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Original Article

Expression status of four mismatch repair proteins in patients with colorectal cancer: clinical significance in 1238 cases

Lizhu Chen[1,2,4], Gang Chen[3,4], Xiongwei Zheng[3,4], Yu Chen[1,2,4],

[1]Cancer Bio-immunotherapy Center, Departments of [2]Medical Oncology, [3]Pathology, Fujian Cancer Hospital and Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, China; [4]Fujian Provincial Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian Province, China

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Abstract: To investigate the expression of mismatch repair proteins (MMR) in colorectal cancer (CRC) and to analyze the correlation between MMR and pathologic features of CRC, immunohistochemistry was used to detect the expression of four MMR proteins (MLHI, PMS2, MSH2 and MSH6). All expression was classified as MMR proficient (pMMR). Absence of one or more of these proteins was classified as MMR deficient (dMMR). Among the 1238 cases of CRC, the four protein expression deletion rates from high to low were: PMS2 5.09% (63/1,238), MLH1 3.47% (43/1,238), MSH6 2.83% (35/1,238), and MSH2 2.10% (26/1,238). The dMMR cases accounted for 8.08% of all CRC cases (100/1,238). The common deletion rates of two or more proteins from high to low were: MLH1/PMS2 41.00% (41/100), MSH2/MSH6 20.00% (20/100), MSH6/PMS 23.00% (3/100), MLH1/MSH2/MSH6/PMS2 1.00% (1/100). dMMR cases were more common than pMMR cases in the ascending colon, T4 stage, stage II group, and poorly-differentiated CRC (P<0.05). MLH1 and PMS2 protein expression deficiency were correlated with tumor site, T stage, and differentiation. The incidence in ileocecum, T4, and poorly differentiated CRC was higher (P<0.05), and these two were positively correlated (P<0.05). The deficiency of MSH2 and MSH6 proteins was correlated with age, tumor site, and TNM stage; it was higher in patients ≤65 years old, in the transverse colon-splenic flexure region, and in stage II CRC (P<0.05), and the two were positively correlated (P<0.05). A co-expression deficiency of MLH1/PMS2 and MSH2/MSH6 was more common. The incidence of dMMR was more common in ascending colon, T4 stage, stage II, and poorly differentiated CRC. This may provide more comparisons and reference data for the molecular mechanism, clinical treatment, and prognosis of CRC.

Keywords: Colorectal cancer, immunohistochemistry, mismatch repair proteins

Introduction

Globally, the incidence of CRC ranks third in malignant tumors and the mortality rate ranks second. In China, there are more than 250,000 new cases of CRC and about 140,000 patients die of CRC every year, making CRC a serious threat to health and quality of life [1, 2]. To reduce morbidity and mortality of CRC we must study the molecular genetic mechanism of occurrence and development of CRC, to find and use effective molecular biomarkers for early diagnosis, stage, typing and prognosis, and improve the prevention, diagnosis and treatment of CRC.

Clinical studies have found that the molecular pathways involved in the occurrence of CRC mainly include chromosomal instability (CIN) and microsatellite instability (MSI) [3]. Of the two pathways, the CIN pathway accounts for about 80%, and the factors that cause CIN are mainly oncogene and tumor suppressor gene mutations, which cause cancer through multiple stages and factors [4]. 90% of hereditary non-polyposis CRCs and 10% to 15% of sporadic CRCs are caused by mismatch repair gene defects [5, 6].

Instability of DNA microsatellites is an important cause of CRC [7]. It refers to the increase of highly repetitive DNA sequences (microsatellites) caused by mutations of mismatch repair genes (MLH1, MSH2, MSH6, PMS1 and PMS2) used to repair DNA replication errors, thus inducing tumors.

There are two kinds of detection methods of MSI: one is using polymerase chain reaction to detect sequence of microsatellite loci in tumor tissue samples. MSI can be determined by comparison with normal DNA sequences. Also, the expression of mismatch repair protein in tumor tissue samples was detected by immunohistochemistry. This has the advantages of being a simpler operation, at lower cost. A significant literature has confirmed immunohistochemical methods to detect the MMR genes with reliability, and the results help to identify specific protein loss and guidance for specific gene germline DNA testing. Therefore, the second method is commonly used for CRC patients for early screeningin clinical practice [8]. We also use immunohistochemistry to detect MSI in patients with advanced CRC.

Materials and methods

Patients

One thousand two hundred and thirty-eight CRC paraffin specimens from 2014 to 2017, archived in the Fujian Cancer Hospital were collected. They were from 731 male and 507 female patients, aged 21~90 years. The median age was 59 years. The experiment was approved by the medical ethics committee of Fujian Cancer Hospital, and the informed consent was obtained.

Methods

The specimens were fixed with formalin solution, dehydrated, and embedded in paraffin. In continuous sections, conventional H&E and immunohistochemical envision staining were carried out. The primary antibodies were rabbit anti-human monoclonal antibody PMS2 (IR-087,), MSH6 (IR086, state machine), mouse anti-human monoclonal antibody MLH1 (IR079) and MSH2 (IR085). Antibodies were purchased from Dako North America, Inc., and used according to the product instructions. The non-neoplastic intestinal epithelial mucosa and interstitial lymphocytes in each section were the internal controls with definite positive nuclear staining. Tris Buffered Saline was used as a blank control instead of primary antibody. The expression of MMR protein in the tumor cell nucleus was determined as positive by definite staining, and any positive tumor cells and the sample was considered as positive [9], while non-staining of the nucleus of the tumor cells was judged to be negative. One or more of the four proteins (MLH1, PMS2, MSH2 and MSH6) missing in expression means that the patient was diagnosed with MMRP expression deficiency (MMR-deficient, dMMR), otherwise is normal (MMR-proficient, pMMR). The pathologic section reading and grading process was independently completed by two senior pathologists by double-blind method.

Statistical analysis

SPSS 19.0 statistical software was used. The counting data was tested by chi-square test and Spearman rank correlation was used for the correlation analysis. Univariate analysis and multivariate logistic regression model were used to analyze the correlation between the expression of mismatch repair protein and the clinicopathologic findings of CRC. $P<0.05$ was considered significant.

Results

The expression of MLH1, MSH2, MSH6, and PMS2 in CRC tissues

The deletion rate of MLH1 protein in one thousand two hundred and thirty-eight CRC tissues was 3.47% (43/1238). The deletion rate of MSH2 protein was 2.10% (26/1238). The deletion rate of MSH6 protein was 2.83% (35/1238). The deletion rate of PMS2 protein was 5.09% (63/1238) (**Table 1**; **Figure 1**).

