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Mismatch repair status in sporadic colorectal cancer: Immunohistochemistry and microsatellite instability analyses

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Abstract

Background and Aim: The aim of the present study was to evaluate associations between mismatch repair (MMR) status and clinicopathological characteristics and prognosis using immunohistochemistry (IHC) and microsatellite instability (MSI) analyses in a prospective cohort of a large number of accumulated samples.

Methods: Tumor tissue samples obtained during curative surgery ($n = 2028$) were analyzed using both MLH1/MSH2 IHC and MSI assays. Clinicopathological parameters and survival outcomes were compared according to IHC and MSI results. The median follow-up period was 43 months (range: 1–85 months).

Results: IHC identified 207 tumor samples (10.2%) with a loss of either MLH1 or MSH2 expression. The MSI analysis identified 203 tumor samples (10%) with high-frequency MSI (MSI-H). Patients with MMR defects were younger, and had tumors characterized by right-colon predilection; large-size, infrequent lymph node metastasis; poorly-differentiated or mucinous histology, and synchronous adenomas ($P < 0.001$–0.008). Patients with MSI-H status had higher 4-year disease-free survival rates than patients with microsatellite stable status (90.8% *vs* 80.6%, $P = 0.001$). A multivariate analysis showed that MSI-H status was a good prognostic factor for recurrence (hazard ratio: 0.48, 95% confidence interval: 0.30–0.83, $P = 0.007$).

Conclusions: Patients with MMR defects had distinct clinicopathological characteristics, including a lower risk of recurrence. IHC and MSI analyses provided complementary information regarding specific clinicopathological parameters and prognosis.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer death worldwide.[1] CRC develops as a consequence of accumulated genetic changes that result in loss of tumor suppressor genes and the activation of oncogenes. In sporadic CRC, these mutations occur through at least two distinct pathways. Chromosomal instability is implicated in approximately 85% of sporadic CRC, and is associated with loss of heterozygosity at multiple tumor suppressor loci, in particular at 5q, 17p, and 18q, as originally reported by Fearon and Vogelstein.[2,3] The second form of genetic instability involves DNA mismatch repair (MMR) defects, which were originally identified in patients with hereditary, non-polyposis colorectal cancer (HNPCC).[4] Patients with HNPCC tend to carry multiple errors in repetitive DNA sequences throughout the genome, a phenomenon termed microsatellite instability (MSI). High-frequency MSI (MSI-H) is observed in approximately 15% of sporadic CRC.[5] Patients with MSI tumors have distinct clinicopathological characteristics, and this type of tumor is generally associated with longer survival in patients with sporadic CRC.[6,7]

MSI analysis and immunohistochemistry (IHC) of MLH1 and MSH2 are effective and widely-accepted methods of identifying MMR defects. MSI analysis is currently considered the gold standard for the assessment of MMR status. The availability of recently-improved molecular tools has enabled the examination of MSI using several candidate markers. MSI analysis is able to identify some MMR defects that cannot be detected by IHC.[8] IHC has a lower sensitivity than MSI analysis, and interpretation of its results might be rendered uncertain by focal or cytoplasmic staining.[9] However, IHC remains an inexpensive, first-line screening tool for the identification of MMR status, and occasionally detects MMR defects that might be missed by MSI analysis (e.g. *MSH6*).[10] The use of MSI analysis appears to

compensate, to some extent, for the limitations of IHC in identifying MMR defects.

Previous studies of CRC, utilizing IHC of MMR proteins and MSI analyses, have identified correlations between MMR status and distinct clinicopathological characteristics.[9–13] The prognostic and predictive roles of MMR status in sporadic CRC and their potential as chemotherapeutic targets remains controversial.[11,14–16] The aim of the present study was to evaluate MMR status using MLH1/MSH2 IHC and MSI analyses in a prospective cohort of more than 2000 patients with sporadic CRC. Associations between MMR status and clinicopathological characteristics and prognosis were assessed using the results of both IHC and MSI analyses.

