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Relationship between Preoperative ^{18}F-Fluorodeoxyglucose Uptake and Epidermal Growth Factor Receptor Status in Primary Colorectal Cancer

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Purpose: Both ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake and epidermal growth factor receptor (EGFR) status are prognostic variables of colorectal cancer (CRC). The aim of this study was to investigate a possible association between ^{18}F-FDG uptake on preoperative positron emission tomography/computed tomography (PET/CT) and EGFR status in primary CRC.

Materials and Methods: Records of 132 patients (66 men and 66 women; mean age=67.1±11.1 years) who underwent ^{18}F-FDG PET/CT for CRC staging and subsequent bowel resection were reviewed. In primary lesions, ^{18}F-FDG uptake was semiquantitatively evaluated in terms of maximum standardized uptake value (SUV_{max}), and EGFR status was determined by immunohistochemistry. Associations of clinicopathological parameters and EGFR status were analyzed by Pearson's chi-square test, multiple logistic regression, and receiver operating characteristic curves.

Results: Eighty-six patients (65.2%) showed EGFR expression. SUV_{max} was significantly lower in EGFR-negative tumors than in EGFR-expressing tumors (10.0±4.2 vs. 12.1±2.1; p=0.012). It was the only significant parameter correlated with EGFR expression (odds ratio=2.457; relative risk=2.013; p=0.038). At the SUV_{max} threshold of 7.5, the sensitivity and specificity for predicting EGFR expression were 84.9% and 40.4%, respectively (area under the curve=0.624; p=0.019).

Conclusion: Preoperative ^{18}F-FDG uptake is slightly correlated with EGFR status in primary CRC. Preoperative SUV_{max} of ^{18}F-FDG may have a limited role in predicting EGFR expression in such tumors because of its poor specificity.

Key Words: Colorectal cancer, ^{18}F-fluorodeoxyglucose, epidermal growth factor receptor

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide.[1] Its prognosis is related to the disease stage at diagnosis and ability to achieve surgical clearance.[2,3] Approximately 80% of the patients present with locoregional disease and 20% show metastatic disease.[4] As with other malignancies, CRC is thought to develop through genetic alterations that cause dysregulation of cell growth. Several molecular markers are available for characterization of CRC and some provide information on the therapeutic response and prognosis.[5] In particular, epidermal growth factor receptor (EGFR) and its downstream signaling pathways regulate key cellular events that drive the progression of many human tumors, including CRC.[6,7] EGFR is overexpressed in 70–80% of the CRCs and is targeted with a monoclonal antibody, such as cetuximab.[8] In many cases, its expression is associated with poor survival.[9-11] Although determination of EGFR status is critical in cancer treatment, it can be confirmed only after pathological examination. Therefore, noninvasive methods would be helpful to predict EGFR expression.

Positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is a useful tool for staging, restaging, thera-

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peutic response monitoring, and prognostication of CRC.[12-14] It shows high sensitivity for detecting metastatic disease.[15,16] Only a few studies have been performed to estimate EGFR status in CRC noninvasively by ^{18}F-FDG PET.

The aim of this retrospective study was to investigate a possible association between ^{18}F-FDG uptake on preoperative PET/CT and EGFR status in primary CRC.

MATERIALS AND METHODS

Subjects

Records of 250 patients with CRC who underwent ^{18}F-FDG PET/CT for staging between 2008 and 2013 at a single institution were reviewed. Those who had received any chemotherapy, radiation therapy, or molecular targeted therapy were excluded. Patients with uncontrolled diabetes mellitus were also excluded. The final analysis included 132 patients. The median duration between preoperative ^{18}F-FDG PET/CT and primary tumor resection was 5.5 days (range=1–20 days). The clinical stage was determined according to the American Joint Committee on Cancer. This study was approved by the Institutional Review Board/Ethics Committee of Hallym University Sacred Heart Hospital, and patient information was de-identified before analysis.