Co-deletion rate of the four mismatch repair proteins in dMMR patients,

The co-deletion rate of MLH1/PMS2 protein was 41.00% (41/100), and the Spearman correlation coefficient was 0.799, $P<0.05$. The co-deletion rate of MSH2/MSH6 protein was 20.00% (20/100), and their Spearman correlation coefficient was 0.689, $P<0.05$. The co-deletion rate of MSH6/PMS2 protein was 3.00% (3/100). The co-deletion rate of MLH1/MSH2/MSH6/PMS2 protein was 1.00% (1/100) (**Table 1**).

Only the MLH1 protein expression was missing in 1.00% (1/100). Only MSH2 protein expression was missing in 5.00% (5/100). Only the expression of MSH6 protein was missing in 11.00% (11/100). Only PMS2 protein expression was missing in 18.00% (18/100).

Table 1. Immunohistochemical expression deletion rates of MLH1, MSH2, MSH6, and PMS2

Characteristic	n	MLH1	MSH2	MSH6	PMS2
Simultaneous expression of two or more proteins was absent in dMMR	1	-	-	-	-
	41	-	+	+	-
	3	+	+	-	-
	20	+	-	-	+
Only one protein expression was absent in dMMR	1	-	+	+	+
	5	+	-	+	+
	18	+	+	+	-
	11	+	+	-	+
pMMR	1138	+	+	+	+
	1238	43	26	35	63

+ expression; - absence.

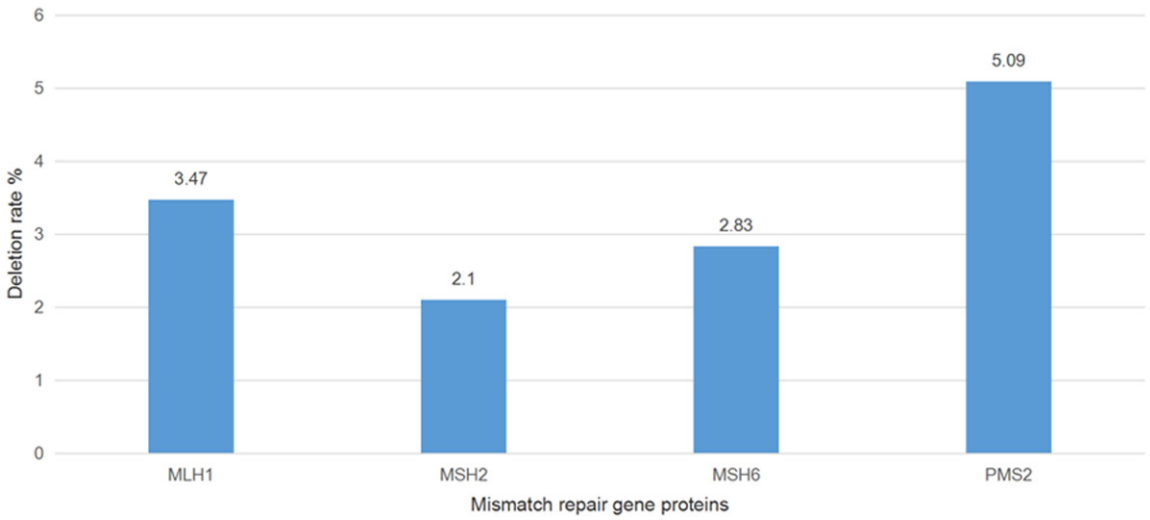

Figure 1. Deletion rate of four mismatch repair gene proteins.

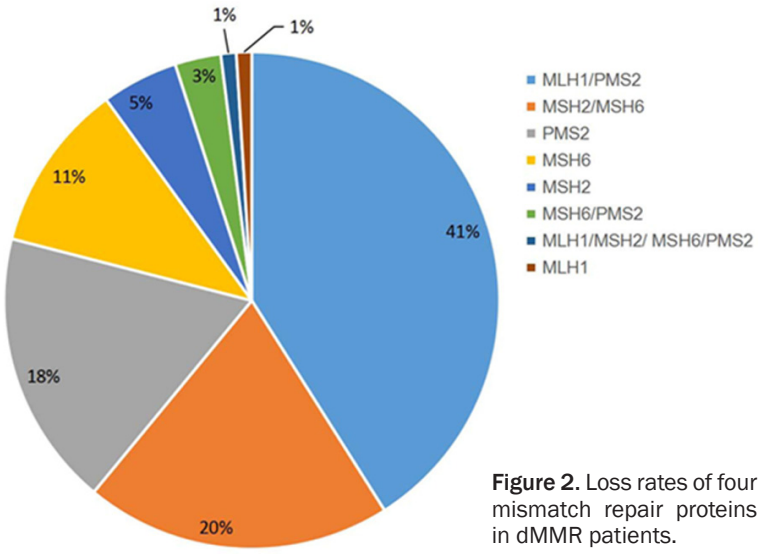

Figure 2. Loss rates of four mismatch repair proteins in dMMR patients.

The sum of the four mismatch repair protein deletions accounted for 8.08% (100/one thousand two hundred and thirty-eight) of all CRC cases (**Figure 2**).

Relationship between protein expression deletion and clinicopathologic features of four mismatch repair genes

Compared with pMMR patients, dMMR patients showed statistically significant differences in the expression of four mismatch repair proteins in tumor location, T stage, TNM

Table 2. Relationship between MSI and clinicopathologic characteristics of CRC

Clinicopathological finding	n	dMMR	pMMR	*P* values
Gender				
Female	507	38	469	0.424
Male	731	62	669	
Age (years) at diagnosis				
≤65 - years old	916	83	833	0.065
>65 - years old	322	17	305	
Tumor site				
Ileocecal junction	40	9	31	0.000
Ascending colon	117	32	85	
Hepatic flexure of transverse colon	48	11	37	
Transverse colon	37	9	28	
Transverse colon splenic flexure	26	7	19	
Descending colon	60	7	53	
Junction of the descending colon and sigmoid colon	4	0	4	
Sigmoid flexure	237	10	227	
Junction of the rectum and sigmoid colon	19	0	19	
Rectum	648	15	633	
Anal canal	2	0	2	
T stage				
1	25	0	25	0.001
2	169	6	163	
3	757	58	699	
4	287	36	251	
N stage				
0	546	53	493	0.074
1	417	34	383	
2	275	13	262	
3	0	0	0	
M stage				
0	1063	89	974	0.565
1	175	11	164	
TNM stage				
I	145	6	139	0.002
II	379	47	332	
III	533	36	497	
IV	181	11	170	
Tumor histologic differentiation				
Poor	78	17	61	0.001
Moderate	1050	71	979	
Well	10	0	10	
Mucinous adenocarcinoma	100	12	88	
General type				
Protrusion	198	20	178	0.738
Ulcerative	682	55	627	
Infiltrating	7	0	7	
Unknown	351	25	326	
Adenocarcinoma or non-adenocarcinoma				
Adenocarcinoma	1235	100	1135	0.776
Non-adenocarcinoma	3	0	3	

