RESEARCH ARTICLE

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High Frequency of *KRAS* Codon 146 and *FBXW7* Mutations in Thai Patients with Stage II-III Colon Cancer

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Abstract

Background: *KRAS*, *NRAS*, and *BRAF* gene mutations are the most clinically relevant and frequently reported in colorectal cancer (CRC). Although data on these genes are frequently reported in several counties, data specific to these genes among Thai population are scarce. The aim of this study was to investigate and identify molecular alterations associated with colon cancer in Thai population, and to determine the impact of these genetic aberrations on clinical outcome. **Methods:** DNA from 108 archived formalin-fixed, paraffin-embedded (FFPE) tissue samples that histologically confirmed adenocarcinoma of stage II-III colon cancer between 2010 and 2012 at Siriraj Hospital (Bangkok, Thailand) were extracted. Gene mutational analysis was performed by next-generation sequencing (NGS) using an Oncomine Solid Tumor DNA kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). **Results:** A total of 22 somatic gene mutations were detected. The mutation frequency observed in *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and *FBXW7* mutations was 47.2%, 1.9%, 1.9%, 12%, and 14.8%, respectively. *KRAS* mutation codon 12, 13, 59, 61, 117, and 146 mutations were identified in 29.6%, 8.3%, 1.8%, 0.9%, 0.0%, and 8.3%, respectively. *KRAS* Exon 4 had better DFS compared with Exon 2 and 3. **Conclusions:** This study is the first to comprehensively report hotspot mutations using NGS in Thai colon cancer patients. The most commonly identified gene mutation frequencies among Thai patients (*KRAS*, *NRAS*, *BRAF*, *TP53*, and *PIK3CA*) were similar to the gene mutation frequencies reported in Western population, except for subgroup of *KRAS* codon 146 and *FBXW7* mutations that had a slightly higher frequency.

Keywords: High frequency- *KRAS* codon 146 mutation- *FBXW7* mutation- colon cancer- Thai patients

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and the second most common cause of cancer-related mortality (Bray et al., 2018). It is well established that colorectal tumorigenesis is a multistep process that involves an accumulation of multiple, successive genetic alterations, including chromosomal abnormalities, gene mutations, and/or epigenetic changes, that transform normal colonic epithelium to colorectal carcinoma (Vogelstein et al, 1988). *APC*, *TP53*, *RAS*, *RAF*, and *PIK3CA* gene mutations are the most commonly reported genetic aberrations in metastatic CRC (mCRC). The prognostic and predictive implications of certain aberrations, including *RAF*, *RAS*, and deficient mismatch repair (dMMR), are well established in CRC, and are now routinely assessed as a component of clinical care (Therkildsen et al., 2014; Rowland et al., 2015). However, the clinical implications of other genetic aberrations in CRC are unclear despite extensive scientific research that has been conducted over the past few decades. Intense research efforts are ongoing to identify novel and reliable biomarkers to help clinicians make personalized treatment decisions in CRC.

Numerous investigations into the mutational status of components in the EGFR-RAS-RAF pathway and the PI3K-AKT pathway have been conducted, and those investigations revealed a diverse distribution pattern of mutations in these genes. However, the rates of these mutations in Thai patients with CRC are not well defined, and only a few mutations have been investigated (i.e., *KRAS* and *BRAF*) (Chaiyapan et al., 2013; Korphaisarn, et al., 2015). Accordingly, the aim of this study was to investigate and identify molecular alterations associated

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with sporadic colon cancer in Thai population, and to determine the impact of these genetic aberrations on clinical outcome.

Materials and Methods

Tissue samples

This single-center retrospective study included formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients diagnosed with stage II-III colon cancer who underwent surgery at Siriraj Hospital during 2010 to 2012. We excluded patients with a known family history of CRC, those suspected of having hereditary or familial CRC, and those who did not receive treatment and follow-up at our center. The study protocol was approved by the Siriraj Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (EC1-755/2558).

