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# Low-Level Microsatellite Instability as a Potential Prognostic Factor in Sporadic Colorectal Cancer

Soo Young Lee, MD, Duck-Woo Kim, MD, Hye Seung Lee, MD, PhD, Myong Hoon Ihn, MD, Heung-Kwon Oh, MD, Byung Soh Min, MD, PhD, Woo Ram Kim, MD, Jung Wook Huh, MD, PhD, Jung-A. Yun, MD, Kang Young Lee, MD, PhD, Nam Kyu Kim, MD, PhD, Woo Yong Lee, MD, PhD, Hee Cheol Kim, MD, PhD, and Sung-Bum Kang, MD, PhD

**Abstract:** Although microsatellite instability-high (MSI-H) colorectal cancers (CRCs) have been shown to exhibit a distinct phenotype, the clinical value of MSI-low (MSI-L) in CRC remains unclear. We designed this study to examine the clinicopathologic characteristics and oncologic implications associated with MSI-L CRCs.

We retrospectively reviewed data of CRC patients from 3 tertiary referral hospitals in Korea, who underwent surgical resection between January 2003 and December 2009 and had available MSI testing results. MSI testing was performed using the pentaplex Bethesda panel. Clinicopathologic features and oncologic outcomes were compared between MSI-L and microsatellite stable (MSS) CRCs; prognostic factors for survival were also examined.

Of the 3019 patients reviewed, 2621 (86.8%) were MSS, and 200 (6.6%) were MSI-L; the remaining 198 (6.6%) were MSI-H. MSI-L and MSS CRCs were comparable in terms of their clinicopathologic features, with the exception of proximal tumor location (MSI-L 30.0% vs MSS 22.1%,  $P=0.024$ ) and tumor size (MSI-L  $5.2 \pm 2.6$  cm vs MSS  $4.6 \pm 2.1$  cm,  $P=0.001$ ). No differences were detected in either 3-year disease-free survival (MSI-L 87.2% vs MSS 82.6%,  $P=0.121$ ) or 5-year overall survival (OS) (MSI-L 74.2% vs MSS 78.3%,  $P=0.131$ ) by univariable analysis. However, MSI-L was an independent prognostic factor for poor OS by Cox regression analysis (hazard ratio 1.358, 95% confidence interval 1.014–1.819,  $P=0.040$ ).

MSI-L may be an independent prognostic factor for OS in sporadic CRCs despite their clinicopathologic similarity to MSS. Further studies investigating the significance of MSI-L in the genesis and prognosis of CRCs are needed.

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**Abbreviations:** AJCC = American Joint Committee on Cancer, ASA = American Society of Anesthesiologists, CEA = carcinoembryonic antigen, CI = confidence interval, CRC = colorectal cancer, DFS = disease-free survival, EMAS = elevated microsatellite alterations at selected tetranucleotide repeats, HNPCC = hereditary nonpolyposis colorectal cancer, HR = hazard ratio, LOH = loss of heterozygosity, m/d = moderately differentiated, MMR = mismatch repair, MSI = microsatellite instability, MSI-H = microsatellite instability-high, MSI-L = microsatellite instability-low, MSS = microsatellite stable, NCCN = National Comprehensive Cancer Network, NCI = National Cancer Institute, OS = overall survival, p/d = poorly differentiated, PCR = polymerase chain reaction, SRC = signet ring cell, w/d = well differentiated.

## INTRODUCTION

Microsatellite instability (MSI) is defined as a change in length of tandemly repeated DNA sequences, caused by a failure of the DNA mismatch repair (MMR) system to correct for such errors during DNA replication.<sup>1,2</sup> MSI, the hallmark of hereditary nonpolyposis colorectal cancer (HNPCC), is recognized as one of the major carcinogenic pathways of colorectal cancer (CRC) and is detected in ~15% of sporadic colorectal cancers.<sup>3,4</sup> CRCs can be classified into 3 groups according to the MSI status: MSI-high (MSI-H), which exhibit  $\geq 30$  to 40% microsatellite marker instability, MSI-Low (MSI-L), which exhibit instability at  $< 30$  to 40% of loci, and microsatellite stable (MSS), which exhibit no unstable markers.<sup>1</sup>

The primary goal of MSI testing is to detect cases of HNPCC, as well as sporadic CRCs developed through the replication error pathway. However, because CRCs with MSI-H display distinctive clinical features including proximal tumor location, poorly differentiated or mucinous histology, large size, lymphocytic infiltration, favorable prognosis, and decreased chemo-responsiveness,<sup>4</sup> MSI testing is also used as a prognostic marker of disease. In contrast to the strong diagnostic value associated with MSI-H tumors, the biologic significance of MSI-L CRCs remains unclear. MSI-L CRCs have often been regarded as indistinct from that of MSS CRCs, making it uncertain whether or not MSI-L CRCs form a clinically unique subgroup.<sup>1,2,5,6</sup> Some studies have reported distinct clinicopathologic features associated with MSI-L CRCs,<sup>7–10</sup> whereas others observed no significant differences between MSI-L and MSS CRCs.<sup>11–13</sup> In terms of oncologic

