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ORIGINAL ARTICLE

Retrospective Cohort Study

Different oncological features of colorectal cancer codon-specific *KRAS* mutations: Not codon 13 but codon 12 have prognostic value

Hong-Min Ahn, Duck-Woo Kim, Hyeon Jeong Oh, Hyung Kyung Kim, Hye Seung Lee, Tae Gyun Lee, Hye-Rim Shin, In Jun Yang, Jeehye Lee, Jung Wook Suh, Heung-Kwon Oh, Sung-Bum Kang

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Abstract

BACKGROUND

Approximately 40% of colorectal cancer (CRC) cases are linked to Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations. *KRAS* mutations are associated with poor CRC prognosis, especially *KRAS* codon 12 mutation, which is associated with metastasis and poorer survival. However, the clinicopathological characteristics and prognosis of *KRAS* codon 13 mutation in CRC remain unclear.

AIM

To evaluate the clinicopathological characteristics and prognostic value of codon-specific *KRAS* mutations, especially in codon 13.

METHODS

This retrospective, single-center, observational cohort study included patients who underwent surgery for stage I-III CRC between January 2009 and December 2019. Patients with *KRAS* mutation status confirmed by molecular pathology reports were included. The relationships between clinicopathological characteristics and individual codon-specific *KRAS* mutations were analyzed. Survival data were analyzed to identify codon-specific *KRAS* mutations as recurrence-related factors using the Cox proportional hazards regression model.

RESULTS

Among the 2203 patients, the incidence of *KRAS* codons 12, 13, and 61 mutations was 27.7%, 9.1%, and 1.3%, respectively. Both *KARS* codons 12 and 13 mutations showed a tendency to be associated with clinical characteristics, but only codon 12 was associated with pathological features, such as stage of primary tumor (T stage), lymph node involvement (N stage), vascular invasion, perineural invasion, tumor size, and microsatellite instability. *KRAS* codon 13 mutation showed no associations (77.2% *vs* 85.3%, P = 0.159), whereas codon 12 was associated with a lower 5-year recurrence-free survival rate (78.9% *vs* 75.5%, P = 0.025). In multivariable analysis, along with T and N stages and vascular and perineural invasion, only codon 12 (hazard ratio: 1.399; 95% confidence interval: 1.034-1.894; P = 0.030) among *KRAS* mutations was an independent risk factor for recurrence.

CONCLUSION

This study provides evidence that *KRAS* codon 13 mutation is less likely to serve as a prognostic biomarker than codon 12 mutation for CRC in a large-scale cohort.

Key Words: Genes; Ras; Codon; Colonic neoplasms; Rectal neoplasms

Core Tip: Based on a large-scale cohort of patients with stage I-III colorectal cancer (CRC), Kirsten rat sarcoma viral oncogene homolog (*KRAS*) codon 13 mutation is less pathogenic and recurrent. Moreover, focusing on the biological effects of codon-specific *KRAS* mutations and minimizing interference with various medical therapies, previous *in vivo* studies demonstrating that *KRAS* codon 13 mutation is less aggressive were translated into clinical outcomes in this study. This may influence many oncologists to consult with patients on their prognosis after surgery. We propose that *KRAS* codon 13 mutation is less likely to serve as a prognostic factor of CRC, compared with codon 12.

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INTRODUCTION

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is one of the downstream molecules of the epidermal growth factor receptor (EGFR) associated with cell proliferation, anti-apoptosis, and survival[1-3]. Abnormal activation of *KRAS*, a well-known oncogene, triggers uncontrolled tumor cell proliferation regardless of the initiating molecular signal from EGFR [4]. Mutations in *KRAS* promote the development of cancer in a variety of organs including the breast, prostate, lung, pancreas, colon, and rectum[1,2]. According to previous reports, approximately 40% of colorectal cancer (CRC) cases are linked to *KRAS* mutations[5-7], which occur more frequently in the proximal rather than in the distal colon[4,8,9]. Clinically, *KRAS* mutations are associated with resistance to anti-EGFR therapy and poor CRC prognosis[10,11].

CRC-related point mutations in *KRAS* occur at different codon locations. In most cases, *KRAS* mutations are detected in codon 12 or 13, whereas mutations in codon 61 or 146 have been reported only in a minority of patients with CRC[12]. Several clinical studies have indicated that *KRAS* codon 12 mutations are associated with metastasis and poor survival in advanced CRC[8,12-14]. *In-vitro* studies comparing cells with *KRAS* codon 12 and 13 mutations have demonstrated stronger transforming activity and resistance to apoptosis in cells with mutations in *KRAS* codon 12 than codon 13[15,16]. Most reports have concluded that *KRAS* codon 12 mutation is a poor prognostic factor following CRC resection. However, the oncological role of *KRAS* codon 13 mutation is controversial. *KRAS* codon 13 mutation has been linked to advanced-stage or lymph node metastasis and has been considered predictive of a higher likelihood of death in several studies[17,18]. In contrast, other investigators have shown no association between *KRAS* codon 13 mutations and tumor progression or CRC prognosis[4,19].

In addition to the controversial prognostic significance of *KRAS* codon 13 mutations, limited information is available regarding the clinical characteristics of codon-specific *KRAS* mutations in CRC. The incidence of codon-specific *KRAS* mutations other than those involving codon 12 (including codon 13) is low. Owing to the infrequency of *KRAS* abnormalities, the pathological features of codon-specific mutations at sites other than codon 12 remain unclear. Owing to the small cohort sizes of previous studies[4,8,12,14,20], the clinical roles of codon-specific *KRAS* mutations in CRC, including codons 12 and 13, are yet to be validated. Moreover, studies on the oncological effects of codon-specific *KRAS* mutations, particularly regarding abnormalities located within minor codons, are limited.

This study was designed to elucidate the clinicopathological characteristics associated with codon-specific *KRAS* mutations in CRC, including codons 12, 13, and 61. The main objective of this study was to determine whether *KRAS* codon 13 mutation could serve as a prognostic biomarker for CRC in a relatively large cohort of individuals.

MATERIALS AND METHODS

Patients

This retrospective observational cohort study was registered at ClinicalTrials.gov (NCT05657210) and reviewed 3144 patients who underwent surgery for CRC between January 2009 and December 2019, with available clinical data on recurrence and survival. All patients underwent routine colon or rectal resection and lymph node dissection according to the tumor location, with or without diverting ileostomies or colostomies. The surgical specimens were submitted to the laboratory for pathological evaluation. Patients with confirmed molecular pathology reports of *KRAS* mutation status were included, whereas those with incomplete data on *KRAS* mutations (n = 368) or microsatellite instability (MSI) status (n = 232) were excluded. Patients with dual or triple *KRAS* mutations (within more than one codon) from pathology reports (n = 2) were excluded. Additionally, to understand the biological importance and minimize the potential influence of systemic therapeutic factors on the prognosis of codon-specific *KRAS* mutations, we excluded patients with stage IV metastatic CRC (n = 339). Finally, data from 2203 eligible patients were collected separately for statistical analysis. This study was approved by the Institutional Review Board (IRB No. B-2203-742-101) of Seoul National University Bundang Hospital and the requirement for informed consent was waived.

