

Retrospective Study

Deficient DNA mismatch repair is associated with favorable prognosis in Thai patients with sporadic colorectal cancer

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Supported by Siriraj Research Development Fund No. 459/2554(EC2).

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Received: June 7, 2014

Peer-review started: June 8, 2014

First decision: June 27, 2014

Revised: July 30, 2014

Accepted: September 18, 2014

Article in press: September 19, 2014

Published online: January 21, 2015

Abstract

AIM: To determine the prognostic significance of deficient mismatch repair (dMMR) and *BRAF* V600E in Thai sporadic colorectal cancer (CRC) patients.

METHODS: We studied a total of 211 out of 405 specimens obtained from newly diagnosed CRC patients between October 1, 2006 and December 31, 2007 at Siriraj Hospital, Mahidol University. Formalin-fixed paraffin-embedded blocks of CRC tissue samples were analyzed for dMMR by detection of MMR protein expression loss by immunohistochemistry or microsatellite instability using polymerase chain reaction (PCR)-DHPLC. *BRAF* V600E mutational analysis was performed in DNA extracted from the same archival tissues by two-round allele-specific PCR and analyzed by high sensitivity DHPLC. Associations between patient characteristics, MMR and *BRAF* status with disease-free survival (DFS) and overall survival (OS) were determined by Kaplan-Meier survival plots and log-rank test together with Cox's proportional hazard regression.

RESULTS: dMMR and *BRAF* V600E mutations were identified in 31 of 208 (14.9%) and 23 of 211 (10.9%) tumors, respectively. dMMR was more commonly found in patients with primary colon tumors rather than rectal cancer (20.4% vs 7.6%, $P = 0.01$), but there was no difference in MMR status between the right-sided and left-sided colon tumors (20.8% vs 34.6%, $P = 0.24$). dMMR was associated with early-stage rather than metastatic disease (17.3% vs 0%, $P = 0.015$). No clinicopathological features such primary site or tumor differentiation were associated with the *BRAF* mutation. Six of 31 (19.3%) samples with dMMR carried the *BRAF*

mutation, while 17 of 177 (9.6%) with proficient MMR (pMMR) harbored the mutation ($P = 0.11$). Notably, patients with dMMR tumors had significantly superior DFS (HR = 0.30, 95%CI: 0.15-0.77; $P = 0.01$) and OS (HR = 0.29, 95%CI: 0.10-0.84; $P = 0.02$) compared with patients with pMMR tumors. By contrast, the *BRAF* V600E mutation had no prognostic impact on DFS and OS.

CONCLUSION: The prevalence of dMMR and *BRAF* V600E in Thai sporadic CRC patients was 15% and 11%, respectively. The dMMR phenotype was associated with a favorable outcome.

Key words: Sporadic colorectal cancer; Mismatch repair; *BRAF*; Overall survival

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Core tip: This study is the first report of prevalence and outcome in sporadic colorectal cancer that harbour deficient mismatch repair (dMMR) and *BRAF* gene mutation in Thai population. The prevalence of dMMR and *BRAF* V600E mutation was 15% and 11%, respectively. This study confirmed the favorable outcome in patients with dMMR tumors, which is consistent to the results of previous reports in Caucasian population. The method we used to detect *BRAF* mutation is allele specific polymerase chain reaction which has the highest sensitivity to detect this mutation when compared to previously reported methods.

Korphaisarn K, Pongpaibul A, Limwongse C, Roothumnong E, Klaisuban W, Nimmannit A, Jinawath A, Akewanlop C. Deficient DNA mismatch repair is associated with favorable prognosis in Thai patients with sporadic colorectal cancer. *World J Gastroenterol* 2015; 21(3): 926-934 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i3/926.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i3.926>

INTRODUCTION

Colorectal tumorigenesis is a multistep process that arises from the accumulation of genetic alterations, including chromosomal abnormalities, gene mutations, and epigenetic changes^[1]. With regards to genetic abnormalities, defective DNA mismatch repair (dMMR) is a type of genomic instability in tumor tissue caused by a failure to correct errors during normal DNA replication^[2]. dMMR can be identified either by the presence of microsatellite instability (MSI) analyzed by polymerase chain reaction (PCR) amplification of microsatellite foci in tumor tissue, or lack of protein expression for any of the MMR genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*, detected by immunohistochemistry (IHC). Tumors with dMMR have been reported in

15%-20% of sporadic colorectal cancers (CRC), and dMMR is associated with distinct clinicopathological features such as proximal tumor site, high grade, early stage, and better prognosis^[3].

