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Clinicopathologic characteristics of resectable colorectal cancer with mismatch repair protein defects in Chinese population

Retrospective case series and literature review

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Abstract

Colorectal cancer (CRC) represents a major malignancy globally, with microsatellite instability as its second top molecular mechanism of carcinogenesis. Immunohistochemical (IHC), whose sensitivity and specificity exceed 90%, is used routinely to detect 4 MMR proteins (MLH1, PMS2, MSH2, and MSH6) for screening mismatch repair system defects. We aimed to assess associations of clinicopathologic characteristics with MMR status in resectable CRC patients.

Stage I-III CRC cases administered surgical resection in Zhejiang Cancer Hospital in 2013 to 2015 were retrospectively analyzed. MLH1, MSH2, MSH6, and PMS2 protein amounts were evaluated immunohistochemically. Clinicopathological information, including age, sex, tumor location, histological subclass, disease stage, regional lymph node (LN) metastasis, American Joint Committee on Cancer (AJCC) 8th edition stage, and survival data were retrospectively reviewed.

A total of 133 CRC cases were assessed, including 74 (55.6%), 45 (33.8%), 55 (41.4%), and 77 (57.9%) not expressing MLH1, MSH2, MSH6, and PMS2, respectively. There were significant associations of MLH1, MSH2, MSH6, and PMS2 proteins with age and sex ($P < .05$). MLH1, MSH2, and MSH6 (but not PMS2) showed positive associations with primary tumor location ($P < .05$). Of the 133 patients, 70 and 63 cases were affected on the right and left sides, respectively; significant associations of primary site with age and sex were observed ($P < .05$). Regarding the MMR status, MLH1, MSH2, and MSH6 protein expression levels were positively associated with primary site ($P < .05$). Five-year overall survival (OS) rates were 84.2% and 79.2% in left-side and right-side cases, respectively; 5-year disease-free survival (DFS) rates were 74.0% and 69.8%, respectively. Survival had no differences between left- and right-side patients in terms of OS ($P = .318$) and DFS ($P = .481$).

These data demonstrate that 4 major dMMR proteins are expressed differently in left- and right-side CRCs, and survival is comparable in right- and left-side resectable CRC cases with dMMR.

Abbreviations: AJCC = American Joint Committee on Cancer, CIN = chromosomal instability, CRC = colorectal cancer, dMMR = mismatch repair protein deficiency, DFS = disease-free survival, IHC = immunohistochemical, LN = lymph node, MMR = mismatched repair, MSI = microsatellite instability, OS = overall survival, PD-1 = programmed cell death 1.

Keywords: immunohistochemistry, left-side colon cancer, microsatellite instability, mismatch repair deficiency, right-side colon cancer

1. Introduction

Colorectal cancer (CRC) represents a major malignancy globally. As reported by Global Cancer Statistics 2020,[1] CRC ranks third in terms of incidence but second in terms of mortality. In China, CRC ranks fifth both in terms of morbidity and mortality according to 2015 data.[2] Therefore, CRC constitutes a great threat to human health. Understanding its clinicopathological characteristics could provide guidance for clinical diagnosis and treatment.

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The occurrence and development of CRC are complex processes. Researches have reported that chromosomal instability (CIN) and microsatellite instability (MSI) are the 2 main molecular pathways of CRC.[3] CIN is a major cause of sporadic CRC.[4] Meanwhile, MSI has been described as the genetic inducer of hereditary nonpolyposis colorectal cancer. Studies have shown that MSI also contributes to the formation and development of sporadic colorectal cancer, which is observed in about 15% of all CRC cases.[5]

MSI refers to the change in length of a microsatellite DNA caused by the insertion or deletion of repetitive units in tumor tissues compared with normal counterparts, with new microsatellite DNA alleles appearing. Mismatched repair (MMR) is the repair of a nucleotide sequence in a DNA molecule that contains mismatched bases. MSI can occur in case of germ line mutations or methylation of MMR genes, and leads to decreased MMR function, which results in failure to repair the mismatch, deletion, or insertion of bases in the DNA sequence.[6]

Studies have reported that mismatch repair proteins include the MutS and MutL groups. MutS comprises MSH2, MSH3, and MSH6, whereas MutL includes MLH1, MLH3, PMS1, and PMS2. Of these, MLH1, MSH2, MSH6, and PMS2 are dominant proteins in MMR.[7] Loss of function of ≥1 mismatched repair proteins can cause MSI, which is also referred to as mismatch repair protein deficiency (dMMR). Therefore, detection of missing mismatched repair proteins could indirectly reflect the MSI status of tumors.