Demographic and clinical characteristics, including age, gender, primary tumor site, tumor staging, date of diagnosis, date of surgery, stage at diagnosis, date of disease recurrence, date of last follow-up, and date of death, were collected. Right-sided colon cancer was defined as cancer in the region from the cecum to the splenic flexure, while left-sided colon cancer was defined as cancer in the region from the descending colon through the rectum. Staging was determined according to AJCC/UICC TMN staging criteria (v.3 2010). Disease-free survival (DFS) was defined as the interval between the date of diagnosis and the date of disease recurrence or death. Overall survival (OS) was defined as the interval between the date of diagnosis and the date of death from any cause. The primary objective of this study was to investigate and identify molecular alterations in Thai patients with sporadic colon cancer using next-generation sequencing (NGS). The secondary aims were to evaluate association between identified aberrations and various demographic and clinicopathologic characteristics, and to identify the factors that significantly affect survival.

Determination of gene mutations

DNA were extracted from FFPE tissue using an automated DNA extraction platform (chemagic™ MSM I Instrument, PerkinElmer, Inc., Waltham, MA, USA). Samples were evaluated using a next-generation sequencing (NGS) platform with 22 gene panels including *EGFR, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7* and *TP53* (Oncomine Solid Tumor DNA kit; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sequencing data were processed and aligned to reference sequences with Torrent Suite software v5.6. Variant calling and filtering have been performed with SEQUENCE Pilot module SeqNext v.4.4 (JSI Medical Systems GmbH, Germany) using default parameter setting of 5% detection limit for distinct variants (Somatic 1 GM). The common variants with allele frequency ≥1% from either 1000G, ExAC or genomAD databases were excluded. All suspected variants were subsequently assessed for pathogenicity with Ensembl Variant Effect Predictor, VEP (8). This study has excluded predicted variants with neutral effects, benign and likely benign variants in ClinVar database, and intronic or synonymous variants which have unavailable supportive data and being unreported in the COSMIC database.

Determination of mismatch repair (MMR) status

MMR status was determined by analysis of MMR protein expression by immunohistochemistry (IHC) or microsatellite instability (MSI) testing. Deficient mismatch repair (dMMR) was defined as the presence of either high-level MSI (MSI-H) or loss of MMR protein expression. Proficient mismatch repair (pMMR) was defined as the presence of either microsatellite stable (MSS)/low-level MSI (MSI-L) or the presence of normal MMR protein expression. Complete details of the IHC analysis of MMR expression and microsatellite instability (MSI) testing have been previously published (Korphaisarn et al., 2015).

Statistical analysis

Patient characteristics and gene mutation frequencies were described using descriptive statistics. Data are presented as number, number and percentage, mean and range, or median and range. Pearson's χ^2 test or Fisher's exact test was used to evaluate associations between *KRAS, NRAS, BRAF, PIK3CA*, and *FBXW7* mutations, and dMMR and clinicopathologic variables. Association between *KRAS* gene mutation and disease-free survival (DFS) was evaluated by Kaplan-Meier estimation and log-rank test. Statistical calculations were performed using SPSS Statistics version 18 (SPSS, Inc., Chicago, IL, USA). P-values less than 0.05 were considered statistically significant.

Results

A total of 108 patients were diagnosed with stage II or III colon adenocarcinoma during 2010 to 2012 at Siriraj Hospital. Tissue blocks were available for all included patients. The median age of subjects was 64 years (range: 30-89), and the ratio of males to females was 1.3:1. Twenty-six patients (24.1%) had stage 2 disease. The majority of primary site tumors were sigmoid colon (40 patients, 37%), followed by ascending colon (23 patients, 21.3%). Patient and tumor characteristics are shown in Table 1.