Methods

Patients, tissue samples, and follow up

A total of 2334 primary CRC patients were prospectively enrolled. All patients underwent curative surgical resection between August 2003 and December 2007 at the Asan Medical Center (Seoul, Korea). Patients categorized as: (i) HNPCC, corresponding to Amsterdam criterion I or II ($n = 22$); (ii) familial adenomatous polyposis (FAP) or attenuated FAP ($n = 13$); (iii) synchronous ($n = 83$) or metachronous CRC ($n = 8$); or (iv) those with a history of preoperative radiochemotherapy ($n = 142$) were excluded. Thirty-eight patients were lost to follow up. Finally, 2028 sporadic CRC patients were analyzed. Tumor samples and samples of normal colonic mucosa excised from a site at least 5 cm away from the tumor border were obtained during surgery. The histology of all samples was assessed by two pathologists. Tumor site was defined as "right colon" for the cecum proximal to the splenic flexure, and "left colon" for the splenic flexure to the rectosigmoid colon. All patients provided written, informed consent, and the study was approved by the Institutional Review Board for Human Genetic and Genomic Research of the University of Ulsan (authorized by the Ministry of Health, Welfare, and Family Affairs, Seoul, Korea), in accordance with the Declaration of Helsinki.

Patients with stages II and III tumors ($n = 1757$) received adjuvant fluoropyrimidine-based chemotherapy. While 1629 (92.7%) patients received 5-fluorouracil (5-FU) plus leucovorin (FL) or capecitabine, the remainder received FL plus oxaliplatin or FL plus irinotecan. The eligibility criteria were: (i) a diagnosis of a histologically-confirmed colorectal adenocarcinoma; (ii) an Eastern Cooperative Oncology Group performance status of 0 or 1; and (iii) age < 75 years. High-risk stage II was defined as T4 lesion, poorly- or mucinous-differentiated histology, bowel obstruction, or lymphovascular invasion.[17,18] Among the 1082 patients with stage II tumors, 321 (29.7%) were high-risk stage II. Of the 770 patients (38%) with rectal cancer, 315 (41%) received postoperative radiation therapy. Patients were followed up postoperatively every 6 months for 2 years, and then annually for 3–5 years. Follow-up investigations included clinical examination, routine blood chemistry, serum carcinoembryonic antigen (CEA) screening, annual colonofiberscopy, chest radiography, and abdominopelvic and chest computed tomography. The median follow-up period was 43 months (range: 1–85 months).

MLH1 and MSH2 IHC

Paraffinized 5-μm-thick tissue sections were mounted onto slides. The sections were deparaffinized in xylene, rehydrated in graded alcohol, and washed in distilled water. Endogenous peroxidase activity was blocked by incubation with 3% H_2O_2. Antigen was retrieved in 10 mM citrate buffer (pH 6.0) by boiling the solution in a microwave for 15 min. Following treatment for 10 min with 10% normal goat serum to block non-specific protein binding, mouse monoclonal antibodies against hMLH1 (G168–15) and hMSH2 (G219–1129; PharMingen, San Diego, CA, USA) were added. Antigen–antibody reactions were visualized using the streptavidin–biotin method and a DAKO LSAB kit (DAKO, Carpinteria, CA, USA). Slides were counterstained with hematoxylin. Normal-matched tissue was used as an internal positive control. Distinct nuclear staining of more than 10% of all nuclei was interpreted as positive staining for hMLH1 and hMSH2. All hematoxylin–eosin and IHC results were confirmed by two pathologists.

MSI

The MSI status of tumor samples was determined using the five-marker Bethesda panel (*BAT25*, *BAT26*, *D5S346*, *D2S123*, and *D17S250*).[19] Polymerase chain reaction products were run on an ABI Prism 310 DNA sequencer (Perkin-Elmer Applied Biosystems Division, Foster City, CA, USA), and analyzed using GeneScan version 3.1 software (Perkin-Elmer, USA). Tumors were classified as: (i) MSI-H, two or more unstable markers; (ii) microsatellite stable (MSS), no unstable markers; or (iii) low-frequency MSI (MSI-L), one unstable marker. MSS and MSI-L tumors were grouped together within the category MSS for the analyses on the basis of their genetic implications.[19]

Statistical analysis

A cross-table analysis employing Pearson's χ^2-test or Fischer's exact test, as appropriate, was used to determine associations between MMR defects (identified by IHC and MSI analyses) and clinicopathological characteristics and recurrence. Potential variables were verified by multivariate analysis using binary logistic regression. Recurrence, disease-free survival (DFS), and overall survival (OS) were used to evaluate clinical outcome. Survival outcomes were compared using the Kaplan–Meier method with a log–rank test, and potential confounders were adjusted for using the Cox regression model. A P-value < 0.05 was considered statistically significant for all analyses, and all calculations were carried out using SPSS software (version 13.0; SPSS, Chicago, IL, USA).

