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Original Article

The utility of histopathological features predicting microsatellite high (MSI-H) colorectal cancer in a limited setting

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Abstract: Background: Colorectal cancer (CRC) is one of the most common cancers worldwide. Approximately 15% of CRCs have microsatellite instability (MSI), which is caused by a dysfunction of the DNA mismatch repair (MMR) genes. The histopathological features have been applied to predict high frequency MSI (MSI-H) in CRCs, and we investigated this association with MSI-H and the histopathological features. Materials and methods: Fifty-four tumors were evaluated based on nine histopathological features associated with MSI-H. DNA was extracted and prepared from the FFPE of tumors and normal colon tissue. Five microsatellite markers (BAT25, BAT26, D2S123, D5S346, and D17S250) were screened. Results: Of the 54 cases, 38 (70.4%) were between 50-60 years old, and 16 (29.6%) were younger than 50 years. Tumors mainly presented in the left-side colon (40 cases, 74.1%). MSI-high (MSI-H) was found in 7 cases, MSI-low (MSI-L) was found in 7 cases, and the rest were microsatellite stable (MSS). The majority of CRC with MSI-H showed a Crohn-like lymphocytic reaction, mucinous features, tumor grade 2, and tumor-infiltrating lymphocytes. Only the mucinous component was significantly associated with the MSI-H ($P < 0.05$). Conclusion: The initial prevalence of MSI-CRCs in our study (via 5 microsatellite markers) was similar to previous studies. Some histopathological features helped to guide the MSI testing. Even though only the mucinous features were related to the MSI-H status, none of MSI-H CRCs lacked any of the of 9 selected histopathological features. Our study was limited by selected age groups and a small sample size. Further studies with more samples including all age groups should be performed to demonstrate the usefulness of the histopathological features in predicting the MSI in CRC.

Keywords: Colorectal cancer (CRC), microsatellite instability (MSI), histopathology, mismatch repair (MMR) genes

Introduction

Colorectal cancer (CRC) is one of the most common cancers in both sexes and represents almost 10% of global cancer incidence [1]. The microsatellite instability (MSI) pathway is one of three major molecular pathways playing a role in carcinogenesis. Approximately 15% of CRC involves a defect in this pathway [2]. The MSI pathway is characterized by mismatch repair (MMR) protein deficiency, arising through a mutation of the MMR genes (*MLH1, MSH2, MSH6,* and *PMS2*) or through a promoter methylation of MLH1 that results in a hypermethylated state [1]. Sporadic MSI-CRCs occur as a result of the epigenetic inactivation of the *MLH1* gene, while Lynch syndrome (LS) is defined by the presence of a germline mutation in the DNA MMR genes or mutations in the *EPCAM* gene [3]. The sporadic MSI-CRCs account for 10% to 15% of CRCs, while LS represents 3% of CRCs [2].

Defects in one of four MMR genes lead to the pathogenesis of MSI-CRC. In the MMR mechanism defects, DNA sequence errors including single base-pair mismatches and small insertion or deletion loops (IDLs) persist. The successful replication of this errant DNA strand results in the permanent fixation of the incorrectly inserted base or an extra repeated sequence bulge in the genome. Therefore, CRC with the defect in the MMR genes indicates the MSI status [2, 4]. Microsatellite stable (MSS) or MSI tumors are usually analyzed via the polymerase chain reaction amplification of 5-10 mononucleotides or higher order microsatellites.

In clinical practice, it is important to identify MSI-H CRC because it has a better overall prognosis when compared to microsatellite stable (MSS) tumors [5]. It is less responsive to 5-fluorouracil-based chemotherapy regimens but has a better response to immunotherapy with PD-L1/PD-1 blocking drugs [6, 7]. Patients with LS are at risk for synchronous and/or metachronous LS-related tumors in other organs including the endometrium, ovaries, duodenum, urinary tract, stomach, pancreas, biliary tree, and brain. Genetic counselling and the close surveillance of at risk patients and their relatives can result in early cancer detection and intervention to decrease the disease-specific mortality [8].