Distribution of KRAS, NRAS, PIK3CA, and BRAF mutations

All of 22 hotspot gene mutations were detected. The mutation frequency observed in *KRAS, NRAS, BRAF,* and *PIK3CA* mutations was 47.2% (51), 1.9% (2), 1.9% (2), and 12.0% (13), respectively. Types of *RAS/BRAF/PIK3CA* mutations are shown in Table 2. Among the *KRAS* gene mutations, 40 (37%) had mutation in exon 2, 2 (1.9%) had mutation in exon 3, and 8 (7.4%) had mutation in exon 4. One tumor sample had mutation in both codon 3 and codon 4. Only *NRAS* mutation in exon 3 (codon Q61K) and *BRAF* mutation at V600E were identified in our cohort (2 samples each). *PIK3CA* mutations were identified in

Table 1. Demographic and Clinicopathologic Characteristics of Study Population

Characteristic	n	%
No. of patients	108	100
Age (yrs), median (range)	64 (30-89)	
Age		
<50 years	15	13.90
>50 years	93	86.10
Gender		
Female	46	42.60
Male	62	57.40
Primary tumor site		
Ascending	23	21.30
Transverse	12	11.10
Descending	11	10.20
Sigmoid	40	37.00
Rectum	19	17.60
Synchronous	3	2.80
Primary tumor site		
Right-sided	36	33.30
Left-sided	70	64.80
Synchronous	2	1.90
Preoperative CEA (ng/dl), median	4.94	
T stage		
T1	1	0.90
T2	6	5.60
T3	74	68.50
T4	27	25.00
N stage		
N0	26	24.10
N1	55	50.90
N2	27	25.00
Stage		
Stage 2	26	24.10
Stage 3	82	75.90
Differentiated		
Well	5	4.60
Moderately	93	86.10
Poorly	7	6.50
No data	3	2.80
LVI and/or PNI		
No	50	46.30
Yes	58	53.70
Margin		
R0	96	88.90
R1	11	10.20
R2	1	0.90
Perforation		
No	104	96.30
Yes	4	3.70
Obstruction		
No	79	73.10
Yes	29	26.90

CEA, carcinoembryonic antigen; LVI, lymphovascular invasion; PNI, perineural invasion

Table 2. Types of *RAS/BRAF/PIK3CA* Mutations Detected in 108 Cases of Thai Colon Cancer

Genes	Exon	Codon	N	%
KRAS	Exon2	G12A/C/D/R/S/V, G13D	40	47.2
	Exon3	A59T, Q61H	2	
	Exon4	A146T/V	8	
	Exon3+4		1	
NRAS	Exon3	Q61K	2	1.9
BRAF	Exon 15	V600E	2	1.9
PIK3CA	Exon9	E542K, E545G/K, Q546L, R537Q	9	12.1
	Exon20	H1047R	4	
FBXW7	Exon5	R278*, R278Q, I272R	5	14.8
	Exon 9	R465C, R465H, E471G	4	
	Exon 10	R479Q, R484M, R505H, R505L	4	
	Exon 11	S582L	3	

13 samples (12.1%). Of these, 9 (8.3%) had mutation in exon 9, while 4 (3.7%) had mutation in exon 20. *PIK3CA* mutations were found to frequently coexist with *KRAS* mutation (84.6% vs. 15.4%; p=0.006).

Mutation frequencies of other genes

In addition to *RAS/RAF/PI3KA* gene mutations, another 18 genes showed at least one mutation, including receptor tyrosine kinase (*RTK*) genes, *RTK* signaling genes, and other known cancer-related genes. The identified gene mutations are, as follows: *RTK* gene mutations: *MET, EGFR, ERBB2, DDR2, ALK, FGFR3, FGFR1, FGFR2,* and *ERBB4; RTK* signaling gene mutations: S*TK11, AKT, PTEN,* and *MAP2K1*; and, other cancer-related gene mutations: *TP53, FBXW7, SMAD4, PTEN, CTNNB1,* and *NOTCH1*. Gene mutation frequency details are shown in Figure 2.

The frequency of *FBXW7* mutations was 14.8% (16). Most of the detected *FBXW7* mutations were missense mutations (15/16, 94%), with arginine missense mutation reported in 10 of 16 samples (Table 2)

Prevalence of dMMR/MSI-H

dMMR/MSI-H was detected in 9 of 108 tumors (8.3%). Interpretation of IHC staining was, as follows: MLH-1 expression and MSH-2 was absent in 1 tumor, and 5 tumors were negative for PMS-2 expression. We were unable to analyze the result in two patients due to tumor

Table 3. Prevalence of dMMR/MSI-H (N=107)