Because of its prognostic and predictive values in some tumors, the MSI/MMR status attracts increasing attention in cancer research. In terms of prognostic value, MSI/MMR-associated mutations have been shown to drive tumorigenesis by inactivating tumor suppressor genes. Clinical data showed that MSI high or dMMR CRC cases have improved clinical outcomes, such that adjuvant chemotherapy is not recommended for such patients with stage II disease.[8] Moreover, clinical trials suggested that solid tumor cases with MSI-high or dMMR are associated with responses to programmed cell death 1 (PD-1) inhibitors.[9] Understanding the associations of MMR status with clinicopathological characteristics in CRC patients would help further guide clinical treatment and explore the pathogenetic mechanisms of this disease.

Here, MLH1, MSH2, MSH6, and PMS2 were assessed for protein expression by IHC to explore the associations of clinicopathologic characteristics with MMR status in resectable CRC patients.

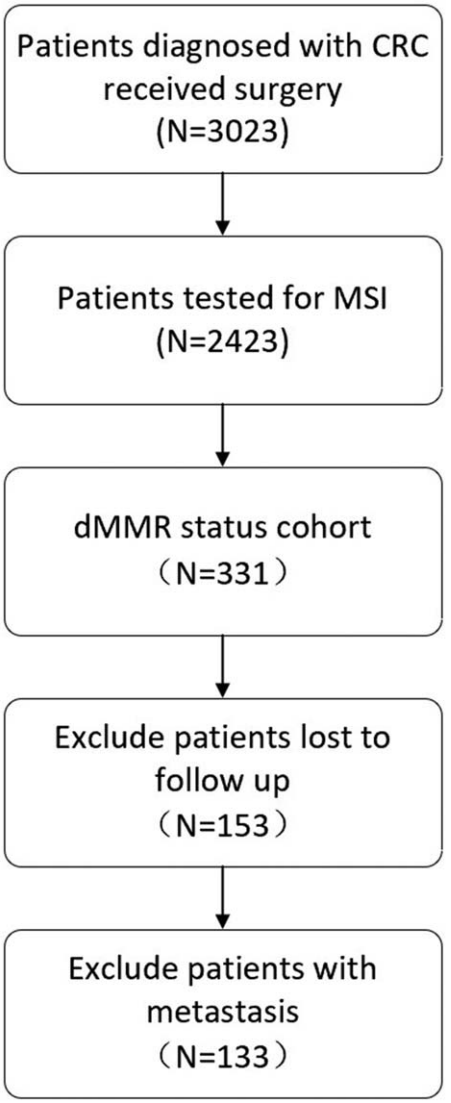

Figure 1. Flowchart of patient selection. CRC = colorectal cancer, dMMR = mismatch repair protein deficiency, MSI = microsatellite instability.

2. Materials and methods

2.1. Patients

Under a human research ethics committee–approved protocol, a single-center study was conducted in Zhejiang Cancer Hospital, whose database comprises >3000 CRC cases administered surgery from January 2013 to December 2015. Written informed consent was provided by each patient before enrolment. Among these cases, a total of 2423 underwent MMR testing by IHC, and 331 (13.7%) had the dMMR status. Exclusion criteria were: undefined disease stage or vital status, metastasis, and loss to follow-up. Finally, 133 patients with stage I-III sporadic colorectal adenocarcinoma were included in this study (Fig. 1). Clinicopathological data, including age, sex, tumor location, histological subclass, disease stage, regional lymph node (LN) metastasis, and American Joint Committee on Cancer (AJCC) 8th edition stage were retrospectively reviewed.

