

## Microsatellite Instability Status of Interval Colorectal Cancers in a Korean Population

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**Background/Aims:** A subset of patients may develop colorectal cancer after a colonoscopy that is negative for malignancy. These missed or de novo lesions are referred to as interval cancers. The aim of this study was to determine whether interval colon cancers are more likely to result from the loss of function of mismatch repair genes than sporadic cancers and to demonstrate microsatellite instability (MSI).

**Methods:** Interval cancer was defined as a cancer that was diagnosed within 5 years of a negative colonoscopy. Among the patients who underwent an operation for colorectal cancer from January 2013 to December 2014, archived cancer specimens were evaluated for MSI by sequencing microsatellite loci. **Results:** Of the 286 colon cancers diagnosed during the study period, 25 (8.7%) represented interval cancer. MSI was found in eight of the 25 patients (32%) that presented interval cancers compared with 22 of the 261 patients (8.4%) that presented sporadic cancers ( $p=0.002$ ). In the multivariable logistic regression model, MSI was associated with interval cancer (OR, 3.91; 95% confidence interval, 1.38 to 11.05). **Conclusions:** Interval cancers were approximately four times more likely to show high MSI than sporadic cancers. Our findings indicate that certain interval cancers may occur because of distinct biological features. (**Gut Liver 2016;10:781-785**)

**Key Words:** Colorectal neoplasms; Microsatellite instability; Colonoscopy

### INTRODUCTION

Colonoscopy is considered the gold standard for the detection and prevention of colorectal cancer. Colorectal adenomatous

polyps are accepted as the precursor lesion for colorectal cancer, so if colonoscopy can detect and remove all polyps, it can effectively reduce the incidence of colorectal cancer. However, colonoscopy is not a perfect examination, and some colorectal cancer is detected between colonoscopic surveillance examinations. Interval cancers have a negative impact on the ability to reduce the incidence of colorectal cancer. The development of interval cancers may represent the limitations of the colonoscopic technique, or differences in tumor biology resulting in rapid tumor growth.

Usually, interval cancer is defined as colorectal cancer that develops within 5 years of a complete colonoscopy. Previous reports have indicated that the incidence of interval cancer ranges from 3% to 8%.<sup>1,2</sup> There are several possible explanations for this discrepancy. First, some tumors may grow rapidly, representing actual new pathology that was not present at the time of the prior colonoscopy. Second, some tumors may have been missed at the time of colonoscopy for various reasons. Third, the neoplastic lesion may have been identified at the time of colonoscopy, but was incompletely resected.<sup>3</sup>

In a previous study, we reported the prevalence, clinicopathologic characteristics, and predictors of interval colorectal cancers in a Korean population.<sup>4</sup>

A previous Western study showed that interval colon cancers are three times more likely to have microsatellite instability (MSI) than sporadic cancers.<sup>5</sup> Colon cancer is associated with loss of function of mismatch repair genes and accelerated tumor growth.<sup>6</sup> In this study, we tested the hypothesis that interval cancers are more likely than sporadic cancers to exhibit MSI in a Korean population.

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## MATERIALS AND METHODS

### 1. Selection of study subjects

We reviewed patients who underwent surgery for colorectal cancer and received a MSI test between January 2013 and December 2014 at Kangbuk Samsung Hospital. All patients underwent the operation at our hospital, but, in most cases, the index colonoscopy (colonoscopy performed before the diagnosis of colon cancer) was not performed at our hospital. We confirmed the previous colonoscopy history through medical records and patient interviews (face-to-face or over the phone). We excluded patients with colon cancer diagnosed within 1 year of an index colonoscopy, and patients with familial adenomatous polyposis, lynch syndrome (hereditary nonpolyposis colorectal cancer), inflammatory bowel disease, or recurred colorectal cancer. Patients were defined as having an interval cancer if they developed colon cancer within 5 years of a complete colonoscopy. Patients were defined as having a sporadic (noninterval) cancer if they were diagnosed with colon cancer on their first recorded colonoscopy or over 5 years after an index colonoscopy. Our institutional review board approved this study.

### 2. Data collection and definitions

Data extracted from patient medical records included comorbidities, lifestyle habits, and personal and family history of colorectal neoplasm (first-degree relatives). The American Joint Committee on Cancer (AJCC) Cancer Staging Manual was used to define and categorize study variables such as TNM cancer stage and histologic grade. The colon was divided into eight segments to describe the tumor location and these were collapsed into two categories: proximal colon (cecum, ascending, hepatic flexure, and transverse colon), and distal colon (splenic flexure, descending colon, and rectum). Mucinous carcinoma was defined as colon cancer with >50% relevant histology.

