The prognostic significance of KRAS and BRAF mutation status in Korean colorectal cancer patients

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Abstract

Background: BRAF and KRAS mutations are well-established biomarkers in anti-EGFR therapy. However, the prognostic significance of these mutations is still being examined. We determined the prognostic value of BRAF and KRAS mutations in Korean colorectal cancer (CRC) patients.

Methods: From July 2010 to September 2013, 1096 patients who underwent surgery for CRC at Seoul St. Mary’s Hospital were included in the analysis. Resected specimens were examined for BRAF, KRAS, and microsatellite instability (MSI) status. All data were reviewed retrospectively.

Results: Among 1096 patients, 401 (36.7%) had KRAS mutations and 44 (4.0%) had BRAF mutations. Of 83 patients, 77 (92.8%) had microsatellite stable (MSS) or MSI low (MSI-L) status while 6 (7.2%) patients had MSI high (MSI-H) status. Patients with BRAF mutation demonstrated a worse disease-free survival (DFS, HR 1.990, CI 1.080–3.660, \( P = 0.02 \)) and overall survival (OS, HR 3.470, CI 1.900–6.330, \( P < 0.0001 \)). Regarding KRAS status, no significant difference was noted in DFS (\( P = 0.0548 \)) or OS (\( P = 0.107 \)). Comparing the MSS/MSI-L and MSI-H groups there were no significant differences in either DFS (\( P = 0.294 \)) or OS (\( P = 0.557 \)).

Conclusions: BRAF mutation, rather than KRAS, was a significant prognostic factor in Korean CRC patients at both early and advanced stages. The subgroup analysis for MSI did not show significant differences in clinical outcome. BRAF should be included in future larger prospective biomarker studies on CRC.

Keywords: BRAF mutation, KRAS mutation, MSI, Colorectal cancer

Background

Colorectal cancer (CRC) is the second most common cancer in females and the third most common cancer in males worldwide [1]. It is one of the most rapidly growing cancers in Korea with an annual increase (from 1999 to 2009) of 6.2% in men and 6.8% in women [2]. Despite advances in CRC treatment and a decline in the mortality rate over the past few decades, CRC remains the second most common cause of cancer death in females and third common cause of cancer death in males [3].

Considerable advances have been made in the characterization of genetic alterations in CRC in support of genome-wide profiling. The Cancer Genome Atlas Network accomplished the largest comprehensive molecular analysis of CRC to date [4]. Based on somatic mutation rates, colorectal adenocarcinomas were classified as hypermutated or non-hypermutated. The hypermutated group had somatic mutations caused by high microsatellite instability (MSI), usually with MLH1 silencing or mismatch repair gene mutations. BRAF and ACVR2A mutations were enriched in hypermutated samples. However, the non-hypermutated group had frequent gene copy number alterations. In addition, APC, TP53, KRAS, and PIK3CA mutations were observed. These are characteristic of chromosomal instability [4].

The v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), a member of the Ras subfamily, is a proto-oncogene that encodes a 21 kDa GTPase located on the short arm of chromosome 12 [5]. The RAS protein activates several downstream signaling cascades...
such as the mitogen-activated protein kinase (MAPK) and PI3K pathways that regulate multiple cellular functions including cell proliferation, differentiation, motility, survival, and intracellular trafficking [6]. KRAS is considered a key downstream component of the epidermal growth factor receptor (EGFR) signaling pathway; therefore, mutations of the gene result in a constitutive activation of the EGFR signaling cascade [5]. KRAS mutations are identified in 30–50% of CRCs and are usually point mutations that occur in codons 12 and 13, less often in codon 61, and very infrequently at other sites such as codons 59, 146, 19, or 20 [5, 7]. KRAS mutation is a well-established biomarker that predicts resistance to therapy using anti-EGFR monoclonal antibodies in metastatic CRC [8]. However, the prognostic value of KRAS mutations in CRC is controversial. Some studies revealed that KRAS mutations are associated with poorer prognosis, while others have reported no association [9–12].

The v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) is a serine/threonine kinase that plays a part in cell proliferation, survival, and differentiation; [13]. Activating BRAF mutations have been detected in various malignant tumors such as melanoma, papillary thyroid cancer, CRC, ovarian cancer, and hairy cell leukemia [13–15]. In CRC, BRAF mutations are reported in 4.7 to 20% of tumors [13, 16]. Usually, BRAF and KRAS mutations are usually mutually exclusive [17]. The most common BRAF mutation, found in over 90% of human cancers, is a glutamic acid for valine substitution at codon 600 in exon 15 (V600E), leading to constitutive activation of the MAPK pathway [18]. The predictive role of BRAF mutation in response to anti-EGFR therapy remains uncertain; however, previous studies found that BRAF mutations are associated with an adverse clinical outcome, especially in advanced stage CRC [16, 19, 20].

In the present study, we comprehensively investigated KRAS and BRAF mutation status in Korean CRC patients. In addition, we analyzed the relationship of KRAS and BRAF mutation with MSI status.