Results

Association of MMR status with specific clinicopathological characteristics

Among the 2028 sporadic CRC samples, the MSI analysis identified MSI-H in 203 patients (10%), MSI-L in 60 patients (3%), and MSS in 1765 patients (87%). IHC identified a loss of MLH1 and/or MSH2 expression in a total of 207 patients (10.2%); 106

Table 1 Annual comparison between the results of MLH1/MSH2 immunohistchemistry (IHC) and microsatellite instability (MSI) analyses

	2003–2004 ($n = 487$)	2005 ($n = 493$)	2006 ($n = 524$)	2007 ($n = 524$)	Total
MLH1/MSH2 IHC+, MSS/MSI-H	403/25	418/26	464/17	461/7	1746/75
MLH1/MSH2 IHC–, MSS/MSI-H	30/29	8/41	16/27	25/31	79/128
Sensitivity (%)†	53.7	61.2	61.4	81.6	63.1
Specificity (%)‡	93.1	98.1	96.7	94.9	95.7

†Sensitivity and ‡specificity: MLH1/MSH2 IHC prediction of high-frequency MSI (MSI-H) status. Overall positive predictive value = 61.8%; negative predictive value = 95.9%; accuracy = 92.4%. MLH1/MSH2 IHC+, positive expression of both MLH1 and MSH2; MLH1/MSH2 IHC–, negative expression of either MLH1 or MSH2; MSS, microsatellite stable status.

patients (5.2%) were MLH1 negative and MSH2 positive, 85 patients (4.2%) were MLH1 positive and MSH2 negative, and 16 patients (0.8%) were negative for both MLH1 and MSH2 expression. The sensitivity of IHC in predicting MSI-H was 63.1%, and the specificity was 95.7%. Rather than remaining constant, the sensitivity of IHC increased annually during the study period (Table 1).

Patients with MSI-H and those with MSS had differing clinicopathological characteristics ($P < 0.001$; Table 2). Patients with MSI-H were younger (< 50 years), and their tumors were characterized by right-colon predilection; large-size (long diameter $\geq$ 6 cm), infrequent lymph node metastasis; poorly-differentiated or mucinous histology; and synchronous adenomas. All variables were verified by multivariate analysis ($P < 0.001$–0.008). With the exception of tumor invasion, associations between significant clinicopathological characteristics and MMR status did not differ between MSI and MLH1/MSH2 IHC assays. Among the MMR-negative patients, the mean age of patients negative for MSH2 expression (55.6 years) was younger than that of patients negative for MLH1 expression (59.6 years, $P = 0.022$).

Survival analyses according to MMR status and adjuvant chemotherapy

A total of 337 patients (16.6%) developed distant metastases and/or local recurrence, and 276 patients (13.6%) died. Patients with MSI-H had higher 4-year DFS rates ($P < 0.001$) and OS rates ($P = 0.016$) than those with MSS (Fig. 1). However, these differences were not evident when stages II and III patients were considered separately according to tumor location. Patients with negative findings of IHC had higher DFS rates ($P = 0.029$), but OS rates were not significantly different ($P = 0.140$). In the multivariate analysis, older age of onset ($\geq$ 50 years), advanced tumor stage, elevated preoperative serum CEA (> 6 ng/mL), lymphovascular invasion, and infiltrative tumors were associated with lower 4-year OS and DFS rates ($P < 0.001$–0.024). The recurrence rate was also significantly lower in patients with MSI-H than in patients with MSS (hazard ratio [HR]: 0.48, 95% confidence interval [CI]: 0.28–0.82, $P = 0.007$; Table 3).

To assess the effect of adjuvant chemotherapy, we analyzed 1102 high-risk stages II and III patients under 75 years of age, 1029 of whom (93.4%) had received adjuvant chemotherapy. A total of 905 patients (87.9%) received FL regimen or capecitabine therapy. Patients who received adjuvant chemotherapy had higher OS rates than patients who did not ($P < 0.001$). However, patients who received adjuvant chemotherapy were younger (57.5 years *vs* 63.6 years, $P < 0.001$). Overall, patients with MSI-H had higher 4-year DFS rates than those with MSS ($P = 0.008$); this was also true for the subpopulation of patients with colon cancer ($P = 0.039$), but not for those with rectal cancer (Table 4). When adjusted for stage and location, these differences were not significant. After stratifying on 5-FU-based chemotherapy, there were no significant survival differences between MSI-H and MSS patients with stage III tumors according to chemotherapeutic regimens (Table 4). IHC results revealed no significant differences in survival between MMR-positive and -negative patients in this group.