2.2. Immunohistochemistry

For immunohistochemical (IHC) staining of MLH1, MSH2, MSH6 and PMS2 in tumor tissue specimens, 4-μm sections obtained after formalin fixation and paraffin embedding underwent xylene dewaxing and rehydration in graded ethyl alcohol. Antigen retrieval was performed with heated 10 mmol/L citrate buffer (pH 6.0). After treatment with 3% hydrogen peroxidase (H_2O_2; Fuzhou Maixin Biotech, China) and blocking (10 minutes at 37°C), the sections were successively incubated with mouse anti-human MLH1 (MAB-0642), anti-human MSH2 (MAB-0291), anti-human MSH6 (MAB-0643), and anti-human PMS2 (MAB-0656) monoclonal primary antibodies (Fuzhou Maixin Biotechnology) for 20 minutes at ambient, respectively, and horseradish peroxidase-linked rabbit anti-mouse IgG (KIT9710; Fuzhou Maixin Biotechnology) for 20 minutes at

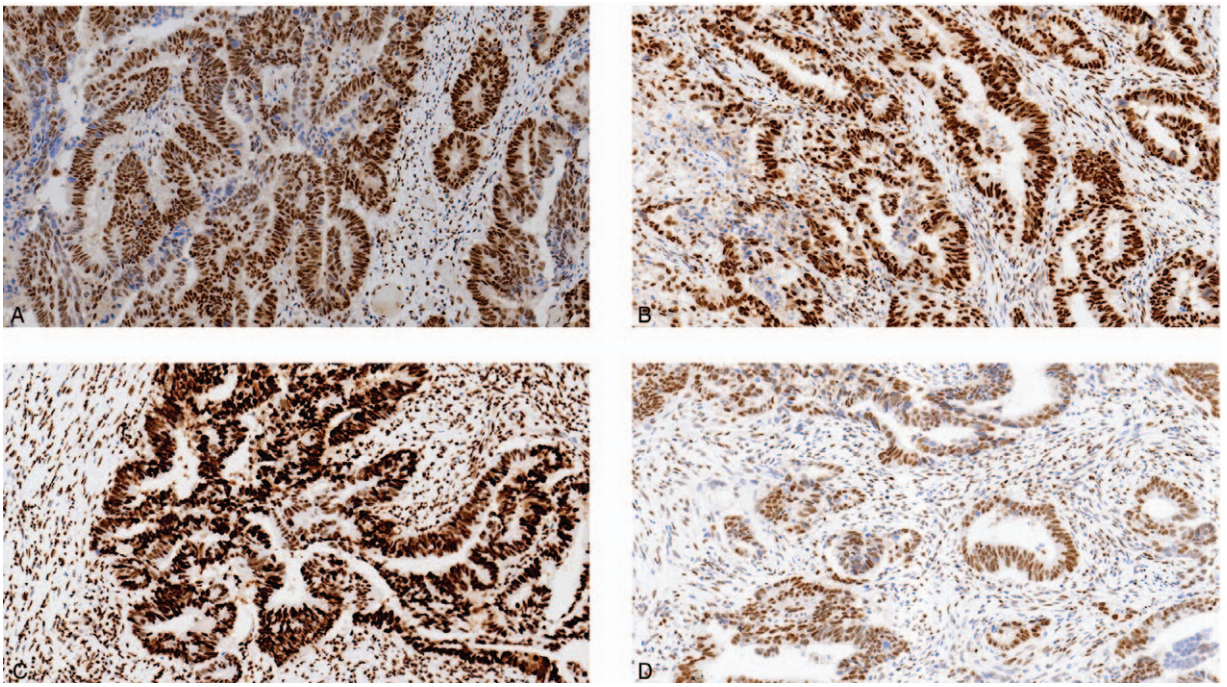

Figure 2. Representative immunohistochemical staining data (×200). Mismatch repair proteins in sporadic colorectal cancer tissue samples were found in the cell nucleus. (A) Positive MLH1 signals; (B) positive MSH2 signals; (C) positive MSH6 signals; (D) positive PMS2 signals.

37°C. After thorough washing, visualization was carried out by 5- to 10-minute incubation with 3,3'-diaminobenzidine. Counterstaining was performed for 5 minutes with hematoxylin at ambient. In negative control samples, primary antibodies were omitted. As shown in Figure 2, the above proteins were detected in the nucleus. Signal intensities were assessed at 200× under a light microscope independently by 2 blinded investigators. Discrepancies required discussion until consensus or a third investigator for review. Non-neoplastic colonic tissues, stroma, and infiltrating lymphocytes normally show positive hematoxylin staining, and served as internal positive controls.