### Methods

#### Patients and treatment

We retrospectively reviewed specimens from 1096 consecutive patients who underwent surgical CRC resection at Seoul St. Mary's Hospital, The Catholic University of Korea, between July 2010 and September 2013. CRC cases with tissue blocks eligible for the KRAS and BRAF mutation testing were included in this study. Two gastrointestinal pathologists reviewed and classified CRC slides according to World Health Organization classification. Clinicopathological parameters were obtained from patient medical records and pathology reports at our institution. Adjuvant chemotherapy was recommended to high-risk (cancer obstruction, perforation, poor differentiation, or lymphovascular/perineural invasion) stage II or stage III CRC patients. According to the BRAF and KRAS mutational status, patients were offered targeted agents as an adjunct to systemic chemotherapy. However, due to insurance coverage issues, only 3 patients received anti-EGFR and only 12 received anti-vascular endothelial growth factor therapy during the study period. Approval for this study was acquired from the Institutional Review Board of the Catholic University of Korea, College of Medicine (KC16RISI0011).

#### DNA isolation and analysis of KRAS and BRAF mutations

For DNA isolation, 10-μm-thick sections from formalin-fixed paraffin-embedded (FFPE) tissue samples were used for each case. Hematoxylin & eosin sections were used as a reference and the largest tumor area was scraped off with a scalpel under a dissecting microscope. Genomic DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen Inc., Valencia, CA) according to the manufacturer's recommendations. Sanger sequencing was performed using an ABI 3730 automated sequencer (Applied Biosystems, Inc., Foster City, CA), to detect the presence of KRAS exon 2 mutations with previously reported primers [21]. Exon 15 of the BRAF gene was amplified by polymerase chain reaction (PCR) using the following forward primer (5′-AATGCTTTGCCTCTGTAGGAAAAT-3′) and reverse primer (5′-TAATCAGTGGAAAAATAGGCTC-3′), resulting in a 209 base pair PCR product. The resultant PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and the appropriate protocol on the QIAcube robotic workstation. Each chromatogram was visually inspected for abnormalities.

#### MSI analysis

Five microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250) recommended by a National Cancer Institute workshop on MSI determined the microsatellite status [22]. PCR analyses were performed and the shift of PCR products from tumor DNA was compared to normal DNA. Tumors with at least 2 of the 5 microsatellite markers displaying shifted alleles were classified as MSI-H, whereas tumors with only 1 marker exhibiting a novel band were classified as MSI-L. Samples in which all microsatellite markers displayed the same patterns in tumor and normal tissues were classified as MSS; subsequently, MSS and MSI-L tumors were grouped for analyses based on genetic implications [22].

#### Statistical analysis

Continuous variables were analyzed by student’s t or Mann-Whitney U test, expressed as the mean ±SD. For categorical variables, χ²-test analysis or Fisher’s exact test was used. Survival analysis was performed by the Kaplan-Meier method. Statistical analysis was performed with SPSS software version 18 (SPSS Inc., Chicago, IL).
and the R programming language (R Core Team 2015, A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, URL http://www.r-project.org/). A P-value of <0.05 was considered significant.

Results

Patient characteristics according to KRAS or BRAF mutation status

The present study included 1092 patients with KRAS and 1096 patients with BRAF mutation data. Tables 1 and 2 summarize the clinicopathological characteristics of patients. A total of 401 patients (36.7%) had KRAS mutations. KRAS mutated CRCs were significantly associated with females (45.1% vs 34.6% with wild-type KRAS; \( P = 0.001 \)), right sided tumors (32.4% vs 21.0%; \( P < 0.001 \)), higher T stage (T4, 15.3% vs 11.0%; \( P = 0.005 \)), well to moderate differentiation (98.7% vs 94.7%; \( P = 0.002 \)), and mucinous adenocarcinoma (9.2% vs 4.9%; \( P = 0.002 \)). BRAF mutations were detected in 44 patients (4.0%). The proportion of BRAF mutation was higher in tumors located in the right colon (56.8% vs 23.9% with wild-type BRAF; \( P = 0.001 \)), with advanced tumor stage (T4, 29.5% vs 11.9%; \( P = 0.005 \)), with lymph node metastasis (N2, 38.6% vs 20.5%; \( P = 0.015 \)), and with lymphatic invasion (65.9% vs 44.0%; \( P = 0.007 \)). BRAF mutated tumors trended toward poorly differentiated histology (10.0% vs 3.6%; \( P = 0.099 \)) and an infiltrative growth pattern (22.7% vs 15.2%; \( P = 0.065 \)) compared to wild-type BRAF tumors, but these were not statistically significant. In addition, gender distribution according to KRAS mutation status did not differ significantly, showing a bimodal distribution pattern along the colorectum. Distributions with respect to tumor sites for all three tumor subgroups (KRAS-mutated, BRAF-mutated and null CRCs), stratified for gender, are shown in Fig. 1a–c.