The *BRAF* gene has 18 exons and encodes a serine/threonine protein kinase belonging to the RAS-RAF-MEK-ERK kinase pathway that is involved in CRC development^[4,5]. The most common activating mutation is found in exon 15 at nucleotide position 1799, whereby a thymine (T) to adenine (A) transversion within codon 600 leads to substitution of valine by glutamate at the amino acid level. This leads to the oncogenic *BRAF* V600E mutation^[6]. *KRAS* and *BRAF* mutations have been reported to be mutually exclusive events within tumors^[6,7]. Several studies reported *BRAF* mutations in 5%-20% of patients with sporadic CRC, with a high frequency in dMMR tumors^[4,8-10]. However, *BRAF* mutations are very rare in CRC patients with hereditary nonpolyposis colorectal cancer (HNPCC)^[11].

Recently, the correlation between dMMR and *BRAF* mutation and CRC prognosis has been widely studied^[8,12,13]. Patients with tumors harboring dMMR were associated with a more favorable survival than those with proficient MMR (pMMR)^[3,14,15]. By contrast, patients with the *BRAF* mutation were associated with a worse clinical outcome, especially patients with pMMR tumors^[12,13,16].

These data, however, all pertain to a Caucasian population, and there is only scarce information available for Asian populations. In this study, we systematically determined the prevalence of dMMR and *BRAF* mutations in Thai patients with sporadic CRC and established correlations with various clinicopathological features to determine their prognostic impact on clinical outcome.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed paraffin-embedded tissue blocks from patients diagnosed with primary colon or rectal adenocarcinoma who underwent surgery between October 1, 2006 and December 31, 2007 were obtained for this study. We excluded patients with a known family history of CRC, those suspected to have hereditary or familial CRC, and those who did not receive treatment and follow-up at our institution. The study protocol was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. This study was supported by the Siriraj Research Development Fund.

Demographic information regarding age, gender, primary tumor site, date of diagnosis, date of surgery, stage at diagnosis, date of disease recurrence, date of last follow-up, and date of death were collected. Staging was classified by AJCC/UICC TMN stage (v.3 2010). Disease-free survival (DFS) is defined as the interval between the date of diagnosis and the date of disease recurrence or death, while overall survival (OS) is the interval between the date of diagnosis and the date of death from any

cause. The primary objective of this study was to determine the prevalence of dMMR and *BRAF* V600E mutations in sporadic CRC patients; the secondary objectives were to examine correlations between MMR status and *BRAF* mutations, and the association of each marker with various clinicopathological characteristics and their prognostic impact on DFS and OS.

Determination of MMR status

MMR status was determined by analysis of MMR protein expression by IHC or MSI testing; dMMR was defined by the presence of either high-level MSI (MSI-H) or loss of MMR protein expression. pMMR was defined by the presence of either microsatellite stable (MSS)/low-level MSI (MSI-L) or the presence of normal MMR protein expression.

IHC analysis of MMR expression

IHC for four MMR proteins, MLH1, MSH2, MSH6 and PMS2, was performed on tissue microarray slides (TMAs). TMAs were assembled from paraffin-embedded tissues using a manual tissue microarrayer (UNITMA Quick-Ray 2 mm-diameter tissue cores). Hematoxylin and eosin stained slides were prepared from paraffin blocks and areas of neoplastic tissue were identified by a gastrointestinal pathologist (A.P.) who selected samples for TMA construction. Duplicated IHC for each MMR protein was performed for each patient sample. Staining for MMR proteins was performed using the following primary antibodies: mouse anti-human MLH-1 (clone G168-728; Cell Marque Corporation, Rocklin, CA), mouse anti-human MSH-2 (clone G219-1129; Cell Marque Corporation, Rocklin, CA), mouse anti-human MSH-6 (clone BC/44; Biocare, Concord, CA), and rabbit anti-human PMS2 (clone EPR3947; Cell Marque Corporation, Rocklin, CA). Loss of MMR protein was defined as the absence of nuclear staining of tumor cells in the presence of positive nuclear staining in normal epithelial cells and lymphocytes. Assessment of IHC staining was performed by the same pathologist (A.P.).