### 3. MSI analysis

We used the presence of MSI to assess the loss of function of mismatch repair gene activity. MSI assays were performed on

DNA extracted from paraffin-embedded tissue blocks. Genomic DNA extraction and MSI testing of DNA samples was conducted at Samsung Medical Center in Seoul. In this study, MSI test was performed by multiplex polymerase chain reaction and analysis with a 3130x1 genetic analyzer. MSI testing of DNA samples was based on five dinucleotide markers (NR27, NR21, BAT26, BAT25, and NR24). Tumors that showed instability in  $\geq 2/5$  of markers tested were classified as a high MSI and 1/5 of markers were classified as low MSI. Tumors that showed instability in 0/5 of markers were designated as microsatellite stable (MSS) cancers. Only high MSI cases were considered MSI positive.

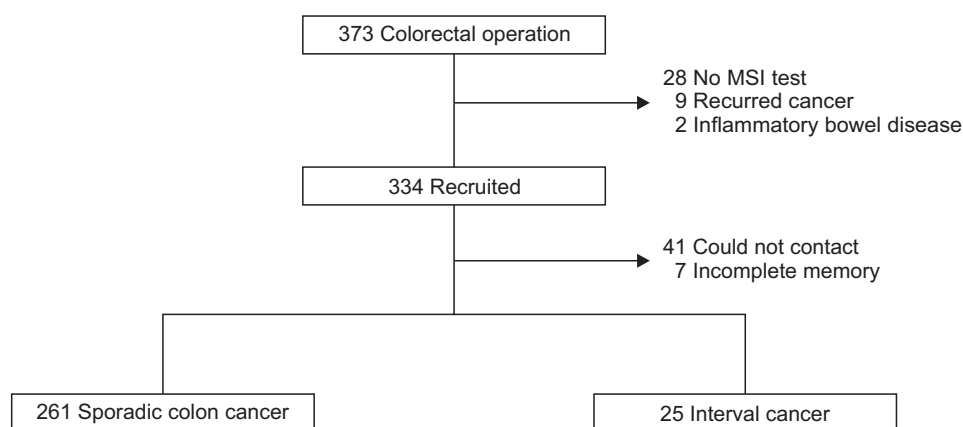
### 4. Statistical analysis

Continuous variables are expressed as the median and range and categorical variables are absolute values or rates. Differences with respect to categorical covariates were evaluated using the chi-square test or Fisher exact test on appropriate cross-tabulations, normally distributed continuous variables using the t-test, and nonnormally distributed continuous variables using the Wilcoxon rank sum test. Logistic regression analysis was used to adjust for confounders (sex, age, and tumor location) to the MSI and interval cancer association. Differences were considered significant when the two-sided p-value was <0.05. SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used for data management and analysis.

## RESULTS

From January 1, 2013 to December 31, 2014, there were 373 patients who underwent an operation for colorectal cancer at our hospital. Among these, 28 patients did not receive a MSI test, nine patients had recurred colorectal cancers, and two patients had inflammatory bowel disease. Of the remaining 334 patients, seven patients had an incomplete memory of prior colonoscopy, and 41 patients were not available for interview (Fig. 1).

In total, there were 286 patients who met the entry criteria for the study. Of these 286 cases, 261 patients had a colonoscopy



**Fig. 1.** Flow chart of the selection of study participants. MSI, microsatellite instability.

at diagnosis (or after more than 5 years) and were classified as having sporadic cancer. A total of 25 individuals had a colonoscopy within 5 years before colorectal cancer diagnosis and were classified as having interval cancers. These interval cancers accounted for 8.7% of all colon cancers.

The interval time between the index colonoscopy and the second colonoscopy (at cancer diagnosis) was the average 35±12 months, with a range of 15 to 57 months. Among the interval cancer group, 18 patients were male and seven were female. The mean age at diagnosis of interval cancer patients was 61±11 years, with a range of 35 to 79 years.

A comparison of baseline characteristics between interval cancers and sporadic cancers is given in Table 1. There were no differences in age, sex, body mass index, risk factors, symptoms, previous abdominal surgery, or family history of colorectal cancer between patients with interval and sporadic cancer groups.

A comparison of tumor characteristics between interval and sporadic patients is shown in Table 2. The results of MSI analysis for the patients were as follows. MSI was demonstrated in eight patients (32%) with interval cancer compared with 22 patients (8.4%) with sporadic cancer ( $p=0.002$ ). After adjusting for confounders, patients with interval cancer were approximately four times more likely to be MSI-positive than sporadic cancers

(odds ratio [OR], 3.9; 95% confidence interval [CI], 1.3 to 11.0) (Table 3). There was a significant difference in the location of the tumor between the two groups, with proximal colon cancers being much more common in the interval cancer group (13/25, 52%) than in the sporadic cancer group (11/261, 29%) in univariate analysis ( $p=0.02$ ). However, this association was not statistically significant in multivariable analysis (OR, 2.58; 95% CI, 0.69 to 4.37;  $p=0.23$ ). There were no significant differences with regard to tumor size, TNM stage, or histologic grade between two groups.