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From the Department of Surgery, Chonnam National University Hwasun Hospital and Medical School, Hwasun (SYL); Department of Surgery, Seoul National University Bundang Hospital, Seongnam (D-WK, MHI, H-KO, S-BK); Korean Hereditary Tumor Registry, Seoul National University College of Medicine, Seoul (D-WK); Department of Pathology, Seoul National University Bundang Hospital, Seongnam (HSL); Department of Surgery, Severance Hospital, Yonsei University College of Medicine (BSM, WRK, KYL, NKK); and Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea (JWH, J-AY, WYL, HCK).

Correspondence: Duck-Woo Kim, Department of Surgery, Seoul National University Bundang Hospital, 166 Gumi-ro, Bundang-gu, Seongnam, Gyeonggi, Korea (e-mail: kdw@snuh.org).

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significance, only a small number of studies examining MSI-L tumors have been published, with conflicting results.<sup>9,10,12–15</sup>

To address these apparent inconsistencies, we conducted an investigation into the clinicopathologic characteristics and oncologic implications of sporadic CRCs with MSI-L.

## METHODS

### Hospital Setting and Study Population

Following review of a prospective database, we included patients subjected to surgery for sporadic CRC and those with available MSI results. All patients underwent radical surgery between January 2003 and December 2009 at 1 of 3 tertiary referral hospitals (Seoul National University Bundang Hospital, Severance Hospital, and Samsung Medical Center), which were 1300 to 2500-bed teaching hospitals in Seoul, Korea. The study period was not the same among the 3 institutions, as routine MSI testing with 5 microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250) was implemented at different points of time: 2007 in Seoul National University Bundang Hospital, 2003 in Severance Hospital, and 2006 in Samsung Medical Center. However, for analyzing long-term oncologic outcomes, we enrolled patients who underwent surgery before 2009. Patients with recurrent CRC, familial adenomatous polyposis, HNPCC, or inflammatory bowel disease were excluded. This study was approved by the appropriate Institutional Review Board at each institution.

The basic principles of radical surgery and neoadjuvant/adjuvant therapy were similar at the 3 institutions. For clinical T3–4 or N1–2 mid-to-low rectal cancers, neoadjuvant chemoradiation was delivered (45.0–50.4 Gy plus 5-fluorouracil/leucovorin), and radical surgery was performed 6 to 8 weeks after completion of neoadjuvant therapy. All patients underwent standard colectomy or proctectomy and regional lymphadenectomy according to tumor location, as described in our previous studies.<sup>16,17</sup> Postoperative adjuvant treatment was determined by the attending physician based on the pathologic stage and the general condition of the patient. For patients with stage II CRC with high-risk features for systemic recurrence, or stage III CRC, 5-fluorouracil-based postoperative adjuvant treatment was recommended in accordance with National Comprehensive Cancer Network (NCCN) guidelines.<sup>18,19</sup> For patients who received neoadjuvant chemoradiation, postoperative adjuvant chemotherapy was recommended for all patients irrespective of surgical pathology results, in accordance with NCCN guidelines.<sup>19</sup> For patients with metastatic disease, cetuximab or bevacizumab was considered. All cases were restaged retrospectively according to the 7th edition of the American Joint Committee on Cancer (AJCC) TNM staging system.

Patients were monitored at regular intervals, as described previously.<sup>17</sup> The detailed follow-up schedule differed slightly among the institutions, although all were based on the NCCN guidelines.<sup>18,19</sup> Disease-free survival (DFS) was determined from the date of surgery to the date of recurrence or death, with patients surviving to at least December 2014 and those lost to follow-up being censored. Overall survival (OS) was defined as the time from the date of surgery to the date of death from any cause with censoring as above. Recurrence was diagnosed via radiological detection of size-increased lesions or histological confirmation.