MSI testing

MSI was analyzed by PCR amplification of microsatellite foci from the five-marker Bethesda panel, which includes two mononucleotide (BAT-25 and BAT-26) and three dinucleotide (D5S346, D2S123, and D17S250) repeats. Samples with instability in two or more of these markers were defined as MSI-H, whereas those with one unstable marker were designated as MSI-L. Samples with no detectable alterations were defined as MSS.

Determination of *BRAF* mutation

Formalin-fixed paraffin-embedded tissues with tumorous regions were macroscopically dissected into 10 μ m-thick sections using microtome blades, and then placed into separate tubes for DNA extraction using a standard phenol/chloroform extraction protocol. Probes were designed to target the most common *BRAF*

mutation, a valine to glutamate transition at amino acid position 600 (V600E). *BRAF* V600E was detected using a previously-described two-round allele specific-polymerase chain reaction (AS-PCR)^[17]. The primary AS-PCR reaction was performed using common-primer pairs (CF; 5'-TAATGCTTGCTCTGATAGGA-3', CR; 5' GGAAAAATAGCCTCAATTCT-3') and a *BRAF* V600E-specific primer (Mt; 5'-AAATAGGTGATTTTGGTCTGGCTACGGA-3'), which is located between common-primer pairs, and CR was used as a reverse primer. The final concentration of AS-PCR reactions comprised 100 ng genomic DNA, 0.02 U/ μ L of Immolase DNA polymerase (Bioline, Taunton, MA), 1 \times buffer, 1.5 mmol/L MgCl₂, 0.2 μ mol/L dNTP, 0.4 μ mol/L of each common primer and 0.8 μ mol/L Mt primer. The reaction was amplified with a Mastercycler pro S (Eppendorf, Eppendorf AG, Hamburg, Germany) using the following protocol: activation at 95 $^{\circ}$ C for 10 min followed by 35 cycles at 95 $^{\circ}$ C for 30 s, 60 $^{\circ}$ C for 30s, 72 $^{\circ}$ C for 30 s and a final extension at 72 $^{\circ}$ C for 10 min. Secondary AS-PCR was amplified under the same conditions as the primary reaction, but genomic DNA template was replaced with 1 μ L of the primary AS-PCR product. Secondary AS-PCR product was analyzed by high-sensitivity denaturing high performance liquid chromatography (HS-DHPLC) (Transgenomic Inc., Foster city, CA) equipped with WAVE Optimized HS Staining Solution I and a fluorescence detector in sizing mode. This assay has been shown to have a detection limit of at least 0.5% V600E-positive tumor cells.

Statistical analysis

Sample size determination was based on a *BRAF* mutation and dMMR estimated prevalence of 15% with a 95%CI of 5%: accounting for 5% of possible cases with no paraffin-embedded tumors tissue blocks, a total sample size of 210 patients was required.

Patient characteristics were described by descriptive statistics. Pearson's χ^2 test was applied to evaluate associations between *BRAF*/MMR status and clinicopathological variables. The association between patient characteristics and either *BRAF* status or MMR status with OS and DFS was explored by Kaplan-Meier estimation and log-rank test together with Cox's proportional hazard regression. Calculations were carried out using SPSS-version 18 software. *P* values of less than 0.05 were considered statistically significant.

RESULTS

A total of 405 patients were diagnosed with colon and rectal adenocarcinoma between October 1, 2006 and December 31, 2007. We investigated 211 patients for whom tissue blocks were available. The median age was 63 years (33-95 years). The ratio of males to females was 1:1. One-hundred and fifty patients (71.1%) had stage II and III disease while 29 patients (13.7%) were diagnosed with stage IV disease. The majority of primary

Table 1 Clinical characteristics of 211 patients with sporadic colorectal cancer *n* (%)