Mutation frequencies in KRAS and BRAF

A KRAS codon 12 mutation was observed in 296 patients. A KRAS codon 13 mutation was observed in 98 patients. Seven other patients had either KRAS codon 14 or 30 mutations. The most frequent amino acid change was Gly12Asp, which accounted for 36.9% of KRAS mutations (148/401). The second most frequent mutation was Gly13Asp (24.2%, 97/401), and the third was Gly12Val (21.9%, 88/401). Table 3 lists detailed nucleotide and codon changes. Regarding BRAF mutations, Val600Glu in exon 15 showed the highest frequency (97.7%, 43/44) (Table 4). In addition, our data revealed 3 KRAS and BRAF co-mutated cases. Among these 3 cases, 2 had Gly13Asp KRAS mutations, 1 had a Gly12Asp mutation, and all BRAF mutations were Val600Glu. All 3 cases had lymph node metastasis and were included in stage III; however, no recurrences or deaths were observed.

Impact of KRAS and BRAF mutations on DFS and OS

After a median follow-up of 29 months, the 5-year disease free survival rate of the study population was 81%. There was no significant difference according to KRAS mutation status; however, DFS trended toward being shorter in patients with KRAS mutations than those with wild-type KRAS (\( P = 0.0548 \)). DFS was also significantly worse in patients with BRAF mutated cancers compared to wild-type BRAF by both univariate (HR 1.98, \( P = 0.0252 \)) and multivariate analyses (HR 2.222) (Fig. 2a and b).

Regarding OS, the 5-year rate was 80%. No significant difference in OS according to KRAS mutation status was revealed (\( P = 0.108 \)). OS was significantly shorter for patients with BRAF mutations than those with wild-type BRAF by univariate analysis (HR 3.46, 95% CI 1.9–6.3, \( P < 0.0001 \)). In the multivariate analysis, BRAF mutations also had a negative impact on OS (HR 4.037, 95% CI 2.172–7.506, \( P < 0.0001 \)) (Fig. 2c and d). In addition, we assessed whether the detrimental effect of KRAS mutations was different according to mutation subtypes and showed that there were no significant differences in DFS (\( P = 0.931 \)) or OS (\( P = 0.816 \)) (Additional file 1: Fig. S1A and B).

Considering KRAS and BRAF mutations together, DFS and OS were significantly more favorable in patients with wild-type KRAS and BRAF compared to patients with mutations in both genes (HR 1.540, 95% CI 1.9–6.3, \( P = 0.0548 \)). DFS was also significantly worse in mutations than those with wild-type KRAS and BRAF compared to patients with mutations in both genes (HR 1.540, 95% CI 1.140–2.080, \( P = 0.0049 \)) and OS (HR 1.860, 95% CI 1.280–2.720, \( P = 0.0010 \)) (Fig. 3a and b).

Subgroup analysis on DFS and OS by stage

In stage I colorectal cancer, BRAF mutations had a negative impact on both DFS (HR 3.936, 95% CI 2.120–7.306, \( P < 0.0001 \)) and OS (HR 4.037, 95% CI 2.172–7.506, \( P < 0.0001 \)). However, KRAS mutations did not demonstrate a significant effect on DFS (HR 1.539, 95% CI 1.039–2.279, \( P = 0.112 \)) or OS (HR 1.555, 95% CI 1.048–2.305, \( P = 0.107 \)) (Fig. 4a and b). In stage II and III colorectal cancer, BRAF mutations had a negative impact on DFS (HR 1.940, 95% CI 1.050–3.570, \( P = 0.0322 \)) and OS (HR 3.320, 95% CI 1.820–6.070, \( P < 0.0001 \)). However, KRAS mutations did not demonstrate a significant effect on DFS (HR 1.250, 95% CI 0.910–1.720, \( P = 0.169 \)) or OS (HR 1.400, 95% CI 0.950–2.070, \( P = 0.0917 \)) (Fig. 4c and d). In stage IV CRC, BRAF mutation status did not show a significant effect on DFS (HR 1.180, 95% CI 0.290–4.870, \( P = 0.82 \)) or OS (HR 2.660, 95% CI 0.950–7.450, \( P = 0.0548 \)). KRAS mutation status also did not demonstrate a significant effect on DFS (HR 1.140, 95% CI 0.670–1.930, \( P = 0.627 \)) or OS (1.410, 95% CI 0.790–2.520, \( P = 0.247 \)) (Fig. 4e and f).

Patient characteristics according to MSI status

MSI test data were available in 83 patients. Univariate analysis was performed according to clinicopathologic factors...
and MSI status. A significant difference was noted in CRC location ($P = 0.037$). MSH-H had a higher frequency in colon cancers of the right side (66.7% vs 23.4%). MSS/MSI-L CRCs were more prevalent on the left (50.6% vs 16.7%). Regarding histological differentiation, a significant difference was noted ($P = 0.012$). MSI-H had higher number of poorly differentiated CRC (1.4% vs 25.0%). Mucinous CRC was observed more frequently in the MSI-H group (6.5% vs 83.3%, $P < 0.001$) (Table 5).

Impact of MSI status on DFS and OS
We compared DFS and OS between MSS/MSI-L and MSI-H groups to evaluate the value of MSI status as a prognostic marker. MSI status did not show a significant difference in DFS ($P = 0.294$) or OS ($P = 0.557$) (Fig. 5a and b).

