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Mismatch Repair Gene Expression as a Predictor of Tumor Responses in Patients With Rectal Cancer Treated With Preoperative Chemoradiation

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Abstract: This study evaluated the predictive and prognostic value of expression of mismatch repair (MMR) protein, including MLH1, MSH2, and MSH6 in rectal cancer patients with preoperative chemoradiotherapy.

MMR protein expression was measured by immunohistochemistry in both pretreatment biopsies (pre-) and pathologic specimens (post-) from 209 patients with locally advanced rectal cancer who underwent preoperative chemoradiotherapy and radical surgery. The patients were followed for a median period of 44 months.

A pathologic complete response (pCR) was observed in 30 patients (14.4%). The expression levels of MLH1, MSH2, and MSH6 were not significantly different between the pCR and non-pCR groups. A multivariate analysis revealed that tumor differentiation, postoperative chemotherapy, and pre-MSH6 expression were independent predictors of overall survival; ypN category and perineural invasion were independent predictors of disease-free survival. The pre-MSH6 expression was significantly associated with tumor budding and expression of all MMR proteins. On multivariate analysis, ypN category and post-MSH6 expression were independent predictors for local recurrence.

In our study, we observed the independent prognostic value of MSH6 expression in pretreatment tissue on overall survival and MSH6 expression after chemoradiation on local recurrence. Constitutive MSH6 expression before and after preoperative therapy may be a useful tool for prediction of oncologic outcome in locally advanced rectal cancer.

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Abbreviations: CEA = carcinoembryonic antigen, DFS = disease-free survival, HNPCC = hereditary nonpolyposis colorectal cancer, MMR = mismatch repair, OS = overall survival, pCR = pathologic complete response, TNM = tumor-node-metastasis.

INTRODUCTION

Pathologic variables related to tumor response to preoperative chemoradiotherapy remain the most important prognostic indicators for oncologic outcomes in patients with locally advanced rectal cancer.[1–3] Identification of additional prognostic factors continues to be an essential aim because patients at the same pathologic stage may reveal a different clinical course. Novel tissue-based prognostic indicators in radiated rectal cancer specimens are currently necessary for developing new molecular-level therapeutic approaches for rectal cancer.[4–6]

It is well known that DNA mismatch repair (MMR) gene abnormalities are associated with approximately 15% of all colorectal cancers.[7] About one-third or fewer of colorectal cancers with altered MMR gene function arise in patients with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, while the rest occur sporadically.[7] Several studies have recently investigated the predictive and prognostic roles of MMR genes in colorectal cancer; however the results are still unclear.[5,8–10]

To our knowledge, this is the first study on the possible predictive and prognostic roles of MMR genes in radiated rectal cancer. This study examined the expression of MLH1, MSH2, and MSH6 in radiated rectal cancer using immunohistochemistry in both pretreatment biopsies and pathologic specimens. The potential predictive and prognostic roles of MMR protein expression were also assessed.

METHODS

A total of 209 consecutive patients who underwent potentially curative resection after preoperative chemoradiation for locally advanced (radiological T3/T4 or N+) rectal cancer located within 10 cm of the anal verge were prospectively enrolled in this study. Patients were excluded if they had metastatic disease, recurrent disease, previous chemotherapy or pelvic radiotherapy, familial adenomatous polyposis, HNPCC, abnormal liver, kidney or bone marrow function. The protocol was approved by the scientific review and ethics committee at our institution and written informed consent was obtained from all the patients before the study.

All patients received preoperative chemoradiotherapy, which included pelvic radiotherapy of the whole pelvis at a dose of 40.4 to 50.4 Gy and concomitant chemotherapy based

