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Clinical Implications of Microsatellite Instability in T1 Colorectal Cancer

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Purpose: The estimation of regional lymph node metastasis (LNM) risk in T1 colorectal cancer is based on histologic examination and imaging of the primary tumor. High-frequency microsatellite instability (MSI-H) is likely to decrease the possibility of metastasis to either regional lymph nodes or distant organs in colorectal cancers. This study evaluated the clinical implications of MSI in T1 colorectal cancer with emphasis on the usefulness of MSI as a predictive factor for regional LNM. **Materials and Methods:** A total of 133 patients who underwent radical resection for T1 colorectal cancer were included. Genomic DNA was extracted from normal and tumor tissues and amplified by polymerase chain reaction (PCR). Five microsatellite markers, BAT-25, BAT-26, D2S123, D5S346, and D17S250, were used. MSI and clinicopathological parameters were evaluated as potential predictors of LNM using univariate and multivariate analyses. **Results:** Among 133 T1 colorectal cancer patients, MSI-H, low-frequency microsatellite instability (MSI-L), and microsatellite stable (MSS) colorectal cancers accounted for 7.5%, 6%, and 86.5%, respectively. MSI-H tumors showed a female predominance, a proximal location and more retrieved lymph nodes. Twenty-two patients (16.5%) had regional LNM. Lymphovascular invasion and depth of invasion were significantly associated with LNM. There was no LNM in 10 MSI-H patients; however, MSI status was not significantly correlated with LNM. Disease-free survival did not differ between patients with MSI-H and those with MSI-L/MSS. **Conclusion:** MSI status could serve as a negative predictive factor in estimating LNM in T1 colorectal cancer, given that LNM was not detected in MSI-H patients. However, validation of our result in a different cohort is necessary.

Key Words: Microsatellite instability, lymph node metastasis, early colorectal cancer, T1, prognosis

INTRODUCTION

Although the basic principle in surgical management of colorectal cancer is wide resection of the primary tumor and adequate regional lymph node dissection, some modifications are appropriate in selected patients. When the cancer is confined to

the mucosal layer, the tumor can be completely removed by an endoscopic procedure or local excision and further lymph node dissection is not necessary since there is no risk of lymph node metastasis (LNM) in these cancers. The same treatment strategy could be applied to invasive carcinoma if the risk of LNM could be predicted. However, the challenge in applying local resection to invasive carcinoma is predicting the presence of LNM. The risk of regional LNM increases in proportion to the depth of invasion. The overall LNM rate of T1 colorectal cancer was reported to range from 6% to 13%,[1-4] which is relatively low compared to 19–28% for T2 and 36–65.4% for T3 and T4 cancer.[5-7]

Currently, when a tumor is found to be confined within the submucosa during a preoperative workup, resection of the primary tumor is attempted. The possibility of LNM is subsequently evaluated based on histologic examination of the primary tumor with regard to features such as depth of invasion, presence of lymphovascular invasion, differentiation, and tumor budding.[8] It is recommended that patients at high risk undergo radical resection due to the possibility of regional LNM. Given that the development of colorectal cancer is known to be the result of accumulating genetic alterations,[9-11] it seems prudent to take into account histologic features of the primary tumor in addition to molecular biological characteristics for the prediction of regional LNM in T1 colorectal cancer. However, to the best of our knowledge, there are limited reports on predicting regional LNM in T1 colorectal cancer using molecular biological characteristics.[12]

MSI is one of the prevalent carcinogenic pathways in colorectal cancer, caused by a mismatch repair gene defect resulting in loss or gain of tandem repeat sequences.[13-16] A high frequency of microsatellite instability (MSI-H) has been found in most patients with hereditary nonpolyposis colorectal cancer (HNPCC), and occurs in approximately 15% of sporadic colorectal cancer cases.[15-18] MSI-H tumors were associated with unique clinicopathological characteristics, such as younger age at onset, proximal location, and frequent peritumoral lymphocytic infiltration.[19-21] Additionally, MSI-H tumors showed a lower incidence of LNM and a better survival rate.[21-24] Most previous studies investigating the relationship between MSI and regional LNM were based on advanced colorectal cancers. However, little is known regarding the specific impact of MSI in early colorectal cancer.