A comparison between MSI-positive cancers and MSI-negative (MSI-low or MSS) cancers was conducted. As shown in Table 4, MSI-positive cancers tended to occur in younger patients (55.9±16.4 years vs 63.4±12.1 years,  $p=0.02$ ), were larger (5.7±2.0 cm vs 4.1±1.9 cm,  $p<0.001$ ), more frequently located in the proximal colon (76% vs 26%,  $p<0.001$ ), more likely to show a family association (20% vs 6%,  $p=0.018$ ), more likely to exhibit mucinous histology (20% vs 2%,  $p<0.001$ ), and more likely

**Table 1.** Baseline Characteristics of Subjects with Interval and Sporadic Colon Cancer

Characteristic	Interval cancer (n=25)	Sporadic cancer (n=261)	p-value
Age, yr	61.3±11.7	62.8±12.9	0.59
Male sex	18 (72)	153 (58)	0.19
Body mass index, kg/m <sup>2</sup>	23.0±2.5	23.0±2.4	0.92
Hypertension	10 (40)	90 (34.5)	0.58
Diabetes mellitus	4 (16)	62 (23)	0.37
Family history of colon cancer*	3 (12)	19 (7)	0.42
Presentation of symptoms	11 (44)	154 (59)	0.14
Abdominal surgery	6 (24)	48 (18)	0.59
Smoking <sup>†</sup>	8 (32)	102 (39)	0.56

Data are presented as mean±SD or number (%).

\*Family history includes only first-degree relatives; <sup>†</sup>Smoking indicates a past or current smoker.

**Table 2.** Clinicopathological Characteristics of Interval and Sporadic Cancers

Characteristic	Interval cancer (n=25)	Sporadic cancer (n=261)	p-value
Tumor size, cm	3.8±2.4	4.3±2.0	0.260
Location (proximal)*	13 (52)	77 (29)	0.020
MSI positivity <sup>†</sup>	8 (32)	22 (8.4)	0.002
TNM stage			0.055
1	9 (36)	55 (21)	
2	8 (32)	72 (27)	
3	6 (24)	101 (38)	
4	2 (8)	33 (12)	
Histologic grade			0.230
Well	3 (13)	14 (5)	
Moderate	19 (86)	232 (91)	
Poor	0	7 (3)	
Mucinous <sup>‡</sup>	3 (12)	8 (3)	0.060

Data are presented as mean±SD or number (%).

MSI, microsatellite instability.

\*Proximal location represents cecum, ascending colon, and transverse colon; <sup>†</sup>MSI positive, tumors with ≥2/5 markers showing MSI; <sup>‡</sup>>50% of tumors with mucinous histology.

**Table 3.** Risk Factors (or Predictive Factors) of Interval Cancer

Variable	Crude analysis			Adjusted analysis		
	OR	95% CI	p-value	OR	95% CI	p-value
MSI*	5.11	1.98–13.18	0.002	3.91	1.38–11.05	0.010
Location <sup>†</sup>	2.58	1.13–5.92	0.020	1.74	0.69–4.37	0.230

Adjusted factors: sex, age, MSI, and location.

OR, odds ratio; CI, confidence interval; MSI, microsatellite instability.

\*MSI positive, tumors with ≥2/5 markers showing MSI; <sup>†</sup>Proximal location: cecum, ascending colon, and transverse colon.

**Table 4.** Characteristics of Cancers by Microsatellite Instability Status

Characteristic	MSI-positive cancer (n=30)	MSI-negative cancer (n=256)	p-value
Age, yr	55.9±16.4	63.4±12.1	0.020
Male sex	21 (70)	150 (58)	0.220
Size, cm	5.7±2.0	4.1±1.9	<0.001
Location (proximal)*	23 (76)	67 (26)	<0.001
Family history <sup>†</sup>	6 (20)	16 (6)	0.018
Mucinous <sup>‡</sup>	6 (20)	5 (2)	<0.001
Histology			0.005
Well	2 (8)	15 (6)	
Moderate	17 (70)	234 (93)	
Poor	5 (20)	2 (1)	
TNM Stage			0.710
1	4 (13)	60 (23)	
2	15 (50)	65 (25)	
3	8 (26)	99 (38)	
4	3 (10)	32 (12)	

Data are presented as mean±SD or number (%).

MSI, microsatellite instability.

\*Cecum, ascending colon, and transverse colon; <sup>†</sup>First-degree relatives; <sup>‡</sup>>50% of tumors with mucinous histology.

to exhibit poor differentiation (20% vs 2%,  $p=0.005$ ) than MSI-negative cancers. There were no differences with respect to sex or TNM stage between the two groups.