At present, a traditional screening program for LS is being changed into universal screening for LS in all cases of newly diagnosed CRCs, as recommended by many organizations [3, 8-10]. The algorithm of the universal screening for LS initially performs MSI testing or MMR protein immunohistochemistry (IHC) testing. Many studies confirm that the universal screening test is a feasible, cost-effective, and more sensitive test than the previous clinical criteria. The universal test identifies cases of LS that may be missed when traditional clinical criteria are used. However, this universal strategy might not be available in all countries. The selective eligibility criteria are essential in countries with limited resources. Many studies have validated the clinical and histological features predicting the microsatellite status of CRCs [2, 11-15]. These include young age, right-side CRCs, a Crohn's like reaction, tumor-infiltrating lymphocytes (TIL), tumor grade, mucinous differentiation, signet ring differentiation, the medullary pattern, tumor border configuration, tumor necrosis, and the AJCC stage.

The purpose of our study was to identify the prevalence of MSI-CRCs in selected Thai patients via five microsatellite markers (BAT25, BAT26, D2S123, D5S346, and D17S250) and to prove the advantage of applying the clinical and histopathological features in the MSI prediction.

Materials and methods

Case selection

We studied 54 primary CRCs in patients below 60 years old (diagnosed at Naresuan University Hospital, Faculty of Medicine, Naresuan University, Thailand between 2005 and 2016). All the cases were resected primary CRCs with available paraffin blocks of tumor and corresponding normal tissue. The entire original hematoxylin and eosin (H&E) slides and the surgical pathology reports of each case were reviewed by a single pathologist (J.S). One paraffin block of a tumor with an advancing edge and normal colonic tissue was selected and sent to the Pathological Diagnostic Unit where H&E slides and ten, 6 micron unstained non-heated sections from each block were prepared for molecular studies. This study was approved by Naresuan University Institutional Review Board (NUIRB). For this retrospective study, formal consent was not required.

Histopathological analysis

All the tumors were reviewed in a blind study by a single gastrointestinal pathologist (J.S) and based on the following histopathologic criteria, including the MSI-High histologic features [12, 16-21].

*Crohn-like lymphocytic reaction (**Figure 1A**):* At the advancing edge of the tumor, a Crohn-like lymphocytic reaction represented significant a host response reaction requiring a minimum of 3 lymphoid nodules [12, 20].

*Mucinous differentiation (**Figure 1B**):* Based on the WHO 2010, adenocarcinomas consisting of more than 50% of the extracellular mucinous component were classified as mucinous adenocarcinoma and adenocarcinoma with a mucinous component if the extracellular mucinous area was less than 50% [20]. The mucinous pools contained tumor clusters, malignant acinar or glandular structures, or individual tumor cells including signet ring cells.

Signet ring cell differentiation: Tumors were classified as Signet ring cell carcinoma if more than 50% of the tumor areas had the presence of signet ring tumor cells that typically displayed prominent intracytoplasmic mucin with displacement and a molding of the nucleus [20] and adenocarcinoma with a signet-ring cell component if the signet ring cells were less than 50%.

*Medullary growth Pattern (**Figure 1C**):* The majority of the tumors had solid sheets with