MMR status	n (%)
pMMR or MSS/MSI-L	98 (91.6%)
dMMR or MSI-H	9 (8.4%)
MLH1	1
MSH2	1
MSH6	0
PMS2	5
No data on IHC	2

dMMR, deficient mismatch repair; MSI-H, microsatellite instability-high; MMR, mismatch repair; pMMR, proficient mismatch repair; MSS, microsatellite stable; MSI-L, microsatellite instability-low; IHC, immunohistochemistry

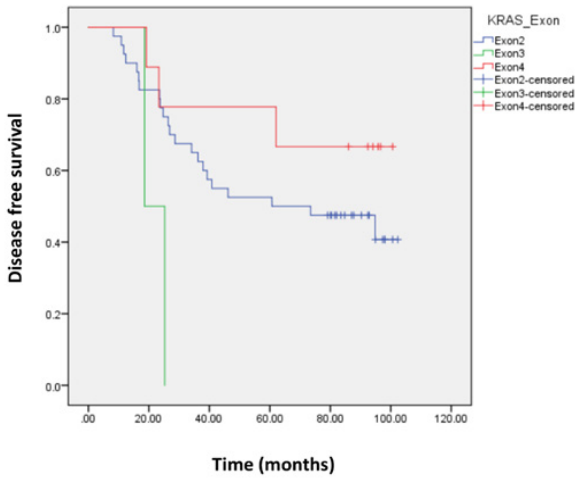

Figure 1. Kaplan-Meier Survival Curves According to *KRAS* Exon Status. Patients with *KRAS* exon 4 mutations had significantly better OS (median OS not reached (NR), 95% confidence interval [CI] NR) than patients with *KRAS* exon 2 and 3 (median OS 60.7 mo, 95% CI 2.1-119.3 mo and median OS 18.6 mo, 95% CI NR, respectively, p=0.047)

loss during TMA construction. Interpretation parameters for IHC of MMR status are shown in Table 3.

Association between KRAS/PIK3CA/FBXW7 mutation and dMMR/MSI-H, demographic, and clinicopathologic factors

Demographic and clinicopathologic factors included gender, age, tumor sidedness, and histologic grade. *PIK3CA* mutations and *dMMR/MSI-H* were more commonly found in patients with right-sided tumor (p=0.03 and p=0.06, respectively). None of the evaluated factors were found to be significantly associated with *KRAS* or *FBXW7* mutations (Table 4).

Survival analysis

The median follow-up time was 84 months (range: 10.9-104.4). At the last follow-up (17 June 2018), there were 66 patients (61.1%) alive and 42 patients (38.9%) deceased. Thirty-five patients (32.4%) had disease recurrence. The estimated 5-year DFS and OS for the entire study population was 59.2% and 70.3%, respectively.

Among the *KRAS* mutations, *KRAS* Exon 4 had better DFS (median DFS, mDFS=NR) than Exons 2 and 3

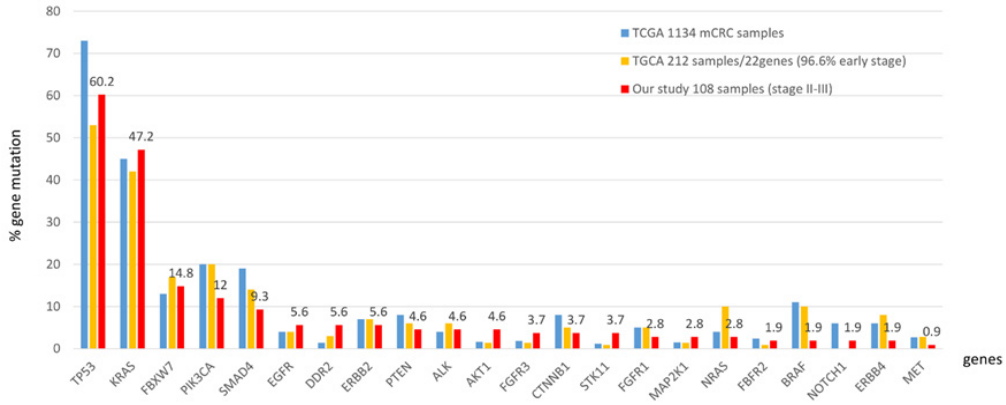

Figure 2. Gene Mutation Frequencies Compared with Different Cohorts

Table 4. Association between *KRAS/PIK3CA/FBXW7* Mutation and MMR Status, and Demographic and Clinicopathologic Factors