Therefore, the aim of this study was to evaluate the clinical implications of MSI in T1 colorectal cancer with emphasis on the usefulness of MSI as a predictive factor for regional LNM.

MATERIALS AND METHODS

Eligibility

Patients who underwent curative resection for T1 colon or rectal adenocarcinoma between January 2005 and May 2011 were selected from our prospectively collected database. Excluded from the initial selection were patients who had undergone preoperative chemotherapy or radiotherapy, patients who were diagnosed with HNPCC or a familial adenomatous polyposis, patients who underwent transanal excision, and patients initially diagnosed with stage IV disease. The inclusion of patients was based on the availability of MSI data, as well as a complete set of clinicopathological information including age, sex, tumor size, histologic grade, lymphovascular invasion, depth of invasion, date of surgery, location of the primary tumor, [proximal colon (cecal, ascending, or transverse colon), distal colon (descending, sigmoid, or rectosigmoid colon), or rectum], date of recurrence, pathologic nodal stage, and total retrieved lymph nodes. After exclusions, 133 patients were included in our analysis. This study was approved by our Institutional Review Board (number: 4-2010-0286).

Sectioning and microdissection

Genomic DNA was extracted from three to five sections of 10-μm-thick formalin-fixed, paraffin-embedded tissue blocks containing tumor and non-neoplastic mucosa using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Separate blocks were used for malignant and non-malignant tissue. Tumor blocks with a tumor area greater than 80% tumor cells were selected based on hematoxylin and eosin stained slides.

Microsatellite instability (MSI)

The MSI status of each tumor was determined with the following five markers: BAT 25, BAT 26, D2S123, D5S346, and D17S250. Fifty nanograms of DNA were amplified in a 20 μL reaction solution containing 2 μL of 10X buffer (Roche, Mannheim, Germany), 1.7 to 2.5 mmol/L of $MgCl_2$, 0.3 μM of each primer pair, 250 μM of deoxynucleotide triphosphate, and 2.5 units of DNA polymerase (Roche). The primer sequences and polymerase chain reaction (PCR) cycles for each marker were adapted from the published data.[25] Fluorescence markers (NED, FAM) were attached to the 5'

end of the forward primer.

All samples were prepared for fragment separation on an ABI Prism 3100 Genetic Analyzer using 0.7 μL of the amplified samples combined with 0.3 μL of GeneScan 500 Size Standard and 9 μL of HiDi Formamide.

MSI was diagnosed when there were aberrant peaks or peak shifts compared to the normal control. A case was categorized as MSI-H if MSI was present at two or more markers, MSI-low (MSI-L) if only one of the five markers showed instability, and microsatellite stable (MSS) if no marker had evidence of MSI.[26] In all of the analyses, MSI-L, and MSS tumors were grouped together and denoted as MSI-L/MSS.

Statistical analysis

All calculations and analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The association of clinicopathological features with MSI status was analyzed using the two-sided Pearson's chi-square test or Fisher's exact test for categorical variables and Student's t-test for continuous variables. Factors associated with LNM were analyzed by logistic regression with forward stepwise selection of variables. Disease-free survival (DFS) was defined as the time from the date of operation to the date of tumor recurrence or last follow-up. Survival analysis was performed with the Kaplan-Meier method. The log-rank test was used to compare survival outcome between groups. A p-value <0.05 was considered to indicate significance.

RESULTS

Patient characteristics

Patients' characteristics are summarized in Table 1. The median age was 60 years (range: 32–84 years). The distribution of tumor locations was as follows: 29 in the proximal colon, 32 in the distal colon and 72 in the rectum. Twenty-two patients (16.5%) had regional LNM. Histologic grade examination revealed only one patient (0.8%) with poorly differentiated adenocarcinoma. The distribution of invasion was 47 sm1, 35 sm2, and 51 sm3. Lymphovascular invasion was detected in 18 patients (13.5%). The median tumor size was 1.9 cm (range: 0.2–8.0 cm).