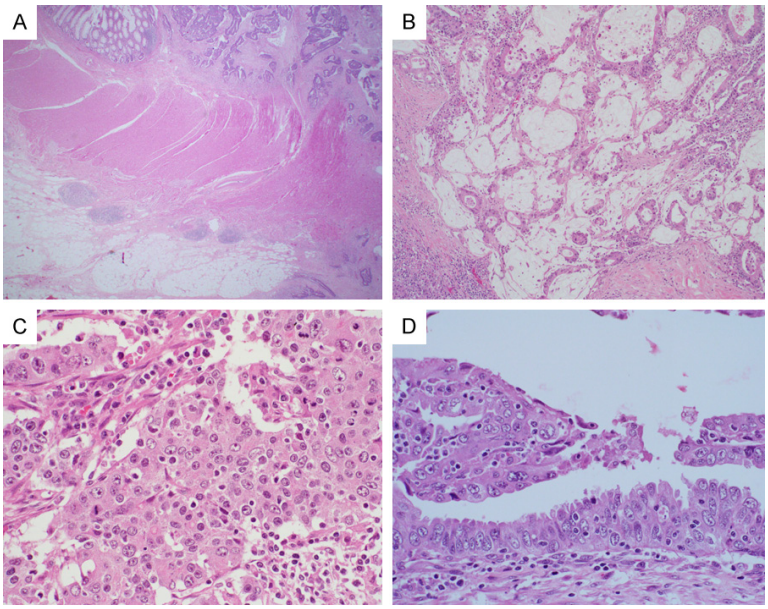

Figure 1. Histopathological features of MSI-H CRCs: (A) Crohn-like lymphocytic reaction, (B) Mucinous differentiation, (C) Medullary growth pattern, and (D) Tumor infiltrating lymphocytes (TILs).

prominent (intraepithelial) lymphoid infiltrate, and the tumor cells typically displayed vesicular nuclei, prominent nucleoli, and abundant eosinophilic cytoplasms and were categorized as Medullary carcinoma [22]. The tumors were classified as carcinoma with focal medullary features if the medullary areas were less than 70% [20].

Tumor grade: Based on the quantitative criteria of the glandular structures, tumor grade was classified in a 4-tiered grading system [23]. Tumors with more than 95% glandular formation were classified as grade 1 or well-differentiated and grade 3 or poorly differentiated with the glandular formation at less than 50%. Tumors with no gland formation or mucin; no squamous or neuroendocrine differentiation were classified as undifferentiated or grade 4.

Histologic Heterogeneity: Tumors with at least 2 different growth patterns, including tumor grade, were classified according to the presence or absence of histologic heterogeneity. Mucinous and non-mucinous tumors were not assigned histologic heterogeneity [12].

Growth pattern at advancing edge: At the advancing edge, tumors with pushing or circumscribed borders and the absence of wildly dissecting tumor glands were described as having an expansile growth pattern, and tumors without a clearly recognized border (irregular or uneven), irregular dissecting glands into underlying structures, and the presence of residual host tissue between infiltrating glands were recognized as having an infiltrative growth pattern [12, 14].

Tumor necrosis: Dirty necrosis is usually present in colorectal cancer. Tumors with less than 10% necrosis were classified as having no tumor necrosis [12].

*Tumor-infiltrating lymphocytes (TIL) (**Figure 1D**):* The quantitation of the Intratumoral T cell Infiltrate in tumors was performed in the area with the most TIL. Five consecutive 40X fields (Olympus BX50 microscope with the UPlanFI objective) were observed. The mean TIL/high-powered field (HPF) for each tumor was calculated by dividing the total number of TIL by 5. Tumors with 2 or more TILs/HPF were classified as positive for TIL [12, 17].

Molecular analysis

DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissues: Up to ten, 6 micron unstained non-heated sections from the tumor paraffin block and the corresponding normal colonic tissue of each case were obtained. Each designated area was identified and marked on a reference H&E slide by the pathologist and then microdissected using a clean scalpel blade. The DNA was extracted using a QIAamp DNA FFPE kit (Qiagen), following the established protocol.

Microsatellite analysis: The microsatellite analysis was performed on the matched tumor and normal DNA samples following the National Cancer Institute (NCI) reference marker panel for the evaluation of MSI in CRC. This panel consists of two mononucleotides (BAT25, BAT26), and three dinucleotide (D2S123, D5S346, and D17S250) repeats [24].

