Poorly Differentiated Colorectal Cancers

Correlation of Microsatellite Instability With Clinicopathologic Features and Survival

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Key Words: Colorectal adenocarcinoma; Microsatellite instability; Prognosis

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ABSTRACT

Objectives: *To evaluate the association of microsatellite instability (MSI) with clinicopathologic features and oncologic outcomes in patients with poorly differentiated colorectal cancer (PD).*

Methods: *Study patients were divided into well-differentiated colorectal cancer (WD) and PD, which were compared according to histologic differentiation and MSI status.*

Results: *Among 1,941 patients, PD was more frequent among microsatellite-unstable tumors (23.6%) than among microsatellite-stable (MSS) tumors (4.2%,* $P < .001$*). Patients with PD had worse 4-year overall survival rates than patients with WD (78.6% vs 88.2%,* $P = 0.010$*). Compared with MSS-PD tumors, MSI-PD tumors were characterized by right-colon predilection, larger size, and infrequent lymph node metastasis (*$P < .001$ *to* $P = .007$*).*

Conclusions: *The clinicopathologic characteristics of PD were closely associated with those of MSI. The outcomes of MSI-PD tumors were better than those of MSS-PD tumors, but this finding did not reach statistical significance.*

Upon completion of this activity you will be able to:
- describe the clinicopathologic characteristics of poorly differentiated colorectal cancer (PD) compared with well-differentiated colorectal cancer.
- list clinicopathologic features that characterize microsatellite-unstable colorectal carcinoma.
- correlate microsatellite instability with clinicopathologic features and oncologic outcomes in patients with PD.

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Questions appear on p 440. Exam is located at www.ascp.org/ajcpcme.

The phenomenon of microsatellite instability (MSI), observed in approximately 15% of sporadic colorectal cancers (CRCs), is caused by DNA mismatch repair (MMR) defects.[1] Colorectal adenocarcinoma with MSI has a characteristic clinicopathologic profile: it typically forms on the right side, has an onset at a younger age and better prognosis, and frequently presents with poorly differentiated histology.[2,3] Histologically, poorly differentiated CRC (PD) exhibits glandular structure in 5% to 50% of tumors,[4] and these tumors represent 4.8% to 23.2% of all colorectal cancers.[5-7] PD has been linked to adverse prognoses in many studies.[6,8]

This gives rise to a paradoxical situation: CRCs with MSI have favorable prognoses but can often exhibit PD morphology, a well-documented adverse prognostic factor. A few studies have shown that patients with MSI-PD exhibit better survival than those with microsatellite-stable (MSS)–PD.

However, because of the small number of samples used in these studies, they did not demonstrate statistically significant differences.[9,10] The purpose of this study was to evaluate the association of MSI with clinicopathologic features and oncologic outcomes, specifically concentrating on the patients with PD in a large cohort.

Materials and Methods

Patients, Tissue Samples, and Follow-Up

A total of 2,028 patients with primary CRC with retrospective analyses, as described in our previous work,[11] were prospectively enrolled. All patients underwent curative surgical resection between August 2003 and December 2007 at the Asan Medical Center (Seoul, Korea). 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. All H&E and immunostained slides were evaluated by 2 pathologists at a 2-headed microscope. Evaluation for degree of differentiation was based on semiquantitative observation by pathologists. In cases with discrepant results, an agreement on a final decision was reached during the time of evaluation. Mucinous adenocarcinomas (n = 84) and signet ring cell carcinomas (n = 3) were excluded. Finally, 1,941 patients with sporadic CRC were analyzed. Tumor site was defined as "right colon" for sites proximal to the splenic flexure and "left colon" for sites from the splenic flexure to the rectosigmoid colon. Of the 1,941 patients enrolled, 1,791 (92.3%) patients received adjuvant fluoropyrimidine-based chemotherapy. Of the 746 patients with rectal cancer, 306 (41.0%) received postoperative radiation therapy. Patients were followed up postoperatively every 6 months for 2 years and then annually for 3 to 5 years. Follow-up investigations included clinical examination, routine blood chemistry, serum carcinoembryonic antigen screening, annual colonofiberscopy, chest radiography, and abdominopelvic and chest computed tomography. The median follow-up period was 41 months (range, 1-85 months).

All patients provided written informed consent, and the study protocol was approved by the Institutional Review Board of Asan Medical Center.

