RESEARCH ARTICLE

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Correlations among KRAS Mutation, Microsatellite Instability, and ^{18}F-FDG Uptake in Colon Cancer

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Abstract

Objective: This study aimed to evaluate the correlation of the maximum standardized uptake value (SUVmax) with the Kirsten ras sarcoma viral oncogene (KRAS) mutation and microsatellite instability (MSI) status in colon cancer. **Methods:** This retrospective study included 195 patients with colon cancer who underwent ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET/CT) before surgery between January 2014 and December 2017. All patients underwent KRAS mutation and MSI analyses using surgical specimens of the primary tumor. The associations of SUVmax with KRAS mutation and MSI were analyzed. **Results:** The SUVmax differed significantly between the microsatellite stable (MSS) and MSI groups (14.5 ± 7.0 vs. 19.1 ± 10.9; P = 0.0249), and between the KRAS wild-type and KRAS mutation groups (14.1 ± 7.6 vs. 17.5 ± 7.9; P = 0.0017). **Conclusions:** SUVmax obtained using ^{18}F-FDG PET/CT showed significant differences in relation to KRAS mutation and MSI status. ^{18}F-FDG PET/CT could be used as a supplemental modality for assessing KRAS mutations and MSI status in colon cancer.

Keywords: Colon cancer- F-18 FDG PET/CT- SUVmax- KRAS- MSI

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Introduction

Approximately 105,000 new colon cancer patients are diagnosed and 53,000 deaths occur due to colon cancer every year in the United States. (Siegel, Miller et al. 2020) Approximately 30%–40% of patients with colorectal cancer have a Kirsten ras sarcoma viral oncogene (KRAS) mutation (Meng et al. 2021). Although the existence of KRAS mutations is related to resistance to anti-epidermal growth factor receptor (EGFR) therapy in colorectal cancer, (Karapetis et al., 2008; Allegra et al., 2009) the prognostic value of these mutations in colon cancer remains debatable (Lee et al., 1996; Roth et al., 2010; Hutchins et al., 2011).

Microsatellite instability (MSI) is characterized by the loss of mismatch repair (MMR) activity. Approximately 15%–25% of colorectal cancers show MSI (Peltomäki 2003). Colon cancers with MSI tend to arise in the proximal colon, are poorly differentiated, and have a better prognosis than those with microsatellite stability (MSS) (Raut et al., 2004; Popat et al., 2005) Checkpoint inhibitors (ICIs) have been recently used for the treatment of various malignancies and have shown good treatment outcomes and relatively fewer side effects in comparison with cytotoxic chemotherapy in certain malignancies. (Robert 2020) However, in colon cancer, their clinical benefit is only obtained in patients with MSI (Le et al., 2015; André et al., 2020).

^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) is widely used for diagnosing tumors, staging, and therapy monitoring in cases of colon cancer (Delbeke and Martin, 2004; Lonneux, 2008; Ben-Haim and Ell, 2009) The maximum standardized uptake value (SUVmax), which reflects glucose metabolism, is the most frequently used parameter for ^{18}F-FDG PET/CT. The correlation between SUVmax and KRAS mutations has been assessed in several studies. However, these studies showed conflicting results and included a relatively small number of patients (Kawada et al., 2012; Chen et al., 2014; Krikelis et al., 2014; Kawada et al., 2015; Lee et al., 2016; Cho et al., 2017; Oner et al., 2017; Kim et al., 2019; Arslan et al., 2020) On the other hand, only two studies have reported the relationship between SUVmax and MSI in colon cancer, and these studies found no significant relationship between SUVmax and MSI (Li et al., 2021; Liu et al., 2021).

Although the prognosis of colon cancer is clearly related to TNM staging, many additional factors, including MSI and KRAS mutations, are known prognostic factors, and assessments of MSI and KRAS mutations are widely used in colorectal cancer. However, sampling of tumor tissue is often difficult in metastatic colon cancer. Moreover, the biopsy samples in such cases may not correctly represent KRAS status because of intratumoral heterogeneity (Baldus et al., 2010). These issues have highlighted the need for complementary examinations to

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support the results of gene profiling. Thus, the purpose of this study was to assess the correlation of KRAS mutations and MSI with 18F-FDG uptake and thereby complement the results of gene profiling.

