Mismatch repair protein expression in patients with stage II and III sporadic colorectal cancer

LIHUA ZHAO

Department of Pathology, Beijing Civil Aviation Hospital, Beijing 100123, P.R. China

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Abstract. Colorectal cancer (CRC) may be classified according to the level of microsatellite instability exhibited by the tumor. The malignant transformation of normal colonic mucosae to carcinomas may be accelerated by the loss or inactivation of DNA mismatch repair (MMR) genes. The present study examined the expression of certain MMR proteins [namely, MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6) and PMS1 homolog 2 (PMS2)] in patients with stage II and III sporadic CRC. The association between the expression of these proteins, and the clinicopathological characteristics of patients with CRC and their tumors, was investigated. MMR protein expression was examined using immunohistochemistry. MLH1, MSH2, MSH6 and PMS2 protein expression was detected in 78.4% (120/153), 75.2% (115/153), 44.4% (68/153) and 79.7% (122/153) of stage II and III sporadic CRCs, respectively. Additionally, the expression of MLH1 and MSH6 was revealed to be significantly higher in stage III tumors when compared with stage II tumors ($P<0.05$). MLH1 and MSH6 negative tumors were larger, poorly differentiated and exhibited extraserosal invasion with infrequent lymph node metastasis ($P<0.05$). Patients with defects in MLH2 and PMS2 also had large tumors that exhibited extraserosal invasion and infrequent lymph node metastasis ($P<0.05$). No statistically significant associations were observed between MLH1, MSH2, MSH6 or PMS2 protein expression and patient age, sex, tumor localization or angiolymphatic invasion status ($P>0.05$). From the present study, it was concluded that MMR protein expression status evaluation may increase the efficiency of MMR testing and be useful in improving the individualized approach to patient monitoring and therapy.

Correspondence to: Dr Lihua Zhao, Department of Pathology, Beijing Civil Aviation Hospital, 1 Gao Jing Jia, Beijing 100123, P.R. China
E-mail: dzhaoli@163.com

Key words: mismatch repair, MutL homolog 1, MutS homolog 2, MutS homolog 6, PMS1 homolog 2, colorectal cancer, immunohistochemistry

Introduction

Colorectal cancer (CRC) is a major global public health concern (1). CRC is the second leading cause of cancer-associated mortality in developed countries, and the sixth to seventh leading cause of cancer-associated mortality in developing countries (2). Comprehensive studies investigating CRC tumorigenesis have made significant progress in previous decades, elucidating the molecular mechanisms governing CRC initiation, development and progression (3).

CRC arises through at least two distinct genetic pathways. One of these pathways involves chromosomal instability (CIN), and the other involves microsatellite instability (MSI) (4). Although the majority of sporadic CRCs exhibit CIN, MSI is only observed in ~15% of all CRCs, and the majority of CRCs with a high frequency of MSI are sporadic (5).

MSI indicates a defective DNA mismatch repair (MMR) system, which is why it is used as a molecular marker. MMR is one of the best understood molecular pathways involved in the pathogenesis of inherited and sporadic cancer. The MMR system serves a function in DNA homeostasis, and is involved in the repair of specific types of errors that occur during DNA replication in dividing somatic cells (6). MSI has been defined as variable microsatellite sequence length caused by insertions or deletions that occur within a tumor, but not in the corresponding normal tissue, and MSI tumors are characterized by an accelerated accumulation of mutations due to a defective MMR system (7).

When MMR is functioning normally, MuS homolog 2 (MSH2) and MutS homolog 6 (MSH6) bind to form the hMutS-α heterodimer, which recognizes and binds to mismatched base pairs, and recruits the MutL homolog 1 (MLH1)/PMS1 homolog 2 (PMS2) hMutL-α heterodimer to repair them. MSH2 and MLH1 are required to stabilize MSH6 and PMS2, respectively, and the loss of either MSH2 or MLH1 results in the degradation of its binding partner (8).