2.3. Statistical analysis

SPSS v19.0 (SPSS Inc, Chicago, IL) was employed for data analysis. The Fisher exact test or χ^2 test was employed for assessing associations of MMR defects (based on IHC data) and tumor site with various clinicopathologic features. In addition, the relationship between the MMR status and tumor site was assessed. Survival was estimated by Kaplan-Meier curves. Two-sided $P < .05$ indicated statistical significance.

3. Results

3.1. Baseline patient features

Table 1 summarizes the clinicopathological features of all 133 patients. Among them, 76 (57.1%) were men, averaging 57.8 years (range 22–87 years); 70 (52.6%) and 63 (47.4%) cases were on the right and left sides, respectively. Overall, 114 (85.7%) patients were diagnosed with adenocarcinoma, whereas 83 (62.4%) and 6 (4.5%) cases were mucinous adenocarcinoma and signet-ring cell carcinoma, respectively. Meanwhile, 64.7% of all patients had T4 stage disease; 23, 19, and only 5 (3.8%) patients had T3, T2, and T1 disease, respectively. More than half of patients had no lymph node metastasis. Clinical stage-III, II, and I cases accounted for 24.1%, 58.6%, and 17.3%, respectively. The MLH1, MSH2, MSH6, and PMS2 proteins were detected immunohistochemically. Among the 133 tumor specimens, 74 (55.6%), 45 (33.8%), 55 (41.4%), and 77 (57.9%) were negative for MLH1, MSH2, MSH6, and PMS2, respectively. There were 74 (55.6%) patients that received adjuvant chemotherapies, including capecitabine single drugs and the CapeOx regimen.

3.2. Associations of clinicopathologic features with the MLH1, MSH2, MSH6, and PMS2 proteins

Associations of MLH1, MSH2, MSH6, and PMS2 expression levels in stage I-III sporadic colorectal adenocarcinoma with various clinicopathologic features are detailed in Table 2. MLH1, MSH2, MSH6, and PMS2 protein expression levels were not significantly associated with the depth of tumor infiltration, regional lymph node metastasis, Cancer (AJCC) 8th edition stage, and histopathologic type. There were significant associations of MLH1, MSH2, MSH6, or PMS2 protein expression levels with age and sex ($P < .05$). MLH1, MSH2, and MSH6 (but not PMS2) protein expression levels showed positive associations with primary tumor site ($P < .05$).

3.3. Associations of primary tumor location with clinicopathological characteristics

In recent years, multiple differences have been revealed between primary right- and left-side CRC. We investigated associations of primary tumor location with clinicopathological characteristics in stage I to III sporadic colorectal adenocarcinoma, as shown in

Table 1

Clinicopathological characteristics of dMMR CRC patients.

Variable	No. of patients (n = 133)	%
Age, y		
Mean	57.8	
Range	22-87	
Sex		
Male	76	57.1
Female	57	42.9
Tumor location		
Right side	70	52.6
Left side	63	47.4
Histology subtype		
Adenocarcinoma	114	85.7
Mucinous adenocarcinoma	83	62.4
Signet-ring cell carcinoma	6	4.5
T stage		
T1	5	3.8
T2	19	14.3
T3	23	17.3
T4	86	64.7
N stage		
N0	101	75.9
N1	22	16.5
N2	10	7.5
AJCC stage		
1	23	17.3
2	78	58.6
3	32	24.1
MMR status		
hMLH1 negative	74	55.6
hMSH2 negative	45	33.8
hMSH6 negative	55	41.4
PMS2 negative	77	57.9
Number of retrived LNs		
Mean	27.7	
SD	16.8	
Chemotherapy		
Yes	74	55.6
No	59	44.4

AJCC = American Joint Committee on Cancer, CRC = colorectal cancer, dMMR = mismatch repair protein deficiency, MMR = mismatched repair.

Table 3. Among all 133 patients, 70 and 63 cases were primary right- and left-side CRC. No significant associations were observed of tumor primary site with the depth of tumor infiltration, regional lymph node metastasis, Cancer (AJCC) 8th edition stage, and histopathologic type. There were significant associations of primary site with age and sex ($P < .05$). Regarding the MMR status, MLH1, MSH2, and MSH6 (but not PMS2) protein expression levels were positively correlated with primary site ($P < .05$).

