However, little is known about the predictors of LNM and prognosis to support the feasibility of local excision in early-stage SRC and MAC. Methods: The Surveillance Epidemiology and End Results Database were used to identify all patients with pT1 adenocarcinomas, including conventional adenocarcinoma (AC), MAC, and SRC. The prevalence of LNM was assessed, and the long-term survival rate in the above three types of colorectal cancer was calculated. Results: SRC accounted for 0.3\% and MAC accounted for 4.4\% of the entire cohort of colorectal adenocarcinomas. Compared to AC, MRC and SRC were more often located in the proximal colon, and exhibited a higher grade. The incidence of LNM in AC, MAC, and SRC was 10.6\%, 17.2\%, and 33.3\% for colon cancers and 14.8\%, 25.9\%, and 46.2\% for rectal cancers, respectively. In patients with lymph nodes resected no less than 12, incidence of LNM in AC, MRC, and SRC was 12\%, 21\%, and 44\% for colon tumors and 17\%, 30\%, and 14\% for rectal tumors, respectively. Although, colon patients MAC showed an entirely worse survival rate than AC, rectum patients MAC showed a similar prognosis to AC. We found that in patients with rectal tumors, SRC had a worse 3 and 5-year prognosis than AC. However, for colon cancers, the prognosis of SRC was similar to that of AC. Histology was not found to be an independent prognostic factor in multivariate survival analysis. Conclusions: MAC and SRC are two distinct subtypes of colorectal cancer that require special attention despite their relatively rare prevalence. pT1 patients with SRC of the rectum and patients with MAC of the colon have higher incidences of LNM, and with these adverse outcomes, local excision is not recommended. Although MAC of the rectum and SRC of colon have a high rate of LNM, the prognosis of these types are similar to that of AC.
Introduction

Primary colorectal signet-ring cell and mucinous carcinoma were first described by Laufman in 1951 and Parham in 1923, respectively [1, 2]. SRC and MAC are distinct from the conventional adenocarcinoma as defined by the World Health Organization colorectal histology classification in 1979. SRCs are relatively rare, accounting for approximately 0.1-2.4% of all primary colorectal cancers, and are characterized by predominantly intracytoplasmic mucin deposition that pushes the nucleus eccentrically. In contrast, MACs (approximately 1.6-25.4%) are composed of an abundant extracellular mucin pool [3, 4]. Although these histological subtypes may represent entities with unique biological behaviors, aggressiveness, and prognosis, in contrast to the TNM staging and grading system, they are currently not included in the clinical classification system or the guidelines for indications of minimally invasive treatment for early colon and rectal cancer, which simply classify SRC and MAC as undifferentiated cancers [5, 6]. SRC is not a simple form of undifferentiated adenocarcinoma. Many studies have shown that patients with early signet-ring cell gastric cancer have a more favorable prognosis than patients with other undifferentiated adenocarcinomas [7, 8]. This phenomenon has been explained by the low possibility of LNM, and it might be considered an indication for endoscopic resection for early gastric cancer with SRC. However, there are few data regarding the indications for local excision of early SRC of colorectal cancer. The present retrospective study based on the surveillance, Epidemiology, and End Results (SEER) database aimed at determining the appropriate management for patients with early SRC and MAC colorectal cancer by analyzing the clinicopathological and long-term survival features of SRC and MAC in early-stage colorectal cancer.

Materials and Methods

Patient selection strategy and tumor characteristics

The study population was obtained from the records of the SEER database (reference number 12224-Nov2013), a program of the National Cancer Institute, which covers approximately 28% of the population of the United States. Cases of colon (C18.0-18.9, C26.0) and rectal cancer (C19.9, C20.9) from 1988 to 2006