MMR-deficient tumors are typically identified via immunohistochemistry (IHC), which detects the loss of expression of one or more MMR proteins (including MLH1, MSH2, MSH6 and PMS2) and MSI testing to identify tumors with elevated MSI (MSI-H) (9). Reduced DNA repair protein expression is associated with MSI; therefore, numerous laboratories routinely use IHC with a panel of antibodies (specific to MLH1, PMS2, MSH2 and MSH6) as surrogate MSI markers (10). IHC has the advantage of being flexible, with

the ability to target any tissue type (including frozen and fixed samples, irrespective of the fixative used) and is performed by clinical pathology laboratories as a routine diagnostic test for MSI in patient tissues (11). Therefore, IHC detection of MMR proteins may be a faster, easier and cheaper method of CRC detection when compared with genetic analysis for MSI.

In the present study, IHC analysis of MLH1, MSH2, MSH6 and PMS2 protein expression was performed to evaluate the prognostic significance of MMR status in patients with a series of stage II and III sporadic CRC.

Materials and methods

Patients and tissues. Tissue samples from 153 patients with primary sporadic colorectal adenocarcinoma who underwent curative resection at the Civil Aviation General Hospital (Beijing, China) were obtained from a prospectively collected database between January 2004 and December 2013. The protocol for the present study was approved by the Institutional Review Board of the Civil Aviation General Hospital, and written informed consent was obtained from all patients in accordance with institutional regulations. All patients included in the present study were diagnosed with stage II or stage III sporadic colorectal adenocarcinoma. None of the patients received prior therapy, including radiotherapy or chemotherapy. All hematoxylin and eosin-stained sections were reviewed for tissue quality, and the highest quality section from each specimen was selected. Following an independent review by two pathologists, all tissues were histologically confirmed to be CRC. Clinical data, including sex and age, were obtained by chart review. Patients with incomplete data were not included in the present study.

Clinicopathological characteristics and histopathological review of tumor samples. The cancer-specific data evaluated for each patient included the tumor stage at presentation, tumor grade, specific histology, tumor location, angiolymphatic invasion status and the number of positive lymph nodes. The stage of each tumor was coded according to the AJCC 6th edition TNM staging system (12) (Table I), as follows: T1, tumor invades submucosa; T2, tumor invades muscularis propria; T3, tumor invades through the muscularis propria into the subserosa, or into nonperitonealized pericolic tissues; T4, tumor directly invades other organs or structures, or perforates visceral peritoneum; N0, no regional lymph node metastasis; N1, metastasis to one to three regional lymph nodes; N2, metastasis to four or more regional lymph nodes; M0, no distant metastasis; and M1, distant metastasis.

IHC analysis of MLH1, MSH2, MSH6 and PMS2 protein expression. IHC analysis of MLH1, MSH2, MSH6 and PMS2 protein expression was performed using a streptavidin-peroxidase IHC kit according to the manufacturer's protocol (Fuzhou Maixin Biotechnology Co., Ltd., Fuzhou, China). IHC staining was performed using 4-μm thick, paraffin-embedded CRC tissue sections mounted on positively charged slides. Tissue sections were deparaffinized with xylene and rehydrated through a gradient alcohol series. Sections were subsequently immersed in 10 mmol/l citrate buffer, pH 6.0, and heated in an autoclave at 121˚C for 5 min for non-enzymatic antigen retrieval. Next, to block the endogenous peroxidase activity, dual endogenous enzyme by incubating the sections in 3% hydrogen peroxide (H_2O_2; Fuzhou Maixin Biotech. Co., Ltd. Fuzhou, China) blocking solution was applied to tissue sections for 10 min at 37˚C. Primary mouse monoclonal antibodies against human MLH1 (MAB-0642), MSH2 (MAB-0291), MSH6 (MAB-0643) and PMS2 (MAB-0656; Fuzhou Maixin Biotechnology Co., Ltd.) were incubated with the tissue sections for 20 min at room temperature. Next, the slides were washed three times using 0.1 M phosphate-buffered saline (PBS; pH 7.4) and incubated with a biotinylated horseradish peroxidase conjugated Rabbit anti-mouse IgG secondary antibodies (working liquid; cat no., KIT9710) (Fuzhou Maixin Biotechnology Co., Ltd.) for 20 min at 37˚C. Slides were washed three times following incubation with the secondary antibody using 0.1 M PBS (pH 7.4). The IHC reaction was visualized by 5-10 min of diaminobenzidine chromogen staining and 5 min hematoxylin counterstaining at room temperature. Negative controls were performed by omission of the primary antibody. Fig. 1 revealed that MLH1, MSH2, MSH6 and PMS2 staining was localized to the cell nuclei. Tissues with positive nuclear staining in the tumor cells were scored as positive. Tissue specimens with a complete absence of nuclear staining were scored as negative. The sections were counterstained with hematoxylin for nuclear counterstaining and examined for the extent and intensity of nuclear staining in tumor cells under a 200x magnification light microscope by two independent observers in a blinded manner. Discordant scores were resolved by review and consensus agreement or use of a third observer. Non-neoplastic colonic tissues, stroma and infiltrating lymphocytes normally demonstrated positive nuclear staining; therefore, these tissues were used as internal positive controls.