Adjuvant/neoadjuvant therapy and follow-ups

All patients who underwent colorectal surgery for curative purposes were recommended adjuvant therapy according to the pathological stage of the cancer. Patients with pathological stage III and high-risk stage II colon cancer are recommended adjuvant chemotherapy. In rectal cancer, patients with pathological stages II and III are treated with adjuvant chemotherapy after surgery. However, in patients with clinical T4 or positive nodes without distant metastasis, preoperative chemoradiation therapy is recommended with long-course radiotherapy (dose of 5040 cGy of radiation over 5 wk; 28 fractions) combined with chemotherapy with 5-fluorouracil/Leucovorin or capecitabine.

According to the cancer monitoring protocol after curative surgery at our facility, patients were evaluated regularly one month after surgery, then every 3 mo for the first 2 years, every 6 mo for the next 3 years, and every 12 mo thereafter for a total of 5 years. Monitoring included measurements of serum carcinoembryonic antigen (CEA) levels every 3 mo; imaging modalities, including computed tomography (CT) (abdomen, pelvis, and chest) every 6 mo; and annual colonoscopy. Cancer recurrence was confirmed histologically or radiologically. The assigned research nurse constantly updated the data on recurrence and death. Information about deaths was double-checked by comparison with the database of the National Health Insurance Service, Korea, which lists the life and death records of Korean people. The registry data were constantly updated and managed by an assigned research nurse in the colorectal surgery department of our hospital.

Data collection

Basic patient clinical information [age, sex, height, weight, and American Society of Anesthesiologists (ASA) score] was collected. Cancer-related clinical characteristics such as primary tumor location, preoperative CEA level, and diverting stoma were included. Data on pathological features were collected based on pathology reports of surgical specimens. The following variables were statistically analyzed: T and N stages, tumor size, lymphatic invasion, vascular invasion, perineural invasion, number of harvested lymph nodes, number of metastatic lymph nodes, MSI status, and *KRAS* mutation status. Codon-specific *KRAS* mutation status was examined for codons 12, 13, and 61.

KRAS mutations were identified from formalin-fixed, paraffin-embedded cancerous tissue obtained from surgical specimens. After deoxyribonucleic acid (DNA) extraction from the tissue, the exons 2 and 3 of the *KRAS* gene were separately amplified by polymerase chain reaction (PCR) using optimized PCR reagents and primers. Codon-specific *KRAS* mutations were identified by pyrosequencing (PyroMark Q24 Mdx, QIAGEN, Hilden, Germany). MSI status was also evaluated using formalin-fixed tissues during surgery. PCR with five markers (*BAT26, BAT25, D5S346, D17S250,* and *D2S123*) followed by fragmentation assay (ABI-3130xl, Thermo Fisher Scientific, MA, United States) was performed to identify the MSI status.

Statistical analysis

Descriptive statistics were used to identify the basic clinicopathological characteristics of the patients, including MSI status frequency and *KRAS* mutations. The differences between wild-type and mutant *KRAS* as well as the mean values of continuous variables, were compared using either the independent t-test or the Mann-Whitney U test according to the results of the Kolmogorov-Smirnov test. Chi-squared or Fisher's exact tests were used to compare categorical variables. Overall survival (OS) and recurrence-free survival (RFS) were calculated from the date of surgery and compared using the Kaplan-Meier method and the log-rank test. For the analysis of risk factors for tumor recurrence, the Cox proportional hazards regression model was used, with the covariance input criterion set at $P < 0.1$. Patients were subdivided based on the primary tumor location (colon *vs* rectum) and MSI status [microsatellite stable (MSS)/MSI-low *versus* MSI-high]. Each subgroup was analyzed for recurrence-related factors using a Cox proportional hazards regression model. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 25.0, for Windows (SPSS, IBM). Descriptive results of continuous variables are expressed as mean ± SD. P value < 0.05 were considered statistically significant.

RESULTS

The present study included 2203 patients who underwent CRC surgery. The clinicopathological characteristics of the patients are shown in Table 1. In terms of MSI status, 1866 patients (84.7%) were identified as MSS, 153 (6.9%) as MSI-low, and 184 (8.4%) as MSI-high (Figure 1A). *KRAS* mutations were detected in 840 patients (38.1%) patients. The incidence of *KRAS* codons 12, 13, and 61 substitutions was 27.7%, 9.1%, and 1.3%, respectively (Figure 1B).

Among the clinical characteristics, female sex, lower ASA score, right-sided colon cancer, higher preoperative CEA levels, and low rates of diverting stoma formation were associated with *KRAS* mutations in codons 12, 13, and 61. Most pathological features, including T stage, N stage, tumor size, lymphatic invasion, perineural invasion, and number of harvested lymph nodes, were associated with *KRAS* mutations, along with molecular features such as MSI status (Table 2).

Analysis of the codon-specific *KRAS* mutational status revealed significant associations of both clinical and pathological characteristics with *KRAS* codon 12 mutations, including female sex, lower ASA score, right-sided colon cancer, preoperative CEA level above the normal range (≥ 5.0 ng/mL), T stage, N stage, MSI status, tumor size, vascular invasion, and perineural invasion. In contrast, only female sex, right-sided colon cancer, high preoperative CEA levels, diverting stoma formation, and no pathological features were significantly correlated with *KRAS* codon 13 mutations. Other than perineural invasion, no clinical characteristics or pathological features were associated with *KRAS* codon 61 mutations (Table 2).

At a mean ± SD follow-up duration of 29.7 mo ± 14.3 mo, and a median of 29 (0-85) months, recurrence within 5 years of curative surgery was observed in 205 (9.3%) among the 2203 patients. Five-year RFS (78.3% *vs* 77.4%, P = 0.130) and OS (89.0% *vs* 89.5%, P = 0.971) rates did not differ significantly between the wild-type and *KRAS* mutant CRC groups. Notably, the 5-year RFS for all codon-specific *KRAS* mutations was statistically different (wild-type, codon 12, and codon 13 mutations: 78.4%, 75.5%, and 85.3%, respectively; P = 0.013; Figure 2A), but the 5-year OS rates were comparable (wild-type, codon 12, and codon 13 mutations: 89.2%, 89.8%, and 86.9%, respectively; P = 0.805; Figure 2B). The 5-year RFS rate of the *KRAS* codon 12 mutation group was significantly lower than that of the patients without codon 12 mutations (78.9% *vs* 75.5%, P = 0.025; Figure 3A). The 5-year RFS rate of the *KRAS* codon 13 mutation group was higher than that of the patients without codon 13 mutations; however, the difference was not statistically significant (77.2% *vs* 85.3%, P = 0.159; Figure 3B). The RFS of the *KRAS* codon 61 mutation group was significantly lower than that of the patients without codon 61 mutations (78.2% *vs* 60.6%, P = 0.039; Figure 3C); however, all cases of recurrence occurred within 2 years of surgery.

In the univariate analysis of recurrence-related factors, cancer location (colon or rectum), preoperative CEA level, diverting stoma, T stage, N stage, MSI status, tumor size, lymphatic invasion, vascular invasion, perineural invasion, number of metastatic lymph nodes, and *KRAS* codon 12 mutations were associated with recurrence. In multivariable analysis, most pathological features, including higher T stage [hazard ratio (HR): 2.620; 95% confidence intervals (CI): 1.479-4.641; P = 0.001], higher N stage (HR: 2.001; 95%CI: 1.399-2.861; P < 0.001), vascular invasion (HR: 1.578; 95%CI: 1.164-2.139; P = 0.003), perineural invasion (HR: 1.684; 95%CI: 1.194-2.376; P = 0.003), and mutation of *KRAS* codon 12 (HR: 1.399; 95%CI: 1.034-1.894; P = 0.030) were identified as independent risk factors of recurrence in multivariable analysis. Among the clinical characteristics, only the presence of a diverting stoma (HR: 1.874; 95%CI: 1.260-2.787; P = 0.002) was independently correlated with recurrence (Table 3).