<table>
<thead>
<tr>
<th>SEER extension code</th>
<th>Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to 2003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Head of polyp</td>
<td>Head of polyp</td>
</tr>
<tr>
<td>14</td>
<td>Stalk of polyp</td>
<td>Stalk of polyp</td>
</tr>
<tr>
<td>15</td>
<td>Polyp, NOS</td>
<td>Polyp, NOS</td>
</tr>
<tr>
<td>16</td>
<td>Submucosa (superficial invasion)</td>
<td>Submucosa (superficial invasion)</td>
</tr>
<tr>
<td>20</td>
<td>Muscularis propria invaded</td>
<td>Muscularis propria invaded</td>
</tr>
<tr>
<td>30</td>
<td>Localized, NOS/confined to colon, NOS</td>
<td>Localized, NOS/confined to colon, NOS</td>
</tr>
<tr>
<td>Since 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>Confined to head of polyp, NOS</td>
<td>Confined to head of polyp, NOS</td>
</tr>
<tr>
<td>140</td>
<td>Confined to stalk of polyp, NOS</td>
<td>Confined to stalk of polyp, NOS</td>
</tr>
<tr>
<td>150</td>
<td>Invasive tumor in polyp, NOS</td>
<td>Invasive tumor in polyp, NOS</td>
</tr>
<tr>
<td>160</td>
<td>Invades submucosa (superficial invasion), including submucosa in the head or stalk of a polyp</td>
<td>Submucosa (superficial invasion), including submucosa in the head or stalk of a polyp</td>
</tr>
<tr>
<td>165</td>
<td>For rectum: Tumor invading submucosa with intraluminal extension to colon and/or anal canal/ anus</td>
<td>For rectum: Tumor invading submucosa with intraluminal extension to colon and/or anal canal/ anus</td>
</tr>
<tr>
<td>170</td>
<td>Stated as T1 with no other information on extension</td>
<td>Stated as T1 with no other information on extension</td>
</tr>
<tr>
<td>300</td>
<td>Localized, NOS/confined to colon, NOS</td>
<td>Confined to rectosigmoid junction, NOS; Confined to rectum, NOS; Localized, NOS</td>
</tr>
</tbody>
</table>

Table 1. Corresponding SEER code of the pathological T1 tumor (TNM7 Map)
Table 2. Corresponding SEER code of the radical surgical Resection

<table>
<thead>
<tr>
<th>SEER Site Specific Surgery Code</th>
<th>G1</th>
<th>Rectum (includes rectosigmoid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to 1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>Partial or subtotal colectomy, but less than hemicolectomy</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>Hemicolectomy or greater (but less than total); right/left colectomy (all of right or left colon and a portion of transverse)</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>Total colectomy</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>Colectomy, NOS</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>Colectomy PLUS partial or total resection of other organs</td>
</tr>
<tr>
<td>Since 1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>Partial colectomy, but less than hemicolectomy (with or without permanent colectomy)</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>Hemicolectomy or greater (but less than total); right or left colectomy</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>Total colectomy</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>Total proctocolectomy</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>Colectomy or colectoproctectomy with an en bloc resection of other organs; pelvic exenteration</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>Colectomy, NOS; Proctectomy, NOS</td>
</tr>
</tbody>
</table>

Fig. 1. (A) study flow diagram from the SEER database.

were identified from the SEER database (SEER*Stat 8.1.5) according to the Site Recode Classifications. Only histologically confirmed malignant tumors of colorectal cancer were included, and lesions were confined to the submucosa (T1). The definition of tumor extension in SEER database can be seen in Table 1. We also limited our analysis to patients underwent surgical resection, corresponding detail have been shown in Table 2. Non-first tumors (those involving more than a single primary tumor or in which the primary cancer was not the first of 2 or more primary malignancies), patients treated with preoperative radiation (downstage Tstage), without information of LNs involvement, and with distant metastasis were excluded. For further details, see Fig. 1. Cancer-specific survival was used as the primary outcome parameter. The histological type groups were categorized as follows using the ICD-0-3 (International Classification of Disease for Oncology, 3rd edition), (ICD-10) coding schema: conventional adenocarcinoma (8010, 8020-
8022, 8140-8141, 8144-8145, 8210-8211, 8220-8221, 8230-8231, 8260-8263), referring to nonmucinous and nonsignet-ring cell tumors; MAC (8470, 8472-8473, 8480-8481); and SRCC (8490) [3, 9]. The “colon” location was defined as cecum, ascending colon, hepatic flexure of the colon, transverse colon, splenic flexure of the colon, descending colon, sigmoid colon, overlapping lesion of the colon, and colon NOS (not otherwise specified). The “rectum” location was defined as the rectosigmoid and rectum [3]. The colon tumors were further divided into three categories: right colon (cecum, ascending colon, and hepatic flexure), transverse colon, and left colon (splenic flexure, descending, and sigmoid colon) tumors. The tumor grades were grouped as follows: Grade I (well differentiated); Grade II (moderately differentiated); Grade III (poorly differentiated); Grade IV (undifferentiated or anaplastic lesions). This study was based on public data from the SEER database, and it did not include interaction with human subjects or the use of personal identifying information. The study was approved by the Review Board of Fudan University Huadong Hospital, Shanghai, China.