Materials and Methods

Patients

This retrospective study included 195 patients with colon cancer who underwent ^{18}F-FDG PET/CT before surgery between January 2014 and December 2017. All patients underwent KRAS mutation and MSI analyses using surgical specimens of the primary tumor. The following patients were excluded from the study: 1) those who were diagnosed with synchronous cancer and 2) those who underwent preoperative chemotherapy or radiotherapy. The clinical stages were determined according to the American Joint Committee on Cancer Manual, 8th edition. (Amin et al., 2017) This study was approved by the Institutional Review Board of our hospital.

FDG PET/CT imaging

PET/CT examinations were performed using a Discovery STE PET/CT scanner (GE Healthcare, Milwaukee, WI, USA). For all patients enrolled in the study, oral intake and intravenous glucose injection were prohibited for at least 6 h before the PET/CT scan. Before injection of 18F-FDG, the blood glucose levels of the patients were assessed. PET/CT scans were obtained when blood glucose level was less than 200 mg/dL. A torso scan was acquired 60 min after intravenous injection of approximately 370 MBq of 18F-FDG. The CT images were acquired using a multidetector CT equipment with the standard protocol and the following parameters: tube voltage, 140 kV; tube current, 60–80 mA; tube rotation time, 0.4 s per rotation; pitch, 0.984; and section thickness, 3.75 mm. Emission PET data were acquired for 2 min per bed. PET images were reconstructed using an ordered-subset expectation maximization iterative reconstruction algorithm with three iterations, 18 subsets, a matrix size of 512 × 512, and a 50-cm transaxial field-of-view (FOV). The PET images were then fused with CT images.

Image analysis

Semi-quantitative image analysis was performed by experienced nuclear medicine physicians on a GE AW 4.6 workstation (GE Healthcare, Milwaukee, WI, USA). For semi-quantitative analysis, a three-dimensional volume of interest (3D VOI) was drawn on the primary tumor by reviewing the patient's clinical history and abdominopelvic CT findings. The SUVmax of the pixels within the 3D VOI was then obtained. SUVmax was calculated automatically using the software. The formula was as follows: maximum activity within VOI (MBq/mL)/ injected 18F-FDG dose (MBq/kg).

KRAS mutation and MSI analysis

DNA was extracted from formalin-fixed paraffin-embedded tumor tissues. KRAS codons 12, 13, and 61 were amplified by polymerase chain reaction (PCR), and KRAS mutations were assessed using the pyrosequencing method according to the manufacturer's instructions. Mononucleotide markers (BAT25 and BAT26) and dinucleotide markers (D2S123, D5S346, and D17S250) specific for microsatellite loci were amplified by multiplex PCR, and MSI status was assessed by capillary electrophoresis.

Statistical analysis

Statistical analyses were performed using MedCalc for Windows, version 20.105 (MedCalc Software, Ostend, Belgium) and R software version 4.1.3. For all statistical comparisons, a P value less than 0.05 was considered significant. The Mann–Whitney U test was used to compare the mean SUVmax in relation to MSI and KRAS status. Receiver operating characteristic (ROC) curve analysis was performed to examine the diagnostic performance of ^{18}F-FDG PET/CT for differentiating MSI and KRAS status.

Results

Characteristics of the study population

The characteristics of the patients included in the study are shown in Table 1. A total of 195 patients (105 male and 90 female patients; mean age, 66.8 ± 11.3 years) were included in this study. The mean SUVmax of the primary tumor was 15.2 ± 7.9. MSI was detected in 29 of the 195 patients (14.9%). KRAS mutations at codons 12 and 13 were found in 62 of the 195 patients (31.8%). Among the

Table 1. Patient Characteristics

Characteristics	Number (%)
Age (years) mean±SD	66.83±11.33
Sex (n (%))	
Male	105 (54)
Female	90 (46)
Histologic grade (n (%))	
Low	26 (13)
Intermediate	162 (83)
High	7 (4)
Stage (n (%))	
I/II	132 (68)
III/IV	63 (32)
KRAS status(n (%))	
Wild-type	133 (68)
Mutation	62 (32)
Microsatellite status(n (%))	
MSS	166 (85)
MSI	29 (15)
CEA (ng/mL) mean±SD	6.56±16.35
CA19-9 (U/mL) mean±SD	23.08±110.98
SUVmax mean±SD	15.16±7.86

SD, standard deviation; KRAS, Kirsten ras sarcoma viral oncogene; MSS, microsatellite stable; MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; SUVmax, maximum standardized uptake value.