Tumor size (HR: 1.100; 95%CI: 1.011-1.198; P = 0.027), vascular invasion (HR: 1.981; 95%CI: 1.362-2.880; P < 0.001), perineural invasion (HR: 1.793; 95%CI: 1.200-2.679; P = 0.004), the presence of metastatic lymph nodes (HR: 1.048; 95%CI: 1.014-1.083; P = 0.006), and *KRAS* codon 12 mutation (HR: 1.496; 95%CI: 1.019-2.196; P = 0.040) were determined as independent risk factors for cancer recurrence when the primary tumor location was in the colon. Perineural invasion (HR: 3.358; 95%CI: 1.885-5.983; P < 0.001), and the presence of metastatic lymph nodes (HR: 1.095; 95%CI: 1.017-1.178; P = 0.016) were independently associated with cancer recurrence when the primary tumor was in the rectum. No codon-specific *KRAS* mutations were associated with recurrent rectal cancer (Table 4).

Among MSS/MSI-low CRC patients, tumor size (HR: 1.117; 95%CI: 1.038-1.202; P = 0.003), vascular invasion (HR: 1.740; 95%CI: 1.282-2.363; P < 0.001), perineural invasion (HR: 2.335; 95%CI: 1.663-3.279; P < 0.001), number of metastatic lymph nodes (HR: 1.050; 95%CI: 1.020-1.081; P = 0.001), and *KRAS* codon 12 mutation (HR: 1.467; 95%CI: 1.077-1.998; P = 0.015) were independent risk factors for cancer recurrence. In contrast, only a high preoperative CEA level (HR: 8.321; 95%CI: 1.387-49.920; P = 0.020) was associated with recurrence in MSI-high CRC. In cases of MSI-high CRC, the *KRAS* codon 12 mutation was statistically irrelevant regarding cancer recurrence, and there were no cases of recurrence during the study period among patients with *KRAS*-mutant CRC involving codons 13 and 61 (Table 5).

DISCUSSION

Among the 2203 patients who underwent curative surgery for stage I-III CRC, the incidence of codon-specific *KRAS* abnormalities was, respectively, 27.7%, 9.1%, and 1.3% for patients with *KRAS* codon 12, 13, and 61 mutations. Only 9.3% (205/2203) recurrences were observed during the 5-year follow-up period. To our knowledge, this study is based on the largest scaled cohort that has ever analyzed not only the oncological impact but also the clinicopathological characteristics of codon-specific *KRAS* mutations in patients with CRC. Most previous studies have reported similar results for *KRAS* codon 12 mutations, but not codon 13, in CRC as a poor oncological factor[4,8,12,14,20]. Despite the minimal oncological effects of minor *KRAS* mutations, such as in codon 61, the data obtained were sufficient to gain statistical power, supporting previous findings that *KRAS* codon 61 mutation is not associated with the clinicopathological features of CRC

Table 1 Clinicopathologic characteristics of the study patients

Clinical characteristics (*n* = 2203)	Value[1]
Age (yr)	64.7 ± 12.2
Sex	
Male	1264 (57.4)
Female	939 (42.6)
Body mass index (kg/m^2)	23.9 ± 3.3
ASA score	
1	575 (26.1)
2	1412 (64.1)
3	211 (9.6)
4	5 (0.2)
Cancer location	
Cecum	46 (2.1)
Ascending colon	386 (17.5)
Hepatic flexure	88 (4.0)
Transverse colon	115 (5.2)
Splenic flexure	18 (0.8)
Descending colon	79 (3.6)
Sigmoid colon	771 (35.0)
Rectum	700 (31.7)
Preoperative CEA (ng/mL)	7.7 ± 42.3
Diverting stoma	
Ileostomy	435 (19.7)
Colostomy	62 (2.8)
T stage	
0	18 (0.8)
1	275 (12.5)
2	383 (17.4)
3	1282 (58.2)
4	245 (11.1)
N stage	
0	1286 (58.4)
1	639 (29.0)
2	278 (12.6)
Tumor size (cm)	4.4 ± 2.4
Lymphatic invasion	597 (27.1)
Vascular invasion	469 (21.3)
Perineural invasion	934 (42.4)
Harvested lymph nodes	45.3 ± 21.2
Metastatic lymph nodes	1.4 ± 2.9
Adjuvant/Neoadjuvant therapy	
Colon	

Adjuvant therapy-stage II	274 (51.4)
Adjuvant therapy-stage III	575 (90.0)
Rectum	
Neoadjuvant therapy	200 (28.6)
Operative first-Adjuvant therapy	264 (52.8)

[1]Results are reported as mean ± SD or as frequency (percent).

ASA: American Society of Anesthesiologists; CEA: Carcinoembryonic antigen; AJCC: American Joint Committee on Cancer.

[21]. An earlier study in a Japanese cohort also identified *KRAS* codon 12, but not codon 13, as an independent risk factor for tumor recurrence in stage I-III CRC. While their results supported the utility of *KRAS* codon 12 mutation as a poor prognostic factor, the correlation between codon-specific *KRAS* mutations and clinicopathological characteristics could not be validated because of the small sample size[20]. In the present study, we analyzed the largest sample group of patients, which provided not only results complementing earlier studies on *KRAS* mutations in CRC, but also additional information on correlations with clinicopathological characteristics and prognostic factors for individual codon-specific *KRAS* mutations.

In addition to resistance to anti-EGFR therapies, such as cetuximab and panitumumab[22], *KRAS* codon 12 mutation in CRC has been established as a poor prognostic factor of survival associated with aggressive behavior[23]. However, the role of *KRAS* codon 13 mutation in CRC remains unclear. Several studies have suggested that *KRAS* codon 13 mutations are associated with advanced-stage disease and metastasis of CRC and potentially serve as a predictive factor for a higher likelihood of death[17,18]. An earlier meta-analysis reported a lower overall survival in patients with *KRAS* codon 13 mutant CRC with no exposure to anti-EGFR therapy than in those treated with targeted therapy[24]. Other studies have demonstrated that *KRAS* codon 13 mutations are not associated with CRC progression[4,19]. Another meta-analysis of metastatic CRC with mutated *KRAS* codon 13 revealed a more significant response to cetuximab than that in patients with other codon-specific *KRAS* mutations[25]. To ascertain the correlation between codon-specific *KRAS* mutations and clinical oncological outcomes throughout the stages of CRC, therapeutic options such as chemotherapy, radiotherapy, and targeted therapy, along with inevitable resistance mechanisms, should be considered[5,26,27].