Histologic Features

According to the 2000 World Health Organization classification,[4] the percentage of a tumor that exhibits formation of gland-like structures can be used to define the tumor grade: well-differentiated (glandular structure >95%); moderately differentiated (glandular structure 50%-95%); poorly differentiated (glandular structure 5%-50%); and undifferentiated (glandular structure <5%). We categorized well- and moderately differentiated samples as well-differentiated CRC (WD) and poorly differentiated and undifferentiated samples as PD. Mucinous adenocarcinomas were defined if more than 50% of the lesion was composed of extracellular mucin that contained malignant epithelium as acinar structures, strips of cells, or single cells. Signet ring cell carcinomas were defined by the presence of more than 50% of tumor cells with prominent intracytoplasmic mucin. These variants of adenocarcinoma were excluded.

MLH1 and MSH2 Immunostaining

Paraffinized 5-μm-thick tissue sections were mounted onto slides. The sections were deparaffinized in xylene, rehydrated in graded alcohols, and washed in distilled water. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide. Antigen was retrieved in 10 mmol/L citrate buffer (pH 6.0) by boiling the solution in a microwave for 15 minutes. After treatment for 10 minutes with 10% normal goat serum to block nonspecific protein binding, mouse monoclonal antibodies against hMLH1 (G168-15) and hMSH2 (G219-1129, PharMingen, San Diego, CA) were added. Antigen-antibody reactions were visualized using the streptavidin-biotin method and a Dako LSAB kit (Dako, Carpinteria, CA). 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.

MSI Analysis

The MSI status of tumor samples was determined using the 5-marker Bethesda panel (*BAT25*, *BAT26*, *D5S346*, *D2S123*, and *D17S250*).[12] Polymerase chain reaction products were run on an ABI Prism 310 DNA Sequencer (Perkin-Elmer Applied Biosystems Division, Foster City, CA) and analyzed using GeneScan version 3.1 software (Perkin-Elmer). Tumors were classified as MSI when 2 or more unstable markers exhibited instability and as MSS when 0 or 1 marker exhibited instability.

Statistical Analysis

A cross-table analysis based on the Pearson χ^2 test or Fischer exact test, as appropriate, was used to determine associations between histologic types and clinicopathologic parameters. Potential variables were verified with 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, with adjustment for potential confounders using the Cox proportional hazards regression model. A P value less than .05 was considered statistically significant for all analyses, and all calculations were carried out using SPSS software (version 18.0, SPSS, Chicago, IL).

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Table 1
Clinicopathologic Characteristics According to Histologic Types

Variables	No.	WD (%)	PD (%)	*P* Value
Sex				
Male	1,162	1,094 (94)	68 (6)	.778
Female	779	731 (94)	48 (6)	
Mean (±SD) age, y	60.0±11.3	60.1±11.1	58.6±13.7	.255
<50	353	328 (93)	25 (7)	.333
≥50	1,588	1,497 (94)	91 (6)	
CEA, ng/mL				
<6	1,646	1,551 (94)	95 (6)	.369
≥6	295	274 (93)	21 (7)	
Mean (±SD) size, cm	5.4±11.3	5.3±1.9	6.9±2.6	<.001
<6	1,218	1,175 (96)	43 (4)	<.001
≥6	723	650 (90)	73 (10)	
Tumor site[a]				
Right colon	487	425 (87)	62 (13)	<.001
Left colon or rectum	1,454	1,400 (96)	54 (4)	
pT				
1/2	129	123 (95)	6 (5)	.511
3/4	1,812	1,702 (94)	110 (6)	
pN				
0	1,049	991 (94)	58 (6)	.367
1/2	892	834 (93)	58 (7)	
Growth type				
Expanding	1,729	1,626 (94)	103 (6)	.919
Infiltrative	212	199 (94)	13 (6)	
Lymphovascular invasion				
Yes	478	418 (87)	60 (13)	<.001
No	1463	1,407 (96)	56 (4)	
MSI status				
MSI	178	136 (76)	42 (24)	<.001
MSS	1,763	1,689 (96)	74 (4)	

CEA, carcinoembryonic antigen; MSI, microsatellite instability; MSS, microsatellite stability; PD, poorly differentiated colorectal adenocarcinoma; pN, pathologic nodal stage; pT, pathologic tumor stage; SD, standard deviation; WD, well- or moderately differentiated colorectal adenocarcinoma.
[a] Right colon indicates that the location is proximal to the splenic flexure; left colon, location is from the splenic flexure to the rectosigmoid colon.