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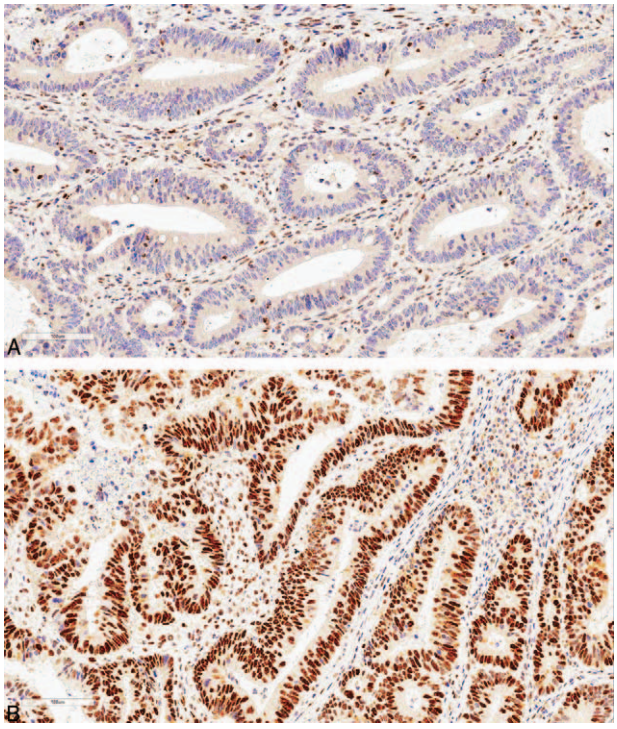

FIGURE 1. Immunohistochemical expression of pretreatment MSH6 expression. A, Low expression. B, High expression.

on a 5-fluorouracil or capecitabine regimen.[11,12] All patients underwent potentially curative radical surgery 6 to 8 weeks after preoperative chemoradiotherapy. Of 209 patients, 195 (92.9%) received postoperative adjuvant chemotherapy. The regimens were as follows: a 5-fluorouracil-based regimen (n = 160, 82.1%), a capecitabine (n = 12, 6.1%), an oxaliplatin-based regimen (n = 13, 6.7%), and other regimens (n = 10, 5.1%).

Immunohistochemistry

Tumor specimens from all 209 patients were obtained during the first biopsy procedure before the initiation of therapy (pre-). In pathologic specimens (post-), a total of 179 patients' specimens were prepared because 30 patients with pathological complete response after chemoradiotherapy were excluded. Immunohistochemical staining methods for DNA MMR genes (hMLH1, hMSH2, and hMSH6) were previously described.[13] Briefly, formalin-fixed, paraffin-embedded tissue block was used for tissue microarrays. Multiple sections (4-μm-thick) were cut from the tissue microarrays and prepared for subsequent immunostaining. Slides were deparaffinized and rehydrated using xylene and ethanol. The activation of endogenous peroxidase was blocked by 3% hydrogene peroxide for 30 minutes. The slides were stained with mouse monoclonal antibodies specific for each MMR protein: hMLH1 (clone G168-15, 1:200; BD Pharmingen, San Diego, CA), hMSH2 (clone FE11, 1:400; Calbiochem, La Jolla, CA), and hMSH6 (clone 44, 1:400; BD Transduction Laboratories, San Diego, CA). Negative controls using normal colonic epithelium adjacent to the tumor and lymphocytes were performed simultaneously. All the slides were evaluated in a blinded manner by 2 experienced gastrointestinal pathologists who had no clinicopathological information.

Statistical Analysis

Statistical evaluation was carried out using the statistical package SPSS for Windows (Version 14.0; SPSS Inc, Chicago, IL). MMR protein expression levels were classified as negative for <10 % nuclear staining, and positive for ≥10% nuclear staining.[9] For positive expression, MMR proteins were divided into low for <90% (median value of MMR expression) nuclear staining, and high for ≥90% nuclear staining (Figure 1). Analysis of clinicopathologic features among the groups was performed using the Student *t* test and the χ^2 test as appropriate. Survival was assessed by the Kaplan–Meier method and differences between curves were evaluated using the log-rank test. The variables with statistically significant *P* values on univariate analysis were entered into a multivariate analysis using the Cox model. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The subjects consisted of 136 men (65.0%), with a median age of 56 years (range, 27–81). Of the 209 patients, 181 (86.6%) underwent sphincter-saving operations. The median number of retrieved lymph nodes from each patient was 10 (range, 1–44).