The survival analysis showed that CRC recurrence, but not overall survival, was associated with codon-specific *KRAS* mutations. Analysis of individual codons showed that *KRAS* codon 12 mutation is an independent risk factor for recurrence, while *KRAS* codon 13 and 61 mutations appeared to be statistically irrelevant. In earlier *in vivo* molecular biology studies, cells with *KRAS* codon 12 and 13 mutations displayed similar morphological changes, but only codon 12 mutants induced anchorage-independent growth, implying a lower aggressiveness of *KRAS* codon 13 mutations[15]. Another *in vitro* study reported that *KRAS* codon 12 mutant cells were more resistant to apoptosis and exhibited enhanced anti-apoptotic molecular signaling relative to codon 13 mutant cells, consistent with the finding that the codon 13 mutation is less aggressive[16]. These *in vivo* results were translated into the clinical outcomes of our study, demonstrating that *KRAS* codon 13 mutation is less aggressive and less likely to serve as a poor prognostic factor for CRC compared with *KRAS* codon 12 mutation.

Interestingly, the prognosis of *KRAS* codon 12 mutant CRC varied based on the primary tumor location in either the colon or rectum. The majority of experiments on tumor location were stratified into right- or left-sided colorectum based on the splenic flexure[28,29]. Even the definition of 'left-sided' differs among studies according to the involvement of the rectum[30,31]. Thus, in the present study, recurrence-related factors were analyzed by subgrouping the tumors into colon and rectum. In the subgroup of tumors located in the colon, patients with *KRAS* codon 12 mutations were estimated to be at a 1.5-fold higher risk of CRC recurrence than those without codon 12 mutations. In contrast, in the rectum, all codon-specific *KRAS* mutations were not linked to recurrence. To the best of our knowledge, this is the first study to investigate the oncological impact of codon-specific *KRAS* mutations based on tumor location (colon or rectum). Our findings support the theory that *KRAS* codon 12 mutation is a poor prognostic factor for colon cancer, but not for rectal cancer.

Previous studies have shown that the combination of *KRAS* mutations and MSI status is a potential prognostic factor in various stages of CRC [26,32-36]. In addition, since MSI status is associated with chemoresistance[37,38], the MSS/MSI-low and MSI-high subgroups were analyzed separately to eliminate the effect of MSI status on prognosis. Interestingly, in the MSS/MSI-low patient subgroup, only *KRAS* codon 12 mutation was statistically related to recurrence, whereas there was no association between codon-specific *KRAS* mutations and recurrence among MSI-high tumors. It is well known that poor oncological outcomes including disease-free and overall survival were reported within MSS tumors combined with *KRAS* mutation[33-36]. To the best of our knowledge, analysis results of codon-specific *KRAS* mutations in MSS/MSI-low and MSI-high tumors have never been reported. Based on our subgroup analysis, *KRAS* codon 12 mutations may be associated with the location of colon and MSS tumors, and not all CRC patients with *KRAS* codon 12 mutations have poor outcomes.

Clarifying the effects of codon-specific *KRAS* mutations on the prognosis of stage IV CRC is a complex issue[5,26,27,39]. A recent study on *KRAS* mutations in CRC with liver metastasis reported that *KRAS* codon 12 mutations were associated with poorer overall survival, while codon 13 was not; however, they also pointed out the exclusion of perioperative management such as anti-epidermal growth factor receptor agents[12]. Among the patients diagnosed with stage IV CRC who underwent surgery in our hospital during the period of the present study, 48.4% had *KRAS* mutations. However, only about half of them (53.1%) underwent surgery with curative intent, whereas the others underwent

Table 2 Univariable analysis[1] of each codon-specific *KRAS* mutation

	KRAS overall[2]			*KRAS* Codon 12			*KRAS* Codon 13			*KRAS* Codon 61		
	WT (%)	MT (%)	*P* value	WT (%)	MT (%)	*P* value	WT (%)	MT (%)	*P* value	WT (%)	MT (%)	*P* value
Age			0.418			0.734			0.698			0.246
< 65 yr	644 (62.8)	382 (37.2)		745 (72.6)	281 (27.4)		936 (91.1)	91 (8.9)		1016 (99.0)	10 (1.0)	
≥ 65 yr	719 (61.1)	458 (38.9)		847 (72.0)	330 (28.0)		1067 (90.7)	110 (9.3)		1160 (98.5)	18 (1.5)	
Sex			**< 0.001**			**< 0.001**			**0.006**			0.238
Male	851 (67.3)	413 (32.7)		961 (76.0)	303 (24.0)		1167 (92.3)	97 (7.7)		1251 (99.0)	13 (1.0)	
Female	512 (54.5)	427 (45.5)		631 (67.2)	308 (32.8)		836 (88.9)	104 (11.1)		924 (98.4)	15 (1.6)	
BMI			0.098			0.347			0.485			0.104
< 25 kg/m^2	853 (60.6)	555 (39.4)		1008 (71.6)	400 (28.4)		1275 (90.6)	133 (9.4)		1386 (98.4)	22 (1.6)	
≥ 25 kg/m^2	510 (64.2)	285 (35.8)		584 (73.5)	211 (26.5)		727 (91.4)	68 (8.6)		789 (99.2)	6 (0.8)	
ASA score			**0.010**			**0.002**			0.942			0.347
1-2	1212 (61.0)	775 (39.0)		1417 (71.3)	570 (28.7)		1806 (90.9)	181 (9.1)		1963 (98.8)	24 (1.2)	
3-4	151 (69.9)	65 (30.1)		175 (81.0)	41 (19.0)		196 (90.7)	20 (9.3)		212 (98.1)	4 (1.9)	
Cancer location (1)[3]			**< 0.001**			**0.002**			**< 0.001**			0.219
Right-sided	329 (51.8)	306 (48.2)		429 (67.6)	206 (32.4)		546 (86.0)	89 (14.0)		624 (98.3)	11 (1.7)	
Left-sided	1034 (65.9)	534 (34.1)		1163 (74.2)	405 (25.8)		1456 (92.9)	112 (7.1)		1552 (98.9)	17 (1.1)	
Cancer location (2)[4]			0.092			0.405			0.117			0.966
Colon	912 (60.7)	591 (39.3)		1078 (71.7)	425 (28.3)		1356 (90.2)	147 (9.8)		1484 (98.7)	19 (1.3)	
Rectum	451 (64.4)	249 (35.6)		514 (73.4)	186 (26.6)		646 (92.3)	54 (7.7)		691 (98.7)	9 (1.3)	
Preoperative CEA			**< 0.001**			**< 0.001**			**0.037**			0.301
< 5.0 ng/mL	1131 (64.7)	616 (35.3)		1299 (74.4)	448 (25.6)		1599 (91.5)	148 (8.5)		1727 (98.9)	20 (1.1)	
≥ 5.0 ng/mL	232 (50.9)	224 (49.1)		293 (64.3)	163 (35.7)		403 (88.4)	53 (11.6)		448 (98.2)	8 (1.8)	
Diverting stoma			**0.001**			0.071			**0.029**			0.131
No	1024 (60.0)	682 (40.0)		1217 (71.3)	489 (28.7)		1538 (90.2)	168 (9.8)		1681 (98.5)	25 (1.5)	
Yes	339 (68.2)	158 (31.8)		375 (75.5)	122 (24.5)		464 (93.4)	33 (6.6)		494 (99.4)	3 (0.6)	
T stage			**0.008**			**0.003**			0.488			0.139