Variable	n	*KRAS*			*PIK3CA*			*FBXW7*			n	*MMR* status		
		Wt	Mt	p	Wt	Mt	p	Wt	Mt	p		pMMR	dMMR	p
		n	n		n	n		n	n			n	n	
Age														
<50 years	15	6	9	0.28	13	2	1	13	2	1	15	11	4	0.02
>50 years	93	51	42		82	11		79	14		92	87	5	
Gender														
Female	46	23	23	0.62	40	6	0.78	37	9	0.23	46	42	4	1
Male	62	34	28		55	7		55	7		61	56	5	
Primary tumor site														
Right-sided	36	18	18	0.58	28	8	0.03	31	5	1	36	30	6	0.06
Left-sided	70	39	31		65	5		59	11		69	66	3	
Differentiation														
Well to mod	99	52	47	1	88	11	0.2	84	15	1	98	90	8	0.48
Poor	7	4	3		5	2		6	1		7	6	1	

A p-value<0.05 indicates statistical significance; MMR, mismatch repair; Wt, wild type; Mt, mutant type; pMMR, proficient mismatch repair; dMMR, deficient mismatch repair; mod, moderate

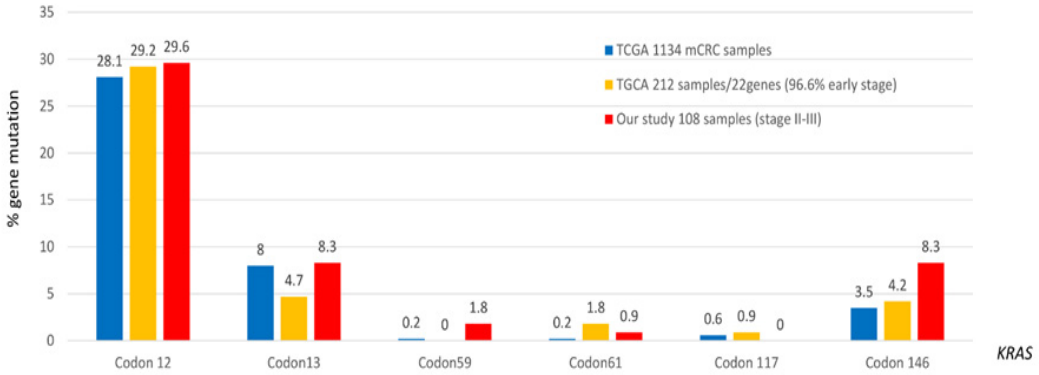

Figure 3. Frequency and spectrum of *KRAS* Mutations

(mDFS: 60.9, 95% CI: 2.1-119.28, and mDFS: 18.6, 95% CI: NR; p=0.047, respectively) (Figure 1).

Discussion

The *KRAS, NRAS, BRAF, TP53*, and *PIK3CA* mutation frequencies in Thai colon cancer were similar to the frequencies reported in Western population. This is the first study to report a slightly higher frequency of *KRAS* codon 146 and *FBXW7* mutation in Thai CRC population.

EGFR signaling plays a key role in the development and progression of CRC. In particular, this receptor triggers downstream signaling cascades, including the RAS-RAF-MAPK and PI3K-AKT pathways, to stimulate cell proliferation, differentiation, survival, and invasion (Peyssonnaux and Eychène, 2001). Gene mutations in the EGFR signaling pathway, such as mutations in *KRAS*, *NRAS*, and *BRAF*, have become an important component of CRC evaluation, and their alterations may determine the therapeutic response to anti-epidermal growth factor receptor (anti-EGFR) therapy.