TABLE 1. Baseline Demographics and Clinical Characteristics Between Pathologic Complete Response (CR) and Non-CR

	CR (n = 30)	Non-CR (n = 179)	*P*
Age, yr			0.382
<55	12 (12.1)	87 (87.9)	
≥55	18 (16.4)	92 (83.6)	
Sex			0.145
Male	16 (11.8)	120 (88.2)	
Female	14 (19.2)	59 (80.8)	
Distance from the anal verge, cm			0.612
≤5	22 (15.2)	123 (84.8)	
>5	8 (12.5)	56 (87.5)	
Pretreatment TNM stage			0.465
II	5 (19.2)	21 (80.8)	
III	25 (13.7)	158 (86.3)	
Histology			0.112
Adenocarcinoma	30 (14.9)	171 (85.1)	
Mucinous carcinoma	0	8 (100)	
Differentiation			0.335
Well + moderate	29 (14.9)	165 (85.1)	
Poor + mucinous	1 (6.7)	14 (93.3)	
Preoperative CEA, ng/mL			0.281
<5	18 (13.6)	114 (86.4)	
≥5	7 (11.7)	53 (88.3)	
Not available	5 (29.4)	12 (70.6)	
Pre-MLH1 expression			0.829
Negative to low	11 (15.1)	62 (84.9)	
High	19 (14.0)	117 (86.0)	
Pre-MSH2 expression			0.224
Negative to low	12 (11.4)	93 (88.6)	
High	18 (17.3)	86 (82.7)	
Pre-MSH6 expression			0.118
Negative to low	9 (10.0)	81 (90.0)	
High	21 (17.6)	98 (82.4)	

CEA = carcinoembryonic antigen.

TABLE 2. Univariate Analysis of the Prognostic Factors for 5-Yr Overall Survival (OS) and Disease-Free Survival (DFS) (n = 209)

	No.	5-Yr OS (%)	*P*	5-Yr DFS (%)	*P*
Age, yr			0.167		0.660
<55	99	92.1		70.9	
≥55	110	86.7		72.7	
Sex			0.431		0.287
Male	136	91.0		73.5	
Female	73	86.3		68.7	
Distance from the anal verge, cm			0.233		0.694
≤5	145	87.1		70.7	
>5	64	94.9		74.7	
Histology			0.066		0.894
Adenocarcinoma	201	89.8		71.8	
Mucinous carcinoma	8	72.9		71.4	
Differentiation			<0.001		0.174
Well + moderate	194	92.2		72.4	
Poor + mucinous	15	49.5		65.0	
Circumferential resection margin, mm			<0.001		0.103
<1	7	57.1		57.1	
≥1	202	90.3		72.4	
Operative method			0.061		0.122
Sphincter-saving	181	91.3		73.7	
Non-sphincter-saving	28	76.2		59.7	
T category			0.018		<0.001
T1 + T2	93	96.5		87.2	
T3 + T4	116	83.9		60.2	
N category			0.026		<0.001
Negative	132	94.2		88.4	
Positive	77	84.6		45.4	
Tumor regression grade			0.091		0.006
CR	30	100		96.3	
Non-CR	179	87.7		68.1	
Number of lymph nodes retrieved			0.040		0.715
<8	68	80.7		73.9	
≥8	141	94.2		71.1	
Lymphovascular invasion			0.014		<0.001
Negative	161	92.5		79.3	
Positive	48	76.0		45.4	
Perineural invasion			0.016		<0.001
Negative	192	90.4		75.2	
Positive	17	76.5		35.3	
Tumor budding			0.353		0.002
Negative	165	89.7		76.5	
Positive	44	88.4		54.3	
Preoperative CEA, ng/mL			0.309		0.595
<5	132	87.6		73.0	
≥5	60	87.7		67.1	
Not available	17	100		76.5	
Postoperative chemotherapy			0.005		0.859
No	16	73.1		73.4	
Yes	193	90.5		71.8	
Pre-MLH1 expression			0.225		0.943
Negative to low	73	86.2		71.0	
High	136	90.8		72.7	
Pre-MSH2 expression			0.237		0.014
Negative to low	105	86.3		63.7	
High	104	93.6		80.7	
Pre-MSH6 expression			0.029		0.692
Negative to low	90	83.8		73.4	
High	119	95.7		71.0	
Post-MLH1 expression			0.066		0.563
Negative to low	53	80.5		64.8	
High	126	91.0		69.9	
Post-MSH2 expression			0.155		0.789
Negative to low	96	84.9		67.3	
High	83	90.6		69.3	
Post-MSH6 expression			0.509		0.923
Negative to low	111	87.0		68.0	
High	68	86.6		69.8	

CEA = carcinoembryonic antigen.