Clinical characteristics of patients with MSI

An evaluation of tumor MSI status revealed MSS in 115 patients (86.5%), MSI-L in 8 patients (6.0%) and MSI-H in 10 patients (7.5%). MSI-L and MSS tumors were grouped together and denoted as MSI-L/MSS. Comparisons of clinicopathological characteristics between the MSI-H and MSI-L/MSS groups are summarized in Table 2. There was a female predominance in the MSI-H group (p=0.005). The predominant location in the MSI-H group was the proximal colon (p=0.001). There was no significant difference between the two groups with regard to the depth of tumor invasion, histologic grade, presence of lymphovascular invasion, or tumor size. In contrast, the number of total retrieved lymph nodes was higher in the MSI-H group (p=0.044). The number of metastatic lymph nodes in the MSI-H group was significantly lower in comparison to the MSI-L/MSS group (p<0.001).

Table 1. Patient Baseline Characteristics

	n (%)
Gender	
Male	73 (54.9)
Female	60 (45.1)
Age (yrs)	
Median (range)	60 (32–84)
Preoperative CEA (ng/mL)	
Median (range)	1.4 (0.0–9.8)
Tumor location	
Proximal colon	29 (21.8)
Distal colon	32 (24.1)
Rectum	72 (54.1)
Depth of invasion	
Sm1	47 (35.3)
Sm2	35 (26.3)
Sm3	51 (38.3)
pN	
Node negative	111 (83.5)
Node positive	22 (16.5)
LN numbers	
<12	48 (36.1)
≥12	85 (63.9)
Histologic grade*	
G1	46 (34.6)
G2	86 (64.7)
G3	1 (0.8)
LVI	
Negative	115 (86.5)
Positive	18 (13.5)
Tumor size (cm)	
Median (range)	1.9 (0.2–8.0)

CEA, carcinoembryonic antigen; LN, lymph node; LVI, lymphovascular invasion.
*Histologic grade: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Table 2. Comparison of Clinicopathological Characteristics between MSI-H Group and MSI-L and MSS Group

	MSI-H group (%)	MSI-L and MSS group (%)	*p* value
Gender			
Male	1 (10)	72 (58.5)	0.005*
Female	9 (90)	51 (41.5)	
Age (yrs)			
Mean (SD)	59.3 (9.4)	59.9 (10.1)	0.841
Preoperative CEA (ng/mL)			
Mean (SD)	1.5 (1.3)	1.7 (1.5)	0.815
Tumor location			
Proximal colon	7 (70)	22 (17.9)	0.001*
Distal colon	0 (0)	32 (26.0)	
Rectum	3 (30)	69 (56.1)	
Depth of invasion			
Sm1	2 (20)	45 (36.6)	0.502*
Sm2	4 (40)	31 (25.2)	
Sm3	4 (40)	47 (38.2)	
pN			
N negative	10 (100)	101 (82.1)	0.213*
N positive	0 (0)	22 (17.9)	
LN numbers			
<12	1 (10)	47 (38.2)	0.094*
≥12	9 (90)	76 (61.8)	
No. of metastatic LNs			
Mean (SD)	0	0.3 (0.7)	<0.001
No. of retrieved LNs			
Mean (SD)	24.9 (12.1)	16.1 (13.1)	0.044
Histologic grade†			
G1	2 (20)	44 (35.8)	0.531*
G2	8 (80)	78 (63.4)	
G3	0 (0)	1 (0.8)	
LVI			
Negative	9 (90)	106 (86.2)	1.0*
Positive	1 (10)	17 (13.8)	
Tumor size (cm)			
<2.0	9 (90)	82 (66.7)	0.169*
≥2.0	1 (10)	41 (33.3)	

CEA, carcinoembryonic antigen; LN, lymph node; LVI, lymphovascular invasion; SD, standard deviation; MSI-H, high-frequency microsatellite instability; MSI-L, low-frequency microsatellite instability; MSS, microsatellite stable.
*Fisher's exact test.
†Histologic grade: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Factors associated with LNM

Histologic grade, lymphovascular invasion, and depth of invasion were significantly associated with LNM by univariate analysis. Multivariate analysis indicated that lymphovascular invasion [hazard ratio (HR), 17.2; 95% confidence interval (CI), 4.8–61.0; $p<0.001$] and depth of invasion (comparing sm1 to sm3: HR, 4.4; 95% CI, 1.1–16.9; $p=0.030$) were independent risk factors for LNM. MSI-H was not correlated with regional LNM by univariate analysis ($p=0.213$, Fisher's exact test). However, there was no LNM in the MSI-H group (Table 3).