Discussion
In this study, we evaluated KRAS and BRAF mutational status in 1096 Korean CRC patients using direct sequencing. To the best of our knowledge, our study is one of the first to report the prognostic significance of KRAS and BRAF mutation status in the Korean CRC population. A major strength of this study was the comprehensive subgroup analysis done according to CRC stage and MSI status with a relatively large sample size.

We uncovered an overall KRAS mutation rate of 36.7% in colorectal cancers, which was consistent with most previous reports [23–26]. We also found that proximal CRCs had a higher percentage of KRAS mutations compared to those at a distal location. This finding is in line with a recent study by Rosty et al. [27]. Furthermore, we

**Table 1 Clinicopathologic characteristics according to KRAS mutation status**

| Patients with KRAS status | $p$-value | $\text{p-value}$ |
|---------------------------|-----------|------------------|
| **Patients with KRAS status** | $N = 1092$ | $N = 1092$ |
| Sex | $N = 691$ | $N = 401$ | $N = 1092$ |
| Male | 452 (65.4%) | 220 (54.9%) | 672 (61.5%) |
| Female | 239 (34.6%) | 181 (45.1%) | 420 (38.5%) |
| Age | 0.771 |
| $< 50$ year | 90 (13.0%) | 49 (12.2%) | 139 (12.7%) |
| $\geq 50$ year | 601 (87.0%) | 352 (87.8%) | 953 (87.3%) |
| Location | $<0.001$ |
| Rt colon | 145 (21.0%) | 130 (32.4%) | 275 (25.2%) |
| Lt colon | 309 (44.7%) | 158 (39.4%) | 467 (42.8%) |
| Rectum | 221 (32.0%) | 107 (26.7%) | 328 (30.0%) |
| Multiple | 16 (2.3%) | 6 (1.5%) | 22 (2.0%) |
| Stage | 0.889 |
| Tis | 15 (2.2%) | 8 (2.0%) | 23 (2.1%) |
| Stage I | 129 (18.8%) | 75 (18.8%) | 204 (18.8%) |
| Stage II | 195 (28.3%) | 112 (28.0%) | 307 (28.2%) |
| Stage III | 256 (37.2%) | 142 (35.5%) | 398 (36.6%) |
| Stage IV | 93 (13.5%) | 63 (15.8%) | 156 (14.3%) |
| T stage | 0.005 |
| T1 | 71 (10.5%) | 25 (6.4%) | 96 (9.0%) |
| T2 | 100 (14.8%) | 77 (19.7%) | 177 (16.6%) |
| T3 | 429 (63.6%) | 229 (58.6%) | 658 (61.8%) |
| T4 | 74 (11.0%) | 60 (15.3%) | 134 (12.6%) |
| N stage | 0.897 |
| N0 | 362 (52.5%) | 207 (51.6%) | 569 (52.2%) |
| N1 | 184 (26.7%) | 106 (26.4%) | 290 (26.6%) |
| N2 | 143 (20.8%) | 88 (21.9%) | 231 (21.2%) |
| M stage | 0.35 |
| M0 | 598 (86.5%) | 338 (84.3%) | 936 (82.7%) |
| M1 | 93 (13.5%) | 63 (15.7%) | 156 (14.3%) |
| Lymphatic invasion | 0.163 |
| Absent | 392 (56.8%) | 209 (52.2%) | 601 (55.1%) |
| Present | 298 (43.2%) | 191 (47.8%) | 489 (44.9%) |
| Venous invasion | 0.055 |
| Absent | 558 (81.0%) | 343 (85.8%) | 901 (82.7%) |
| Present | 131 (19.0%) | 57 (14.2%) | 188 (17.3%) |
| Perineural invasion | 0.123 |
| Absent | 537 (77.8%) | 294 (73.5%) | 831 (76.2%) |
| Present | 153 (22.2%) | 106 (26.5%) | 259 (23.8%) |
| Differentiation | 0.002 |
| Well/Moderate | 629 (94.7%) | 374 (98.7%) | 1003 (96.2%) |
| Poor | 35 (5.3%) | 5 (1.3%) | 40 (3.8%) |
| Histology | 0.008 |
| Non-mucinous adenocarcinoma | 657 (95.1%) | 364 (90.8%) | 1021 (93.5%) |
| Mucinous adenocarcinoma | 34 (4.9%) | 37 (9.2%) | 71 (6.5%) |
| Recur | 0.143 |
| Recur | 593 (85.8%) | 330 (82.3%) | 923 (84.5%) |
| Non-recur | 98 (14.2%) | 71 (17.7%) | 169 (15.5%) |
| Expire | 0.219 |
| Expire | 629 (91.0%) | 355 (88.5%) | 984 (90.1%) |
| Non-Expire | 62 (9.0%) | 46 (11.5%) | 108 (9.9%) |
| Neoadjuvant Tx | 0.217 |
| No | 605 (87.6%) | 364 (90.8%) | 969 (88.7%) |
| CTx | 31 (4.5%) | 10 (2.5%) | 41 (3.8%) |
| RT | 2 (0.3%) | 0 (0.0%) | 2 (0.2%) |
| CCRT | 53 (7.7%) | 27 (6.7%) | 80 (7.3%) |
found that the frequencies of \textit{KRAS} mutations showed a bimodal distribution pattern along the colorectum. Consistent with previous studies, our data indicated that the frequency of \textit{KRAS} mutated tumors was highest in the cecum (60\%) \cite{27, 28}. (Fig. 1a–c) The data emphasized the regional differences between proximal and distal CRCs with respect to clinicopathological and molecular pathogenesis \cite{29}. In addition, we saw a bimodal distribution pattern in both male and female patients, which was different from Rosty et al. who showed that the frequencies of \textit{KRAS} mutated carcinoma were diverse in different colorectal segments between male and female subjects \cite{27}. Like CRCs with \textit{BRAF} mutations, \textit{KRAS}-mutated carcinomas had an increased frequency of the mucinous feature. Several others have also reported this finding \cite{27, 30}.

