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 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

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...
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 intrinsic 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 χ² 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...
Asian Pacific Journal of Cancer Prevention, Vol 20

Gene Mutations in Thai Colon Cancer Patients

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Gene Mutations in Thai Colon Cancer Patients

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: STK11, AKT, PTEN, and MAP2K1;
- 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 1. Demographic and Clinicopathologic Characteristics of Study Population

| Characteristic              | n  | %   |
|----------------------------|----|-----|
| No. of patients            | 108| 100 |
| Age (yrs), median (range)  | 64 | (30-89) |
| Gender                     |    |     |
| Female                     | 46 | 42.6|
| Male                       | 62 | 57.4|
| Primary tumor site         |    |     |
| Ascending                  | 23 | 21.30|
| Transverse                 | 12 | 11.10|
| Descending                 | 11 | 10.20|
| 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/RAF/PI3KA 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|
|       | Exon15| V600E              | 2  | 1.9|
| BRAF  |       |                    |    |    |
|       | Exon9| E542K, E545G/K, Q546L, R537Q | 9 | 12.1|
|       | Exon20| H1047R            | 4  |    |
| FBXW7 | Exon5| R278*, R278Q, 1272R| 5  | 14.8|
|       | Exon9| R465C, R465H, E471G | 4  |    |
|       | Exon10| R479Q, R484M, R505H, R505L | 4  |    |
|       | Exon11| S582L            | 3  |    |

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
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 (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, \(p=0.047\)).

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

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

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
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, 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; Zahraei 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 Ngamphaiboone, et al. who reported a frequency of 7% in Thai CRC samples (Ngamphaiboone 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 findings 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 compared 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., 2015), 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 \textit{KRAS}. The frequency of \textit{BRAF} mutations varies widely from 1.6\% to 26.0\% (Kim et al., 2008; Kohonen-Corish et al., 2014). The \textit{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 \textit{BRAF}-mutated samples harbored a concurrent \textit{KRAS} mutation, which confirms that these mutations are mutually exclusive.

Phosphatidylinositol-4,5-biphosphonate 3-kinases (\textit{PIK3CA}s), 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 \textit{PIK3CA}s occur in exon 9 (60-65\%) or exon 20 (20-25\%), and can co-occur with \textit{KRAS} or \textit{BRAF} mutations (Sartore-Bianchi et al., 2009; De Roock et al., 2010). In this study, the frequency of \textit{PIK3CA} mutation was 12\% (13 samples). Nine samples had mutation in exon 9, while 4 samples had mutation in exon 20. We demonstrated that \textit{PIK3CA} mutations were more commonly found in right-sided tumor (p=0.03), which is consistent with data from the previous cohort (Jauhri et al., 2017). We also confirmed significant association between \textit{PIK3CA} mutations and \textit{KRAS} mutations (p=0.006), which was similar to previous findings (Day et al., 2013; Rosty et al., 2013; Zhang et al., 2015).

\textit{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 \textit{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 \textit{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 \textit{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 \textit{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 (\textit{KRAS}, \textit{NRAS}, \textit{BRAF}, TP53, and \textit{PIK3CA}) were similar to the gene mutation frequencies reported in Western population, except subgroup of \textit{KRAS} codon 146 and \textit{FBXW7} mutations that had a slightly higher frequency.

References

Alam TS, Jeong D, Son MW, et al (2014). The \textit{BRAF} mutation is associated with the prognosis in colorectal cancer. \textit{J Cancer Res Clin Oncol}, \textit{140}, 1863-71.

Akhoondi S, Sun D, von der Lehr N, et al (2007). \textit{FBXW7}/\textit{hCDC4} is a general tumor suppressor in human cancer. \textit{Cancer Res}, \textit{67}, 9006-12.

Asaka S, Arai Y, Nishimura Y, et al (2009). Microsatellite instability-low colorectal cancer acquires a \textit{KRAS} mutation during the progression from Dukes’ A to Dukes’ B. \textit{Carcinogenesis}, \textit{30}, 494-9.

Bozzao C, Varvara D, Piglionica M, et al (2012). Survey of \textit{KRAS}, \textit{BRAF} and \textit{PIK3CA} mutational status in 209 consecutive Italian colorectal cancer patients. \textit{Int J Biol Markers}, \textit{27}, e566-74.

Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. \textit{CA Cancer J Clin}, \textit{68}, 394-424.

Cancer Genome Atlas Network (2012). Comprehensive molecular characterization of human colon and rectal cancer. \textit{Nature}, \textit{487}, 330-7.

Cao J, Ge MH, Ling ZQ (2016). Fbxw7 tumor suppressor: A vital regulator contributes to human tumorigenesis. \textit{Medicine (Baltimore)}, \textit{95}, e2496.

Chaiyapan W, Duangpakdee P, Boonmirottanapon T, Kanngern S, Sangkhathat S (2013). Somatic mutations of K-ras and \textit{BRAF} in Thai colorectal cancer and their prognostic value. \textit{Asian Pac J Cancer Prev}, \textit{14}, 329-32.