Variables	Value
No. of patients	211 (100)
Median age (yr, range)	63 (33-95)
Age (yr)	
≤ 50	30 (14.2)
> 50	181 (85.8)
Sex	
Female	105 (49.8)
Male	106 (50.2)
Site	
Right-sided	43 (20.8)
Left-sided	73 (34.6)
Rectum	91 (43.1)
Synchronous lesions	4 (1.9)
Stage	
I	32 (15.2)
II	65 (30.8)
III	85 (40.3)
IV	29 (13.7)
Bowel wall invasion	
pT1	4 (1.9)
pT2	44 (20.8)
pT3	146 (69.2)
pT4	17 (8.1)
Lymph node metastasis	
pN0	105 (49.8)
pN1	60 (28.4)
pN2	46 (21.8)
Distant metastasis	
No	182 (86.3)
Yes	29 (13.7)
Invasion	
NO	118 (55.9)
LVI	47 (22.3)
PNI	15 (7.1)
Both LVI/PNI	31 (14.7)
Differentiation	
Well	32 (15.2)
Moderately	171 (81)
Poorly	8 (3.8)

pT: Pathological tumor stage; pN: Pathological nodal stage; ALI: Angiolymphatic invasion; PNI: Perineural invasion.

site tumors were rectal (91 patients, 43.1%), followed by left-sided colon tumors (73 patients, 34.6%). Patient and tumor characteristics are shown in Table 1.

Prevalence of dMMR and the BRAF V600E mutation

Of the 211 patients, IHC for MMR proteins and MSI detection was analyzed in 164 and 47 tumors, respectively. dMMR was identified in 10 out of 164 tumors and 21 out of 44 tumors; therefore dMMR was detected in a total of 31 out of 208 tumors (14.9%). We were unable to analyze the results in three patients because of unamplified DNA by PCR. Of the 10 patients with dMMR, interpretation of the IHC staining was as follows: MLH-1 expression was absent in four tumors, while six tumors were negative for MSH-2 expression. Interpretation parameters for IHC of MMR status is shown in Table 2.

The BRAF V600E mutation was identified in 23 of 211 patients (10.9%). The prevalence of the BRAF

Table 2 Interpretation of immunohistochemistry for mismatch repair status

MMR mutation	IHC staining			
	MLH-1	MSH-2	MSH-6	PMS-2
MLH-1	-	+	+	-
MSH-2	+	-	-	+
MSH-6	+	+	-	+
PMS-2	+	+	+	-

MMR: Mismatch repair; IHC: Immunohistochemistry.

Table 3 Prevalence of mismatch repair and BRAF status *n* (%)

Variables	All cases
MMR status	<i>n</i> = 211
IHC method	164 (77.73)
pMMR	154
dMMR	10
MLH-1	4
MSH-2	6
MSH-6	0
PMS-2	0
MSI method	44 (20.85)
MSI-H	21
MSI-L/MSI-S	23
Unknown	3 (1.42)
BRAF status	
Wild type	188 (89.1)
Mutation	23 (10.9)

pMMR: Proficient mismatch repair; dMMR: Deficient mismatch repair; MSI-H: High-level MSI; MSI-L: Low-level MSI; MSS: Microsatellite stable.

mutation and dMMR with specific types of mutations is shown in Table 3.

Association between dMMR/BRAF mutation and clinicopathological factors

The association of dMMR and BRAF mutations with clinicopathological factors was evaluated by Pearson's χ^2 test. These factors included gender, age, site of the primary tumor, UICC stage, lymphovascular or/and perineural invasion, histologic grade, pT, pN, and M stage. dMMR was more commonly found in patients with primary colon tumors rather than rectal cancer (20.4% *vs* 7.6%, *P* = 0.01) but no difference in MMR status was found between right-sided and left-sided colon tumors (*P* = 0.24). dMMR was associated with early stage rather than metastatic disease (17.3% *vs* 0%, *P* = 0.015). None of the abovementioned factors were found to be significantly associated with the BRAF mutation (Table 4).

Association between dMMR and BRAF mutation

Two-hundred and eight tumor samples with data for both MMR and the BRAF mutation were available: 25/31 cases (80.6%) with dMMR were negative for BRAF V600E, and 6/31 (19.4%) were positive for BRAF mutation; whereas 160/177 cases (90.4%) with pMMR were negative for BRAF mutation, and 17/177 (9.6%)

Table 4 Association between mismatch repair/*BRAF* status and clinicopathological factors