Using the 7th UICC tumor-node-metastasis (TNM) staging system, 26 and 183 patients had clinical stage II and stage III tumors, respectively, and 30, 49, 51, 70, and 9 patients had pathological complete response (pCR), stage I, stage II, stage

TABLE 3. Multivariate Analysis of the Prognostic Factors for 5-Yr Overall Survival (OS) and Disease-Free Survival (DFS)

	Hazard Ratio (95% CI)	*P*
OS		
Differentiation	11.443 (3.218–40.695)	<0.001
Circumferential resection margin	0.295 (0.066–1.312)	0.109
ypT category	1.946 (0.447–8.464)	0.375
ypN category	1.147 (0.329–3.998)	0.830
No. of lymph node retrieved	0.431 (0.155–1.196)	0.106
Lymphovascular invasion	2.233 (0.688–7.252)	0.181
Perineural invasion	2.764 (0.743–10.282)	0.129
Postoperative chemotherapy	0.067 (0.018–0.260)	<0.001
Pre-MSH6 expression	0.254 (0.080–0.807)	0.020
DFS		
ypT category	1.488 (0.711–3.118)	0.292
ypN category	3.856 (1.914–7.767)	<0.001
Tumor regression grade	2.608 (0.330–20.611)	0.363
Lymphovascular invasion	1.375 (0.757–2.497)	0.295
Perineural invasion	2.154 (1.055–4.397)	0.035
Tumor budding	1.109 (0.593–2.073)	0.746
Pre-MSH2 expression	0.585 (0.330–1.036)	0.066

CI = confidence interval (95%).

TABLE 4. Clinicopathological Parameters According to Level of Pre-MSH6 Expression

	Low (n = 90)	High (n = 119)	*P*
Age, yr			0.918
<55	43 (47.8)	56 (47.1)	
≥55	47 (52.2)	63 (52.9)	
Sex			0.054
Male	52 (57.8)	84 (70.6)	
Female	38 (42.2)	35 (29.4)	
Distance from the anal verge, cm			0.297
≤5	59 (65.6)	86 (72.3)	
>5	31 (34.4)	33 (27.7)	
Histology			0.744
Adenocarcinoma	87 (96.7)	114 (95.8)	
Mucinous carcinoma	3 (3.3)	5 (4.2)	
Differentiation			0.770
Well + moderate	83 (92.2)	111 (93.3)	
Poor + mucinous	7 (7.8)	8 (6.7)	
T category			0.156
T1 + T2	35 (38.9)	58 (48.7)	
T3 + T4	55 (61.1)	61 (51.3)	
N category			0.964
Negative	57 (63.3)	75 (63.0)	
Positive	33 (36.7)	44 (37.0)	
Tumor regression grade			0.118
CR	9 (10.0)	21 (17.6)	
Non-CR	81 (90.0)	98 (82.4)	
Lymphovascular invasion			0.824
Negative	70 (77.8)	91 (76.5)	
Positive	20 (22.2)	28 (23.5)	
Perineural invasion			0.500
Negative	84 (93.3)	108 (90.8)	
Positive	6 (6.7)	11 (9.2)	
Tumor budding			<0.001
Negative	82 (91.1)	83 (69.7)	
Positive	8 (8.9)	36 (30.3)	
Preoperative CEA, ng/mL			0.857
<5	55 (61.1)	77 (64.7)	
≥5	27 (30.0)	33 (27.7)	
Not available	8 (8.9)	9 (7.6)	
Pre-MLH1 expression			0.005
Negative to low	41 (45.6)	32 (26.9)	
High	49 (54.4)	87 (73.1)	
Pre-MSH2 expression			<0.001
Negative to low	67 (74.4)	38 (31.9)	
High	23 (25.6)	81 (68.1)	
Post-MLH1 expression			<0.001
Negative to low	42 (46.7)	23 (19.3)	
High	48 (53.3)	96 (80.7)	
Post-MSH2 expression			<0.001
Negative to low	64 (71.1)	46 (38.7)	
High	26 (28.9)	73 (61.3)	
Post-MSH6 expression			<0.001
Negative to low	81 (90.0)	42 (35.3)	
High	9 (10.0)	77 (64.7)	

CEA = carcinoembryonic antigen; CR = complete response.