In the current study, we revealed that the G > A transition, followed by G > T transversion were the predominant types of \textit{KRAS} mutations, and the substitution of aspartate for glycine at codon 12 was the most frequent change. Others have also identified the G > A transition and the glycine to aspartate transition on codon 12 as the most frequent type of \textit{KRAS} activating mutation \cite{31–33}. For codon 13, the 38G > A transition was the most frequent type, which was similar to the findings of other studies \cite{23, 34}.

\textit{KRAS} mutations were associated with a higher tumor stage (pT) in this study. However, there were no differences in risk of recurrence, DFS or OS in patients according to their \textit{KRAS} mutation status. These findings are in agreement with those by Rosty et al.; however, the prognostic roles of \textit{KRAS} mutations are still being debated \cite{27, 34, 35}.

| Table 2 Clinicopathologic characteristics according to \textit{BRAF} mutation status | Patients with \textit{BRAF} status | p-value |
| --- | --- | --- |
| | Negative \((N = 1052)\) | Positive \((N = 44)\) | Total \((N = 1096)\) |
| Sex | | | 0.149 |
| Male | 652 (62.0\%) | 22 (50.0\%) | 674 (61.5\%) |
| Female | 400 (38.0\%) | 22 (50.0\%) | 422 (38.5\%) |
| Age | | | 0.375 |
| < 50 year | 131 (12.5\%) | 8 (18.2\%) | 139 (12.7\%) |
| ≥ 50 year | 921 (87.5\%) | 36 (81.8\%) | 957 (87.3\%) |
| Location | | | 0 |
| Rt colon | 252 (24.0\%) | 25 (56.8\%) | 277 (25.3\%) |
| Lt colon | 455 (43.3\%) | 14 (31.8\%) | 469 (42.8\%) |
| Rectum | 324 (30.8\%) | 4 (9.1\%) | 328 (29.9\%) |
| Multiple | 21 (2.0\%) | 1 (2.3\%) | 22 (2.0\%) |
| Stage | | | 0.226 |
| Tis | 23 (2.2\%) | 0 (0.0\%) | 23 (2.1\%) |
| StageI | 205 (19.6\%) | 5 (11.4\%) | 210 (19.2\%) |
| StageII | 323 (30.9\%) | 12 (27.3\%) | 335 (30.7\%) |
| StageIII | 496 (47.4\%) | 27 (61.4\%) | 523 (47.9\%) |
| T stage | | | 0.006 |
| T1 | 93 (9.1\%) | 3 (6.8\%) | 96 (9.0\%) |
| T2 | 173 (16.9\%) | 4 (9.1\%) | 177 (16.6\%) |
| T3 | 637 (62.1\%) | 24 (54.5\%) | 661 (61.8\%) |
| T4 | 122 (11.9\%) | 13 (29.5\%) | 135 (12.6\%) |
| N stage | | | 0.015 |
| N0 | 553 (52.7\%) | 17 (38.6\%) | 570 (52.1\%) |
| N1 | 282 (26.9\%) | 10 (22.7\%) | 292 (26.7\%) |
| N2 | 215 (20.5\%) | 17 (38.6\%) | 232 (21.2\%) |
| M stage | | | 0.451 |
| M0 | 307 (29.0\%) | 0 (0.0\%) | 307 (27.5\%) |
| M1 | 1 (25.0\%) | 0 (0.0\%) | 1 (25.0\%) |
| Lymphatic invasion | | | 0.007 |
| Absent | 588 (56.0\%) | 15 (34.1\%) | 603 (55.1\%) |
| Present | 462 (44.0\%) | 29 (65.9\%) | 491 (44.9\%) |
| Venous invasion | | | 0.109 |
| Absent | 873 (83.2\%) | 32 (72.7\%) | 905 (82.8\%) |
| Present | 176 (16.8\%) | 12 (27.3\%) | 188 (17.2\%) |
| Perineural invasion | | | 0.451 |
| Absent | 804 (76.6\%) | 31 (70.5\%) | 835 (76.3\%) |
| Present | 246 (23.4\%) | 13 (29.5\%) | 259 (23.7\%) |
| Differentiation | | | 0.081 |
| Well | 96 (9.5\%) | 2 (5.0\%) | 98 (9.4\%) |
| Moderate | 875 (86.9\%) | 34 (85.0\%) | 909 (86.8\%) |
The reported frequency of \textit{BRAF} mutations in different populations varies widely. In this study, \textit{BRAF} mutations were found in 4.0\% of colorectal cancers, which is slightly lower than previous reports worldwide [36–50]. In general, a lower incidence has been noted in Asian populations such as China, Japan, and Saudi Arabia [37–39]. Interestingly, two studies from Korea showed higher \textit{BRAF} mutation rates of 15.9\% and 9.6\% [40, 41]. The study cohort by Kim et al. consisted of advanced CRC patients, which might have influenced the higher mutation rate in their study [41]. Ahn et al. used the PNA-clamp real-time PCR method for the detection of \textit{BRAF} mutations, which is known to be superior to direct sequencing in sensitivity and might have caused differences in the mutation rate among study groups [40, 51]. In addition, the enrolled patients of the study by Tsai et al. were under 30 years of age and distinct from other studies [47].