Survival analysis

We compared disease-free survival between patients with MSI-H and those with MSI-L/MSS (Fig. 1). There was no significant difference in three-year DFS between the two groups (100% in the MSI-H group; 93.7% in the MSI-L/MSS group; $p=0.542$) (Fig. 1).

Table 3. Factors Associated with Lymph Node Metastasis in T1 Colorectal Carcinoma

	Univariate		Multivariate	
	(%)	*p* value	HR (95% CI)	*p* value
Gender				
Male	10/73 (13.7)	0.330	N/A	
Female	12/60 (20.0)			
Age (yrs)				
<60	11/61 (18.0)	0.670	N/A	
≥60	11/72 (15.3)			
Tumor location				
Proximal colon	3/29 (10.3)	0.405*	N/A	
Distal colon & rectum	19/104 (18.3)			
Preoperative CEA (ng/mL)				
<5.0	21/129 (16.3)	0.519*	N/A	
≥5.0	1/4 (25.0)			
Histologic grade†				
G1	3/46 (6.5)	0.024	N/S	N/S
G2 & G3	19/87 (21.8)			
LVI				
Negative	11/115 (9.6)	<0.001*	1	<0.001
Positive	11/18 (61.1)		17.2 (4.8–61.0)	
Tumor size (cm)				
<2.0	17/91 (18.7)	0.328	N/A	
≥2.0	5/42 (11.9)			
Depth of invasion				
Sm1	4/47 (8.5)	0.007	1	
Sm2	3/35 (8.6)		0.7 (0.1–4.1)	0.710
Sm3	15/51 (29.4)		4.4 (1.1–16.9)	0.030
Microsatellite status				
MSI-H	0 /10 (0)	0.213*	N/A	
MSI-L & MSS	22/123 (17.9)			

CEA, carcinoembryonic antigen; LVI, lymphovascular invasion; N/A, not applicable; N/S: non significant; HR, hazard ratio; CI, confidence interval; MSI-H, high-frequency microsatellite instability; MSI-L, low-frequency microsatellite instability; MSS, microsatellite stable.
Factors with *p* value less than 0.2 in univariate analysis were entered into multivariate analysis.
*Fisher's exact test.
†Histologic grade: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

DISCUSSION

The major finding of this study was that MSI status could be used as a negative predictive marker in estimating LNM in T1 colorectal cancer given that there was no LNM in MSI-H patients.

The standard treatment for colorectal cancer is complete resection of the primary tumor with regional lymphadenectomy due to the potential risk of regional LNM. However, in T1 colorectal cancer, regional lymphadenectomy can be omitted without deterioration of oncologic outcomes for selected patients with little risk of regional LNM. The prediction of regional LNM was made based on preoperative imaging and histologic characteristics of the primary tumor such as depth of tumor invasion, tumor differentiation and lymphovascular invasion.[8] In current practice, even after complete removal of the primary tumor, an additional radical resection is recommended for patients found to have high LNM risk upon histologic examination. However, a large-scale multicenter study found that the actual rate of LNM was only 14–23% according to respective high risk factors.[1] For this reason, a great deal of effort has gone into reducing potential over-treatment by precise prediction of LNM risk.