Table I. Tumor classification.

Stage	T Stage	N Stage	M Stage
I	T1 or T2	N0	M0
IIa	T3	N0	M0
IIb	T4	N0	M0
IIIa	T1 or T2	N1	M0
IIIb	T3 or T4	N1	M0
IIIc	Any T	N2	M0
IIId	Any T	N3	M0
IIIc	Any T	N4	M0
IV	Any T	Any N	M1

T, tumor; N, lymph node; M, metastasis.

Statistical analysis. The Kruskal-Wallis test was applied (differentiation, tumor size, invasive depth and lymph node metastasis which are 3 categories). Cross-table analysis employing χ^2 test, as appropriate, was used to analyze associations between MMR defects (identified by IHC) and sex, ages, type, angiolymphatic invasion characteristics. Potential variables were verified by multivariate analysis using binary logistic regression. $P<0.05$ was considered to indicate a

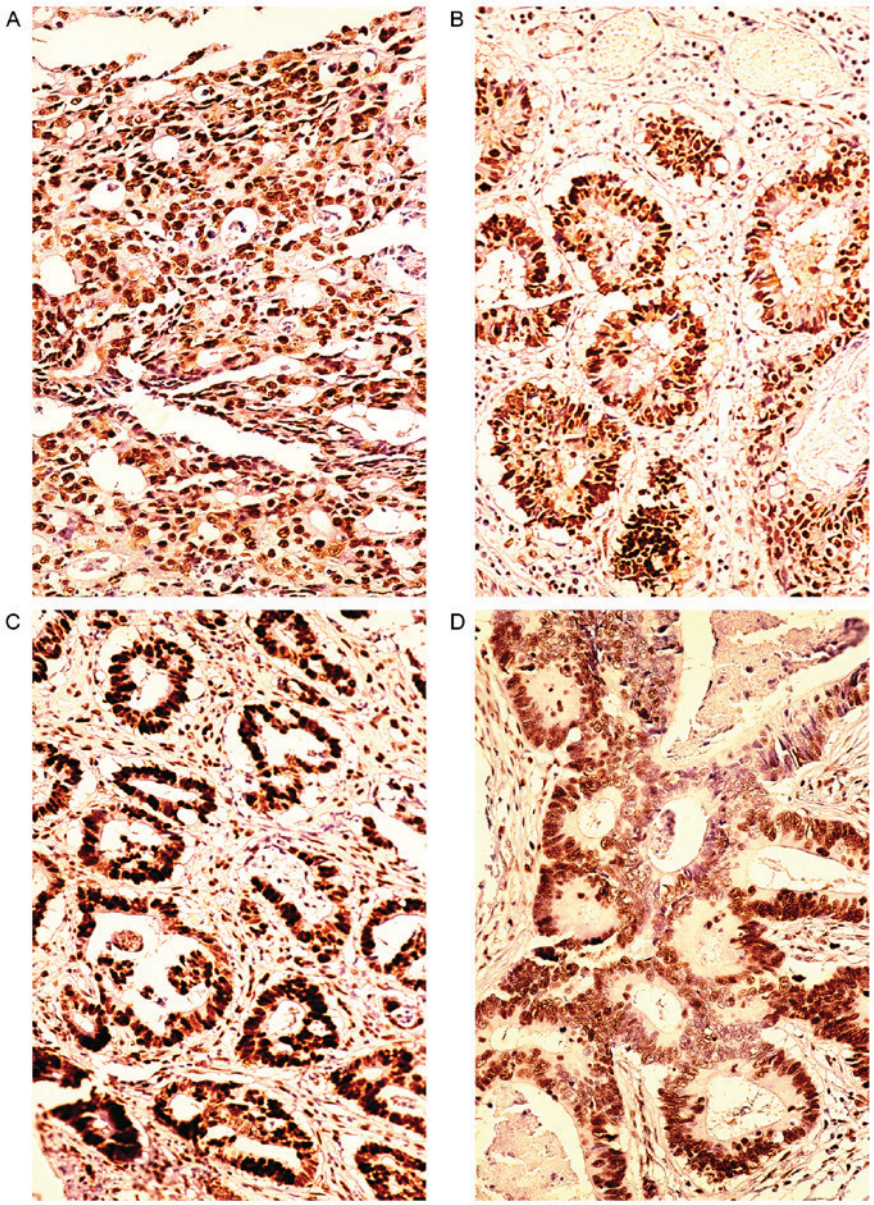

Figure 1. Representative examples of immunostaining (x400 magnification). The positive expression of mismatch repair proteins in sporadic colorectal cancer tissues was localized to the cell nucleus. (A) Positive expression of MutL homolog 1; (B) positive expression of MutS homolog 2; (C) positive expression of MutS homolog 6; (D) the positive expression of PMS1 homolog 2. MMR, mismatch repair; CRC, colorectal cancer; MLH1, MutL homolog 1; MSH2, MutS homolog 2; MSH6, MutS homolog 6; PMS2, PMS1 homolog 2.

statistically significant difference for all analyses, and all calculations were performed using SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA).

Results

Patient demographics. A total of 153 patients with CRC were included in the present study. Out of these, 51 patients were ≤50 years old. A further 102 were >50 years old. Patient age at diagnosis ranged between 23-84 years, with a mean age of 58 years. Out of the 153 patients included in the present study, 97 (63.0%) were males and 56 (37.0%) were females.