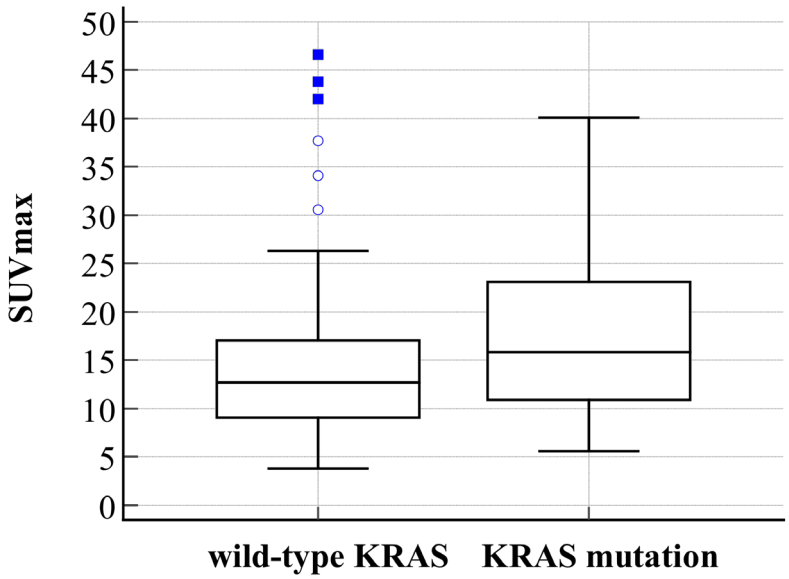

Figure 1a. Analysis of SUVmax according to the KRAS Status. SUVmax was significantly higher in patients with KRAS mutation than in those with wild-type KRAS (P = 0.0017; Mann–Whitney U test).

195 patients, 132 had stage I–II disease while 63 patients had stage III-IV disease. The mean carcinoembryonic antigen (CEA) and carbohydrate antigen (CA)19-9 levels were 6.6 ± 16.3 and 23.1 ± 111.0, respectively. The mean body mass index (BMI) was 28.6 ± 49.7. Twenty-six tumors were low-grade, 162 were intermediate-grade, and seven were high-grade.

Relationship between SUVmax and MSI and KRAS status

The SUVmax was 14.5 ± 7.0 in the MSS group and 19.1 ± 10.9 in the MSI group, and the SUVmax differed significantly between the two groups (P = 0.0249) (Fig. 1a). SUVmax was 14.1 ± 7.6 in the KRAS wild-type group and 17.5 ± 7.9 in the KRAS mutation group, and the SUVmax differed significantly between the two groups (P = 0.0017) (Figure 1b).

ROC curve analysis

In the ROC curve analysis for differentiation of the KRAS mutation group from the KRAS wild-type group, a cutoff SUVmax of 14.8 yielded the highest accuracy (sensitivity, 59.7%; specificity, 63.9%; and area under curve [AUC], 0.64) (Figure 2a). For the differentiation of MSI from MSS, a cutoff SUVmax of 10.1 yielded the highest accuracy (sensitivity, 93.1%; specificity, 31.3%; and AUC, 0.63) (Figure 2b).

Discussion

KRAS mutations are found in 30%-40% of patients with colon cancer and are related to resistance to

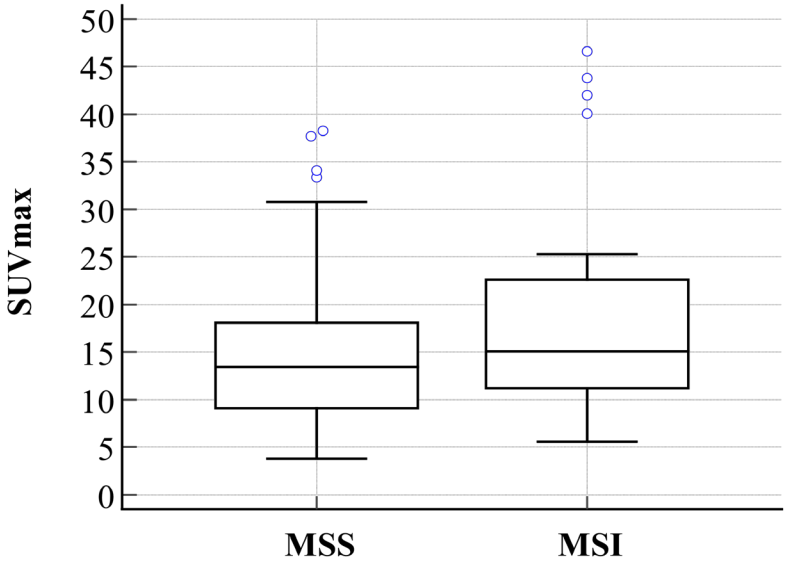

Figure 1b. Analysis of SUVmax According to the Microsatellite Status. SUVmax was significantly higher in patients with microsatellite instability (MSI) than in those with microsatellite stability (MSS; P = 0.0249; Mann–Whitney U test).