Discussion

Many CRC studies have compared defective MMR and intact MMR tumors, finding that defective MMR status is associated with distinct clinicopathological characteristics and better survival rates.[4,6,7,11,20,21] These findings might therefore suggest treatment options in addition to surgery, according to MMR status in CRC. MSI analysis and IHC of MLH1/MSH2 are widely-accepted methods for identifying MMR defects. In the present study, the sensitivity of IHC in detecting MSI-H was lower than that reported in previous studies.[11–13] False interpretation of IHC results might result from technical limitations or the occurrence of focal or cytoplasmic staining.[9,10] To find the cause of this decreased sensitivity, we reviewed all processes that influence the accuracy of IHC. One major technical problem encountered was the delayed fixation of samples during the early study period, which resulted in year-to-year differences in sensitivity.[22] There are several possible explanations for the finding of MSI-H status with positive MLH1 and MSH2 expression in 75 patients, including technical problem of IHC, the possibility of immunological reactivity to a functionally-inactive mutant protein, the presence of a heterozygous wild-type allele, or the presence of a *MSH6* mutation.[21] The accuracy of IHC might also have been limited by the fact that only two IHC antibodies were used. Nevertheless, in view of its high specificity and accuracy, IHC remains an important screening tool, and its limitations can be compensated for by the addition of MSI analyses.

The clinicopathological characteristics of patients with defective MMR were distinct, and included an association with younger age, right-colon predilection, infrequent lymph node involvement, poor differentiation or mucinous histology, and synchronous adenomas. These data confirm and extend the findings of other MSI and IHC studies of MMR status.[11,20,21,23] Younger age of onset was more closely associated with *MSH2* mutation than with *MLH1* mutation, in accordance with the findings of previous studies.[13,24] Interestingly, the proportion of MSH2-negative patients in the current study (49%) was higher than that in previous reports (15–

Table 2 Clinicopathological characteristics according to microsatellite instability (MSI) and immunohistchemistry (IHC) status

Characteristics	*n*	MSI status			MLH1/MSH2 expression		
		MSS *n* (%)	MSI-H *n* (%)	*P*-value	Positive *n* (%)	Negative *n* (%)	*P*-value
Sex							
Male	1210	1092 (90)	118 (10)	0.651	1099 (91)	111 (9)	0.073
Female	818	733 (90)	85 (10)		722 (88)	96 (12)	
Age, years (mean ± SD)	59.9 ± 11	60.2 ± 11	56.7 ± 12	< 0.001	60.1 ± 11	58.1 ± 12	0.017
< 50 years	382	320 (84)	62 (16)	< 0.001	328 (86)	54 (14)	0.006
≥ 50 years	1646	1505 (91)	141 (9)		1493 (91)	153 (8)	
Tumor site							
Right colon	524	385 (74)	139 (26)	< 0.001	414 (79)	110 (21)	< 0.001
Left colon	734	690 (94)	44 (6)		678 (92)	56 (8)	
Rectum	770	750 (97)	20 (3)		729 (95)	41 (5)	
CEA							
< 6 ng/mL	1715	1538 (90)	177 (10)	0.306	1538 (90)	177 (10)	0.761
≥ 6 ng/mL	313	287 (92)	26 (8)		283 (90)	30 (10)	
Size							
< 6 cm	1250	1195 (96)	55 (4)	< 0.001	1166 (93)	84 (7)	< 0.001
≥ 6 cm	778	630 (81)	148 (19)		655 (84)	123 (16)	
pT							
1/2	130	121 (93)	9 (7)	0.289	124 (95)	6 (5)	0.025
3/4	1898	1704 (90)	194 (10)		1697 (89)	201 (11)	
pN							
0	1100	952 (87)	148 (13)	< 0.001	958 (87)	142 (13)	< 0.001
1/2	928	873 (94)	55 (6)		863 (93)	65 (7)	
pM							
0	1980	1779 (90)	201 (10)	0.225	1776 (90)	204 (10)	0.474
1	48	26 (96)	2 (4)		45 (94)	3 (6)	
Histology							
WD/MD	1815	1681 (93)	134 (7)	< 0.001	1674 (92)	141 (8)	< 0.001
PD/MUC	213	144 (68)	69 (32)		147 (69)	66 (31)	
Growth type							
Expanding	1804	1617 (90)	187 (10)	0.156	1616 (90)	188 (10)	0.414
Infiltrative	224	208 (93)	16 (7)		205 (92)	19 (8)	
Lymphovascular invasion							
Yes	500	1380 (90)	148 (10)	0.392	1376 (90)	152 (10)	0.497
No	1528	445 (89)	55 (11)		445 (89)	55 (11)	
Synchronous adenoma							
Yes	650	609 (94)	41 (6)	< 0.001	603 (93)	47 (7)	0.002
No	1378	1216 (88)	162 (12)		1218 (88)	160 (12)	