Tumor histology. Of the 153 patients with CRC included in the present study, 92 had stage II and 61 had stage III cancer. The localization of all tumors was noted (right colon, defined as cecum through transverse colon; left colon, defined as descending colon through rectum; and rectum). Information concerning morphological features (tumor size, tumor grade, angiolymphatic invasion, tumor differentiation and depth of invasion) was also collected. Tumor localization was as follows: 47/153 (31.0%) were located in the left colon, 38/153 (25.0%) were located in the right colon and 68/153 (44.0%) were located in the rectum. Of these, 21/153 (14.0%) patients had tumors that were smaller than 3 cm, 92/153 (60.0%) had tumors between 3 and 5 cm and 40/153 (26.0%) had tumors larger than 5 cm.

IHC analysis of MLH1, MSH2, MSH6 and PMS2 protein expression. MLH1, MSH2, MSH6 and PMS2 protein expression in stage II and III CRC was examined (Table II). Of the 153 tumors included in the present study, 120 (78.4%) were

Table II. MLH1, MSH2, MSH6 and PMS2 protein expression in stage II and III colorectal cancers.

	Stage II (92)		Stage III (61)			
Protein	- (%)	+ (%)	- (%)	+ (%)	χ^2	P-value
MLH1	27 (29.3)	65 (70.7)	6 (9.88)	55 (90.2)	8.225	0.004
MSH2	26 (28.2)	66 (71.8)	12 (19.7)	49 (80.3)	1.449	0.229
MSH6	61 (66.3)	31 (33.7)	24 (39.3)	37 (60.7)	10.798	0.001
PMS2	22 (23.9)	70 (76.1)	9 (14.8)	52 (85.2)	1.904	0.168

MLH1, MutL homolog 1; MSH2, MutS homolog 2; MSH6, MutS homolog 6; PMS2, PMS1 homolog2.

Table III. MLH1 protein expression and the clinicopathological characteristics of patients and their tumors.