In this study cohort, we revealed that \textit{BRAF} mutation was significantly associated with poorer DFS and OS in colorectal cancers. In addition, \textit{BRAF} mutational status was an independent prognostic factor for DFS and OS in multivariate analysis, which is consistent with previous studies (Table 5). Moreover, we compared different tumor stages and found that \textit{BRAF} mutations were also associated with poorer DFS and OS in both stage I and stage II/III subgroups. However, there was no significant association between \textit{BRAF} mutation and survival in the stage IV subgroup. Yaeger et al. recently showed that \textit{BRAF} mutation confers a poor prognosis in metastatic CRC patients [42]. This discrepancy may come from the relatively small study population in this metastatic setting, ethnic distinctions and subsequent differences in \textit{BRAF} mutation rates. Further studies in a larger population data are needed to confirm this result. Nevertheless, our findings highlight that the clinical meaning of \textit{BRAF} mutation is similar to Korean CRC patients, even if the

| Table 3 Frequency of Mutations in \textit{KRAS} exon2 |
|-----------------------------------------------|
| \textit{KRAS} codon 12                        |
| c.34G > A          | Gly12Ser  | 16 |
| c.34G > C          | Gly12Arg  | 2  |
| c.34G > T          | Gly12Cys  | 31 |
| c.35G > A          | Gly12Asp  | 148|
| c.35G > T          | Gly12Asp  | 1  |
| c.35G > T          | Gly12Val  | 88 |
| c.38G > A          | Gly12Asp  | 5  |
| c.35G > C          | Gly12Ala  | 11 |

| \textit{KRAS} codon 13                        |
|-----------------------------------------------|
| c.35G > A          | Gly13Asp  | 1  |
| c.38G > A          | Gly13Asp  | 97 |
| c.37G > T          | Gly13Cys  | 2  |
| c.36G > T          | Gly13Val  | 2  |
| c.38_39 GC > TT   | Gly13Val  | 1  |

| \textit{KRAS} codon 14                        |
|-----------------------------------------------|
| c.40G > A          | Val14lle  | 1  |

| \textit{KRAS} codon 30                        |
|-----------------------------------------------|
| c.90C > T          | Asp30Asp  | 1  |

| Table 4 Frequency of \textit{BRAF} Mutations |
|---------------------------------------------|
| \textit{BRAF} codon 600                     |
| c.1799 T > A      | Val600Glu | 43 |
| c.1796 C > G      | Thr599Arg | 1  |
mutation frequency is lower than in western patients. Importantly, we revealed that \textit{BRAF} mutation status is important in predicting the prognosis of early CRCs, which is one of the novel findings of our study. Our findings support a role for \textit{BRAF} mutation in the natural history of CRC because only rare cases in our study cohort received targeted therapy other than the standard chemotherapy regimen after resection.

We found that only 0.3\% ($n = 3$) of \textit{KRAS} mutated CRC cases harbored \textit{BRAF} mutations. Of these, two cases showed \textit{KRAS} mutations at codon 13 (38G $>$ A) with the remaining mutation at codon 12 (35G $>$ A),

![Fig. 2](image1)

\textbf{Fig. 2} Kaplan-Meier curves for disease-free survival and overall survival according to \textit{KRAS} or \textit{BRAF} mutation status. \textbf{a} Disease-free survival (DFS) according to \textit{KRAS} status, \textbf{b} DFS according to \textit{BRAF} status, \textbf{c} Overall survival (OS) according to \textit{KRAS} status and \textbf{d} OS according to \textit{BRAF} status

![Fig. 3](image2)

\textbf{Fig. 3} Kaplan-Meier curves for DFS and OS according to \textit{KRAS} mutation status in combination with \textit{BRAF}. \textbf{a} DFS according to \textit{KRAS} mutation status in combination with \textit{BRAF} and \textbf{b} OS according to \textit{KRAS} mutation status in combination with \textit{BRAF}
and all three cases had the BRAF V600E mutation. The concomitant occurrence of KRAS and BRAF mutations is very rare in CRCs (< 1%), which imply that they may play a role in different tumor subtypes [11, 52].