### MSI Analysis

MSI testing was performed on specimens after surgical resection. DNA was extracted from paraffin-embedded tumor

and surrounding normal tissues for each patient. Diagnoses were confirmed via microscopic examination, with each area identified on a reference H&E-stained slide, and microdissected using a scalpel to ascertain the presence of adequate neoplastic tissue. Five microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250) were used to determine microsatellite status; markers were selected based upon the recommendations of a National Cancer Institute (NCI) workshop on MSI.<sup>1</sup> Polymerase chain reaction (PCR) analyses were performed as described previously,<sup>20</sup> and the shift of PCR products from tumor DNA was compared to that of DNA from normal colonic mucosa. Tumors with at least 2 of the 5 microsatellite markers displaying shifted alleles were classified as MSI-H, whereas tumors with only 1 marker exhibiting a shifted allele were classified as MSI-L. Samples in which all microsatellite markers displayed identical patterns in tumor and normal tissues were classified as MSS.

### Statistical Analyses

Categorical variables were compared using the  $\chi^2$  or Fisher's exact test; continuous variables were compared using Student's *t* test. Because MSI-H tumors are widely known to have better prognosis, we excluded MSI-H tumors in survival analyses to compare the oncologic outcome between MSI-L and MSS CRCs exclusively. Survival rates were estimated and compared using the Kaplan–Meier method and log-rank test. Cox regression was utilized for the multivariable survival analysis, and all variables with  $P < 0.15$  on Kaplan–Meier survival analysis were entered into the multivariable proportional hazards model. The assumption of proportional hazards was verified by examination of log cumulative hazard plots for parallelism. The predictive discrimination of each prognostic model was examined by calculating Harrell's concordance index (C-index). All results were considered significant at  $P < 0.05$ . Statistical analyses were carried out using SPSS software version 21.0 (IBM Inc., Armonk, NY).

## RESULTS

Of the 3019 sporadic CRC patients identified in our review, 200 (6.6%) had MSI-L CRCs, 198 (6.6%) had MSI-H CRCs, and the remaining 2621 (86.8%) had MSS CRCs. The clinicopathologic characteristics of each group are shown in Table 1. MSI-H tumors were associated with young age, proximal tumor location, large tumor size, poor differentiation, and N0 and M0 stages. The majority of features associated with oncologic outcome, including tumor stage, were similar between MSI-L and MSS; however, some differences were observed. A statistically significant difference in tumor location was observed between MSI-L and MSS tumors, with 30.0% of MSI-L tumors located in the right colon, compared to only 22.1% for MSS ( $P = 0.024$ ). MSI-L tumors were also larger in their longest diameter (mean 5.2 cm) relative to MSS tumors (mean 4.6 cm;  $P = 0.001$ ).

The median follow-up time for all patients was 55 months (range 0–129), with 3-year DFS seen in 83.6% of patients, and a 5-year OS of 78.9%. Kaplan–Meier survival analysis showed that preoperative carcinoembryonic antigen, tumor size, TNM stage, lymphatic, venous, perineural invasion, and tumor differentiation were associated with both DFS and OS (Table 2). Rectal cancer was associated with shorter DFS times, whereas patient age and American Society of Anesthesiologists (ASA) score were associated only with OS. MSI-H tumors showed better DFS ( $P = 0.004$ ) and OS ( $P < 0.001$ ) compared to MSS