PET/CT

Whole-body PET was performed in a PET/CT scanner (Gemini TF-64, Philips Medical Systems, Cleveland, OH, USA) after intravenous injection of about 5.18 MBq/kg (0.14 mCi/Kg) of ^{18}F-FDG. All patients fasted for at least 6 h previously and presented with a blood glucose level lower than 150 mg/dL. After approximately 1 h, CT images were acquired from the base of the skull to the upper thigh. PET images were obtained immediately thereafter and coregistered with the CT images, allowing the display of PET, CT, and PET/CT images.

Standardized uptake values (SUVs) of ^{18}F-FDG were calculated as follows: SUV=[decay-corrected activity (kBq)/tissue volume (mL)]/[injected ^{18}F-FDG activity (kBq)/body mass (g)]. The region of interest (ROI) was placed manually around the primary tumor and maximum SUV (SUV_{max}) within the ROI was used to minimize partial-volume effects.

Immunohistochemistry

Immunostaining was carried out in an automated tissue staining system (BenchMark XT; Ventana Medical Systems, Inc., Tucson, AZ, USA) using validated protocols.[17] Endogenous peroxidase activity was blocked by hydrogen peroxide before antibody incubation. A combination of ethylenediaminetetraacetic acid and boric acid in Tris buffer (CC1 reagent; Ventana Medical Systems) was applied to tissue sections for antigen retrieval, as needed, before incubation with the primary antibody (anti-EGFR, prediluted; Ventana Medical Systems). The tissue sections were washed and incubated with the primary antibody and then with horseradish peroxidase-conjugated multimer antibody reagent (ultraView universal HRP multimer; Ventana Medical Systems). Antigen detection was performed using diaminobenzidine (ultraView universal DAB chromogen; Ventana Medical Systems), and sections were counterstained with hematoxylin. Tumor cells were considered as EGFR-positive when their staining was more marked than that of the adjacent normal epithelium.

Statistical analysis

Continuous variables were analyzed using Student's t-test, and the results are expressed as mean±standard deviation. Associations of SUV_{max}, tumor size, and carcinoembryonic antigen (CEA) level (ng/mL) were tested by bivariate Pearson's correlation test. To identify factors associated with EGFR status, Pearson's chi-square test was used in univariate analysis. Multivariate logistic regression was applied to identify associations of clinicopathological parameters and EGFR status. Receiver operating characteristic curves and Youden's index were used to identify threshold values of SUV_{max}, tumor size, and CEA level with the highest accuracy for predicting EGFR

Table 1. Patient Characteristics

Characteristic	Subcategory	n	%
Age (yrs)	<64	46	34.8
	≥64	86	65.2
Gender	Male	66	50
	Female	66	50
Tumor stage	T1	7	5.3
	T2	17	12.9
	T3	96	72.7
	T4	12	9.1
Lymph node metastasis	Absent	62	47.0
	Present	70	53.0
Distant metastasis	Absent	116	87.9
	Present	16	12.1
Histopathologic diagnosis	Adenocarcinoma	124	93.9
	Mucinous carcinoma	6	4.5
	Signet ring cell carcinoma	2	1.5
Differentiation	Well differentiated	48	36.4
	Moderately differentiated	79	59.8
	Poorly differentiated	5	3.8
Lymphovascular invasion	Absent	82	62.1
	Present	50	37.9
Perineural invasion	Absent	117	88.6
	Present	15	11.4
P53 status	Negative	32	24.2
	Positive	100	75.8
EGFR status	Negative	46	34.8
	Positive	86	65.2

EGFR, epidermal growth factor receptor.