Table 1. Personal data and clinical features of the 54 selected patients and their MSI-status

Features	NO. (%)	MSS NO. (%)	MSI-L NO. (%)	MSI-H NO. (%)
Age				
50 to 60	38 (70.4)	30 (79)	4 (10.5)	4 (10.5)
below 50	16 (29.6)	10 (62.5)	3 (18.75)	3 (18.75)
Sex				
Male	31 (57.4)	23 (74.2)	4 (12.9)	4 (12.9)
Female	23 (42.6)	17 (74)	3 (13)	3 (13)
Location				
Right-side colon	14 (25.9)	9 (64.3)	2 (14.3)	3 (21.4)
Left-side colon	40 (74.1)	30 (75)	4(10)	6 (15)
AJCC stage				
Stage 1	6 (11.1)	15 (68.2)	3 (13.6)	4 (18.2)
Stage 2 (A to C)	16 (29.6)			
Stage 3 (A to C)	29 (53.7)	25 (78.1)	4 (12.5)	3 (9.4)
Stage 4 (A to C)	3 (5.6)			

Fifty to one hundred nanograms of DNA was amplified separately with a forward primer labelled with fluorescent dye and the specific unlabeled reverse primers [25], and Amplitaq Gold® 360 Master Mix (Life Technologies) followed the protocol as described previously [25]. The PCR products were pooled, and the size of each of the PCR amplicons was analyzed using capillary electrophoresis (3500 Genetic Analyzer, Life Technologies). The shift of the PCR products from tumor DNA was compared to the size of the DNA from the corresponding normal colonic mucosa. The size of each fluorescent PCR product was calculated using GeneMapper® software (Applied Biosystems).

In accordance with the National Cancer Institute (NCI) consensus [24], the interpretation criteria require at least 5 loci (≥ 30-40% of analyzed loci). If more than 1 of the 5 loci showed instability, the tumor was interpreted as MSI-H; with only 1 unstable locus, it was interpreted as MSI-L. A sample with no instability at five loci would be MSS [16].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for PC was used for the data analysis. The evaluation of the metric and categorical parameters was performed using descriptive statistics including the means, minimums, maximums, frequencies, and percentages. The sensitivities, specificities, positive predictive values, and negative predictive values for the assessment of the histologic features of MSI-H were calculated against the gold standard of MSI PCR results. A logistic regression analysis was performed to correlate the histologic features of MSI-H with the MSI PCR results. The level of statistical significance was set to p-value < 0.05.

Results

Personal data and clinical features of the selected patients and MSI-status

A total of 54 resected colorectal carcinomas was initially selected based on the Revised Bethesda guidelines [16]. They were divided into two age groups. 16 (29.6%) of the selected patients were younger than 50 years, and 38 (70.4%) were between 50 and 60-years-old. Of these, 31 (57.4%) were males and 23 (42.6%) were females. Left-sided CRC was predominant (40 patients, 74.1%), but 14 patients (25.9%) presented right-side CRC. The majority of the patients were at stages 2 (29.6%) and 3 (53.7%) according to the AJCC 8th edition [23] *(**Table 1**)*.

The determination of MSI using the 5-marker panel (BAT25, BAT26, D2S123, D5S346, and D17S250) with matched normal DNA revealed 40 (74%) MSS, 7 (13%) MSI-L, and 7 (13%) MSI-H. The number of CRCs with MSI-H were not clearly different between the two age groups, but in each group, the percentage of patients younger than 50 years (18.8%) was higher than it was in the age group between 50 and 60-years-old (10.5%). For gender, the numbers and percentages of CRCs with MSI-H were similar; 4 (12.9%) cases in males and 3 (13%) cases in females. 6 (15 %) of the 40 left-side CRCs and 3 (21.4%) of the 14 right-side CRCs had MSI-H status. The numbers and percentages of low AJCC stage (I and II) with MSI-H were higher than the high stage (III and IV). Four (18.2%) CRCs with low AJCC stages and 3 (9.4%) CRCs with high stages were MSI-H *(**Table 1**)*.