**TABLE 1.** Correlation Between Microsatellite Status and Clinicopathologic Variables

Characteristics		MSS (n = 2621)	MSI-H (n = 198)	P*	MSI-L (n = 200)	P*
Sex	Male	1595 (60.9%)	107 (54.0%)	0.059	112 (56.0%)	0.176
	Female	1026 (39.1%)	91 (46.0%)		88 (44.0%)	
Age (yr)		61.0 ± 11.4	58.2 ± 13.9	0.006	62.3 ± 11.3	0.120
ASA score	1,2	2415 (93.9%)	184 (95.8%)	0.274	173 (92.0%)	0.305
	3,4	157 (6.1%)	8 (4.2%)		15 (8.0%)	
Location	Right colon	579 (22.1%)	113 (57.1%)	<0.001	60 (30.0%)	0.024
	Left colon	870 (33.2%)	48 (24.2%)		54 (27.0%)	
	Rectum	1172 (44.7%)	37 (18.7%)		86 (43.0%)	
Preop CEA (ng/mL)	< 5	1800 (70.5%)	163 (84.9%)	<0.001	130 (66.3%)	0.221
	≥ 5	754 (29.5%)	29 (15.1%)		66 (33.7%)	
Size (cm)		4.6 ± 2.1	6.0 ± 2.7	<0.001	5.2 ± 2.6	0.001
T stage	0	21 (0.8%)	1 (0.5%)	0.624	2 (1.0%)	0.576
	1	175 (6.7%)	11 (5.6%)		10 (5.0%)	
	2	345 (13.2%)	24 (12.1%)		25 (12.5%)	
	3	1756 (67.0%)	143 (72.2%)		144 (72.0%)	
	4	324 (12.4%)	19 (9.6%)		19 (9.5%)	
	5	1301 (49.7%)	142 (71.7%)		105 (52.5%)	
N stage	1	766 (29.3%)	41 (20.7%)	<0.001	58 (29.0%)	0.651
	2	550 (21.0%)	15 (7.6%)		37 (18.5%)	
	3	2234 (85.2%)	186 (93.9%)		173 (86.5%)	
M stage		387 (14.8%)	12 (6.1%)	0.001	27 (13.5%)	0.626
TNM stage	0	19 (0.7%)	1 (0.5%)	<0.001	2 (1.0%)	0.717
	I	405 (15.5%)	30 (15.2%)		26 (13.0%)	
	II	812 (31.0%)	106 (53.5%)		70 (35.0%)	
	III	998 (38.1%)	49 (24.7%)		75 (37.5%)	
	IV	387 (14.8%)	12 (6.1%)		27 (13.5%)	
Lymphatic invasion	Negative	1764 (68.0%)	157 (80.5%)	<0.001	143 (71.5%)	0.306
	Positive	830 (32.0%)	38 (19.5%)		57 (28.5%)	
Venous invasion	Negative	2003 (77.2%)	169 (86.7%)	0.002	159 (79.5%)	0.457
	Positive	591 (22.8%)	26 (13.3%)		41 (20.5%)	
Perineural invasion	Negative	2303 (88.8%)	187 (95.9%)	0.002	173 (86.5%)	0.328
	Positive	291 (11.2%)	8 (4.1%)		27 (13.5%)	
Differentiation	w/d, m/d	2462 (94.5%)	167 (85.2%)	<0.001	183 (91.5%)	0.077
	p/d, SRC, mucinous	143 (5.5%)	29 (14.8%)		17 (8.5%)	
Adjuvant chemotherapy	Not performed	638 (25.2%)	52 (26.8%)	0.613	57 (29.1%)	0.225
	Performed	1897 (74.8%)	142 (73.2%)		139 (70.9%)	

ASA = American Society of Anesthesiologists, CEA = carcinoembryonic antigen, m/d = moderately differentiated, MSI-H = microsatellite instability-high, MSI-L = microsatellite instability-low, MSS = indicates microsatellite stable, p/d = poorly differentiated, SRC = signet ring cell, TNM = tumor-node-metastasis, w/d = well differentiated.

\* Compared to MSS.

tumors (Fig. 1), but MSI-L was not significantly associated with DFS ( $P=0.121$ ) or OS ( $P=0.131$ ) by univariable analysis (Fig. 1) (Table 2). However, after adjusting for confounders via multivariable Cox regression, MSI-L was associated with poorer OS (hazard ratio [HR] = 1.354, 95% confidence interval [CI] 1.011–1.815,  $P=0.042$ ), as well as other factors (Table 3), although it was not an independent prognostic factor for DFS (HR = 0.704, 95% CI 0.462–1.074,  $P=0.104$ ). The C-index of each model was 0.676 for DFS and 0.805 for OS. The prognostic model for OS that included MSI had higher C-index (0.805) than the model without MSI (0.804).

Figure 2 shows the distribution of unstable microsatellite markers in MSI-L CRCs. The majority of the 200 MSI-L tumors (97.0%) showed instability at dinucleotide repeats (D5S346 13.5% [27/200], D17S250 34.5% [69/200], D2S123 49.0% [98/200]), compared with only 3.0% which

displayed instability at a mononucleotide repeat (BAT26 1.0% [2/200], BAT25 2.0% [4/200]).

## DISCUSSION

The analyses presented here constitute the largest study to date examining the clinicopathologic features and oncologic outcomes associated with MSI-L CRC. In terms of clinicopathologic features, sporadic CRCs with MSI-L were broadly similar to those with MSS, with the only exceptions being that of tumor location and tumor size. However, among oncologic outcomes, MSI-L was shown to be an independent predictor of lower OS.

MSI-H CRCs have been reported to demonstrate more frequent association with proximal location, poorly differentiated or mucinous histology, large size, favorable stage, and

**TABLE 2.** Univariable Analysis of the Prognostic Factors for 3-Year Disease-Free Survival (3Y DFS) and 5-Year Overall Survival (5Y OS)