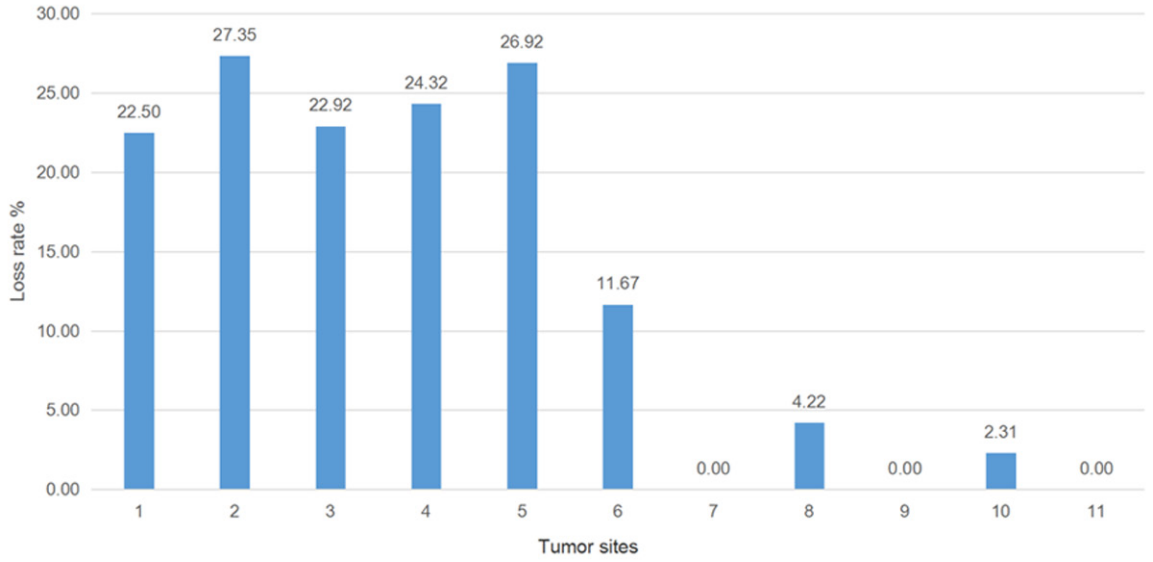

Figure 3. Loss rate of four mismatch repair proteins in different tumor sites. 1. Ileocecal group. 2. Ascending colon group. 3. Transverse colon hepatic flexure group. 4. Transverse colon group. 5. Transverse colon and splenic flexure group. 6. Descending colon group. 7. Descending colon sigmoid junction group. 8. Sigmoid group. 9. Rectosigmoid junction group. 10. Rectum group. 11. The anal group.

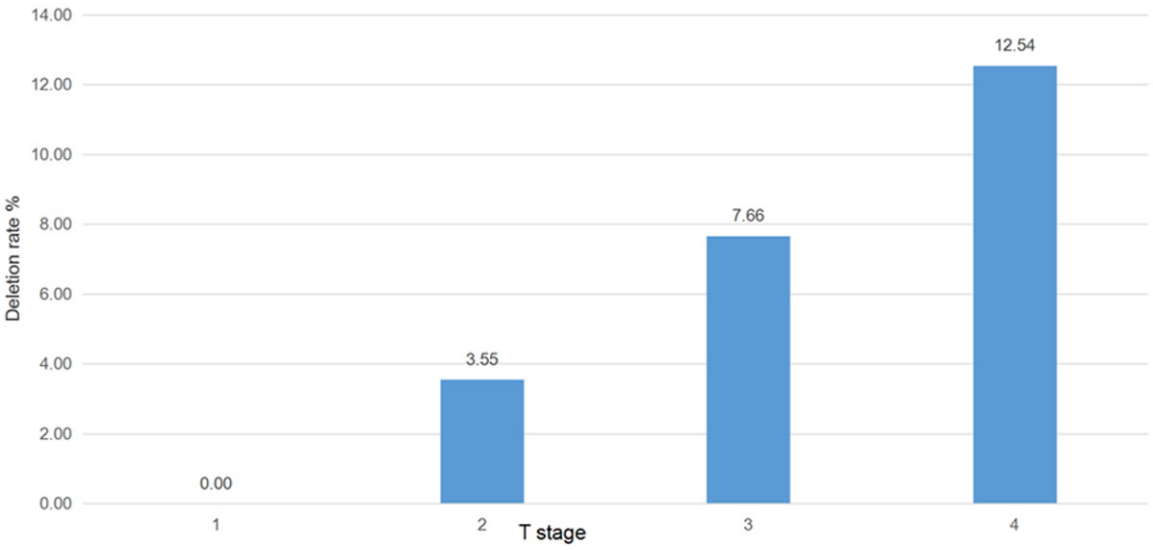

Figure 4. Deletion rate of four mismatch repair proteins in T stage.

stage and degree of tumor differentiation (**Table 2**, P<0.05).

The expression deletion rate of the four mismatch repair proteins in the ileocecal group was 22.50%, the ascending colon group was 27.35%, the transverse colon hepatic flexure group was 22.92%, and the transverse colon group was 24.32%. In the transverse colon and splenic flexure group, the expression deletion rate was 26.92%, the descending colon group was 11.67%, the descending colon sigmoid junction group was 0%, and the sigmoid group was 4.22%. Expression deletion rate was 0% in the rectosigmoid junction group, the rectum group was 2.31%, and the anal group was 0%. The difference was statistically significant (P<0.05) (**Figure 3**).

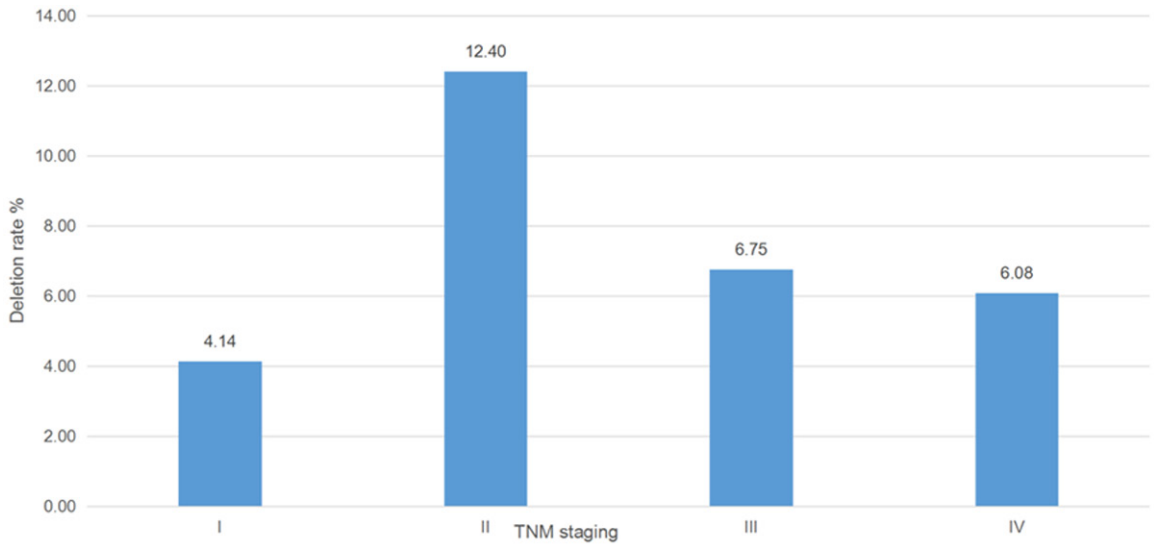

Figure 5. Deletion rate of four mismatch repair proteins in TNM staging.