		MLH1			
Variable	n (153)	- (%)	+ (%)	χ^2	P-value
Sex					
Male	97	24 (24.7)	73 (75.3)	1.578	0.209
Female	56	9 (16.1)	47 (83.9)		
Age, years					
≤50	51	13 (25.5)	38 (74.5)	0.695	0.404
>50	102	20 (19.6)	82 (80.4)		
Type					
Ulcerated	139	3 (22.3)	108 (77.7)	0.843	0.487
Protruded	14	2 (14.2)	12 (85.8)		
Tumor size, cm					
<3	21	2 (9.5)	19 (90.5)	11.267	0.004
3-5	92	15 (16.3)	77 (83.7)		
>5	40	16 (40)	24 (60.0)		
Localization					
Right colon	38	9 (23.7)	29 (76.3)	2.300	0.317
Left colon	47	13 (27.7)	34 (72.3)		
Rectum	68	11 (16.2)	57 (83.8)		
Differentiation					
Well	36	4 (11.1)	32 (88.9)	7.814	0.020
Moderate	64	11 (17.2)	53 (82.8)		
Poor	53	18 (33.9)	35 (66.1)		
Invasive depth					
Intra muscularia	11	2 (18.2)	9 (81.8)	10.408	0.005
Intra subserosa	112	18 (16.1)	94 (83.9)		
Extra subserosa	30	13 (43.3)	17 (56.7)		
Angiolymphatic invasion					
Without	104	19 (18.3)	85 (81.7)	2.090	0.148
With	49	14 (28.6)	35 (71.4)		
Lymph node metastasis					
Without	44	17 (38.7)	27 (61.4)	10.586	0.005
1-3	73	11 (15.1)	62 (84.9)		
≥4	36	5 (13.9)	31 (86.1)		

MLH1, MutL homolog 1.

Table IV. MSH2 protein expression and the clinicopathological characteristics of patients and their tumors.

		MSH2			
Variable	n	- (%)	+ (%)	χ^2	P-value
Sex					
Male	97	27 (27.8)	70 (72.2)	1.276	0.259
Female	56	11 (19.6)	45 (80.4)		
Age, years					
≤50	51	16 (31.4)	35 (68.6)	1.751	0.186
>50	102	22 (21.6)	80 (78.4)		
Type					
Ulcerated	139	35 (25.2)	104 (74.8)	0.096	0.757
Protruded	14	3 (21.4)	11 (78.6)		
Tumor size, cm					
<3	21	3 (14.3)	18 (85.7)	9.246	0.010
3-5	92	18 (19.6)	74 (80.4)		
>5	40	17 (42.5)	23 (57.5)		
Localization					
Right colon	38	9 (23.6)	29 (76.3)	1.099	0.577
Left colon	47	13 (27.7)	34 (72.3)		
Rectum	68	15 (22.1)	57 (79.2)		
Differentiation					
Well	36	8 (22.2)	28 (77.8)	3.368	0.186
Moderate	64	25 (39.1)	39 (60.9)		
Poor	53	15 (28.3)	38 (71.7)		
Invasive depth					
Intra muscularia	11	2 (18.2)	9 (81.8)	6.824	0.033
Intra subserosa	112	23 (20.5)	89 (79.5)		
Extra subserosa	30	13 (43.3)	17 (56.7)		
Angiolymphatic invasion					
Without	104	21 (20.2)	83 (79.8)	3.752	0.053
With	49	17 (34.6)	32 (65.4)		
Lymph node metastasis					
Without	44	17 (38.6)	27 (61.4)	6.452	0.040
1-3	73	15 (20.5)	58 (79.5)		
≥4	36	6 (16.7)	30 (83.3)		

MSH2, MutS homolog 2.

positive for MLH1, 115 (75.2%) were positive for MSH2, 68 (44.4%) were positive for MSH6 and 122 (79.7%) were positive for PMS2. Of the 120 MLH1 positive tumors, 65 (54.0%) were stage II and 55 (46.0%) were stage III. Of the 115 MSH2 positive tumors, 66 (57.0%) were stage II and 49 (43.0%) were stage III. Of the 68 MSH6 tumors, 31 (46.0%) were stage II and 37 (54.0%) were stage III. Of the 122 PMS2 positive tumors, 70 (57.0%) were stage II and 52 (43.0%) were stage III.

Clinicopathological characteristics associated with MLH1, MSH2, MSH6 and PMS2 protein expression. Associations between the expression of MLH1, MSH2, MSH6 and PMS2 in stage II and stage III sporadic CRC and several standard clinicopathological patient and tumor characteristics are detailed in Tables III-VI. No significant associations were observed between MLH1, MSH2, MSH6 or PMS2 protein expression and sex, age, tumor location, tumor type or angiolymphatic invasion. There were significant associations between MLH1 and MSH6 protein expression and larger tumor size, poor differentiation, infrequent lymph node metastasis and invasive depth (P<0.05). MSH2 and PMS2 protein expression was positively associated with larger tumor size, tumor invasion depth and infrequent lymph node metastasis (P<0.05).