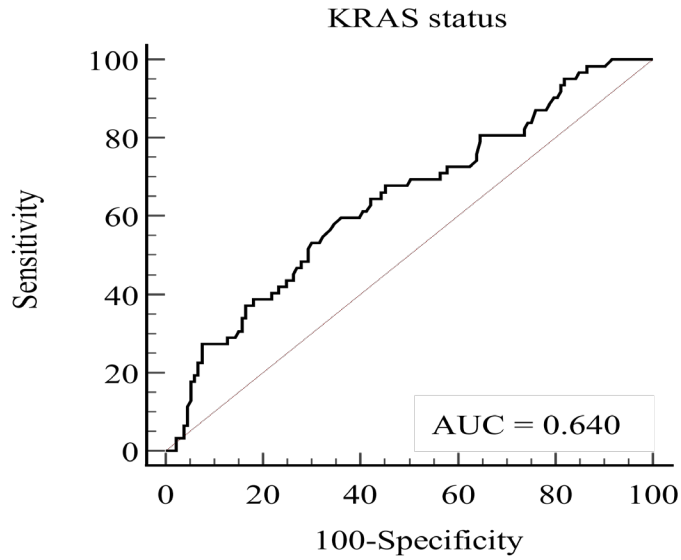

Figure 2a. ROC Curve of SUVmax for Distinguishing KRAS Mutation from Wild-Type KRAS. AUC, area under curve; KRAS, Kirsten ras sarcoma viral oncogene; SUVmax, maximum standardized uptake value; ROC, receiver operating characteristic curve.

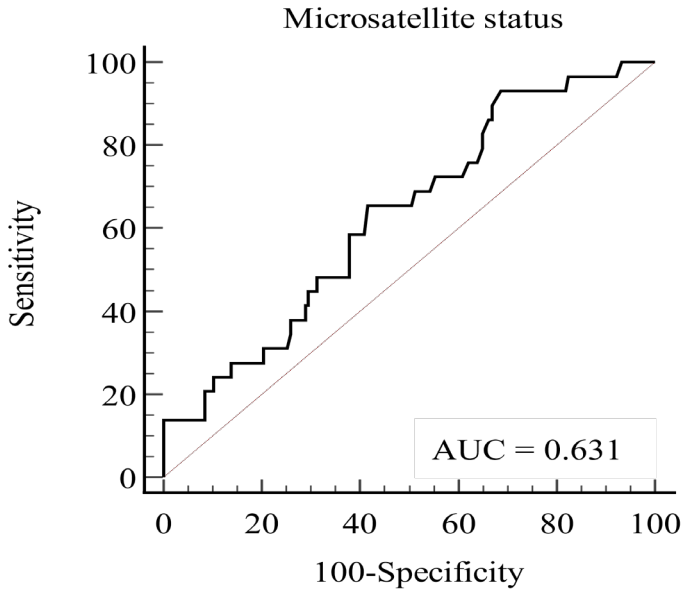

Figure 2b. ROC Curve of SUVmax for Distinguishing MSI from MSS. AUC, area under curve; MSS, microsatellite stability; MSI, microsatellite instability; SUVmax, maximum standardized uptake value; ROC, receiver operating characteristic curve.

anti-EGFR therapy. Therefore, KRAS mutation analysis is indispensable before initiation of anti-EGFR therapy. However, due to the difficulties in sampling metastatic tumor tissue and the intratumoral heterogeneity of KRAS mutation status in a single tumor sample, complementary examinations are needed to support the results of gene profiling. (Baldus et al., 2010). In this study, we aimed to evaluate the correlation of SUVmax with KRAS mutation and MSI status. Significant differences were observed between the SUVmax of tumors with KRAS mutation and wild-type KRAS, and between the SUVmax of tumors with MSI and MSS.