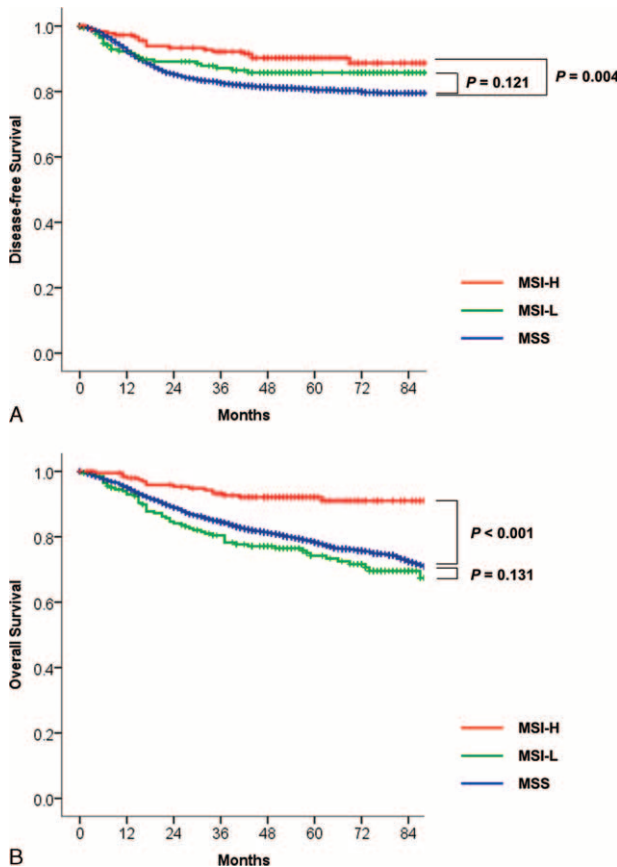
		No.	3Y DFS (%)	P	No.	5Y OS (%)	P
Sex	Male	1466	82.6	0.581	1707	77.5	0.490
	Female	941	83.5		1114	78.8	
Age (yr)	< 70	1818	83.3	0.153	2141	81.1	< 0.001
	≥ 70	589	81.7		680	67.4	
ASA score	1, 2	2205	83.2	0.158	2588	78.6	0.002
	3, 4	149	79.7		172	68.7	
Location	Colon	1305	85.6	<0.001	1563	77.8	0.447
	Rectum	1102	79.8		1258	78.3	
Preoperative CEA (ng/mL)	< 5	1772	86.3	<0.001	1930	84.3	< 0.001
	≥ 5	576	72.8		820	63.1	
Tumor size (cm)	< 5	1427	84.6	0.004	1578	82.0	< 0.001
	≥ 5	965	80.2		1226	72.5	
T stage	0, 1, 2	568	94.3	<0.001	578	94.1	< 0.001
	3, 4	1839	79.4		2243	73.8	
N stage	0	1330	90.9	<0.001	1406	89.3	< 0.001
	1, 2	1073	73.0		1411	66.7	
M stage	0				2407	86.2	< 0.001
	1				414	29.7	
Lymphatic invasion	Negative	1722	86.9	<0.001	1907	84.2	< 0.001
	Positive	660	71.9		887	63.9	
Venous invasion	Negative	1925	85.8	<0.001	2162	82.8	< 0.001
	Positive	457	70.0		632	61.0	
Perineural invasion	Negative	2165	84.3	<0.001	2476	79.7	< 0.001
	Positive	217	66.8		318	62.4	
Differentiation	w/d, m/d	2277	83.3	0.003	2645	79.0	< 0.001
	p/d, SRC, mucinous	114	73.4		160	58.5	
Adjuvant chemotherapy	Not performed	624	87.5	0.003	695	79.4	0.928
	Performed	1729	81.4		2036	78.7	
Microsatellite status	MSS	2234	82.6	0.121	2621	78.3	0.131
	MSI-L	173	87.2		200	74.2	

ASA = American Society of Anesthesiologists, CEA = carcinoembryonic antigen, DFS = indicates disease-free survival, m/d = moderately differentiated, MSI-L = microsatellite instability-low, MSS = microsatellite stable, OS = overall survival, p/d = poorly differentiated, SRC = signet signet ring cell, TNM = tumor-node-metastasis, w/d = well differentiated.

better prognosis,<sup>3</sup> which is consistent with our results. However, among published studies, comparisons between MSI-L and MSS CRCs remain controversial. Several studies examining CRCs with MSI-L were able to identify clinicopathologic features distinct from those of MSS;<sup>7–10</sup> however, these results could not be replicated in all studies.<sup>11–13</sup> This inherent lack of reproducibility between studies raises a fundamental question as to whether MSI-L should be regarded as a distinct genetic subgroup.<sup>5,6</sup> It is undeniable that some portion of MSI-L is misdiagnosed due to technical factors including PCR amplification errors, different microsatellite markers, and cut-off levels.<sup>21–24</sup> Alternatively, MSI-L may also occur as a result of random mutational events during neoplastic cell evolution.<sup>24</sup> However, these hypotheses are not sufficient to explain the specificity of MSI-L tumors.<sup>24</sup> Several lines of molecular evidence have been presented to suggest that MSI-L forms a biologically discrete subgroup,<sup>11,24–26</sup> including increased frequency of *KRAS* mutation, lower frequency of 5q loss of heterozygosity (LOH), distinct gene expression

profiles in cDNA microarrays, and others. Here, we showed that patients with MSI-L tumors had worse overall prognosis relative to those with MSS tumors despite broadly similar clinicopathologic features, consistent with the notion of MSI-L CRCs as a distinct biological entity. Future investigations using whole genome sequencing may be necessary to determine the genetic significance of MSI-L, similar to what was done for MSI-H tumors.<sup>27</sup>