In the present study, we evaluated *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* somatic mutation frequencies in a Thai cohort of 108 stage II-III colon cancer patients. The frequency rates were 47.6%, 1.9%, 1.9%, and 12%, respectively. In 45 samples (41.6%), no mutations were detected in the *KRAS, NRAS, BRAF*, or *PIK3CA* genes, but mutations were detected in one of the other genes analyzed. No mutations were detected in any of the targets analyzed in 8 samples. The gene mutation frequencies in all analyzed genes compared with data from The Cancer Genome Atlas (TCGA) database (Cancer Genome Atlas Network, 2012) are shown in Figure 2.

The frequency of *KRAS* mutations varies worldwide, ranging from 13% to 56% (Roth et al., 2010; Montomoli et al., 2012; Murtaza et al., 2014; Zahrani et al., 2014; Peeters et al., 2015). The frequency of *KRAS* mutations (47.2%) in the current study was consistent with the published data from Asian countries (Asaka et al., 2009; Lin et al., 2011; Ahn et al., 2014; Tong et al., 2014), Western countries (Lamy et al., 2011; Vaughn et al., 2011; Bozzao et al., 2012), and the *TCGA* database (Cancer Genome Atlas Network, 2012) – but not with all reports (Liou et al., 2011; Chaiyapan et al., 2013; Phipps et al., 2013; Rosty et al., 2013). The wide variability of results among studies may be attributed to ethnicity, geographical distribution, and the techniques used in previous studies.

In this study, the majority of *KRAS* mutations occurred in codon 12 or 13 (78.4%). *KRAS G12D* was the most common mutation, followed by *G12V, G13D, G12C*, and *G12S* mutations, which was consistent with prior studies (Tong et al., 2014; Zhang et al., 2015). Interestingly, the present study demonstrated a high frequency of *KRAS* codon 146 mutation (8.3%) when compared to previously reported rates (1.2-3.8%) (Edkins et al., 2006; Yanus et al., 2013; Imamura et al., 2014; Tong et al., 2014; Osumi et al., 2016). A TCGA dataset (Cancer Genome Atlas Network, 2012) from 212 sequenced CRC cases showed *KRAS* codon 146 mutation in 4.2% of cases (Figure 2). However, our data are consistent with the most recent data from Ngamphaiboon, et al. who reported a frequency of 7% in Thai CRC samples (Ngamphaiboon et al., 2015). This data was slightly different from those studies in Western populations, which suggests that race may play a role in *KRAS* mutation patterns. Moreover, these finding highlight the importance of extended *RAS* mutational analysis in all patients before the initiation anti-EGFR treatment in Thai population. Our study also demonstrated a better DFS in *KRAS* exon 4 compare with exons 2 and 3 (Figure 1). Imamura et al. also reported that *KRAS* codon 12 mutation had worse prognosis when compared with *KRAS* wild type (Colorectal cancer specific mortality, HR=1.45, 95%CI 1.12-1.87, P=0.0048) but not in *KRAS* codon 61 or 146 (Imamura et al., 2014), reflecting that not all *KRAS* mutations were created equal prognosis. However, due to small sample size in this study, further study is needed to confirm this result.

Discovery of Neuroblastoma RAS Viral Oncogene Homolog (*NRAS*), which is a member of the *RAS* oncogene family, was reported within the last few years. Although a substantial amount of data has been reported on *KRAS* gene mutations, relative little data has been reported on *NRAS* mutations. In the present study, *NRAS* mutations were detected in 1.9% of tumors compared with 4.3% from Taiwan (Chang et al., 2016), 0.9-4.2% from China (Shen et al., 2013; Zhang et al., 2015; Shen et al. 2016), 2.7-4.5% from Japan (Ogura et al., 2014; Kawazoe et al., 2015; Osumi et al., 2016), 2.3% from Korea (Lee et al. 2015), 3.6-4.6% from Italy (Foltran et al., 2015; Malapelle et al., 2016), and 2.2-5.1% from the United States (Irahara et al., 2010; Vaughn et al., 2011).