Variable	MMR status (n = 208)			<i>BRAF</i> status (n = 211)		
	pMMR	dMMR	P value	Wild type	Mutant	P value
Gender						
Female	87	16	0.800	94	11	0.844
Male	90	15		94	12	
Age (yr)						
≤ 50	24	6	0.397	29	1	0.151
> 50	153	25		159	22	
Site						
Right-sided	31	11	0.037 ^a	36	7	0.510
Left-sided	59	12		67	6	
Rectum	84	7		81	10	
Synchronous lesions	3	1		4	0	
Stage						
I	25	7	0.053	30	2	0.552
II	55	8		58	7	
III	68	16		73	12	
IV	29	0		27	2	
Invasion						
No	99	17	0.910	106	12	0.701
LVI or PNI or both	78	14		82	11	
Differentiation						
Well-moderately	172	28	0.067	180	23	0.313
Poorly	5	3		8	0	
Bowel wall invasion						
pT1	3	1	0.511	4	0	0.410
pT2	34	9		39	5	
pT3	126	18		128	18	
pT4	14	3		17	0	
Lymph node metastasis						
pN-	88	15	0.891	96	9	0.280
pN+	89	16		92	14	
Distant metastasis						
No	148	31	0.015 ^a	161	21	0.456
Yes	29	0		21	2	

^aP < 0.05 vs control. MMR: Mismatch repair; pT: Pathological tumor stage; pN: Pathological nodal stage; ALI: Angiolymphatic invasion; PNI: Perineural invasion.

were positive for *BRAF* mutation (P = 0.11) (Table 5).

Survival analysis

The median follow-up time was 56.7 mo. At the last follow-up (December 5, 2012), there were 133 patients alive and 78 patients deceased. Estimated 5-year survival for the entire population was 64.5%.

Univariate analysis by Kaplan-Meier survival analysis and log rank test was performed using clinical parameters and known prognostic factors to evaluate their significance with DFS and OS. These factors included age, gender, primary tumor site, UICC stage, T stage, regional lymph node involvement, distant metastasis, histological grade, angiolymphatic (LVI) and/or perineural invasion (PNI), MMR status, and the *BRAF* V600E mutation. Factors with statistical significance for DFS were UICC stage (P < 0.01), pT3 (P = 0.01), regional lymph node involvement (P < 0.01), and LVI and/or PNI (P < 0.01). Factors that were statistically significant for OS were UICC stage, pT3, pT4, regional lymph node involvement, and LVI and/or PNI (P

Table 5 Association between mismatch repair status and *BRAF* V600E

<i>BRAF</i>	MMR status		
	dMMR	pMMR	P value
Normal	25	160	0.11
V600E	6	17	

MMR: Mismatch repair; dMMR: Deficient mismatch repair; pMMR: Proficient mismatch repair.

Table 6 Univariate analysis of prognostic factors influencing disease-free survival and overall survival

Variable	n	DFS		OS	
		Median survival (mo)	P value	Median survival (mo)	P value
Gender					
Female	105	NR		NR	
Male	106	NR	0.528	NR	0.640
Age (yr)					
≤ 50	30	NR		NR	
> 50	181	NR	0.695	NR	0.424
Site					
Right-sided	43	NR		NR	
Left-sided	73	NR		NR	
Rectum	91	NR		NR	
Synchronous lesions	4	NR	0.682	NR	0.788
Stage					
I	32	NR		NR	
II	65	NR	< 0.0001 ^a	NR	< 0.0001 ^a
III	85	66.7	< 0.0001 ^a	NR	< 0.0001 ^a
IV	29	10.4	< 0.0001 ^a	21.6	< 0.0001 ^a
Invasion					
No invasion	118	NR		NR	
LVI or PNI or both	93	52.8	0.002 ^a	57.13	< 0.0001 ^a
Differentiation					
Well-moderately	203	NR		NR	
Poorly	8	29.83	0.575	40.67	0.306
Bowel wall invasion					
pT1-T2	48	NR		NR	
pT3	146	62.23	0.011 ^a	NR	< 0.0001 ^a
pT4	17	66.73	0.122	66.733	< 0.0001 ^a
Lymph node metastasis					
pN-	105	NR		NR	
pN+	106	27.57	< 0.0001 ^a	54	< 0.0001 ^a
Distant metastasis					
No	182	-	-	NR	< 0.0001 ^a
Yes	29	-	-	21.6	
<i>BRAF</i> status					
Wild type	188	NR	0.794	NR	0.465
Mutation	23	NR		NR	
MMR status					
pMMR	177	NR	0.004 ^a	NR	0.006 ^a
dMMR	31	NR		NR	

^aP < 0.05 vs control. DFS: Disease-free survival; OS: Overall survival; NR: Not reached; ALI: Angiolymphatic invasion; PNI: Perineural invasion; pT: Pathological tumor stage; pN: Pathological nodal stage; pMMR: Proficient mismatch repair; dMMR: Deficient MMR.