T0-2	446 (66.0)	230 (34.0)		517 (76.5)	159 (23.5)		610 (90.2)	66 (9.8)		671 (99.3)	5 (0.7)	
T3-4	917 (60.1)	610 (39.9)		1075 (70.4)	452 (29.6)		1392 (91.2)	135 (8.8)		1504 (98.5)	23 (1.5)	
N stage			**0.012**			**0.046**			0.617			0.094
N0	824 (64.1)	462 (35.9)		950 (73.9)	336 (26.1)		1172 (91.1)	114 (8.9)		1274 (99.1)	12 (0.9)	
N1-2	539 (58.8)	378 (41.2)		642 (70.0)	275 (30.0)		830 (90.5)	87 (9.5)		901 (98.3)	16 (1.7)	
MSI status			**0.003**			**0.001**			0.458			0.973
MSS	1138 (61.0)	728 (39.0)		1327 (71.1)	539 (28.9)		1701 (91.2)	165 (8.8)		1842 (98.7)	24 (1.3)	
MSI-low	90 (58.8)	63 (41.2)		110 (71.9)	43 (28.1)		135 (88.2)	18 (11.8)		151 (98.7)	2 (1.3)	
MSI-high	135 (73.4)	49 (26.6)		155 (84.2)	29 (15.8)		166 (90.2)	18 (9.8)		182 (98.9)	2 (1.1)	
Tumor size (cm)	4.3 ± 2.4	4.6 ± 2.3	**0.005**	4.3 ± 2.5	4.6 ± 2.1	**0.001**	4.4 ± 2.3	4.6 ± 2.7	0.837	4.4 ± 2.4	4.5 ± 2.1	0.708
Lymphatic invasion			**0.005**			0.099			0.080			0.302
No	1022 (63.6)	584 (36.4)		1176 (73.2)	430 (26.8)		1470 (91.5)	136 (8.5)		1589 (98.9)	18 (1.1)	
Yes	341 (57.1)	256 (42.9)		416 (69.7)	181 (30.3)		532 (89.1)	65 (10.9)		587 (98.3)	10 (1.7)	
Vascular invasion			0.090			**0.047**			0.614			0.061
No	1057 (61.0)	677 (39.0)		1236 (71.3)	498 (28.7)		1573 (90.7)	161 (9.3)		1716 (99.0)	18 (1.0)	
Yes	306 (65.2)	163 (34.8)		356 (75.9)	113 (24.1)		429 (91.5)	40 (8.5)		459 (97.9)	10 (2.1)	
Perineural invasion			**0.003**			**0.027**			0.387			**0.048**
No	819 (64.5)	450 (35.5)		940 (74.1)	329 (25.9)		1159 (91.3)	110 (8.7)		1258 (99.1)	11 (0.9)	
Yes	544 (58.2)	390 (41.8)		652 (69.8)	282 (30.2)		843 (90.3)	91 (9.7)		917 (98.2)	17 (1.8)	
Harvested LN	44.5 ± 20.6	46.5 ± 22.1	**0.040**	45.0 ± 21.1	45.9 ± 21.6	0.500	45.0 ± 20.9	47.9 ± 24.1	0.079	45.2 ± 21.3	47.7 ± 16.6	0.208
Metastatic LN	1.4 ± 3.1	1.3 ± 2.6	0.420	1.4 ± 3.1	1.3 ± 2.4	0.406	1.4 ± 2.9	1.4 ± 3.1	0.832	1.4 ± 2.9	1.4 ± 1.9	0.149

[1]The continuous variables were compared using either independent *t*-test or Mann-Whitney *U* test; the categorical variables were compared using Chi-square or Fisher's exact test.
[2]Kirsten rat sarcoma viral oncogene homolog overall indicates at least one mutation within codon 12, 13, or 61.
[3]Right-sided: From the cecum to distal 2/3 transverse colon; Left-sided: From the splenic flexure to rectum.
[4]Rectum: Below the pelvic inlet (an imaginary line drawn from the sacral promontory to the pubic symphysis).
KRAS: Kirsten rat sarcoma viral oncogene homolog; WT: Wild-type; MT: Mutation; BMI: Body mass index; ASA: American Society of Anesthesiologists; CEA: Carcinoembryonic antigen; MSI: Microsatellite instability; MSS: Microsatellite stable; LN: Lymph node.

palliative treatment. Additionally, there is a wide range of variations in the metastatic burden and forms of treatment for these patients. Therefore, in the present study, we excluded stage IV disease to focus on the biological importance and prognostic impact of codon-specific *KRAS* mutations in stage I-III CRC.

Table 3 Univariable and Cox regression analyses of *KRAS* mutations for determination of recurrence-related factors

	Recurrence			Multivariable Cox regression analysis[2]			
	Absent[1] (*n* = 1998)	Present[1] (*n* = 205)	*P* value	HR	95%CI Lower	95%CI Upper	*P* value
Age (yr)			0.716				
< 65	933 (46.7)	93 (45.4)		-	-	-	-
≥ 65	1065 (53.3)	112 (54.6)		-	-	-	
Sex			0.616				
Male	1143 (57.2)	121 (59.0)		-	-	-	-
Female	855 (42.8)	84 (41.0)		-	-	-	
BMI			0.094				
< 25 kg/m^2	1266 (63.4)	142 (69.3)		-	-	-	-
≥ 25 kg/m^2	732 (36.6)	63 (30.7)		-	-	-	
ASA score			0.980				
1-2	1802 (90.2)	185 (90.2)		-	-	-	-
3-4	196 (9.8)	20 (9.8)		-	-	-	
Cancer location (1)[3]			0.860				
Right-sided	577 (28.9)	58 (28.3)		-	-	-	-
Left-sided	1421 (71.1)	147 (71.7)		-	-	-	
Cancer location (2)[4]							
Colon	1376 (68.9)	127 (62.0)	**0.043**	1.000			
Rectum	622 (31.1)	78 (38.0)		1.053	0.718	1.545	0.791
Preoperative CEA			**< 0.001**				
< 5.0 ng/mL	1607 (80.4)	140 (68.3)		1.000			
≥ 5.0 ng/mL	391 (19.6)	65 (31.7)		1.158	0.849	1.579	0.354
Diverting stoma			**< 0.001**				
No	1568 (78.5)	138 (67.3)		1.000			
Yes	430 (21.5)	67 (32.7)		1.874	1.260	2.787	**0.002**
T stage			**< 0.001**				
T0-2	659 (33.0)	17 (8.3)		1.000			
T3-4	1339 (67.0)	188 (91.7)		2.620	1.479	4.641	**0.001**
N stage			**< 0.001**				
N0	1230 (61.6)	56 (27.3)		1.000			
N1-2	768 (38.4)	149 (72.7)		2.001	1.399	2.861	**< 0.001**
MSI status			**0.037**				
MSS	1680 (84.1)	186 (90.7)		0.855	0.342	2.138	0.738
MSI-low	143 (7.2)	10 (4.9)		1.284	0.643	2.566	0.479
MSI-high	175 (8.8)	9 (4.4)		1.000			
Tumor size (cm)	4.3 ± 2.4	4.9 ± 2.1	**< 0.001**	0.997	0.927	1.074	0.944
Lymphatic invasion			**< 0.001**				
No	1493 (74.7)	113 (55.1)		1.000			
Yes	505 (25.3)	92 (44.9)		1.324	0.977	1.793	0.070
Vascular invasion			**< 0.001**				