CEA, carcinoembryonic antigen; left colon, location is from splenic flexure to rectosigmoid colon; MD, moderately differentiated; MSI-H, high-frequency MSI; MSS, microsatellite stable status; MUC, mucinous; PD, poorly differentiated; pM, pathologic metastatic stage; pN, pathologic nodal stage; pT, pathologic tumor stage; right colon, location is proximal to splenic flexure; WD, well differentiated.

20%).[25] One possible reason for this difference is that we used strict criteria to exclude HNPCC, a hereditary CRC, that does not fulfill Amsterdam criteria, might be involved to this study. An alternative possibility is racial differences found between Asia and the West in the frequency of MSH2 positivity.

Defective MMR was associated with larger and more advanced tumors, in accordance with the findings of several previous studies.[6,20,23,26] The MMR system has been implicated in signaling events that activate cell-cycle checkpoint or apoptosis, an observation that might partially explain the occurrence of overproliferation in tumors with defective MMR.[27] The extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signal-transduction pathway might also contribute to tumor growth through the upregulation of anti-apoptotic activity. This signaling pathway, which is downstream of RAS/RAF, is thought to be activated by defective MMR. There is increasing evidence that the activation of the ERK/MAPK pathway is involved in the differentiation, proliferation, and progression of CRC.[28] The clinicopathological characteristics of defective MMR tumors contrast with those of tumors resulting from chromosomal instability, such as those associated with FAP, which show left-colon predilection, pathogenic *APC*, *KRAS*, and *TP53* mutations, and aggressive behavior.[5,29]

The present study demonstrated that MSI-H status is a good prognostic factor only for recurrence, not for OS. The absence of a good prognostic value for OS might be attributable in part to a