Beyond clinical features, a number of studies have examined the impact of MSI-L on oncologic outcomes,<sup>9,10,12–15</sup> although the results have been inconsistent (Table 4). Some studies described poorer survival in patients with MSI-L tumors,<sup>12–15</sup> whereas others failed to observe any significant differences<sup>9</sup> or favorable oncologic outcomes<sup>10</sup> as compared to patients with MSS tumors. This apparent inconsistency between studies may be due to differences in study design, as each study used a different surrogate for oncologic outcome, including OS<sup>9,10,12–14</sup>, DFS,<sup>9</sup> cancer-specific survival,<sup>12</sup> and recurrent distant metastasis.<sup>15</sup> Furthermore, these



**FIGURE 1.** Kaplan–Meier survival curve depicting oncologic outcomes of sporadic CRC patients according to microsatellite status (MSI). (A) Disease-free survival. (B) Overall survival. CRC = colorectal cancer, MSI = microsatellite instability.

studies have a number of weaknesses, such as a small number of MSI-L tumors,<sup>9,10,12–15</sup> inclusion of MSI-H tumors when comparing survival outcome,<sup>10,13</sup> or inclusion only of patients with stage II or III tumors.<sup>12,14,15</sup> All of these differences make it difficult to draw solid conclusions. The present large multicenter study identified MSI-L as an independent prognostic factor for OS, consistent with previous reports of poor OS in patients with MSI-L tumors.<sup>13,14</sup>

It is noteworthy that MSI-L was an independent prognostic factor for OS, but not for DFS, despite the strong association generally seen between these 2 outcomes. Actually, a previous study also demonstrated reduced cancer-specific survival but no difference in overall survival of MSI-L compared with MSS.<sup>12</sup> One of the possible reasons is that significant but subtle impact of MSI-L on survival might bring about this discrepancy between survival surrogates. In this case, OS is considered the more definitive end point due to its obvious clinical importance and unambiguous nature,<sup>28</sup> whereas DFS is not always an appropriate surrogate of OS. Although DFS has been shown to exhibit a strong association with OS in adjuvant colon cancer studies,<sup>29</sup> any statistical association between DFS and OS would be weakened if survival after recurrence were

prolonged.<sup>28,30</sup> We are unable to explain why MSI-L was associated with OS but not DFS; however, in this instance we think that this lack of correlation does not weaken the prognostic significance of MSI-L, as the most significant association was seen with the more important survival endpoint.

Another statistical issue is that the prognostic effect of MSI-L only emerges when using the multivariable model. Frequently, a variable that is not significantly associated with an outcome by univariable analysis can be identified as an independent risk factor in multivariable analysis after adjusting for the effects of the other variables. Whereas such an observation on its own may not be sufficient to prove an association, we think that the comprehensive nature of this study and the strong statistical power provided by use of a large, unbiased, multicenter study population is sufficient to demonstrate MSI-L as a potential prognostic factor in sporadic CRCs.

Previous studies have presented evidence demonstrating a distinct genetic pathway underlying the development of MSI-L tumors. MSI-L phenotype was reported to be strongly associated with serrated or hyperplastic polyps with an alternative histological pathway.<sup>25,31</sup> Asaka et al<sup>32</sup> suggested that MSI-L have different timing and frequency of the *KRAS* mutation, which may influence the oncologic outcome. Some other studies demonstrated different levels of LOH at 1p32, 8p12–22, 5q, and 18q, all of which may be associated with prognosis.<sup>10,11,25</sup> Low expression of O<sup>6</sup>-methylguanine DNA methyltransferase due to promoter hypermethylation is associated with both *KRAS* and *p53* mutation, which may result in poor survival.<sup>10,14</sup> Recently, some authors surmised that hypoxia associated with loss of *MSH3* may be related to the recurrence of MSI-L tumors.<sup>15</sup> Further translational studies are needed to identify the distinct genetic pathways driving MSI-L oncogenesis, which may provide insights into potential adjuvant therapy responsiveness, along with targeted therapy of MSI-L CRCs.