Table 2
Clinicopathologic Characteristics of Patients With PD According to MSI status

Variables	No.	MSS (%)	MSI (%)	*P* Value
Sex				
Male	68	48 (71)	20 (29)	.070
Female	48	26 (54)	22 (46)	
Mean (±SD) age, y	58.6±13.7	58.0±13.7	59.6±13.9	.547
<50	25	18 (72)	7 (28)	.335
≥50	91	56 (61)	35 (39)	
CEA, ng/mL				
<6	95	58 (61)	37 (39)	.191
≥6	21	16 (76)	5 (24)	
Mean (±SD) size, cm	6.9±2.6	6.0±2.2	8.5±2.5	<.001
<6	43	37 (86)	6 (14)	<.001
≥6	73	37 (51)	36 (49)	
Tumor site[a]				
Right colon	62	27 (44)	35 (56)	<.001
Left colon or rectum	54	47 (87)	7 (13)	
pT				
1/2	6	6 (100)	0 (0)	.058
3/4	110	68 (62)	42 (38)	
pN				
0	58	30 (52)	28 (48)	.007
1/2	58	44 (76)	14 (24)	
Growth type				
Expanding	103	65 (63)	38 (37)	.665
Infiltrative	13	9 (69)	4 (31)	
Lymphovascular invasion				
Yes	60	39 (65)	21 (35)	.780
No	56	35 (63)	21 (37)	

CEA, carcinoembryonic antigen; MSI, microsatellite instability; MSS, microsatellite stability; PD, poorly differentiated colorectal adenocarcinoma; pN, pathologic nodal stage; pT, pathologic tumor stage; SD, standard deviation.
[a] Right colon indicates that the location is proximal to the splenic flexure; left colon, location is from the splenic flexure to the rectosigmoid colon.

Results

Association of PD With MSI Status and Clinicopathologic Characteristics

Based on histologic differentiation, 120 patients (6%) were categorized as WD, 1,705 (88%) as moderately differentiated, and 116 (6%) as PD. Adenocarcinomas with a mucinous component were found in 124 patients (6%). MSI analyses identified MSI in 178 patients (9%) and MSS in 1,763 patients (91%). Among 42 patients with PD and MSI, immunostaining revealed a loss of MLH1 and/or MSH2 expression in 32 patients (76%): 19 patients (45%) were MLH1-negative and MSH2-positive, 10 patients (24%) were MLH1-positive and MSH2-negative, and 3 patients (7%) were negative for both MLH1 and MSH2 expression.

Patients with PD had different clinicopathologic characteristics from those with WD **Table 1**. Patients with PD were younger (<50 years), and their tumors were characterized by right-colon predilection, large size, frequent lymphovascular invasion (LVI), and higher frequency of MSI ($P < .001$). Among patients with PD, the MSI group was characterized by right-colon predilection, larger size, and remarkably infrequent lymph node metastasis relative to the MSS group ($P < .001$-$.007$) **Table 2**. LVI and lymph node metastasis were closely correlated with each other in the PD group as well as the WD group ($P < .001$).

Survival Analyses According to Histologic Types and MSI Status

A total of 324 patients (16.7%) developed distant metastases and/or local recurrence, and 266 patients (13.7%) died. Although the 4-year DFS rates did not differ between patients with PD and those with WD (76.3% vs 81.9%, $P = .226$), patients with PD had worse 4-year OS rates than patients with WD (78.6% vs 88.2%, $P = .010$) **Figure 1**. Specifically for 116 patients with PD, patients with MSI-PD had higher 4-year DFS and OS rates than patients with MSS-PD, which did not reach statistical significance **Figure 2**. Among these patients, 103 patients (88.8%) received adjuvant chemotherapy. In terms of tumor location, patients with right-colon tumor site and PD had a higher DFS rate than patients with left-colon/rectum tumor site and PD (89.3% vs 61.9%, $P = .006$). Patients with MSI-WD showed higher