### Statistical analysis

Clinicopathological variables in the analysis included age at diagnosis, gender, race, tumor site, therapy (radiation and surgery), tumor size, and tumor grade. Univariate analysis was performed using the Pearson chi-squared method or Fisher’s exact test for categorical variables and a Kruskal-Wallis test for continuous variables. The Kaplan-Meier method was employed to calculate the cancer-cause-specific death rate, and the difference between the survival curves was analyzed using a log-rank test. Meanwhile, 3 and 5-year survival rates between groups were calculated by log-log transformation for survival function. A Cox proportional hazards model was used in the multivariate analysis [10]. All of the reported tests were 2-sided, and p values < 0.05 were considered statistically significant. All statistical analysis was conducted using R statistical software, version 3.1.3, with survival and rms libraries and SPSS version 17.0 for windows (SPSS Inc. Chicago, IL, USA).

### Results

#### Study cohort

A total cohort of 21,463 patients matching the inclusion criteria were analyzed in this study, most of whom had AC (95%). The incidence of SRC was extremely rare and accounted for 0.3% (62) of all of the patients, whereas that of MAC was 4.4% (934). The patients’ median age was 68 years (range: 20-100 years). The median numbers of resected lymph nodes during the surgery were ten. Lymphadenectomy of 9777 patients (45.5%) was considered to be adequate (with lymph nodes examined no less than 12). Among the entire cohort, males (n = 11,016) were more common than females (n = 10,447) and more tumors were located in the colon (n = 15,987, 74%) than in the rectum (n = 5,339, 25%). The race of the population-based study was predominantly white and made up of 81.2% in AC, 88.4% in MAC, and 88.7% in SRC, respectively. For further details, see Table 3.

#### Clinicopathological characteristics of the patients and the tumors

SRC were found more frequently to present with younger age compared to AC and MAC, more common in patients younger than 40 years old (p < 0.021; Table 3), and this comparison was similar in colon patients after stratifying the lesion by tumor location (Table 4). There was a propensity for MAC (72.6%) and SRC (77.1%) to be located in the proximal colon (large bowel proximal to the splenic flexure) in contrast to AC (56.5%; p < 0.001). Compared to AC, the percentage of tumor size exceeding 20 mm of MAC was higher; however, there was a similar distribution with regard to the tumor size between SRC and AC. Colon MAC exhibited a female predominance and rectal SRC cancers was more frequently diagnosed in male (Table 4), compared with the AC group. In terms of tumor differentiation grade, the SRC patients (80.7%) were diagnosed with high-grade tumors (Grades III and IV) more frequently than the MAC (10.5%) and AC (7.5%; p < 0.001) patients.
Risk factors for lymph node metastasis

Univariate analysis of risk factors for lymph node metastasis (LNM) found age, grade, tumor size and histological type to be significant factors regardless of tumor location (Table 5). Overall, the rate of LNM was 12.0% (2,573 out of 21,463). Among these 2,573 patients, 2,379 (92.5%) patients had AC, 22 patients (0.8%) had SRC and 172 patients (6.7%) had MAC. SRC had a higher rate of LNM (35.5%) than MAC (18.4%) and AC (11.6%). When patients were stratified by site, a similar phenomenon was also seen. In the colon, SRC had an LNM rate of 33.3%, MAC had a rate of 17.2%, and AC had a rate of 10.6%. In the rectum, SRC had an LNM rate of 46.2%, MAC had a rate of 25.9%, and AC had a rate of 14.8% (Table 6). The multivariate analysis revealed that the age, race, tumor size, location, histological type, and grade were independent factors predicting LNM (Table 7). When patients were stratified by site, similar results were found in the colon patients. However, race was not an independent factor in the patients with rectal tumors (data not shown).
Survival analysis