< 0.01 for all) (Table 6). Patients with dMMR tumors were found to have significantly better DFS (P < 0.01) and OS (P < 0.01) (Figure 1). There was no significant difference in either DFS or OS with respect to adjuvant chemotherapy

Table 7 Independent risk factors correlating with disease-free survival and overall survival of stage I-IV colorectal cancer patients by Cox's proportional hazard regression analysis

Variable	Adjusted analysis for DFS			Adjusted analysis for OS		
	HR	95%CI	P value	HR	95%CI	P value
Stage						
Stage I ¹						
Stage II	1.23	0.43-3.62	0.702	1.47	0.40-5.40	0.559
Stage III	4.03 ^a	1.57-10.32	0.004 ^a	4.94 ^a	1.50-16.27	0.009 ^a
Stage IV	-			32.64 ^a	9.57-111.27	< 0.001 ^a
Invasion						
No invasion ¹						
LVI/PNI/both	1.17	0.75-1.81	0.486	1.36	0.85-2.19	0.204
Differentiation						
Well-moderate ¹						
Poorly	2.57	0.87-7.43	0.083	3.78	1.27-11.21	0.017 ^a
MMR status						
pMMR ¹						
dMMR	0.30	0.15-0.77	0.013 ^a	0.29 ^a	0.10-0.84	0.023 ^a

¹Reference. ^a $P < 0.05$ vs control. DFS: Disease-free survival; OS: Overall survival; HR: Hazard ratio; MMR: Mismatch repair; pMMR: Proficient MMR; dMMR: Deficient MMR.

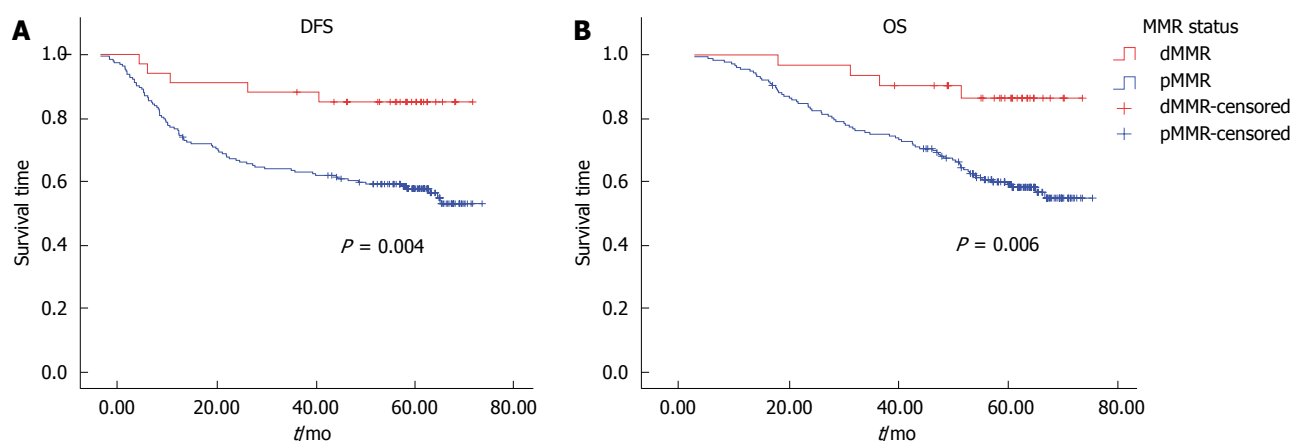


Figure 1 Kaplan-Meier survival curve of colorectal cancer patients according to mismatch repair status. A: Disease-free survival (DFS); B: Overall survival (OS); MMR: Mismatch repair; pMMR: Proficient MMR; dMMR: Deficient MMR.

in dMMR patients ($P = 0.35, 0.21$, respectively). However, there was no significant difference in either DFS or OS with respect to *BRAF* mutation (Figure 2).