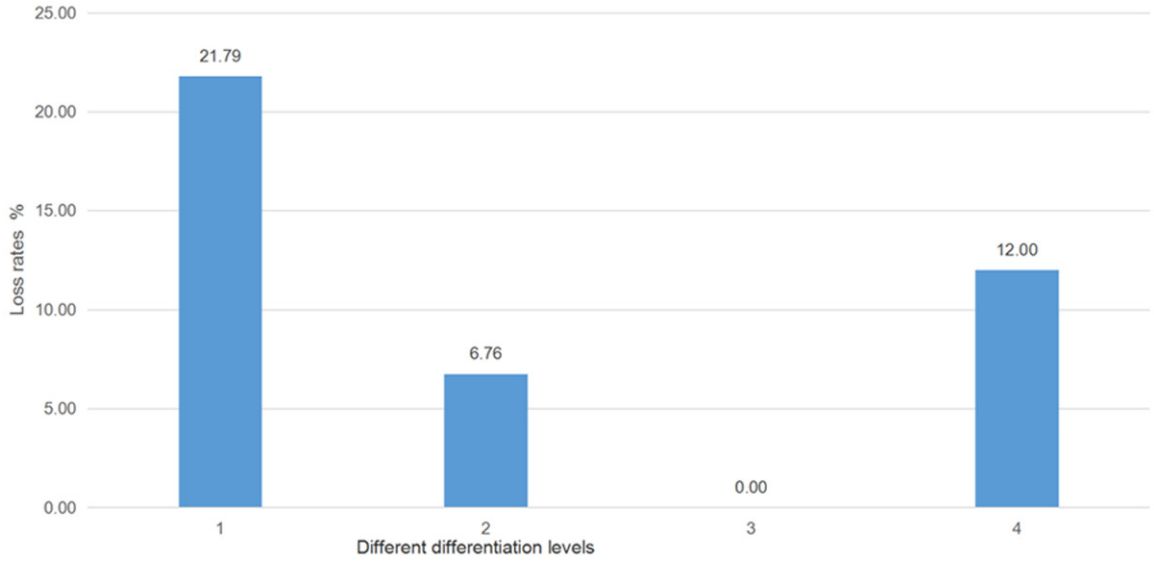

Figure 6. Loss rates of four mismatch repair proteins at different differentiation levels. 1. Poorly differentiated group. 2. Moderately differentiated group. 3. Well differentiated group. 4. Mucinous adenocarcinoma group.

The deletion rate in T4 group (12.54%) was significantly higher than that in the T3 group (7.66%), and T2 group (3.55%) (P<0.05) (**Figure 4**).

The deletion rate in TNM stage II group (12.40%) was significantly higher than that in group III (6.75%), group IV (6.08%), and group I (4.14%) (P<0.05) (**Figure 5**).

The deletion rate in the poorly differentiated group (21.79%) was significantly higher than that in the mucinous adenocarcinoma group (12.00%), the moderately differentiated group (6.76%) and the highly differentiated group (0%) (P<0.05) (**Figure 6**).

There was no significant difference in the rate between the patient's age, gender, M stage, N stage, gross type, and adenocarcinoma or non-adenocarcinoma (**Table 2**, P>0.05).

MLH1 protein expression deletion in tumors

Compared with pMMR patients, in dMMR patients the MLH1 protein deletion was signifi-

Table 3. Relationship of MHL1/MSH2/MSH6/PMS2 expression with clinicopathologic features of CRC

Clinicopathologic feature	Number	MLH1				MSH2				MSH6				PMS2			
		-	+	x^2	p	-	+	x^2	p	-	+	x^2	p	-	+	x^2	p
Gender				2.117	0.146			0.02	0.887			2.284	0.131			0.003	0.958
Female	507	13	494			11	496			10	497			26	481		
Male	731	30	701			15	716			25	706			37	694		
Age at diagnosis (years)				0.176	0.675			4.63	0.031			3.979	0.046			0.495	0.482
≤ 65 - years old	916	33	883			24	892			31	885			49	867		
> 65 - years old	322	10	312			2	320			4	318			14	308		
Tumor site				44.841	0.000			47.464	0.000			51.503	0.000			64.446	0.000
Ileocecal junction	40	8	32			2	38			1	39			8	32		
Ascending colon	117	8	109			10	107			13	104			17	100		
Hepatic flexure of transverse colon	48	4	44			1	47			3	45			7	41		
Transverse colon	37	5	32			2	35			3	34			6	31		
Transverse colon splenic flexure	26	2	24			4	22			4	22			3	23		
Descending colon	60	3	57			2	58			3	57			4	56		
Junction of descending colon and sigmoid colon	4	0	4			0	4			0	4			0	4		
Sigmoid flexure	237	3	234			4	233			5	232			5	232		
Junction of rectum and sigmoid colon	19	0	19			0	19			0	19			0	19		
Rectum	648	10	638			1	647			3	645			13	635		
Anal canal	2	0	2			0	2			0	2			0	2		
T stage				9.051	0.029			4.34	0.227			6.636	0.084			10.429	0.015
1	25	0	25			0	25			0	25			0	25		
2	169	5	164			1	168			1	168			5	164		
3	757	20	737			17	740			25	732			34	723		
4	287	18	269			8	279			9	278			24	263		
N stage				3.015	0.221			5.063	0.08			4.834	0.089			1.955	0.376
0	546	24	522			16	530			19	527			33	513		
1	417	13	404			8	409			13	404			19	398		
2	275	6	269			2	273			3	272			11	264		
3	0	0	0			0	0			0	0			0	0		
M stage				0.857	0.354			0.567	0.451			0.217	0.641			1.163	0.281
0	1063	39	1024			21	1042			31	1032			57	1006		
1	175	4	171			5	170			4	171			6	169		
TNM stage				4.233	0.237			11.651	0.009			8.403	0.038			6.514	0.089
I	145	5	140			1	144			1	144			5	140		
II	379	19	360			15	364			18	361			28	351		
III	533	15	518			5	528			12	521			24	509		
IV	181	4	177			5	176			4	177			6	175		
Tumor histologic differentiation				8.804	0.032			3.123	0.373			7.791	0.051			10.953	0.012
Poor	78	8	70			4	74			6	72			10	68		