Poorly differentiated tumors demonstrated lower MLH1 expression when compared with moderately and well differentiated tumors, with a statistically significant difference between them (poor vs. moderate, P=0.036; poor vs. well-differentiated, P=0.014). MLH1 positive expression was

Table V. MSH6 protein expression and the clinicopathological characteristics of patients and their tumors.

Variable	n	MSH6 - (%)	MSH6 + (%)	χ^2	P-value
Sex					
Male	97	49 (50.5)	48 (49.5)	2.727	0.099
Female	56	36 (64.2)	20 (35.7)		
Age, years					
≤50	51	32 (62.7)	19 (37.7)	1.601	0.206
>50	102	53 (51.9)	49 (48.0)		
Type					
Ulcerated	139	77 (55.4)	62 (44.6)	0.016	0.900
Protruded	14	8 (57.1)	6 (42.9)		
Tumor size, cm					
<3	21	6 (28.5)	15 (71.4)	7.951	0.019
3-5	92	53 (57.6)	39 (42.4)		
>5	40	26 (65)	14 (35.0)		
Localization					
Right colon	38	26 (68.4)	12 (31.6)	3.908	0.142
Left colon	47	26 (55.3)	21 (44.7)		
Rectum	68	33 (48.5)	35 (51.5)		
Differentiation					
Well	36	14 (38.9)	22 (61.1)	7.305	0.026
Moderate	64	35 (54.7)	29 (45.3)		
Poor	53	36 (67.9)	17 (32.1)		
Invasive depth					
Intra muscularia	11	4 (36.6)	7 (63.6)	5.818	0.05
Intra subserosa	112	59 (52.7)	53 (47.3)		
Extra subserosa	30	22 (73.3)	8 (26.7)		
Angiolymphatic invasion					
Without	104	63 (60.6)	41 (39.4)	3.316	0.069
With	49	22 (44.9)	27 (55.1)		
Lymph node metastasis					
Without	44	31 (70.5)	13 (29.5)	8.905	0.012
1-3	73	39 (53.4)	34 (46.6)		
≥4	36	15 (41.7)	21 (58.3)		

MSH6, MutS homolog 6.

detected in 32/36 (88.9%) well differentiated tumors, 53/64 (82.8%) moderately differentiated tumors and 35/53 (66.0%) poorly differentiated tumors.

A statistically significant association was observed between MLH1 expression and tumor size (χ^2=11.267; P=0.004). MLH1 expression was frequently observed in tumors <3 cm, and 19/21 (90.5%) tumors were positive for MLH1. In tumors between 3-5 cm, 77/92 (83.7%) tumors were MLH1 positive. In tumors >5 cm, 24/40 (60.0%) were identified to be MLH1 positive.

Negative expression of MLH1 was associated with the invasive depth of tumors, and the differences in expression were statistically significant (χ^2=10.408, P=0.005). MLH1 expression was observed in 9/11 (81.8%) intra muscularia, 94/112 (83.9%) intra subserosa and 17/30 (56.7%) extra subserosa tumors.

MLH1 expression was higher in tumors with multiple lymph node metastases than in tumors without lymph node metastasis, with a statistically significant difference between them (χ^2=10.586; P=0.005). MLH1 expression was detected in 27/44 (61.4%) of tumors without lymph node metastasis, 62/73 (84.9%) of tumors with one to three lymph node metastases and 31/36 (86.1%) of tumors with four or more lymph node metastases.

MSH2 protein expression was observed in 18/21 (85.7%) of tumors <3 cm, 74/92 (80.4%) of tumors between 3 and 5 cm and 23/40 (57.2%) of tumors >5 cm. These differences were statistically significant (χ^2=9.246; P=0.01).

Table VI. PMS2 protein expression and the clinicopathological characteristics of patients and their tumors.