TABLE 5. Predictive Factors of Local Recurrence Identified Using Univariate and Multivariate Analyses

	Univariate Analysis	Multivariate Analysis		
	P	*P*	HR	95% CI
Age	0.916			
Sex	0.075			
Distance from the anal verge	0.965			
Histology	0.768			
Differentiation	0.069			
Circumferential resection margin	0.049	0.087	0.268	0.059–1.212
Operative method	0.082			
ypT category	0.079			
ypN category	<0.001	0.005	4.800	1.615–14.268
Tumor regression grade	0.072			
Number of lymph nodes retrieved	0.899			
Lymphovascular invasion	0.027	0.776	1.156	0.427–3.130
Perineural invasion	0.186			
Tumor budding	0.008	0.154	2.040	0.766–5.429
Preoperative CEA	0.836			
Postoperative chemotherapy	0.113			
Pre-MLH1 expression	0.270			
Pre-MSH2 expression	0.881			
Pre-MSH6 expression	0.147			
Post-MLH1 expression	0.903			
Post-MSH2 expression	0.294			
Post-MSH6 expression	0.049	0.035	2.727	1.075–6.916

CI = confidence interval.

III, and stage IV cancers, respectively. The expression levels of MSH2 and MSH6 between prechemoradiotherapy and postchemoradiotherapy were significantly different (86.9 ± 16.7 and 82.6 ± 22.6, $P = 0.017$; 79.7 ± 26.6 and 72.2 ± 31.1, $P < 0.001$, respectively), but MLH1 expression was similar between the 2 periods (90.4 ± 14.5 and 90.3 ± 17.8, $P = 0.953$), excluding 30 patients with pathological complete response. The pretreatment clinical characteristics of patients in the pCR and non-pCR groups are shown in Table 1; however, there was no statistical difference between the 2 groups including in expression of MMR proteins.

During the study follow-up (median of 44 months, range of 2–87 months), the factors associated with shorter overall survival in univariate analysis were differentiation, circumferential resection margin, ypT category, ypN category, number of lymph nodes retrieved, lymphovascular invasion, perineural invasion, postoperative chemotherapy, and prechemoradiotherapy MSH6 expression (Table 2). The factors associated with lower disease-free survival in univariate analysis were ypT category, ypN category, tumor regression grade, lymphovascular invasion, perineural invasion, tumor budding, and prechemoradiotherapy MSH2 expression (Table 2). No significant association was observed between overall survival and disease-free survival and postchemoradiotherapy MMR protein expression (Table 2). A multivariate analysis revealed that tumor differentiation ($P < 0.001$), postoperative chemotherapy ($P < 0.001$), and pre-MSH6 expression ($P = 0.020$) were independent predictors