We analyzed the MSI status in 83 CRC patients and revealed a frequency of 7.2% for MSI-H, which appears somewhat lower than reports from western countries [53]. In line with our findings, a recent multicenter study by Oh et al. showed low frequencies of MSI-H in Korean CRC patients [53]. This result suggested ethnic differences in the molecular characteristics of colorectal tumorigenesis including MSI status. MSI is known to be associated with better

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**Fig. 4** Kaplan-Meier curves for DFS and OS according to KRAS or BRAF status in CRC patients with different stage. **a** DFS according to KRAS or BRAF status in CRC patients with stage I. **b** OS according to KRAS or BRAF status in CRC patients with stage I. **c** DFS according to KRAS or BRAF status in CRC patients with stage II and III. **d** OS according to KRAS or BRAF status in CRC patients with stage II and III. **e** DFS according to KRAS or BRAF status in CRC patients with stage IV. **f** OS according to KRAS or BRAF status in CRC patients with stage IV.
clinical outcome in early stage CRCs than MSS cancers [54, 55]. In the present study, MSI status did not have significant prognostic value on DFS and OS; however, a tendency toward worse survival was observed in MSS and MSI-L cases. 

*BRAF* activating mutations correlated with poor survival in MSS CRC. *BRAF* mutations occur in about 40% of MSI CRCs; however, it was unclear if it had a prognostic impact in this setting [45]. A recent study revealed that both *BRAF* and *KRAS* mutations are associated with poorer survival in MSI CRC patients compared to those with wild-type *BRAF* and *KRAS* genes [45]. However, we could not draw any meaningful conclusion about the *BRAF* and/or *KRAS* status in MSI CRC cohorts because the mutated cases in this study were rare.

A limitation of this study is the insufficiency of data on the efficacy of an EGFR-blocking antibody according to *KRAS* and *BRAF* mutation status due to only rare cases being treated by EGFR targeted therapy at our institution during the study period. In addition, the sample size was too small to evaluate the significance of the MSI status with infrequent *KRAS* and *BRAF* mutation subtypes. Subsequent translational studies from different cohorts are needed to confirm our data. Nevertheless, a strong point of this study is the relative large study cohort which reduce selection bias. We revealed *BRAF* mutation as an independent prognostic marker for CRCs throughout all stages.

**Conclusion**

In conclusion, our study demonstrated that *BRAF* mutation, occurring at a low frequency, was a significant prognostic factor in Korean CRC patients. Our data suggests that molecular features that include *KRAS* and *BRAF* mutations as well as MSI status in CRC patients are

| Patients with MSI status | p-value | N=77 | N=6 | N=83 |
|--------------------------|---------|------|-----|------|
| Sex | 0.482 | | | |
| Male | | 44 (57.1%) | 2 (33.3%) | 46 (55.4%) |
| Female | | 33 (42.9%) | 4 (66.7%) | 37 (44.6%) |
| Age | 0.608 | | | |
| < 50 year | | 13 (16.9%) | 0 (0.0%) | 13 (15.7%) |
| ≥ 50 year | | 64 (83.1%) | 6 (100.0%) | 70 (84.3%) |
| Location | 0.037 | | | |
| Rt colon | | 18 (23.4%) | 4 (66.7%) | 22 (26.5%) |
| Lt colon | | 39 (50.6%) | 1 (16.7%) | 40 (48.2%) |
| Rectum | | 17 (22.1%) | 0 (0.0%) | 17 (20.5%) |
| Multiple | | 3 (3.9%) | 1 (16.7%) | 4 (4.8%) |
| Stage | 0.642 | | | |
| Stagl | | 14 (18.2%) | 2 (33.3%) | 16 (19.3%) |
| Stagell | | 27 (35.1%) | 2 (33.3%) | 29 (34.9%) |
| StageIII | | 36 (46.8%) | 2 (33.3%) | 38 (45.8%) |
| T stage | 0.984 | | | |
| T1 | | 9 (11.7%) | 1 (16.7%) | 10 (12.0%) |
| T2 | | 13 (16.9%) | 1 (16.7%) | 14 (16.9%) |
| T3 | | 39 (50.6%) | 3 (50.0%) | 42 (50.6%) |
| T4 | | 16 (20.8%) | 1 (16.7%) | 17 (20.5%) |
| N stage | 0.788 | | | |
| N0 | | 41 (53.2%) | 4 (66.7%) | 45 (54.2%) |
| N1 | | 14 (18.2%) | 1 (16.7%) | 15 (18.1%) |
| N2 | | 22 (28.6%) | 1 (16.7%) | 23 (27.7%) |
| Lymphatic invasion | 0.971 | | | |
| Absent | | 46 (59.7%) | 3 (50.0%) | 49 (59.0%) |
| Present | | 31 (40.3%) | 3 (50.0%) | 34 (41.0%) |
| Venous invasion | 0.378 | | | |
| Absent | | 58 (75.3%) | 6 (100.0%) | 64 (77.1%) |
| Present | | 19 (24.7%) | 0 (0.0%) | 19 (22.9%) |
| Perineural invasion | 0.248 | | | |
| Absent | | 53 (68.8%) | 6 (100.0%) | 59 (71.1%) |
| Present | | 24 (31.2%) | 0 (0.0%) | 24 (28.9%) |
| Differentiation | 0.012 | | | |
| Well | | 13 (17.8%) | 0 (0.0%) | 13 (16.9%) |
| Moderate | | 59 (80.8%) | 3 (50.0%) | 62 (78.0%) |
| Poor | | 1 (1.4%) | 1 (16.7%) | 2 (2.6%) |
| Histology | <0.001 | | | |
| Non-mucinous adenocarcinoma | | 72 (93.5%) | 1 (16.7%) | 73 (88.0%) |
| Mucinous adenocarcinoma | | 5 (6.5%) | 5 (83.3%) | 10 (12.0%) |