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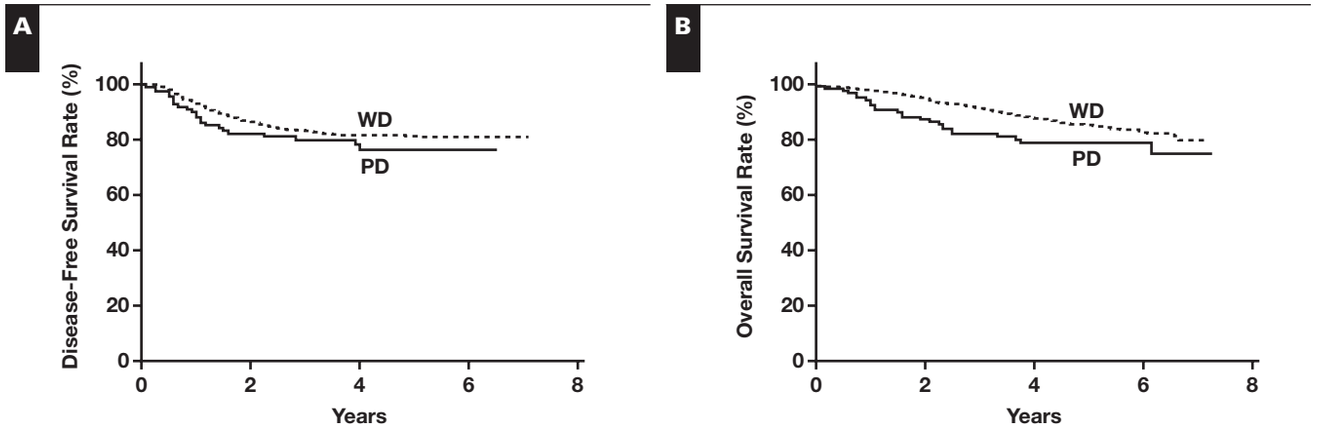

Figure 1 Survival curves according to histologic types. **A**, Disease-free survival rate ($P = .226$). **B**, Overall survival rate ($P = .010$). WD, well-differentiated colorectal adenocarcinoma; PD, poorly differentiated colorectal adenocarcinoma.

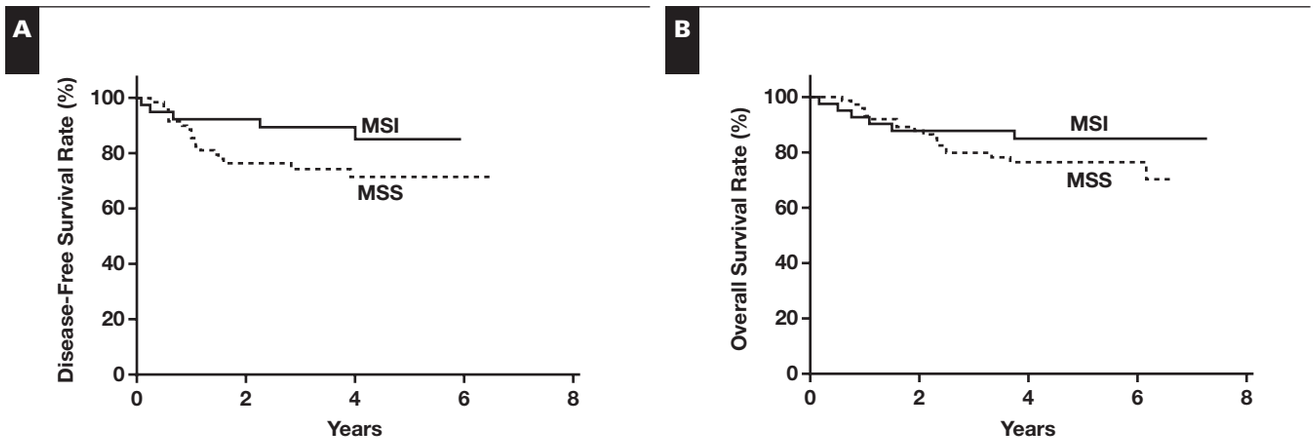

Figure 2 Survival curves of poorly differentiated adenocarcinomas according to microsatellite instability status. **A**, Disease-free survival rate ($P = .112$). **B**, Overall survival rate ($P = .393$). MSI, microsatellite instability; MSS, microsatellite stability.