The histologic features of 54 selected patients correlated with MSI-status

A total of 54 consecutively resected colorectal carcinomas were evaluated for their histologic

Table 2. Histologic features of the 54 selected patients and MSI-status

Histologic Features	N (%)	MSI status		
		MSS (%)	MSI-L (%)	MSI-H (%)
Crohn-like lymphocytic reaction				
Presence (> 3 lymphoid nodules per section)	39 (72.2)	27 (69.2)	6 (15.4)	6 (15.4)
Absence	15 (27.8)	13 (86.6)	1(6.7)	1 (6.7)
Mucinous features				
Presence	20 (37)	10 (50)	4 (20)	6 (30)
Absence	34 (63)	30 (88.2)	3 (8.8)	1 (3)
Signet ring cell features				
Presence	6 (11.1)	6 (100)	-	-
Absence	48 (88.9)	34 (70.8)	7 (14.6)	7 (14.6)
Medullary features				
Presence	9 (16.7)	6 (66.7)	-	3 (33.3)
Absence	45 (83.3)	34 (75.5)	7 (15.6)	4 (8.9)
Tumor differentiation				
Well (G1)	6 (11.1)	3 (50.0)	2 (33.3)	1 (16.7)
Moderate (G2)	46 (85.2)	36 (78.2)	5 (10.9)	5 (10.9)
Poor and undifferentiated (G3 + G4)	2 (3.7)	1 (50)	-	1 (50)
Histologic Heterogeneity				
Presence	2 (3.7)	1 (50)	-	1 (50)
Absence	52 (96.3)	39 (75)	7 (13.5)	6 (11.5)
Growth pattern at advancing edge				
Infiltrative	53 (98.1)	39 (73.6)	7 (13.2)	7 (13.2)
Expansile/Pushing	1 (1.9)	1 (100)	-	-
Tumor Necrosis				
Presence	46 (85.2)	36 (78.3)	6 (13)	4 (8.7)
Absence	8 (14.8)	4 (50)	1 (12.5)	3 (37.5)
Tumor-infiltrating lymphocyte: Mean/HPF				
Absence (< or = 2/HPF)	18 (33.3)	15 (83.3)	2 (11.1)	1 (5.6)
Presence (> 2/400X HPF)	36 (66.7)	25 (69.4)	5 (13.9)	6 (16.7)

HPF, high-power field.

features before and without any knowledge of the final MSI PCR results. At least nine histological features were studied in each case *(**Table 2**)*. Four out of the 9 features are known to be related to MSI-H histological features in the Revised Bethesda guidelines, including Crohn-like lymphocytic reaction, mucinous differentiation, medullary growth pattern, and signet ring cell differentiation. The five remaining features were previously analyzed in the literature and showed an association with MSI-H [12, 17]. The limited numbers of the 54 CRCs presented distinct histologic features that included 6 (11.1%) CRCs showing signet ring differentiation, 9 (16.7%) with medullary features, 8 (14.8%) with unusual tumor grades (G1, G3, or G4), 2 (3.7%) with histologic heterogeneity, 1 (1.9%) with an expansile growth pattern at the advancing edge, and 8 (14.8%) with no tumor necrosis. Based on the MSI status, a majority of the 7 CRCs with MSI-H displayed Crohn-like lymphocytic reactions as well as mucinous differentiations and tumor-infiltrating lymphocytes (TILs).

Based on the revised Bethesda guidelines, three patients who were older than 50-years-old and who lacked any MSI-H associated histological features were excluded. The correlation of the clinical and histologic features of the CRCs with MSI PCR results were analyzed via the logistic regression method. The sensitivity, specificity, positive predictive value, and negative predictive value of each of the MSI-H and

Table 3. Logistic regression analysis of each of the clinical and histologic features in predicting MSI-H status

Histologic Features	Sensitivity	Specificity	PPV	NPV	Odds Ratio	95% confidential interval (lower-upper)	*p*-value
Crohn-like lymphocytic reaction	85.71	32.43	19.35	92.30	2.222	0.237-20.830	0.484
Any mucinous differentiation	85.71	72.97	37.50	96.42	16.200	1.728-151.850	0.015*
Any signet cell differentiation	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Medullary growth Pattern	42.85	83.78	33.33	88.57	3.875	0.685-21.934	0.126
Unusual tumor grades (G1/G3 + G4)	28.57	89.18	33.33	86.84	3.330	0.474-22.977	0.228
Histologic heterogeneity	14.28	97.29	50	85.71	6.000	0.329-109.419	0.226
Expansile growth pattern at advancing edge	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Absence of tumor necrosis	42.85	89.19	42.85	89.19	6.187	1.001-38.243	0.050
Tumor-infiltrating lymphocyte	85.71	37.83	20.68	93.33	3.652	0.397-33.587	0.253

*statistically significant at *p*-value < 0.05; n/a, not applicable; NPV, negative prediction value; PPV, positive prediction value.