^{18}F-FDG PET/CT is widely used for the diagnosis, staging, and therapy monitoring of colon cancer. (Delbeke and Martin, 2004; Lonneux, 2008; Ben-Haim and Ell, 2009). Several previous studies have reported the relationship between 18F-FDG uptake and KRAS mutation status. Most studies have reported that SUVmax differed significantly according to KRAS mutation status (Kawada et al., 2012; Chen et al., 2014; Kawada et al., 2015; Lee et al., 2016; Cho et al., 2017; Arslan et al., 2020). Although a few studies have reported that SUVmax did not differ significantly according to KRAS mutation status, (Krikelis et al., 2014; Oner et al., 2017) these studies were performed with a relatively small number of patients. In this study, the SUVmax was significantly higher in patients with KRAS mutations than in those with wild-type KRAS. A previous study reported that colorectal cell lines with KRAS mutation showed higher expression of glucose transporter 1 (GLUT-1) than that of wild-type

KRAS (Yun et al., 2009; Iwamoto et al., 2014). Since the GLUT protein is an important factor for the accumulation of glucose in tumor cells, this finding can explain the relationship between SUVmax and KRAS mutation status (Avril, 2004). In this study, although the SUVmax values of tumors with KRAS mutations and wild-type KRAS were significantly different, the diagnostic accuracy of SUVmax for differentiation of patients with KRAS mutation and those with wild-type KRAS was relatively low (AUC, 0.64). A previous meta-analysis also reported that ^{18}F-FDG PET/CT might not be useful in predicting the status of KRAS mutations (Kim et al., 2019).

MSI is characterized by the loss of MMR activity. Approximately 15%–25% of colorectal cancers show MSI (Peltomäki, 2003). Colon cancers with MSI tend to arise in the proximal colon, are poorly differentiated, and have a good prognosis (Raut et al., 2004; Popat et al., 2005). Furthermore, ICIs showed clinical benefit only in patients with MSI (Le et al., 2015, André et al., 2020). Only two studies have reported the relationship between SUVmax and MSI in colon cancer, and these included a small number of patients with MSI, which showed no significant relationship between SUVmax and MSI (Li et al., 2021; Liu et al., 2021). In this study, the SUVmax of tumors with MSI was significantly higher than that of tumors with MSS. A previous study reported a relationship between the SUVmax and MSI in stomach cancer. The authors of that study suggested that tumors with MSI may show increased FDG accumulation, potentially due to inflammation (Chung et al., 2013). However, no previous studies have evaluated the signaling pathway of glucose metabolism in MSI tumors. Future studies investigating the signaling pathways of glucose metabolism leading to increased SUVmax in MSI tumors are needed. In this study, the SUVmax of tumors with MSI and MSS was significantly different, and the diagnostic accuracy of SUVmax for differentiating between patients with MSI and those with MSS was relatively low (AUC, 0.63).

Machine learning methods have been widely used to predict cancer recurrence, lymph node metastasis status, and other clinically important factors for treatment decisions with high diagnostic accuracy. In this study, SUVmax was significantly correlated with KRAS mutations and MSI. Although KRAS mutation and MSI could not be predicted accurately with only SUVmax, in future studies, machine learning models using more clinical factors that are related to KRAS and MSI may predict the KRAS status and MSI more precisely.

Our study had certain limitations. First, patients with distant metastasis are usually not candidate for surgery. KRAS mutation and MSI analyses using surgical specimens of the primary tumor wasn't conducted for those patients. Thus, patients with distant metastasis could not be sufficiently included. Second, although the findings showed significant differences in SUVmax between KRAS mutation and wild-type KRAS, and between MSI and MSS, the overlapping range of SUVmax values between the groups was wide, and diagnostic accuracy was relatively low. Future studies using machine learning models with more clinically relevant factors, including SUVmax, may show higher diagnostic accuracy for the differentiation of patients with KRAS mutations and wild-type KRAS, and patients with MSI and MSS.

In conclusion, SUVmax using ^{18}F-FDG PET/CT showed significant differences according to KRAS mutation and MSI status. ^{18}F-FDG PET/CT could be used as a supplemental modality for assessing KRAS mutations and MSI status in colon cancer.

Author Contribution Statement

SS contributed to the study design, data analysis, and writing of the manuscript. SJ contributed to data collection and analysis. JS contributed to data analysis and interpretation and supervised the finding of this work. All authors discussed the results and contributed to final manuscript.

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Ethics statement

The study was approved by the Institutional Review Board of Inje University Busan Paik Hospital (IRB No. 2021-11-017) and was performed in accordance with the ethical standards proposed in the 1964 Declaration of Helsinki and its later amendments.

Data Availability

Participants of this study did not agree for their data to be shared publicly, sharing the data is not available.

Study Registration

This study was not registered in any registering dataset.

Conflict of Interest

The authors declare no other potential conflicts of interest relevant to this article.

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