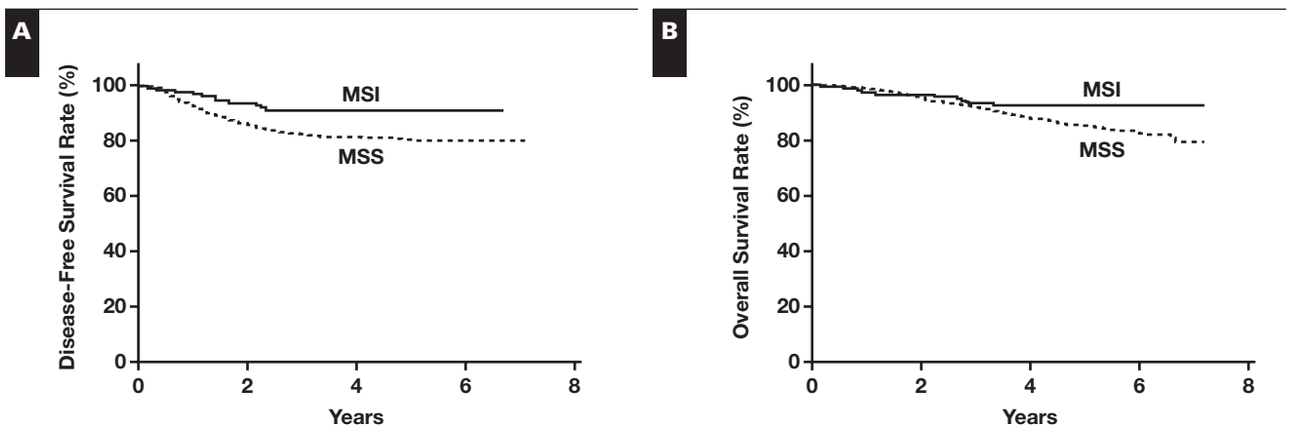

Figure 3 Survival curves of well-differentiated adenocarcinomas according to microsatellite instability status. **A**, Disease-free survival rate ($P = .008$). **B**, Overall survival rate ($P = .047$). MSI, microsatellite instability; MSS, microsatellite stability.

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DFS and OS rates than those with MSS-WD (P = .008 and P = .047, respectively) **Figure 3**.

Among patients with PD, univariate analyses revealed significant variables that influenced DFS and/or OS rates: age over 50 years, tumor localization in the left colon and rectum, MSS status, infiltrative tumor growth, lymph node metastasis, LVI, and absence of adjuvant chemotherapy (P < .001-.037). In multivariate analysis, MSI status was not a significant factor, but lymph node metastasis was identified as an independent prognostic factor for recurrence and survival in patients with PD **Table 3**.

Discussion

To date, few studies have been performed on the relationships between infrequent histologic types and MSI, perhaps because histologic subtypes such as PD, mucinous, and signet ring cell are rare; furthermore, MSI analyses are not routinely used in clinical practice. In addition to histopathologic examinations of CRC specimens, MSI analyses and immunostaining have routinely been added at our institution since 2003,[11] which made it possible to include a large number of samples from CRC patients with MSI and PD.

There is little controversy on the distinct clinicopathologic characteristics and poor prognosis of PD.[6,8] In contrast, MSI tumors generally have distinct characteristics and good prognoses.[11,13-15] Previous studies have indicated that PD is highly associated with MSI.[2,9,15,16] Among various characteristics of MSI and PD, we found that tumors with PD and MSI share 2 characteristics, namely right-colon predilection and large size, consistent with the results of the previous study.[10] The MMR system, which has defects that are involved in MSI, has been implicated in signaling events that activate the cell cycle checkpoint or apoptosis. This might partially explain the occurrence of overproliferation in tumors with defective MMR.[17] Mutations of *BRAF*, a serine-threonine kinase of the Raf family, are frequently observed in sporadic CRC with MSI.[18] Previous investigations of canonical molecular changes in colorectal tumorigenesis have also revealed that right-colon predilection and large tumor size are closely related with *BRAF* mutations.[19]