No	1615 (80.8)	119 (58.0)		1.000			
Yes	383 (19.2)	86 (42.0)		1.578	1.164	2.139	**0.003**
Perineural invasion			**< 0.001**				
No	1211 (60.6)	58 (28.3)		1.000			
Yes	787 (39.4)	147 (71.7)		1.684	1.194	2.376	**0.003**
Harvested LN	45.3 ± 21.2	44.9 ± 21.4	0.705	-	-	-	-
Metastatic LN	1.2 ± 2.6	3.3 ± 4.5	**< 0.001**	1.028	0.995	1.061	0.095
KRAS Codon 12							
Wild-type	1459 (73.0)	133 (64.9)	**0.013**	1.000			
Mutation	539 (27.0)	72 (35.1)		1.399	1.034	1.894	**0.030**
KRAS Codon 13							
Wild-type	1809 (90.5)	193 (94.1)	0.088	1.000			
Mutation	189 (9.5)	12 (5.9)		0.637	0.350	1.160	0.140
KRAS Codon 61							
Wild-type	1975 (98.8)	200 (97.6)	0.176	1.000			
Mutation	23 (1.2)	5 (2.4)		1.950	0.790	4.812	0.147

[1]Results are reported as mean ± SD or as number (percent).
[2]No values indicated variables do not match the covariance input criterion (P < 0.1 in univariable analysis).
[3]Right-sided: From the cecum to distal 2/3 transverse colon; Left-sided: From the splenic flexure to rectum.
[4]Rectum: Below the pelvic inlet (an imaginary line drawn from the sacral promontory to the pubic symphysis).
KRAS: Kirsten rat sarcoma viral oncogene homolog; HR: Hazard ratio; CI: Confidence interval; BMI: Body mass index; ASA: American Society of Anesthesiologists; CEA: Carcinoembryonic antigen; MSI: Microsatellite instability; MSS: Microsatellite stable; LN: Lymph node.

Table 4 Cox regression analyses of recurrence-related factors in subgroups based on tumor location in the colon and rectum

	Colon (n = 1503)		Rectum (n = 700)	
	HR (95%CI)	P value	HR (95%CI)	P value
Preoperative CEA ≥ 5.0 ng/mL	1.290 (0.860-1.933)	0.218	1.215 (0.705-2.220)	0.444
Diverting stoma (+)	0.903 (0.394-2.067)	0.809	1.249 (0.755-2.065)	0.386
T3-4 stage (*vs* T0-2)	1.211 (0.737-1.991)	0.450	1.079 (0.601-1.939)	0.799
N1-2 stage (*vs* N0)	1.241 (0.863-1.784)	0.244	1.126 (0.649-1.954)	0.674
Tumor size (cm)	1.100 (1.011-1.198)	**0.027**	1.077 (0.948-1.223)	0.256
Lymphatic invasion	1.342 (0.919-1.960)	0.128	0.971 (0.562-1.676)	0.915
Vascular invasion	1.981 (1.362-2.880)	**< 0.001**	1.401 (0.841-2.334)	0.195
Perineural invasion	1.793 (1.200-2.679)	**0.004**	3.358 (1.885-5.983)	**< 0.001**
Metastatic LN	1.048 (1.014-1.083)	**0.006**	1.095 (1.017-1.178)	**0.016**
KRAS Codon 12 mutation	1.496 (1.019-2.196)	**0.040**	1.492 (0.902-2.466)	0.119
KRAS Codon 13 mutation	0.831 (0.412-1.678)	0.606	0.481 (0.146-1.578)	0.227
KRAS Codon 61 mutation	2.385 (0.730-7.795)	0.150	2.270 (0.511-10.088)	0.282

KRAS: Kirsten rat sarcoma viral oncogene homolog; HR: Hazard ratio; CI: Confidence interval; CEA: Carcinoembryonic antigen; MSI: Microsatellite instability; MSS: Microsatellite stable; LN: Lymph node.

In two patients in our cohort, *KRAS* mutations were detected at two or more codon sites. The first patient was a 75-year-old male who underwent surgery for descending colon cancer and was pathologically diagnosed with stage III (pT3N1M0) colon cancer with codon 12 and 13 *KRAS* mutations. The second patient was a 60-year-old female who underwent surgery for sigmoid colon cancer diagnosed as stage I (pT1N0M0) with both codon 12 and 61 *KRAS* mutations. Both patients survived for more than 5 years after surgery with no recurrence or metastasis. In a previous

Table 5 Cox regression analyses of recurrence-related factors in subgroups based on microsatellite instability status: Microsatellite stable/microsatellite instability-low *versus* microsatellite instability-high

	MSS/MSI-low (*n* = 2019)		MSI-high (*n* = 184)[1]	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Preoperative CEA ≥ 5.0 ng/mL	1.178 (0.839-1.653)	0.344	8.321 (1.387-49.920)	**0.020**
Rectal cancer (*vs* colon cancer)	1.238 (0.850-1.804)	0.266	-	-
Diverting stoma (+)	1.069 (0.707-1.617)	0.752	2.431 (0.139-42.442)	0.543
T3-4 stage (*vs* T0-2)	1.175 (1.038-1.202)	0.407	0.284 (0.020-4.032)	0.353
N1-2 stage (*vs* N0)	1.190 (0.879-1.610)	0.260	1.000 (0.151-6.643)	1.000
Tumor size (cm)	1.117 (1.038-1.202)	**0.003**	0.991 (0.713-1.379)	0.960
Lymphatic invasion	1.242 (0.909-1.698)	0.174	1.154 (0.149-8.923)	0.891
Vascular invasion	1.740 (1.282-2.363)	**< 0.001**	0.009 (0.000-29.277)	0.255
Perineural invasion	2.335 (1.663-3.279)	**< 0.001**	0.538 (0.049-5.909)	0.613
Metastatic LN	1.050 (1.020-1.081)	**0.001**	1.442 (0.865-2.402)	0.160
KRAS Codon 12 mutation	1.467 (1.077-1.998)	**0.015**	2.508 (0.406-15.510)	0.323
KRAS Codon 13 mutation	0.713 (0.390-1.301)	0.270	-	-
KRAS Codon 61 mutation	2.265 (0.915-5.605)	0.077	-	-

[1]No values: Due to a small sample size, the hazard ratio and confidence interval were not pre.

MSI: Microsatellite instability; MSS: Microsatellite stable; HR: Hazard ratio; CI: Confidence interval; CEA: Carcinoembryonic antigen; MSI: Microsatellite instability; MSS: Microsatellite stable; LN: Lymph node.