Cancer-specific survival (CSS) was calculated and stratified by histological subtypes and tumor location. The median follow-up interval was 60 months (range: 0-287 months). In total, 1,547 (7%) patients died from colorectal cancer during the follow-up period. The 3-year CSS rate was 96.0% for AC (colon: 96.0%; rectum: 95.8%), 94.3% for MAC (colon: 94.1%; rectum: 95.2%), and 90.1% for SRC (colon: 92.4%; rectum: 82.5%). The 5-year CSS rate was 93.3% for AC (colon: 93.8%; rectum: 92.2%), 90.7% for MAC (colon: 90.5%; rectum: 91.6%), and 85.6% for SRC (colon: 89.2%; rectum: 73.3%) (Table 8). For all patients MAC showed an entirely worse survival rate than AC (Fig. 2A). For colon patients, MAC also showed an entirely worse survival rate than AC (Fig. 2B). However, for rectum patients, MAC showed a similar prognosis to AC (Fig. 2C). Comparison of entire survival curves between SRC and AC failed to pick up significant differences. Low incidence of SRC could attribute to the intersection of two curves, which caused the log-rank test's negative and Grambsch-Therneau test also demonstrated the proportional hazards assumption of Log-rank test is not available. So, we used cloglog transformation for survival function and focused our interest on the comparison at fixed point in time. Adjusted for tumor location, we found that in patients...
with rectal tumors, SRC had a worse 3 and 5-year prognosis than AC. However, for colon cancers, the prognosis of SRC was similar to that of AC (p = 0.218 for 3 year; p = 0.204 for 5 year, Table 8). Adjusted for histology, we found that patients with AC, tumors located in rectum had a worse 5-year prognosis than those located in colon (Fig. 3A). Differently, tumors located in rectum from patients with MAC and SRC had a similar prognosis to tumors located in colon (Fig. 3B and 3C). The patients with MAC, prognosis of these patients in proximal lesion was worse than in distal (Fig. 4).

**Multivariate survival analysis**

After adjusting for age, race, tumor size and grade, we performed a multivariate survival analysis stratified by tumor site and histological type was not an independent prognostic factor regardless of tumor site (Table 9). For patients with colon or rectal tumors, African Americans had a higher risk of death compared with whites and other races. High-grade adenocarcinomas, larger tumor size and older patients tended to increase the risk of death in either the colon or the rectum.

**Discussion**

Achievement of cure and a better quality of life should both be considered when devising a treatment strategy. Although traditional surgical resection TME (total mesorectal excision) and CME (complete mesocolic excision) achieve excellent oncological control and long-term survival for colorectal cancer patients, these approaches are also associated with substantial morbidity and mortality [11, 12].
Particularly for distal rectal cancer patients, permanent colostomy is distressing and some patients develop inferiority or depression.

The management of early-stage colorectal cancer remains controversial and lacks sufficient evidence to guide decision making. Although local excision, as an alternative to TME and CME, shows advantages in terms of preserving anal function, fewer complications and shorter hospital stays compared with the curative procedure, it is inferior to radical resection for oncological outcomes and does not adequately remove the regional lymph nodes. Previous studies reported that LNM occurs in 6.3% to 17% of T1 and 10% to 22% of T2 colorectal cancers [13, 14]. Our analysis, based on population data, demonstrated that the rate of lymph node metastasis of T1 colorectal cancers was 12.0%. The risk of LNM in patients with colon cancers is 11.0%, with a similar rate for patients with rectal cancer (15.1%), which was consistent with previous reports [15-17]. Identifying a subgroup of patients for whom local excision would be safe is crucial when planning a tailored strategy. The National Comprehensive Cancer Network describes malignant lesions with T1, Grades I or II, no angiolymphatic invasion, and negative margin of resection as safe for endoscopic removal. However, the guidelines do not mention the histology subtypes. The JSCCR Guideline (Japanese Society for Cancer of the Colon and Rectum) simply classifies mucinous adenocarcinoma and signet-ring cell carcinoma into the poorly differentiated group [18].

Because of the rare occurrence of SRC and MAC, the clinical evaluation of these cancer types is difficult, in particular at the early stage. The SEER database provides a sufficiently
large sample size to conduct such an evaluation. To our knowledge, our study is the only large-cohort study to investigate the possibility of local excision in early-stage colorectal patients with MAC and SRC.

Consistent with a majority of previous large-cohort retrospective analyses from the US, Europe and Asia, MAC and SRC had a propensity to be located in the proximate colon, to present in younger patients, to exhibit a worse differentiated grade and higher likelihood of LNM, and to be found in advanced TNM stages [5, 6, 9, 19]. In contrast to these studies, we focused our analysis on patients, whose lesions were confined to the submucosa and aimed to expand the indication for local excision, enabling individually tailored regimens for these patients. Our study will aid in the planning of individually tailored regimens. For example, a subgroup of patients had a chance to preserve their bowel function, reduce their morbidity or mortality rates and lead a better quality of life. With an increasing number of patients having early cancers of the colon and rectum, it is imperative to alter how we assess colorectal cancers. There are many methods for treating colorectal cancer patients, including endoscopic submucosal dissection, transanal endoscopic microsurgery, laparoscopically assisted endoluminal resection, laparoscopic colectomy, and open radical resections, each of which has specific indications. Patients with colorectal cancer should be evaluated by a multidisciplinary team and subjected to the least invasive treatment modality required to obtain cost-effective, patient-friendly cancer remedies.