Moderate	1050	31	1019			20	1030			24	1026			45	1005		
High	10	0	10			0	10			0	10			0	10		
Mucinous adenocarcinoma	100	4	96			2	98			5	95			8	92		
General type				1.158	0.763			2.832	0.418			2.066	0.559			2.218	0.528
Protruding type	198	7	191			7	191			8	190			10	188		
Ulcerative type	682	26	656			14	668			16	666			39	643		
Infiltrating type	7	0	7			0	7			0	7			0	7		
Unknown	351	10	341			5	346			11	340			14	337		
Adenocarcinoma or non-adenocarcinoma				0.000	1.000			0.000	1.000			0.000	1.000			0.000	1.000
Adenocarcinoma	1235	43	1192			26	1209			35	1200			63	1172		
Non-adenocarcinoma	3	0	3			0	3			0	3			0	3		

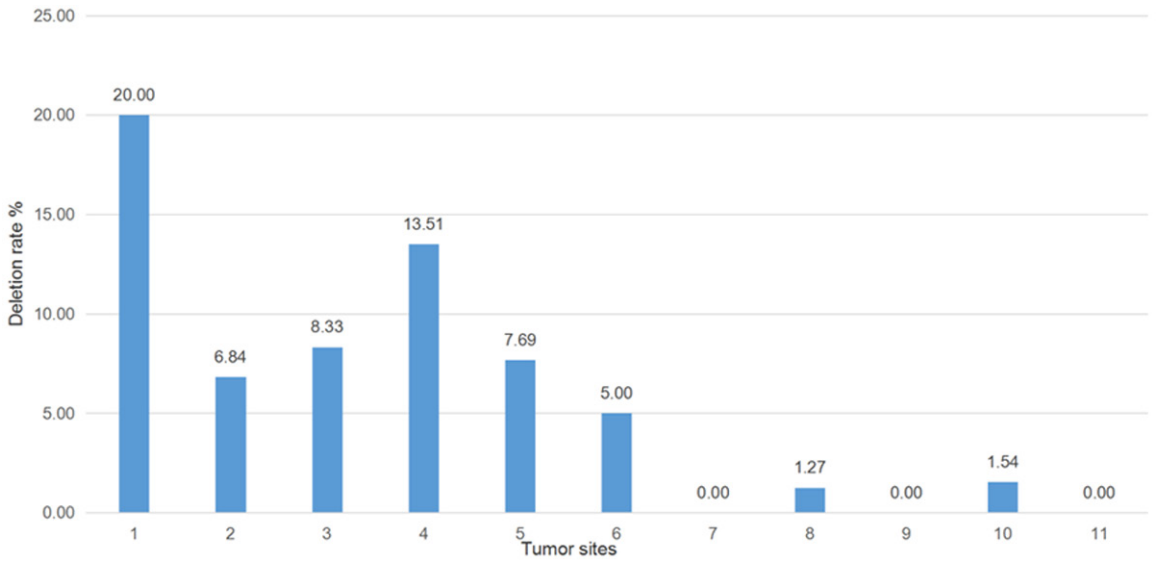

Figure 7. Deletion rate of MLH1 protein expression in different tumor sites. 1. Ileocecal group. 2. Ascending colon group. 3. Transverse colon hepatic flexure group. 4. Transverse colon group. 5. Transverse colon and splenic flexure group. 6. Descending colon group. 7. Descending colon sigmoid junction group. 8. Sigmoid group. 9. Rectosigmoid junction group. 10. Rectum group. 11. Anal group.

cantly different from tumor site, T stage, and differentiation (**Table 3**, P<0.05).

The MLH1 protein deletion rate in the ileocecal group was 20.00%, in the ascending colon group was 6.84%, in the transverse hepatic flexure group was 8.33%, in the transverse colon group was 13.51%, in the transverse colon lack of splenic flexure group was 7.69%, in the descending colon group was 5.00%, in the junction of the descending colon and sigmoid group was 0%, in the sigmoid colon group was 1.27%, in the junction of the rectum and sigmoid colon 0%, in the expression of rectum group was 1.54%, and in the anal canal group the loss rate was 0% (**Figure 7**). Therefore, the loss of expression was most frequently found in the ileocecal area (P<0.05).

Distribution in T stage: deletion rates in T1 group (0.00%), T2 group (2.25%), T3 group (2.64%), and T4 group (6.27%). The deletion rate in the T4 group was higher than that of other groups (**Table 3**, P<0.05).

Distribution in the degree of tumor differentiation: deletion rate in the poor differentiation group was 10.26%, in the medium differentiation group 2.95%, in the high differentiation group 0.00%, and in the mucinous adenocarcinoma group 4.00%. The expression deletion rate in the poor differentiation group was higher than that in other groups (**Table 3**, P<0.05).

MSH2 protein expression deletion in tumors

There were statistically significant differences between dMMR patients and PMMR patients in the expression of MSH2 protein in age, tumor site, and TNM stage (**Table 3**, P<0.05).

The deletion rate at age at ≤65 years old (2.62%) was higher than that at age of >65 years old (0.62%). The difference was significant (P<0.05).

The MSH2 protein expression deletion rate in the ileocecal group was 5.00%, in the ascending colon group was 8.55%, in the transverse hepatic flexure group was 2.08%, in the transverse colon group was 5.41%, in the transverse colon splenic flexure group expression rate 15.38%, in the descending colon group was 3.33%, in the junction of the descending colon and sigmoid group was 0%, in the sigmoid colon group was 1.69%, in the junction of the rectum and sigmoid colon group was 0%, in the rectum group was 0.15%, and in the anal canal group was 0% (**Figure 8**). Therefore, a lack of expression was most frequently found in the transverse colon splenic flexure (P<0.05).