Multivariate Cox proportional hazard regression analysis of factors influencing DFS and OS was performed using the factors mentioned previously. The independent risk factor for worse DFS was stage III (HR = 4.03, 95%CI: 1.57-10.32, $P < 0.01$) and risk factors for worse OS were stage III (HR = 4.94, 95%CI: 1.50-16.27, $P < 0.01$), stage IV (HR = 32.64, 95%CI: 9.57-111.27, $P < 0.01$) and poor differentiation (HR = 3.78, 95%CI: 1.27-11.21, $P = 0.02$). dMMR remained a significant prognostic factor for longer DFS (HR = 0.30, 95%CI: 0.15-0.77, $P = 0.01$) and OS (HR = 0.29, 95%CI: 0.10-0.84, $P = 0.02$) (Table 7).

DISCUSSION

In this study, we performed an analysis of MMR status and the *BRAF* V600E mutation in sporadic CRC using archival paraffin-embedded tissue blocks from patients

diagnosed between 2006 and 2007. All patients included in the study underwent resection of their primary tumor to ensure sufficient tumor tissues would be available for the analysis. Therefore, the incidence of stage IV disease in this study (14%) was lower than previously reported from our institution in the same period (32%)^[18]. Because we only selected patients who had undergone primary tumor resection, selection bias might have been introduced by not using all of the patients. The primary objective was to determine the prevalence of each biomarker. Sample size determination was based on *BRAF* mutation and dMMR estimated incidences of 15% as reported in previous studies^[3,4,8-10].

IHC is an excellent method to determine MMR status. This method is inexpensive, technically simple with demonstrably good correlation with MSI-H^[9,14]. Review of the literature suggested that IHC has an overall sensitivity of approximately 90% and specificity of greater than 99% in detecting MSI in sporadic CRC^[19]. In our study, we used antibodies against all four MMR

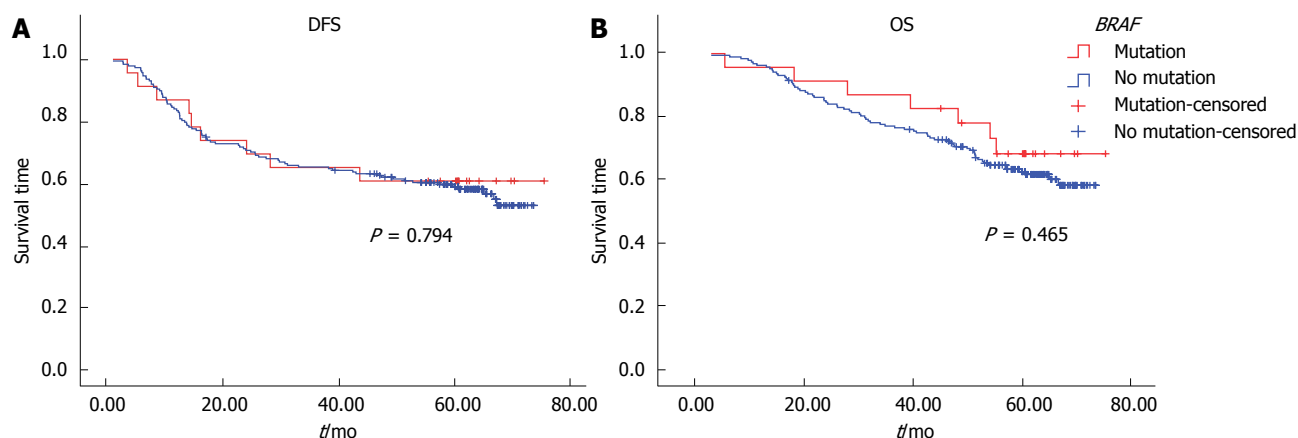


Figure 2 Kaplan-Meier survival curve of colorectal cancer patients according to the presence of the *BRAF* mutation. A: Disease-free survival (DFS); B: Overall survival (OS).

proteins to increase detection sensitivity for specific loss of protein expression other than MLH-1 and MSH-2, the two most common dMMR genes. TMA is an approach that allows high-throughput IHC to be performed on large numbers of small punched-out tissue cores from different tumors^[20], thus providing time- and cost-saving benefits. TMA-IHC for MMR protein expression was validated in tumors from HNPCC patients and found to have concordant results when compared to MSI analysis and conventional IHC on whole slides^[21].

Approximately 15%-20% of sporadic CRC carried dMMR. The proportion of samples with dMMR was higher in tumors located in or above the splenic flexure, stage II rather than stage III disease, poorly differentiated tumors, and those of mucinous subtype^[9]. Several studies, including a meta-analysis, found that patients with dMMR tumors had significantly better survival compared with that of pMMR patients^[9,14,22].