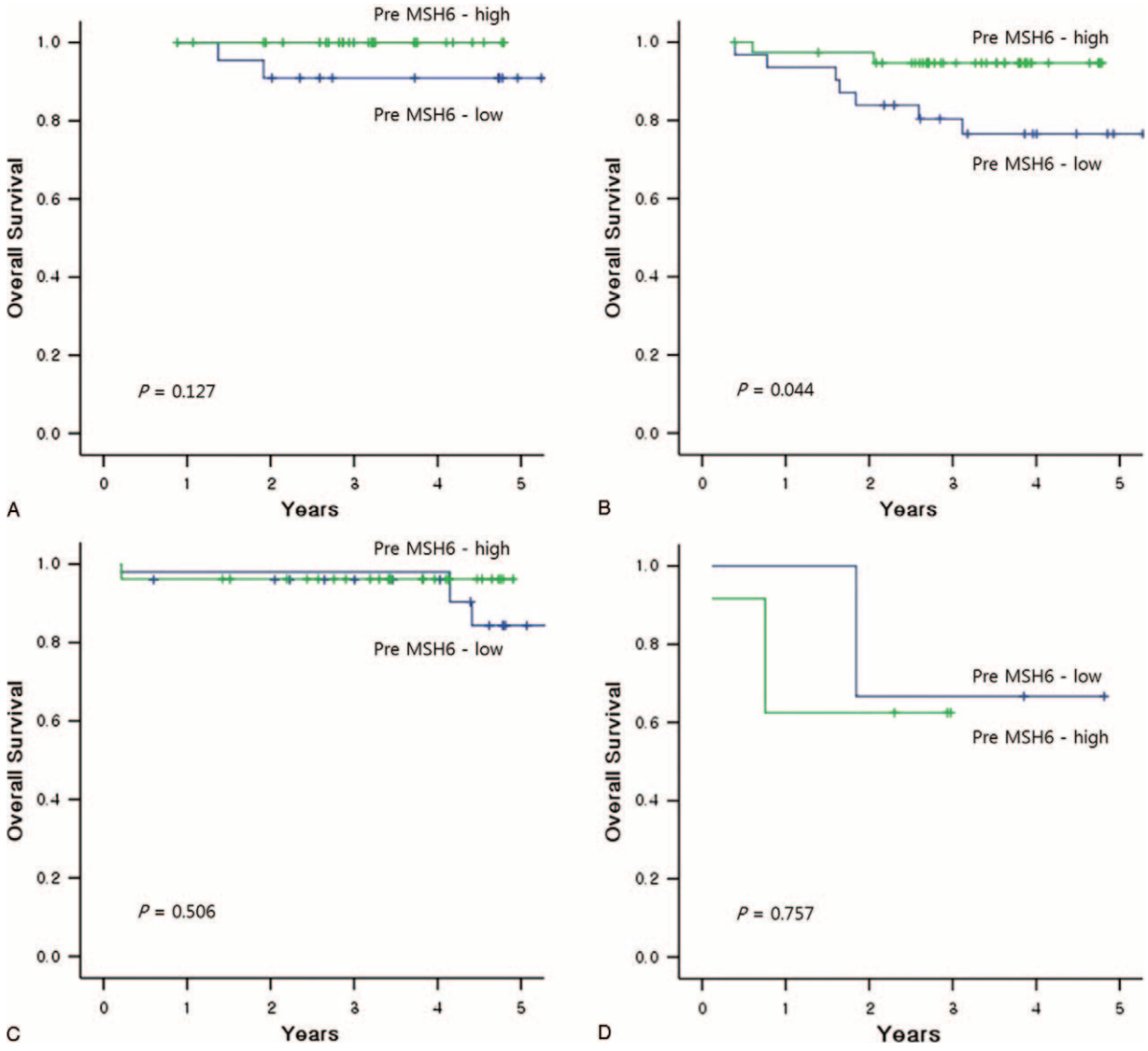

FIGURE 2. Overall survival according to the expression of pre-MSH6 and pathological tumor-node-metastasis stage (A) stage I, (B) stage II, (C) stage III, and (D) stage IV.

of overall survival; ypN category ($P < 0.001$) and perineural invasion ($P = 0.035$) were independent predictors of disease-free survival in patients with rectal cancer after preoperative chemoradiotherapy (Table 3).

The correlations between tumor prechemoradiotherapy-MSH6 expression and the clinicopathological features of rectal cancer are summarized in Table 4. The pre-MSH6 expression was significantly associated with tumor budding ($P < 0.001$) and expression of all MMR proteins (all $P < 0.05$). A multivariate analysis revealed that ypN category ($P = 0.005$) and postchemoradiotherapy MSH6 expression ($P = 0.035$) were independent predictors of local recurrence-free survival (Table 5). When the low and high pre-MSH6 expression groups were subdivided according to the pathological TNM stage, the 5-year overall survival rate differed between the 2 groups only for the patients with stage III cancer (Figure 2). For stage I, II, and IV cancers, the 5-year overall survival rate did not differ between the 2 groups.

