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

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ORIGINAL ARTICLE

Retrospective Study

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

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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*

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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)

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

Variables	Value
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.

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- **Prevalence of dMMR and the BRAF V600E mutation**

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	*n* = 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.

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- **Association between dMMR/BRAF mutation and clinicopathological factors**

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

Variable	MMR status (n = 208)			*BRAF* 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	

[a]$P < 0.05$ *vs* control. MMR: Mismatch repair; pT: Pathological tumor stage; pN: Pathological nodal stage; ALI: Angiolymphatic invasion; PNI: Perineural invasion.

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- **Association between dMMR and BRAF mutation**

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

	MMR status		
BRAF	**dMMR**	**pMMR**	***P* value**
Normal	25	160	0.11
V600E	6	17	

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

- **Survival analysis**

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	
BRAF 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	

[a]*P* < 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.

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- **Survival analysis**

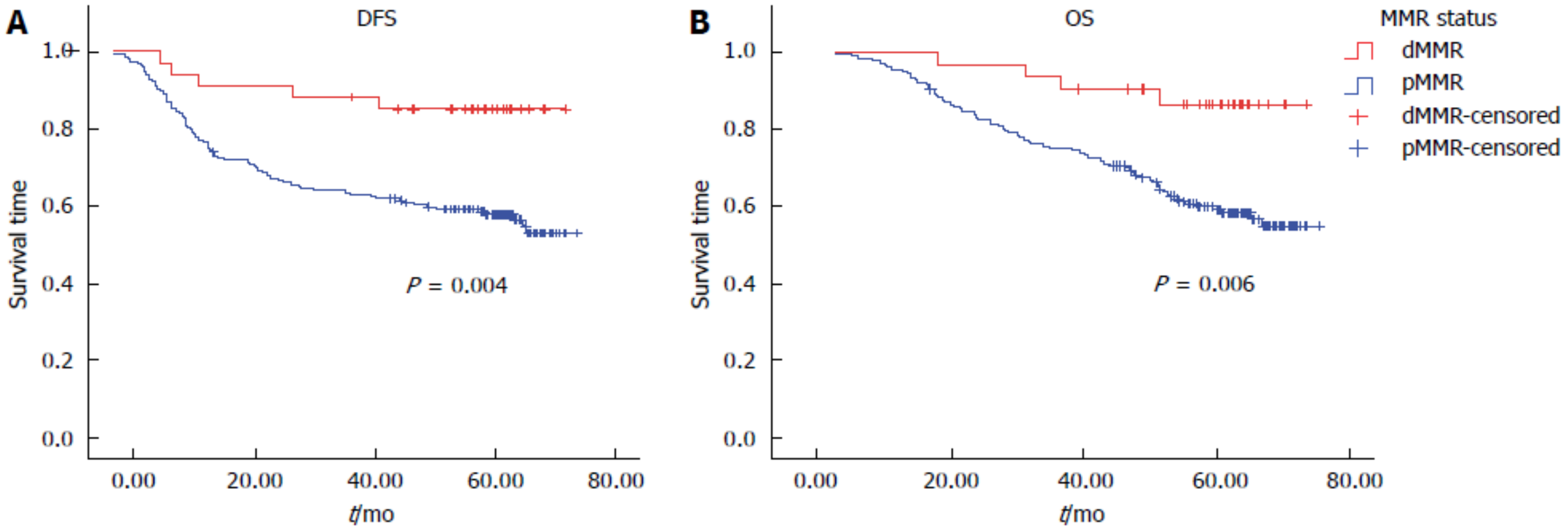

Figure 1 Kaplan-Meier survival curve of colorectal cancer patients according to mismatch repair status. A: Disease-free survival (DFS); B: Overall survival (OS); MMR: Mismatch repair; pMMR: Proficient MMR; dMMR: Deficient MMR.

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- **Survival analysis**

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≤ 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	
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Distant metastasis					
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Mutation	23	NR		NR	
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pMMR	177	NR	0.004[a]	NR	0.006[a]
dMMR	31	NR		NR	

[a]$P < 0.05$ *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.

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- **Survival analysis**

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]

[1]Reference. [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.

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- **Survival analysis**

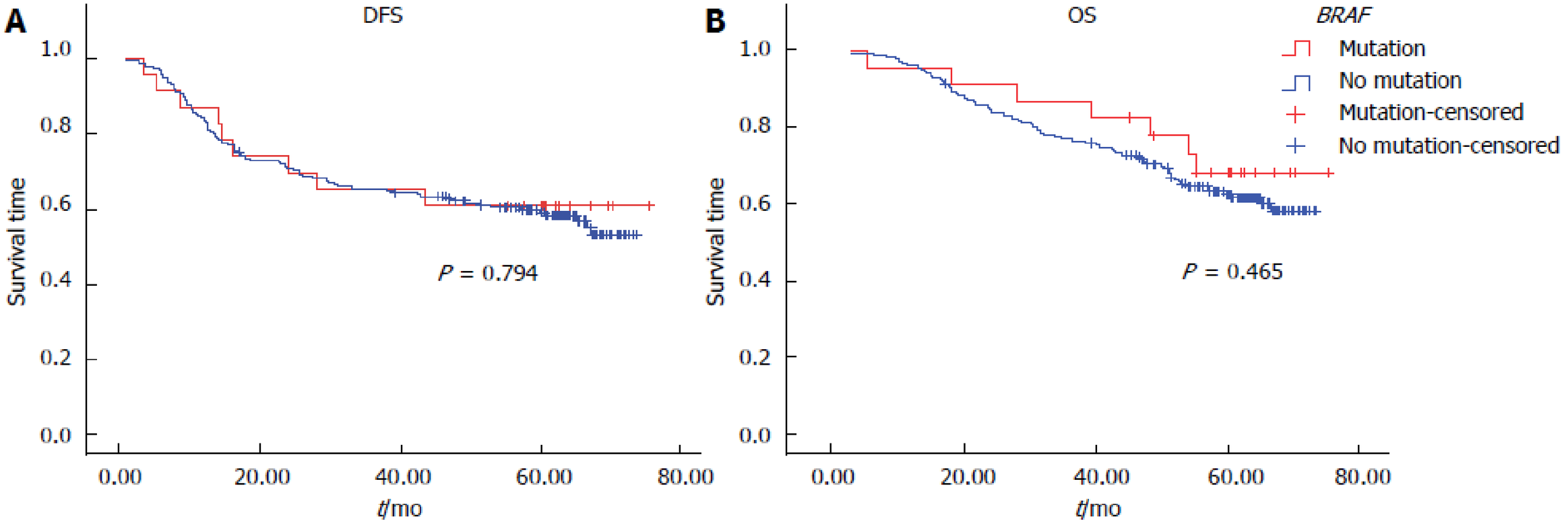

Figure 2 Kaplan-Meier survival curve of colorectal cancer patients according to the presence of the *BRAF* mutation. A: Disease-free survival (DFS); B: Overall survival (OS).