3.4. Overall survival and disease-free survival in dMMR patients with different primary tumor sides

Among the 133 patients, 28 died, including 10 and 18 with left-side and right-side disease, respectively. A total of 38 cases recurred, including 19 each with left-side and right-side primary tumors. Overall survival (OS) and disease-free survival (DFS) were analyzed in all 133 dMMR CRC patients. Five-year OS rates were 84.2% and 79.2% in left- and right-side cases, respectively. Five-year DFS rates were 74.0% and 69.8% in left- and right-side cases, respectively. Patient survival showed no significant differences between left- and right-side cases in terms of OS ($P = .318$) and DFS ($P = .481$) (Fig. 3).

4. Discussion

With recent advances in molecular biology, it is gradually admitted that the molecular classification of CRC may be closely associated with clinicopathological features, biological behaviors, treatment, and prognosis; therefore, it is necessary to carry out molecular detection and classification for CRC patients. In 1997, the NCI recommended MSI detection by PCR, detecting 5 loci (the 2 single nucleotide markers bat-25 and bat-26, and the 3 double nucleotide markers D23123, D5S346, and D17S250), to determine the status of MSI.[5] However, PCR is complicated, time-consuming, and costly, and not convenient for clinical application. Compared with PCR, IHC staining is cheaper and less time-consuming. Pathologists can routinely detect MMR proteins by IHC, providing guidance for subsequent genetic testing and clinical diagnosis.[10] Therefore, in recent years, IHC has been mostly used to detect MMR protein expression levels for MSI status prediction, with sensitivity and specificity >90%.[11] Therefore, IHC can be used as one of the screening methods applied for MSI status detection.

MMR proteins are expressed in the nucleus, and more expressed in one-third of submucosal lacunae cells, stromal inflammatory cells, and epithelial cells under physiological conditions. When the double allele of MSI in tumor cells is inactivated, IHC could not detect MMR proteins in tumor cells. If a certain MMR protein was completely missing in the tumor cell nucleus, it was considered to indicate an expression deletion.

In this study, we performed IHC to test the expression levels of MMR dominant proteins (MLH1, MSH2, MSH6, and PMS2), whose associations with the clinicopathological characteristics of CRC were assessed. The results showed that in all 2423 CRC patients, 331 had MMR protein expression deletion, indicating a deletion rate of 13.7%, which was relatively close to findings by Khoo et al (14.4%).[12] Other studies have shown that the MMR protein deficiency rate varies from 4.3% to 20%,[13–15] which suggests that the proportion of dMMR CRC varies by country, race, and stage. Meanwhile, dMMR CRC has unique clinical characteristics, including early-onset age, right-side preference, and histological types of mucinous adenocarcinoma and low-differentiated adenocarcinoma.[16] In this study, the median age at onset was <60 years, with a minimum of 22 years; females were more affected than males, the right side more affected than the left, and mucinous adenocarcinoma accounted for more than half of all cases, corroborating previous studies. As shown above, MLH1 and PMS2 loss was more common than MSH2 and MSH6 loss, in agreement with Khan et al.[17] We also found that most patients had very deep tumor infiltration, but less lymph node metastasis in dMMR CRC.

As shown above, MLH1, MSH2, MSH6, and PMS2 expression loss had no associations with the depth of tumor infiltration, regional lymph node metastasis, Cancer (AJCC) 8th edition stage, and histopathologic type, which indicates that mutations of these 4 proteins exist in the initial stage of CRC tumor development and continue to affect CRC tumor progression. Patients with loss of MLH1 and PMS2 were common among right-side CRC cases, whereas cases with loss of MSH2 and MSH6 were common in the left-side CRC group.

Table 2

Clinicopathological characteristics associated with MLH1, MSH2, MSH6, and PMS2 protein expression.