Table 4. Results of the MSI testing of the 14 patients with MSI-L (No.1-7) and MSI-H (No.8-14)

No.	Age	Clinical data	BAT25	BAT26	D2S123	D5S346	D17S250
1	48	Left-side, stage IIA			x		
2	52	Left-side, stage IIIA			x		
3	47	Left-side, stage IIIB					x
4	46	Left-side, stage I			x		
5	57	Right-side, stage IIIc			x		
6	50	Right-side, stage IIB			x		
7	53	Left-side, stage IIIC				x	
Percentage of instability in MSI-L			0%	0%	71.4%	14.3%	14.3%
8	45	Right-side, stage IIA	x			x	
9	50	Left-side, stage IIA	x	x			x
10	37	Right-side, stage I	x				x
11	51	Left-side, stage IIA	x		x		x
12	50	Left-side, stage IIB	x	x			
13	50	Right-side, stage IIIB	x	x			
14	31	Left-side, stage IIIC	x	x		x	
Percentage of instability in MSI-H			100%	71.4%	14.3%	28.5%	42.9%

X, MSI detected in that marker.

MSI-H associated histologic features were calculated. In our study, any mucinous differentiation was a statistically significant predictor of MSI-H *(**Table 3**)*. This feature had a sensitivity of 85.71%, a specificity of 72.97%, a positive predictive value of 37.5%, and a negative predictive value of 96.42%.

MSI status

Regarding the MSI testing, neither BAT25 nor BAT26 was positive for MSI-L. Out of the 7 CRCs with MSI-H, BAT25 was found in 7 of the tumors, BAT26 instability was found in 5 of the tumors, and D2S123, D5S346, and D17S250 were found in 1, 2, and 3 of the tumors respectively. Five tumors with MSI-H presented both BAT25 and BAT26 instabilities together, and two of them were only BAT25 positive but not BAT26 positive *(**Table 4**)*.

Discussion and conclusion

The determination of the MSI status in colorectal cancers identifies not just the inherited cancers. MSI-H colorectal cancers include Lynch syndrome (HNPCC) and sporadic colorectal cancers that have better prognoses and specific clinical implications. MSI-H colorectal cancers have a better survival rate compared to MSS colorectal cancer [5]. Moreover, MSI status might predict the responses of chemotherapy and immunotherapy [6, 7]. In our setting, the universal screening approach has limita-

tions, including the availability of testing, high costs, and treatment options. Selecting the appropriate primary CRC cases to perform the additional testing would be reasonable and cost effective. We evaluated the correlation of the selected clinical and histopathological features and the MSI status in order to predict MSI-H CRCs which would be useful for treatment planning [2, 11-15].

The overall incidence of MSI-H colorectal cancers in our study was similar to the incidence reported in earlier studies [16, 17] including both LS and the sporadic cases. The study was initially focused on patients aged 60 or under at the time of their cancer diagnosis. The patients in the younger age group (less than 50-years-old) had a higher percentage of MSI-H colorectal cancer than the older (between 50 and 60-years-old) group, a finding that corresponds with previous studies [12, 17, 18, 26-28]. However, the gender distribution of MSI-H CRCs was not significantly different. Also, the percentage of MSI-H in the right-side CRCs was slightly higher than the left-side cancers. The difference between the Asian and Western populations was mentioned in a literature review [29] and in Asian studies [30, 31]. The percentage of the low AJCC stage MSI-H CRCs in our study was higher than the high AJCC stage group, but it did not reach the level of statistical significance. However, this evidence might support a better prognosis in this phenotype of colorectal cancers.