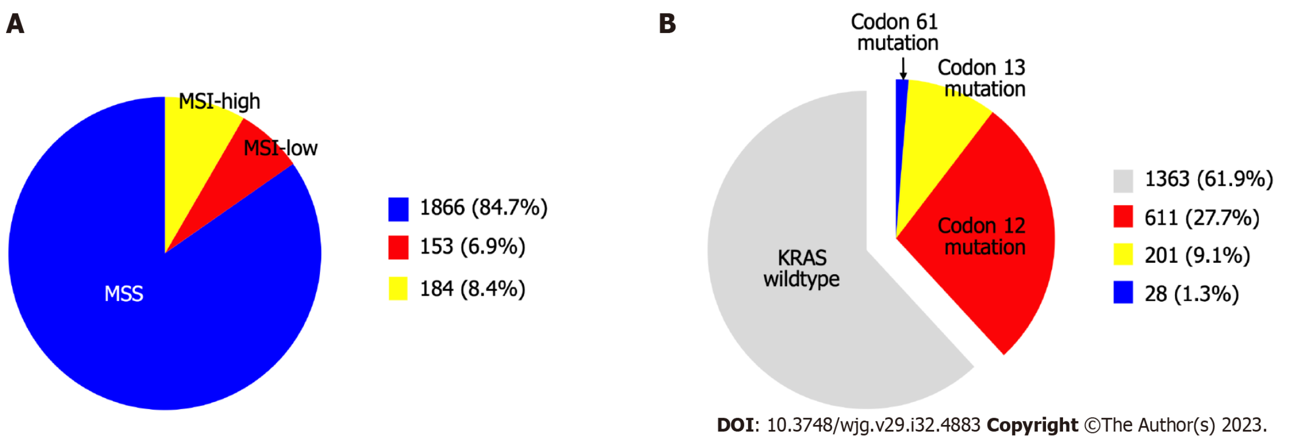

Figure 1 Incidence of microsatellite instability status and Kirsten rat sarcoma viral oncogene homolog mutations. A: Microsatellite instability status; B: Mutations of the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene in relation to baseline characteristics. *KRAS*: Kirsten rat sarcoma viral oncogene homolog; MSS: Microsatellite stable; MSI: Microsatellite instability.

study, 12 patients with two or more codon mutations among 505 CRC *KRAS* mutation cases were reported but were eventually excluded from the analysis[21]. For the same reason, these two patients were excluded from the current study despite our intellectual curiosity.

The present study had several limitations. First, *BRAF* mutation, a biomarker related to the prognosis of CRC after surgery, was omitted from our analysis. According to previous studies on CRC biomarkers, both *BRAF* and MSI status have an important prognostic impact on recurrence and survival[34,40]. Unfortunately, a large amount of data was collected without knowledge of the *BRAF* mutation status because of alterations in routine molecular examinations by our facility during the study period. Second, since *KRAS* mutations were evaluated using postoperative specimens for both colon and rectal cancer, it may be audacious to conclude that the *KRAS* codon 12 mutation is a prognostic factor in rectal cancer. In advanced rectal cancer, trimodality therapy comprises chemoradiation followed by surgery, which takes at least 1-2 mo. This delay may affect the oncological outcome; therefore, the prognostic value of codon-specific *KRAS* mutations according to the primary tumor site should be carefully interpreted. Third, uncontacted patients without follow-up could have missing data on recurrence and survival despite constantly updating the clinical data by the

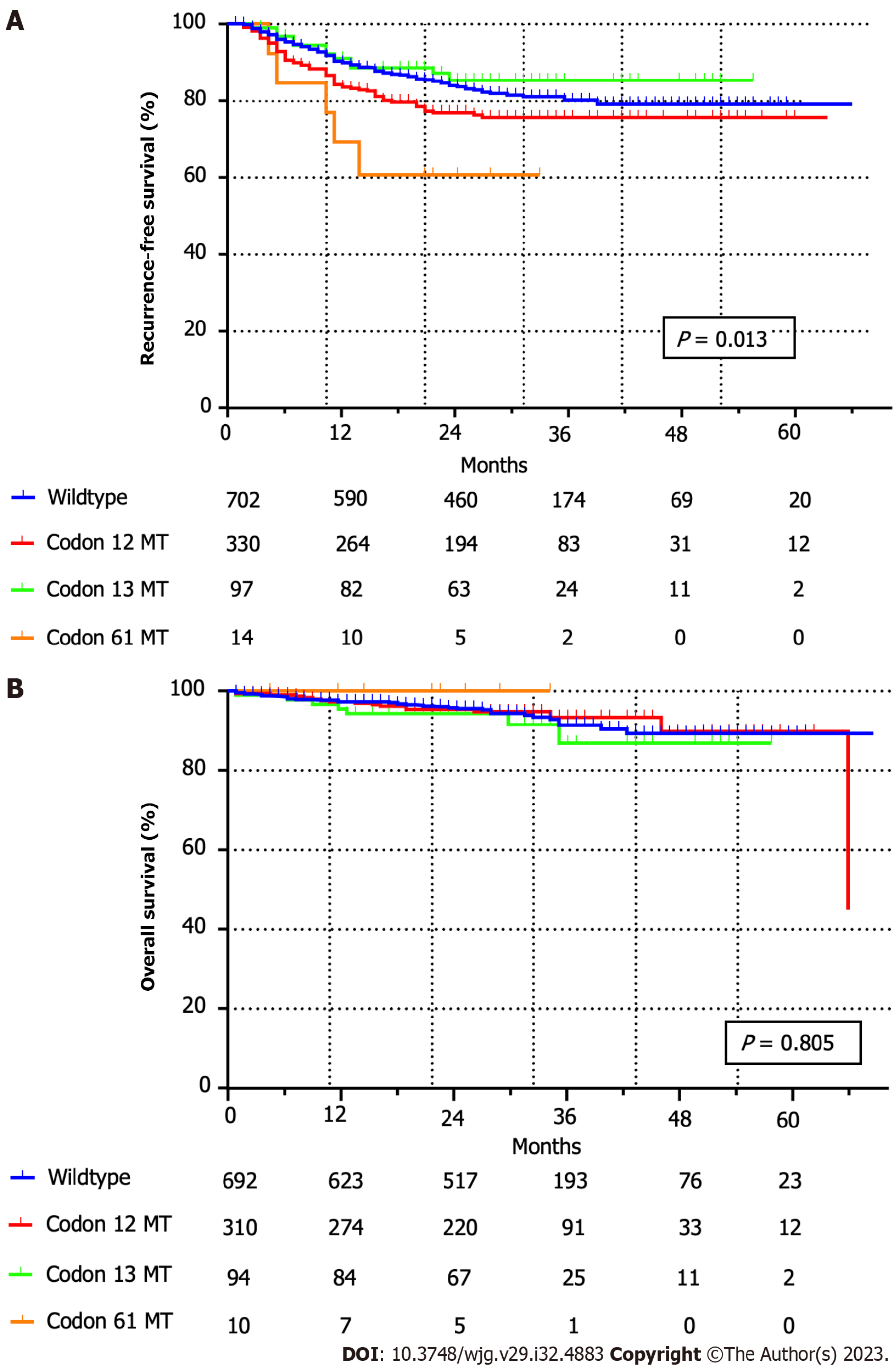

Figure 2 Comparative survival analysis between colorectal cancer samples with wild-type and Kirsten rat sarcoma viral oncogene homolog mutation. Blue lines indicate wild-type Kirsten rat sarcoma viral oncogene homolog (*KRAS*). Other lines represent codon-specific *KRAS* mutations of codon 12 (red), 13 (green), and 61 (orange). A: Recurrence-free survival; B: Overall survival rates were compared using a log-rank test. WT: Wild-type; MT: Mutation.

assigned research nurses in our department. The refusal to revisit after a few follow-ups could have produced missing data in our cohort, and double-checking with the National Health Insurance database might have reduced the error as much as possible. Unfortunately, these efforts could not separate other causes of death from cancer-related ones. Fourth, this study had a retrospective and single-center design, which could have led to selection bias. Despite this, the present study was based on a large-scale cohort with a relatively well-organized CRC registry of patients who underwent surgery, and is the largest cohort study ever that analyzed codon-specific KRAS mutations.