Variable	n	PMS2		χ^2	P-value
		- (%)	+ (%)		
Sex					
Male	97	20 (20.6)	77 (79.4)	0.021	0.885
Female	56	11 (19.6)	45 (80.4)		
Age, years					
≤50	51	14 (27.4)	37 (72.5)	2.448	0.118
>50	102	17 (16.7)	85 (83.3)		
Type					
Ulcerated	139	29 (20.9)	110 (79.1)	0.341	0.559
Protruded	14	2 (14.3)	12 (85.7)		
Tumor size, cm					
<3	21	2 (9.5)	19 (90.5)	7.718	0.021
3-5	92	15 (16.3)	77 (83.7)		
>5	40	14 (35.0)	26 (65.0)		
Localization					
Right colon	38	8 (21.1)	30 (78.9)	1.525	0.466
Left colon	47	12 (25.5)	35 (74.5)		
Rectum	68	11 (16.2)	57 (83.8)		
Differentiation					
Well	36	5 (13.9)	31 (86.1)	3.378	0.185
Moderate	64	11 (17.2)	53 (82.8)		
Poor	53	15 (28.3)	38 (71.7)		
Invasive depth					
Intra muscularia	11	2 (18.2)	9 (81.8)	12.308	0.002
Intra subserosa	112	16 (14.3)	96 (85.7)		
Extra subserosa	30	13 (43.3)	17 (56.7)		
Angiolymphatic invasion					
Without	104	18 (17.3)	86 (82.7)	1.754	0.185
With	49	13 (26.5)	36 (73.5)		
Lymph node metastasis					
Without	44	15 (34.1)	29 (65.9)	9.256	0.010
1-3	73	12 (16.4)	61 (83.6)		
≥4	36	4 (11.1)	32 (88.9)		

PMS2, PMS1 homolog 2.

MSH2 negative expression was also associated with the invasive depth of tumors, and the differences in expression were statistically significant (χ^2=6.824; P=0.033). MSH2 protein expression was observed in 9/11 (81.5%) intra muscularia, 89/112 (79.5%) intra subserosa and 17/30 (56.7%) extra subserosa tumors.

MSH2 expression was detected in 27/44 (61.4%) tumors without lymph node metastasis, 58/73 (79.5%) with one to three metastases and 30/36 (83.8%) with four or more lymph node metastases. These differences in expression were statistically significant (χ^2=7.305; P=0.026).

MSH6 expression was more common in well-differentiated tumors than in moderately or poorly differentiated tumors, and this difference was statistically significant (poor vs. moderately differentiated, P=0.145; poor vs. well differentiated, P=0.007). MSH6 expression was detected in 22/36 (61.1%) well differentiated, 29/64 (45.3%) moderately differentiated and 17/54 (32.1%) poorly differentiated tumors.

MSH6 expression was observed in 15/21 (71.4%) tumors <3 cm, 39/92 (42.4%) tumors between 3-5 cm and 14/40 (35.0%) tumors >5 cm. These differences were statistically significant (χ^2=5.818; P=0.05).

The frequency of MSH6 positive tumors significantly decreased with increasing invasive depth, and this difference in expression was revealed to be statistically significant (χ^2=6.051, P=0.049). MSH6 expression was observed in 7/11 (63.6%) intra muscularia, 53/112 (47.3%) intra subserosa and 8/30 (26.7%) extra subserosa tumors.

MSH6 expression was detected in 13/44 (29.5%) tumors without lymph node metastasis, 34/73 (46.6%) of tumors with one to three metastases and 21/36 (58.3%) of tumors with

four or more lymph node metastases. These differences were statistically significant (χ^2=8.905; P=0.012).

Larger tumors were less frequently positive for PMS2 protein expression, and this difference was statistically significant (χ^2=7.718; P=0.021). PMS2 expression was observed in 19/21 (90.5%) tumors <3 cm, 77/92 (83.7%) tumors between 3 and 5 cm and 26/40 (65.0%) tumors >5 cm.

The frequency of PMS2 positive tumors was significantly lower in tumors, which had invaded out of the subserosa, compared with those that had not, and this difference was statistically significant (χ^2=12.308; P=0.002). PMS2 expression was observed in 9/11 (81.8%) intra muscularia, 96/112 (85.7%) intra subserosa and 17/30 (56.7%) extra subserosa tumors.

PMS2 expression was observed in 29/44 (65.9%) tumors without lymph node metastasis, 61/73 (83.6%) tumors with one to three metastases and 32/36 (88.9%) tumors with four or more lymph node metastases. These differences were statistically significant (χ^2=9.256; P=0.010).