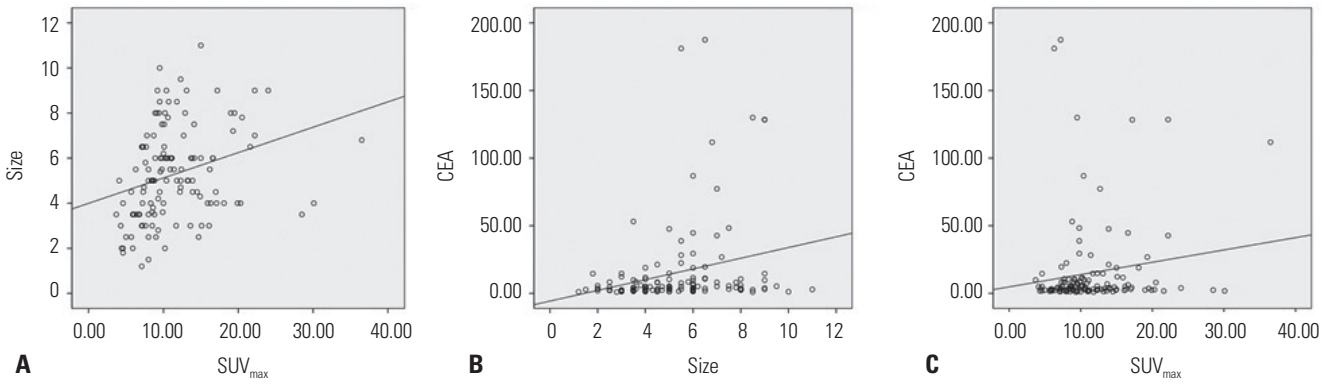

Fig. 1. Relationships of SUV_{max}, tumor size, and CEA level in primary CRC. (A) SUV_{max} showed a significant positive linear correlation with tumor size (r=0.293; p=0.001). (B) Tumor size and CEA level showed a significant positive linear correlation (r=0.253; p=0.003). (C) No correlation was noted between CEA level and SUV_{max} (r=0.151; p=0.083). SUV_{max}, maximum standardized uptake value; CEA, carcinoembryonic antigen; CRC, colorectal cancer.

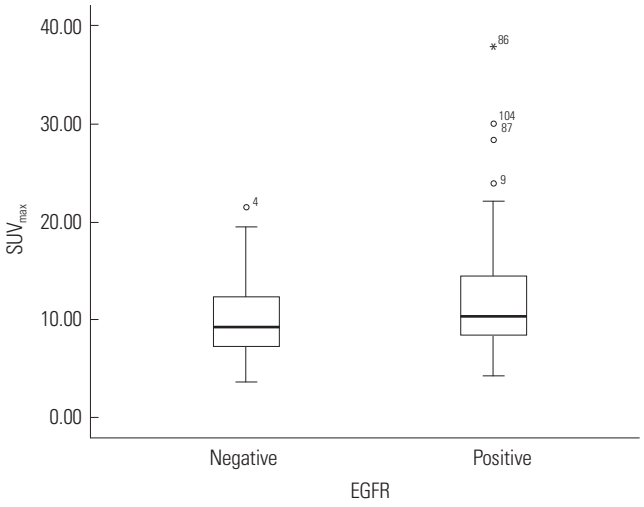

Fig. 2. Relationship of SUV_{max} and EGFR status in primary CRC. EGFR-expressing tumors had higher SUV_{max} than EGFR-negative tumors. SUV_{max}, maximum standardized uptake value; EGFR, epidermal growth factor receptor; CRC, colorectal cancer.