It is well known that LVI, a poor prognostic factor, appears to be correlated with PD, lymph node metastasis, and systemic metastasis.[20] Lymphatic spread of cancer cells is an important early event in metastasis to lymph nodes by carcinomas. Little is known about the mechanisms by which tumor cells gain entry into the lymphatic system. However, many researchers have suggested the greater importance of lymphangiogenesis relative to pre-existing peritumoral lymphatics.[21,22] The mitogen-activated protein kinase (MAPK) signal transduction pathway may partially contribute to tumor invasion and metastasis.[23] Mutations in MEK, which is downstream in the MAPK signaling pathway, are frequently observed in colorectal tumors with LVI.[19] In this study, the PD group exhibited more frequent LVIs than the WD group, although both groups had similar proportions of lymph node metastasis. Interestingly, we found that the MSI-PD group had a lower incidence of lymph node metastasis than the MSS-PD group, irrespective of the high prevalence of LVI in the PD group. Among various markers of MSI, allelic loss and MSI at CRC-specific loci were significantly associated with LVI and PD.[24] Although the mechanism is unknown, one possible explanation is that lymph node metastases through LVI possibly appear to be blocked by host immune responses, such as the Crohn-like lymphoid reaction and tumor-infiltrating lymphocytes, both of which are known as characteristic histologic features of MSI tumors.[16,25]

Kazama et al[10] reviewed 53 patients with PD and showed that MSI-PD tended to be associated with a better prognosis than MSS-PD without reaching statistical significance (P = .15). Although larger samples (n = 116) were used in our study, we also could not demonstrate definite statistical differences for survival (P = .112). However, because

Table 3
Multivariate Analysis of DFS and OS in Patients With PD[a]

	DFS		OS	
Parameters	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value**
Age, ≥50 y vs <50 y	2.29 (0.67-7.88)	.187	6.36 (0.83-48.92)	.076
Sex, female vs male	0.57 (0.24-1.37)	.208	0.68 (0.30-1.59)	.382
Location, left colon/rectum vs right colon	2.67 (0.88-8.15)	.084	2.43 (0.86-6.85)	.094
MSI status, MSS vs MSI	1.15 (0.35-3.70)	.821	0.76 (0.25-2.28)	.625
Growth type, infiltrative vs expanding	1.79 (0.64-4.98)	.266	1.64 (0.55-4.87)	.375
Lymph node metastasis, yes vs no	4.53 (1.41-14.58)	.011	4.84 (1.50-15.61)	.008
Lymphovascular invasion, yes vs no	1.71 (0.63-4.59)	.291	1.38 (0.54-3.53)	.505
Adjuvant chemotherapy, no vs yes	1.20 (0.15-9.63)	.864	10.95 (3.60-33.25)	<.001

CEA, carcinoembryonic antigen; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; MSI, microsatellite instability; MSS, both microsatellite stability; OS, overall survival; PD, poorly differentiated colorectal adenocarcinoma.
[a] HR and P value were calculated using Cox proportional hazards regression.

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there were a large number of patients in the MSS group, we could compare survival differences among the 4 groups categorized by MSI and PD status. In these survival analyses, we found that MSI tumors had better prognoses, irrespective of histologic type. A recent study reported that PD patients with proximal tumor locations had better survival than those with distal tumor locations.[6] The results of our study suggest that a high proportion of MSI explains the better survival of patients with PD in proximal locations.

Whether patients with MSI tumors respond to fluorouracil (5-FU)–based adjuvant chemotherapy remains a matter of controversy. Recent studies have demonstrated that patients with MSI tumors do not appear to benefit from 5-FU–based adjuvant chemotherapy.[14,26,27] However, in this study, we could not conclude that adjuvant chemotherapy is beneficial for MSI-PD patients because we did not randomize chemotherapy, and most patients received adjuvant chemotherapy. However, withholding adjuvant chemotherapy from these patients might be considered for these 2 reasons: (1) better survival outcomes of MSI-PD patients than those with MSS-WD and (2) negative aspects of chemotherapy, including adverse toxic events and costly expenses.

The current study included inevitable limitation due to the relatively small number of PD patients reaching statistical significance for the subgroup analysis. Conclusively, in addition to generally accepted knowledge, we found that MSI-PD exhibited distinct features compared with MSS-PD: right-colon predilection, larger tumor size, and infrequent lymph node metastasis. Although MSI status is not an independent predictor of survival, outcomes for MSI-PD tumors were better than those for MSS-PD. This indicates that MSI-PD is a clinically distinct and less aggressive subtype that exhibits remarkably infrequent lymph node metastasis.

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