Chang SC, Lin PC, Lin JK, et al (2016). Mutation spectra of common cancer-associated genes in different phenotypes of colorectal carcinoma without distant metastasis. \textit{Ann Surg Oncol}, \textit{23}, 849-55.

Day FL, Jorissen RN, Lipton L, et al (2013). \textit{PIK3CA} and \textit{PTEN} gene and exon mutation-specific clinicopathologic and molecular associations in colorectal cancer. \textit{Clin Cancer
Asian Pacific Journal of Cancer Prevention, Vol 20

Lamy A, Blanchard F, Le Pessot F, et al (2011). Prognostic role of KRAS, NRAS, BRAF and PIK3CA mutations in advanced colorectal cancer. *Cancer Biol Ther*, 5, 928-32.

Foltran L, De Maglio G, Pella N, et al (2015). Prognostic role of KRAS, NRAS, BRAF and PIK3CA mutations in advanced colorectal cancer. *Future Oncol*, 11, 629-40.

Kawazoe A, Shitara K, Fukuoka S, et al (2015). A retrospective analysis of clinicopathological, molecular, and prognostic associations of KRAS codon 61 and codon 146 mutations in colorectal cancer: cohort study and literature review. *Mol Cancer*, 13, 135.

Irahara N, Baba Y, Nosho K, et al (2010). NRAS mutations are rare in colorectal cancer. *Diagn Mol Pathol*, 19, 157-63.

Jauhri M, Bhatnagar A, Gupta S, et al (2017). Prevalence and coexistence of KRAS, BRAF, PIK3CA, NRAS, TP53, and APC mutations in Indian colorectal cancer patients: Next-generation sequencing-based cohort study. *Tumour Biol*, 39, 101428371692265.

Kadokawa S, Kakuta M, Takahashi S, et al (2015). Prognostic value of KRAS and BRAF mutations in curatively resected colorectal cancer. *World J Gastroenterol*, 21, 1275-83.

Kawazoe A, Shiota K, Fukukawa S, et al (2015). A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. *BMJ Cancer*, 15, 258.

Kemp Z, Rowan A, Chambers W, et al (2005). CDC4 mutations occur in a subset of colorectal cancers but are not predicted to cause loss of function and are not associated with chromosomal instability. *Cancer Res*, 65, 11361-6.

Kim YH, Kakar S, Cun L, Deng G, Kim YS (2008). Distinct CpG island methylation profiles and BRAF mutation status in serrated and adenomatous colorectal polyps. *Int J Cancer*, 123, 2587-93.

Kohen-Corish MR, Tseung J, Chan C, et al (2014). KRAS mutations and CDK2A promoter methylation show an interactive adverse effect on survival and predict recurrence of rectal cancer. *Int J Cancer*, 134, 2820-8.

Korpaisarn K, Pongpaibul A, Limwongse C, et al (2015). Deficient DNA mismatch repair is associated with favorable prognosis in Thai patients with sporadic colorectal cancer. *World J Gastroenterol*, 21, 926-34.

Korpaisarn K, Morris VK, Overman MJ, et al (2017). *FBXW7* missense mutation: a novel negative prognostic factor in metastatic colorectal adenocarcinoma. *Oncotarget*, 8, 39268-79.

Lamy A, Blanchard F, Le Pessot F, et al (2011). Metastatic colorectal cancer KRAS genotyping in routine practice: results and pitfalls. *Mod Pathol*, 24, 1090-100.

Lee WS, Lee JN, Baek JH, Park YH (2015). RAS status in Korean patients with stage III and IV colorectal cancer. *Clin Transl Oncol*, 17, 751-6.

Lin CH, Lin JK, Chang SC, et al (2011). Molecular profile and copy number analysis of sporadic colorectal cancer in Taiwan. *J Biomed Sci*, 18, 36.

Liou JM, Wu MS, Shun CT, et al (2011). Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population. *Int J Colorectal Dis*, 26, 1387-95.

Luey N, Toon CW, Sisson L, et al (2014). A further investigation of combined mismatch repair and BRAFV600E mutation specific immunohistochemistry as a predictor of overall survival in colorectal carcinoma. *PLoS One*, 9, e106105.

Malapelle U, Pisapia P, Sgariglia R, et al (2016). Less frequently mutated genes in colorectal cancer: evidences from next-generation sequencing of 653 routine cases. *J Clin Pathol*, 69, 767-71.

Meng WJ, Sun XF, Tian C, et al (2007). Microsatellite instability did not predict individual survival in sporadic stage II and III rectal cancer patients. *Oncology*, 72, 82-8.

Montgomery J, Hamilton-Dutoit SJ, Froslev T, Taylor A,Ericshen R (2012). Retrospective analysis of KRAS status in metastatic colorectal cancer patients: a single-center feasibility study. *Clin Exp Gastroenterol*, 5, 167-71.