In the present study, the majority of instability in MSI-L CRCs was associated with dinucleotide repeats. This observation is consistent with previous observations of dinucleotide marker instability in a majority of MSI-L CRCs.<sup>12,32</sup> However, the classification of microsatellite status is, to some degree, dependent on the markers used for detection.<sup>33</sup> Here, we used the most widely used 5 markers recommended by the NCI, although other many studies have been performed to improve the microsatellite panel for the detection of MMR defects.<sup>4,34,35</sup> The revised Bethesda guidelines recommended the use of 5 mononucleotide repeats for the detection of MSI-H tumors due to the high sensitivity of this assay,<sup>2,34</sup> and a simplified mononucleotide panel (BAT-25 and BAT-26) is still used for convenience. However, using only mononucleotide markers would miss possible MSI-L tumors, as they are insensitive for detecting MSI-L cancers.<sup>24,36</sup> On the other hand, the revised Bethesda guidelines also recommend that a secondary mononucleotide repeat, such as BAT40, should be tested if only dinucleotide repeats are mutated. BAT40, which has been shown to be mutated in >75% of MSI-H patients,<sup>12</sup> was not tested in this study, raising the possibility that some MSI-H tumors may have been erroneously classified as MSI-L in this study due to the panel utilized. A comprehensive microsatellite panel which includes dinucleotide markers and is sensitive for both MSI-H and MSI-L tumors is warranted for the accurate diagnosis on CRC patients.<sup>24</sup>

Interestingly, MSI-H and MSI-L tumors accounted for only 6.6% of all colorectal cancers respectively, which appears lower than that reported previously from Western countries (15–25%).<sup>20</sup> However, the frequencies of MSI-H and MSI-L

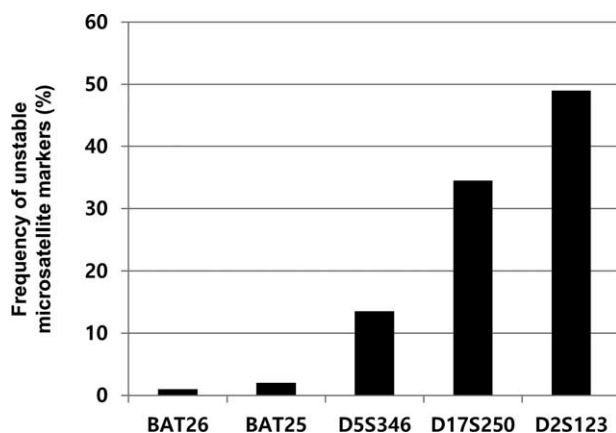
**TABLE 3.** Multivariable Analysis of the Prognostic Factors for Disease-Free Survival and Overall Survival

	Hazard Ratio (95% CI)	P
Disease-free survival		
Location (rectum vs colon)	1.527 (1.257–1.854)	< 0.001
Preoperative CEA ( $\geq 5$ ng/mL vs < 5 ng/mL)	1.591 (1.298–1.950)	< 0.001
Tumor size ( $\geq 5$ cm vs < 5 cm)	0.984 (0.806–1.200)	0.870
T stage (3, 4 vs 0, 1, 2)	2.145 (1.509–3.048)	< 0.001
N stage (1, 2 vs 0)	2.196 (1.748–2.759)	< 0.001
Lymphatic invasion (positive vs negative)	1.101 (0.822–1.476)	0.518
Venous invasion (positive vs negative)	1.335 (0.991–1.799)	0.057
Perineural invasion (positive vs negative)	1.494 (1.136–1.967)	0.004
Differentiation (p/d, SRC, mucinous vs w/d, m/d)	1.346 (0.918–1.972)	0.128
Adjuvant chemotherapy (performed vs not performed)	1.035 (0.810–1.322)	0.784
Microsatellite status (MSI-L vs MSS)	0.704 (0.462–1.074)	0.104
Overall survival		
Age ( $\geq 70$ yr vs < 70 yr)	2.101 (1.763–2.505)	< 0.001
ASA (3, 4 vs 1, 2)	1.449 (1.063–1.974)	0.019
Preoperative CEA ( $\geq 5$ ng/mL vs < 5 ng/mL)	1.476 (1.241–1.755)	< 0.001
Tumor size ( $\geq 5$ cm vs < 5 cm)	1.030 (0.869–1.222)	0.730
T stage (3, 4 vs 0, 1, 2)	1.967 (1.371–2.823)	< 0.001
N stage (1, 2 vs 0)	1.541 (1.253–1.895)	< 0.001
M stage (1 vs 0)	5.472 (4.547–6.586)	< 0.001
Lymphatic invasion (positive vs negative)	1.420 (1.122–1.798)	0.004
Venous invasion (positive vs negative)	1.204 (0.956–1.517)	0.114
Perineural invasion (positive vs negative)	1.083 (0.866–1.355)	0.484
Differentiation (p/d, SRC, mucinous vs w/d, m/d)	1.534 (1.172–2.010)	0.002
Microsatellite status (MSI-L vs MSS)	1.354 (1.011–1.815)	0.042

Variables with  $P < 0.15$  on univariable survival analysis were entered into each model.