SRC of colorectal adenocarcinomas share common features of those that are typically expressed in SRC of gastric cancer [19]. However, as opposed to our expectation based on early gastric SRC research, the incidence of LNM in patients with MAC and SRC is high. LNM is associated with 18.4% of patients with MAC and 35.5% of patients with SRC, with similar rates when the patients are divided by site (MAC: colon 17.2%, rectum 25.9%; SRC: colon 33.4%, rectum 46.2%). Moreover, this result may underestimate the true incidence of LNM because of inadequate lymphadenectomy. Of all of the patients, 54.5% represented an insufficient number of resected lymph nodes (fewer than 12). When limiting our analysis in patients with adequate lymph node removed, the incidence of LNM was higher.
Patients with SRC experienced reduced 3 and 5 survival rates in rectum patients, and the patients with MAC showed reduced 3 and 5 survival rates in colon patients. Rectum patients with MAC and colon patients with SRC showed no difference compared to those with AC. After adjusting for age, race, grade, tumor size, N stage, therapy regimen and site, a multivariate analysis also did not find histology to be an independent prognostic factor.

The aggressive behavior and reduced survival associated with SRC has been demonstrated by many previous studies, and many researchers attribute this phenomenon to a greater propensity of SRC histology to reach advanced stage, driven by the absence of the expression of adhesive molecules such as E-cadherin and β-catenin, which causes the tumor cells to have a more aggressive behavior and to disperse to other organs [20, 21]. Our research demonstrated high prevalence of LNM of SRC in both colon and rectum tumor, while didn’t find the adverse prognosis of SRC in colon cancer.

However, the prognostic implications of MAC remain controversial. It has been reported that MAC was associated with a poorer prognosis, but others did not find that MAC adversely influenced the clinical outcomes [22]. An increasing number of studies have found that the prognostic role for MAC is more dependent on the tumor stage or the specific tumor site [3, 9].
Some researchers attribute these discrepancies to geographical variations, patient selection, differences in the defined histological criteria for MAC, and molecular alterations [19, 23]. Leopoldo et al. and Liu et al. identified two subtypes of mucinous adenocarcinoma of colorectal cancer [23, 24]. The MAC with MSI (microsatellite instability) phenotype presented an earlier TNM stage, a lower frequency of cancer recurrence and better survival compared with conventional adenocarcinoma and MSS (microsatellite stable) mucinous tumors. Compared to MAC in rectal lesions, MAC in colon lesions is associated with a higher MSI, a loss of hMlh1 expression, and a lower or absent p27 expression, and MSI mucinous adenocarcinomas tend to be located in the proximal colon [25-27]. Interestingly, in contrast to previous studies, we analyzed the rate of LNM according to the subdivided colon sites and found the proximal lesion to be associated with a higher incidence of LNM, and prognosis of these patients was worse than in distal.

Several inherent limitations of the SEER database were involved in this study. First, the white race predominated, and the lack of groups such as Asians and Hispanics in this study requires that the findings be generalized with caution. Second, the SEER database does not contain information on the adjuvant therapy and the family history, which may be directly related to patients' outcomes. HNPCCs (hereditary nonpolyposis colorectal cancers) have a propensity to exhibit colloid pathology, and compared with the matched stage of sporadic cancer, these specific tumors are associated with improved survival rates [28]. The status of angioinvasion and lymphatic invasion, recurrence and MSI data are also not captured in the SEER database, and therefore, the impact of this information were not included in this study. Third, preoperative radiation may increase the proportion of mucin in primary tumors. The increasing component of mucin reflects the sensitivity to radiotherapy and improves prognosis.

**Conclusion**

MAC and SRC are not common forms of adenocarcinomas, especially early-stage colorectal carcinomas. The histological subtypes and differentiated grades should be
considered separately. Pre- and postoperative routine pathological reports should include information about the histology. Our data suggest that pT1 patients with SRC and patients with MAC of the rectum have a high incidence of LNM, and with these adverse outcomes, local excision is not recommended. Although MAC of the rectum and SRC of the colon have a high rate of LNM, the prognosis of these types is similar to that of AC.

**Disclosure Statement**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

**References**