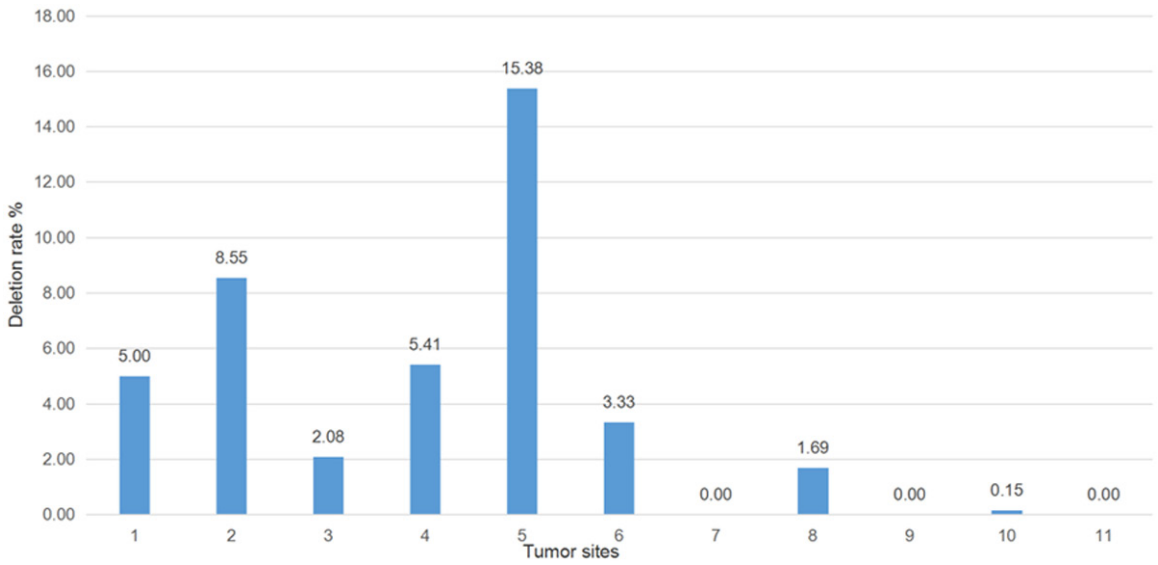

Figure 8. Deletion rate of MSH2 protein expression in different tumor sites. 1. Ileocecal group. 2. Ascending colon group. 3. Transverse colon hepatic flexure group. 4. Transverse colon group. 5. Transverse colon and splenic flexure group. 6. Descending colon group. 7. Descending colon sigmoid junction group. 8. Sigmoid group. 9. Rectosigmoid junction group. 10. Rectum group. 11. Anal group.

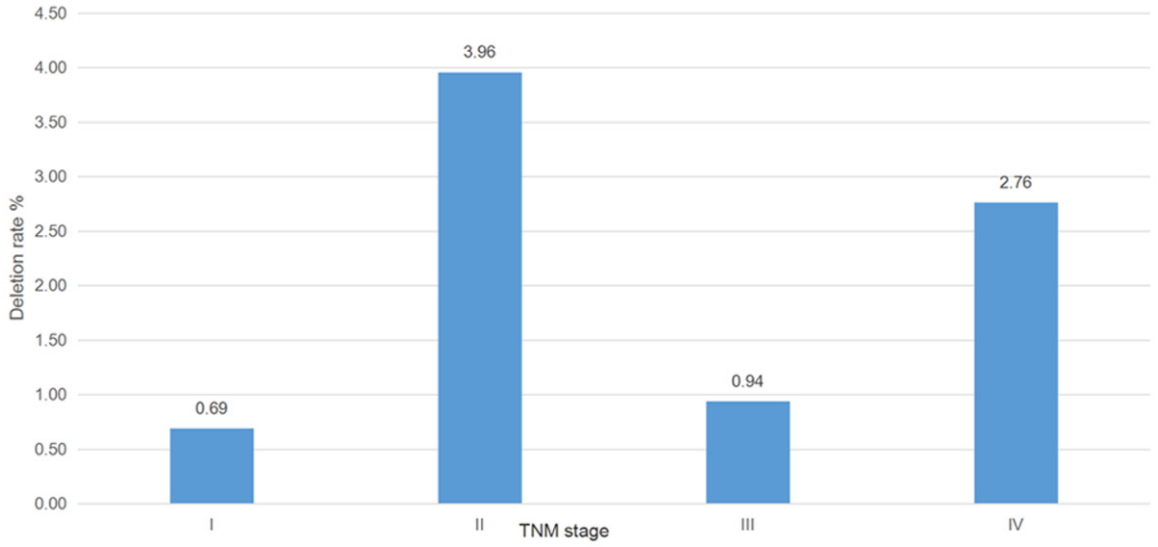

Figure 9. Deletion rate of MSH2 protein expression in TNM stage.

In TNM stage, the expression deletion rate of MSH2 in group II (3.96%) was significantly higher than that in group I (0.69%), group III (0.94%) and group IV (2.76%) ($P<0.05$) (**Figure 9**).

MSH6 protein deletion in tumors

There were significant differences in the deletion rate of MSH6 protein between dMMR patients and PMMR patients in terms of age, tumor site and TNM stage (**Table 3**, $P<0.05$).

The deletion rate of MSH6 protein at age ≤65 (3.38%) was higher than that at age >65 (1.24). The difference was statistically significant ($P<0.05$).

The deletion rate of MSH6 protein in the ileocecal group was 2.50%, in the ascending colon group was 11.11%, in the transverse hepatic flexure group was 6.25%, in the transverse colon group was 8.11%, in the transverse colon splenic flexure group expression rate 15.38%,

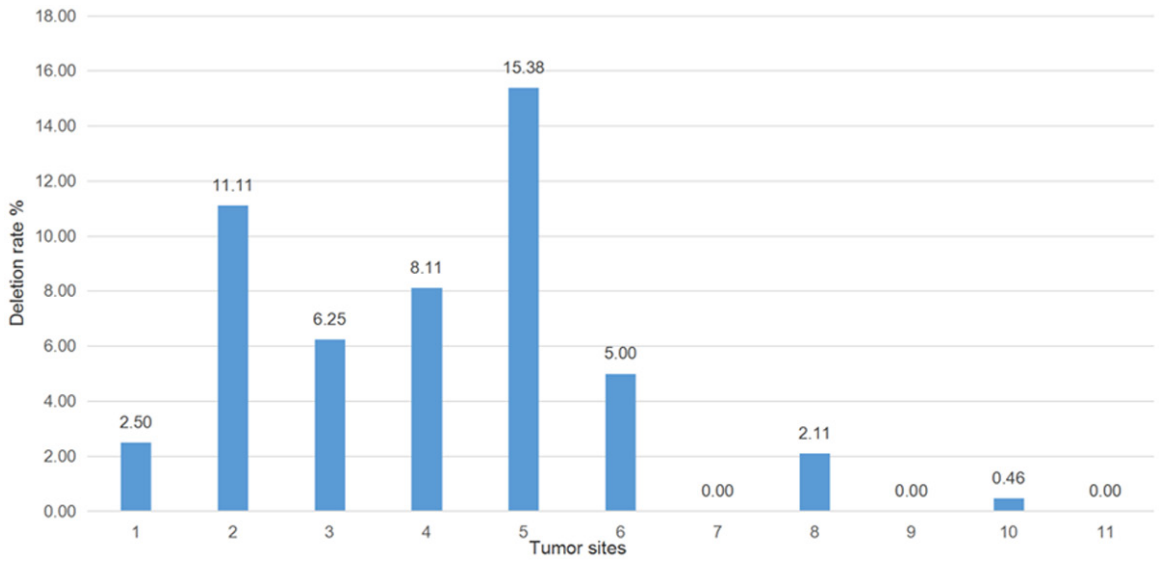

Figure 10. Deletion rate of MSH6 protein expression in different tumor sites. 1. Ileocecal group. 2. Ascending colon group. 3. Transverse colon hepatic flexure group. 4. Transverse colon group. 5. Transverse colon and splenic flexure group. 6. Descending colon group. 7. Descending colon sigmoid junction group. 8. Sigmoid group. 9. Rectosigmoid junction group. 10. Rectum group. 11. Anal group.