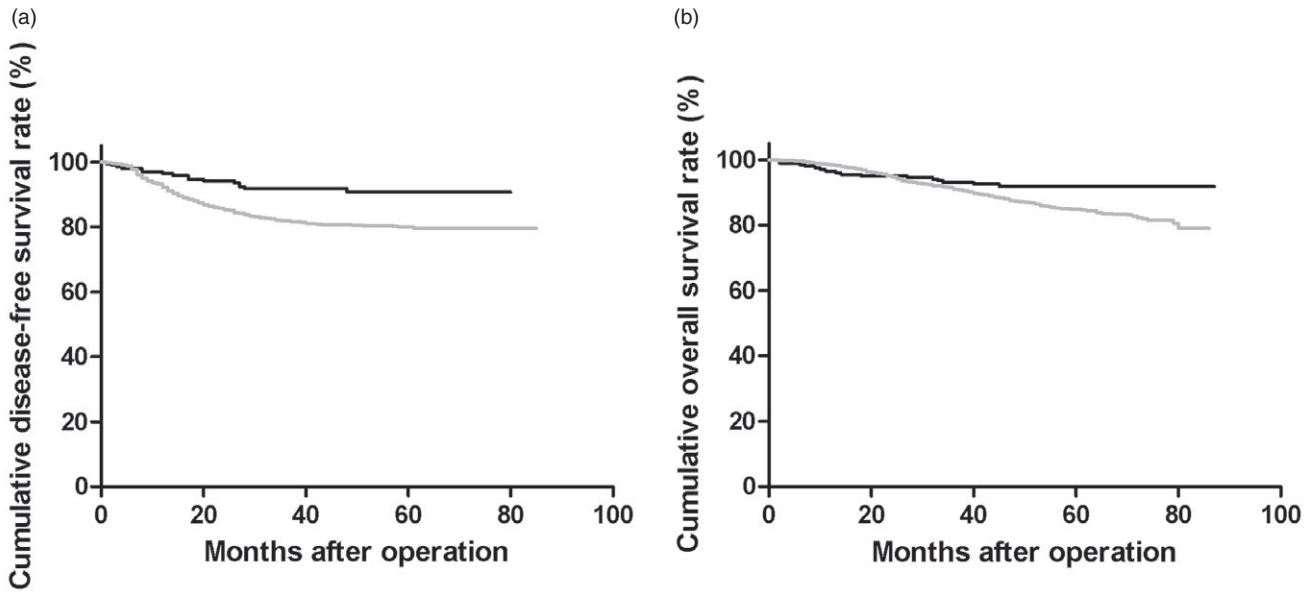

Figure 1 Disease-free survival (a) and overall survival (b) periods according to microsatellite instability (MSI) status. —, high-frequency MSI; —·—, microsatellite stable status.

Table 3 Multivariate analysis of disease-free survival (DFS) and overall survival (OS)

Parameters	DFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age, ≥ 50 years *vs* < 50 years	1.41 (1.05–1.89)	0.021	1.67 (1.17–2.36)	0.004
Sex, male *vs* female	0.89 (0.71–1.11)	0.292	0.96 (0.76–1.23)	0.762
Location, colon *vs* rectum	1.08 (0.86–1.34)	0.510	1.04 (0.82–1.33)	0.738
MSI status, MSI-H *vs* MSS	0.48 (0.28–0.82)	0.007	0.65 (0.39–1.10)	0.111
CEA, ≥ 6 ng/mL *vs* < 6 ng/mL	1.53 (1.18–1.97)	0.001	1.39 (1.04–1.84)	0.024
Growth type, infiltrative *vs* expanding	1.77 (1.35–2.34)	< 0.001	1.82 (1.34–2.48)	< 0.001
Lymph node metastasis, yes *vs* no	2.36 (1.83–3.05)	< 0.001	2.48 (1.87–3.29)	< 0.001
Distant metastasis, yes *vs* no	4.18 (2.85–6.15)	< 0.001	2.80 (1.81–4.33)	< 0.001
Lymphovascular invasion, yes *vs* no	1.98 (1.58–2.48)	< 0.001	2.15 (1.67–2.76)	< 0.001
Histology, PD/MUC *vs* WD/MD	1.26 (0.89–1.80)	0.194	1.60 (1.11–2.30)	0.012
Adjuvant chemotherapy, no *vs* yes	1.07 (0.73–1.59)	0.726	2.79 (2.04–3.80)	< 0.001

Hazard ratios (HR) and *P*-values were calculated by Cox proportional regression. CEA, carcinoembryonic antigen; CI, confidence interval; MD, moderately differentiated; MSI-H, high-frequency microsatellite instability; MSS, microsatellite stable status; MUC, mucinous; PD, poorly differentiated; WD, well differentiated.

relatively short follow-up period. A number of studies have investigated the prognostic significance of MSI-H in CRC.[4,6,11] A meta-analysis of 32 studies, which included a total of 7642 patients, estimated a combined HR for OS in association with MSI-H of 0.65 (95% CI: 0.59–0.71).[7] The precise mechanism underlying the correlation between MMR defects and a favorable prognosis is unclear. However, proposed causes of the attenuated tumor behavior observed in defective MMR tumors include intense lymphocytic infiltration, an increased rate of apoptosis, and the infrequent occurrence of allelic loss or mutations of *TP53, DCC*, and *KRAS*.[30] Unlike MSI status, IHC status was not an independent prognostic factor for recurrence, probably due to the low sensitivity of IHC.

Over a median follow-up period of 43 months, patients receiving adjuvant chemotherapy showed better OS rates than those who did not. Adjuvant 5-FU chemotherapy has been shown to significantly improve OS and DFS in stage III and possibly high-risk stage II patients.[18] In this latter study, nodal status, T stage, and grade were independent prognostic factors for both DFS and OS, whereas age was only a significant prognostic factor for OS. In the present study, patients who did not receive adjuvant chemotherapy were older or had a poorer performance status than patients who received adjuvant chemotherapy. Since there was no difference in DFS, the improved OS of patients receiving adjuvant chemotherapy might reflect an age-associated sample selection bias rather than effects of chemotherapy.