Murtaza BN, Bibi A, Rashid MU, et al (2014). Spectrum of K ras mutations in Pakistani colorectal cancer patients. *Braz J Med Biol Res*, 47, 35-41.

Ngamphaiboon N, Arsa L, Ruangekawit P, et al (2015). High incidence of KRAS codon 146 in expanded RAS test of colorectal cancer patients using next generation sequencing in Thai population. *Abstract. Eur J Cancer*, 51, 81.

Nitsche U, Rosenberg R, Balmert A, et al (2012). Integrative marker analysis allows risk assessment for metastasis in stage II colon cancer. *Ann Surg*, 256, 763-71.

Ogura T, Kakuta M, Yatsuoka T, et al (2014). Clinicopathological characterization and prognostic impact of colorectal cancers with NRAS mutations. *Oncol Rep*, 32, 50-6.

Osumi H, Shinozaki E, Suemaga M, et al (2016). RAS mutation is a prognostic biomarker in colorectal cancer patients with metastasectomy. *Int J Cancer*, 139, 803-11.

Peeters M, Kafatos G, Taylor A, et al (2015). Prevalence of RAS mutations and individual variation patterns among patients with metastatic colorectal cancer: A pooled analysis of randomised controlled trials. *Eur J Cancer*, 51, 1704-13.

Peyssonnaux C, Eychene A (2001). The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell*, 93, 53-62.

Phipps AI, Buchanan DD, Makar KW, et al (2013). *KRAS*-mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers. *Br J Cancer*, 108, 1757-64.

Rosty C, Young JR, Walsh MD, et al (2013). PIK3CA activating mutation in colorectal carcinoma: associations with molecular features and survival. *PLoS One*, 8, e54799.

Roth AD, Tejpar S, Delorenzi M, et al (2010). Prognostic role of *KRAS* and *BRAF* in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol*, 28, 466-74.

Rowland A, Dias MM, Wiese MD, et al (2015). Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. *Br J Cancer*, 112, 1888-94.

Samuels Y, Velchelescu VE (2004). Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle*, 3, 1221-4.

Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al (2009). Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. *PLoS One*, 4, e7287.

Shen H, Yuan Y, Hu HG, et al (2011). Clinical significance of K-ras and BRAF mutations in Chinese colorectal cancer patients. *World J Gastroenterol*, 17, 809-16.

Shen Y, Wang J, Han X, et al (2013). Effectors of epidermal growth factor receptor pathway: the genetic profiling of KRAS, BRAF, PIK3CA, NRAS mutations in colorectal cancer characteristics and personalized medicine. *PLoS One*, 8, e11628.

Shen Y, Han X, Wang J, et al (2016). Prognostic impact of...
mutation profiling in patients with stage II and III colon cancer. Sci Rep, 6, 24310.
Soliman AS, Bondy ML, El-Badawy SA, et al (2001). Contrasting molecular pathology of colorectal carcinoma in Egyptian and Western patients. Br J Cancer, 85, 1037-46.
Spruck CH, Strohmaier H, Sangfelt O, et al (2002). hCDC4 gene mutations in endometrial cancer. Cancer Res, 62, 4535-9.
Taniguchi H, Uehara K, Nakayama K, et al (2016). The location of colorectal cancer (right- vs. left-sided colon and rectum) affects the prevalence of BRAF V600E, non-V600E and PIK3CA mutations: a prospective registration study in the Aichi Cancer Network. Ann Oncol, 27, 569P.
Therkildsen C, Bergmann TK, Henrichsen-Schnack T, Ladelund S, Nilbert M (2014). The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. Acta Oncol, 53, 852-64.
Tong JH, Lung RW, Sin FM, et al (2014). Characterization of rare transforming KRAS mutations in sporadic colorectal cancer. Cancer Biol Ther, 15, 768-76.
Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS (2011). Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. Genes Chromosomes Cancer, 50, 307-12.
Vogelstein B, Fearon ER, Hamilton SR, et al (1988). Genetic alterations during colorectal-tumor development. N Engl J Med, 319, 525-32.
Xiao H, Yoon YS, Hong SM, et al (2013). Poorly differentiated colorectal cancers: correlation of microsatellite instability with clinicopathologic features and survival. Am J Clin Pathol, 140, 341-7.
Yanus GA, Belyaeva AV, Ivantsov AO, et al (2013). Pattern of clinically relevant mutations in consecutive series of Russian colorectal cancer patients. Med Oncol, 30, 686.
Zahran A, Kandil M, Badar T, et al (2014). Clinico-pathological study of K-ras mutations in colorectal tumors in Saudi Arabia. Tumori, 100, 75-9.
Zhang J, Zheng G, Yang Y, et al (2015). Molecular spectrum of KRAS, NRAS, BRAF and PIK3CA mutations in Chinese colorectal cancer patients: analysis of 1,110 cases. Sci Rep, 5, 8678.