In this study, the role of MSI was investigated as one of the predictive factors for LNM in T1 colorectal cancer, since we feel that in the prediction of early metastasis, biological

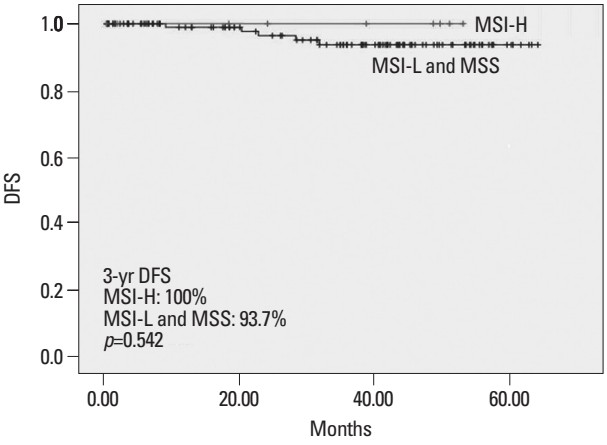

Fig. 1. Comparison of survival between MSI-H group and MSI-L/MSS group. There was no difference of survival outcomes between "MSI-H group" and "MSI-L/MSS group" (mean follow-up periods: 31 months). DFS, disease-free survival; MSI-H, high-frequency microsatellite instability; MSI-L, low-frequency microsatellite instability; MSS, microsatellite stable.

characteristics should be taken into account in addition to conventional pathologic examination. MSI-H cancers are reported to have a decreased likelihood of metastases to either regional LNs or distant organs.[22,23,27] Interestingly, regional LNM was not identified in patients with MSI-H T1 colorectal cancers in this study. Although not statistically significant, the prominent difference in LNM between the MSI-H group (0%) and MSI-L/MSS group (17.9%) indicate the possibility that the lack of an observed association between MSI status and LNM may have been the result of a type II error related to insufficient sample size. In the studied cohort, lymphovascular invasion, and depth of invasion were identified as independent risk factors for LNM by multivariate analysis, consistent with other reports.[8] Although this study was not substantial enough to make definite conclusions, our results suggest that MSI has the potential to be used as a predictive factor for LNM in T1 colorectal cancer.

Colorectal cancers with MSI-H have distinct clinicopathological features. MSI-H cancers tend to be associated with a slight predominance in females, proximal tumor location, large tumor size, greater depth of tumor invasion, and poor histology.[20,21,23,24] Our data also demonstrated that MSI-H T1 colorectal cancer showed a female predominance, proximal tumor location and more retrieved lymph nodes.

The total number of retrieved lymph nodes in colorectal cancer is known to be positively correlated with good prognosis.[28-30] Many studies demonstrated an association between MSI-H status and higher total lymph node counts.[31] However, there are discrepancies among some studies. While Belt, et al.[32] reported that high lymph node retrieval was associated with MSI-H tumors especially in stage III colon cancer and not in stage II colon cancer, the association was not evident in stage III colorectal cancer in another series.[33] It was reported that this discrepancy might have originated from different uses of panels in defining MSI-H, different incidences of MSI-H phenotype, and limited numbers of MSI-H cases.[26,34] In our study, although the frequency of MSI-H was relatively low (7.5%), a higher lymph node harvest was observed in MSI-H tumors (p=0.044), confirming the positive correlation of MSI-H and retrieved lymph node numbers. In the sub-group analysis of stage I patients (n=111), there was still a trend toward increased total retrieved lymph nodes in MSI-H tumors (MSI-H: mean of 24.9; MSI-L/MSS: mean of 16; p=0.062; data not shown).

The incidence of regional LNM was 16.5% in the current study, higher than in previous reports (6.3% to 13%).[1-4] This difference may be the result of patient selection bias in this study. Most of the included patients were candidates for radical surgery because of the presence of risk factors for regional LNM or technical factors such as incomplete resection or difficulty in complete resection of the primary tumor. These factors could have contributed to the relatively high incidence of regional LNM; however, the fact that all included patients underwent radical surgery is a unique aspect of this study. For this reason, the presence of cancer metastasis to regional lymph nodes was confirmed by pathological examination. It is also noteworthy that there was no regional LNM in patients with MSI-H, even among high risk patients. In conclusion, given that there was no LNM in patients with MSI-H tumors, MSI status could serve as a negative predictive factor in estimating LNM in T1 colorectal cancer. Although this study showed the possibility of negative predictive power of MSI-H in LNM, the sample size is relatively small. Further large scale studies are required to confirm our observation.

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