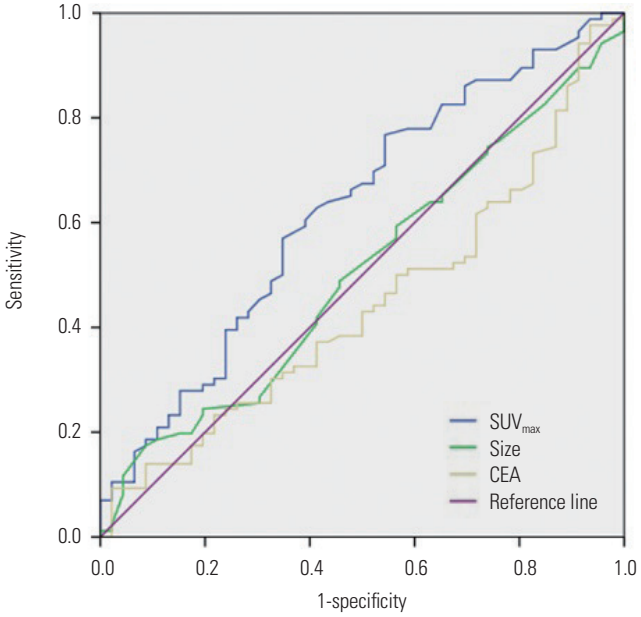

Fig. 3. ROC curves of SUV_{max}, tumor size, and CEA level for predicting EGFR expression in primary CRC. SUV_{max}: AUC=0.624, p=0.019; tumor size: AUC=0.506, p=0.909; CEA level: AUC=0.445, p=0.296. ROC, receiver operating characteristic; SUV_{max}, maximum standardized uptake value; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; CRC, colorectal cancer; AUC, area under the curve.

expression. Values of p<0.05 were considered significant. All analyses were performed in SPSS software (version 21.0; IBM Corp., Armonk, NY, USA).

RESULTS

Table 1 shows the patient characteristics. Sixty-six patients (50%) were men, and the median age was 67 years (range=35–89 years). Twenty-four lesions were staged as T1–2 (18.2%) and 108 as T3–4 (81.8%). Lymph node metastases were detected in 70 patients (53.0%). Further, 16 patients showed distant metastases (12.1%): the most common site was the liver (10 cases, 62.5%), followed by the lungs (5 cases) and bone (1 case). Curative bowel resection was performed for single hepatic metastasis, while palliative bowel resection was performed for distant metastases, because of bowel obstruction.

PET showed ^{18}F-FDG hypermetabolism in all the primary tumors. The SUV_{max} ranged from 3.7 to 36.5 (median, 11.4±5.3). Tumor size ranged from 1.5 to 11.0 cm (median, 5.3±2.0) on pathological examination. Tumor size showed significant positive correlations with SUV_{max} (r=0.293; p=0.001) (Fig. 1A) and CEA level (r=0.253; p=0.003) (Fig. 1B). No correlation was noted between CEA level and SUV_{max} (r=0.151; p=0.083) (Fig. 1C). EGFR expression was found in 86 patients (65.2%). Mean SUV_{max} was significantly higher in EGFR-expressing tumors than in EGFR-negative tumors (12.1±5.7 vs. 10.0±4.2; p=0.012) (Fig. 2).

At the SUV_{max} threshold of 7.5, the sensitivity and specificity for predicting EGFR expression were 84.9% and 40.4%, respectively [area under the curve (AUC)=0.624; p=0.019]. Tumor size (AUC=0.506; p=0.909) and CEA level (AUC=0.445; p=0.296) were not predictive of EGFR expression (Fig. 3).

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Table 2. Relationships between EGFR Status and Clinicopathological Parameters

Characteristic	Subcategory	EGFR expression (n=86)	Odds ratio	95% CI		Univariate *p* value	Multivariate *p* value
				Lower	Upper		
Age (yrs)	<64	33	0.633	0.291	1.373	0.245	0.363
	≥64	53					
Gender	Male	42	1.143	0.558	2.340	0.715	0.512
	Female	44					
Tumor stage	T1–2	16	0.921	0.361	2.349	0.863	0.620
	T3–4	70					
Lymph node metastasis	Absent	42	0.806	0.392	1.656	0.557	0.587
	Present	44					
Distant metastasis	Absent	76	0.877	0.297	2.589	0.812	0.783
	Present	10					
Histopathologic diagnosis	Adenocarcinoma	79	3.987	0.475	33.45	0.171	0.148
	Others	7					
Differentiation	Well differentiated	50	0.900	0.426	1.901	0.426	0.705
	Moderately to poorly differentiated	36					
Lymphovascular invasion	Absent	50	1.646	0.769	3.520	0.197	0.196
	Present	36					
Perineural invasion	Absent	77	0.779	0.259	2.344	0.656	0.469
	Present	9					
SUV_{max}	>7.5	73	2.457	1.038	5.816	0.038	0.041
	≤7.5	13					
Tumor size (cm)	≤5	44	1.136	0.554	2.330	0.727	0.707
	>5	42					
CEA level (ng/mL)	≤5	50	0.720	0.554	2.330	0.370	0.210
	>5	36					

EGFR, epidermal growth factor receptor; CI, confidence interval; SUV_{max}, maximum standardized uptake value; CEA, carcinoembryonic antigen.