Discussion

MSI was first reported in 1993, as the presence of thousands of somatic alterations in the length of DNA microsatellite repeats in sporadic and familial colorectal tumors (13). MSI is the result of defects in the MMR system and the MSH2, MLH1, PMS2 and MSH6 genes (14).

The MMR system recognizes and corrects base-pair mismatches and small nucleotide (1-4 base pair) insertion or deletion mutations within the duplex DNA that arise from nucleotide misincorporation during DNA replication (15,16). A properly functioning MMR system is essential for the maintenance of genomic stability, and the mutation rates in tumor cells with MMR deficiencies are 100- to 1,000-fold higher than in normal cells (17). Consequently, a malfunctioning MMR system leads to genome-wide instability.

Previous studies have identified that colorectal tumors with MSI accumulate mutations at microsatellite sequences in the coding regions of tumor progression genes (15). MSI is detected in tumor tissue by examining a panel of five markers: BAT25, BAT26, D2S123, D5S346 and D17S250, known as the Bethesda markers. This method requires specific laboratory expertise and equipment, and is routinely used in clinical pathology laboratories (18). It has been demonstrated that loss of MLH1, MSH2, PMS2 and MSH6 protein expression is associated with a defective MMR system. Therefore, IHC analysis of the expression of these markers may potentially be used to detect MSI tumors (19).

In the present study, MLH1, MSH2, MSH6 and PMS2 expression was observed at 70.7, 71.7, 33.7 and 76.1% of stage II colon cancer tissues, respectively. MLH1, MSH2, MSH6 and PMS2 expression was higher in stage III tumors, at 90.2, 80.3, 60.7 and 85.2% respectively. The differences in expression were statistically significant for MLH1 and MSH6, and were significantly higher in stage III colon cancer than in stage II tumors (χ^2=8.225 and 10.798, respectively; P<0.05). MMR deficiency in sporadic cancers is primarily due to MLH1 expression loss due to the somatic hypermethylation of its promoter; however, in the present study, MMR deficiencies in examined sporadic CRCs were primarily due to the loss of MSH6. In the present study, MLH1 protein was detected in 70.7% of stage II and 90.2% of stage III colon cancer samples, and MSH6 protein was detected in 33.7% of stage II and 60.7% of stage III colon cancer samples.

In the present study, in tumors >5 cm, MLH1, MSH2, MSH6 and PMS2 expression was lower than the expression of these proteins observed in the tumors <5 cm. This result is in accordance with the results of several previous studies (20). For example, Lanza *et al* (11) demonstrated that patients with MMR defective colorectal tumors were younger and had tumors that were localized in the right-colon. MMR defective colorectal tumors exhibited infrequent lymph node metastasis, were larger and were poorly differentiated or of mucinous histology. Additionally, patients with MMR colorectal tumors had distinct clinicopathological characteristics, including a lower risk of recurrence. In another study by Sinicrope *et al* (21), the prevalence of a defective MMR system in stage II and III colon cancers was 15%, and the MMR phenotype was significantly associated with higher tumor stage, proximal site, poor or undifferentiated histology, female sex and older age.

In the present study, it was observed that the loss of MLH1, MSH2, MSH6 and PMS2 expression was associated with advanced tumors. The loss of MLH1 and MSH6 protein expression was significantly associated with large, poorly differentiated tumors characterized by extraserosal invasion and infrequent lymph node metastasis. Similarly, the loss of MLH2 and PMS2 protein expression was significantly associated with large tumors characterized by extraserosal invasion and infrequent lymph node metastasis. No significant associations between the loss of MLH1, MSH2, MSH6 or PMS2 expression and the age, sex, tumor location or angiolymphatic invasion of patients with CRC were observed.

Unlike patients with intact MMR, patients with MMR deficient colon cancers do not benefit from 5-fluorouracil-based adjuvant therapy (22). Therefore, the identification of patients with MMR deficient tumors is critical for the selection of an appropriate and effective treatment strategy. The results of the present study may help improve patient outcomes by assisting the identification of patients who possess CRCs exhibiting defective MMR. The use of this information in clinical decision-making would represent an important step toward individualized cancer therapy.

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