DISCUSSION

We evaluated the possible predictive and prognostic roles of MMR genes before and after preoperative therapy in patients who received preoperative chemoradiation and curative surgery for locally advanced rectal cancer. In this study, the independent prognostic value of MSH6 expression in pretreatment tissue on overall survival and MSH6 expression after chemoradiation on local recurrence were observed. Our study is the first to explore that MMR protein expression could be an independent prognostic factor for long-term oncologic outcomes in radiated rectal cancer patients, although it did not have a predictive effect for radiation response.

Evaluation of the MMR protein expression in colorectal cancer is useful for the identification of patients at risk for Lynch syndrome; it may provide prognostic information as MSI has been shown to be correlated with better prognosis in patients with colorectal cancer.[14] Immunohistochemistry has been found to have a sensitivity of 83% and specificity of 89%

for patients with mutations in MLH1, MSH2, or MSH6, so it offers the advantage of directly assessing the MMR gene that is likely to be mutated.[15] MSH6-defective tumors are characterized by rectal location and less typical MMR-defective histology, including poor differentiation, mucinous component, peritumoral lymphocytes, and Crohn-like lymphocytic reactions.[16–18] In discordance with previous reports,[16–18] MSH6 expression was not correlated with poorly differentiated and mucinous carcinoma in our study; interestingly, it was significantly correlated with tumor budding. The present study is interesting because it highlights the prognostic role of MMR protein expression in patients with preoperative chemoradiation therapy for rectal cancer. MSH6 protein expression in pretreatment biopsy tissue is an independent predictor for overall survival and MSH6 expression in resection specimens is an independent predictor for local recurrence in our analysis. Moreover, a trend for a better disease-free survival rate was observed in tumors with high expression of MSH2 ($P = 0.014$ in univariate analysis and $P = 0.066$ in multivariate analysis). Our data are in line with the observation that high activity of the DNA-mismatch repair system is associated with sensitivity of cancer cells to DNA-damaging therapies.[19,20]

In the present study with a median follow-up period of 44 months, we observed that patients receiving postoperative chemotherapy showed better overall survival rates than those who did not. In colon cancer, fluorouracil-based adjuvant chemotherapy has been shown to significantly improve overall and disease-free survivals in stage III and high-risk stage II patients.[21] Although all patients who underwent preoperative chemoradiation were recommended for postoperative adjuvant chemotherapy irrespective of their pathologic stage, the prognostic role of postoperative chemotherapy in this cohort remains unclear.[22–24] In our study, patients who did not receive postoperative therapy were older or had poorer performance status than those who received it. Because no difference in disease-free survival between the 2 groups was found, the improved overall survival of patients receiving therapy may reflect an age-related sample selection bias rather than the effects of chemotherapeutic agents.

The present study had some limitations. The lack of standardization of immunohistochemical procedures, the limited numbers of patients, and the retrospective nature of this study represent major limitations for the clinical applicability of this information. Moreover, the dynamics of protein expression before and after treatment should be interpreted with caution with regard to the potential association with outcome. We believe that further investigations with larger sample sizes are necessary to assess the prognostic role of MMR gene expression in patients with rectal cancer after preoperative chemoradiation, and to validate their possible value as novel therapeutic targets. In conclusion, the immunohistochemical detection of expression of MMR proteins in both pretreatment biopsy tissue and resected specimens has a significant prognostic impact on survival and recurrence in patients with rectal cancer after preoperative chemoradiation. We believe that baseline and posttreatment expression of MMR proteins, especially MSH6, may enhance the prognostic stratification in rectal cancer patients with preoperative chemoradiotherapy.