EGFR status showed no difference with respect to most of the clinicopathological parameters (Table 2). Only SUV_{max} was significantly associated with EGFR expression (p=0.038). Multivariate logistic regression also revealed SUV_{max} as an independent predictor of EGFR expression (p=0.041, odd ratio=2.457, confidence interval 95% 1.038–5.816).

DISCUSSION

The utility of ^{18}F-FDG PET has been studied extensively in colon cancer. ^{18}F-FDG is metabolized similarly to glucose and transported intracellularly; once phosphorylated, ^{18}F-FDG-6-phosphate is not processed by the glycolytic pathway and accumulates preferentially in cells with high glucose uptake, such as tumor cells. The accuracies of ^{18}F-FDG PET in the assessment of primary tumors and detection of metastases of CRC have been demonstrated.[16,18] However, incidental physiological bowel uptake or inflammation may yield a false-positive finding.[19] In addition, ^{18}F-FDG PET lacks resolution to evaluate the depth of tumor penetration through the bowel wall.[20] Nevertheless, focal intense hypermetabolism is highly suggestive of colon cancer.[21]

Abdel-Nabi, et al.[22] demonstrated that ^{18}F-FDG PET has a sensitivity of 100%, a specificity of 43%, and positive and negative predictive values of 90% and 100%, respectively, for primary CRC. Moreover, Mukai, et al.[23] demonstrated that the ^{18}F-FDG PET true positive rate is 95.8% in CRC. In the present study, all the primary tumors were seen on ^{18}F-FDG PET scans, and a significant correlation was found between size and ^{18}F-FDG uptake of the primary tumor (r=0.298; p=0.001). This finding is similar to that of Gu, et al.,[24] who showed that SUV_{max} is significantly related to size and depth of invasion of the primary tumor. They explained this result by the increasing number of tumor cells with increased tumor size. Na, et al.[25] also demonstrated that the degree of ^{18}F-FDG uptake is associated with tumor size in primary CRC. The authors consider that ^{18}F-FDG uptake of the primary tumor is correlated with macroscopic and microscopic tumor growth.

Activation of the proto-oncogene encoding EGFR may contribute to transformation of cellular phenotypes and create tumor cells with substantial growth and survival advantages.[26] Recently, EGFR status has received much attention because EGFR and some downstream components serve as targets for anticancer therapies. Preclinical data indicate that pharmacological blockade of EGFR is an effective therapeutic strategy in

advanced CRC.[27] Although EGFR status is a useful tool to predict the therapeutic response, data on its association with ^{18}F-FDG uptake in CRC are insufficient and contradictory. Weihua, et al.[28] suggested that glucose uptake is associated with EGFR expression. However, Na, et al.[25] found no significant difference in ^{18}F-FDG uptake according to EGFR status in CRC. In the present study, ^{18}F-FDG uptake tended to be higher in primary tumors expressing EGFR. The results also suggest that SUV_{max} of the primary tumor could indicate EGFR status, despite the low sensitivity and specificity. Larger-scale studies are needed to validate these conclusions.

There were several limitations in this study. First, because of its retrospective nature, selection bias was unavoidable. Second, *KRAS* and *BRAF* mutations, potential biomarkers of resistance to anti-EGFR monoclonal antibodies,[29,30] were not analyzed. ^{18}F-FDG uptake with respect to *KRAS* and *BRAF* mutation status should be investigated. Third, we could not evaluate the correlation between EGFR and the molecular markers directly related with FDG uptake such as glucose transporter and hexokinase. Finally, we also could not evaluate the prognostic significance of FDG uptake and EGFR status of primary CRC because of lack of follow-up data. Further large-scale studies are needed.

In summary, preoperative ^{18}F-FDG uptake is slightly correlated with EGFR status in primary CRC. Preoperative SUV_{max} of ^{18}F-FDG may have a limited role in predicting EGFR expression in such tumors because of its poor specificity.

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