Table 5 Clinicopathologic characteristics according to MSI status (Continued)
Table 6 Studies on BRAF mutation status in colorectal cancer patients

| Reference (year) | Country | BRAF mutation % (n) | BRAF mutation type (%) | Methods | Prognostic value | Comments |
|------------------|---------|---------------------|------------------------|---------|-----------------|----------|
| Pai et al. (2012) [36] | USA     | 11.0 (20)           | V600E (100)            | real-time PCR | Significant     | Stage I-IV proficient DNA mismatch repair |
| Kadowaki et al. (2015) [37] | Japan   | 4.9 (40)            | V600E (80)             | PCR combined with restriction enzyme digestion | Significant | Stage I-IV independent of MSI status |
| Chen et al. (2014) [38] | China   | 4.2 (9)             | V600E (88.9)           | direct sequencing | Significant | Stage I-IV |
| Siraj et al. (2014) [39] | Saudi Arabia | 2.5 (19)     | V600E (89.5)           | direct sequencing | No prognostic significance | Stage I-IV |
| Ahn et al. (2014) [40] | Korea   | 15.9 (26)           | V600E (100)            | PNA clamp real-time PCR | Significant | Stage I-IV |
| Kim et al. (2014) [41] | Korea   | 9.6 (13)            | N/A                    | direct sequencing | Significant | Stage III-IV |
| Yaeger et al. (2014) [42] | USA     | 5 (92)              | V600E (96.7)           | mass spectrometry-based assay | Significant | Metastatic colorectal cancers |
| Eklof et al. (2013) [43] | Sweden  | 17.9 (35)           | V600E (100)            | allelic discrimination assay | Significant No prognostic significance | Stage I-IV two different cohorts |
| Renaud et al. (2015) [44] | France  | 10.6 (19)           | V600E (100)            | direct sequencing | Significant | Metachronous lung metastasis |
| de Cuba et al. (2015) [45] | Netherlands | 51.0 (73)     | V600E (100)            | high resolution melting and sequencing | Significant | Stage II and III microsatellite instable colon cancers |
| Foltran et al. (2015) [46] | Italy   | 5.2 (10)            | V600E (100)            | pyrosequencing | Significant | Metastatic colorectal cancers |
| Tsai et al. (2015) [47] | Taiwan  | 18.6 (11)           | V600E (100)            | direct sequencing | Significant | Stage I-IV early-onset colorectal cancers |
| Saridaki et al. (2013) [48] | Greece  | 8.2 (41)            | V600E (100)            | real-time PCR | Significant | Metastatic colorectal cancers |
| Kalady et al. (2012) [49] | USA     | 11.7 (56)           | V600E (98.2)           | direct sequencing | Significant | Stage I-IV |
| Farina-Sarasqueta et al. (2010) [50] | Netherlands | 19.9 (59)       | V600E (100)            | real-time PCR | Significant | Stage I-IV |
| Present case | Korea   | 4.0 (44)            | V600E (97.7)           | direct sequencing | Significant | Stage I-IV Significant prognostic implications through all stages |
important in future clinical trials. Further large translational studies are required to validate the significance of both BRAF and/or KRAS mutation status in MSI CRCs.

Additional files

Additional file 1: Fig. S1. Kaplan-Meier curves for DFS and OS between KRAS mutation at codon 12 and 13. A. DFS between KRAS mutation at codon 12 and 13 and B. OS between KRAS mutation at codon 12 and 13. (PPTX 266 kb)

Abbreviations

BRAF: v-Raf murine sarcoma viral oncogene homolog B1; CI: Confidence interval; CRC: Colorectal cancer; DFS: Disease free survival; EGFR: Epidermal growth factor receptor; FFPE: Formalin-fixed paraffin-embedded; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MAPK: Mitogen-activated protein kinase; MSI: Microsatellite instability; OS: Overall survival

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Availability of data and materials

The dataset presented in this investigation is available by request from the corresponding author.

Authors’ contributions

SHL conceptualized and designed this study. DDW collected the clinicopathologic data and performed the data analysis. SHL and DDW interpreted the analysis results and drafted the manuscript. DDW, JIL, ILK, STO, ESJ, SHL were involved in revising the manuscript and providing critical reviews. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Catholic University of Korea, Seoul St. Mary’s Hospital, College of Medicine (KC16RIS00111) and written informed consent was obtained by all patients.

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