	MLH1			MSH2			MSH6			PMS2		
Variable	**Negtive**	**Positive**	***P***	**Negtive**	**Positive**	***P***	**Negtive**	**Positive**	***P***	**Negtive**	**Positive**	***P***
Age, y												
Mean	61.19	53.58	<.01	51.67	60.95	<.01	53.02	61.19	<.01	61.64	52.55	<.01
SD	12.10	13.37		13.69	12.68		13.60	12.82		12.74	13.34	
Sex												
Male	36	40	.03	31	45	.05	39	37	<.01	38	38	.03
Female	38	19		14	43		16	41		39	18	
Tumor location												
Right side	46	24	.01	18	52	.04	21	49	<.01	46	24	.05
Left side	28	35		27	36		34	29		31	32	
T stage												
T1	1	4	.09	2	3	.53	2	3	.54	3	2	.36
T2	7	12		8	11		10	9		8	11	
T3	14	9		5	18		7	16		16	7	
T4	52	34		30	56		36	50		50	36	
N stage												
N0	53	48	.40	36	65	.25	45	56	.28	56	45	.32
N1	14	8		8	14		8	14		13	9	
N2	7	8		1	9		2	8		8	2	
AJCC stage												
1	8	15	.07	9	14	.68	11	12	.39	11	12	.42
2	45	33		27	51		34	44		45	33	
3	21	11		9	23		10	22		21	11	
Histology subtype												
Adenocarcinoma	64	50	.78	38	76	.77	47	67	.94	67	47	.62
Mucinous adenocarcinoma	43	40	.25	31	52	.27	39	44	.09	43	40	.07
Signet-ring cell carcinoma	2	4	.26	4	2	.08	3	3	.66	2	4	.21

AJCC = American Joint Committee on Cancer.

Table 3

Association Between Primary Tumor Location and Clinicopathological Characteristics.

	Right side		Left side		
Variable	**No. of patients (n = 70)**	**%**	**No. of patients (n = 63)**	**%**	***P***
Age, y					
Mean	60.40		54.90		.02
SD	12.10		14.90		
Sex					
Male	36	51.43	40	63.49	.02
Female	34	48.57	23	36.51	
T stage					
T1	2	2.86	3	4.76	.10
T2	6	8.57	13	20.63	
T3	16	22.86	7	11.11	
T4	46	65.71	40	63.49	
N stage					
N0	50	71.43	51	80.95	.05
N1	11	15.71	11	17.46	
N2	9	12.86	1	1.59	
AJCC stage					
1	8	11.43	15	23.81	.12
2	42	60.00	36	57.14	
3	20	28.57	12	19.05	
Histology subtype					
Adenocarcinoma	60	85.71	54	85.71	1.00
Mucinous adenocarcinoma	45	64.29	38	60.32	.64
Signet-ring cell carcinoma	4	5.71	2	3.17	.48
MMR status					
hMLH1 negative	46	65.71	28	44.44	.01
hMSH2 negative	18	25.71	27	42.86	.04
hMSH6 negative	21	30.00	34	53.97	.01
PMS2 negative	46	65.71	31	49.21	.05

AJCC = American Joint Committee on Cancer, MMR = mismatched repair.