Whether patients with MSI-H tumors respond to 5-FU-based adjuvant chemotherapy remains a matter of controversy. No prospective randomized studies have yet been performed to compare

Table 4 Survival analysis according to microsatellite instability (MSI) status in patients treated with adjuvant chemotherapy

Parameters	No. of patients (% of colon)	All		Colon		Rectum	
		DFS,%	OS,%	DFS,%	OS,%	DFS,%	OS,%
All patients, MSI-H/MSS	108(92)/921(57)	**88.4/77.1(2)**	92.6/87.2	**88.4/79.2(1)**	97.9/88.8	88.9/74.2	100/85.1
According to stage							
High-risk stage II, MSI-H/MSS	62(97)/211(70)	91.5/82.9	100/97.6	94.5/85.8	100/95.2	100/76.1	100/92.1
Stage III, MSI-H/MSS	46(85)/710(54)	84.1/75.4	82.6/85.1	83.9/76.7	79.5/86.3	85.7/73.8	100/83.7
According to chemo-regimens							
FL or capecitabine, MSI-H/MSS	93(90)/812(54)	**90.0/77.1(2)**	**95.7/87.3(2)**	**90.2/79.4(1)**	**95.2/89.3(1)**	89.9/74.5	100/85.0
High-risk stage II, MSI-H/MSS	59(97)/202(68)	91.2/82.2	100/94.0	91.0/84.9	100/95.0	100/76.1	100/92.1
Stage III, MSI-H/MSS†	34(79)/610(49)	87.6/75.4	88.2/85.0	88.4/76.8	85.2/86.5	85.7/74.1	100/83.6
FOLFOX or FOLFIRI, MSI-H/MSS‡	15(100)/109(86)	79.0/77.6	73.3/87.7	79.0/79.3	73.3/87.8	NA/66.7	NA/86.7

P-value symbol: 1, < 0.01–0.05; 2, < 0.001–0.01; others not significant. There was no significant difference in survival between †stage III patients with 5-fluorouracil (5-FU) plus leucovorin (FL) or capecitabine and ‡FOLFOX (FL plus oxaliplatin) or FOLFIRI (FL plus irinotecan) according to MSI status. ‡FOLFOX or FOLFIRI: 112 patients (90%) were stage III. 5-FU-based chemotherapy in patients with high-risk stage II and stage III tumors, whose age was under 75 years. DFS, disease-free survival (4 years); MSI-H, high-frequency MSI; MSS, microsatellite stable status; NA, not available; OS, overall survival (4 years).

chemotherapy outcome in MSI-H and MSS patients.[31] There is therefore no consensus that chemotherapy should be withheld in MSI-H patients.[25] Early reports have indicated that 5-FU adjuvant chemotherapy is beneficial for patients with MSI-H tumors, but these studies were either small or non-randomized.[32,33] The results of *in vitro* studies showed that MMR-deficient cells are less sensitive to 5-FU than MMR-intact cells.[34,35] Recent, well-powered, case-control studies have demonstrated that patients with MSI-H tumors do not appear to benefit from 5-FU-based adjuvant chemotherapy.[36–38] However, in the current study, 5-FU-based adjuvant chemotherapy seemed to benefit patients with MSI-H colon cancers. Because our survival analysis of chemotherapy effects might have a possible sample-selection bias, the actual benefit of chemotherapy for patients with MSI-H should be confirmed by further prospective randomized studies.

This study had two major limitations. First, IHC and MSI analyses were performed more as a service test rather than as a lab investigation, which also contributed to annual differences in the sensitivity of IHC. Because of year-to-year variations in IHC results, it is difficult to determine whether the complementarity of MSI and IHC could be of use in clinical practice over a long period. Second, as we did not randomize chemotherapy, and grouping was weighted toward patients receiving adjuvant chemotherapy, the ability to evaluate the actual benefit of chemotherapy was limited. In an attempt to account for this problem, a large number of samples was collected.

In conclusion, patients with MMR defects had distinct clinicopathological characteristics, and a significantly lower risk of recurrence. The MSI analysis was very accurate and useful in determining MMR status and its association with specific clinicopathological parameters and outcomes. IHC was an efficient tool for evaluating MMR status as an initial screen, notwithstanding less sensitivity compared to MSI.

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