Many studies confirm the distinct histologic features of MSI-H CRCs [2-4, 11, 12, 16, 17, 20, 21]. In our study, nine histopathological features, including Crohn-like lymphocytic reaction, tumor-infiltrating lymphocyte (TIL), mucinous features, signet-ring cell feature, medullary feature, tumor differentiation, histologic heterogeneity, growth pattern at advancing edge, and tumor necrosis were selected and evaluated for the initial prediction of MSI status in CRCs. Of all 54 primary CRCs, most cases have Crohn-like lymphocytic reaction and TIL. The presence of Crohn-like lymphocytic reaction and TIL (2 or more TILs/HPF) were not statistically significant in the MSI-H status, but it yielded highly negative predictive values (92.30% and 93.33%, respectively). However, Crohn-like lymphocytic reaction and TILs were reported to be common histopathological features that usually show an interpretative error [3], and there is still no standard cut-off point for TIL. Variable cut-off values for TIL have been used in previous studies [12, 17, 21, 32].

Based on the MSI-H histology in the revised Bethesda Guidelines for HNPCC, there were few cases of CRCs with any signet ring differentiation or medullary growth patterns. The MSI-H CRCs in our study were significantly related to mucinous differentiation which was a good discriminator between MSI-H and MSS colorectal cancers (odds ratio: 16.2, P = 0.015). Expansile or pushing advanced tumor borders and histologic heterogeneity were rarely present. Most of our cases were moderately differentiated CRCs. Well differentiated or poorly differentiated/undifferentiated adenocarcinoma were unusual histologic grades in our study. We found few cases lacking tumor necrosis. Both CRCs with unusual tumor grades and a lack of necrosis were insignificantly related to the MSI-H status, but had a high specificity (89.18 and 89.19%, respectively) and a negative predictive value (86.84 and 89.19, respectively). All of the MSI-H CRCs presented at least two or more MSI-H selected histopathological features. Crohn-like lymphocytic reactions, mucinous differentiations, and tumor-infiltrating lymphocytes (> 2 TIL/HPF) presented in almost all MSI-H CRCs.

Regarding the 5-microsatellite panel, the mononucleotide marker is commonly instable in MSI-H [33] and the dinucleotide complex, i.e. D17S250 is more unstable than the noncomplex dinucleotide repeat, i.e., D2S123 [34]. Our results corresponded to previous studies indicating that the D2S123 instability is more frequent than the D17S250 instability in MSI-H [35, 36]. However a previous study on Thai CRC patients found the microsatellite instability of BAT-26 in all CRCs [36], while we found BAT-26 instable in 71.4% of MSI-H cases and BAT-25 in all MSI-H colorectal cancers. The BAT26 stability might be related to a large intragenic *MSH2* deletion [37].

Using the 5-marker Bethesda panel, dinucleotide repeats seem to be more sensitive than mononucleotide repeats. Based on the results of the dinucleotide repeats, the MSI status might be an under or overestimation [16]. In our study, 85.7% of the MSI cases showed instability in the dinucleotide repeats (D2S123,

D5S346, and D17S250). Five out of 7 MSI-H tumors had an instability in one dinucleotide repeat, but the instability of the dinucleotide repeats was present in all MSI-L tumors. To prove the equivocal cases, additional MSI markers should be tested. The revised Bethesda guidelines recommend additional mononucleotide markers. For example, NR-21, NR-24, BAT-40, TGF-BetaR, and D18S58 were further studied and the result of MSI-L was shifted to MSI-H [38].

In conclusion, nine histopathological features were selected to evaluate their correlation with MSI status, and only the mucinous feature was significantly related to MSI-H colorectal cancers. Initially, we would like to select the appropriate cases for MSI-testing. The identification of MSI-H colorectal cancers is important for therapeutic selection. However, MSI-H tumors might also be Lynch syndrome or sporadic MSI-H colorectal cancers. Further molecular studies will be the next step.

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Disclosure of conflict of interest

None.

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