BRAF is a member of the *RAF* gene family, and it

acts as a downstream effector of activated *KRAS*. The frequency of *BRAF* mutations varies widely from 1.6% to 26.0% (Kim et al., 2008; Kohonen-Corish et al., 2014). The *BRAF* V600E mutation frequency of 1.9% observed in this study was consistent with various Asian studies (Shen et al., 2011; Hsieh et al., 2012), but is lower than several Western studies (Vaughn et al., 2011; Phipps et al., 2013; Luey et al., 2014) and our previous study (Korphaisarn et al., 2015). These differences in mutation frequencies may be attributable to different sample selections, ethnicity, geographical distributions, and investigative techniques used. None of the *BRAF*-mutated samples harbored a concurrent *KRAS* mutation, which confirms that these mutations are mutually exclusive.

Phosphatidylinositol-4,5-biphosphonate 3-kionases (*PIK3CAs*), which is a family of lipid kinases, can activate the AKT signaling pathway and facilitate cellular growth and proliferation (Samuels and Velculescu, 2004). More than 80% of *PIK3CAs* occur in exon 9 (60-65%) or exon 20 (20-25%), and can co-occur with *KRAS* or *BRAF* mutations (Sartore-Bianchi et al., 2009; De Roock et al., 2010). In this study, the frequency of *PIK3CA* mutation was 12% (13 samples). Nine samples had mutation in exon 9, while 4 samples had mutation in exon 20. We demonstrated that *PIK3CA* mutations were more commonly found in right-sided tumor (p=0.03), which is consistent with prior studies (Jauhri et al., 2017). We also confirmed significant association between *PIK3CA* mutations and *KRAS* mutations (p=0.006), which was similar to previous findings (Day et al., 2013; Rosty et al., 2013; Zhang et al., 2015).

FBXW7 is a tumor suppressor gene on human chromosome 4q that encodes the substrate recognition components of SKP1-Cullin1-F-box protein ubiquitin E3 ligase complexes (Spruck et al., 2002). These specific E3 ligase complexes negatively regulate the intracellular abundance of an expanding list of key oncogenic proteins. Therefore, the loss of *FBXW7* function results in accumulation of its substrates, which leads to oncogenesis and progression of multiple cancers, including CRC (Cao et al., 2016). In CRC, the frequency of *FBXW7* mutations has been shown to vary from 6% to 10% (Kemp et al., 2005; Akhoondi et al., 2007; Malapelle et al., 2016; Korphaisarn et al., 2017). In the present study, the frequency of *FBXW7* mutations was 14.8%, which is slightly higher than the previously reported rates. However, it is consistent with the data from TCGA database (Cancer Genome Atlas Network, 2012). Most of the detected *FBXW7* mutations were arginine missense, which is consistent with data from the previous cohort (Korphaisarn et al., 2017).

In this study, we found that 8.4% (9/107) of patients harbored dMMR tumors detected by both TMA-IHC and MSI analysis, which is comparable to some rates reported in stage II-III CRC in the literature (Meng et al., 2007; Xiao et al., 2013; Kadowaki et al., 2015), but not all reports (Soliman et al., 2001; Nitsche et al., 2012).

Optimizing treatment decision-making in CRC patients is difficult, because of the heterogeneity of the disease relative to tumor biology, clinical response, and racial differences. Accordingly, future studies should investigate tumor biomarkers that correlate with clinical outcome to facilitate optimized treatment for individual patient.

Limitations

This study has some limitations. First and consistent with the retrospective nature of the study, some patient data may have been incomplete or missing. Second, the sequencing panels used were limited to hotspot regions; therefore, the possible presence of mutations outside of these regions cannot be excluded. Third, the collected data were from a single center. Lastly, the small sample size of our study may have yielded insufficient statistical power to identify all significant differences and associations. However, to the best of our knowledge, this is the first study to comprehensively investigate molecular aberrations in Thai patients with colon cancer.

In conclusion, this study is the first to comprehensively report hotspot mutations using next-generation sequencing in Thai stage II-III colon cancer patients. The most commonly identified gene mutation frequencies among Thai colon cancer patients (*KRAS*, *NRAS, BRAF, TP53,* and *PIK3CA*) were similar to the gene mutation frequencies reported in Western population, except subgroup of *KRAS* codon 146 and *FBXW7* mutations that had a slightly higher frequency.

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