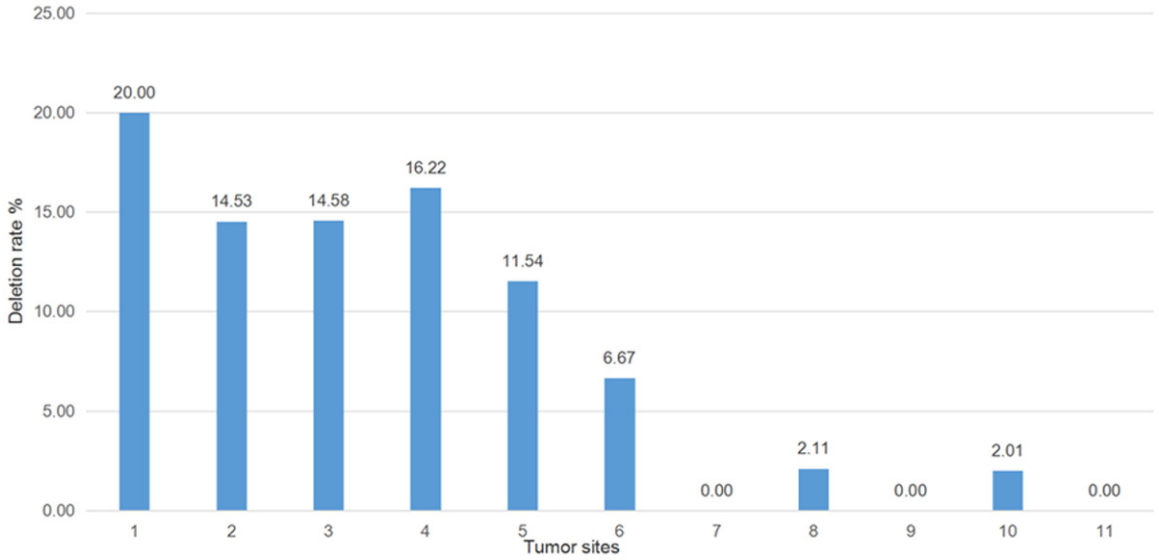

Figure 11. Deletion rate of PMS2 protein expression in different tumor sites. 1. Ileocecal group. 2. Ascending colon group. 3. Transverse colon hepatic flexure group. 4. Transverse colon group. 5. Transverse colon and splenic flexure group. 6. Descending colon group. 7. Descending colon sigmoid junction group. 8. Sigmoid group. 9. Rectosigmoid junction group. 10. Rectum group. 11. Anal group.

in the descending colon group was 5.00%, in the junction of the descending colon and sigmoid group was 0%, in the sigmoid colon group was 2.11%, in the junction of the rectum and sigmoid colon group was 0%, in the rectum group was 0.46%, and in the anal canal group was 0% (**Figure 10**). Therefore, the lack of expression was most frequently found in the transverse colon and splenic flexure. The difference was significant ($P<0.05$).

The deletion rate by TNM stage: in the stage I group was 0.69%, in the stage II group was 4.75%, in the stage III group was 0.38%, and in

Table 4. Logistic regression analysis of dMMR in one thousand two hundred and thirty-eight patients with CRC

Variate	Single factor analysis		Multiple-factor analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender	0.559 (0.327-0.958)	0.034	/	/
Age	1.788 (1.044-3.062)	0.034	/	/
Tumor site	0.717 (0.672-0.765)	0.000	0.706 (0.658-0.757)	0.000
T stage	1.923 (1.374-2.690)	0.000	1.823 (1.191-2.788)	0.006
N stage	0.712 (0.539-0.941)	0.017	/	/
M stage	0.734 (0.384-1.403)	0.350	/	/
TNM stage	0.886 (0.702-1.117)	0.307	/	/
Differentiation	0.896 (0631-1.271)	0.537	/	/
General type	0.894 (0.733-1.090)	0.269	/	/
Adenocarcinoma or non-adenocarcinoma	0.000 (0.000-0.000)	0.999	/	/

the stage IV group was 2.21%. The deletion rate in stage II group was higher than that in other groups. The difference was significant (P<0.05).

PMS2 protein expression deletion in tumors

The dMMR patients and pMMR patients showed significant differences in the deletion rate of PMS2 protein in tumor site, T stage, and differentiation (**Table 3**, P<0.05).

Its deletion rate of the PMS2 protein in the ileocecal group was 20.00%, in the ascending colon group was 14.53%, in the transverse hepatic flexure group was 14.58%, in the transverse colon group was 16.22%, in the transverse colon splenic flexure group was 11.54%, in the descending colon group was 6.67%, in the junction of the descending colon and sigmoid group was 0%, in the sigmoid colon group was 2.11%, in the junction of the rectum and sigmoid colon group was 0%, in the rectum group was 2.01%, and in the anal canal group was 0% (**Figure 11**). Therefore, the loss of expression was most frequently found in the ileocecal area (P<0.05).

Distribution of the deletion rate of PMS2 protein in T stage: in T1 group was 0.00%, in T2 group was 2.96%, in T3 group was 4.49%, and in the T4 group was 8.36%. The difference was statistically significant (P<0.05).

Distribution in the degree of tumor differentiation: the deletion rate of PMS2 protein in the poor differentiation group was 12.82%, in the medium differentiation group was 4.29%, in the high differentiation group was 0.00%, and in the mucinous adenocarcinoma group was 0.08%. The poor differentiation group rate was significantly higher than in other groups (P<0.05).

Logistic regression analysis of dMMR with CRC

Multivariable logistic analysis was used to evaluate the correlation of the mismatch repair protein deficiency with clinical pathological characteristics of CRC. The above significant (P<0.05) factors were introduced into the multivariate logistic regression model. The result shows that lack of mismatch repair protein expression in patients with CRC was closely related to the tumor site, and T stage. This suggests that the detection of mismatch repair protein expression deficiency is beneficial to the risk and prognosis assessment of patients with CRC (**Table 4**).

Discussion

MSI is mainly caused by mismatch repair gene defects. Until now we found a total of nine mismatch repair proteins, but mainly four kinds, MLHl, PMS2, MSH2 and MSH6, were studied. These proteolytic products are all nucleic acid hydrolases. This hydrolytic enzyme can correct the mismatched bases in the process of DNA replication and make the gene more authentic in the process of DNA replication.