ASA = American Society of Anesthesiologist, CEA = carcinoembryonic antigen, CI = indicates confidence interval, m/d = moderately differentiated, MSI-L = microsatellite instability-low, MSS = microsatellite stable, p/d = poorly differentiated, SRC = signet ring cell, TNM = tumor-node-metastasis, w/d = well differentiated.

were not largely different among the 3 institutions (MSI-H, 3.4–7.4%; MSI-L, 4.1–7.2%). In the previous study, we suggested a possible explanation that ethnic differences in the molecular characteristics of colorectal carcinogenesis could



**FIGURE 2.** Distribution of unstable microsatellite markers in MSI-L colorectal cancers. The most frequent unstable marker of MSI in our MSI-L tumors was D2S123 (49%). MSI = microsatellite instability, MSI-L = microsatellite instability-low.

affect the MSI status.<sup>20</sup> This large multicenter study confirmed low frequencies of MSI-H and MSI-L in Korean patients with colorectal cancer.

The present study has several limitations. First, as described above, the classification of microsatellite status is limited by the number of markers used, which may have resulted in misclassifications. Second, we did not review individual chemotherapy regimens in detail. Although the proportion of adjuvant chemotherapy was not different between MSI-L and MSS groups, detailed regimens, including target therapy, could have affected oncologic outcomes. Lastly, because of the retrospective study design, we could not evaluate mutations of *BRAF* and *KRAS*. Nevertheless, the great strength of our study is that we used large, consecutive, and multicenter study populations to reduced selection bias, and showed MSI-L to be a poor prognostic factor for all stage sporadic CRCs. Further translational research should focus on the genetic mechanisms underlying MSI-L tumorigenesis using clinically discrete groups in terms of oncologic outcome. These efforts would be the most likely to bring about a positive impact on the diagnosis and management of CRCs.

In conclusion, we identified MSI-L as an independent prognostic factor for OS in sporadic CRCs despite clinicopathologic similarities between MSI-L and MSS tumors. Further clinical and translational studies are needed to investigate the significance of MSI-L in the genesis and prognosis of CRCs.

**TABLE 4.** Clinicopathologic Features and Oncologic Outcomes of Patients With Microsatellite Instability-Low (MSI-L) Colorectal Cancers: Comparison of the Present Study With Previous Studies

First Author, Publication Year	Country	No. of Patients	MSI-L, n (%)	Control Group	Microsatellite Markers	Clinicopathologic Features	Oncologic Outcomes
<i>Present study</i>	Korea	3019	200 (6.6)	MSS	Bethesda panel*	Right sided, larger size	Poorer (OS)
Wright, 2005 <sup>12</sup>	Australia	255	33 (15.8)	MSS	11 markers <sup>†</sup>	No difference	Poorer (CSS)
Kohonen-Corish, 2005 <sup>14</sup>	Australia	183	51 (27.9)	MSS	Bethesda panel* + MYCL1	—	Poorer (OS)
Kim, 2009 <sup>9</sup>	Korea	657	30 (4.6)	MSS, MSI-H	Bethesda panel*	Poor histology, larger size, early stage, mucinous carcinoma	No difference (OS, DFS)
Azzoni, 2011 <sup>10</sup>	Italy	184	22 (12.0)	MSS, MSI-H	7 markers <sup>‡</sup>	No difference	Better (OS)
Garcia, 2012 <sup>15</sup>	America	133	20 (15.0)	MSS	Bethesda panel* + D18S64, D18S69, 7 EMAST markers <sup>§</sup>	—	Poorer (distant metastasis)

CSS = cancer-specific survival, DFS = disease-free survival, EMAST = elevated microsatellite alterations at selected tetranucleotide repeats, MSI-H = microsatellite instability-high, MSI-L = indicates microsatellite instability-low, MSS = microsatellite stable, OS = overall survival.  
 \* BAT25, BAT26, D2S123, D5S346, D17S250.  
 † BAT25, BAT26, BAT40, D2S123, D5S346, D10S197, D17S250, D18S69, c-myb, L-myc.  
 ‡ D18S58, D18S61, BAT26, BAT50, D8S254, D4S2397, MYCL.  
 § MYCL1, D20S82, D20S85, L17835, D8S321, D9S242, D19S394.

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