In this study, we performed IHC staining of 211 cases. MSI analysis was completed in 47 cases with uninformative IHC staining results. The final result indicated 14.9% (31/208) of sporadic CRC patients harbored dMMR tumors detected by both TMA-IHC and MSI analysis (10/164 and 21/47 cases, respectively), which was comparable with 11.3% (36 of 318) found in sporadic CRC patients in South Korea^[23] and in that reported in the literature^[3]. dMMR was more commonly found in patients with primary colon tumors rather than rectal cancer, but there was no difference in the prevalence of dMMR in right-sided and left-sided colon tumors. This might be related to the small number of patients included in the study and the fact that the majority (78%) of patients had left-sided tumors. We did not find any association between dMMR and high grade tumors ($P = 0.067$). This result should be interpreted carefully because the majority were moderately differentiated tumors, accounting for 79.1% of cases, while only 15.1 and 3.8% were well and poorly differentiated tumors, respectively. However, this study confirmed that dMMR was independently associated with favorable outcome in both DFS and OS.

Recently *BRAF* mutations have been widely studied

with respect to their prognostic and predictive value in CRC. Studies have shown that the *BRAF* mutation was associated with a poor outcome in CRC patients with pMMR but not with dMMR^[9,10]. The prevalence of the *BRAF* mutation in this study was 10.9%, which was comparable with Caucasian patients, but the result was higher than that reported by studies in Taiwan (1.7%), South Korea (4.5%) and Japan (6.5%)^[24-26]. The main difference between our study and others is that our data were analyzed using allele-specific PCR to detect *BRAF* V600E, which is more highly sensitive in detecting this mutation than other methods. Several other approaches are suitable for evaluating this *BRAF* mutation, including direct sequencing, real-time PCR with melt curve analysis, and AS-PCR^[27]. The *BRAF* mutation was strongly associated with dMMR, right-sided and poorly differentiated tumors^[9]. A recent study showed that the *BRAF* V600E mutation was independently associated with worse DFS in patients with stage III colon cancer receiving adjuvant chemotherapy^[15]. In this study, there was no association between the *BRAF* mutation and any pathological features, no significant difference in the prevalence of the *BRAF* mutation in dMMR and pMMR tumors, and also no prognostic impact for either DFS or OS. This is likely a result of too few patients analyzed to determine the prognostic impact.

In conclusion, we found that the prevalence of dMMR and the *BRAF* V600E mutation in Thai sporadic CRC patients was 15 and 11%, respectively. However, only the dMMR phenotype was associated with favorable DFS and OS in our study cohort.

ACKNOWLEDGMENTS

The authors are grateful to Professor Peter Hokland for his kind help in critically reading our manuscript.

COMMENTS

Background

Colorectal tumorigenesis is a multistep process, which can arise from the

accumulation of genetic alterations. DNA mismatch repair (dMMR) and *BRAF* gene have been widely studied in this disease. The correlation between dMMR and *BRAF* mutation and colorectal cancer (CRC) prognosis has been reported. Patients with tumors harboring dMMR were found to be associated with a more favorable survival than those having proficient MMR (pMMR). In contrast, *BRAF* mutation was found to be associated with worse clinical outcome especially in patients with pMMR tumors. Unfortunately, most of these data have been studied in western country, and there is only scarce information in the Asian population.

Research frontiers

In this study, the authors have systematically determined the prevalence of dMMR and *BRAF* mutation in sporadic CRC patients and established the correlations between MMR status and *BRAF* mutation, association with various clinicopathological features, and prognostic impact on the outcome of Thai patients with sporadic CRC.

Innovations and breakthroughs

The method the authors used to detect *BRAF* mutation is allele specific polymerase chain reaction which has the highest sensitivity to detect this mutation when compared to previously reported methods. This study is the first reported data in Thai population.

Applications

This study confirmed the favorable outcome in patients with dMMR tumors, which is consistent to the results of previous reports in Caucasian population.

Peer review

This is a good retrospective study in which this is the first reported data in Thai population. The method that used to detected *BRAF* mutation has the highest sensitivity to detect this mutation when compare to previously reported studies and the result of the study can imply that patients with dMMR tumors had a significantly better survival in both Asian and Caucasian population.

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P- Reviewer: Wang YD S- Editor: Qi Y L- Editor: A
E- Editor: Wang CH





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ISSN 1007-9327