REFERENCES

1. Garcia-Aguilar J, Hernandez de Anda E, Sirivongs P, et al. A pathologic complete response to preoperative chemoradiation is associated with lower local recurrence and improved survival in rectal cancer patients treated by mesorectal excision. *Dis Colon Rectum.* 2003;46:298–304.
2. Habr-Gama A, Perez RO, Nadalin W, et al. Long-term results of preoperative chemoradiation for distal rectal cancer correlation between final stage and survival. *J Gastrointest Surg.* 2005;9:90–99.
3. Huh JW, Jung EJ, Park YA, et al. Sphincter-preserving operations following preoperative chemoradiation: an alternative to abdominoperineal resection for lower rectal cancer? *World J Surg.* 2008;32:1116–1123.
4. Huh JW, Lee JH, Kim HR. Pretreatment expression of 13 molecular markers as a predictor of tumor responses after neoadjuvant chemoradiation in rectal cancer. *Ann Surg.* 2014;259:508–515.
5. Bertolini F, Bengala C, Losi L, et al. Prognostic and predictive value of baseline and posttreatment molecular marker expression in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys.* 2007;68:1455–1461.
6. Garcia-Aguilar J, Chen Z, Smith DD, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. *Ann Surg.* 2011;254:486–492.
7. Aaltonen LA, Peltomaki P, Leach FS, et al. Clues to the pathogenesis of familial colorectal cancer. *Science.* 1993;260:812–816.
8. Yoon YS, Yu CS, Kim TW, et al. Mismatch repair status in sporadic colorectal cancer: immunohistochemistry and microsatellite instability analyses. *J Gastroenterol Hepatol.* 2011;26:1733–1739.
9. Kim HR, Kim HC, Yun HR, et al. An alternative pathway in colorectal carcinogenesis based on the mismatch repair system and p53 expression in Korean patients with sporadic colorectal cancer. *Ann Surg Oncol.* 2013;20:4031–4040.
10. Garrity MM, Burgart LJ, Mahoney MR, et al., North Central Cancer Treatment G. Prognostic value of proliferation, apoptosis, defective DNA mismatch repair, and p53 overexpression in patients with resected Dukes' B2 or C colon cancer: a North Central Cancer Treatment Group Study. *J Clin Oncol.* 2004;22:1572–1582.
11. Park CH, Kim HC, Cho YB, et al. Predicting tumor response after preoperative chemoradiation using clinical parameters in rectal cancer. *World J Gastroenterol.* 2011;17:5310–5316.
12. Lee H, Park HC, Park W, et al. Negative impact of pretreatment anemia on local control after neoadjuvant chemoradiotherapy and surgery for rectal cancer. *Radiat Oncol J.* 2012;30:117–123.
13. Huh JW, Kim HC, Kim SH, et al. Mismatch repair system and p53 expression in patients with T1 and T2 colorectal cancer: predictive role of lymph node metastasis and survival. *J Surg Oncol.* 2014;109:848–852.
14. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol.* 2005;23:609–618.
15. Palomaki GE, McClain MR, Melillo S, et al. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet Med.* 2009;11:42–65.
16. Klarskov L, Holck S, Bernstein I, et al. Challenges in the identification of MSH6-associated colorectal cancer: rectal location, less typical histology, and a subset with retained mismatch repair function. *Am J Surg Pathol.* 2011;35:1391–1399.
17. Alexander J, Watanabe T, Wu TT, et al. Histopathological identification of colon cancer with microsatellite instability. *Am J Pathol.* 2001;158:527–535.
18. Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. *Gastroenterology.* 2004;127:17–25.

19. Lage H, Dietel M. Involvement of the DNA mismatch repair system in antineoplastic drug resistance. *J Cancer Res Clin Oncol.* 1999;125:156–165.
20. Fujieda S, Tanaka N, Sunaga H, et al. Expression of hMSH2 correlates with in vitro chemosensitivity to CDDP cytotoxicity in oral and oropharyngeal carcinoma. *Cancer Lett.* 1998;132:37–44.
21. Gill S, Loprinzi CL, Sargent DJ, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol.* 2004;22:1797–1806.
22. Huh JW, Kim HR. Postoperative chemotherapy after neoadjuvant chemoradiation and surgery for rectal cancer: is it essential for patients with ypT0-2N0? *J Surg Oncol.* 2009;100:387–391.
23. Bosset JF, Collette L, Calais G, et al., Trial ERG. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med.* 2006;355:1114–1123.
24. Das P, Skibber JM, Rodriguez-Bigas MA, et al. Clinical and pathologic predictors of locoregional recurrence, distant metastasis, and overall survival in patients treated with chemoradiation and mesorectal excision for rectal cancer. *Am J Clin Oncol.* 2006;29:219–224.