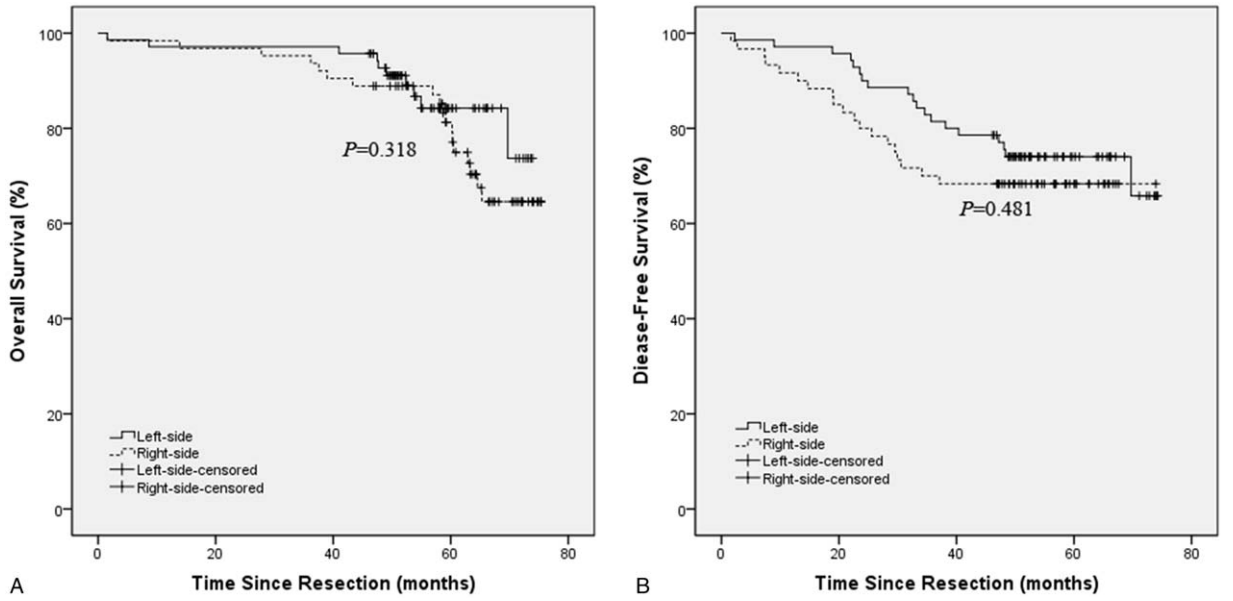

Figure 3. Kaplan-Meier curves for (A) overall survival (OS) and (B) disease-free survival (DFS) in 133 patients with mismatch repair protein deficiency.

Next, we evaluated the associations of primary tumor side with clinicopathological characteristics in these patients. Recently, more and more studies have reported that right- and left-side CRC significantly differ in epidemiological, clinical, and histological indexes.[18,19] Multiple clinical trials suggested that cases with right-side CRC have markedly superior PFS, OS, and ORR compared with those with left-side lesions.[20,21] Clinico-biological evidences support that right- and left-side CRCs have divergent carcinogenetic mechanisms. Right-side lesions are more likely diploid with mucinous histology, elevated MSI, CpG island methylation, and mutated BRAF. Left-side lesions are commonly infiltrated and constricted, with CIN and aneuploidy. By assessing >77,000 colon adenocarcinoma cases from the SEER database, Meguid et al[22] revealed that right-side CRC patients are markedly older and less likely to be male, in agreement with our findings that the primary tumor site had significant associations with age and sex. However, Meguid et al revealed an elevated proportion of right-side lesion cases with node-positive disease compared with left-side counterparts. An analysis of 17,641 patients by Benedix et al[23] also yielded the same results, and patients with right-side colon cancer had increased depth of tumor infiltration than left-side cancer patients. In contrast, this study demonstrated that there were no associations of depth of tumor infiltration, regional lymph node metastasis, Cancer (AJCC) 8th edition staging, histopathologic type, and primary tumor site with dMMR. In addition, no significant differences in PFS and OS between the right- and left-side lesion groups were found in patients with dMMR. We speculate that MMR gene mutations are beneficial and confer improved survival to cancer patients, and could reverse the prognosis of patients with right-side CRC. The National Comprehensive Cancer Network (NCCN) guidelines and other studies supported that stage-II patients with MSI-H show an improved prognosis.[24,25] Interestingly, in stage-III and IV CRC cases, whether MSI-high (MSI-H) predicts good prognosis remains largely controversial. Some reports confirmed MSI-H remains a good prognostic marker with improved survival in cancer.[26,27] Meanwhile, others consider MSI-H as a deleterious parameter in cancer-related survival.[28] A mechanistic research suggested that dMMR cases have markedly increased somatic mutations and substantial infiltration of lymphocytes.[29] It was previously demonstrated that MSI-H CRC is associated with tumor-infiltrating lymphocytes, and immune reactions are very critical to patient survival.[30] This might explain why prognosis in right-side colon lesions in dMMR patients is not worse than that of the left ones.

However, due to potential limitations in this study, bias was inevitable. First, this was a single-center retrospective trial. In addition, the sample size was relatively small and the follow-up time was short. Further mechanistic assessments are required to determine dMMR status' role in improving prognosis in cases of right CRC.

In conclusion, deficiency of MLH1, MSH2, MSH6, and PMS2 at the protein level is not uncommon. By detecting the expression levels of MMR proteins, dMMR could be preliminarily determined for CRC, and MMR proteins were found to be closely associated with clinicopathological characteristics in CRC. To the best of our knowledge, this study firstly revealed no marked survival differences between right- and left-side lesions in resectable CRC patients with dMMR.

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Author contributions

Jingjing Li, MD: drafting the article, revising it critically for intellectual content, final approval of the version to be submitted.
Qi Xu, MM: acquisition of data.
Cong Luo MD: interpretation of data.
Lei Chen, MD: calibration and analysis of data.
Jieer Ying, MD: the conception and design of the study.

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