In the occurrence and development of CRC, 90% of MMR gene mutations are mainly caused by the inactivation of MLHl and MSH2 [10], and the methylation of the MLHl promoter mainly leads to the high expression of MSI [11]. MSI

can cause the mutation/inactivation of the MMR gene. In addition, the MSH6 mutation alone can only lead to partial loss of MMR function, but missense mutation of MSH2 leads to inactivation of the MSH2/msh6-dependent MMR gene. These two conditions result in the functional loss of the MMR system and the inability to repair the mismatched bases in a timely manner during DNA replication, thus leading to the activation of oncogenes and the inactivation and accumulation of tumor suppressor gene mutations, resulting in occurrence of CRC.

In this study, MMR protein detection was carried out by expanding the sample size, and the results showed that there were 100 cases of MMR protein deletion in one thousand two hundred and thirty-eight cases, with the deletion rate of 8.08%, which was lower than the results of the Hispanic population from Puerto Rico reported by De Jesus-Monge et al. (10.24%) [12], and the Chinese population reported by Ye et al. (9.9%) [13]. However, in a study on Mashhad, the loss rate was only 4.3% [14], and Cheah reported 14.8% in Malaysia and India [15]. It can be seen that the proportion of dMMR in CRC varies from country to country and race to race.

Amira et al. [8] reported that the miss rates of MLH1, MSH2, MSH6, and PMS2 were 15%, 21%, 13% and 15%, respectively. Our experiment showed that the missing rates of MLH1, MSH2, MSH6, and PMS2 were 3.47%, 2.10%, 2.83% and 5.09%, respectively. According to the research results of Kim et al. [16]. in Koreans, the missing rates of MLH1, MSH2 and MSH6 were 3.7%, 2% and 5.2%, respectively. This shows that the types and proportions of deleted proteins are different among the studies. The MMR protein deletion rate in CRC was poorer in China and Korea. In China, MLH1 and PMS2 proteins were mainly absent. These results suggest that there are differences in the mutated genes between different populations and regions. This further reflects the necessity of formulating the CRC diagnosis and treatment norms in China.

Among the 100 dMMR patients with CRC, 41 cases had combined MLH1 and PMS2 deletions (41.00%), and 20 cases had combined MSH2 and MSH6 deletions (20.00%). The single deletion rate of MLH1 protein was 1.0%, of MSH2 protein was 5.00%, of MSH6 protein was 11.00%, and of PMS2 protein was 18.00%. This is basically consistent with proportions reported in the literature [14].

We also found 41 cases of MLH1 and PMS2 co-expression loss, and 20 cases of MSH2 and MSH6 co-expression loss. The correlation was positive ($P<0.05$). This indicates that MLH1 and PMS2, MSH2, and MSH6 are often co-expressed or missing. The MMR system is a safety net in the body that maintains the integrity and stability of genetic material. The MMR system may involve the participation of multiple molecules and enzymes in DNA repair. Among them, MSH2 and MLH1 are the main participants, and they often play a role in dimer formation with their homologous MMR proteins. MLH1 is homologous to PMS2, and MSH2 is homologous to MSH6 and MSH3 [17]. The MLHl protein combines with the PMS2 protein to form a dimer, which jointly participates in the activation of endonuclease by the body. The MSH2 protein binds to the MSH6 protein and plays a role in the process of mismatch repair as a complex protein [18, 19]. This repair mechanism mentioned above may be a good explanation for our experimental results.

In this group, patients with MLH1 protein deletion did not always possess a PMS2 protein deletion, and patients with MSH2 protein deletion did not always have a MSH6 protein deletion. Therefore, this finding is consistent with Shia [20] reporting that screening for MMR in CRC requires screening not only MLH1 and MSH2, but also PMS2 and MSH6 protein expression needs to be screened.

We found that the MMR protein deficiency was related to the tumor site and the depth of invasion, and it was significantly higher in the dMMR group in ascending colon (27.35%) and transverse colon splenic flexure (26.9%) than in other sites. The missing rate of MLHl and PMS2 expression in ileocecal (20.00%) was higher than that in other sites, while the loss of MSH2 and MSH6 expression in transverse colon and splenic flexure (15.38%) was higher than in other sites. It may be that the proximal colon (cecum, ascending colon) is farther away from the anus and the outside world, which is less affected by external factors and more susceptible to genetic factors. Therefore, CRC caused by the lack of MMR expression is more likely to

occur in the proximal colon. The characteristics of the tumor site may be a significant feature of CRC caused by the absence of MMR expression, but further studies are needed to draw more consistent conclusions.

Our study showed that MLH1 and PMS2 deletion was more common in poorer-differentiated proximal CRC tissues, which was consistent with other studies [21, 22]. The deficiency of MSH2 and MSH6 protein expression is more common in patients aged ≤65 years old than in the pMMR group, which is of great significance for clinical screening of Lynch syndrome and guiding its family members to make early diagnosis and prevent the occurrence of CRC or parenteral cancer.

This study showed statistically significant differences between the dMMR group and the pMMR group in tumor site, infiltration depth, TNM stage, and degree of differentiation ($P<0.05$). This is consistent with other studies [23].

MMR defects may not always cause MSI. For example, sporadic endometrial cancer is not caused by mismatch repair defects, and may be caused by the positive MSI of other tumor-related gene mutations, or the existence of other undetected repair genes.

Overall, tumorigenesis is a complex process with multiple genes, multiple factors and multiple steps, which is the result of the combined action of oncogenes, tumor suppressor genes, and other related repair systems. The major causes of CRC may be the lack of MLH1, MSH2, MSH6 and PMS2 expression. Immunohistochemistry was used to detect the expression deficiency of them in CRC patients, and dMMR or pMMR was preliminarily determined for CRC, and the missing mismatch protein type could be suggested. It is hoped that this provides more comparison and reference data for clinical practice. However, the relationship between the protein expression of the four mismatch repair genes and the clinical findings needs to be confirmed by collecting more case tissues and further detailed studies. The expression of mismatch repair protein is absent in some CRC tissues, and is closely related to tumor site and T stage, which may be valuable for clinical treatment and prognosis of CRC.

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Disclosure of conflict of interest

None.

Abbreviations

CRC, Colorectal cancer; MMR, Mismatch repair; MSI, Microsatellite instability; MSI-H, Microsatellite instability-high; PCR, Polymerase chain reaction.

Address correspondence to: Yu Chen, Department of Medical Oncology, Fujian Cancer Hospital and Fujian Medical University Cancer Hospital, 420 Fu'ma Road, Fuzhou 350000, Fujian, China. Tel: +86-13859089836; E-mail: chenyu1980@fjmu.edu.cn

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