## DISCUSSION

To our knowledge, this is the first study to evaluate the prevalence of MSI in Korean patients with interval colorectal cancer. The prevalence of MSI was 32% in interval cancers compared to 8.4% in sporadic cancers. We found that interval cancers were about four times more likely than sporadic cancers to be MSI positive. MSI was independently associated with interval cancers.

The interval cancer rate of 8.7% in this study was comparable to that reported in previous Western studies. However, this results show a relatively high proportion of interval cancer than previous our study (6.2%).<sup>4</sup> The higher proximal location rate of interval cancer (52%) was similar to that of previous studies, but only in univariate analysis.<sup>2,5,7,8</sup> There were no significant differences with regard to age, sex, tumor stage and mucinous histology between the two groups in other studies.<sup>2,9</sup> These differences are thought to be due to insufficient number of samples.

There are several explanations for the development of interval cancer. Basically, it can either be a technical problem with colonoscopy or a biological problem. In terms of technical problems associated with colonoscopy, it was impossible to observe the whole colon mucosa because of poor bowel preparation. As bowel preparation improves, the polyp detection rate increases.<sup>7</sup> Inadequate bowel preparation has been hypothesized to be a

risk factor for missed interval cancers. Second, the colonoscopy exam duration may not be sufficient. It was reported that colonoscopy withdrawal time has been associated with polyp detection rate.<sup>10,11</sup> The quality control guidelines of colonoscopy recommend a minimum of six minutes or more.<sup>12</sup> Third, incomplete resection of the polyp could be a reason for interval cancer. The causes of incomplete resection are large polyp size, sessile type, and location in a difficult area for endoscopic resection. In particular, if the polyp is more than 20 mm, the potential for incomplete resection increases.<sup>13,14</sup>

Finally, a rapidly growing polyp can be a cause of interval cancer. The progression from adenoma to carcinoma is generally thought to span 10 years according to the time interval of the adenoma-carcinoma sequence.<sup>15</sup> However, several studies have reported that some patients experience rapid progression to cancer from a small adenoma.<sup>16,17</sup> There are some rapid pathways for colon cancer development: the mismatch repair pathway and the serrated pathway. Mismatch repair defects associated with MSI tumors lead to a rapid accumulation of mutations necessary for tumorigenesis and result in accelerated tumor growth. Hereditary nonpolyposis colorectal cancer is known as a fast growing tumor in association with a DNA mismatch repair (MMR) gene.<sup>18</sup> The majority of MSI cancers are sporadic cancers due to loss of function of MMR gene activity from hypermethylation of the hMLH1 mismatch repair gene.<sup>19</sup> MSI, even in patients without Lynch syndrome is associated with proximal tumor location and improved survival compared with MSS cancers and more rapid lesion growth.<sup>20,21</sup> Tumors that arise from sessile serrated polyps also may contribute to interval cancers because of their proximal colon predominance and difficulty of detection at colonoscopy.<sup>1,22</sup> These flat-type lesions in particular have a tendency to invade the submucosal layer even when they are small. Sessile serrated polyps were often associated with mutations in the BRAF oncogene, which is not typically seen in traditional adenomas.<sup>23</sup> This BRAF mutation has been tightly linked to a specific DNA methylation aberrancy of CpG islands broadly referred to as the CpG island methylator phenotype,<sup>24</sup> and CIMP-high/BRAF-mutation are associated with sessile serrated adenoma as well as with MSI.<sup>25,26</sup> Therefore, we should always be aware of these types of lesions.

This study had some strength. This is the first report on the high prevalence of MSI in interval colorectal cancers in Koreans, and the third result worldwide.<sup>5,27</sup> We reported the prevalence of interval cancers and MSI positive cancers in a generalized group.

The present study also had several limitations. First, we only analyzed a small number of patients with interval cancer in tertiary single center. Selection bias was inevitable. Our results may not be representative of prevalence of interval cancer in Korea. A large sample study is essential to further discuss the features of interval cancer. Second, most of the index colonoscopies were not performed at our hospital, so most of the information about index colonoscopy was obtained from patient's

memory, some interval cancers were misclassified as sporadic cancer, or vice versa. There may be recall bias. Third, we did not know all the results of index colonoscopy, for instance, withdrawal time of examination, degree of bowel preparation, presence of adenoma or other lesions, and department of endoscopist. Therefore, we lacked the ability to distinguish the cause of interval cancers.

In conclusion, this study suggests that tumor biology may play an important role in the pathogenesis of interval cancer. Mismatch repair pathway defects are present in a significant proportion of interval colon cancer cases. Further research is needed to clarify the role of this pathway in the etiology of interval cancers.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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