CONCLUSION

Most of the *KRAS* mutations in our study involved *KRAS* codons 12 and 13. Notably, *KRAS* codon 12 mutation was significantly associated with pathological features closely related to cancer recurrence and had a poor prognostic impact

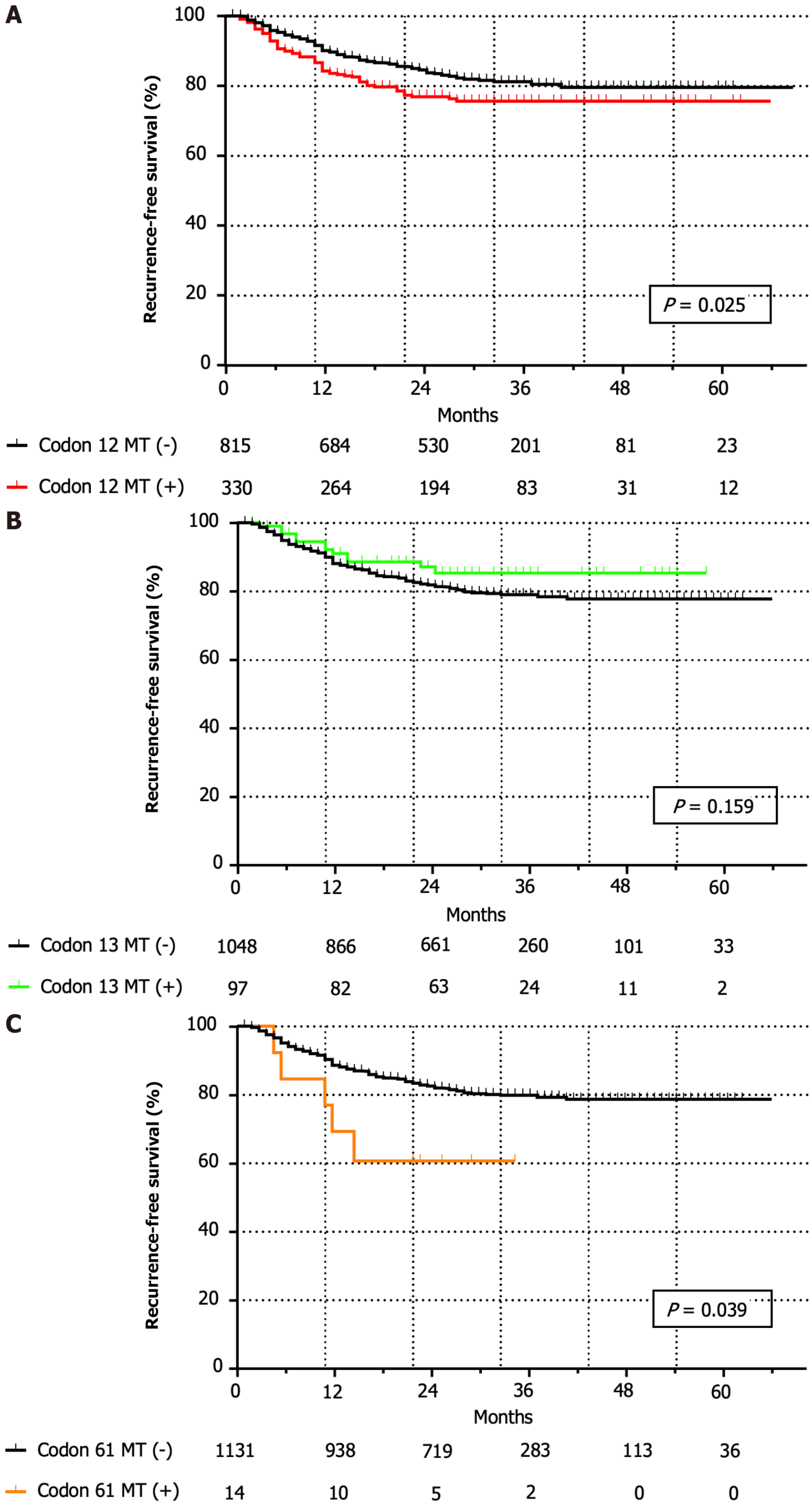

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Figure 3 Survival analysis of each codon-specific Kirsten rat sarcoma viral oncogene homolog mutation in colorectal cancer. Colored lines indicate codon-specific Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations. A: The red line indicates the recurrence-free survival (RFS) of patients with *KRAS* codon 12 mutations; B: The green line indicates the RFS of patients with *KRAS* codon 13 mutations; C: The orange line indicates the RFS of patients with *KRAS* codon 61 mutations. MT: Mutation.

in patients with MSS tumors, or those located in the colon but not in the rectum. Given its irrelevance to pathological features and recurrence, we propose that *KRAS* codon 13 mutation is less likely to serve as a prognostic factor for CRC.

ARTICLE HIGHLIGHTS

Research background

Abnormal activation of Kirsten rat sarcoma viral oncogene homolog (*KRAS*), a well-known oncogene, triggers uncontrolled tumor cell proliferation. Approximately 40% of colorectal cancer (CRC) are linked to *KRAS* mutations. CRC -related point mutations in *KRAS* occur at different codon locations. *KRAS* codon 12 or 13 mutations are detected in a majority of CRC patients, whereas mutations in codon 61 or 146 have been reported only in a minority.

Research motivation

KRAS mutations are associated with poor CRC prognosis, especially *KRAS* codon 12 mutation, which is associated with metastasis and poorer survival. However, the clinicopathological characteristics and prognosis of *KRAS* codon 13 mutation in CRC remain controversial.

Research objectives

This study aimed to evaluate the clinicopathological characteristics and prognostic value of codon-specific *KRAS* mutations, especially in codon 13.

Research methods

This retrospective, single-center, observational cohort study included patients who underwent surgery for stage I-III CRC. The relationships between clinicopathological characteristics and individual codon-specific *KRAS* mutations were analyzed. By using the Cox proportional hazards regression model, survival analysis were performed to identify codon-specific *KRAS* mutations as recurrence-related factors.

Research results

Both *KARS* codons 12 and 13 mutations showed a tendency to be associated with clinical characteristics, but only codon 12 was associated with pathological features. *KRAS* codon 13 mutation showed no associations, whereas codon 12 was associated with a lower 5-year recurrence-free survival rate. In multivariable analysis, only codon 12 (HR: 1.399; 95% confidence interval: 1.034-1.894; P = 0.030) among *KRAS* mutations was an independent risk factor for recurrence. This may influence many oncologists to consult with patients on their prognosis after surgery.

Research conclusions

KRAS codon 12 mutation was significantly associated with pathological features closely related to cancer recurrence and had a poor prognostic impact in patients with microsatellite stable tumors, or those located in the colon but not in the rectum. On the other hand, *KRAS* codon 13 mutation is irrelevant to pathological features and recurrence, which consider less likely to serve as a prognostic factor for CRC.

Research perspectives

Focusing on the biological effects of codon-specific *KRAS* mutations, *KRAS* codon 13 mutation is less pathogenic and recurrent, Based on a large-scale cohort of patients with stage I-III CRC. This study's results may influence not only the prognosis but also the management of CRC patients individually. Therefore, the therapeutic usage and needs of codon-specific *KRAS* mutation in CRC should be considered in future studies.

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FOOTNOTES

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Institutional review board statement: This study was reviewed and approved by the Institutional Review Board of Seoul National University Bundang Hospital (Approval No. B-2203-742-101).

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